Aquatic Ecosystems

Interactivity of Dissolved Organic Matter

Edited by
Stuart E. G. Findlay • Robert L. Sinsabaugh

Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

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Preface

Dissolved organic matter (DOM) is the major form of organic matter in almost all aquatic ecosystems. It plays significant roles in aquatic food webs, mediates the availability of dissolved nutrients and metals, and modifies the optical properties of water bodies. The contribution of DOM to global carbon budgets, including potential storage in the deepsea and responsiveness to climate change, is only beginning to be addressed. Because of the importance of DOM for a host of different questions, active research areas are extremely diverse and each discipline is applying new conceptual models and analytical approaches. Chapters in this volume describe the state of the art for a range of topics and synthesize the progress made via diverse approaches.

The scope is limited to issues relevant to the sources and bioavailability of DOM and the interface between DOM and several ecosystem-level processes such as nutrient/toxicant transformations and retention. This volume contains overviews of (1) the supply of DOM to aquatic ecosystems and variation in DOM composition, (2) processes mediating the transfer of

DOM into microbial food webs, and (3) illustrations of diverse approaches to synthesis of knowledge on these topics. Of necessity, the book does not explicitly include the large literature on geochemistry (e.g., DOM–dissolved metal interactions) or water quality (e.g., generation of halogenated dissolved organic compounds) but focuses on DOM supply, diversion to food webs, and effects on aquatic ecosystems. There is also some restriction in geographic scope, dealing primarily with the terrestrial–estuarine segments of DOM generation and transport, and we do not try to encompass, for instance, all the current research on DOM sources and composition in the world's oceans (see Hansell and Carlson, 2002).

Dissolved organic matter is commonly a major term in carbon, energy, and nutrient budgets for aquatic ecosystems and, as such, can have broad effects on food webs, heterotrophy, and nutrient retention/release. Motivation for this synthesis of knowledge about DOM is driven by both the quantitative importance of DOM in material budgets *and* the large number of significant interactions between DOM and other ecosystem processes and components. A consequence of the appreciation that DOM is linked directly and indirectly to many ecosystem processes and components is that DOM metabolism can affect nutrient balances of aquatic ecosystems. In the simplest case, loadings of bioavailable dissolved organic carbon into nutrient-rich receiving waters can lead to significant conversion of inorganic nutrients to organic forms, changing the inorganic nutrient concentrations and ratios delivered to ecosystems further downstream. Conversely, the organic nutrients can be released by microbial metabolism of DOM with obvious effects on the likelihood of eutrophication of downstream waters. The supply of total organic carbon (largely DOC) relative to inorganic nutrients can shift the net ecosystem metabolism from heterotrophy to autotrophy by fueling either respiration or primary production. Therefore, understanding the supply and availability of DOM and its interactions with inorganic nutrients affects whole-ecosystem processes such as respiration as well as the likelihood that an ecosystem will retain or export nutrients.

There is also growing evidence that the nature of DOM will affect microbial community structure and function. As we apply the new tools to identify microbial taxa in natural ecosystems, we can begin to delimit the relative importance of trophic and edaphic factors in influencing microbial community structure. The apparent two-way link between microbial community composition and DOM composition allows for further complexity in the dynamics of both DOM and bacterial groups.

The control exerted by specific landscape elements (wetlands, certain soil characteristics) and hydrology on DOM delivery to aquatic ecosystems is becoming clearer, providing a nice example of how physical structure affects material movements among ecosystems. Somewhat less appreciated are the mechanisms by which DOM can alter small-scale physical characteristics, ranging from light penetration to augmenting the physical heterogeneity of

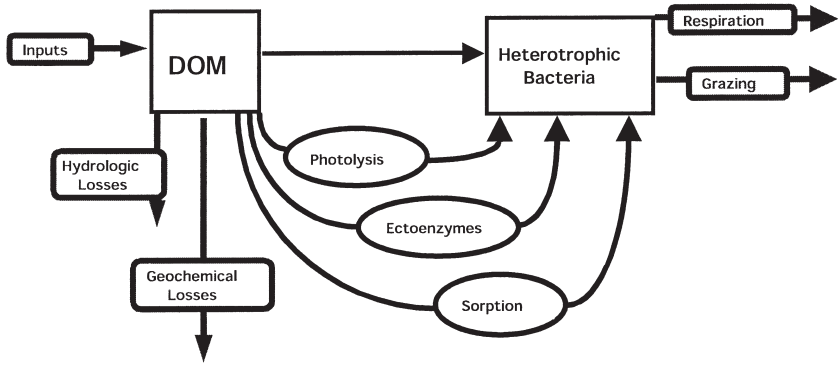
the aqueous medium. A major theme derived from this volume is that DOM takes part in multiple interactions with external and internal physical processes, interacts with cycling of other elements and compounds, and concurrently influences, and is influenced by, microbial communities.

This volume is organized into three major sections: (1) Sources and Composition, (2) Transformation and Regulation, and (3) Approaches to Synthesis.

DOM is supplied to aquatic ecosystems from both external (allochthonous) and internal (autochthonous) sources, and differences in the ultimate origin combined with differential transformation during transport may lead to significantly different behavior of autochthonous versus allochthonous DOM. The actual composition of DOM is highly variable, but, in general, this material contains a large proportion of dissolved humic materials together with a host of simpler compounds. Although there has been substantial analytical progress in describing components of DOM, there are no general rules for knowing the level of specificity necessary to answer particular questions.

There are four major transformation pathways leading from the DOM pool into the microbial loop: direct uptake and photolysis-, ectoenzyme-, and sorption-mediated uptake (Fig. 1). Each of these pathways or processes is regulated by a combination of intrinsic and extrinsic factors. Intrinsic factors are elements of the pathway itself and include DOM characteristics, enzyme kinetics, and microbial diversity. For instance, the uptake characteristics of the resident microbial community will affect which monomers are assimilated from the pool of DOM. Conversely, the composition of the DOM pool is likely to affect which microbial consortia are present and active at any given time.

The volume concludes with a series of chapters dealing with modeling (theoretical, conceptual, and empirical) to illustrate the diverse approaches to synthesizing this rapidly growing field.



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SECTION

ONE

SOURCES AND COMPOSITION

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1

Supply of Dissolved Organic Matter to Aquatic Ecosystems: Autochthonous Sources

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I. INTRODUCTION

The photosynthetic production of dissolved organic matter (DOM) in marine and freshwater ecosystems is a major source of metabolic substrates

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for heterotrophic microorganisms and influences both the activity and the composition of aquatic microbial communities (Pomeroy, 1974; Azam and Cho, 1987). Such internally produced (autochthonous) DOM is essentially derived from algae and macrophytes. The algal component, which is typically dominated by phytoplankton in lentic ecosystems and periphyton in lotic ecosystems, has received the lion's share of attention as an autochthonous source of DOM (Baines and Pace, 1991; Münster, 1993). This attention is not unwarranted given the biologically labile nature of many molecules released by algae to the environment and the large impact of these molecules on many aquatic systems (i.e., marine, estuarine, and some freshwaters). Macrophytes, on the other hand, which include liverworts, mosses, and vascular plants attached to or rooted in the substrata of lakes and streams (Sculthorpe, 1967), have received much less attention as autochthonous sources of organic carbon in natural waters. This is somewhat surprising because macrophytes can be dominant photosynthetic organisms in many water bodies and thereby also a large contributor of DOM.

In this chapter, we review and discuss the role of autochthonous sources of DOM in surface waters. Our focus is on the input of DOM from algae and macrophytes within aquatic ecosystems and largely confined to the major pools of DOM potentially available for use as substrates by heterotrophic bacteria. Hence, we will not consider phytoplankton/macrophyte release of specific volatile organics, vitamins, antibiotics, toxic compounds, or enzymes, unless they fall within the scope stated above.

II. ALGAL SOURCES OF DISSOLVED ORGANIC MATTER

A. Algal Production of Organic Compounds

Microalgae are important sources of organic matter in most aquatic systems as they transform solar energy into reduced carbon compounds. Upon cell death, the photosynthetically fixed organic substances are released to the surrounding water, either directly as dissolved compounds or as particulate detritus that can act as a secondary source of DOM (Azam and Cho, 1987). A similar release of phytoplankton-derived DOM can result from herbivore grazing. With grazing, a significant fraction of phytoplankton biomass can be released to the surrounding medium rather than being used by the grazer (Lampert, 1978).

In addition to production of DOM through various processes following phytoplankton cell death, a fraction of the photosynthetically fixed carbon is also released as dissolved compounds from actively growing cells (Fogg, 1983; Baines and Pace, 1991). The organic compounds that are released or lost from phytoplankton through these different mechanisms either are directly available for use by heterotrophic microorganisms or can be transformed to such substrates through various enzymatic and abiotic

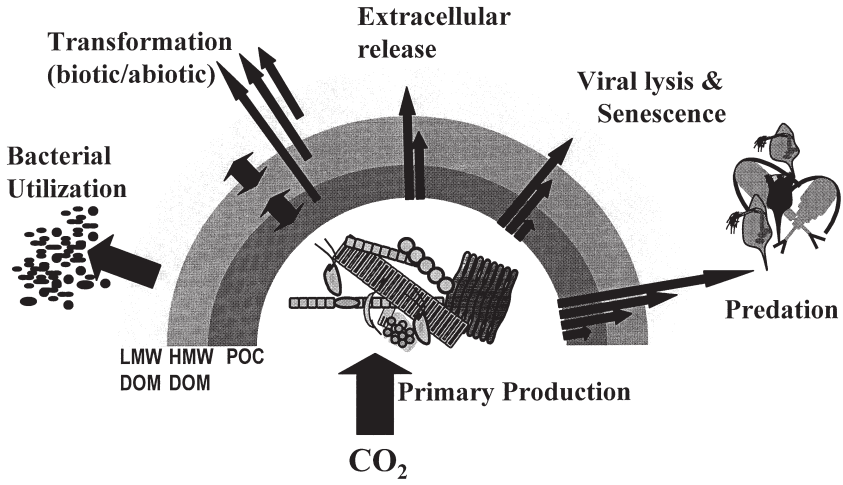

FIGURE 1 Fate and major transformation pathways of phytoplankton- and macrophyte-derived DOM in aquatic systems. Arrows indicate fluxes; POC denotes particulate organic matter; LMW and HMW DOM refer to the monomeric (low molecular weight) and polymeric (high molecular weight) fractions, respectively.

(e.g., photolysis) processes (Fig. 1). The global importance of the net carbon transfer from phytoplankton to heterotrophic microorganisms can be illustrated by some recent budget estimates of the fate of marine autotrophic production (Duarte and Cebrián, 1996). This study presents a compilation of data from 154 reports of marine net primary production and associated carbon flows (decomposition, herbivory, export, and storage) and concludes that 36% of the total net primary production is directly lost through microbial decomposition of dissolved and particulate organic matter, whereas a slightly larger fraction (52%) is transferred to herbivores through predation (Duarte and Cebrián, 1996). However, there are also examples of uncoupling between primary producers and heterotrophic microorganisms in aquatic systems (e.g., lotic systems; Findlay *et al.*, 1993; Rosenfeld and Hudson, 1997). Variations in the strength of this interaction could have a major influence on the fate of photosynthetically produced organic material, at least locally.

The major biopolymers in phytoplankton, as in all living cells, are proteins, polymeric sugars, and lipids, whereas a substantial fraction of the total cellular biomass (10–35%) consists of various monomeric compounds of low molecular weight (<600 Da; Morris, 1981; Siuda and Wcisko, 1990; Hama, 1992; Lignell and Lindqvist, 1992). It should be noted that there is considerable variation in the contribution of these different molecules to total biomass both among different phytoplankton species and among populations in different physiological states (Morris, 1981; Biddanda and Benner, 1997).

Nutrient availability influences the distribution of biomass among cellular constituents, with high nutrient availability resulting in more protein rich cells (Hama and Honjo, 1987; Hama, 1988), whereas elevated light and increased ultraviolet (UV) irradiation produces a lower protein content (Morris and Skea, 1978; Goes *et al*., 1995, 1996). From these studies and others, it is evident that environmental conditions have a strong effect on the quality of photosynthetically produced organic compounds in phytoplankton. Interestingly, variation in the contribution of these major biomolecules to total biomass among phytoplankton species and among different physiological states does not seem to result in any major alteration in the elemental composition of phytoplankton cells. Widely different phytoplankton groups in different growth phases (stationary vs. exponential growth phase) typically have C : N ratios close to the Redfield ratio (C : N 6.6; Goldman *et al*., 1979; Morris, 1981; Biddanda and Benner, 1997).

B. Pathways for Algal Dissolved Organic Matter Production

Principally, all cell constituents may be released to the surrounding water as cells either senesce (gradual aging process following cell death) or lyse due to viral infection (Fuhrman, 1992; Bratbak *et al*., 1994; Murray and Eldridge, 1994). The latter process mediates a very rapid and efficient transfer of cell constituents to the DOM pool or to particulate detritus (Wommack and Colwell, 2000; see Chapter 16). Predation is another important cause of algal cell death. Predatory ingestion typically results in part of the cell material being released as DOM or particulate material due to "sloppy feeding." This process can be a significant mechanism for release of phytoplankton cell material as DOM (Jumars *et al*., 1989). Part of the ingested algal biomass will also be rapidly released through direct excretion from the predators, and such a release can stimulate both algal and bacterial growth in aquatic systems (Persson, 1997; Vanni and Layne, 1997). Still, compared to viral lysis and nonpredatory cell death, herbivore consumption grazing generally diverts a lot of the algal-produced DOM from being utilized by the microbial component of aquatic food webs (Fig. 1). This predatory bypass could be a key component in regulating carbon fluxes, particularly in marine systems. Duarte and Cebrián (1996) estimated that the flux of carbon into grazers could account for 40–57% of the total organic carbon fixed by marine phytoplankton. Release of DOM from algal-derived particulate detritus is largely regulated by extracellular enzymatic degradation (Biddanda, 1988), and the role of this process is discussed further in one of the following chapters of this volume (see Chapter 13). The quantitative importance of the different DOM-releasing processes described above is likely to vary as a result of the chemical and biological character of the ecosystem; the balance among the different mechanisms may have a pro-

found influence on the amount and quality of phytoplankton biomass that is transferred to the DOM pool.

The release of DOM to the surrounding water from actively growing phytoplankton has been recognized for some time. Since DOM release was first reported some 70 years ago for a culture of marine *Clamydomonas* sp. (Braarud and Føyn, 1930), it has been shown to be universal, though highly variable, among phytoplankton species and aquatic systems (Hellebust, 1965; Fogg, 1983). High release rates of dissolved organic compounds reported in some of the early studies may need to be interpreted with caution and may have been due to experimentally introduced artifacts. For example, Sharp (1977) points out several critical factors in the methods commonly employed to study this phenomenon (use of cultures, bottle effects, ^{14}C–Na_2CO_3 purity, filtration-induced damage to cells). In spite of these methodological pitfalls, it is generally accepted that phytoplankton-mediated release of DOM is a normal component of photosynthesis (Smith *et al*., 1977; Mague *et al*., 1980; Baines and Pace, 1991).

Extracellular release of DOM may occur via passive leakage across cell membranes or through active excretion by the algae. It should be noted that neither the function nor the exact mechanism of the release of organic carbon from phytoplankton is fully understood, but extracellular release can be a substantial loss of photosynthetically fixed organic carbon and can occur at high energetic cost for the cell (Fogg *et al*., 1965). One frequently hypothesized explanation for exudation is that it is a means for the algae to dispose of excess reducing power (reduced organic compounds) that periodically overwhelm the cell's ability to synthesize new cell material (Fogg, 1983). This possible explanation, however, is contradicted by the fact that exudates are also released from phytoplankton under dark conditions (i.e., when no photosynthesis occurs). Therefore, a more likely explanation is that dissolved exudates are simply released to the surrounding water by passive, diffusion-driven leakage across cell membranes. Alternatively, that there is another, unknown role of the released exudates. This could, for example, be related to the fact that exudates create nutrient- and bacteria-enriched microzones around the algae (Azam and Cho, 1987), possibly affecting grazing pressure and nutrient availability. Algae that generate an enriched microzone may also reduce their exposure to infectious viruses by more than 50% simply by maintaining a surrounding layer with high abundances of bacteria and microflagellates which are able to remove the viral particles (Murray, 1995).

The vast majority of studies assessing the quantity of extracellularly released organic compounds from primary producers have relied on different experimental protocols based on the ^{14}C-tracer technique developed to measure photosynthesis in marine waters (Steeman-Nielsen, 1952). Using this technique, the extracellular DOM release by phytoplankton has been studied in both pure cultures and natural water samples. From pure culture

studies, it has been shown that the fraction of total primary production released as extracellular organic matter varies substantially among algal species, even under identical growth conditions (range 0–25% of net primary production; Hellebust, 1965; Fogg, 1971). The size of the phytoplankton cells has also been identified as one potentially important factor determining the fraction of photosynthetically produced organic compounds released extracellularly; in a comparison of extracellular release of DOM from picoeukaryotes and nanoplanktonic algae, the smaller cells released a substantially higher fraction of their total primary production as exudates (Malinsky-Rushansky and Legrand, 1996).

For lentic ecosystems, the fraction (%) of photosynthetically fixed organic carbon released extracellularly (PER; percentage extracellular release) varies dramatically (1–99.9% of net primary production; Table I). PER has been shown to be negatively related to total primary production (Anderson and Zeutschel, 1970; Mague *et al*., 1980; Vegter and Visscher, 1984; Morán and Estrada, 2000). Hence, phytoplankton in oligotrophic waters should release a larger fraction of their photosynthetically fixed organic carbon as dissolved compounds than algae in more productive environments. However, this pattern does not seem to be universal, and there are several studies that report either very weak or no correlation at all between productivity and PER (e.g., Williams and Yentsch, 1976; Wolter,

TABLE I Selected Reports of Percentage Extracellular Release (PER) of Photosynthetically Fixed Carbon in Cultures, Freshwaters, and Marine Waters

System	*PER range (%)*	*Reference*
Coastal Atlantic Ocean	0–23	Williams and Yentsch (1976)
	4–30	Mague *et al*. (1980)
	~23	Norrman, *et al*. (1995)
Coastal Pacific Ocean	11–17	Anderson and Zeutschel (1970)
	8–9	Iturriaga and Zsolnay (1983)
Baltic Sea	0.8–23	Larsson and Hagström (1982)
	2–17	Lignell (1990)
North Sea	<1–80	Lancelot (1983)
Southern Ocean	3–47	Morán and Estrada (2000)
Temperate lakes	6–40	Sundh (1991)
	30.5–99	Sell and Overbeck (1992)
	<1–62	Vegter and Visscher (1984)
	5–46	Søndergaard *et al*. (1985)
Phytoplankton cultures		
Axenic marine phytoplankton	2.5–35	Hellebust (1965)
Chaetoceros affinis cultures	15–37	Obernosterer and Herndl (1995)
±axenic marine phytoplankton	4–29	Malinsky-Rushansky and Legrand (1996)
Nonaxenic marine phytoplankton	11–32	Biddanda and Benner (1997)

1982). In a large survey using data from 16 different studies (both lakes and marine waters), Baines and Pace (1991) concluded that, for the freshwater dataset, there appeared to be a negative correlation between total primary production and PER (from 5 to 40% with decreasing productivity), whereas no such general pattern could be observed for the marine and estuarine dataset. Furthermore, for both types of ecosystems, the absolute extracellular release was primarily related to the total photosynthetic production of carbon rather than to the total phytoplankton biomass, indicating that release is closely associated with actively growing phytoplankton cells.

Nutrient deficiency has also been suggested to be an important regulator of algal release of DOM, with an increased release of DOM when one or more nutrients are in low supply (Fogg, 1983; Obernosterer and Herndl, 1995). Sharp (1977) showed that even small experimental manipulations in nutrient concentrations induced increased release of algal DOM, but attributed this to "cultural shock" rather than an impact of nutrient deficiency *per se*. These studies are also supported by field data from the North Sea (Lancelot, 1983) where *in situ* nitrogen availability was negatively correlated to the release of exudates from marine algal communities dominated by either dinoflagellates or *Phaeocystis* sp. However, even if these observations strongly support the concept of elevated algal excretion under nutrient deficiency, this concept does not seem to be valid for all groups of phytoplankton (e.g., diatoms; Lancelot, 1983).

Light intensity is another factor suggested to play a major role in regulating algal release of DOM (Hellebust, 1974). On the one hand, elevated release of DOM has been observed upon sudden exposure of natural water and algal cultures to high light levels (Fogg *et al*., 1965; Sharp, 1977; Mague *et al*., 1980). The physiological explanation for this elevated extracellular release is currently unclear but could include membrane damage (Hellebust, 1974) or inhibition of certain metabolic processes. On the other hand, ambient fluctuations in light levels appear to influence extracellular release primarily by modulating primary productivity, whereas the relative release of extracellular DOM (PER) is less affected (Nalewajko, 1966; Lancelot, 1983).

Autochthonous production of DOM in lotic ecosystems has received far less attention; however, a similar release of DOM from algae likely occurs. In a survey of organic matter budgets for 29 streams drawn from most of the major biomes, in-stream primary production accounted for more than 40% of total carbon inputs in eight streams and more than 80% of inputs in five streams (Webster and Meyer, 1997). The contribution of algae to stream carbon budgets is undoubtedly greatest in streams with open stream channels or during periods of high light as during early spring or autumn. For example, in Deep Creek in the Great Basin Desert of Idaho, algal production accounted for 75–85% of total energy inputs into the stream (Minshall, 1978). Similarly, in Sycamore Creek in the Sonoran Desert of Arizona, benthic algae production accounted for 99% of organic matter

inputs (Grimm, 1987; Jones *et al*., 1997; Schade and Fisher, 1997). In the most extreme case, algal growth accounts for nearly 100% of organic matter inputs in streams of McMurdo Dry Valleys of Antarctica (McKnight and Tate, 1997). The high contribution of algal growth to stream organic matter budgets is not restricted to desert streams, but also occurs during periods of high light in mesic regions. In White Clay Creek, a piedmont stream in Pennsylvania, algal biomass is as high as 100 mg Chlorophyll-*a* m^{-2} during vernal algal blooms that occur before trees leaf out and block sunlight penetrating to the stream channel (Kaplan and Bott, 1982).

Presumably, this high contribution of in-stream production to stream carbon budgets translates to DOM inputs. As evidence, DOC concentration in White Clay Creek exhibited a diel change with an afternoon maximum as much as 40% greater than the daily minimum due to photosynthetic fixation and subsequent extracellular release (Kaplan and Bott, 1982, 1989). DOC release for two species of benthic algae averaged 78 μg C mg Chlorophyll-a^{-1} h^{-1} and was as high as 132 μg C mg Chlorophyll-a^{-1} h^{-1} (Kaplan and Bott, 1982). As in lentic ecosystems, bacteria in streams rapidly assimilate the input of DOM from periphyton. In White Clay Creek, bacterial activity tracked the increase in stream DOM with an increase in activity of 1.4 to 3.0 times from morning to afternoon. Similarly, in Sycamore Creek, ecosystem respiration in the stream sediments was over threefold greater during the day than at night (Jones *et al*., 1995). Respiration measurements in Sycamore Creek were conducted in the field and laboratory to control for temperature effects, indicating that diel changes in sediment activity were indeed due to changes in DOM supply. Moreover, sediment respiration in Sycamore Creek is highest in regions of hydrologic downwelling, where surface water flows into underlying sediments. This high respiration is presumably due to an influx of algal-derived DOM (Jones *et al*., 1995).

C. Chemical Composition of Algal-Derived Dissolved Organic Matter

Our knowledge of the molecular-level chemical composition of extracellularly released compounds from phytoplankton originates almost exclusively from studies of pure cultures of different types of phytoplankton (reviewed by Hellebust, 1974). One of the few exceptions is a study by Kaplan and Bott (1989) in which acetate concentration reached maximum concentration in late afternoon during a vernal algal bloom. The authors suggest that algal excretion could be the source of this compound, but alternative processes, such as bacterial fermentation, could also explain this pattern. We would also like to add that a photochemical source is likely to contribute to this diurnal pattern because acetate is one of the major low-molecular-weight compounds produced from photolysis of natural organic matter (Bertilsson and Tranvik, 2000; see Chapter 10).

Extracellularly released low-molecular-weight DOM from algal cultures is mainly composed of one or more of the following groups of compounds: (i) monomeric sugars, (ii) carboxylic acids, (iii) amino acids, and (iv) alditols. Additionally, phytoplankton release of other organic compounds such as ketones and aldehydes has been reported (Jalliffier-Merlon, *et al.*, 1991; Nuccio *et al.*, 1995). In an early study, Hellebust (1965) screened the photosynthetic release of exudates from a wide range of algal cultures and found considerable variability among species although alditols such as glycerol and mannitol, as well as a range of amino acids and some sugars (glucose, arabinose), were the major products in many cultures. This early study was performed using a combination of the ^{14}C tracer technique and subsequent thin layer chromatography combined with autoradiographic detection to visualize and quantify exudates. The use of such a technique could have resulted in a possible exclusion of several compounds from the analysis and a high risk for inaccurate identification of detected compounds. A similar separation was used to measure the release of individual amino acids and sugars from some planktonic algal cultures (Maršálek and Rojičková, 1996). Four sugars (xylose, galactose, glucose, and arabinose) were produced in equal amounts among the different cultures, whereas five amino acids were reported (glycine, cysteine, glutamine, glutamic acid, and proline). In a study of a hot spring isolate of *Synechococcus* sp., a multidimensional reverse-phase liquid chromatography technique was used to screen the organic compounds released from a *Synechococcus* culture (Teiser, 1993). A wide range of low-molecular-weight organic acids, sugars, and sugar alcohols was released, with fructose, adonitol, and formic, acetic, oxalic, β-hydroxypyruvic, succinic, citric, and glycolic acids among the most abundant.

An alternative approach for screening the composition of phytoplankton exudates is to use either ^{14}C-tracer methods combined with chemical fractionation (Hama and Handa, 1987; Siuda and Wcisko, 1990; Sundh, 1991) or colorimetric methods (Obernosterer and Herndl, 1995; Biddanda and Benner, 1997) to characterize the contribution of different classes of organic compounds (carbohydrates and amino acids in polymeric or monomeric forms) to the total pool of exudates. These studies revealed that monomeric and combined carbohydrates were the major components of exudates, typically accounting for 20–90% of the total extracellularly released DOM.

As previously noted, organic compounds released from phytoplankton consist not only of low-molecular-weight compounds but also of high-molecular-weight polymeric organic compounds (Fogg, 1977). Early experimental results indicate that compounds with a molecular weight higher than 1500 Da may even dominate the pool of extracellularly released organic compounds (Nalewajko and Schindler, 1976). The experimental approach to demonstrate the size distribution of extracellularly released DOM varies. Many researchers have combined the commonly used ^{14}C–$NaHCO_3$ tech-

nique to measure primary production with a size fractionation of the ^{14}C-labeled DOC released from the phytoplankton (McKinley *et al*., 1977). Using this approach in a eutrophic lake, low-molecular-weight DOM (<700 Da) dominated the extracellularly released fraction under daylight conditions (typically constituting more than 70% of the total extracellularly released organic carbon; Søndergaard and Schierup, 1982), whereas high-molecular-weight compounds were the dominant products under dark conditions. Such a differential release of extracellular organic compounds during a diurnal cycle has also been observed in an axenic culture of the chlorophyte *Ankistrodesmus* (Vieira and Myklestad, 1986).

The finding that low-molecular-weight compounds dominate the pool of organic carbon released from actively photosynthesizing cells has also been observed in other eutrophic lakes (Hama and Handa, 1987) as well as in coastal waters (Iturriaga and Zsolnay, 1983) and an estuarine system (Jensen, 1983). In two of these studies (Jensen, 1983; Hama and Handa, 1987), the relative contribution of high-molecular-weight compounds increased with incubation time. This was interpreted as a need for longer incubation times to obtain a uniform labeling of cellular biomass and a resulting incorporation of ^{14}C also into larger, more complex polymers. A similar conclusion was made earlier by Saunders and Storch (1971) upon observing a time lag between radiolabels appearing in lake phytoplankton and in extracellularly released material. We would like to add that the pattern could also emerge if excreted low-molecular-weight compounds were being more rapidly utilized by heterotrophic bacterioplankton (see below). Finally, some data indicate substantial seasonal variability in the molecular size distribution of excretion (Lignell, 1990), with a large release of high-molecular-weight material associated with algal blooms.

A few studies have used nontracer methods to study the amount and quality of DOM released by phytoplankton. Kepkay *et al*. (1993) used ultrafiltration with a cutoff of 10,000 Da to separate various size classes of DOM and combined this fractionation with a subsequent analysis of the DOC in the different fractions using high-temperature catalytic oxidation. Their results support findings obtained using the tracer approach in that low-molecular-weight compounds are the dominant extracellularly released carbon compounds. However, it should be noted that DOM with a molecular weight less than 10,000 Da also includes many polymeric compounds. A limitation of this *in situ* methodology is the problem of discriminating the bulk DOM from newly released material from the phytoplankton. In the study mentioned above, the concentration of carbon in size fractions was studied during an episodic algal bloom, thereby allowing the authors to monitor bloom-related changes in the dissolved organic matter. Note, however, that the measures will be a composite of a multitude of processes, including grazing, cellular lysis, and bacterial metabolic processes, and release for a single time point or during nonbloom periods is virtually impossible

to monitor. In another recent study, ^{1}H-NMR (nuclear magnetic resonance) combined with gas chromatography–mass spectrophotometric analysis was used to study the chemical composition of high-molecular-weight exudates (>1000 Da; operationally defined as the material retained using tangential flow ultrafiltration with a 1000 Da cutoff) from different algal cultures (Aluwihare and Repeta, 1999). Their study indicates that the pool of high-molecular-weight algal exudates was between 7 and 37% of the total extracellular release and that high-molecular-weight exudates are comprised largely of polysaccharides that are structurally and compositionally similar to the microbially recalcitrant acyl-heteropolysaccharides commonly found in seawater.

D. Biological Availability of Algal-Derived Dissolved Organic Matter

The DOM released from algal cells has long been recognized as a high-quality substrate for bacteria (Cole *et al*., 1982). This is reflected not only in the rapid turnover of a large fraction of the DOM that is released from phytoplankton (Biddanda, 1988; Kirchman *et al*., 1991; Malinsky-Rushansky and Legrand, 1996; Petit *et al*., 1999), but also in elevated bacterial numbers and activity associated with algal blooms and high phytoplankton biomass (particularly in environments with negligible terrestrial or littoral influence; Nalewajko *et al*., 1980; Chróst *et al*., 1989; Gajewski and Chróst, 1995). Consequently, current estimates of the release of photosynthetically fixed organic matter may be severe underestimates due to concurrent bacterial uptake of released exudates (with the exception of studies involving axenic cultures or where adequate corrections are made). The fate of DOM utilized by bacteria is either a loss through mineralization or incorporation into bacterial biomass that can act as a source of nutrients and energy for higher trophic levels (Pomeroy, 1974; Hagström *et al*., 1988; Weiss and Simon, 1999). This simultaneous bacterial removal of organic compounds released by phytoplankton also means that the exudates that are most available to bacteria will largely escape chemical identification (Fig. 2).

The most commonly used approach to quantify the concurrent flux of algal exudates to heterotrophic bacteria is to combine the ^{14}C-tracer method with a differential filtration step in which free-living bacteria are physically separated from phytoplankton and excreted DOM (reviewed by Baines and Pace, 1991). The data presented in this review indicate that, on average, 46% of the excreted DOM is incorporated by bacteria. One limitation of this approach is that bacteria attached to particles are largely excluded from the analysis, but this can, to some extent, be overcome by monitoring the distribution of heterotrophic bacteria in both size fractions (Søndergaard *et al*., 1985).

In the case of eukaryotic phytoplankton, specific prokaryotic inhibitors may be used to block bacterial uptake. The application of this technique in

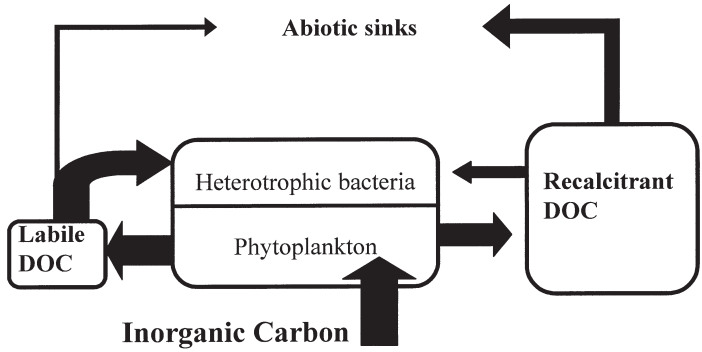

FIGURE 2 Relationship between biological availability of autochthonously produced DOM (labile/recalcitrant) and (i) concentration *in situ* and (ii) uptake by heterotrophic microorganisms. Box sizes indicate ambient concentrations, whereas arrow thicknesses indicate transformation rates.

estuarine waters indicated that a major fraction, between 34 and 90% of the released exudates, was utilized by bacteria during a short-time incubation (Jensen, 1983). By calculating the bacterial carbon demand using two different growth estimates, similar results were obtained in five lakes and a coastal water (between 55 and 84% were utilized by bacteria; Søndergaard *et al.*, 1985). Another possible way to circumvent this methodological problem is to measure the flux of ^{14}C–Na_2CO_3 into particulate and dissolved fractions over a time course and then fit the data to a three-compartment kinetic model (Wiebe and Smith, 1977; Morán and Estrada, 2000). Using this method in different open ocean waters, the estimated flux of exudates into bacteria was in the same range as in the studies of Jensen (1983) and Søndergaard et al. (1985). Clearly, there is a need to develop improved and standardized methods to measure both the transfer of carbon from phytoplankton to heterotrophic bacteria and the quality of the entire pool of released exudates, even the fractions that are rapidly utilized by accompanying microorganisms. In particular, aquatic environments with a high spatial and temporal heterogeneity such as wetlands, benthic mats, and biofilms, present a huge methodological challenge.

Long-term decomposition experiments have shown that a substantial fraction of the algal-derived DOM is resistant to bacterial mineralization over a period of several years (Fry *et al.*, 1996), and to the best of our knowledge, there are no reports of a complete bacterial utilization of exudates released from phytoplankton. It is uncertain whether this is due to the original properties of the phytoplankton-derived DOC or whether diagenetic changes during the course of the incubation render the material recalcitrant. Some data suggest that a fraction of algal exudates released is indeed more resistant to microbial degradation (e.g., high-molecular-weight acyl-heteropolysaccharides; Aluwihare and Repeta, 1999). This concept has

some support from earlier studies. For example, in a coastal water sample, phytoplankton exudates with a molecular weight greater than 500 Da were degraded more slowly than exudates of lower molecular weight (Iturriaga and Zsolnay, 1983).

In conclusion, the release of exudates appears to be controlled mainly by the total primary production (photosynthetic activity), and although very little is known about the detailed chemical composition of algal exudates, existing studies have shown that carbohydrates, organic acids, and dissolved and free amino acids may be significant constituents. However, to be able to fully understand the role of algal sources of DOM, there is clearly a need for more studies on the release and chemical composition of algal exudates, particularly as the availability of various fractions of the released DOM to bacterial utilization seems to vary extensively (Norrman *et al.*, 1995).

III. MACROPHYTE PRODUCTION OF DISSOLVED ORGANIC MATTER

At whole lake scales, littoral zones are a major component of autochthonous DOM production and important sources of labile organic matter for aquatic bacteria. Of the approximately one billion lakes in the world, the littoral zone accounts for more than 95% of lake surface area in nearly 99.8% of all lakes (Wetzel, 1983; Fig. 3a). The importance of shallow waters is even more marked when the bounds of lakes are expanded to include wetlands. With such an expanded view, the littoral zone and wetlands comprise more than 95% of the area in 99.999% of all lakes (Fig. 3a). Clearly, shallow waters are a dominant feature of lentic ecosystems.

Most lakes and many streams and rivers have a large proportion of their basins potentially available to macrophytes and epiphytes. Of the world's lakes, over 99.9% have surface areas that are smaller than 0.5 km^2 and have average depths less than 3.2 m (Wetzel, 1983). In addition to providing zones available for macrophyte colonization and growth, primary production rates in wetlands and littoral zones are among the highest reported for any ecosystem, considerably greater than for pelagic environments. Production by macrophytes ranges from approximately 0.15 to 15 kg organic matter m^{-2} yr^{-1} compared with less than 1 kg organic matter m^{-2} yr^{-1} in pelagic zones (Moss, 1998). Given the extent of habitat available for colonization, and the high production rates, macrophytes and littoral zones are potentially important sources of autochthonous DOM.

As with phytoplankton, macrophytes generate DOM either through extracellular release of photosynthate or following aging and subsequent release of dissolved and particulate constituents to the surrounding water. Information about extracellular release of DOM from macrophytes is scant. Available data indicate that extracellular release varies widely, with esti-

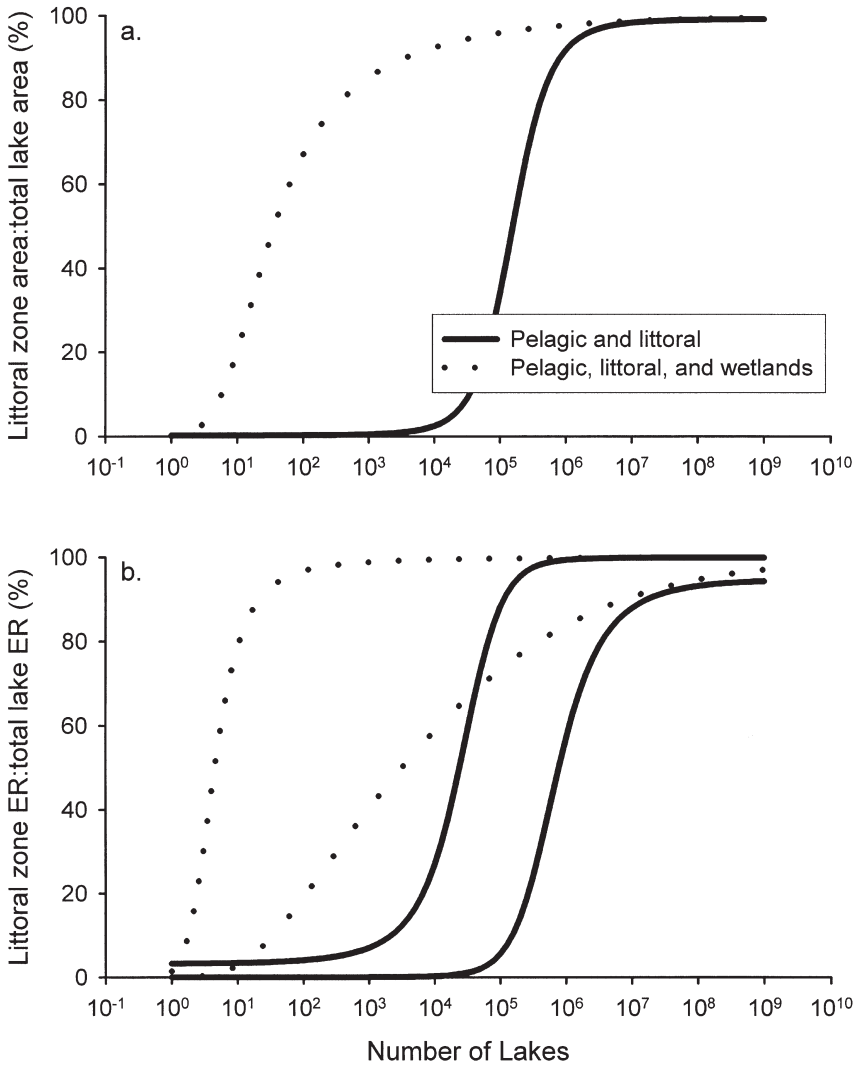

FIGURE 3 Proportion of lake area accounted for by littoral zones for the world's lakes (a), and the proportion of extracellular (ER) dissolved organic matter inputs derived from littoral zones (b; see text for description of the model). The solid lines illustrate relationships in which lake boundaries are restricted to littoral and pelagic zones and the dotted lines illustrate patterns in which lake boundaries are expanded to include adjacent wetlands. In (b), the two sets of lines illustrate the range in the contribution of littoral zones to total lake ER with variation in rates of primary production for phytoplankton (0.1–2.0 kg organic matter m^{-2} yr^{-1}) and macrophytes (0.6–3.8 kg organic matter m^{-2} yr^{-1}). The relationship between littoral zone area and number of lakes is from Wetzel (1983).

mates ranging from 0 to 44.5% of net primary production (Wetzel, 1969; Wetzel and Manny, 1972; Hough and Wetzel, 1975; Wetzel and Penhale, 1979). Moreover, loss of DOM through extracellular release occurs in both fresh and marine ecosystems at comparable rates (Wetzel and Penhale, 1979). Based on the limited available data, extracellular release from macrophytes averages 4 to 10% of net primary production (Wetzel and Manny, 1972).

In addition to extracellular release, a pulse of DOM is liberated from macrophytes following cell death. As with extracellular release of DOM from macrophytes, little information is available regarding DOM release upon senescence of macrophytes. Based on available information, a sizable proportion of the degradation products from macrophytes appears to be DOM. In *Spartina alterniflora*, DOC accounted for 50–60% of the degradation products from the lignin fraction of lignocellulose detritus (Moran and Hodson, 1990), and autolytic production of DOM may range from 30–40% of net production by macrophytes (Otsuki and Wetzel, 1974). Interestingly, production of autolytic DOM appears to increase in more senescent tissue. DOC released from *Juncus effusus* culms varied from approximately 50 μg C cm^{-2} of leaf area from fresh tissue to approximately 250 μg C cm^{-2} of leaf area from culms that were 50–75% senescent (Mann and Wetzel, 1996).

The DOM released from macrophytes, like the DOM released from phytoplankton, is quite labile. Of the DOM generated by *S. alterniflora*, 12% was decomposed within 16 hours and 30% was lost after 30 days (Moran and Hodson, 1989). With *J. effusus*, 31–52% of the DOM released was decomposed within 24 hours (Mann and Wetzel, 1996). Further, DOM from senescent culms was broken down significantly faster than that from fresh, photosynthetic leaves. Hence, senescent *J. effusus* culms not only generated more DOM, but generated DOM that was more labile. Moreover, much of the DOM derived from macrophytes is incorporated into bacterial biomass. Of the labile organic matter produced by *J. effusus* culms, 17–34% was incorporated into bacterial biomass (Mann and Wetzel, 1996). Similarly, in an experiment examining microbial uptake of DOC leachate from alligatorweed (*Alternanthera philoxeroides*) in the Ogeechee River, 53% of carbon was incorporated into bacterial biomass (Findlay *et al.*, 1986).

In addition to a preponderance of shallow waters and habitats available for macrophyte growth, production by macrophytes is, on average, eightfold greater than that by phytoplankton on an areal basis (Wetzel, 1983), suggesting a potentially large input of autochthonous DOM from macrophytes into lakes. To summarize the role of macrophytes in freshwater ecosystems, we conducted a simple modeling exercise to evaluate the potential contributions of DOM from macrophytes at whole lake scales. Our analysis weighted macrophyte production and extracellular release by the proportion of the world's lakes that are littoral or wetland zones. Assuming phyto-

plankton production ranges from 0.1 to 2.0 kg organic matter m^{-2} yr^{-1} and macrophyte production ranges from 0.6 to 3.8 kg organic matter m^{-2} yr^{-1} (range of mean values from Wetzel, 1983), and extracellular release rates of 13 and 5%, respectively, most autochthonous inputs of DOM into lentic systems are from macrophytes. Restricting lake boundaries to the littoral and pelagic zones reveals that in 95% of all lakes more than 94.4% of extracellular inputs are derived from macrophytes (Fig. 3b). Expanding boundaries to include wetlands indicates that in 95% of all lakes more than 97% of extracellular inputs are derived from macrophytes (Fig. 3b).

This major input of DOM from macrophytes is not restricted to lakes, but is also realized in other aquatic ecosystems. DOM export from watersheds in lotic ecosystems is directly related to annual runoff, but significantly greater in swamp-draining streams compared with upland-draining streams (Mulholland and Kuenzler, 1979; see Chapter 2 and 6). In the Hudson Estuary, planktonic bacterial production is 3 to 6 times greater than primary production (Findlay *et al.*, 1992). DOC derived from submerged aquatic plants in part supports the difference in bacterial carbon uptake and planktonic primary production.

Whereas uncertainty in the rates used in this brief analysis are considerable, the point is quite clear that at whole lake scales the vast majority of lakes have a large input of extracellular DOM from macrophytes. This analysis, however, does not discount the input of DOM into pelagic zones from phytoplankton. Clearly, in large lakes and oceanic systems, much of pelagic DOM will originate from phytoplankton. Moreover, DOM within pelagic and littoral zones is likely of localized origins and much of the DOM generated by macrophytes is undoubtedly decomposed within littoral zones before being transported to pelagic zones. Interestingly, in spite of a growing body of information highlighting the role macrophytes and wetlands play in DOM inputs into aquatic ecosystems, phytoplankton have received the lion's share of attention as sources of autochthonous DOM.

IV. SUMMARY

It is clear that autochthonous DOM of algal and macrophyte origin is an important contributor to the total pool of organic matter in most aquatic environments. The relative importance of these two sources (algae and macrophytes) varies between systems with a higher proportion of algal input in large lakes and marine waters, whereas macrophytes can be major contributors to DOM in smaller lakes where the littoral zone dominates the system. Production of DOM from aquatic algae and macrophytes occurs by several different mechanisms: (i) predatory grazing, (ii) cell death and senescence, (iii) viral lysis, and (iv) extracellular release. The balance among these mechanisms not only determines the temporal and spatial scale of the

DOM input, but also regulates the quantity of the plant biomass that is transformed to DOM and the quality of the dissolved material that is released.

Both algae and macrophytes release a significant proportion of their net primary production as dissolved organic matter during active growth. This material consists mainly of biologically labile, low-molecular-weight compounds. Despite an extensive literature on the quantitative and qualitative aspects of extracellular release of DOM from primary producers, we still know very little about the physiological explanation for this release or the effect it may have on the primary producers themselves and associated heterotrophic communities. This uncertainty may be due in part to the lack of standardized methodology in experimental methods, making qualitative and quantitative comparisons among various studies difficult. If we are to fully understand the role of this globally and locally important link in the carbon cycle, there is a clear need for future research in this area, for example, more complete chemical assessments of the various organic compounds that are being released from primary producers and how their physiology as well as environmental factors affect the quantity and quality of the released material. In addition, a more in-depth assessment of the functional links between heterotrophic microorganisms and aquatic primary producers, including kinetic studies of DOM release, transformation and uptake, as well as population level coupling between primary producers and heterotrophic microorganisms, may help us understand the influence of autochthonously produced DOM on aquatic ecosystems.

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REFERENCES

Aluwihare, L. I., and D. J. Repeta. 1999. A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae. *Marine Ecology Progress Series* **186:**105–117.

Anderson, G. C., and R. P. Zeutschel. 1970. Release of dissolved organic matter by marine phytoplankton in coastal and offshore areas of the northeast Pacific Ocean. *Limnology and Oceanography* **15:**402–407.

Azam, F., and B. C. Cho. 1987. Bacterial utilization of organic matter in the sea. *In* "Ecology of Microbial Communities" (M. Fletcher, C. R. G. Gray, and J. G. Jones, Eds.), pp. 261–281. Cambridge Univ. Press, Cambridge, UK.

Baines, S. B., and M. L. Pace. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. *Limnology and Oceanography* **36:**1078–1090.

Bertilsson, S., and L. J. Tranvik. 2000. Photochemical transformation of organic matter in lakes. *Limnology and Oceanography* **45:**753–762.

Biddanda, B. A. 1988. Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. II. Microbial metabolism. *Marine Ecology Progress Series* **42:**89–95.

Biddanda, B., and R. Benner. 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnology and Oceanography* **42:**506–518.

Braarud, T., and B. Føyn. 1930. Beitrage zur Kenntnis des Stoffwechsel in Meeren. I. *Matematik för Naturvetare* **14:**1–24.

Bratbak, G. F., F. Thingstad, and M. Heldal. 1994. Viruses and the microbial loop. *Microbial Ecology* **28:**209–221.

Chróst, R. J., U. Münster, H. Rai, D. Albrecht, P. K. Witzel, and J. Overbeck. 1989. Photosynthetic production and exoenzymatic degradation of organic matter in the euphotic zone of a eutrophic lake. *Journal of Plankton Research* **11:**223–242.

Cole, J. J., G. E. Likens, and D. L. Strayer. 1982. Photosynthetically produced dissolved organic carbon: An important carbon source for planktonic bacteria. *Limnology and Oceanography* **27:**1080–1090.

Duarte, C. M., and J. Cebrián. 1996. The fate of autotrophic production. *Limnology and Oceanography* **41:**1758–1766.

Findlay, S., L. Carlough, M. T. Crocker, H. K. Gill, J. L. Meyer, and P. J. Smith. 1986. Bacterial growth on macrophyte leachate and fate of bacterial production. *Limnology and Oceanography* **31:**1335–1341.

Findlay, S., M. L. Pace, D. Lints, and K. Howe. 1992. Bacterial metabolism of organic carbon in the tidal freshwater Hudson Estuary. *Marine Ecology Progress Series* **89:**147–153.

Findlay, S., K. Howe, and D. Fontvielle. 1993. Bacterial–algal relationships in streams of the Hubbard Brook. *Ecology* **74:**2326–2336.

Fogg, G. E. 1971. Extracellular products of algae in freshwaters. *Archiv für Hydrobiologie* **5:**1–25.

Fogg, G. E. 1977. Excretion of organic matter by phytoplankton. *Limnology and Oceanography* **22:**576–577.

Fogg, G. E. 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica Marina* **26:**3–14.

Fogg, G. E., C. Nalewajko, and W. D. Watt. 1965. Extracellular products of phytoplankton photosynthesis. *Proceedings of the Royal Society of London Series B* **162:**517–534.

Fry, B., C. S. Hopkinson, and A. Nolin. 1996. Long-term decomposition of DOC from experimental diatom blooms. *Limnology and Oceanography* **41:**1344–1347.

Fuhrman, J. 1992. Bacterioplankton roles in cycling of organic matter: The microbial food web. *In* "Primary Productivity and Biogeochemical Cycles in the Sea" (P. G. Falkowski and A. D. Woodhead, Eds.), pp. 361–383. Plenum, New York.

Gajewski, A. J., and R. J. Chróst. 1995. Production and enzymatic decomposition of organic matter by microplankton in a eutrophic lake. *Journal of Plankton Research* **17:**709–728.

Goes, J. I., N. Handa, S. Taguchi, T. Hama, and H. Saito. 1995. Impact of UV radiation on the production patterns and composition of dissolved free and combined amino acids in marine phytoplankton. *Journal of Plankton Research* **17:**1337–1362.

Goes, J. I., N. Handa, S. Taguchi, T. Hama, and H. Saito. 1996. Metabolism of neutral monosaccharide constituents of storage and structural carbohydrates in natural assemblages of marine phytoplankton exposed to ultraviolet radiation. *Limnology and Oceanography* **41:**1478–1489.

Goldman, J. C., J. J. McCarthy, and D. G. Peavey. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* **279:**210–215.

Grimm, N. B. 1987. Nitrogen dynamics during succession in a desert stream. *Ecology* **68:**1157–1170.

Hagström, Å., F. Azam, A. Andersson, J. Wikner, and F. Rassoulzadegan. 1988. Microbial loop in an oligotrophic pelagic marine ecosystem: Possible roles of cyanobacteria and nanoflagellates in the organic fluxes. *Marine Ecology Progress Series* **49:**171–178.

Hama, T. 1988. ^{13}C-GC-MS analysis of photosynthetic products of the phytoplankton population in the regional upwelling area around the Izu Islands, Japan. *Deep Sea Research* **35:**91–110.

Hama, T. 1992. Primary productivity and photosynthetic products around the Kuroshio warm-core ring. *Deep Sea Research* **39:**279–293.

Hama, T., and N. Handa. 1987. Pattern of organic matter production by natural phytoplankton population in a eutrophic lake. 2. Extracellular products. *Archiv für Hydrobiologie* **109:**227–243.

Hama, T., and T. Honjo. 1987. Phytoplankton products and nutrient availability in phytoplankton population from Gokasho Bay, Japan. *Journal of Experimental Marine Biology and Ecology* **112:**251–266.

Hellebust, J. A. 1965. Excretion of some organic compounds by marine phytoplankton. *Limnology and Oceanography* **10:**192–206.

Hellebust, J. A. 1974. Extracellular products. *In* "Algal Physiology and Biochemistry" (W. D. P. Stewart, Ed.), pp. 838–863. Univ. of California Press, Berkeley.

Hough, R. A., and R. G. Wetzel. 1975. The release of dissolved organic carbon from submersed aquatic macrophytes: Diel, seasonal, and community relationships. *Verhandlungen der Internationale Vereiningung für Theoretische und Angewandte Limnologie* **19:**939–948.

Iturriaga, R., and A. Zsolnay. 1983. Heterotrophic uptake and transformation of phytoplankton extracellular products. *Botanica Marina* **26:**375–381.

Jalliffier-Merlon, E., J. C. Marty, V. Denant, and A. Saliot. 1991. Phytoplankton sources of volatile aldehydes in the river Rhone estuary. *Estuarine Coastal and Shelf Science* **32:**463–482.

Jensen, L. M. 1983. Phytoplankton release of extracellular organic carbon, molecular weight composition and bacterial assimilation. *Marine Ecology Progress Series* **11:**39–48.

Jones, J. B., S. G. Fisher, and N. B. Grimm. 1995. Vertical hydrologic exchange and ecosystem metabolism in a Sonoran Desert stream. *Ecology* **76:**942–952.

Jones, J. B., J. D. Schade, S. G. Fisher, and N. B. Grimm. 1997. Organic matter dynamics in Sycamore Creek, a desert stream in Arizona, USA. *In* "Stream Organic Matter Budgets" (J. R. Webster and J. L. Meyer, Eds.), pp. 78–81. *Journal of the North American Benthological Society* **16:**3–161.

Jumars, P. A., D. H. Penry, J. A. Baross, M. J. Perry, and B. W. Frost. 1989. Closing the microbial loop: Dissolved carbon pathway to heterotrophic bacteria from incomplete digestion and absorption in animals. *Deep Sea Research* **4:**483–495.

Kaplan, L. A., and T. L. Bott. 1982. Diel fluctuations of DOC generated by algae in a piedmont stream. *Limnology and Oceanography* **27:**1091–1100.

Kaplan, L. A., and T. L. Bott. 1989. Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: Effect of temperature, water chemistry and habitat. *Limnology and Oceanography* **34:**718–733.

Kepkay, P., S. E. H. Niven, and T. G. Milligan. 1993. Low molecular weight and colloidal DOC production during a phytoplankton bloom. *Marine Ecology Progress Series* **100:**233–244.

Kirchman, D. L., Y. Suzuki, C. Garside, and H. W. Ducklow. 1991. High turnover of dissolved organic carbon during a spring phytoplankton bloom. *Nature* **352:**612–614.

Lampert, W. 1978. Release of dissolved organic carbon by grazing phytoplankton. *Limnology and Oceanography* **23:**831–834.

Lancelot, C. 1983. Factors affecting phytoplankton extracellular release in the Southern Bight of the North Sea. *Marine Ecology Progress Series* **12:**115–121.

Larsson, U., and Å. Hagström. 1982. Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic eutrophication gradient. *Marine Biology* **67:**57–70.

Lignell, R. 1990. Excretion of organic carbon by phytoplankton: Its relation to algal biomass, primary productivity and bacterial secondary production in the Baltic Sea. *Marine Ecology Progress Series* **68:**85–99.

Lignell, R., and K. Lindqvist. 1992. Effect of nutrient enrichment and temperature on intracellular partitioning of $^{14}CO^2$ in a summer phytoplankton community in the northern Baltic. *Marine Ecology Progress Series* **86:**273–281.

Mague, T. H., E. Friberg, D. J. Hughes, and I. Morris. 1980. Extracellular release of carbon by marine phytoplankton; a physiological approach. *Limnology and Oceanography* **25:**262–279.

Malinsky-Rushansky, N. Z., and C. Legrand. 1996. Excretion of dissolved organic carbon by phytoplankton of different sizes and subsequent bacterial uptake. *Marine Ecology Progress Series* **132:**249–255.

Mann, C. J., and R. G. Wetzel. 1996. Loading and utilization of dissolved organic carbon from emergent macrophytes. *Aquatic Botany* **53:**61–72.

Maršálek, B., and R. Rojíčková. 1996. Stress factors enhancing production of algal exudates: A potential self-protective mechanism? *Zeitschrift für Naturforschung* **C51:**646–650.

McKinley, K. R., A. K. Ward, and R. G. Wetzel. 1977. A method for obtaining more precise measures of excreted organic carbon. *Limnology and Oceanography* **22:**570–573.

McKnight, D. M., and C. M. Tate. 1997. Canada Stream: A glacial meltwater stream in Taylor Valley, South Victoria Land, Antarctica. *In* "Stream Organic Matter Budgets" (J. R. Webster and J. L. Meyer, Eds.), pp. 14–17. *Journal of the North American Benthological Society* **16:**3–161.

Minshall, G. W. 1978. Autotrophy in stream ecosystems. *BioScience* **28:**767–771.

Moran, M. A., and R. E. Hodson. 1989. Formation and bacterial utilization of dissolved organic carbon derived from detrital lignocellulose. *Limnology and Oceanography* **34:**1034–1047.

Moran, M. A., and R. E. Hodson. 1990. Contributions of degrading *Spartina alterniflora* lignocellulose to the dissolved organic carbon pool of a salt marsh. *Marine Ecology Progress Series* **62:**161–168.

Morán, X. A. G., and M. Estrada. 2000. Phytoplankton DOC and POC production in the Bransfield and Gerlache straits as derived from kinetic experiments of ^{14}C incorporation. *Deep Sea Research*, in Press.

Morris, I. 1981. Photosynthetic products, physiological state, and phytoplankton growth. *In* "Physiological Bases of Phytoplankton Ecology". *Canadian Bulletin of Fisheries and Aquatic Sciences* **210:**83–102.

Morris, I., and W. Skea. 1978. Products of photosynthesis in natural populations of marine phytoplankton from the Gulf of Maine. *Marine Biology* **47:**303–312.

Moss, B. 1998. "Ecology of Fresh Waters: Man and Medium, Past to Future". Blackwell Sci. Oxford, 557 pp.

Mulholland, P. J., and E. J. Kuenzler. 1979. Organic carbon export from upland and forest wetland watersheds. *Limnology and Oceanography* **24:**960–999.

Münster, U. 1993. Concentrations and fluxes of organic carbon substances in the aquatic environment. *Antonie van Leeuwenhoek* **63:**243–274.

Murray, A. G. 1995. Phytoplankton exudation: Exploitation of the microbial loop as a defense against algal viruses. *Journal of Plankton Research* **17:**1079–1094.

Murray, A. G., and P. M. Eldridge. 1994. Marine viral ecology: Incorporation of bacteriophage into the microbial planktonic food web paradigm. *Journal of Plankton Research* **16:**627–641.

Nalewajko, C. 1966. Photosynthesis and excretion in various planktonic algae. *Limnology and Oceanography* **11:**1–10.

Nalewajko, C., and D. W. Schindler. 1976. Primary production, extracellular release, and heterotrophy in two lakes in the ELA, northwestern Ontario. *Journal of the Fisheries Research Board of Canada* **33:**219–226.

Nalewajko, C., K. Lee, and P. Fay. 1980. Significance of algal extracellular products to bacteria in lakes and in cultures. *Microbial Ecology* **6:**199–207.

Norrman, B., U. L. Zweifel, C. S. Hopkinson, and B. Fry. 1995. Production and utilization of dissolved organic carbon during an experimental diatom bloom. *Limnology and Oceanography* **40:**898–907.

Nuccio, J., P. J. Seaton, and R. J. Kieber. 1995. Biological production of formaldehyde in the marine environment. *Limnology and Oceanography* **40:**521–527.

Obernosterer, I., and G. J. Herndl. 1995. Phytoplankton extracellular release and bacterial growth: Dependence on the inorganic N : P ratio. *Marine Ecology Progress Series* **116:**247–257.

Otsuki, A., and R. G. Wetzel. 1974. Release of dissolved organic matter by autolysis of a submersed macrophyte, *Scirpus subterminalis*. *Limnology and Oceanography* **19:**842–845.

Persson, A. 1997. The effects of predation and excretion on aquatic food webs. *Oikos* **79:**137–146.

Petit, M., G. P. Alves, and P. Lavandier. 1999. Phytoplankton exudation, bacterial reassimilation and production for three diel cycles in different trophic conditions. *Archiv für Hydrobiologie* **146:**285–309.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. *Bioscience* **24:**499–504.

Rosenfeld, S., and J. J. Hudson. 1997. Primary production, bacterial production, and invertebrate biomass in pools and riffles in southern Ontario streams. *Archiv für Hydrobiologie* **139:**301–316.

Saunders, G.W., and T.A. Storch. 1971. Coupled oscillatory control mechanism in a planktonic system. *Nature* **230:**58–60.

Schade, J. D., and S. G. Fisher. 1997. The influence of leaf litter on a Sonoran Desert stream ecosystem. *Journal of the North American Benthological Society* **16:**612–626.

Sculthorpe, C. D. 1967. "The Biology of Vascular Plants." St. Martin's, New York.

Sell, A. F., and J. Overbeck. 1992. Exudates: Phytoplankton–bacterioplankton interactions in Plussee. *Journal of Plankton Research* **14:**1199–1215.

Sharp, J. H. 1977. Excretion of organic matter by marine phytoplankton: Do healthy cells do it? *Limnology and Oceanography* **22:**381–399.

Siuda, W., and R. Wcisko. 1990. Production and utilization of organic matter by microplankton in an eutrophic lake. *Archiv für Hydrobiologie* **34:**67–73.

Smith, W. O., R. T. Barber, and S. A. Huntsman. 1977. Primary productivity off the coast of northwest Africa: Excretion of dissolved organic matter and its heterotrophic uptake. *Deep Sea Research* **24:**35–47.

Søndergaard, M., and H.-H. Schierup. 1982. Release of extracellular organic carbon during a diatom bloom in lake Mossø: Molecular weight fractionation. *Freshwater Biology* **12**:313–320.

Søndergaard, M., B. Riemann, and N. O. G. Jørgensen. 1985. Extracellular organic carbon (EOC) released by phytoplankton and bacterial production. *Oikos* **45:**323–332.

Steeman-Nielsen E. 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *Journal du Conseil Permanent International de la Mer* **18:**117–140.

Sundh, I. 1991. The dissolved organic carbon released from lake phytoplankton — biochemical composition and bacterial utilization. Ph.D. Dissertation, Uppsala University, Sweden, 21 pp.

Teiser, M. L. O. 1993. Extracellular low molecular weight organic compounds produced by *Synechococcus* sp. and their roles in the food web of alkaline hot spring microbial mat communities. Ph. D. Dissertation, University of Oregon, Eugene, 197 pp.

Vanni, M., and C. Layne. 1997. Nutrient recycling and herbivory as mechanisms in the top-down effect of fish on algae in lakes. *Ecology* **78:**21–40.

Vegter, F., and P. R. M. D. Visscher. 1984. Extracellular release by phytoplankton during photosynthesis in lake Grevelingen (SW Netherlands). *Netherlands Journal of Sea Research* **18:**260–272.

Vieira, A. A. H., and S. Myklestad. 1986. Production and extracellular carbohydrate in cultures of *Ankistrodesmus densus* Kors. (Cholorophyceae). *Journal of Plankton Research* **8:**985–994.

Webster, J. R., and J. L. Meyer. 1997. Organic matter budgets for streams: A synthesis. *In* "Stream Organic Matter Budgets" (J. R. Webster and J. L. Meyer, Eds.). pp. 141–161. *Journal of the North American Benthological Society* **16:**3–161.

Weiss, M., and M. Simon. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: The relative significance of amino acids and carbohydrates. *Aquatic Microbial Ecology* **17:**1–12.

Wetzel, R. G. 1969. Factors influencing photosynthesis and excretion of dissolved organic matter by aquatic macrophytes in hard-water lakes. *Verhandlungen der Internationale Vereiningung für Theoretische und Angewandte Limnologie* **17:**72–85.

Wetzel, R. G. 1983. "Limnology," 2nd ed. Saunders College, Philadelphia, 860 pp.

Wetzel, R. G., and B. A. Manny. 1972. Secretion of dissolved organic carbon and nitrogen by aquatic macrophytes. *Verhandlungen der Internationale Vereiningung für Theoretische und Angewandte Limnologie* **18:**162–170.

Wetzel, R. G., and P. A. Penhale. 1979. Transport of carbon and excretion of dissolved organic carbon by leaves and roots/rhizomes in seagrasses and their epiphytes. *Aquatic Botany* **6:**149–158.

Wiebe, W. J., and D. F. Smith. 1977. Direct measurement of dissolved organic carbon release by phytoplankton and incorporation by microheterotrophs. *Marine Biology* **42:**213–223.

Williams, P. J. LeB., and C. S. Yentsch. 1976. An examination of photosynthetic production, excretion of photosynthetic products, and heterotrophic utilization of dissolved organic compounds with reference to results from a coastal subtropical sea. *Marine Biology* **35:**31–40.

Wolter, K. 1982. Bacterial incorporation of organic substances released by natural phytoplankton populations. *Marine Ecology Progress Series* **7:**287–295.

Wommack, K. E., and R. R. Colwell. 2000. Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* **64:**69–114.

2

Sources, Production, and Regulation of Allochthonous Dissolved Organic Matter Inputs to Surface Waters

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Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

I. INTRODUCTION

Allochthonous dissolved organic matter is a source of organic carbon, nitrogen, phosphorus, and sulfur to aquatic systems that is derived from the surrounding terrestrial ecosystem. Dissolved organic matter (DOM) is defined as that portion of organic material that passes through an average filter with a pore size of less than 0.7 μm (range used 0.22–1.22 μm). Therefore, DOM comprises a continuum of small organic molecules to highly polymeric humic substances. Molecular weights of dissolved organic compounds typically range from 100 to 100,000 Da. The transfer of organic material to aquatic ecosystems is sometimes readily observed, such as when leaves fall into a stream or lake and leach dissolved organic matter (e.g., McDowell and Fisher, 1976; McCart *et al*., 1995; France *et al*., 1996). In most cases, however, allochthonous inputs to freshwaters are dominated by direct inputs of dissolved organic matter associated with the advective transport of surface water or groundwater. When considered in the context of a whole watershed, most of these advective inputs have passed through or over the soil before entering surface water, because only a small fraction typically enters as direct precipitation or surface runoff into streams or lakes. Worldwide, riverine transport of organic carbon to the oceans is estimated at between 0.4 and 0.9 Pg C yr^{-1} (Schlesinger and Melack, 1981; Degens, 1982; Meybeck, 1982; Degens, *et al*. 1991; Aitkenhead and McDowell, 2000). This estimated flux of riverine organic carbon is 1–2 orders of magnitude smaller than the movement of carbon between the ocean and the atmosphere (90 Pg C yr^{-1}) and between the vegetation and the atmosphere (110 Pg C yr^{-1}) (Schneider, 1989; Dixon and Turner, 1991). However, the flux of dissolved organic carbon (DOC) could, in certain circumstances, contribute a large term to net ecosystem production (NEP) fluxes (e.g., Kling *et al*., 1992). Furthermore, Hope *et al*. (1994) suggested that the export of riverine carbon to the ocean is likely underestimated based on three major sources of error in existing data, including (a) lack of storm event sampling (e.g., Grieve, 1984), (b) horizontal and vertical variation in aquatic particulate organic carbon (Curtis *et al*., 1979), and (c) infrequent sampling of carbon as free CO_2 in streams and rivers (Dawson *et al*., 1995).

Riverine DOC concentration and export have been linked to watershed attributes in several studies. Watershed soil C : N ratio (Aitkenhead and

McDowell, 2000), watershed carbon density (Hope *et al*., 1997, Aitkenhead *et al*., 1999), percentage peat cover (Dillon and Molot, 1997; Aitkenhead *et al*., 1999), watershed basin size and slope (Clair *et al*., 1994), watershed hydrology (Grieve, 1991a; Boyer *et al*., 1996), and percentage wetlands (Eckhardt and Moore, 1990) have all been successful in predicting riverine DOC export and concentration (see also Chapter 6). Dissolved organic carbon and nitrogen concentration and flux and, consequently, allochthonous inputs to surface waters are likely to change in the future. For example, changes in soil carbon and nitrogen pools are likely to occur as a result of anticipated changes in temperature and global distribution of precipitation (Manabe and Wetherald, 1980; Rind *et al*., 1990) and would likely affect DOC production and transport. Increasing atmospheric CO_2 is also expected to increase DOC flux in surface waters (Clair *et al*., 1999). Finally, change in land use (e.g., Nielson *et al*., 1999; Ross *et al*., 1999, Khomutova *et al*., 2000) such as conversion from indigenous forest to pasture can cause measurable alterations in soil carbon and nitrogen pools (Ross *et al*., 1999).

The flux of DOC from terrestrial landscapes to surface runoff has wide-ranging consequences for aquatic chemistry and biology. DOC affects the complexation, solubility, and mobility of metals (Perdue *et al*., 1976; Driscoll *et al*., 1988; Martell *et al*., 1988; see Chapter 8) as well as the adsorption of pesticides to soils (Senesi, 1992; Worrall *et al*., 1997). Formation of trihalomethanes when drinking water is disinfected with chlorine, a world-wide threat to water supplies, is also linked to DOC concentrations (Siddiqui *et al*., 1997). DOC attenuates ultraviolet-B (UV-B) radiation and thus provides some protection to aquatic biota from exposure to harmful UV radiation (e.g., Williamson and Zagarese, 1994). Finally, DOC affects the heat balance and thus stratification in lakes, which is an important constraint for aquatic organisms with limited habitats (Schindler *et al*., 1996, 1997).

The major objective of this chapter is to examine and synthesize the published literature with respect to sources and production of terrestrially derived DOC, its relationship with dissolved organic nitrogen (DON), and the mechanistic controls on their export from terrestrial ecosystems to surface waters. With the exception of wet precipitation (which is ranked by continental landmass), we have classified data for throughfall and soil solution under biome type. Where possible, we have shown mean and standard deviations of some biomes to illustrate the amount of variance within and between biomes. Relationships between DOC and DON are illustrated using only those studies that report both DOC and DON concentration. Because most research on DOC and DON has been accomplished in relatively undisturbed areas, particularly forests, this chapter concentrates on the aspect of diffuse-source allochthonous inputs to surface waters and not point-source inputs from urban and agricultural areas. Recent work by Westerhoff and Anning (2000), however, indicates that more research on effluent or point-source DOC as a contributor to riverine allochthonous inputs may be

necessary. When they compared DOC concentrations among effluent-dependent (>90% treated domestic wastewater) rivers, controlled/uncontrolled streams, and ephemeral, intermittent, and perennial streams, they found significantly greater DOC concentrations in effluent-dependent streams and reservoir outflows.

II. SOURCE, PRODUCTION, AND FRACTIONS OF DISSOLVED ORGANIC MATTER

A. Source and Production

Dissolved organic matter (DOM) is produced as precipitation moves through the atmosphere, washes through vegetation, infiltrates the soil organic horizon, and percolates downward through mineral soil horizons. Dissolved organic matter is typically the product of dissolved atmospheric dust and gases, throughfall, root exudate, leaf and root litter, and the primary and secondary metabolites of microorganisms. Whereas DOM is produced in the organic horizon, there are also measurable losses in organic and mineral soil. Dissolved organic carbon is lost through both carbon mineralization (e.g., Qualls and Haines, 1992a; Yano *et al*., 1998, 2000) and DOC adsorption to soil particles (e.g., Kaiser and Zech, 1998; Qualls, 2000; Kaiser *et al*., 2001). There is evidence of increasing low-molecular-weight (LMW) dissolved organic carbon and decreasing high-molecular-weight (HMW) dissolved organic carbon as precipitation passes through the forest canopy, infiltrates and percolates through the soil column, and enters stream water at Hubbard Brook, suggesting microbial processing of HMW dissolved organic material (Fig. 1). Schindler and Krabbenhoft (1998) found a decrease in pH and a corresponding rapid decrease in the molecular weight of DOC when examining chemical changes in pore waters as groundwater flows through the hyporheic zone and discharges to a stream in a temperate forest of northern Wisconsin. They suggest that aliphatic compounds (organic acids) are likely generated in the hyporheic zone. Thus, the flux of DOM to surface waters at any one time relies on the solution flow path through the soil, which is determined by soil texture and antecedent soil moisture. Recent research also suggests that weathering of shale and consequent microbial reworking of adsorbed organic carbon is an additional allochthonous source of dissolved and particulate organic carbon to surface waters (Petsch *et al*., 2001; Raymond and Bauer, 2001).

B. Forms of Dissolved Organic Carbon and Nitrogen

Organic forms of carbon, nitrogen, sulfur, and phosphorus (typically less than 0.7 μm in diameter) contribute to bulk DOM. Humic substances,

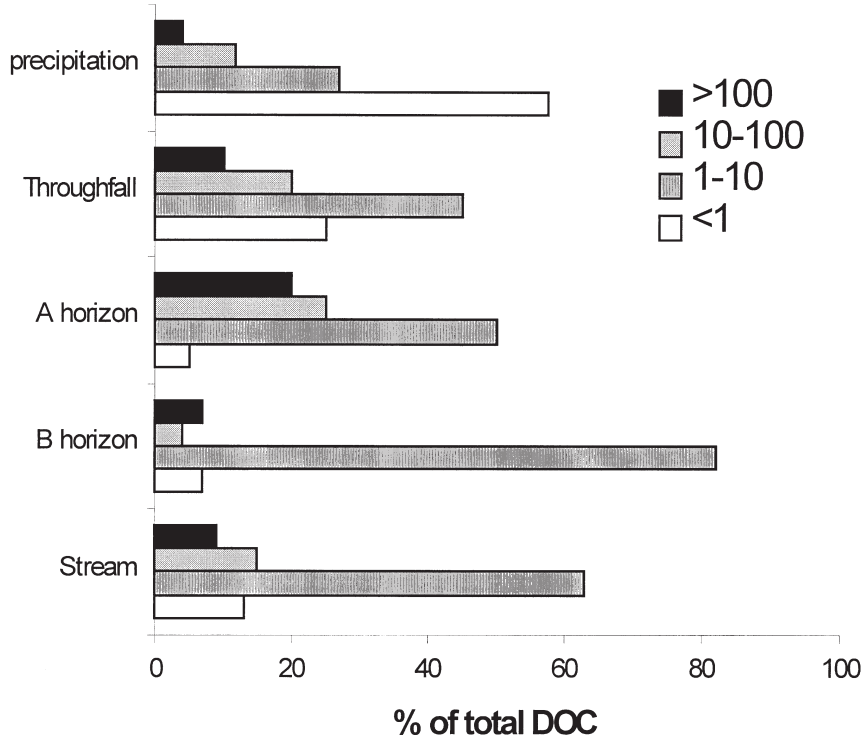

FIGURE 1 Molecular weight distribution (thousands of daltons) of dissolved organic carbon in wet precipitation, throughfall, A and B horizon soil solution, and stream water at Hubbard Brook, New Hampshire. Source: Adapted from McDowell (1982) and Cole *et al.* (1984).

defined as a series of relatively high molecular weight, yellow- to black-colored substances formed by secondary synthesis reactions, make up 50% of DOC. Most of these humic substances are fulvic acids, resulting from the microbial degradation of plant and animal remains that include a substantial portion of fatty acids (Schnitzer and Neyroud, 1975), and are high in aliphatic and carboxyl groups. Fulvic acids are further subdivided into hydrophilic and hydrophobic acids; hydrophobic is a generic relative term as all DOC is hydrophilic or dissolved in water. The remainder of the humic substance is humic acid, which contains a greater proportion of aromatic groups such as methoxyls and phenolics. Of the remaining DOC, 30% is in the form of macromolecular hydrophilic acids, and approximately 20% is identifiable low-molecular-weight (LMW) organics such as carbohydrates (e.g., glucose, fructose, and melizitoze), carboxylic acids, and amino acids (Herbert and Bertsch, 1995; see also Chapters 3–5 and 9). The ratio of fulvic to humic acids is generally 10 : 1 in low-colored surface waters, 5 : 1

in highly colored surface waters, and 1 : 3 in most interstitial soil solution (Malcolm, 1993). Methods to fractionate DOM into its component parts include fractionation by solubility, chemical class, or molecular weight. Methods include alkali and acid extraction (hydrolysis) (Swift, 1985), XAD-8 resin at pH 2 (Leenheer, 1981), gel permeation, chromatography, ultrafiltration, and centrifugation (e.g., Hayes *et al*., 1975; Buffle *et al*., 1978; Aiken, 1984). Fluorescence is able to differentiate between plant- and microbial-synthesized DOM (Stewart and Wetzel, 1980; McKnight *et al*., 2001). Individual low-molecular-weight organic acids are frequently analyzed via high-performance liquid chromatography (HPLC) to identify and quantify specific organic acids such as oxalic, citric, acetic, malic, aconitic, and succinic acids (Fox and Comerford, 1990; Fox, 1995). Cross-polarization magic angle spinning ^{13}C NMR can quantify the functional groups of DOC derived from different sources.

Of the organic nitrogen in soil, approximately one third to one half remains structurally unidentified (Parsons and Tinsley, 1975; Paul and Clark, 1989; Schnitzer, 1991). Based on chemical fractionation, approximately 50% of that organic nitrogen is hydrolyzable (Paul and Clark, 1989). Soil solution DON likely includes free amino acids, amino sugars, amines, amides, peptides, and intracellular enzymes derived from animal and plant decomposition (David *et al*., 1998). Dissolved organic phosphorus (DOP) has greater mobility than inorganic phosphorus probably because it is not so readily adsorbed by clays or calcium carbonate layers in the soil. The major source of DOP is animal waste and sewage sludge; consequently, manured fields and application of biosolids to soils with a sandy texture are assumed to provide a high rate of infiltration to the water table, allowing the phosphorus to migrate relatively quickly to surface waters and thereby accelerating eutrophication.

C. Analysis of Dissolved Organic Carbon and Nitrogen

Analytical methods for measuring the individual fractions, particularly sulfur and phosphorus, can be complex. Accordingly, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) tend to be the major fractions of dissolved organic matter determined in precipitation, throughfall, and soil and stream waters and are the only organic nutrients considered in this chapter. Dissolved organic carbon is typically measured with high-temperature combustion followed by the detection of CO_2, and DON is quantified by deducting dissolved inorganic nitrogen (DIN) from total dissolved nitrogen (TDN). Methods to measure total nitrogen include the Kjeldahl method, persulfate digestion, and thermal oxidization. These techniques describe the concentration of organic carbon and nitrogen in solution and not their structural forms.

D. Concentration and Flux of Dissolved Organic Carbon and Nitrogen

Synthesis of concentrations and fluxes of DOC and DON in precipitation, throughfall, and soil organic and mineral horizons are often reported in the published literature (Willey *et al*., 2000; Michalzik *et al*., 2001; Neff and Asner, 2001; Neff *et al*., 2002). Dissolved organic matter from these sources contributes DOM to groundwater and surface water. Leaf litter leachate is documented as a major source of DOC in the soil water of forested watersheds (Hongve, 1999) and in surface waters (Wetzel and Manny, 1972; McDowell and Fisher, 1976). However, specific quantitative allocation of DOC and DON from roots, leaf litter, and the forest floor in soil solution remains uncertain.

E. Precipitation

Wet precipitation can be considered an allochthonous source of DOM to larger rivers and lakes where precipitation falls directly onto the water surface. Concentrations and fluxes of DOC and DON in wet precipitation were reviewed by Willey *et al*. (2000) and Neff *et al*. (2002). We expanded their data to present wet precipitation DOC and DON concentration and flux (Table I, Fig. 2). Mean dissolved organic carbon concentrations and fluxes range from 0.8 ± 0.3 (SD) mg L^{-1} in tropical islands to 3.1 ± 2.1 mg L^{-1} in Europe and from 20.5 ± 21.6 kg ha^{-1} yr^{-1} in North America to 34.5 ± 0.7 kg ha^{-1} yr^{-1} in Australasia, respectively (Table I). Dissolved organic nitrogen concentrations and fluxes range from 0.1 ± SD 0.1 mg L^{-1} in Australasia and South America to 1.75 mg L^{-1} in Africa and 1.6 kg ha^{-1} yr^{-1} in Australasia to 10.5 kg ha^{-1} yr^{-1} in Africa (Table I). Precipitation DOC : DON ratios range from 7.0 (Costa Rica) to 18.0 (Europe), which may indicate relatively high proportions of potentially marine-derived nitrogeneous compounds in precipitation DOM in Costa Rica or volatile organic N emissions from trees as the source of precipitation DON, compared to a greater proportion of carbonaceous compounds derived from industry throughout Europe.

The source of DOC and DON in precipitation is likely "rain-out" and "wash-out" of pollen and organic dust particles from the atmosphere and mixed-phase reactions that produce DOC and DON from gaseous precursors such as peroxyacetyl nitrate (PAN). Cornell *et al*. (1995) suggest that urea is an important component of atmospheric organic nitrogen. Aerosol amine-N, free amino acids, and urea may also be injected into the atmosphere from marine and agricultural environments or biomass burning (Lobert *et al*., 1991; Milne and Zita, 1993; Schade and Crutzen, 1995). These compounds are unlikely to be formed *in situ* due to the oxidizing conditions of the atmosphere (Milne and Zita, 1993; Schade and Crutzen, 1995). Global deposition of reduced atmospheric organic nitrogen is estimated to be within the range of 14–40 Tg N per year (Neff *et al*., 2000). Weathers *et al*.

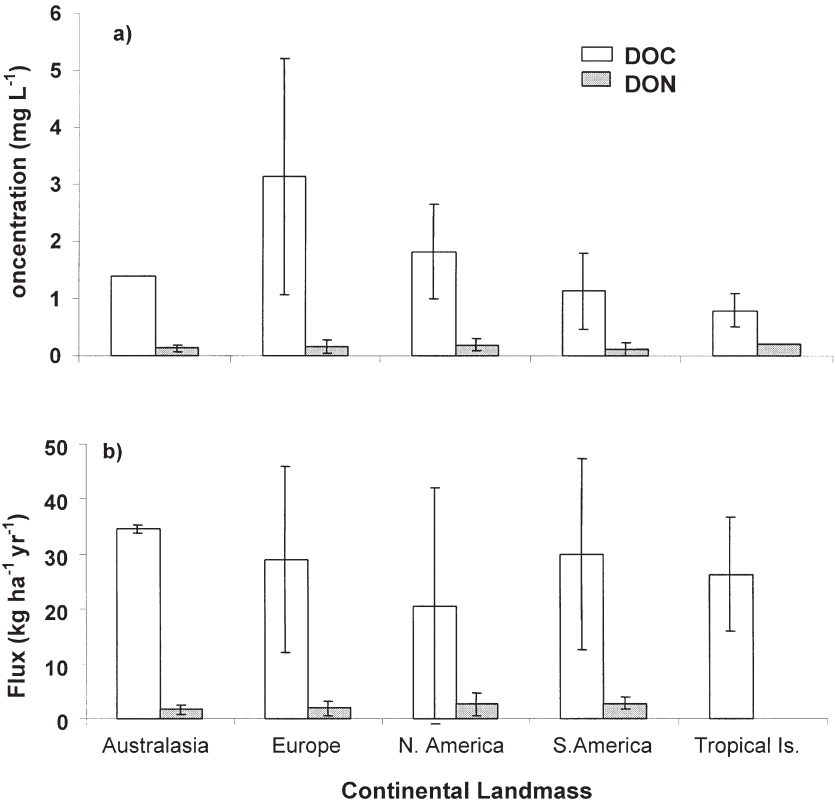

FIGURE 2 Dissolved organic matter (a) concentration (mg L^{-1}) and (b) flux (kg ha^{-1} yr^{-1}) in wet precipitation over continental landmasses. Source of data: Mean values and standard deviations from data in Table I.

(2000) report that high-elevation, coniferous forests receive 300% greater atmospheric N deposition (44 kg N ha^{-1} yr^{-1}) than do lower elevation forests (11.1 kg ha^{-1} yr^{-1}). Their findings were within the range of global N deposition reported by Neff *et al.* (2002).

F. Throughfall

As precipitation moves through vegetation, it is enriched with DOC and DON. This is likely the result of removal of the dry deposition of organic materials such as pollen and dust, aphid honeydew, and insect exudate from the leaf surface and leaching from sources inside the leaf itself. Throughfall DOM as an allochthonous source to aquatic systems would occur when branches overhang smaller streams. Concentrations of throughfall DOC are typically an order of magnitude higher than that of precipitation DOC and

TABLE I Concentration and Flux of DOC and DON in Precipitation Ranked by Continental Landmass

Landmass	*DOC* *(mg L^{-1})*	*DON*	*DOC* *(kg ha^{-1} yr^{-1})*	*DON*	*Location*	*Data source*
Africa		2.7			Kampala, Uganda	Visser (1964)
		0.8	10.5		Durban, South Africa	Simpson and Hemens (1978)
Australasia	1.4		34.0		Westland, New Zealand	Moore (1989)
	1.4		35.0		Reefton, New Zealand	Moore and Jackson (1989)
		0.2		2.2	Taupo 1, New Zealand	Timperley *et al.* (1985)
		0.1		1.0	Taupo 2, New Zealand	Timperley *et al.* (1985)
		0.1			New Zealand	Wilson (1959)
Europe	2.4				Keilator, Scotland	Aitkenhead (1995)
				3.3	Grizedale Forest, England	Carlisle *et al.* (1966)
		0.1			Czech Republic	Cornell *et al.* (1995)
		0.3			East Anglia, UK	Cornell *et al.* (1995)
	7.6		58.0		Hohe Matzen, Bavaria	Guggenberger and Zech (1993)
	4.6		42.0		Oberwarmensteinach, Bavaria	Guggenberger and Zech (1993)
	4.0		36.4		Wulfersreuth, Bavaria	Guggenberger and Zech (1993)
	1.6	0.1	20.0	0.9	Alptal, Switzerland	Hagedorn *et al.* (2000)
				1.3	Gotenberg, Sweden	Malmqvist (1978)
	3.0				Beaujolais Mountains, France	Marques and Ranger (1997)
	2.5		14.0		Sweden	Neumann *et al.* (1959)
	1.9		20.0		Dutch Delta, Netherlands	Nguyen *et al.* (1990)
			13.0		Jutland, Denmark	Nielson *et al.* (1999)
	0.6				Afon Cyff, Wales, UK	Reynolds *et al.* (1989)
Japan		0.2		6.3	Biwa Lake, Japan	Timperley *et al.* (1985)

(*continued*)

TABLE I (*continued*)

Landmass	*DOC* ($mg\ L^{-1}$)	*DON* ($mg\ L^{-1}$)	*DOC* ($kg\ ha^{-1}\ yr^{-1}$)	*DON* ($kg\ ha^{-1}\ yr^{-1}$)	*Location*	*Data source*
South America	0.8				Amazon, South America	Andreae *et al.* (1990)
				3.9	Manaus, Brazil	Brinkman (1983)
		0.3			Maraba, Brazil	Cornell *et al.* (1995)
		0.04			Recife, Brazil	Cornell *et al.* (1995)
	0.7	0.1	28.5	3.4	La Selva, Costa Rica	Eklund *et al.* (1997)
				2.5	Lake Calado, Amazon, South America	Lesack and Melack (1996)
			13.4	1.3	Lake Valencia, Venezuela	Lewis (1981)
	1.9	0.05	48.0		Lake Calado, South America	Williams *et al.* (1997)
Tropical islands		0.2			Bermuda, West Indies	Cornell *et al.* (1995)
		0.2			Tahiti	Cornell *et al.* (1995)
	1.0		33.6		Luquillo Mountains, Puerto Rico	McDowell (1998)
	0.6		19.0		El Verde, Puerto Rico	McDowell *et al.* (1990)
North America		0.2		1.1	Alberta, Canada	Caiazza *et al.* (1978)
			15.5	1.9	Hubbard Brook, New Hampshire	Campbell *et al.* (2000)
			14.1	1.4	Cone Pond, New Hampshire	Campbell *et al.* (2000)
			17.1	1.8	Sleepers River, Vermont	Campbell *et al.* (2000)
			11.6	1.3	Lyle Brook, Vermont	Campbell *et al.* (2000)
				1.3	Sierra Nevada, California	Chorover *et al.* (1994)

	0.1			North Carolina	Cornell *et al.* (1995)
		13.8	0.6	Harvard Forest, Massachusetts	Currie *et al.* (1996)
2.0		4.9		Mont St Hilaire, Canada	Dalva and Moore (1991)
1.5				Olympic National Park, Washington	Edmonds *et al.* (1991)
			1.7	Sangre de Cristo Mountains, New Mexico	Gosz (1980)
	0.2	10.3	1.2	Como Creek, Colorado	Grant and Lewis (1982)
	0.4	89.2	5.4	Gainesville, Florida	Hendry and Brezonik (1980)
3.4	0.3	39.9	3.5	Chesapeake Bay, District of Columbia	Jordan *et al.* 1995
			4.0	Walker Branch, Tennessee	Kelly and Meagher (1986)
1.5		3.0		Schefferville, Canada	Koprivnjak and Moore (1992)
1.1		12.0		Hubbard Brook, New Hampshire	Likens *et al.* (1983)
1.9		19.0		Ithaca, New York	Likens *et al.* (1983)
1.1		16.0		Hubbard Brook, New Hamsphire	McDowell and Likens (1988)
1.1				Howland, Maine	McLaughlin *et al.* (1996)
	0.2		4.4	Harp Lake, Ontario, Canada	Nichols and Cox (1978)
2.9				Bear Brook, Maine	Norton *et al.* (1999)
	0.1		0.7	Morehead, North Carolina	Peierls and Paerl (1997)
2.9				Iowa, Wisconsin	Quideau and Bockheim (1997)
			2.8	Coastal Plain, Florida	Riekerk (1983)
1.0				Harp Lake, Canada	Schiff *et al.* (1990)
	0.1		8.7	Lewes, Delaware	Scudlark *et al.* (1998)
			4.3	Coweeta, North Carolina	Swank and Waide (1987)
			1.5	Cascade Mountains, Oregon	Swank *et al.* (1987)
			2.3	Minnesota	Verry and Timmons (1977)
1.4		21.0		Wilmington, North Carolina	Willey *et al.* (2000)

TABLE II Concentration and Flux of DOC and DON in Throughfall Ranked by Biome

Biome	*DOC* ($mg\ L^{-1}$)	*DON* ($mg\ L^{-1}$)	*DOC* ($kg\ ha^{-1}\ yr^{-1}$)	*DON* ($kg\ ha^{-1}\ yr^{-1}$)	*Location*	*Data source*
Cool conifer	6.0				Ewich Forest, Scotland	Aitkenhead (1995)
	14.0	0.3			Loch Vale, Coloraedo	Arthur and Fahey (1993a)
	8.8				#1 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	7.3				#2 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	4.3				#3 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	2.7				#4 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	4.4				#5 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	11.6				Landes of Goscony, France	Colina-Tejada *et al.* (1996)
	24.7	0.7	139.0	3.5	Harvard Forest, Massachusetts	Currie *et al.* (1996)
	9.6	0.4	71.0	3.6	Huntington Forest, New York	David and Driscoll (1984)
	4.5				Birkenes, Norway	Easthouse *et al.* (1992)
	8.4				Olympic National Park, Washington	Edmonds *et al.* (1991)
	12.0				Olympic National Park, Washington	Edmonds *et al.* (1991)
		0.6		1.2	Medicine Bow Mountains, Wyoming	Fahey *et al.* (1985)
			101.2		Howland, Maine	Fernandez *et al.* (1995)
	13.4				Piocorto, Galicia, Spain	Fernandez-Sanjuro *et al.* (1997)
		0.3		3.5	Whiteface Mountains, New York	Friedland *et al.* (1991)
	21.4		128.0		Hohe Matzen, Bavaria, Germany	Guggenberger and Zech (1993)
	10.0		70.4		Wulfersreuth, Bavaria, Germany	Guggenberger and Zech (1993)
	11.6		93.0		Oberwarmensteinach, Bavaria	Guggenberger and Zech (1993)
	9.6				Alptal, Switzerland	Hagedorn *et al.* (2000)
	48.5		32.0		Schefferville, Canada	Koprivnjak and Moore (1992)
	14.4				Beaujolais Mountains, France	Marques and Ranger (1997)
	15.0				Beaujolais Mountains, France	Marques and Ranger (1997)
	13.8				Beaujolais Mountains, France	Marques and Ranger (1997)
	15.2	0.8	84.1	3.4	PROTOS Waldstein, Germany	Michalzik and Matzner (1999)
	16.0		280.0		Westland, New Zealand	Moore (1989)

	16.1				Howland, Maine	McLaughlin *et al.* (1996)
			120.0		Jutland, Denmark	Nielsen *et al.* (1999)
		0.4		1.8	Eastern Finland	Piirainen *et al.* (1998)
	12.1				Iowa, Wisconsin	Quideau and Bockheim (1997)
	11.6				Iowa, Wisconsin	Quideau and Bockheim (1997)
	7.5				Calhoun Forest, South Carolina	Richter and Markewitz (1996)
	10.0				Fichtelgebirge, Bavaria, Germany	Schwesig and Matzner (2000)
Cool deciduous	9.9		41.1		Maglehems, Ora, Sweden	Bergkvist and Folkeson (1992)
			91.5		Coastal Plain, North Carolina	Brinson *et al.* (1980)
				5.0	Grizedale Forest, England	Carlisle *et al.* (1966)
	29.0	0.6	117.0	2.7	Harvard Forest, Massachusetts	Currie *et al.* (1996)
	9.1		18.6		Mont St Hilaire, Canada	Dalva and Moore (1991)
	13.2		22.4		Mont St Hilaire, Canada	Dalva and Moore (1991)
	4.8				Huntington Forest, New York	David and Driscoll (1984)
	23.1				Hubbard Brook, New Hampshire	Eaton *et al.* (1973)
	6.5				Sanche, Galicia, Spain	Fernandez-Sanjuro *et al.* (1997)
	7.9		12.2		Oceana, Great Lakes Region	Leichty *et al.* (1995)
	5.5		10.6		Alberta, Great Lakes Region	Leichty *et al.* (1995)
	11.9		47.3		Hubbard Brook, New Hampshire	McDowell and Likens (1988)
	20.9		340.0		Reefton, New Zealand	Moore and Jackson (1989)
			212.0		Coastal Plain, North Carolina	Mulholland (1981)
			39.0		Jutland, Denmark	Nielsen *et al.* (1999)
	9.5	0.2	120.0	3.9	Coweeta, North Carolina	Qualls *et al.* (1991)
	6.2				Tomakomi Forest, Japan	Shibata *et al.* (2001)
	4.3				Wolfheze, Netherlands	Tietema and Wessell (1994)
Tropical forest	6.2		127.0		Luquillo Mountains, Puerto Rico	McDowell (1998)
Cool mixed forests	14.6		20.5		Mont St Hilaire, Canada	Dalva and Moore (1991)
Warm mixed forests	35.0				Sandhill, South Carolina	Dosskey and Bertsch (1997)

are remarkably similar from cool coniferous and deciduous forests (12.6 ± 8.5 mg L^{-1} from cool coniferous canopies and 11.6 ± 7.6 mg L^{-1} from cool deciduous canopies; Fig. 5). DON concentrations also increase in throughfall and typically average 0.5 ± 0.2 mg L^{-1} in cool coniferous forests and 0.4 ± 0.3 mg L^{-1} in cool deciduous stands. Antecedent moisture and rainfall dynamics, however, will have a strong influence on the concentration of DOM collected at any one time. Individual reports of throughfall DOC flux are variable and range from 10.6 kg C ha^{-1} yr^{-1} in a cool deciduous forest in Canada to 340 kg C ha^{-1} yr^{-1} in a Manuka scrub in New Zealand (Moore and Jackson, 1989; Leichty *et al*., 1995). Fewer flux measurements of DON have been recorded but they range from 1.2 kg ha^{-1} yr^{-1} in the Medicine Bow Mountains of Wyoming to 5.0 kg ha^{-1} yr^{-1} in the Grizedale Forest of northern England (Carlisle *et al*., 1966; Fahey *et al*., 1985; Table II). Aphid-infested forests may enhance DOC and DON concentrations significantly. Approximately 400–700 kg ha^{-1} yr^{-1} fresh mass of honeydew in conifer and 800 kg ha^{-1} yr^{-1} in hardwood forests have been reported in European forests (Michalzik *et al*., 1999). DOC : DON ratios increase significantly as precipitation in cool temperate climates passes through forest canopies as throughfall. DOC : DON ratios averages 31 ± 12.4 in throughfall of coniferous canopies increasing to 47.9 ± 0.6 in throughfall of deciduous canopies.

G. Forest Floor and Organic Soil

Depending on factors such as watershed slope, depth of water table, antecedent soil moisture, and barriers to precipitation and throughfall infiltration, DOM from the forest floor and organic soil horizon can contribute a large flux of allochthonous DOM to surface waters. Soil solution for DOM analysis is typically collected with zero-tension lysimeters placed at the boundary between organic soil and mineral soil.

Concentrations and flux of DOC are extremely variable with depth in the forest floor and organic (Oa) soil horizons. Average annual soil solution DOC concentrations in soil organic horizons range between 7.2 ± 4.0 mg L^{-1} in cool grasslands and 36.9 ± 23.3 mg L^{-1} in cool coniferous forests (Table III). Although DOC concentrations are shown to be lower in the organic horizons of tropical forests (Table III), this is likely related to the depth of solution collector in this biome, which has an extremely shallow organic horizon. Concentrations of DON in organic soil horizons average 1.56 ± 1.2 mg L^{-1} in cool deciduous forests and 1.1 ± 0.3 mg L^{-1} in cool coniferous forests (Table III). Overall, DOC : DON ratios increase slightly relative to those in throughfall and range between 39 ± 16 in cool coniferous forests and 37 ± 6 in cool deciduous forests. Some studies report a relatively low DOC : DON ratio in organic soil horizons (e.g., MacLean *et al*., 1999; Michalzik and Matzner, 1999). These lower DOC : DON ratios are likely due to local environmental factors such as high aphid infestation or permafrost conditions.

TABLE III Concentration and Flux of DOC and DON in Forest Floor and Organic Soil Horizons

Biome	*DOC* ($mg\ L^{-1}$)	*DON* ($mg\ L^{-1}$)	*DOC* ($kg\ ha^{-1}\ yr^{-1}$)	*DON* ($kg\ ha^{-1}\ yr^{-1}$)	*Soil order*	*Location*	*Data source*
Cool conifer	28.3	0.4			Alf	Loch Vale, Colorado	Arthur and Fahey (1993b)
	74.2				Inc/Ult	#1 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	54.1				Inc/Ult	#2 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	52.4				Spod	#3 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	57.4				Spod	#4 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	15.5				Spod	#5 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
			108.0		Inc	Snake River, Colorado	Brooks *et al.* (1999)
			190.0		Inc	Deer Creek, Colorado	Brooks *et al.* (1999)
	47.7	1.2	398.0	9.5	Inc	Harvard Forest, Massachusetts	Currie *et al.* (1996)
	103.0				Spod	Howland, Maine	Dai *et al.* (1996)
	51.6				Spod	Huntington Forest, New York	David and Driscoll (1984)
	10.9				Spod	Birkenes, Norway	Easthouse *et al.* (1992)
	5.5				Hist	Birkenes, Norway	Easthouse *et al.* (1992)
	10.8				Ent	Olympic National Park, Washington	Edmonds *et al.* (1991)
	21.2				Inc	Sor, Galicia, Spain	Fernandez-Sanjuro *et al.* (1997)
		1.5		6.2	Inc/Alf	Medicine Bow Mountains	Fahey *et al.* (1985)
	77.6				Spod	Howland, Maine	Fernandez *et al.* (1995)
	9.4				Spod	Plynlimon, Wales, UK	Fiebig *et al.* (1990)
		0.8		9.1	Spod	Whiteface Mountains, New York	Friedland *et al.* (1991)
	14.5				Hist	Loch Dee, Scotland	Grieve (1990b)
	4.9				Hist	Loch Dee, Scotland	Grieve (1990b)
	12.3				Hist	Loch Dee, Scotland	Grieve (1990b)
	27.7		146.4		Inc	Wulfersreuth, Bavaria, Germany	Guggenberger and Zech (1993)
	26.6		169.3		Spod	Oberwarmensteinach, Bavaria	Guggenberger and Zech (1993)
	54.4		380.0		Spod	Hohe Matzen, Bavaria, Germany	Guggenberger and Zech (1993)
			103.0	0.3	Spod	Klosterhede, Denmark	Gundersen *et al.* (1998)
			350.0	17.0	Spod	Aber, Wales, UK	Gundersen *et al.* (1998)

(*continued*)

TABLE III (*continued*)

Biome	*DOC* (*mg L^{-1}*)	*DON* (*mg L^{-1}*)	*DOC* (*kg ha^{-1} yr^{-1}*)	*DON* (*kg ha^{-1} yr^{-1}*)	*Soil Order*	*Location*	*Data source*
			130.0	7.0	Spod	Speuld, Netherlands	Gundersen *et al.* (1998)
			190.0	6.0	Spod	Ysselsteyn, Netherlands	Gundersen *et al.* (1998)
	15.3	0.6			Gley	Alptal, Switzerland	Hagedorn *et al.* (2000)
	33.1	1.0			Gley	Alptal, Switzerland	Hagedorn *et al.* (2000)
	41.5		250.0		Hist	Schefferville, Canada	Koprivnjak and Moore (1992)
	32.4				Camb	Lysina, Slavkov, Czech Republic	Kram *et al.* (1997)
	87.6				Camb	Pluhuv Bor, Slavkov, Czech Republic	Kram *et al.* (1997)
	65.0				Spod	Höglwald, Bavaria, Germany	Kreutzer (1995)
	33.0				Spod	Howland, Maine	Lawrence and David (1996)
	16.9				Inc	Cone Pond, New Hampshire	Lawrence and David (1996)
	12.0	1.2			Inc	Caribou-Poker Creek, Arkansas	MacLean *et al.* (1999)
	12.0	1.1			Inc	Caribou-Poker Creek, Arkansas	MacLean *et al.* (1999)
	45.0				Inc	Beaujolais Mountains, France	Marques and Ranger (1997)
	43.7				Inc	Beaujolais Mountains, France	Marques and Ranger (1997)
	43.6				Inc	Beaujolais Mountains, France	Marques and Ranger (1997)
		1.6		11.8	Camb	Niedersachsen, Germany	Matzner (1988)
			475.0	12.0	Inc	Harvard Forest, Massachusetts	McDowell *et al.* (1998)
	51.0				Spod	Lake Michigan, Michigan	McLaughlin *et al.* (1996)
	37.8	1.2	114.7	4.7	Spod	Waldstein, Bavaria, Germany	Michalzik and Matzner (1999)
	55.7		836.0		Inc	Westland, New Zealand	Moore (1989)
			456.0		Spod	Jutland, Denmark	Nielsen *et al.* (1999)
		0.9		2.6	Spod	Eastern Finland	Piirainen *et al.* (1998)
	23.9				Moll	Iowa, Wisconsin	Quideau and Bockheim (1997)
	31.1				Moll	Iowa, Wisconsin	Quideau and Bockheim (1997)
	42.6	1.2			Spod	Gardsjon, Sweden	Raastad and Mulder (1999)
	31.0				Spod	Fichtelbirge, Bavaria, Germany	Schwesig and Matzner (2000)
			106.0		Moll	Medicine Bows, Wyoming	Yavitt and Fahey (1986)

Cool deciduous	23.2	0.6			Inc	Harvard Forest, Massachusetts	Aitkenhead-Peterson (2000)
	26.2	0.8	225.0	6.1	Inc	Harvard Forest, Massachusetts	Currie *et al.* (1996)
	27.2				Spod	Panther, New York	Cronan and Aiken (1985)
	46.0				Spod	Mont St Hilaire, Canada	Dalva and Moore (1991)
	32.4				Spod	Huntington Forest, New York	David and Driscoll (1984)
	21.2				Inc	Sor, Galicia, Spain	Fernandez-Sanjuro *et al.* (1997)
	18.7				Spod	Woods Lake, New York	Geary and Driscoll (1996)
	5.7				Spod	Woods Lake, New York	Geary and Driscoll (1996)
	28.0		21.3		Spod	Oceana, Great Lakes Region, Canada	Leichty *et al.* (1995)
	26.2		10.3		Spod	Alberta, Great Lakes Region, Canada	Leichty *et al.* (1995)
	37.5		262.8		Spod	Hubbard Brook, New Hampshire	McDowell and Likens (1988)
			302.0	7.8	Inc	Harvard Forest, Massachusetts	McDowell *et al.* (1998)
	23.0	3.1			Inc	Caribou-Poker Creek, Arkansas	MacLean *et al.* (1999)
		2.0		12.5	Camb	Niedersachsen, Germany	Matzner (1988)
	46.0		690.0		Spod	Reefton, New Zealand	Moore and Jackson (1989)
			255.0		Moll	Jutland, Denmark	Nielsen *et al.* (1999)
	22.3				na	Tomakomi Forest, Japan	Shibata *et al.* (2001)
Cool grassland		221.0			Inc	Snake River, Colorado	Brooks *et al.* (1999)
		205.0			Inc	Deer Creek, Colorado	Brooks *et al.* (1999)
		285.0			Inc	Deer Creek, Colorado	Brooks *et al.* (1999)
	10.0				Inc	Green Lake, Colorado	Litaor (1988)
	4.3				Moll	Iowa, Wisconsin	Quideau and Bockheim (1997)
Cool mixed forest	10.5				Spod	Tegernsee Alps, Bavaria, Germany	Bäumler and Zech (1998)
	23.8				Spod	Woods, New York	Cronan and Aiken (1985)
	21.0				Spod	Sagamore, New York	Cronan and Aiken (1985)
	49.2				Spod	Mont St Hilaire, Canada	Dalva and Moore (1991)
	60.0				Spod	Bear Brook, Maine	David *et al.* (1999)
	17.5				Spod	Harp Lake, Canada	Schiff *et al.* (1990)

(*continued*)

TABLE III (*continued*)

Biome	*DOC* ($mg\ L^{-1}$)	*DON* ($mg\ L^{-1}$)	*DOC* ($kg\ ha^{-1}\ yr^{-1}$)	*DON* ($kg\ ha^{-1}\ yr^{-1}$)	*Soil Order*	*Location*	*Data source*
Heath/moorland	5.2				Hist	Green Burn, Scotland	Grieve (1990a)
	32.0				Inc	Green Lake, Colorado	Litaor (1988)
			187.0		Spod	Jutland, Denmark	Nielsen *et al.* (1999)
Warm deciduous			220.0		And	Adelaide, Australia	Stevens *et al.* (1999)
			30.0		And	Adelaide, Australia	Stevens *et al.* (1999)
Peatland		0.5			Hist	N. Pennines, England	Adamson *et al.* (1998)
	30.0				Hist	Schefferville Fens, Canada	Moore (1988)
	11.6				Hist	Sept-Iles bog, Canada	Moore (1988)
	37.7				Hist	Crystal Bog, Wisconsin	Marin *et al.* (1990)
Warm conifer				13.9	Inc	Sta. Coloma de Farners, Spain	Cortina *et al.* (1995)
	34.0		251.0		Ult	Calhoun, South Carolina	Richter and Markewitz (1996)
	33.0	0.8	420.0	10.0	Ult	Coweeta, North Carolina	Qualls *et al.* (1991)
Warm mixed forest	25.5		128.0		Ult	Atlantic Plain, South Carolina	Dosskey and Bertsch (1997)
Tropical forest	5.3		92.0		Ult	Luquillo Mountains, Puerto Rico	McDowell (1998)
	1.4				Ox	Lake Calado, South America	Williams *et al.* (1997)

Soil orders: Alf, alfisol; And, andisol; Cam, cambisol; Gley, gleysol; Hist, histosol; Inc, inceptisol; Moll, mollisol; Ox, oxisol; Spod, spodosol; Ult, ultisol.

An ongoing experiment at the Harvard Forest Long Term Ecological Research site offered the opportunity to quantify organic soil solution DOC and DON from roots, litterfall, and the organic horizon. The experiment is a plot-scale manipulation of organic matter sources in a northern hardwood stand. Treatments include doubling or removing of leaf litter, removal of roots, and removal of the organic horizon. By comparing treatments and control, the importance of various sources of organic matter in producing soil solution dissolved organic matter can be estimated. From this experiment, Aitkenhead-Peterson (2000) estimated that 88% (± unknown percentage) of bulk soil solution DOC is derived from the organic horizon, with the remainder supplied by leaf litter leachate (7% ± SD 4.6%) and root exudate and decay (15% ± SD 1.7%) (Fig. 3a). The sum is greater than 100%; this is due to only one lysimeter producing soil solution in the "no input" treatments so that a standard deviation could not be calculated. Partitioning soil solution DON in a similar fashion was impossible because when either leaf litter or root input was excluded, DON concentrations increased (Fig. 3b). When DOC and DON concentrations in the treatments were averaged by season, DOC concentrations were significantly reduced with leaf litter and root exclusion in the fall but not in the summer when an increase in DOC was apparent (Fig. 3c). DON concentrations were significantly increased with leaf litter and root exclusion in the summer, but not in the fall (Fig. 3d). The increase in DON concentrations is possibly due to the reduction in immobilization when litter and root input was removed. Mean annual DOC and DON concentrations in this study were strongly and significantly correlated with fungal biomass (Fig. 4a and 4b), suggesting that fungi may control the production of both DOC and DON either directly or indirectly.

Production of extracellular enzymes by fungal biomass should result in a direct increase in both DOC and DON concentrations the enzymes themselves, as well as a potential increase in DOC concentrations due to the action of the extracellular enzymes on organic matter. Three factors are likely to influence production and activity of extracellular enzymes: (a) microbial activity and its logical dependence on nutrient content, (b) the accumulation of soil organic matter, and (c) the mineralization of soil organic matter (Trasar-Cepeda *et al*., 2000). The potential importance of fungal-mediated DOC production derived from extracellular enzyme activity was demonstrated in a microcosm study using beech leaves from a hardwood forest floor near Copenhagen (Møller *et al*., 1999). Net DOC production and β-*N*-acetylglucosaminidase and cellulase activities were all significantly greater with *Humicola* sp. fungi than with either bacteria alone or a combination of fungi and bacteria. Furthermore, several other studies have found correlations between soil enzyme activity and DOC production, soil properties, or nitrogen fertilization. For example, Carreiro *et al*. (2000) report significantly higher cellulase activity in forested soils that have been fertilized with nitrogen than in unfertilized forest soils. Ajwa *et al*. (1999) report that

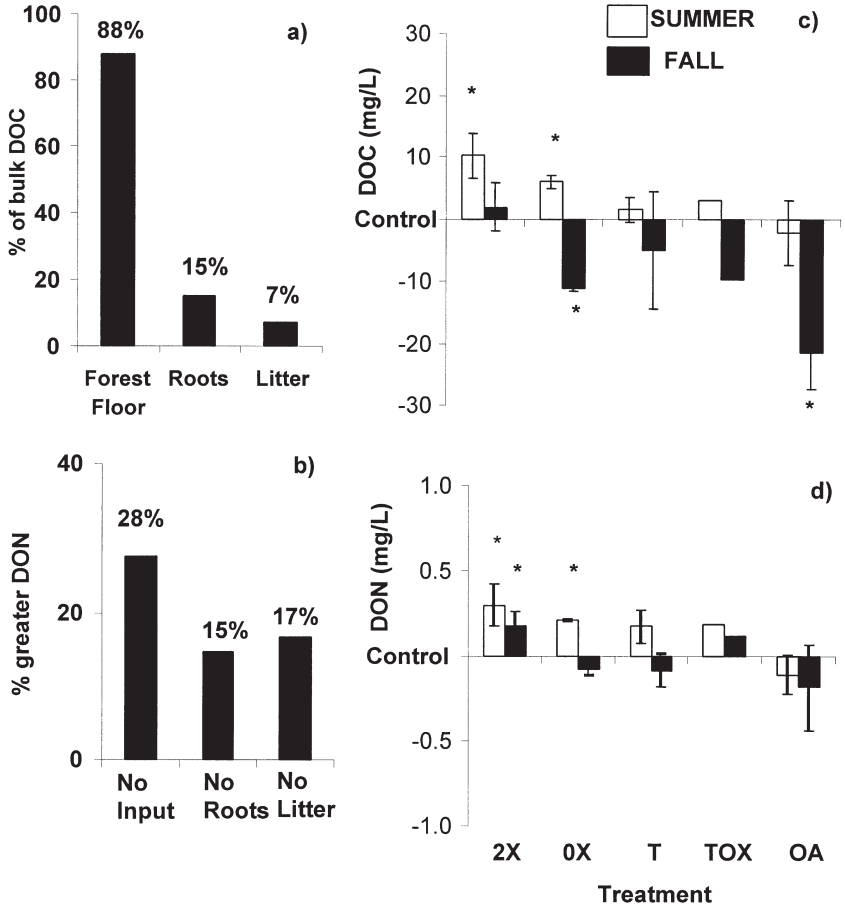

FIGURE 3 (a) Magnitude of various DOC sources in forest floor soil solution, (b) percentage increase in DON concentrations with litter removal, (c) differences in seasonal DOC concentrations for each treatment compared to the control plot (control plot set at zero), (d) differences in seasonal DON concentrations compared to the control plot (control set at zero). 2*X*, double leaf litter input; 0*X*, zero leaf litter input; *T*, no root input; *TOX*, no leaf litter + no root input; *OA*, no Oa horizon. (*p <0.05). Source of data: Aitkenhead-Peterson (2000).

significantly increased β-glucosidase activity of was found in tall grass prairie soil under long-term nitrogen fertilization. Both studies suggest a microbial response to carbon limitation. Beta-glucosidase activity was strongly and significantly correlated to DOC concentration in wetland field studies (Freeman *et al*., 1997). Trasar-Cepeda *et al*., (2000) report on several types of extracellular enzymes responsible for hydrolyzing DOM across 40 different soils in Spain. Although this latter study was specifically aimed at reporting biochemical parameters, the authors also report percentages of carbon and

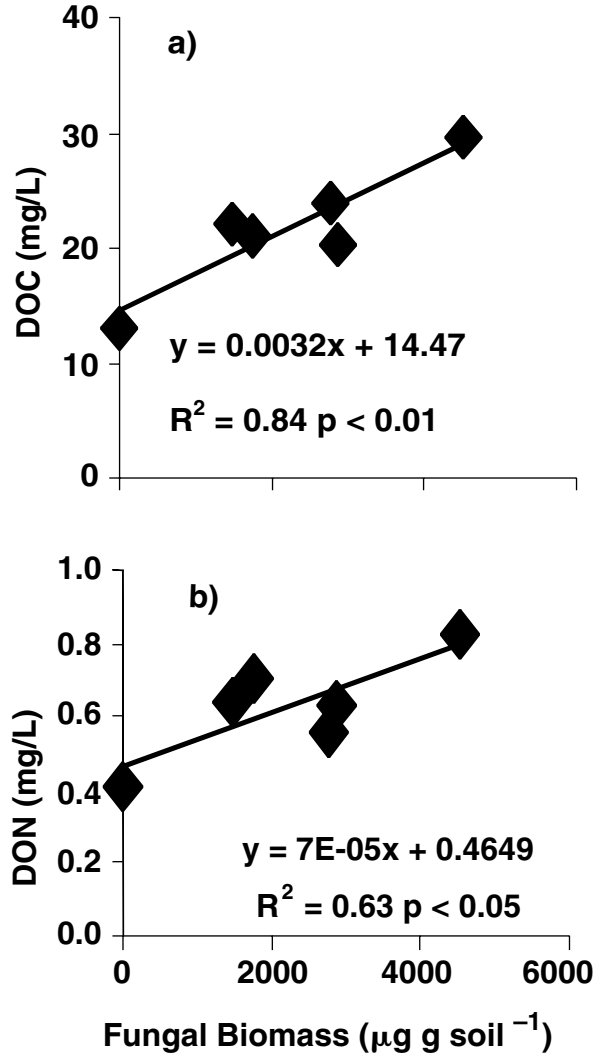

FIGURE 4 Relationship between fungal biomass and (a) annual DOC concentrations and (b) annual DON concentrations at the DIRT plot study in the Harvard Forest, Massachusetts. (Source of data: Aitkenhead-Peterson (2000).

nitrogen for the soils tested for enzyme activity. Using statistical analysis on their dataset, we found that cellulase activity was strongly and positively correlated to both soil carbon ($r = 0.56$ $n = 40$) and nitrogen ($r = 0.54$ $n = 40$). Dinesh *et al.* (1998) also report significant positive relationships between soil enzyme activity and organic sulfur, phosphorus, and carbon in a study of soil enzymes in mangrove swamps. They further found that soils with a

high carbon content stimulated microbial activity and enzyme synthesis. These examples of the recent advances in enzyme activity measurement and their correlation with soil or litter properties suggest that fungal and bacterial synthesis and release of enzymes may be a significant contributor to soil solution DOC and DON. Because DOM is not strictly "soluble" and typically represents the portion of organic material that passes through a 0.7-μm filter, the supposition that extracellular enzymes as well as viruses and some bacteria contribute to bulk soil solution DOM is feasible. Leaching from micro- and macroorganism cells and tissue may also produce a proportion of soil solution DON. When Yavitt and Fahey (1984) examined soil solution from biocide-treated microcosms, they observed a 10-fold increase in DON concentrations that they considered the result of cell lysis and death.

Both abiotic (chemical and physical) and biotic (biological) processes have been postulated to control DOM production and loss. Litterfall inputs, wet precipitation volume, and soil characteristics have all been reported to have an effect on DOM production in, and leaching losses from organic soil horizons. Some studies report a positive correlation between litterfall input and soil solution DOC concentration (Lundström, 1993; Casals *et al*., 1995; Currie *et al*., 1996; Gundersen *et al*., 1998). Yet Michalzik *et al*. (2001) report no correlation between litterfall carbon and nitrogen input and DOC or DON when analyzing several plot studies on a regional scale. Cycles of drying and rewetting of the forest floor, however, are almost certain to have an effect on DOC release and microbial processes (Lodge *et al*., 1994; Casals *et al*., 1995). In both plot-scale and laboratory studies, DOM release is enhanced with increasing water flux (Kalbitz and Knappe, 1997; Tipping *et al*., 1999). Soil pH is positively related to DOC concentration in the forest floor (Michalzik *et al*., 2001). This may be due to favorable environmental conditions for microbial degradation (Andersson *et al*., 1999) or increased deprotonation of functional groups, resulting in increased solubility of DOC (Tipping and Hurley, 1988). Positive correlations among DOC leaching, carbon mineralization, and C : N content of the forest floor are consistent with the biotic influence on DOC production, consumption, or both. Whereas Michalzik *et al*. (2001) report no relationship between flux of DOC and DON and soil C : N ratio, or soil carbon and nitrogen pools at the field scale, several authors have reported a relationship between DOC concentration and soil carbon and nitrogen at both field and microcosm scales (Gödde *et al*.,1996; Currie and Aber, 1997; Tipping *et al*., 1999; Aitkenhead and McDowell, 2000). Several studies have investigated the correlation between DOC and CO_2 flux under both laboratory and field conditions (Seto and Yanagiya, 1983; Gödde *et al*., 1996; Brooks *et al*., 1999). Soil respiration is indicative of microbial activity and is the product of approximately 33% root respiration and 67% microbial respiration in a North American mixed hardwood forest (Bowden *et al*., 1993). There are differing opinions, however, as to whether DOC production is dependent on

microbial activity measured by CO_2 or vice versa. For example, a laboratory incubation study using volcanic and alluvial soils under various land uses in the Tama Basin, Tokyo, used CO_2 flux as the dependent variable, reasoning that CO_2 flux is dependent on concentrations of labile carbon (Seto and Yanagiya, 1983). Evidence that soil solution DOC is a major energy source for soil heterotrophic activity is supported by the work of Qualls and Haines (1992a) and Yano *et al.* (1998, 2000). In a field study in Colorado, Brooks *et al.* (1999) used DOC as the dependent variable, proposing that heterotrophic activity (measured by respiration) is a source of DOC production. This latter view is supported by the work of McLatchey and Reddy (1998) who found significant relationships among soil enzyme activity, microbial biomass, and carbon mineralization.

Seasonal influences on DOM production and release reflect abiotic (temperature and moisture) and biotic (vegetal growth and enhanced microbial activity) controls on allochthonous inputs. DOC and DON concentrations in both throughfall and organic soil solution vary seasonally with highest concentrations in the growing season when temperatures are warmer and high-intensity/short-duration storms are frequent (Dai *et al.*, 1996). Seasonal effects also influence streamwater DOM concentrations (e.g., Grieve, 1991b).

H. Mineral Soil

The contribution of mineral soil solution DOM to allochthonous inputs will depend on watershed slope, depth of water table, antecedent soil moisture, and barriers to organic soil solution infiltration of the mineral soil. Simply, if soil solution from the organic soil infiltrates and percolates mineral soil, then a large reduction in DOM concentration will occur and this is reflected in stream water base flow chemistry. When storm flow occurs, the increase in allochthonous inputs is related to hydrologic flow through organic horizons. Soil solution in mineral soil horizons is typically collected with suction or tension lysimeters because the flow of water is not gravitational but controlled largely by matric and osmotic potentials.

Dissolved organic carbon and nitrogen concentrations and flux decrease significantly as water percolates down from the forest floor and soil organic horizons to the mineral soil. Concentrations of DOC in mineral soil average 13.2 ± 10.8 mg L^{-1} in cool coniferous forests and 11.7 ± 12.0 mg L^{-1} in cool deciduous forests (Table IV). The large variance in mineral soil DOC concentration may be related to adsorption/desorption processes in the soil. Study sites experiencing exceptionally high mineral soil solution DOC, such as the Campina site in South America (McClain *et al.*, 1997), Reefton in New Zealand (Moore and Jackson, 1989), and the watershed of Lake Michigan (McLaughlin *et al.*, 1996), may also be where DOC desorption is occurring. Mineral soil solution DOC concentrations vary tremendously within biome

TABLE IV Concentration and Flux of DOC and DON in Mineral Soil Horizons

Biome	*DOC* (*mg L*$^{-1}$)	*DON* (*mg L*$^{-1}$)	*DOC* (*kg ha*$^{-1}$ *yr*$^{-1}$)	*DON* (*kg ha*$^{-1}$ *yr*$^{-1}$)	*Soil Order*	*Location*	*Data source*
Cool conifer	26.1	0.5		3.0	Alf	Loch Vale, Colorado	Arthur and Fahey (1993b)
	39.4	1.0			Alf	Loch Vale, Colorado	Arthur and Fahey (1993b)
	14.7				Inc/Ult	#1 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	19.3				Spod	#3 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	5.8				Spod	#4 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	5.3				Spod	#5 Cape Blanco, Oregon	Bockheim and Langley-Turnbaugh (1997)
	26.0	0.8	167.0	5.4	Inc	Harvard Forest, Massachusetts	Currie *et al.* (1996)
	6.0				Spod	Huntington Forest, New York	David and Driscoll (1984)
	3.4				Spod	Birkenes, Norway	Easthouse *et al.* (1992)
	0.6				Hist	Birkenes, Norway	Easthouse *et al.* (1992)
	2.9				Inc	Olympic National Park, Washington	Edmonds *et al.* (1991)
		0.5		1.2	Inc/Alf	Medicine Bow Mountains, Wyoming	Fahey *et al.* (1985)
	3.8				Spod	Howland, Maine	Fernandez *et al.* (1995)
	7.9				Spod	Howland, Maine	Fernandez *et al.* (1995)
	4.0				Inc	Piocorto, Galicia, Spain	Fernandez-Sanjuro *et al.* (1997)
		0.2		2.1	Spod	Whiteface Mountains, New York	Friedland *et al.* (1991)
	11.2				Hist	Loch Dee, Scotland	Grieve (1990b)
	2.7				Hist	Loch Dee, Scotland	Grieve (1990b)
	8.7				Hist	Loch Dee, Scotland	Grieve (1990b)
	2.5		9.6		Inc	Wulfersreuth, Bavaria	Guggenberger and Zech (1993)
	3.8		28.2		Spod	Oberwarmensteinach, Bavaria	Guggenberger and Zech (1993)
	31.2		187.0		Spod	Hohe Matzen, Bavaria	Guggenberger and Zech (1993)
			51.0		Spod	Klosterhede, Denmark	Gundersen *et al.* (1998)
			43.0		Spod	Aber, Wales, U.K.	Gundersen *et al.* (1998)
			23.0		Spod	Speuld, Netherlands	Gundersen *et al.* (1998)

			190.0		Spod	Ysselsteyn, Netherlands	Gundersen *et al.* (1998)
	9.1	0.3			Gley	Alptal, Switzerland	Hagedorn *et al.* (2000)
	9.7	0.4			Gley	Alptal, Switzerland	Hagedorn *et al.* (2000)
	17.0		100.0		Hist	Schefferville, Canada	Koprivnjak and Moore (1992)
	16.0				Spod	Höglwald, Bavaria	Kreutzer (1995)
	32.6				Inc	Beaujolais Mountains, France	Marques and Ranger (1997)
	6.2				Inc	Beaujolais Mountains, France	Marques and Ranger (1997)
	13.0				Inc	Beaujolais Mountains, France	Marques and Ranger (1997)
	42.0				Spod	Lake Michigan, Michigan	McLaughlin *et al.* (1996)
	24.7	0.2	85.8	0.2	Spod	Waldstein, Bavaria, Germany	Michalzik and Matzner (1999)
	13.0				Gley	Aberfoyle, Scotland	Miller *et al.* (1990)
	3.0				Spod	Aberfoyle, Scotland	Miller *et al.* (1990)
	11.8		177.0		Inc	Westland, New Zealand	Moore (1989)
			143.0		Spod	Jutland, Denmark	Nielsen *et al.* (1999)
	8.8				Moll	Iowa, Wisconsin	Quideau and Bockheim (1997)
	17.9				Moll	Iowa, Wisconsin	Quideau and Bockheim (1997)
		0.3		0.2	Spod	Eastern Finland	Piirainen *et al.* (1998)
Cool deciduous	3.0	0.4			Inc	Harvard Forest, Massachusetts	Aitkenhead-Peterson (2000)
	12.2		37.8		Cam	Maglehems Ora, Sweden	Bergkvist and Folkeson (1992)
	6.8				Spod	Panther, New York	Cronan and Aiken (1985)
	21.0	0.6	123.0	3.2	Inc	Harvard Forest, Massachusetts	Currie *et al.* (1996)
	16.6				Spod	Mont St Hilaire, Canada	Dalva and Moore (1991)
	6.0				Spod	Huntington Forest, New York	David and Driscoll (1984)
	3.8				Cam	Sanche, Galicia, Spain	Fernandez-Sanjuro *et al.* (1997)
	7.6				Spod	Woods Lake, New York	Geary and Driscoll (1996)
	4.3				Spod	Woods Lake, New York	Geary and Driscoll (1996)
	5.9		23.0		Spod	Hubbard Brook, New Hampshire	McDowell and Likens (1988)
	45.7		686.0		Spod	Reefton, New Zealand	Moore and Jackson (1989)
			24.0		Moll	Jutland, Denmark	Nielsen *et al.* (1999)
	7.0				na	Tomakomi Forest, Japan	Shibata *et al.* (2001)

(*continued*)

TABLE IV (*continued*)

Biome	*DOC* ($mg\ L^{-1}$)	*DON* ($mg\ L^{-1}$)	*DOC* ($kg\ ha^{-1}\ yr^{-1}$)	*DON* ($kg\ ha^{-1}\ yr^{-1}$)	*Soil order*	*Location*	*Data source*
Cool grassland	2.6				Moll	Iowa, Wisconsin	Quideau and Bockheim (1997)
Cool mixed forests	2.7				Spod	Tegernsee Alps, Bavaria	Bäumler and Zech (1998)
	6.7				Spod	Woods, New York	Cronan and Aiken (1985)
	6.2				Spod	Sagamore, New York	Cronan and Aiken (1985)
	19.4				Spod	Mont St Hilaire, Canada	Dalva and Moore (1991)
	1.8				Spod	Bear Brook, Maine	David *et al.* (1999)
	2.9				Spod	Harp Lake, Canada	Schiff *et al.* (1990)
Heath/moorland	3.3				Hist	Green Burn, Scotland UK	Grieve (1990a)
			16.0		Spod	Jutland, Denmark	Nielsen *et al.* (1999)
Warm mixed forests	1.8		6.0		Ult	Atlantic Plain, South Carolina	Dosskey and Bertsch (1997)
Tropical forests	35.9				Spod	Campina, South America	McClain *et al.* (1997)
	3.5				Ox	Barro Branco, South America	McClain *et al.* (1997)
	1.5				Ox	Barro Branco, South America	McClain *et al.* (1997)
	2.4		42.7		Ult	Luquillo Mountains, Puerto Rico	McDowell (1998)
	0.6				Ox	Lake Calado, South America	Williams *et al.* (1997)
Warm conifer	1.5		9.0		Ult	Calhoun Forest, South Carolina	Richter and Markewitz (1996)

Soil orders: Alf, alfisol; And, andisol; Cam, cambisol; Gley, gleysol; Hist, histosol; Inc, inceptisol; Moll, mollisol; Ox, oxisol; Spod, spodosol; Ult, ultisol.

type. For example, within the cool conifer biome, mineral soil DOC concentrations range from 3.8 mg L^{-1} in a coniferous stand on a spodosol in Bavaria (Guggenberger and Zech, 1993) to 42.0 mg L^{-1} in a coniferous stand on a spodosol in the United States (McLaughlin *et al*., 1996). The reduction in DOC between organic and mineral soil at these two coniferous sites is 86% and 17%, respectively. The large variation in mineral soil solution DOC may also be related to the depth of the solution collector. The majority of studies included in Table IV sampled soil solution at 30–40 cm, which is the maximum value for the mineral soil DOC contribution to surface waters. Dissolved organic carbon concentrations are typically lower in groundwater than those for upper mineral soils because the opportunities for further adsorption and microbial degradation are increased with travel time. Nevertheless, one of the most consistent findings over the past two decades has been that the flux of DOC in the mineral horizon is almost always less than the flux through the overlying organic horizon. This conclusion holds true across many biomes and across vegetation types within a geographic region (coniferous vs. hardwood) (e.g., David and Driscoll, 1984). We believe this is due to the nearly universal ability of mineral soils to adsorb a significant amount of DOC from soil solution, resulting in a net sink of carbon or a stored pool which may be later released through microbial respiration (e.g., Petsch *et al*., 2001).

Dissolved organic nitrogen also shows some reduction in mineral soil solution relative to the organic soil horizon. Although few data are currently available for mineral soil solution DON concentration and flux, DON reduction in mineral soil is comparable to that of DOC (Fig. 5).

III. REGULATION OF ALLOCHTHONOUS DISSOLVED ORGANIC CARBON AND DISSOLVED ORGANIC NITROGEN

Delivery of terrestrially derived DOC and DON to surface waters depends on the balance between production and loss from solution, and the opportunity for hydrological transport (e.g., Boyer *et al*., 1996; Yano *et al*., 2000; Kaiser *et al*., 2001). The labile fractions of both DOC and DON are typically consumed by soil microbes (Hadas *et al*., 1992; Qualls and Haines 1992a; Yano *et al*., 1998). The hydrophobic fractions are typically sorbed to mineral soil (e.g., Jardine *et al*., 1989; Dai *et al*., 1996; Kaiser and Zech 1998; Qualls, 2000), and the balance is found in stream water. Variations in flow path such as lateral versus vertical can result in fairly large (five-fold) changes in the concentration of DOC delivered to surface waters over short time intervals (hours to days) (Boyer *et al*., 1996). In the absence of wetlands, hydrologic flow paths control DOC transfer to surface water because discharging water can pass through different soil horizons depending on antecedent moisture conditions. For example, at Hubbard Brook, concentrations

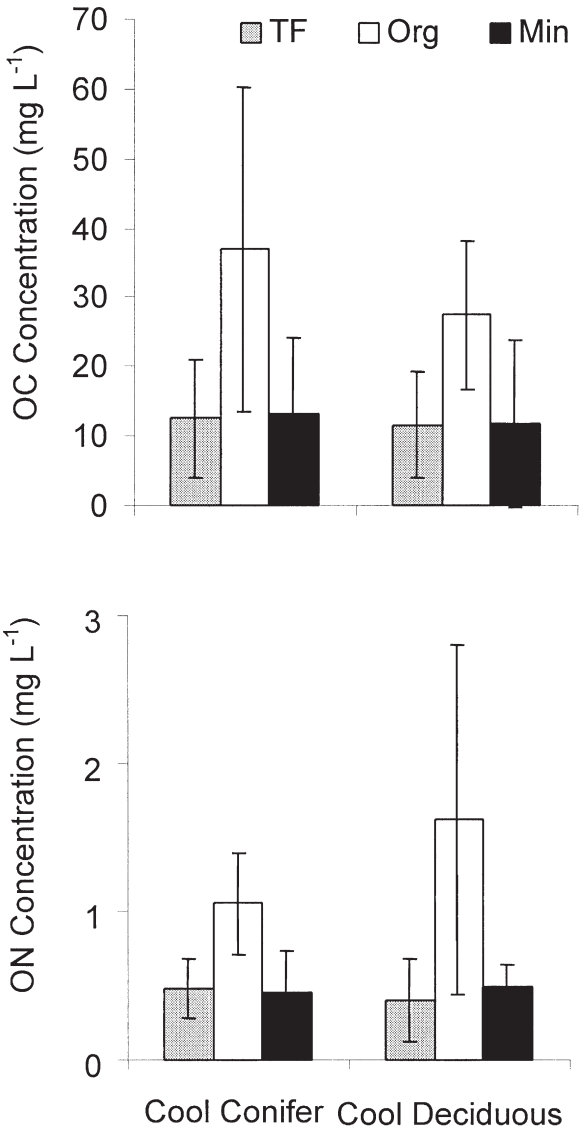

FIGURE 5 Mean concentrations of DOC and DON in throughfall, forest floor and organic soil horizons, and mineral soil B horizons in cool conifer and cool deciduous biomes. Error bars indicate 1 standard deviation of the mean.

of DOC in stream flow varied up to five-fold during storms, likely due to a change in flow path (McDowell and Wood, 1984). Additional sources of DOC to surface waters include the contribution of DOC from near stream sediments and the hyporheic zone and valley bottom wetlands.

A. Adsorption of Dissolved Organic Carbon and Dissolved Organic Nitrogen in Mineral Soil Horizons

Individual investigators have reported marked decreases in DOC concentration during its movement from organic to mineral horizons to stream water. Particularly large decreases in DOC concentration between the organic and mineral soil horizons occur in spodosols (McDowell and Wood, 1984; Koprivnjak and Moore, 1992; Vance and David, 1992; Guggenberger and Zech, 1993). Decreases in DOC have also been reported in inceptisols and ultisols (Qualls *et al*., 1991; McDowell, 1998), alfisols (Chittleborough *et al*., 1992; Donald *et al*., 1993), and oxisols (McClain *et al*., 1997). Whereas the mineral horizon of spodosols typically adsorbs DOC, there are also reported occurrences of DOC desorption (Ferrier *et al*., 1990; Dahlgren and Marrett, 1991; McClain *et al*., 1997; Qualls, 2000). Rivers displaying atypical DOC export include the Rio Negro, a tributary of the Amazon (McClain *et al*., 1997), watershed 9 at Hubbard Brook (Campbell *et al*., 2000), and the black water rivers of South Carolina (Dosskey and Bertsch, 1994). In both the Rio Negro and the black water rivers of South Carolina, the forests are on sandy soil. McClain's work demonstrates the effect of soil texture on DOC stabilization; soluble materials simply flow through sandy soils and cannot be stabilized. Qualls (2000) suggests two simple overriding factors that determine whether DOM will be adsorbed: (a) hydrologic factors and (b) geochemical factors. If the soil solution bypasses the mineral soil, a hydrological short circuit occurs and DOM will not be adsorbed. Geochemically, sandy soils will experience greater losses of DOM to aquatic ecosystems than will soils high in oxy-hydroxides or certain clays.

The mechanism by which DOC is adsorbed to mineral surfaces is debatable. Anion exchange, ligand exchange, water and cation bridging, hydrophobic interactions, van der Waals forces, dipole–dipole interactions, π–π bonds between aromatic or highly conjugated groups, and hydrogen bonding (nonionic compounds) are all mechanisms that potentially contribute to DOM retention (Parfitt *et al*., 1977; Tipping, 1981; Jardine *et al*., 1989; Jekel, 1991; Pignatello, 1993; Gu *et al*., 1995). Although many of these mechanisms are speculative, rather than proven, carbon bound by ligand exchange is most likely to be protected from microbial metabolism, and carbon bound by anion exchange carbon is most likely rereleased. Qualls (2000) examined potential mechanisms of DOC adsorption to organic and mineral soil and suggested that hydrogen bonding and possibly van der Waals forces are the most important in controlling sorption of soluble organic matter in the forest floor. The most likely mechanism of sorption in ultisol mineral soil appears to be ligand exchange on iron and aluminum oxy-hydroxides (Qualls, 2000). Parfitt *et al*. (1977) also report that ligand exchange was the major mechanism of fulvic acid adsorption to goethite based on infrared spectroscopic evidence.

Whereas several specific soil attributes are advocated as being responsible for DOC sorption in the mineral soil (Table V), it appears that the greater the clay or aluminum and iron oxide content of a soil, the greater its adsorptive capacity for DOC. For example, there is a positive correlation between *m* (the measure of the affinity of a substance for the sorbent or the partition coefficient) and soil clay content, dithionite extractable iron (Fe_d), and oxalate extractable aluminum (Al_o) (Moore *et al*., 1992; Nelson *et al*., 1993; Kaiser and Zech, 1998). Direct measurements of the surface area of soil particles also correlate very well with DOC adsorption capacity (Nelson *et al*., 1993). Furthermore, Nelson *et al*. (1993) report that riverine DOC concentrations are negatively correlated to the clay content of watershed

TABLE V Soil Attributes Potentially Responsible for DOC Adsorption in Mineral Soils

Soil attribute	*Source*
Clay content	Fahey and Yavitt (1988)
	Kaiser and Zech (1996)
	Nelson *et al*. (1990)
	Nelson *et al*. (1993)
	Inoue and Wada (1968)
%C in B horizon	McCracken (1998)
	Jardine *et al*. (1989)
	McDowell and Wood (1984)
	Kaiser *et al*. (2000)
Goethite and gibbsite	Sibanda and Young (1986)
	Tipping (1981)
Soil mineral surface area	Mayer (1994a, b)
	Tipping (1981)
HCl extractable Fe and Al	McDowell and Wood (1984)
Oxalate-extractable Al and Fe	Dalva and Moore (1991)
	Moore *et al*. (1992)
	Qualls (2000)
Dithionite-extractable Fe	Jardine *et al*. (1989)
	Moore *et al*. (1992)
	Qualls (2000)
Mn oxides	Tipping and Heaton (1983)
Temperature	Jardine *et al*. (1989)
pH	Jardine *et al*. (1989)
	Kennedy *et al*. (1996)
	Vance and David (1989)
	Rustad *et al*. (1993)
Phyllosilicate amorphous Al and Si surface coatings	Schultess and Huang (1991)
Soil solution chemistry	
(ionic strength and $[NO_3^-\ SO_4^{2-}]$	Evans *et al*. (1988)
	Guggenberger and Zech (1992)
Source of DOC	Moore and Matos (1999)

soils. This suggests that watersheds with greater soil clay content will have decreased surface water DOC concentrations.

Certain fractions of DOC also appear to be preferentially adsorbed to secondary minerals. Hydrophobic acids such as tannic acid are the carbon fraction most commonly adsorbed to mineral soil (Leenheer, 1980; Dai *et al*., 1996; Kennedy *et al*., 1996; Kaiser and Zech, 1998). In contrast, hydrophilic acids are the fraction most commonly observed to desorb from mineral surfaces (Kaiser and Zech, 1998). Higher N content in hydrophilic compounds compared to hydrophobic compounds suggests that DON is unlikely to be adsorbed in the mineral soil to the same extent as DOC. Evidence to support this would lie in a decrease in the DOC : DON ratio as soil solution percolates through the soil column. Whereas Qualls and Haines (1991) report a decrease in the DOC : DON ratio in mineral soil relative to organic soil horizons, we found no compelling evidence to support decreasing mineral soil solution the DOC : DON ratios in our synthesis. To date, there are no direct measures of DON adsorption in mineral soils and we have to assume from our synthesis that DON is no less susceptible to adsorption than DOC. Support for this assumption lies in the continuing relationship between DOC and DON in precipitation through to mineral soil solution (Fig. 6).

Soil pH affects the ability of mineral soil to adsorb carbon in laboratory studies (Jardine *et al*., 1989). At high pH, the adsorption capacity of a soil diminishes and DOC flux increases, whereas, at low pH, DOC adsorption to mineral soil is high and DOC flux is reduced. Evidence to support a pH

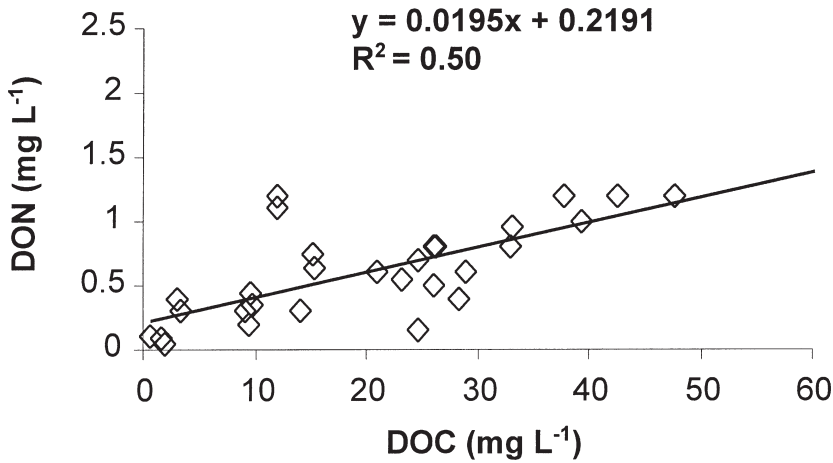

FIGURE 6 Relationship between DON and DOC in precipitation, throughfall, organic soil solution, and mineral soil solution. Data points are from individual studies (Tables I–IV) that reported both DOC and DON concentrations.

control on either the sorption ability or the solubilization of DOC is shown in several studies (e.g., Jardine *et al*., 1989; Tipping and Woof, 1990; Andersson *et al*., 1999). Tipping and Woof (1990) calculated that an increase of 0.5 pH units would result in a 50% increase in DOM. Inversely, in low-pH, reducing conditions, there is no preference for hydrophobic over hydrophilic DOC adsorption, suggesting that a reduction in DOM export would occur (Hagedorn *et al*., 2000). These studies suggest that a reduction in soil pH generated by nitrogen deposition is likely to decrease allochthonous inputs to surface waters. However, on a regional field scale, Michalzik *et al*. (2001), analyzing data from several studies, report no correlation between mineral soil solution DOC and soil pH.

Several methods are used to quantify DOC sorption: (a) measuring adsorption isotherms using continuous, unsaturated flow through intact soil cores (Qualls and Haines, 1992b), (b) initial mass relationships (Taylor and Kunishi, 1971; McDowell and Wood, 1984; Nodvin *et al*., 1986), and (c) Freundlich and Langmuir isotherms. Initial mass relationships were developed because the release of indigenous DOM could not be analyzed by either Freundlich or Langmuir isotherms (Kaiser *et al*., 2000). The majority of recent studies on DOC adsorption have adopted the mass isotherm approach. The initial mass isotherm describes the relationship between the amount of DOC adsorbed or released to the initial amount of DOC added to the soil. This approach is useful because it describes the reactive soil pool or the amount of substance present in the soil that can be readily solubilized or released as well as the partition coefficient which is a measure of the affinity of the substance in solution for the soil. The partition coefficient is not affected by sample storage or preparation or differing soil : solution ratios (Kaiser *et al*., 2001). This aspect will enable partition coefficient comparisons of a range of soils on a global scale, which may lead to greater insight into the effects of adsorption on allochthonous inputs to surface waters.

B. Loss of Soil Solution Dissolved Organic Carbon and Dissolved Organic Nitrogen through Immobilization and Mineralization

Relatively little research has been performed on the bioavailability of DOC in soil solution (Qualls and Haines, 1992a; Boyer and Groffman, 1996; Yano *et al*., 1998; 2000) compared to the large number of bioassays conducted for lake, stream, and marine systems (see Chapter 17). Labile or biodegradable DOC in soil solution ranges from 16 to 68% (Zsolnay and Steindl, 1991) and is dependent on soil depth and land use. Almost all DOC can be considered to be potentially labile if the time scale is long enough (e.g., Petsch *et al*., 2001). We define labile soil solution DOC as that which is immobilized and mineralized either as a first response (2–3 hours) (Yano *et al*., 1998) or over a relatively short time span (Qualls and Haines, 1992a).

Typically less than 27% of forest floor soil solution DOC is bioavailable (Qualls and Haines, 1992a; Boyer and Groffman, 1996; Yano *et al.*, 1998). This fraction corresponds extremely well to the 24% glucose found in Norway spruce DOM (Guggenberger *et al.*, 1994) and suggests that glucose may be the limiting factor in bulk soil solution DOC. Qualls and Haines (1992a) used flask incubations to investigate the biodegradability of DOC and DON in throughfall, soil solution obtained from several soil horizons, and stream water. Their method was to aerate inoculated solutions in flasks for a period of 134 days, measuring the DOC content at intervals to monitor reductions in the concentration of DOC. They report that the highest proportion of labile carbon was in throughfall (48.7%) with lower proportions of labile DOC in organic soil solution (27%). Yano *et al.* (1998, 2000) quantified bioavailable DOC in throughfall and Oa horizon soil solution using a flowthrough bioreactor method. They found that between 50 and 75% of throughfall is labile. Yano *et al.* (1998) found a similar percentage of labile DOC to that of Qualls and Haines (1992a) in soil solution of Oa horizons in both hardwood and pine forests (17–45%). They also report a significant seasonal effect on the quantity of labile DOC, which may be due to enhanced production of LMW DOC by roots during the growing season. Nitrogen fertilization also appears to affect the percentage of labile DOC in soil solution. Yano *et al.* (2000) report a higher proportion of biodegradable DOC in hardwood stands undergoing high nitrogen fertilization than in forest stands with no nitrogen fertilization.

C. Flow Paths and Wetlands

Large fluxes of allochthonous DOM to surface waters occur when there is a barrier precluding water infiltration of the soil column (i.e., from the organic to mineral soil horizon). Barriers to infiltration of the soil column include the occurrence of an ortstein horizon (cemented horizon containing spodic materials) and fragipan (a brittle pan underlying spodic, cambic, or argillic horizons) in the soil profile, particularly in humid temperate ecosystems with spodosols. In arid and humid tropical ecosystems, a duripan (silica cemented horizon) may act as an infiltration barrier. In subarctic and arctic ecosystems, the depth of permafrost can regulate the infiltration capacity of a soil. In watersheds where a barrier to infiltration occurs, the concentrations of DOC appearing in surface waters can be quite high (e.g., MacLean *et al.*, 1999). Furthermore, in watersheds where the slope is steep and the soil shallow, DOC export can also be exceptionally high because water is less likely to contact the underlying mineral soil. Saturated soils such as wetlands (Eckhardt and Moore, 1990) and peatlands (Dillon and Molot, 1997; Aitkenhead *et al.*, 1999) also tend to produce runoff with higher DOC concentrations (see Chapter 6). Saturated soils can be due to a barrier to infiltration or due to a perched or seasonally high water table.

D. Prediction of Soil Solution and Riverine Dissolved Organic Carbon Flux Using Watershed Soil Properties

Recent research shows that when organic soil solution concentrations are averaged over a growing season or year, 94% of the variance in forest floor soil solution DOC concentration in coniferous forests can be explained by soil C : N ratio (Aitkenhead and McDowell, 2000). In a laboratory setting, DOC also exhibits a strong correlation with soil C : N ratio in some studies (Gödde *et al*., 1996; Kalbitz and Knappe, 1997) but not in others (Michel and Matzner, 1999). At a coarser, biome scale, average biome soil C : N proved to be an excellent predictor of average biome riverine DOC export (Aitkenhead and McDowell, 2000). Unfortunately, most researchers interested in soil solution DOC and DON typically do not measure surface water DOC and DON and vice versa, so we are unable to test mineral soil solution DOC as a potential predictor of surface water DOC concentration and flux. Instead, we have illustrated the difference between a tropical rain forest and a temperate forest in terms of DOC flux through each potential allochthonous source (Fig. 7). Both studies used tension lysimeters for "organic" and "mineral" horizons. The flux of DOC in precipitation and throughfall is 3 times greater in a tropical rain forest compared to a

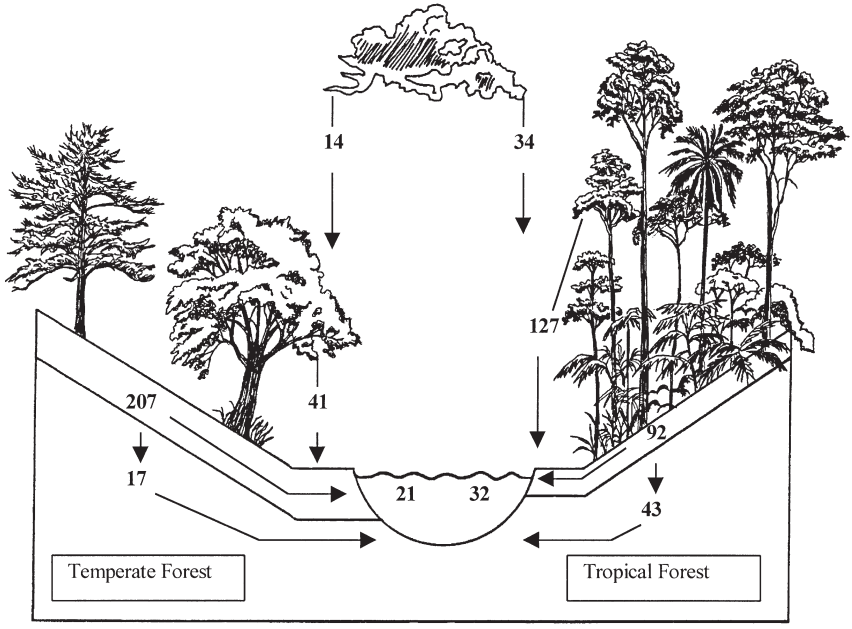

FIGURE 7 Flux of dissolved organic carbon in precipitation, throughfall, organic soil horizons, and mineral soil horizons in a cool temperate and wet tropical forest (kg ha). Source of data: McDowell and Likens (1988) and McDowell (1998). Illustration by L. Isaacson, University of New Hampshire.

temperate forest. This is due to increased rainfall in rain forest biomes. The temperate forest displays a doubling of DOC flux in the organic soil compared to the rain forest, which has reduced DOC flux. This is due to the different organic horizon depths between the two biomes. Soil solution sampling was at 40 cm depth and 80 cm depth for the tropical rain forest and 15 cm and 30 cm for the temperate forest. Adsorption of DOC in the mineral horizon is apparent in both biomes with a large reduction in DOC flux. In the tropical biome, further loss of DOC occurs prior to its appearance in stream water. In the temperate forest, however, DOC flux increases relative to mineral soil solution DOC flux. This may indicate greater autochthonous DOC in temperate streams compared to tropical streams.

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REFERENCES

Adamson, J. K., W. A. Scott, and A. P. Rowland. 1998. The dynamics of dissolved nitrogen in a blanket peat dominated catchment. *Environmental Pollution* **99:**69–77.

Aiken, G. R. 1984. Evaluation of ultrafiltration for determining molecular weight of fulvic acid. *Environmental Science and Technology* **18:**978–981.

Aitkenhead, J. A. 1995. Spatial and temporal variations in sources, and controls on DOC and major ions in streamwater draining forested and moorland catchments. B.Sc. Dissertation, University of Stirling.

Aitkenhead, J. A., and W. H. McDowell. 2000. Soil C : N ratio as a predictor of annual riverine DOC flux at local and global scales. *Global Biogeochemical Cycles* **14:**127–138.

Aitkenhead-Peterson, J. A. 2000. Source, production and export of dissolved organic carbon and nitrogen. Ph.D. Dissertation, University of New Hampshire.

Aitkenhead, J. A., D. Hope, and M. F. Billett. 1999. The relationship between dissolved organic carbon in streamwater and soil organic carbon pools at different spatial scales. *Hydrological Process* **13:**1289–1302.

Ajwa, H. A., C. J. Dell, and C. W. Rice. 1999. Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. *Soil Biology and Biochemistry* **31:**769–777.

Andersson, S., I. Nilsson, and I. Valeur. 1999. Influence of dolomitic lime on DOC and DON leaching in a forest soil. *Biogeochemistry* **47:**297–317.

Andreae, M. O., R. W. Talbot, H. Berresheim, and K. M. Beecher. 1990. Precipitation chemistry in central Amazonia. *Journal of Geophysical Research* **95:**16987–16999.

Arthur, M. A., and T. J. Fahey. 1993a. Throughfall chemistry in an Engelmann spruce–subalpine fir forest in north central Colorado. *Canadian Journal of Forest Research* **23:**738–742.

Arthur, M. A., and T. J. Fahey. 1993b. Controls on soil solution chemistry in a subalpine forest in north-central Colorado. *Soil Science Society of America Journal* **57:**1122–1130.

Bäumler, R., and W. Zech. 1998. Soil solution and impact of forest thinning in mountain forests in the Bavarian Alps. *Forest Ecology and Management* **108:**231–238.

Bergkvist, B., and L. Folkeson. 1992. Soil acidification and element fluxes of a *Fagus sylvatica* forest as influenced by simulated nitrogen deposition. *Water, Air and Soil Pollution* **65:**111–133.

Bockheim, J. G., and S. Langley-Turnbaugh. 1997. Biogeochemical cycling in coniferous ecosystems on different aged marine terraces in coastal Oregon. *Journal of Environmental Quality* **26:**292–301.

Bowden, R. D., K. J. Nadelhoffer, R. D. Boone, J. M. Melillo, and J. B. Garrison. 1993. Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperate mixed hardwood forest. *Canadian Journal of Forest Research* **23:**1402–1407.

Boyer, E. W., G. M. Hornberger, K. E. Bencala, and D. McKnight. 1996. Overview of a simple model describing variation of dissolved organic carbon in an upland catchment. *Ecological Modeling* **86:**183–188.

Boyer, J. N., and P. M. Groffman. 1996. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. *Soil Biology and Biochemistry* **28:**783–790.

Brinkman, W. L. F. 1983. Studies on hydrobiogeochemistry of a tropical lowland forest system. *Geojournal* **11:**89–101.

Brinson, M. M., H. D. Bradshaw, R. N. Holmes, and J. B. Elkins, Jr. 1980. Litterfall, stemflow and throughfall nutrient fluxes in an alluvial swamp forest. *Ecology* **61:**827–835.

Brooks, P. D., D. M. McKnight, and K. E. Bencala. 1999. The relationship between soil heterotrophic activity, soil dissolved organic carbon (DOC) leachate, and catchment-scale DOC export in headwater catchments. *Water Resources Research* **35:**1895–1902.

Buffle, J., P. Deladoey, and W. Haerdi. 1978. The use of ultrafiltration for separation and fractionation of organic ligands in fresh waters. *Analytica Chimica Acta* **101:**339–357.

Caiazza, R., K. D. Hage, and D. Gallup. 1978. Wet and dry deposition of nutrients in central Alberta. *Water, Air and Soil Pollution* **9:**309–314.

Campbell, J. L., J. W. Hornbeck, W. H. McDowell, D. C. Buso, J. B. Shanley, and G. E. Likens. 2000. Dissolved organic nitrogen budgets for upland forested ecosystems in New England. *Biogeochemistry* **49:**123–142.

Carlisle, A. A., H. F. Brown, and E. J. White. 1966. The organic matter and nutrient elements in the precipitation beneath a sessile oak (*Quercus petraea*) canopy. *Journal of Ecology* **54:**87–98.

Carreiro, M. M., R. L. Sinsabaugh, D. A. Repert, and M. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* **81:**2359–2365.

Casals, P., J. Romanya, J. Cortina, J. Fons, M. Bode, and V. R. Vallejo. 1995. Nitrogen supply rate in Scots pine (*Pinus sylvestris*) forests of contrasting slope and aspect. *Plant and Soil* **169:**67–73.

Chittleborough, D. J. K., R. J. Smettem, E. Cotaris, and F. W. Leaney. 1992. Seasonal changes in pathways of dissolved organic carbon through a hillslope soil (Xeralf) with contrasting texture. *Australian Journal of Soil Research* **30:**465–476.

Chorover, J. P., M. Vitousek, D. A. Everson, A. M. Esperanza, and D. Turner. 1994. Solution chemistry profiles of mixed conifer forests before and after fire. *Biogeochemistry* **26:**115–144.

Clair, T. A., T. L. Pollock, and J. M. Ehrman. 1994. Exports of carbon and nitrogen from river basins in Canada's Atlantic provinces. *Global Biogeochemical Cycles* **8:**441–450.

Clair, T. A., J. M. Ehrman, and K. Higuchi. 1999. Changes in freshwater carbon exports from Canadian terrestrial basins to lakes and estuaries under a $2 \times CO_2$ atmospheric scenario. *Global Biogeochemical Cycles* **13:**1091–1097.

Cole, J. J., W. H. McDowell, and G. E. Likens. 1984. Sources and molecular weight of "dissolved" organic carbon in an oligotrophic lake. *Oikos* **42:**1–9.

Colina-Tejada, A., A. Ambles, and P. Jambu. 1996. Nature and origin of soluble lipids shed into the soil by rainwater leaching a forest cover of *Pinus maritima* sp. *European Journal of Soil Science* **47:**637–643.

Cornell, S., A. Rendell, and T. Jickells. 1995. Atmospheric inputs of dissolved organic nitrogen to the oceans. *Nature* **376:**243–246.

Cortina, J., J. Romanya, and W. R. Vallejo. 1995. Nitrogen and phosphorous leaching from the forest floor of a mature *Pinus radiata stand*. *Geoderma* **66:**321–330.

Cronan, C. S., and G. R. Aiken. 1985. Chemistry and transport of soluble humic substances in forested watersheds of the Adirondack Park, New York. *Geochimica et Cosmochimica Acta* **45:**1697–1705.

Currie, W. S., and J. D. Aber. 1997. Modeling leaching as a decomposition process in humid montane forests. *Ecology* **78:**1844–1860.

Currie, W. S., J. D. Aber, W. H. McDowell, R. D. Boone, and A. H. Magill. 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. *Biogeochemistry* **35:**471–505.

Curtis, W. F., R. H. Meade, C. F. Nordin, Jr., N. B. Price, and E. R. Sholkovitz. 1979. Non-uniform vertical distribution of fine sediment in the Amazon River. *Science* **280:**381–383.

Dahlgren, R. A., and D. J. Marrett. 1991. Organic carbon sorption in arctic and subalpine spodosol B horizons. *Soil Science Society of America Journal* **55:**1382–-1390.

Dai, K. O. H., M. B. David, and G. F. Vance. 1996. Characterization of solid and dissolved carbon in a spruce fir spodosol. *Biogeochemistry* **35:**339–365.

Dalva, M., and T. R. Moore. 1991. Sources and sinks of dissolved organic carbon in a forested swamp catchment. *Biogeochemistry* **15:**1–19.

David, M. B., and C. T. Driscoll. 1984. Aluminum speciation and equilibria in soil solutions of a haplorthod in the Adirondack Mountains (New York USA). *Geoderma* **33:**297–318.

David, M., B. A. M. Cupples, G. B. Lawrence, G. Shi, K. Vogt, and P. M. Wargo. 1998. Effect of chronic nitrogen additions on soil nitrogen fractions in red spruce stands. *Water, Air and Soil Pollution* **105:**183–192.

David, M., G. Vance, and J. Kahl. 1999. Chemistry of dissolved organic carbon at Bear Brook watershed, Maine: Stream water response to $(NH_4)2SO_4$ additions. *Environmental Monitoring and Assessment* **55:**149–163.

Dawson, J. C., D. Hope, M. S. Cresser, and M. F. Billett. 1995. Downstream changes in free carbon dioxide in an upland catchment from northeast Scotland. *Journal of Environmental Quality* **24:**699–706.

Degens, E. T. 1982. Transport of carbon and minerals in major world rivers. 1. *In* "Proceedings of a Workshop Arranged by the Scientific Committee on Problems of the Environment (SCOPE) and the United Nations Environment Programme (UNEP), Hamburg University."

Degens, E. T., S. Kempe and J. E. Richey. 1991 "Biogeochemistry of Major World Rivers." Wiley, Chichester.

Dillon, P. J., and L. A. Molot. 1997. Effect of landscape form on export of dissolved organic carbon, iron and phosphorous from forested stream catchments. *Water Resources Research* **33:**2591–2600.

Dinesh, R. B., R. Shomew, R. Shome, and A. K. Bandyopadhyay. 1998. Soil enzymes in mangroves: Activities and their relation to soil properties. *Current Science* **75:**510–512.

Dixon, R. K., and D. P. Turner. 1991. The global carbon cycle and climate change: Responses and feedbacks from below ground systems. *Environmental Pollution* **73:**245–262.

Donald, R. G., D. W. Anderson, and J. W. B. Stewart. 1993. Potential role of dissolved organic carbon in phosphorus transport in forested soils. *Soil Science Society of America Journal* **57:**1611–1618.

Dosskey, M. G., and P. M. Bertsch. 1994. Forest sources and pathways of organic matter transport to a blackwater stream: A hydrologic approach. *Biogeochemistry* **24:**1–19.

Dosskey, M. G., and P. M. Bertsch. 1997. Transport of dissolved organic matter through a sandy forest soil. *Soil Science Society of America Journal* **61:**920–927.

Driscoll, C. T., R. D. Fuller, and D. M. Simone. 1988. Longitudinal variations in trace metal concentrations in a northern forested ecosystem. *Journal of Environmental Quality* **17:**101–107.

Easthouse, K., B. J. Mulder, N. Christophersen, and H. M. Seip. 1992. Dissolved organic carbon fractions in soil and streamwater during variable hydrological conditions at Birkeness, Southern Norway. *Water Resources Research* **28:**1585–1596.

Eaton, J. S., G. E. Likens, and F. H. Bormann. 1973. Throughfall and stemflow chemistry in a northern hardwood forest. *Journal of Ecology* **61:**495–508.

Eckhardt, B., and T. R. Moore. 1990. Controls on dissolved organic carbon concentrations in streams, southern Quebec. *Canadian Journal of Fisheries and Aquatic Sciences* **47:**1537–1544.

Edmonds, R. L., T. B. Thomas, and J. J. Rhodes. 1991. Canopy and soil modification of precipitation chemistry in a temperate rain forest. *Soil Science Society of America Journal* **55:**1685–1693.

Eklund, T. J., W. H. McDowell, and C. M. Pringle. 1997. Seasonal variation of tropical precipitation chemistry: La Selva, Costa Rica. *Atmospheric Environment* **31:**3903–3910

Evans, A., Jr., L. W. Zelazny, and C. E. Zipper. 1988. Solution parameters influencing dissolved organic carbon levels in three forest soils. *Soil Science Society of America Journal* **52:**1789–1792.

Fahey, T. J., and J. B. Yavitt. 1988. Soil solution chemistry in Lodgepole pine (*Pinus contorta* sp. *latifolia*) ecosystems, southeastern Wyoming, USA. *Biogeochemistry* **6:**91–118.

Fahey, T. J., J. B. Yavitt, J. A. Pearson, and D. H. McKnight. 1985. The nitrogen cycle in lodgepole pine forests, southeastern Wyoming. *Biogeochemistry* **1:**257–275.

Fernandez, I. J., G. B. Lawrence, and Y. Son. 1995. Soil solution chemistry in a low elevation spruce fir ecosystem, Howland, Maine. *Water, Air and Soil Pollution* **84:**129–145.

Fernandez-Sanjuro, M. J., V. Fernandez Vega, and E. Garcia-Rodeja. 1997. Atmospheric deposition and ionic concentration in soils under pine and deciduous forests in the river Sor catchment (Galicia, NW Spain). *Science of the Total Environment* **204:**125–134.

Ferrier, R. C., J. D. Miller, T. A. B. Walker, and H. A. Anderson. 1990. Hydrochemical changes associated with vegetation and soils. *In* "The Surface Waters Acidification Programme" (B. J. Mason, Ed.). Cambridge Univ. Press, Cambridge, UK.

Fiebig, D. M., M. A. Lock, and C. Neal. 1990. Soil water in the riparian zone as a source of carbon for a headwater stream. *Journal of Hydrology* **116:**217–237.

Fox, T. R. 1995. The influence of low-molecular-weight organic acids on properties and processes in forest soils. *In* "Carbon Forms and Functions in Forest Soils" (W. W. McFee and J. M. Kelly, Eds.). Soil Science Society of America, Madison, WI.

Fox, T. R., and N. B. Comerford. 1990. Low molecular weight organic acids in selected forest soils of the southeastern USA. *Soil Science Society of America Journal* **54:**1139–1144.

France, R., H. Culbert, and R. Peters. 1996. Decreased carbon and nutrient input to boreal lakes from particulate organic matter following riparian clear-cutting. *Environmental Management* **20:**579–583.

Freeman, C., G. Liska, N. J. Ostle, M. A. Lock, S. Hughes, B. Reynolds, and J. Hudson. 1997. Enzymes and biogeochemical cycling in wetlands during a simulated drought. *Biogeochemistry* **39:**177–187.

Friedland, A. J., E. K. Miller, J. J. Battles, and J. F. Thorne. 1991. Nitrogen deposition, distribution and cycling in a subalpine spruce-fir forest in the Adirondacks, New York, USA. *Biogeochemistry* **14:**31–55.

Geary, R. J., and C. T. Driscoll. 1996. Forest soil solutions: Acid/base chemistry and response to calcite treatment. *Biogeochemistry* **32:**195–220.

Gödde, M., M. B. David, M. J. Christ, M. Kaupenjohann, and G. F. Vance. 1996. Carbon mobilization from the forest floor under red spruce in the northeastern USA. *Soil Biology and Biochemistry* **28:**1181–1189.

Gosz, J. R. 1980. Nutrient budget studies for forests along an elevational gradient in New Mexico. *Ecology* **61:**515–521.

Grant, M. C., and W. M. Lewis. 1982. Chemical loading rates from precipitation in the Colorado Rockies. *Tellus* **34:**74–88.

Grieve, I. C. 1984. Concentrations and annual loading of dissolved organic matter in a small moorland stream. *Freshwater Biology* **14:**533–537.

Grieve, I. C. 1990a. Variations in chemical composition of the soil solution over a four-year period at an upland site in southwest Scotland. *Geoderma* **46:**351–362.

Grieve, I. C. 1990b. Soil and soil solution chemical composition at three sites within the Loch Dee catchment, SW Scotland. *Journal of Soil Science* **41:**269–277.

Grieve, I. C. 1991a. A model of dissolved organic carbon concentration in soil and stream waters. *Hydrological Processes* **5:**301–307.

Grieve, I. C. 1991b. Dissolved organic carbon trends in small streams, land use effects and models of temporal variation. *In* "Sediment and Stream Water Quality in a Changing Environment: Trends and Explanation." Proceedings of the Vienna Symposium, August, 1991. IAHS Publication, No. 203, pp. 201–208.

Gu, B., J. Schmitt, Z. Chen, L. Liang, and J. F. McCarthy. 1995. Adsorption and desorption of differing organic matter fractions on iron oxide. *Geochimica et Cosmochimica Acta* **59:**219–229.

Guggenberger, G., and W. Zech. 1992. Retention of dissolved organic carbon and sulfate in aggregated acid forest soils. *Journal of Environmental Quality* **21:**643–653.

Guggenberger, G., and W. Zech. 1993. Dissolved organic carbon control in acid forest soils of the Fichtelgebirge (Germany) as revealed by distribution patterns and structural composition analysis. *Geoderma* **59:**109–129.

Guggenberger, G., W. Zech, and H. R. Schulter. 1994. Formation and mobilization of dissolved organic matter: Evidence from chemical structural studies of organic matter fractions in acid forest floor solutions. *Organic Geochemistry* **21:**51–66.

Gundersen, P., B. A. Emmett, O. J. Kjonaas, C. J. Koopmans, and A. Tietema. 1998. Impact of nitrogen deposition on nitrogen cycling in forests: A synthesis of NITREX data. *Forest Ecology and Management* **101:**37–55.

Hadas, A., M. Sofer, J. A. E. Molina, P. Barak, and C. E. Clapp. 1992. Assimilation of nitrogen by soil microbial populations: NH_4 versus organic N. *Soil Biology and Biochemistry* **24:**137–143.

Hagedorn, F., P. Schleppi, P. Waldner, and H. Fluhler. 2000. Export of dissolved organic carbon and nitrogen from Gleysol dominated catchments: The significance of water flow paths. *Biogeochemistry* **50:**137–161.

Hayes, M. H. B., R. S. Swift, R. E. Wardle, and J. K. Brown. 1975. Humic materials from organic soil: A comparison of extractants and properties of extracts. *Geoderma* **13:**231–245.

Hendry, C. D., and P. L. Brezonik. 1980. Chemistry of precipitation at Gainesville, Florida. *Environmental Science and Technology* **14:**843–849.

Herbert, B. E., and P. M. Bertsch. 1995. Characterization of dissolved and colloidal organic matter in soil solution: A review. *In* "Carbon Forms and Functions in Forest Soils" (W. W. McFee and J. M. Kelly, Eds.), pp. 63–88, Soil Science Society of America, Madison, WI.

Hongve, D. 1999. Production of dissolved organic carbon in forested catchments. *Journal of Hydrology* **224:**91–99.

Hope, D., M. F. Billett, and M. S. Cresser. 1994. A review of the export of carbon in river water: Fluxes and processes. *Environmental Pollution* **84:**301–324.

Hope, D., M. F. Billett, R. Milne, and T. A. W. Brown. 1997. Exports of organic carbon in British rivers. *Hydrological Processes* **11:**325–344.

Inoue, T., and K. Wada. 1968. Adsorption of humified clover extracts by various clays. *International Congress of Soil Science Transactions* **9:**289–298.

Jardine, P. M., N. L. Weber, and J. F. McCarthy. 1989. Mechanisms of dissolved organic carbon adsorption on soil. *Soil Science Society of America Journal* **53:**1378–1385.

Jekel, M. R. 1991. Particle stability in the presence of pre-ozonated humic acids. *Aqua* **40:**18–24.

Jordan, T. E., D. L. Correll, D. E. Weller, and N. M. Goff. 1995. Temporal variation in precipitation chemistry on the shore of the Chesapeake Bay. *Water, Air and Soil Pollution* **83:**263–284.

Kaiser, K., and W. Zech. 1996. Nitrate, sulfate, and biphosphate retention in acid forest soils affected by natural dissolved carbon. *Journal of Environmental Quality* **25:**1325–1331.

Kaiser, K., and W. Zech. 1998. Rates of dissolved organic matter release and sorption in forest soils. *Soil Science* **163:**714–725.

Kaiser, K., L. Haumaier, and W. Zech. 2000. The sorption of organic matter in soils as affected by the nature of soil carbon. *Soil Science* **165:**305–313.

Kaiser, K., M. Kaupenjohann, and W. Zech. 2001. Sorption of dissolved organic carbon in soils: Effects of soil sample storage, soil-to-solution ratio, and temperature. *Geoderma* **99:**317–328.

Kalbitz, K., and S. Knappe. 1997. Einfluß der Bodeneigenschaften auf die Friesetzung der gelösten organischen Substanz (DOM) aus dem Oberboden. *Zeitschrift für Pflanzenernfür und Bodenkunde* **160:**475–483.

Kelly, J. M., and J. F. Meagher. 1986. Nitrogen input/output relationships for three forested watersheds in eastern Tennessee. *In* "Watershed Research Perspectives" (D. L. Correll, Ed.). Smithsonian Press, Washington, DC.

Kennedy, J., M. F. Billett, D. Duthie, A. R. Fraser, and A. F. Harrison. 1996. Organic matter retention in an upland humic podzol: The effects of pH and solute type. *European Journal of Soil Science* **47:**615–625.

Khomutova, T. E., L. T. Shirshova, S. Tinz, W. Rolland, and J. Richter. 2000. Mobilization of DOC from sandy loamy soils under different land use (Lower Saxony, Germany). *Plant and Soil* **219:**13–19.

Kling, G. W., G. W. Kipphut, and M. C. Miller. 1992. The flux of CO_2 and CH_4 from lakes and rivers in arctic Alaska. *Hydrobiologia* **240:**23–36.

Koprivnjak, J. F., and T. R. Moore. 1992. Sources, sinks and fluxes of dissolved organic carbon in sub-arctic fen catchments. *Arctic and Alpine Research* **24:**204–210.

Kram, P., J. Hrupka, B. S. Wenner, C. T. Driscoll, and C. E. Johnson. 1997. The biogeochemistry of base cations in two forest catchments with contrasting lithology in the Czech Republic. *Biogeochemistry* **37:**173–202.

Kreutzer, K. 1995. Effects of forest liming on soil processes. *Plant and Soil* **168/169:**447–470.

Lawrence, G. B., and M. B. David. 1996. Chemical evaluation of soil solution in acid forest soils. *Soil Science* **161:**298–313.

Leenheer, J. A. 1980. Study of sorption of complex organic solute mixtures on sediments by dissolved organic carbon fractionation analysis. *In* "Contaminants and Sediments." (R. A. Baker, Ed.). Ann Arbor Science, Ann Arbor, MI.

Leenheer, J. A. 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and waste waters. *Environmental Science and Technology* **15:**578–587.

Leichty, H. O., E. Kuuseoks, and G. D. Mroz. 1995. Dissolved organic carbon in northern hardwood stands with differing acidic inputs and temperature regimes. *Journal of Environmental Quality* **24:**927–933.

Lesack, L. F. W., and J. M. Melack. 1996. Mass balance of major solutes in a rainforest catchment in the Central Amazon: Implications for nutrient budgets in tropical rainforests. *Biogeochemistry* **32:**115–152.

Lewis, W. M., Jr. 1981. Precipitation chemistry and nutrient loading by precipitation in a tropical watershed. *Water Resources Research* **17:**169–181.

Likens, G. E., E. S. Edgerton, and J. N. Galloway. 1983. The composition and deposition of organic carbon in precipitation. *Tellus* **35B:**16–24.

Litaor, M. I. 1988. Soil solution chemistry in an alpine watershed, Front Range, Colorado, USA. *Arctic and Alpine Research* **20:**485–491.

Lobert, J. M., D. H. Scharffe, W. M. Hao, T. A. Kuhlbusch, P. Warneck, and P. J. Crutzen. 1991. Experimental evaluation of biomass burning emissions: Nitrogen and carbon containing compounds. *In* "Global Biomass Burning: Atmospheric, Climatic and Biospheric Implications" (J. S. Levine, Ed.). MIT Press, Cambridge, MA.

Lodge, D. J., W. H. McDowell, and C. P. McSwiney. 1994. The importance of nutrient pulses in tropical forests. *Trends in Ecology and Evolution* **9:**384–387.

Lundström, U. S. 1993. The role of organic acids in the soil solution of a podzolized soil. *Journal of Soil Science* **44:**121–133.

MacLean, R. M., W. Oswood, J. G. Irons III, and W. H. McDowell. 1999. The effect of permafrost on stream biogeochemistry: A case study of two streams in the Alaskan (U.S.A.) taiga. *Biogeochemistry* **47:**239–267.

Malcolm, R. K. 1993. Concentration and composition of dissolved organic carbon in soils, streams and groundwaters. *In* "Organic Substances in Soil and Water: Natural Constituents and Their Influence on Contaminant Behavior." (A. J. Beck, K. C. Jones, M. H. B. Hayes, and U. Mingelgrin, eds.), pp. 19–30, Royal Society of Chemistry, Cambridge, UK.

Malmqvist, P. A. 1978. Atmospheric fallout and street cleaning — effects on urban storm water and snow. *Progress in Water Technology* **10:**495–505.

Manabe, S., and R. T. Wetherald. 1980. On the distribution of climate change resulting from an increased CO_2 content of the atmosphere. *Journal of Atmospheric Science* **37:**99–118.

Marin, L. E., T. K. Kratz, and C. J. Bowser. 1990. Spatial and temporal patterns in the hydrogeochemistry of a poor fen in northern Wisconsin. *Biogeochemistry* **1:**63–76.

Marques, R., and J. Ranger. 1997. Nutrient dynamics in a chronosequence of Douglas fir (*Pseudotsuga menziesii* (Mirb,) Franco) stands in the Beaujolais Mounts (France). *Forest Ecology and Management* **91:**255–277.

Martell, A. E., R. J. Motekaitis, and R. M. Smith. 1988. Structure stability relationships of metal complexes and metal speciation in environmental aqueous solutions. *Environmental Toxicology and Chemistry* **7:**417–434.

Matzner, E. 1988. Der Stoffumsatz zweier Waldökosysteme im Solling, Ber. d. Forschungszentrums Waldökosysteme/Waldsterben d. Uni Gottingen. Report Reihe A, Bd. 40, pp. 1–217.

Mayer, L. M. 1994a. Relationships between mineral surfaces and organic carbon concentrations in soils and sediments. *Chemical Geology* **114:**347–363.

Mayer, L. M. 1994b. Surface area control of organic carbon accumulation in continental shelf sediments. *Geochimica et Cosmochimica Acta* **58:**1271–1284.

McCart, E., A. M. Gordon, N. K. Kaushik, B. Lazerte, E. Mallory, R. W. Bachmann, J. R. Jones, R. H. Peters, and D. M. Soballe. 1995. Characterization of selected allochthonous organic carbon inputs within a terrestrial–aquatic ecotone in Algonquin Park, Ontario. *Lake Reserve Management* **11:**168.

McClain, M. E., J. E. Richey, J. A. Brandes, and T. P. Pimentel. 1997. Dissolved organic matter and terrestrial–lotic linkages in the central Amazon basin of Brazil. *Global Biogeochemical Cycles* **11:**295–311.

McCracken, K. L. 1998. Dynamics of dissolved organic carbon during weathering and soil formation. Ph.D. Dissertation, University of New Hampshire, Durham.

McDowell, W. H. 1982. Mechanisms controlling the organic chemistry of Bear Brook, NH. Ph.D. Dissertation, Cornell University, Ithaca, NY.

McDowell, W. H. 1998. Internal nutrient fluxes in a Puerto Rican rain forest. *Journal of Tropical Ecology* **14:**521–536.

McDowell, W. H., and S. G. Fisher. 1976. Autumnal processing of dissolved organic matter in a small woodland stream ecosystem. *Ecology* **57:**561–569.

McDowell, W. H., and G. E. Likens. 1988. Origin, composition and flux of dissolved organic carbon in the Hubbard Brook valley. *Ecological Monographs* **58:**177–195.

McDowell, W. H., and T. Wood. 1984. Podzolization: Soil processes control dissolved organic carbon in streamwater. *Soil Science* **137:**23–32.

McDowell, W. H., C. Gines-Sanchez, C. E. Asbury, and C. R. Ramos-Perez. 1990. Influence of sea salt aerosols and long-range transport on precipitation chemistry at El Verde, Puerto Rico. *Atmospheric Environment* **24A:**2813–2821.

McDowell, W. H., W. S. Currie, J. D. Aber, and Y. Yano. 1998. Effects of chronic nitrogen amendment on production of dissolved organic carbon and nitrogen in forest soils. *Water, Air and Soil Pollution* **105:**175–182.

McKnight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography* **46:**38–48.

McLatchey, G. P., and K. R. Reddy. 1998. Regulation of organic matter decomposition and nutrient release in a wetland soil. *Journal of Environmental Quality* **27:**1268–1274.

McLaughlin, J. W., G. Liu, M. F. Jurgensen, and M. R. Gale. 1996. Organic carbon characteristics in a spruce swamp five years after harvesting. *Soil Science Society of America Journal* **60:**1228–1236.

Meybeck, M. 1982. Carbon, nitrogen and phosphorous transport in world rivers. *American Journal of Science* **282:**401–450.

Michalzik, B., and E. Matzner. 1999. Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. *European Journal of Soil Science* **50:**579–590.

Michalzik, B., T. Mueller, and B. Stadler. 1999. Aphids on Norway spruce and their effects on forest floor solution chemistry. *Forest Ecology and Management* **118:**1–10.

Michalzik, B., K. Kalbitz, J. H. Park, S. Solinger, and E. Matzner. 2001. Fluxes and concentrations of dissolved organic carbon and nitrogen — a synthesis for temperate forests. *Biogeochemistry* **52:**173–205.

Michel, K., and E. Matzner. 1999. Release of dissolved organic carbon and nitrogen from forest floors in relation to solid phase properties, respiration and N-mineralization. *Journal of Plant Nutrition and Soil Science* **62:**645–652.

Miller, J. D., H. A. Anderson, R. C. Ferrier, and T. A. B. Walker. 1990. Hydrochemical fluxes and their effects on stream acidity in two forested catchments in central Scotland. *Forestry* **63:**311–330.

Milne, P. J., and R. G. Zita. 1993. Amino acid nitrogen in atmospheric aerosols: Occurrence, sources and photochemical modification. *Journal of Atmospheric Chemistry* **16:**361–398.

Møller, J., M. Miller, and A. Kjoller. 1999. Fungal–bacterial interaction on beech leaves: Influence on decomposition and dissolved organic matter quality. *Soil Biology and Biochemistry* **31:**367–374.

Moore, T. R. 1988. Dissolved iron and organic matter in northern peatlands. *Soil Science* **145:**70–76.

Moore, T. 1989. Dynamics of dissolved organic carbon in forested catchments, Westland New Zealand. *Water Resources Research* **25:**1321–1330.

Moore, T. R., and R. J. Jackson. 1989. Dynamics of dissolved organic carbon in forested and disturbed catchments, Westland, New Zealand. 2. Larry River. *Water Resources Research* **25:**1331–1339.

Moore, T. R., and L. Matos. 1999. The influence of source on the sorption of dissolved organic carbon by soils. *Canadian Journal of Soil Science* **79:**321–324.

Moore, T. R., W. de Souza, and J. F. Koprivnjak. 1992. Controls on the sorption of dissolved organic carbon by soils. *Soil Science* **154:**120–129.

Mulholland, P. J. 1981. Organic carbon flow in a swamp stream ecosystem. *Ecological Monographs* **51:**307–322.

Neff, J. C., and G. Asner. 2001. Dissolved organic carbon in terrestrial ecosystems: Synthesis and a model. *Ecosystems* **4:**29–48.

Neff, J. C., E. A. Holland, F. J. Dentener, W. H. McDowell, and K. M. Russell. 2002. Atmospheric organic nitrogen: Implications for the global N cycle. *Biogeochemistry* **57/58:**99–136.

Nelson, P. N., E. Cotsaris, J. M. Oades, and D. B. Burnsill. 1990. The influence of soil clay on dissolved organic matter in stream waters. *Australian Journal of Marine and Freshwater Research* **41:**761–774.

Nelson, P. N., J. A. Baldock, and J. M. Oades. 1993. Concentration and composition of dissolved organic carbon in streams in relation to catchment soil properties. *Biogeochemistry* **19:**27–50.

Neumman, G. H., S. Fonselius, and I. Wahlman. 1959. Measurements on the content of non-volatile organic material in atmospheric precipitation. *International Journal of Air Pollution* **2:**132–141.

Nguyen, V. D., A. G. A. Merks, and P. Valenta. 1990. Atmospheric deposition of acid, heavy metals and dissolved organic carbon and nutrients in the Dutch delta area in 1980–1986. *Science of the Total Environment* **99:**77–91.

Nichols, K. H., and C. M. Cox. 1978. Atmospheric nitrogen and phosphorous loading to Harp Lake, Ontario, Canada. *Water Resources Research* **14:**589–592.

Nielson, K. E., U. L. Ladekarl, and P. Nornberg. 1999. Dynamic soil processes on heathland due to changes in vegetation to oak and sitka spruce. *Forest Ecology and Management* **114:**107–116.

Nodvin, S. C., C. T. Driscoll, and G. E. Likens. 1986. Simple partitioning of anions and dissolved organic carbon in a forest soil. *Soil Science* **142:**27–35.

Norton, S., J. Kahl, I. Fernandez, T. Haines, L. Rustad, S. Nodvin, J. Scofield, T. Strickland, H. Erickson, P. Wiggington, and J. Lee. 1999. The Bear Brook Watershed, Maine (BBWM), USA. *Environmental Monitoring and Assessment* **55:**7–51.

Parfitt, R. L., A. R. Fraser, and V. C. Farmer. 1977. Adsorption on hydrous oxides. III. Fulvic acid and humic acid on goethite, gibbsite and imogolite. *Journal of Soil Science* **28:**289–296.

Parsons, J. W., and J. Tinsley. 1975. Nitrogenous Substances. *In* "Soil Components" (J. E. Geiseking, Ed.), pp. 263–304. Springer-Verlag, New York.

Paul, E., and F. E. Clark. 1989. "Soil Microbiology and Biochemistry." Academic Press, San Diego.

Peierls, B. J., and H. W. Paerl. 1997. Bioavailability of atmospheric organic nitrogen deposition to coastal phytoplankton. *Limnology and Oceanography* **42:**1819–1823.

Perdue, E. M., K. C. Beck, and J. Helmut-Reuter. 1976. Organic complexes of iron and aluminium in natural waters. *Nature* **260:**418–420.

Petsch, S. T., T. I. Eglinton, and K. J. Edwards. 2001. ^{14}C-dead living biomass: Evidence for microbial assimilation of ancient organic carbon during shale weathering. *Science* **292:**1127–1131.

Pignatello, J. J. 1993. Recent advances in sorption kinetics. *In* "Organic Substances in Soil and Water: Natural Constituents and Their Influences on Contaminant Behavior" (A. J. Beck, K. C. Jones, M. H. B. Hayes, and U. Mingelgrin, Eds.), pp. 128–140. Royal Society of Chemistry, Cambridge, UK.

Piirainen, S., L. Finer, and M. Starr. 1998. Canopy and soil retention of nitrogen deposition in a mixed boreal forest in eastern Finland. *Water, Air and Soil Pollution* **105:**165–174.

Qualls, R. G. 2000. Comparison of the behavior of soluble organic and inorganic nutrients in forest soils. *Forest Ecology and Management* **138:**29–50.

Qualls R. G., and B. L. Haines. 1991. Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. *Soil Science Society of America Journal* **55:**1112–1123.

Qualls, R. G., and B. L. Haines. 1992a. Biodegradability of dissolved organic matter in forest throughfall, soil solution and streamwater. *Soil Science Society of America Journal* **56:**578–586.

Qualls, R., and B. L. Haines. 1992b. Measuring adsorption isotherms using continuous, unsaturated flow through intact soil cores. *Soil Science Society of America Journal* **56:**456–460.

Qualls, R. G., B. L. Haines, and W. T. Swank. 1991. Fluxes of dissolved organic nutrients and humic substances in a deciduous forest. *Ecology* **72:**254–266.

Quideau, S. A., and J. G. Bockheim. 1997. Biogeochemical cycling following planting to red pine on a sandy prairie soil. *Journal of Environmental Quality* **26:**1167–1175.

Raastad, I. A., and J. Mulder. 1999. Dissolved organic matter (DOM) in acid forest soils at Gardsjon (Sweden): Natural variabilities and effects of increased input of nitrogen and of reversal of acidification. *Water, Air and Soil Pollution* **114:**199–219.

Raymond, P. A., and J. E. Bauer. 2001. Riverine export of aged terrestrial organic matter to the North Atlantic Ocean. *Nature* **409:**497–500.

Reynolds, B., M. Hornung, and S. Hughes. 1989. Chemistry of streams draining grassland and forest catchments at Plynlimon, mid-Wales. *Hydrological Science Journal* **34:**667–686.

Richter, D. D., and D. Markewitz. 1996. Carbon changes during the growth of loblolly pine on formerly cultivated soil. The Calhoun Experimental Forest, USA. *NATO Advanced Studies Institute Series* **138:**397–407.

Riekerk, H. 1983. Impacts of silviculture on flatwoods runoff, water quality and nutrient budgets. *Water Resources Bulletin* **19:**73–70.

Rind, D., R. Goldberg, J. Hansen, C. Rosenzweig, and R. Reudy. 1990. Potential evapotranspiration and the likelihood of future drought. Journal of *Geophysical Research* **95:**9983–10004.

Ross, D. J., K. R. Tate, N. A. Scott, and C. W. Feltham. 1999. Land-use change: Effects on soil carbon, nitrogen and phosphorous pools and fluxes in three adjacent ecosystems. *Soil Biology and Biochemistry* **31:**803–813.

Rustad, L. E., I. J. Fernandez, R. D. Fuller, M. B. David, S. C. Nodvin, and W. A. Halterman. 1993. Soil solution response to acidic deposition in a northern hardwood forest. *Agriculture Ecosystems and Environment* **47:**117–134.

Schade, G. W., and P. J. Crutzen. 1995. Emission of aliphatic amines from animal husbandry and their reactions: Potential source of N_2O and HCN. *Journal of Atmospheric Chemistry* **22:**319–346.

Schiff, S. L., R. Aravena, S. E. Trumbore, and P. J. Dillon. 1990. Dissolved organic carbon cycling in forested watersheds: A carbon isotope approach. *Water Resources Research* **26:**2949–2957.

Schindler, J. E., and D. P. Krabbenhoft. 1998. The hyporheic zone as a source of dissolved organic carbon and carbon gases to a temperate forested stream. *Biogeochemistry* **43:**157–174.

Schindler, D. W., P. J. Curtis, B. R. Parker, and M. P. Stainton. 1996. Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes. *Nature* **379:**705–708.

Schindler, D. W., P. J. Curtis, S. E. Bayley, B. R. Parker, K. G. Beaty, and M. P. Stainton. 1997. Climate induced changes in the dissolved organic carbon budgets of boreal lakes. *Biogeochemistry* **36:**9–28.

Schlesinger, W. H., and J. M. Melack. 1981. Transport of organic carbon in the world's rivers. *Tellus* **33:**172–187.

Schneider, S. H. 1989. The changing climate. *Scientific American* **261:**70–79.

Schnitzer, M. 1991. Soil organic matter — the next 75 years. *Soil Science* **151:**41–58.

Schnitzer, M., and J. A. Neyroud. 1975. Further investigations on the chemistry of fungal "humic acids". *Soil Biochemistry* **7:**349–356.

Schultess, C. P., and C. P. Huang. 1991. Humic and fulvic acid adsorption by silicon and aluminum oxide surfaces on clay minerals. *Soil Science Society of America Journal* **55:**34–42.

Schwesig, D., and E. Matzner. 2000. Pools and fluxes of mercury and methylmercury in two forested catchments in Germany. *Science of the Total Environment* **260:**213–223.

Scudlark, J. R., K. M. Russell, J. N. Galloway, T. M. Church, and W. C. Keene. 1998. Organic nitrogen in precipitation at the mid-Atlantic U.S. coast — methods, evaluation and preliminary measurements. *Atmospheric Environment* **32:**1719–1728.

Senesi, N. 1992. Binding mechanisms of pesticides to soil humic substances. *Science of the Total Environment* **123/124:**63–76.

Seto, M., and K. Yanagiya. 1983. Rate of CO_2 evolution from soil in relation to temperature and amount of dissolved organic carbon. *Japanese Journal of Ecology* **33:**199–205.

Shibata, H., H. Mitsuhashi, Y. Miyake, and S. Nakano. 2001. Dissolved and particulate carbon dynamics in a cool-temperate forested basin in northern Japan. *Hydrological Processes* **15:**1817–1828.

Sibanda, H. M., and S. D. Young. 1986. Competitive adsorption of humus acids and phosphate on goethite, gibbsite and two tropical soils. *Journal of Soil Science* **37:**197–204.

Siddiqui, M. S., G. L. Amy, and B. D. Murphy. 1997. Ozone enhanced removal of natural organic matter from drinking water sources. *Water Research* **31:**3098–3106.

Simpson, D. E., and J. Hemens. 1978. Nutrient budget for a residential stormwater catchment in Durban, South Africa. *Progress in Water Technology* **10:**631–643.

Stevens, D. P., J. W. Cox, and D. J. Chittleborough. 1999. Pathway of phosphorous, nitrogen and carbon movement over and through texturally differentiated soils, South Australia. *Australian Journal of Soil Science* **37:**679-693.

Stewart, A. J., and R. G. Wetzel. 1980. Fluorescence: Absorbance ratios as a molecular weight tracer of dissolved organic matter. *Limnology and Oceanography* **25:**559–564.

Swank, W. T. and J. B. Waide. 1987. Characterization of baseline precipitation and stream chemistry and nutrient budgets for control watersheds. *In* "Forest Hydrology and Ecology at Coweeta" (W. T. Swank and D. A. Crossley, Eds.). Springer-Verlag, New York.

Swank, W. T., L. Swift, Jr., and J. E. Douglass. 1987. Streamflow changes associated with forest cutting, species conversions, and natural disturbances. *In* "Forest Hydrology and Ecology at Coweeta" (W. T. Swank and D. A. Crossley, Jr., Eds.). pp. 297–312. Springer-Verlag, New York.

Swift, R. S. 1985. Fractionation of soil humic substances. *In* "Humic Substances in Soil, Sediment, and Water: Geochemistry, Isolation, and Characterization" (G. R. Aiken, Ed.), pp. 387–408. Wiley, New York.

Taylor, A. A., and H. M. Kunishi. 1971. Phosphate equilibria on stream sediment and soil in a watershed draining an agricultural region. *Journal of Agriculture and Food Chemistry* **19:**827–831.

Tietema, A., and W. W. Wessell. 1994. Microbial activity and leaching during initial oak leaf decomposition. *Biology and Fertility of Soils* **18:**49–54.

Timperley, M. H. R., J. V. Timperley, M. Kawashima, and M. Ishigami. 1985. Organic nitrogen compounds in atmospheric precipitation: Their chemistry and availability to phytoplankton. *Canadian Journal of Fisheries and Aquatic Sciences* **42:**1171–1177.

Tipping, E. 1981. The adsorption of aquatic humic substances by iron oxides. *Geochimica et Cosmochima Acta* **45:**191–199.

Tipping, E., and M. J. Heaton. 1983. The adsorption of aquatic humic substances by two oxides of manganese. *Geochimica et Cosmochima Acta* **47:**1393–1397

Tipping, E., and M. A. Hurley. 1988. A model of solid–solution interactions in acidic organic soils, based on the complexation properties of humic substances. *Journal of Soil Science* **39:**505–519.

Tipping, E., and C. Woof. 1990. Humic substances in acid organic soils: Modeling their release to the soil solution in terms of humic charge. *Journal of Soil Science* **41:**573–586.

Tipping, E., C. Woof, E. Rigg, A. F. Harrison, P. Ineson, K. Taylor, D. Benham, J. Poskitt, A. P. Rowland, R. Bol, and D. D. Harkness. 1999. Climatic influences on the leaching of dissolved organic matter from upland UK moorland soils, investigated by a field manipulation experiment. *Environment International* **25:**83-95.

Trasar-Cepeda, C. M., C. Leiros, and F. Gil-Sotres. 2000. Biochemical properties of acid soils under climate vegetation (Atlantic oakwood) in an area of the European temperate humid zone (Galicia, NW Spain): Specific parameters. *Soil Biology and Biochemistry* **32:**747–755.

Vance, G. F., and M. M. David. 1989. Effect of acid treatment on dissolved organic carbon retention by a spodic horizon. *Soil Science Society of America Journal* **53:**1242–1247.

Vance, G. F., and M. B. David. 1992. Dissolved organic carbon and sulfate sorption by spodosol mineral horizons. *Soil Science* **154:**136–144.

Verry, E. S., and D. R. Timmons. 1977. Precipitation nutrients in the open and under two forests in Minnesota. *Canadian Journal of Forest Research* **7:**112–119.

Visser, S. A. 1964. Origin of nitrates in tropical rainwater. *Nature* **201:**35–36.

Weathers, K. C., G. M. Lovett, G. E. Likens, and R. Lathrop. 2000. The effect of landscape features on deposition to Hunter Mountain, Catskill Mountains, New York. *Ecological Applications* **10:**528–540.

Westerhoff, P., and D. Anning. 2000. Concentrations and characteristics of organic carbon in surface water in Arizona: Influence of urbanization. *Journal of Hydrology* **236:**202–222.

Wetzel, R. G., and B. A. Manny. 1972. Decomposition of dissolved organic carbon and nitrogen compounds from leaves in an experimental hard-water stream. *Limnology and Oceanography* **17:**927–931.

Willey, J. D., R. J. Kieber, M. S. Eyman, and G. Brooks Avery, Jr. 2000. Rainwater dissolved organic carbon: Concentrations and global flux. *Global Biogeochemical Cycles* **14:**139–148.

Williams, M. R., T. R. Fisher, and J. M. Melack. 1997. Solute dynamics in soil water and groundwater in a central Amazon catchment undergoing deforestation. *Biogeochemistry* **38:**303–335.

Williamson, C. E., and H. E. Zagarese. 1994. The impact of UV-B radiation on pelagic freshwater ecosystems. *Archiv für Hydrobiologie Beiheft* **43:**9–11.

Wilson, A. T. 1959. Organic nitrogen in New Zealand snows. *Nature* **183:**318–319.

Worrall, F., A. Parker, J. E. Rae, and A. C. Johnson. 1997. A study of the sorption kinetics of isoproturon on soil and subsoil, the role of dissolved organic carbon. *Chemosphere* **34:**87–97.

Yano, Y., W. H. McDowell, and N. E. Kinner. 1998. Quantification of biodegradable dissolved organic carbon in soil solution with flow-through bioreactors. *Soil Science Society of America Journal* **62:**1556–1564.

Yano, Y., W. H. McDowell, and J. D. Aber. 2000. Biodegradable dissolved organic carbon in forest soil solution and effects of chronic nitrogen deposition. *Soil Biology and Biochemistry* **32:**1743–1751.

Yavitt, J. B., and T. J. Fahey. 1984. An experimental analysis of solution chemistry in a lodgepole pine forest floor. *Oikos* **43:**222–234.

Zsolnay, A., and H. Steindl. 1991. Geovariability and biodegradability of the water-extractable organic material in an agricultural soil. *Soil Biology and Biochemistry* **23:**1077–1082.

3

Trace Organic Moieties of Dissolved Organic Material in Natural Waters

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I. INTRODUCTION

In many freshwater aquatic systems, dissolved organic material (DOM) represents a major pool of organic material. Processes that produce, consume, and transform DOM are important in the overall cycling of carbon, energy, and nutrients in these aquatic ecosystems. Current research has

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focused on understanding the role of these processes in aquatic ecosystem dynamics and in linking aquatic ecosystems to changes in climate, land use, atmospheric deposition, and vegetation in their surrounding catchments.

From the perspective of environmental chemistry, however, DOM concentration is a convenient bulk property of natural water that is easily measured (Thurman, 1985). The research focus has been on understanding DOM as comprising chemically distinct classes of compounds that interact with specific chemical constituents, such as trace metals and organic contaminants, or which can be utilized as microbial substrates for growth or energy transfer. One successful strategy in aquatic ecology is to employ new chemical tools developed by environmental chemists for the purpose of understanding the role of DOM in aquatic ecosystem dynamics. The measurement of trace organic moieties within DOM has the potential to be useful in this context.

A conceptual "DOM pie" diagram (Fig. 1) represents the chemical view of the DOM pool of a typical riverine sample. The specific chemical classes or wedges can be separated based upon several different operational fractionation schemes (Thurman and Malcolm, 1981; Aiken, 1985; Aiken *et al.*, 1992; Benner, 1998). For typical natural waters, the dominant fraction comprising DOM is fulvic acid, which is defined as the yellow, moderate molecular weight organic acid fraction of aquatic humic substances that is soluble at all pH values (Aiken *et al.*, 1985). In surface waters receiving

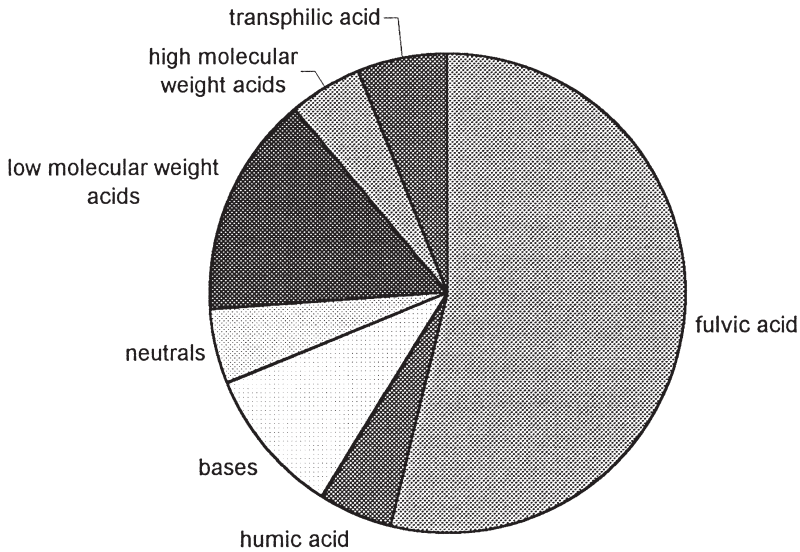

FIGURE 1 DOM pie diagram based on distribution of DOC in a typical river with a DOC concentration of 5 mg C/L (adapted from Thurman, 1985). Fulvic acids typically constitute the largest percentage of DOC, followed by low-molecular-weight organic acids.

DOM from the leaching of soils and vegetation in their catchments, fulvic acid is typically 45–65% of the DOM; in wetlands fulvic acid is typically 80–90% of the DOM; and in groundwaters and surface waters with mainly autochthonous microbial sources of DOM fulvic acid is typically 10–30% of the DOM (Thurman, 1985).

In the DOM pie diagram in Figure 1, all three aqueous humic fractions (fulvic acid, humic acid, and transphilic acid) are heterogeneous mixtures of compounds that have similar chemical properties in terms of acidic functionality, hydrophobic character, and molecular weight (Leenheer *et al.*, 1989). The importance of organic acids in DOM is to be anticipated because of the great enhancement (by factors of about 10^4) of organic solubility imparted by ionized carboxyl groups. In fractionation schemes based only on molecular weight, organic molecules that are neutrals, acids, and bases may be initially assigned to the same wedge of a DOM pie, and further fractionation may be used to separate based on functional group content or hydrophobicity.

The conceptual DOM pie illustrates the chemical heterogeneity of DOM. The different wedges may be derived predominantly from different pools of precursor organic material in soils, litter, plankton, suspended organic matter, or sediment. However, the different precursor pools are also subject to different biogeochemical transformations or loss processes (such as photolysis or microbial uptake), which increase the difficulty of establishing a link between a particular precursor pool and a specific chemical class of DOM. Thus, the complexity of the DOM pie suggests that searching for an ecologically meaningful tracer for DOM as a "whole" may be a poorly conceived endeavor.

The conceptual DOM pie does provide a framework for considering potential ecologically useful "tracers" within different DOM wedges. For the higher or moderate molecular weight organic fractions, such as fulvic acid, a tracer may be a trace moiety that is covalently bound to some subset of organic molecules within a fraction. Specifically, in this chapter we define a trace moiety as one that is not present in every organic molecule within a fraction. In this context, "moiety" can refer to a heteroatom (O, N, P, S), an isotope of carbon or of a heteroatom, or a functional group that may contain a heteroatom or an isotope and can be thought of more generally as referring to a chemical "thing" that can be measured. For example, in ^{13}C-NMR or ^{15}N-NMR spectroscopy of humic substances, the distribution of isotopically heavier carbon or nitrogen atoms among different moieties is assumed to be the same as that of the bulk carbon and nitrogen atoms. In low-molecular-weight fractions, the organic tracer may be a specific identifiable organic molecule. For example, glucose could be useful as a tracer for the pool of dissolved simple sugars in DOM (McKnight *et al.*, 1993). Trace moieties may be of interest either because (1) they are representative of properties of most of the molecules in the fraction and may

indicate a DOM source or (2) they are representative of trace reactive functional groups and may indicate dominant biogeochemical processes.

To maintain a focus on the use of tracers in DOM fractions, this chapter will present only brief descriptions of studies of bulk DOM properties, and will focus primarily on the use of trace moieties from the fulvic acid fraction in freshwater aquatic environments. In addition to being a major DOM fraction, fulvic acid is biogeochemically reactive in natural waters (see Maranger and Pullin, Chapter 8; Chin, Chapter 7; Moran and Covert, Chapter 10). Furthermore, current fractionation methods allow for relatively straightforward isolation of small quantities of fulvic acid from small volume filtered water samples (100–200 mL) in a reproducible manner, as well as for isolation of larger preparative quantities of material. We present examples to illustrate the use of particular trace moieties but do not present a comprehensive review of each trace moiety.

In addition to considering the use of tracers in the context of a DOM fraction, it is important to match the process for which a tracer is desired with the trace moiety to be measured. The usefulness of measuring a particular trace moiety in a field study will greatly depend upon the question being asked. One reason for careful selection of a trace moiety is that the extra analytical effort involved can be substantial, and there are few methods that can be easily incorporated in a routine manner into field studies. In this chapter we consider the use of trace organic moieties to answer two primary questions: (1) what are the sources of fulvic acid? and (2) What is the importance of the biogeochemical processes controlling fulvic acid concentration and chemistry or the chemistry of other solutes (nutrients or metals, for example)? For both of these questions, it will be critical to obtain a range of other hydrologic and biogeochemical data, such as mass balance of DOM and fulvic acid, because none of the current tracers are definitive by themselves. The emphasis in this chapter is placed on the use of the tracer rather than on reviewing details of the analytical methods.

II. TRACERS OF THE SOURCE OF DOM IN AQUATIC ECOSYSTEMS

Much insight can be gained about the dynamics of an aquatic ecosystem by constructing an elemental budget, such as for C or N. In carbon budgets of many aquatic ecosystems, the DOC pool represents a major pool or flux of organic carbon that is being transported through the system. Thus, using tracers in DOC fractions can provide important information on C budgets of aquatic ecosystems. This approach was pioneered by Ertel and co-workers (1984, 1986), who determined lignin degradation products in fulvic acids from different sites in the Amazon River to quantify the importance of allochthonous sources of DOM in this large river. Since these studies, the measurement of lignin degradation products, carbohydrates, and amino

acids in fulvic acids or ultrafilterable DOM has been useful in studies following the flux of riverine DOM into coastal areas (Hedges *et al.*, 1994). Furthermore, Ertel *et al.*, (1993) used lignin and carbohydrate biomarkers in fulvic acids in a subalpine bog to determine that the peat in the bog contributed more to the dissolved fulvic acid in the bogwater than fresh wetland plant leachates (see Benner, Chapter 5).

In the following section, we discuss three classes of tracers that have recently proved useful for assessing sources of DOC and in constructing both DOC and DON budgets for aquatic ecosystems. Measurements of these tracers could be combined with measurements of the established lignin and carbohydrate tracers in a field study. Our intent is to provide related examples rather than a thorough literature review of each class of tracer.

A. Isotopic Tracers

The stable isotopes ^{13}C and ^{15}N as well as the radioactive isotope ^{14}C have the potential to serve as tracers for sources of fulvic acid if there is sufficient difference in isotopic signature between the possible organic sources in the catchment of a lake, stream, or wetland. The measurement of ^{13}C is particularly attractive because the measurement is relatively inexpensive and can be very precise. However, using ^{13}C measurements to successfully resolve sources can be difficult because of the overlap of ^{13}C values for different DOC precursor materials (Schiff *et al.*, 1997; Raymond and Bauer, 2001). The measurement of ^{13}C is reported as $\delta^{13}C$ in parts per thousand (‰),

$$\delta^{13}C = [(R_{sample}/R_{PDB}) - 1]*1000,$$

where R_{sample} is the $^{13}C/^{12}C$ ratio for the sample and R_{PDB} is the $^{13}C/^{12}C$ ratio for the international PeeDee Belemnite standard. Terrestrial plants have a wide range of $\delta^{13}C$ values with C3 plants (trees, most shrubs, and grasses) ranging from –20 to –35‰. Stable carbon isotope values for freshwater DOC have a reported range between –18 and –46‰ (Schiff *et al.*, 1990; Wang *et al.*, 1998).

Measurements of ^{13}C have been used to investigate DOC cycling in headwater streams (Schiff *et al.*, 1990; Baron *et al.*, 1991; Schiff *et al.*, 1997; McKnight *et al.*, 1997; Elder *et al.*, 2000; Palmer *et al.*, 2001) as well as a wide variety of river and estuarine environments (Wang *et al.*, 1998; Raymond and Bauer, 2001). These studies demonstrate that the sources and processes controlling streamwater DOC (e.g., Palmer *et al.*, 2001) and downstream changes in DOC precursor material (e.g., Elder *et al.*, 2000) can be resolved in cases where the $\delta^{13}C$ values of source material are relatively well constrained. Measurements of $\delta^{13}C$ can also act as tracers of the fate of DOC in soils. Schiff *et al.* (1990) showed that the downward movement of DOC in the soil profile has associated $\delta^{13}C$ isotopic effects possibly due

to the preferential decomposition or sorption of selected compounds. Several studies have used $\delta^{13}C$ as a tracer for the fulvic acid fraction of DOC and have found that $\delta^{13}C$ values for the fulvic fraction are generally 1–3‰ lighter than the $\delta^{13}C$ of bulk DOC (Schiff *et al.*, 1990; Baron *et al.*, 1991).

As an example of using $\delta^{13}C$ to infer source, Figure 2 presents data from a study in the Loch Vale watershed in Rocky Mountain National Park, which compares the $\delta^{13}C$ with the C : N ratio in large and small particulates, colloids, and fulvic acids from several lakes and streams in the catchment. The small particulates represented primarily planktonic algae and bacteria and had characteristics similar to those of the colloids, which were retained by ultrafiltration through a 100 Da membrane. This result suggests that algae and bacteria in the lakes and streams produced the colloids. In contrast, the large particulates comprised primarily soil particles and plant debris and had a lighter isotopic signature and lower N content compared with the small particulates. The samples that were most isotopically depleted and had the lowest N content were all four of the fulvic acid samples, consistent with degradation of plant material and leaching of soils in the catchment being the primary sources of fulvic acid in the midsummer, when these samples were collected. Measurement of $\delta^{13}C$ in fulvic acids from the lakes in a seasonal study showed that the $\delta^{13}C$ varied during snowmelt and with depth in the lakes, with more enriched $\delta^{13}C$ values in the surface

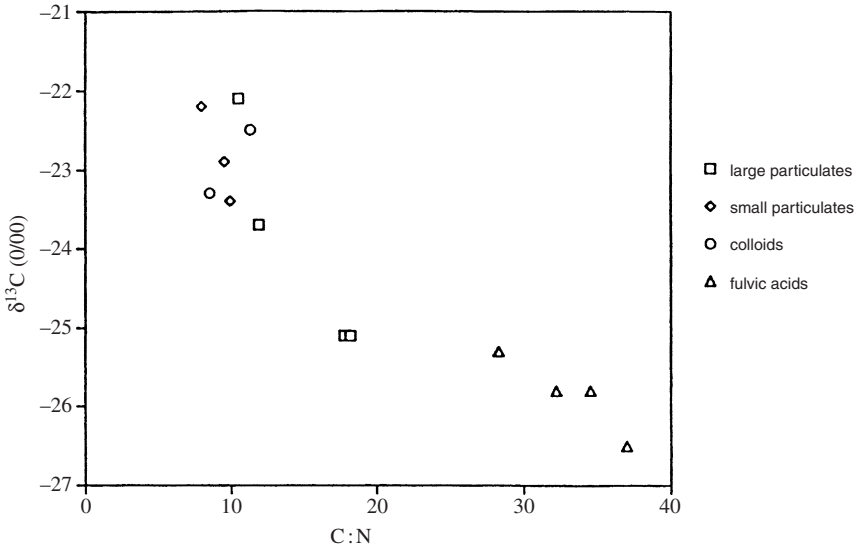

FIGURE 2 Comparison of ^{13}C to C : N ratio in Loch Vale surface waters (from McKnight *et al.*, 1997). The fulvic acids as well as some of the large particulates are clustered between –25 and –27‰, the range for plant-derived carbon. In contrast, all of the small particulates and colloids had ^{13}C values in the heavier range, potentially indicating a microbial or algal source.

waters during snowmelt and even more depleted values at the bottom of the lake under ice cover (Baron *et al*., 1991).

Measurement of ^{14}C may provide another possible tracer if one potential source of DOM, such as a groundwater, may be older than modern carbon. ^{14}C is reported in parts per thousand (‰),

$$^{14}C = [(A_{SN}/A_{abs}) - 1] * 1000,$$

where A_{SN} is the specific activity of a sample (proportional to the $^{14}C/C$ ratio) and A_{abs} is the absolute ^{14}C activity in the international isotopic standard (NBS oxalic acid) (Wang *et al*., 1998). A potential constraint is that the measurement of ^{14}C is generally much more expensive; therefore it may not be practical to incorporate ^{14}C measurements into a field study involving large numbers of samples. Knowledge of the ^{14}C age of the potential end members is critical for this approach to be useful. However, ^{14}C typically has a larger dynamic range and less overlap among sources than ^{13}C (Raymond and Bauer, 2001). As a result, ^{14}C can be useful as a tool to constrain input and removal processes affecting DOM in freshwater systems. Carbon cycling studies in headwater streams often use ^{14}C in conjunction with ^{13}C (Schiff *et al*., 1990, and 1997; Palmer *et al*., 2001). This dual-isotope approach allows for a more robust interpretation of DOC sources and sinks.

Measurements of ^{14}C have rarely been used as tracers to study sources of individual DOC fractions. Aiken *et al*. (1996) used ^{14}C measurements of fulvic acid in a permanently ice-covered closed basin lake in Antarctica to investigate sources of DOC in the lake. Sampling along a depth profile in the lake showed that the fraction of modern carbon decreased with depth and confirmed that the high concentrations of fulvic acid in the bottom waters represented an old source, with an age of around 3000 years, which was diffusing into the upper water column (Fig. 3).

The measurement of ^{15}N is reported as $\delta^{15}N$ in parts per thousand (‰),

$$\delta^{15}N = [(R_{sample}/R_{atm}) - 1] * 1000,$$

where R_{sample} is the $^{15}N/^{14}N$ ratio for the sample and R_{atm} is the $^{15}N/^{14}N$ ratio for the N_2 in the atmosphere. There are a large number of studies that use ^{15}N tracer additions to study nitrogen dynamics in aquatic ecosystems (e.g., Peterson *et al*., 2001). However, there are relatively few measurements of the natural abundance of ^{15}N in dissolved organic matter in freshwater ecosystems (Kendall, 1998). Elder *et al*. (2000) used ^{15}N measurements in conjunction with ^{13}C measurements to infer sources of DOM in stream-water in northern Wisconsin. Kendall *et al*. (2001) used a similar dual-isotope approach with ^{15}N and ^{13}C to investigate the sources of DOM in four large river systems in the United States. Examining the ^{15}N value of an individual DOM fraction has the potential to simplify the process of attributing DOM to particular precursor materials.

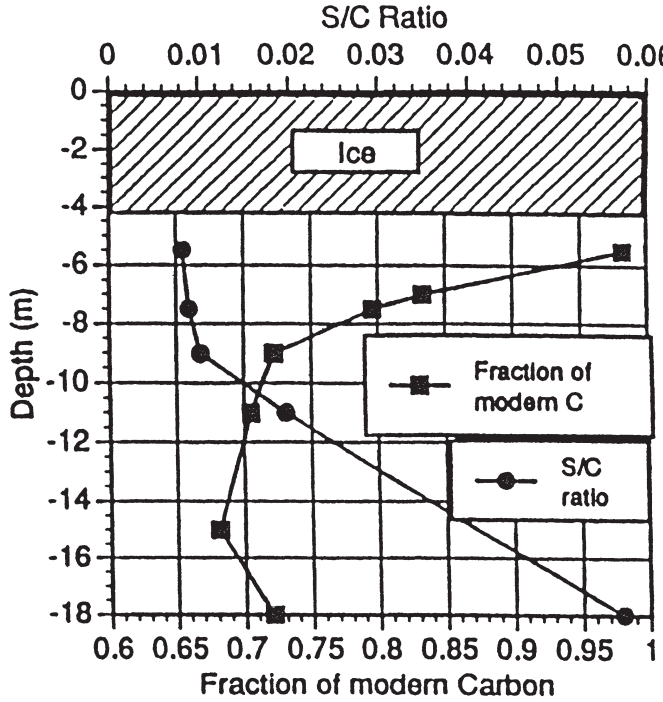

FIGURE 3 Depth profiles for ^{14}C age and sulfur-to-carbon (S/C) ratios for fulvic acid samples isolated from Lake Fryxell, a permanently ice-covered lake in the McMurdo Dry Valleys, Antarctica (from Aiken *et al.*, 1996). In the upper water column, the fulvic acids have a modern signal. This young fulvic acid derives from perennial algal mats in glacial meltwater streams. At depth, the fulvic acids are quite old (about 3000 years) and are possibly derived from organic material in sediments. The ratio of S/C increases with depth as conditions in the water column become anoxic.

Here we present one example to illustrate the utility of ^{15}N for providing information about the source of dissolved organic nitrogen in a freshwater ecosystem. The ^{15}N contents of fulvic acid fractions of different size varied slightly and were similar to the value for the ^{15}N content of the synfulvic acid fraction in a eutrophic coastal pond in Antarctica (Table I) (Brown *et al.*, 2002). Continuous production of mucilage by the chlorophyte population in the pond and diffusion of DOM from the sediments are the two main DOM sources; these data suggest that the same source predominates for all of these fulvic acid fractions.

B. Fluorescence as a Tracer

In addition to the absorbance of visible and ultraviolet light, fulvic acids are major fluorophores in natural waters. Analysis of the fluorescence of

TABLE I Elemental Analyses for December 1997 Isolated DOM Fractions from Pony Lake, Cape Royds, Antarctica (Brown *et al*., in prep.), with C : N, and C : H, Isotopic Composition (Values Corrected for Ash Content).

		Elemental analysis (%)					*Atomic ratios*	
Sample	$\delta^{15}N$	*C*	*H*	*O*	*N*	*S*	*C : N*	*C : H*
Synfulvic acid								
Whole sample	11.1	43.2	5.6	40.4	6.9	3.9	7.3	0.64
Fulvic acid								
Whole sample	10.5	47.4	5.6	36.4	7.6	3.1	7.3	0.71
Large	11.4	48.3	5.7	35.6	7.2	3.2	7.9	0.70
Middle	10.5	49.5	6.0	34.6	6.7	3.3	8.6	0.69
Small	9.9	46.1	5.3	36.2	8.5	3.9	6.4	0.72

Note: Ultrafiltration was used to fractionate the DOM prior to fulvic acid isolation, and the size was determined by high-pressure size exclusion chromatography. The middle and large size fractions had molecular weights corresponding to a range of 1260 to 1470 amu (atomic mass units), and those in the small size fraction had molecular weights of about 1000 amu.

fulvic acids has shown that not all fulvic acid molecules contain fluorophores; thus fluorophores in aquatic fulvic acid fit our definition of trace organic moiety. Excitation-emission matrices (EEMs) typically reveal the presence of two fluorophores in fulvic acids. The main fluorophore (referred to as the "upper" maxima) has an excitation peak at about 320 to 340 nm and an emission peak near 400 nm. The second fluorophore, the "lower" peak, exists at excitation wavelengths shorter than 280 nm with an emission peak near 400 nm (Coble *et al*., 1990; De Sousa Sierra, 1994; Mobed *et al*., 1996). Although the chemical structure of the two types of fluorophores is not known, quinone moieties may be important (Klapper *et al*., in 2002). Because fluorescence is relatively easy to measure, any insight into organic carbon sources that can be gained from fluorescence measurements would be valuable. Mopper and Schultz (1993) studied fluorescence spectra of marine DOM and found that it could be categorized into two distinct groups: one with "protein-type" fluorescence and one with "humic-type" fluorescence. These two classes of DOM were characterized by differing excitation and emission spectra and demonstrated the utility of fluorescence for studying the nature and distribution of aquatic DOM fractions. Pullin and Cabaniss (1997) showed that synchronous fluorescence spectra provided a sensitive measurement for detecting the mixing of water from the Cuyahoga River into Lake Erie.

We found consistent differences in the fluorescence properties of fulvic acids from streams where fulvic acids are terrestrially derived and from lakes where fulvic acids are microbially derived (McKnight *et al*., 2001). The upper maximum in microbially derived samples is more sharply defined and

occurs at lower excitation and emission wavelengths than the upper maximum in terrestrially derived samples. We developed a fluorescence index (FI) based on the ratio of the emission intensity at a wavelength of 450 nm to the emission intensity at 500 nm, obtained with an excitation of 370 nm. This index has a value of about 1.9 for microbially derived fulvic acids and a value of about 1.4 for terrestrially derived fulvic acids.

The use of fluorescence spectroscopy in a study of the seasonal yield of DOC and DON in the surface waters of an alpine/subalpine catchment is illustrated in Figure 4. The catchment is Green Lakes Valley in the Colorado Rocky Mountains, which has an annual hydrologic cycle dominated by snowmelt in the spring (May–June). In the alpine lake algal populations become abundant in the summer in both the water column and benthos (Waters, 1999). The percentage of the DOC that was fulvic acid peaked during spring snowmelt (early June 1999) and decreased through the summer at both alpine and forested sampling sites. This decrease was accompanied by an increase in the FI, indicating the increasing importance of a microbially derived source of fulvic acid in late July and August 1999. The increase in the FI was more pronounced at the alpine Green Lake 4 than at lower elevation stream sampling sites (Fig. 4). Figure 5 shows the full EEM spectra for a late ice cover (May 2000) and summer snowmelt (early July

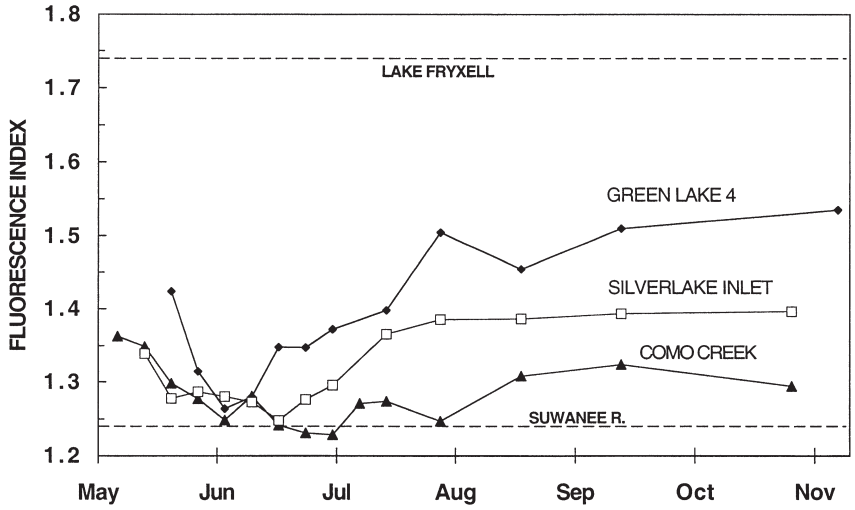

FIGURE 4 Change in fluorescence index during the period of spring snowmelt and in summer in lakes and streams in Green Lakes Valley, Colorado. All three sampling sites show a dip in the FI coincident with snowmelt runoff and the flushing of DOC from near-surface soil layers. Later in the season, the DOC in Green Lake 4, which is surrounded by talus, is derived from benthic algal mats and organic rich sediments. The DOC in the forested lakes and streams, Silver Lake and Como Creek, retain more of a terrestrially derived character, indicating that runoff from the subalpine forest continues to be a dominant source of dissolved fulvic acid.

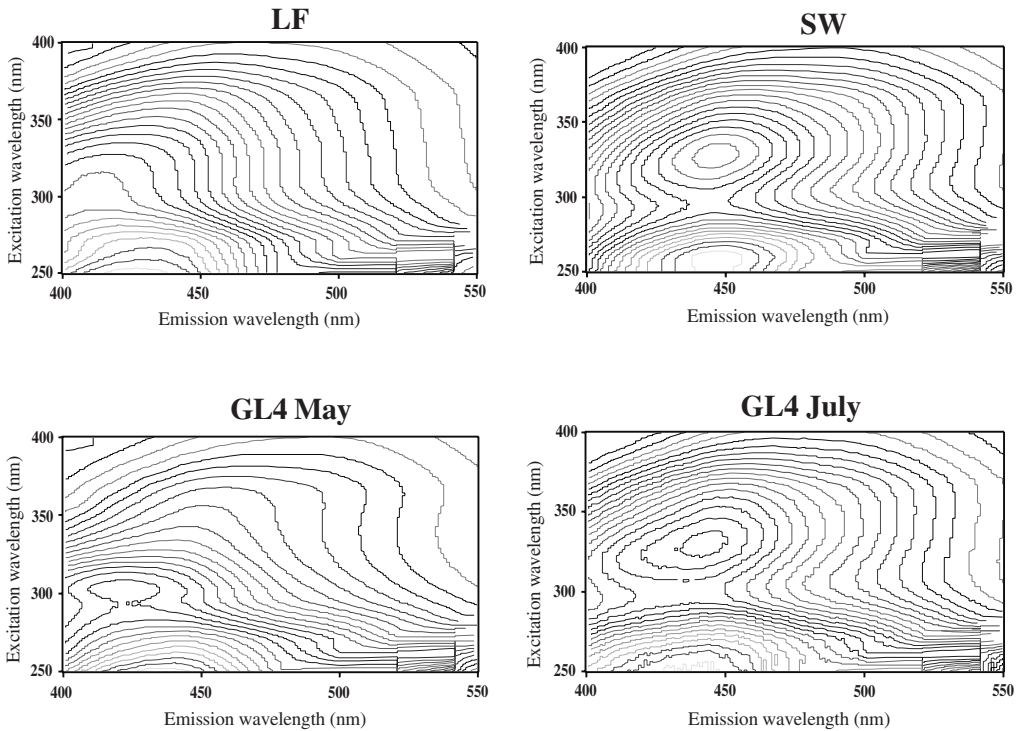

FIGURE 5 EEM spectra for isolated fulvic acids from Green Lake 4 (GL4) during the late ice-cover period (May), and during summer snowmelt (July): comparison with EEMs of Suwannee Stream (SW) and Lake Fryxell (LF) fulvic acids. The fluorescence spectrum for the Green Lake 4 fulvic acid is similar to that of the microbially derived Lake Fryxell fulvic acid in May and then shifts toward the spectrum of the terrestrially derived Suwannee Stream fulvic acid during snowmelt, when meltwater is flushing soluble organic carbon from catchment soils.

2000) fulvic acid from Green Lake 4 in comparison with the EEM for the two reference samples used as end members for the FI, the Lake Fryxell and Suwannee River fulvic acids. During ice cover, the EEM for GL4 resembles the Lake Fryxell fulvic acid, probably reflecting the decomposition of the algal material under ice cover in winter. During summer snowmelt, there is a shift in the EEM peak that more closely resembles the EEM of the Suwannee River sample, indicating that there is a change in the source of fulvic acids in surface waters.

In the same way that $\delta^{13}C$ measurements of potential source organic material were useful in interpreting the $\delta^{13}C$ of bulk dissolved fulvic acid, the fluorescence characteristics of fulvic acid sources may be useful in interpreting the fluorescence characteristics of aquatic fulvic acid. One potential source of DOM is diffusion from lake sediments (Ishiwatari, 1985). Analysis of diatoms in a sediment core from the alpine lake (Green Lake 4) has shown that benthic species of the genus *Fragilaria* became abundant beginning around 1950, coincident with the introduction of N fertilizer use in the nearby plains (Waters, 1999). This increase in benthic diatoms in the sediment record is matched by an increase in the FI of fulvic acids extracted from the sediment and by a twofold increase in organic carbon content of the sediments (Fig. 6). Thus, one source of the microbially derived fulvic acids in the lake under ice cover and in summer could be DOM diffusing from the sediments; another source could be rapid production of fulvic acids from the growth of phytoplankton.

In the Front Range of the Rocky Mountains, the N enrichment of alpine catchments from increased atmospheric N deposition from agricultural and urban development on the plains is an important environmental and resource issue (Williams *et al.*, 1996). The difference in N content (about 0.5–1.0% for terrestrially derived DOM vs. about 2–3% for microbially derived DOM) could be significant in terms of estimating the contribution of dissolved fulvic acid flux to the yield of N from alpine and subalpine catchments in the Rocky Mountains. The example for this alpine catchment illustrates the potential usefulness of the fluorescence index in field studies addressing applied issues related to environmental management.

C. Biogeochemically Important Heteroatoms as Tracers: Nitrogen and Phosphorus

Both N and P are important heteroatoms in DOM from the perspective of understanding biogeochemical cycles within catchments. All organisms require N and P for growth, and these nutrients are present in detrital organic material in soil and sediments. Thus, the incorporation of N- and P-containing moieties is likely to occur as an inherent aspect of DOM production and represents a potentially important pathway by which these

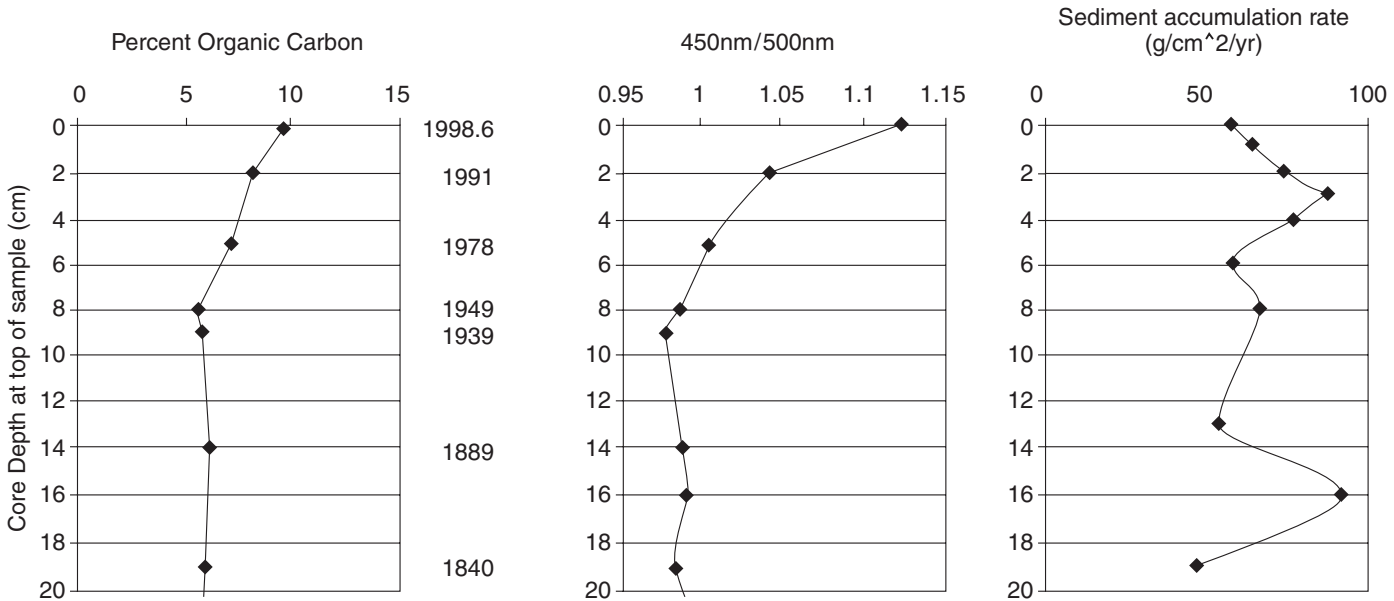

FIGURE 6 Sediment profiles for organic carbon content and fluorescence index for Green Lake 4. Organic carbon content and the FI signal both demonstrate consistent increases, indicating a greater microbial DOM contribution during the past 50 years. These changes occurred in association with increases in benthic diatoms, and nitrogen deposition from fertilizer use and urban development.

nutrients can be lost from terrestrial ecosystems (Hedin *et al*., 1995; Vitousek *et al*., 1998). As a result, measurement of the N and P content of DOM can potentially provide important information about DOM sources and important pathways in nutrient cycling.

We have a general understanding of the relationship between sources of fulvic acid and the N content of fulvic acid (McKnight *et al*., 1994). In terrestrial ecosystems with abundant lignin-containing plants, the degradation of lignin results in fulvic acids with a relatively high aromatic ring content (aromaticity) and a relatively low N content. Aromaticity can be quantified as the relative abundance of aromatic (AR) and aliphatic (AL-I) carbons in a ^{13}C-NMR spectrum and can be inferred by measuring the molar absorptivity at 254 or 280 μm (Chin *et al*., 1994). Aromatic moieties are generally recalcitrant to microbial degradation, and their relatively high abundance in dissolved fulvic acid may explain the slow turnover of fulvic acid in aquatic ecosystems. Lignin, although rich in aromatic rings, does not contain N, and thus precursor lignaceous organic material acts to dilute or lower the N content of fulvic acid. A comparison of fulvic acids from a wide range of ecosystems shows that there is a relationship between AR/AL-I ratios and C:N atomic ratios of DOM, with microbially derived fulvic acids having lower ratios than fulvic acids derived from soil and higher plants (Fig. 7).

Although there is a general relationship between aromaticity and N content of dissolved fulvic acids, perturbations to the N cycle in a particular environment may cause deviations from the trend shown in Figure 7. For example, increased N loading to watersheds may cause the terrestrial ecosystem to become N saturated (Aber *et al*., 1998). Ecosystem N saturation could result in elevated N content in both fulvic acids and the low-molecular-weight, nonhumic DOM fractions. In Figure 7, it is interesting to note that for the Loch Vale watershed, which is located on the east of the continental divide and receives N loading from industrial and agricultural activities along the Colorado Front Range, the fulvic acids have lower C : N ratios compared with fulvic acids from west of the continental divide (e.g., Deer Creek). This difference in C : N ratios suggests that increased N deposition may be altering the relative N content of fulvic acids in headwater streams in Colorado. The relationship between N deposition and the N content of fulvic acids could be further explored by examining fulvic acids from a broader range of watersheds.

There are relatively few studies that have measured the P content of dissolved organic material; however, available information appears to indicate that the P content of DOM can act as a broad indicator of the conditions under which the DOM was formed. The P content of dissolved fulvic acids and colloids in aquatic ecosystems has been determined by elemental analysis (McKnight *et al*., 1985; 1997). The P content of fulvic acids varies by more than an order of magnitude across different ecosystems (Table II). Fulvic acids from Thoreau's Bog in Massachusetts have a high P content compared

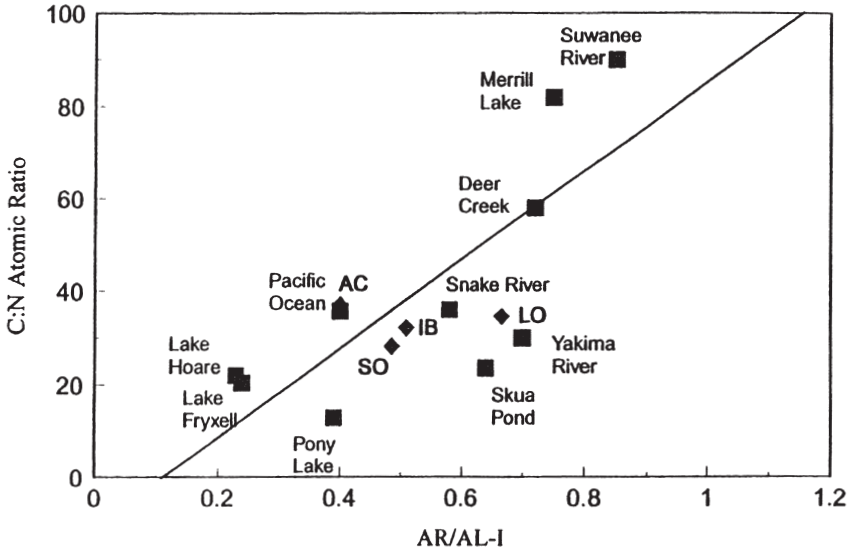

FIGURE 7 Diagram of C : N vs. AR:AL-I, where AR:AL-I is the ratio of aromatic (AR) to aliphatic (AL-I) carbons, as determined from a quantitative ^{13}C-NMR spectrum. The solid line is a simple linear regression ($r^2 = 0.52$, $n = 14$, $p = 0.003$). The position of fulvic acids collected in Rocky Mountain National Park in Colorado is shown relative to other aquatic fulvic acids (from McKnight *et al*., 1997). SO, Sky Pond Outlet; AC, Andrews Creek; IB, Icy Brook; LO, Loch Vale Outlet. Other fulvic acids are described in McKnight *et al*. (1992 and 1994).

with fulvic acids from the nearby Shawsheen River and a number of headwater catchments in the Colorado Front Range.

The variability of the P content of fulvic acids appears to be a function of both the hydrologic conditions and the nutrient status of the aquatic system in which the fulvic acids were formed. McKnight *et al*. (1985) hypothesized that the P in Thoreau's Bog fulvic acids was in the form of organic phosphate esters or inositol phosphates, which are major phosphorus products from the breakdown of plant material. The solubility of these P-containing moieties would be greater in bogwater than in most surface

TABLE II Comparison of Organic P Content of Fulvic Acids from Different Aquatic Ecosystems

Fulvic acids	*% P by weight*	*Reference*
Thoreau's Bog	0.73	McKnight *et al*. (1985)
Shawsheen R	0.15	McKnight *et al*. (1985)
Loch Vale	0.04	McKnight *et al*. (1997)
N. Boulder Ck, CO (June)	0.04	Hood (unpublished data)
N. Boulder Ck, CO (September)	0.09	Hood (unpublished data)

waters because of both low calcium concentrations and the probable complexation of iron and aluminum by fulvic acids at low pH. As a result, the P content of Thoreau's Bog fulvic acids appears to reflect the unique wetland environment in which these fulvic acids were formed. Similarly, the increase in the P content of fulvic acids in North Boulder Creek between June (peak snowmelt runoff) and September (fall baseflow) corresponds with seasonal changes in the source of aquatic DOM within the catchment. Thus, monitoring changes in the P content of individual DOM fractions over time can provide information about changes in the source or processing of DOM at the catchment scale.

III. BIOGEOCHEMICAL PROCESSES: INTERACTIONS WITH MINERAL SURFACES

Although much of the DOM in natural waters is produced through leaching and degradation of organic material in the catchment, there is not a direct correspondence between the concentration and chemical composition of DOM in soil interstitial water in upper soil horizons and the concentration and composition of DOM in the receiving stream or lake (see also Aitkenhead-Peterson *et al.*, Chapter 2). These relationships were examined by Wallis (1979), who proposed that losses of DOM along flowpaths in soils and in riparian sediments were important controlling processes. Further studies were conducted by McDowell and Likens (1984), Cronan and Aiken (1985), Fiebig *et al.* (1990), and Mulholland and Hill (1997). Along soil, shallow groundwater, and riparian flowpaths, DOM contacts mineral surfaces such as clays and oxides that can remove the more hydrophobic and, typically, higher molecular weight fractions of DOM, including fulvic acid. Easthouse *et al.* (1992) demonstrated a vertical gradient of DOC fractions with hydrophobic acids dominating in the O horizon and hydrophilic acids dominating in the E and B horizons in the soil profile of a forested catchment in Norway. Similarly, DON concentrations measured in soil horizons of lodgepole forests were several orders of magnitude higher than surface water concentrations in subalpine ecosystems in the Colorado/Wyoming Front Range (Hood, 2002). In addition to sorption, another important removal mechanism is microbial uptake of DOM. These two removal mechanisms result in either long-term storage of organic carbon or rapid mineralization. Thus, tracers that indicate the extent of either sorption or microbial removal would be particularly useful in constructing carbon budgets and in studying such issues as carbon sequestration in soils in response to increasing atmospheric CO_2 levels and linkages between terrestrial and aquatic ecosystems.

Because of the continuous exchange of water between the stream and its hyporheic zone, similar surface reactions and microbial processes that alter DOM en route to streams are also at work in stream ecosystems. These

processes act as a "final polish" on the chemical composition of DOM in the stream, and the relative importance of chemical sorption vs. microbial uptake has consequences for stream ecosystem dynamics and would be worth tracking with the use of tracers.

A. Tracers of the Extent of Chemical Fractionation by Sorption by Oxides

It has long been known that in soils the movement of leachate from the forest floor through lower soil horizons with abundant iron and aluminum oxides results in the removal of dissolved organic fractions, especially fulvic acid, through sorption reactions (Stevenson, 1985; Gu *et al*., 1996). Iron oxides are also important reactive surfaces in streams and lakes, as they are abundant in zones with strong redox gradients. Ferrous iron diffusing from anoxic lake or hyporheic sediments or from an anoxic hypolimnion can be rapidly oxidized in an oxic water column and the ferric iron precipitated as hydrous iron oxide. Furthermore, photoreduction of the iron oxides can then drive daytime dissolution and subsequent regeneration of the hydrous oxide surface at night. These fresh oxide surfaces are much more reactive in DOM sorption than more crystalline oxides. Thus knowledge of the chemical fractionation due to oxide sorption may be useful in evaluating the extent of these reactions with the use of DOM tracers in natural systems.

DOM sorption reactions have been studied by soil and aquatic chemists, and the extent of sorption has been shown to be dependent upon the molecular size, aromaticity, and carboxylic acid content of fulvic acid (Zhou *et al*., 2001; McKnight *et al*., 1992). Table III shows the differences in chemical characteristics for the sorbable and non-sorbable fractions of fulvic acid in the Suwannee River, which is used as the site for the reference aquatic fulvic acid of the International Humic Substances Society (IHSS). These results show that the $\delta^{13}C$ signatures of the sorbable and nonsorbable fulvic acid fractions were different by more than 1 per mL, in addition to higher aromaticity and carboxyl content (which was measured by ^{13}C-NMR spectroscopy of isolated fulvic acid). These differences in $\delta^{13}C$ exceed the analytical precision of the measurement and suggest that it may be possible to use $\delta^{13}C$ as tracers for sorption on iron oxides in a stream system if the $\delta^{13}C$ signature of the sources were clearly resolved.

The results in Table III show that a significant difference also exists in the $\delta^{15}N$ signatures of the sorbable and nonsorbable fractions. These results also suggest a potential for tracing removal of N-containing fulvic acid by sorption; however, microbial processes are also likely to influence the $\delta^{15}N$ signature. A more definitive approach may be to examine specific N-containing functional groups in fulvic acids. For example, amino acid residues are commonly found in fulvic acid, incorporated into the structure through covalent bonds, and account for about 5% of the N content of fulvic acids. Amino acids range in their formation constants for forming complexes with iron, from values as high as 10^5 for glutamic acid to only

TABLE III Differences in Chemical Properties of Suwannee River Fulvic Acid Sorbed onto Iron Oxide (Sorbable) and Suwannee River Fulvic Acid Remaining in Solution (Nonsorbable)

				Elemental content (% by weight)					*Carbon distribution (%)*				
Sample	*SUVA* $(mg\ C)^{-1}$	$\delta^{15}N$	$\delta^{13}C$	*C*	*H*	*O*	*N*	*S*	*Al-I*	*Al-II*	*Al-III*	*AR*	*C-I*
Sorbable	.049	–4.1	–27.5	45.6	4.2	44.1	0.7	.35	35	17	8	20	17
Nonsorbable	.025	–5.5	–28.9	51.4	5.2	39.4	0.5	.36	49	16	6	12	13

10^2 for glycine. In a study of the sorption of fulvic acids onto iron and aluminum oxides formed in the confluence of a stream system, McKnight *et al.* (1992) found that the amino acids that formed strong complexes were enriched in the sorbed fulvic acids compared with the fulvic acid that remained in solution. Thus, amino acid moieties have the potential to act as a tracer of the sorption potential of fulvic acids.

IV. BIOGEOCHEMICAL PROCESSES: SURFACE WATER TO SEDIMENTS

In the water column and sediments of lakes and streams biogeochemical processes can change the concentration and composition of DOM (Steinberg and Muenster, 1985). The chemical changes can be manifested as a change in the distribution among the major fractions represented in the DOM pie (Fig. 1) and as changes in the chemistry of the major fractions. One of the most important biogeochemical processes is clearly the uptake of DOM by microorganisms, which supports the microbial loop and higher trophic levels (Moran and Hodson, 1990). Studies have shown that microbial uptake results in the progressive decrease in the larger molecular weight fractions of the ultrafilterable DOM (UDOM) and accumulation of smaller molecular weight material, as well as loss of carbohydrate moieties. Additionally, there are processes other than direct microbial uptake that can also modify the DOM pool, and an example is discussed in the following section. In field studies, DOM tracers may be useful, along with other data, in the evaluation of the extent of biogeochemical processes that have altered different DOM fractions.

A. Tracers of Photolysis of Aquatic Fulvic Acid

Aquatic ecosystems are synchronized with the diurnal photocycle through photosynthesis by algae and macrophytes and through photochemical re-

actions with dissolved and particulate metals and DOM. Recent studies have shown that photolysis of DOM releases nutrients (N and P) to algae and bacteria, providing a link between primary production and DOM photolysis (see Moran and Covert, Chapter 10). Photolysis of fulvic acid results in changes in the chemistry of the bulk of the fulvic acid, such as decreases in aromaticity and specific UV absorbance (Kouassi and Zika, 1992; Wetzel *et al*., 1995; Moran and Zepp, 1997). However, these chemical changes may also be caused by mixing with fulvic acid from other sources or by sorption on hydrous iron oxides and therefore may not be definitive indicators of the modification by photolysis.

There are several trace moieties that are also decreased by photolysis, and when measured together these changes may be useful in assessing the importance of photolysis in an aquatic ecosystem. In a study of the changes in fulvic acid chemistry in Spirit Lake, Washington, following the Mt. St. Helens eruption, McKnight *et al*. (1988) observed a progressive loss and eventual disappearance of phenolic hydroxyl moieties in the fulvic acid in the lake, which was attributed to the cumulative effect of photolysis over several years. In the Spirit Lake study, phenolic hydroxyl was determined through derivatization of isolated fulvic acids with ^{13}C-enriched diazomethane and measurement by ^{13}C-NMR spectroscopy, a labor-intensive method (Fig. 8). However, a reasonable estimate of phenolic hydroxyl content can also be obtained through a simple pH titration (Perdue, 1985), which could be done using the fulvic acid isolated from small volume samples. These measurements would be practical to include in a field study, and the interpretation of changes in phenolic hydroxyl could be verified by more detailed spectroscopic characterization of a smaller set of samples.

Fluorophores are another type of trace moiety that is lost through photolysis (Pullin and Cabaniss, 1997; Langford and Bruccoleri, 2000). The decrease in fluorescence by photolysis is confirmed by the observation that the fluorescence intensity per mg DOC is a good indicator of the production of hydrogen peroxide from DOM photolysis in a wide range of freshwaters (Scully and Lean, 1994). As discussed above, the relative fluorescence intensity of the fulvic acid fraction is fairly simple to measure. Furthermore, measurement of FI and/or δ^{13}C may allow for differentiation between a decrease in fluorescence intensity per mg C due to mixing with fulvic acid from microbial material and a decrease due to photolysis.

B. Tracers of Incorporation of Organic Sulfur in Fulvic Acid under Anoxic Conditions

Similar to the N content of fulvic acid, the S content can reflect differences in the S content of precursor organic material. However, the S content can also be influenced strongly by the chemical conditions of the environment in which the fulvic acid is generated. Fulvic acids from reducing

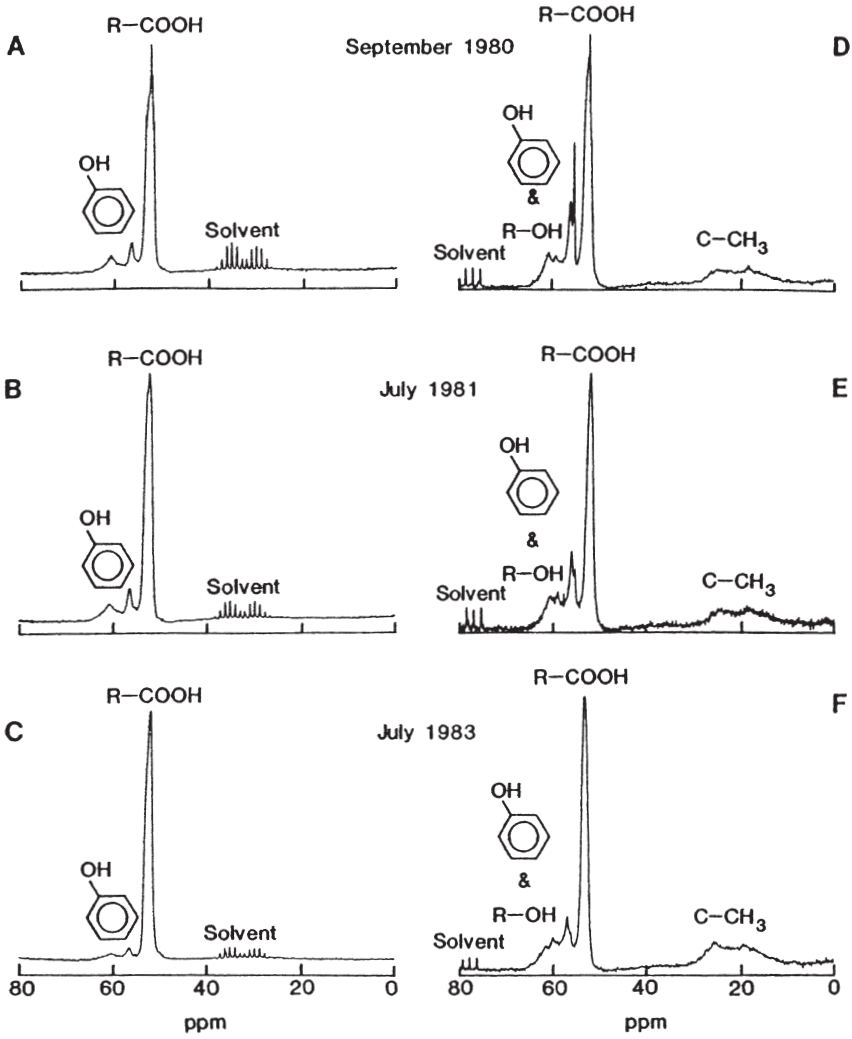

FIGURE 8 Change in phenolic hydroxyl content of fulvic acids from Spirit Lake during the several years after the eruption of Mt. St. Helens (from McKnight *et al*., 1988). The measurement of phenolic hydroxyl content is by derivitization with ^{13}C-enriched diazomethane that is quantitatively measured by ^{13}C-NMR spectroscopy.

environments with high concentrations of dissolved sulfide, such as occur in wetlands and in lake sediments or bottom waters, can have S contents that are higher than those of fulvic acids generated under oxic conditions. The influence of reducing conditions on S content is illustrated in Figure 3, which shows how the sulfur-to-carbon (S/C) ratio of fulvic acid changes

with depth in Lake Fryxell, Antarctica. The S/C ratio increases with depth as a result of changes in the redox conditions in the water column toward anoxic reducing conditions at depth (Aiken *et al*., 1996). The chemical form of S in fulvic acid is not well known, but analysis using soft ionization-pyrolysis mass spectroscopy and XANES has shown that sulfone and sulfide groups are both important moieties. The methods using soft ionization-pyrolysis mass spectroscopy require only small (mg) quantities of material and have the potential to be incorporated into field studies. In this context, such results, in combination with the use of sulfur isotopes, could provide insights into the biogeochemical history of fulvic acid at different sampling sites in a watershed, river, or lake system through interpretation of the different types of S-containing groups. In a study by Brüchert (1998) of fulvic acids in sediments from Watson Bay, St. Andrew's, FL, a significant fraction of S bound to fulvic acids was traced to the nearby pulp mill industry and to "biosynthetic" planktonic sources in the bay. In the uppermost centimeters of sediment, the dominant form in fulvic acids is likely to be sulfide (Brüchert, 1998) with prevalent thiol, polysulfide, and polythionate groups (Vairavamurthy *et al*., 1994).

V. CONCLUSIONS

The inherent chemical complexity of DOM presents many challenges to understanding the role of DOM in C and N cycling and other processes in aquatic ecosystems. The measurement of trace organic moieties in major fractions of DOM, such as fulvic acids, can provide valuable data for understanding sources and biogeochemical pathways. In field studies, multiple lines of evidence can be critical for definitive interpretation of results. The tracer approaches outlined in this chapter should be used in conjunction with mass balance and flux measurements, for example.

In this chapter, we have presented a variety of tracers that may prove useful in understanding both the character and the source of dissolved organic matter. A summary of the inputs of DOM to the aquatic system along with the tracers appropriate for individual inputs is presented in Figure 9. The figure indicates that the tracers are appropriate for assessing specific aspects of the biogeochemical history and composition of DOM in aquatic ecosystems. For example, measurements of the fluorescence and $\delta^{13}C$ are both useful for discriminating between terrestrial and microbial sources of fulvic acids. In contrast, changes in DOM quality due to photolysis can be assessed by measuring changes in either phenolic hydroxyl content or UV absorbance. Finally, measurement of the sulfur content and sulfur isotopes in fulvic acids may prove useful for evaluating the redox conditions under which fulvic acids were formed. Used individually or in concert, the measurement of trace organic moieties in dissolved organic material has the potential

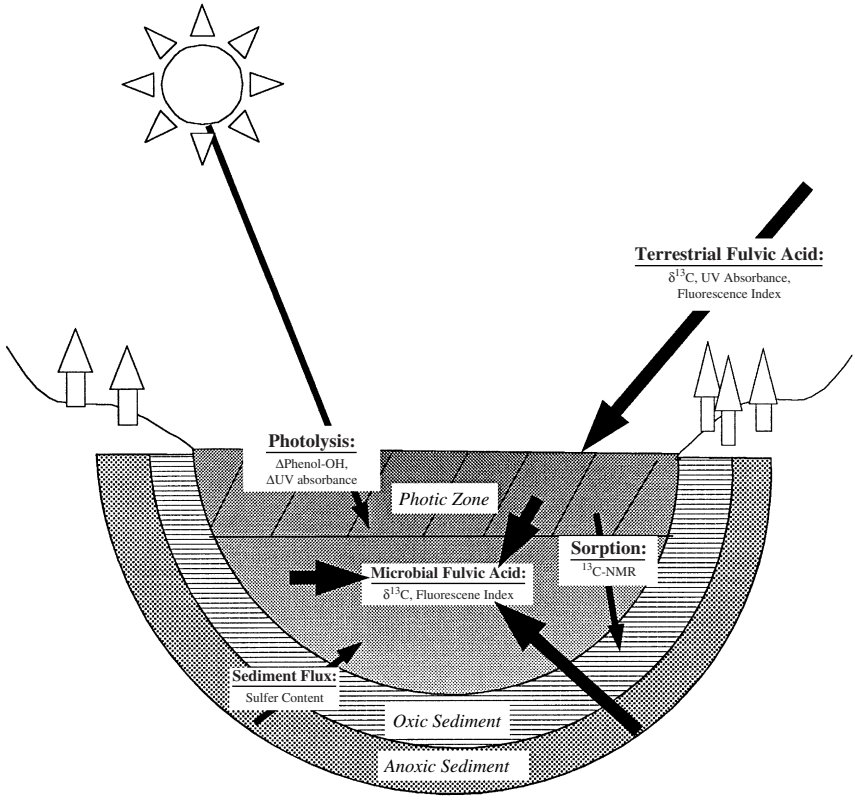

FIGURE 9 Organic matter inputs to the aquatic ecosystem and potential tracers of these inputs.

to provide new insights into the mechanisms behind the production, transformation, and consumption of DOM at the catchment scale.

Ultimately, the usefulness of tracer methods will partially depend upon how readily they can be incorporated into a field study. Methods that can be applied to filtered water samples are less labor intensive than those requiring some type of fractionation, such as the use of small-volume XAD-8 columns or ultrafiltration. However, column or ultrafiltration fractionation can be streamlined to make them practical for field studies, and the better resolution of DOM chemistry may make the extra effort worthwhile. If fulvic acid or high molecular weight fractions are isolated in a study, these can be saved for potential subsequent analysis of trace moieties as motivated by initial results. Finally, the overall question being addressed in a particular experimental or field study will determine which tracer methods, if any, are included.

REFERENCES

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Bernston, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems — Hypotheses revisited. *Bioscience* **48:**921–934.

Aiken, G. R. 1985. Isolation and concentration techniques for aquatic humic substances. *In* "Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation, and Characterization" (G. R. Aiken, D. M. McKnight, R. L. Wershaw, and P. MacCarthy, Eds.), pp. 363–385.

Aiken, G. R., D. M. McKnight, R. L. Wershaw, and P. MacCarthy. 1985. An introduction to humic substances in soil, sediment, and water. *In* "Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation, and Characterization" (G. R. Aiken, D. M. McKnight, R. L. Wershaw, and P. MacCarthy, Eds.), pp. 1–9.

Aiken, G. R., D. M. McKnight, K. A. Thorn, and E. M. Thurman. 1992. Isolation of hydrophilic organic acids from water using nonionic macroporous resins. *Organic Geochemistry* **18:**567–573

Aiken, G. R., D. M. McKnight, R. Harnish, and R. L. Wershaw. 1996. Geochemistry of aquatic humic substances in the Lake Fryxell Basin, Antarctica. *Biogeochemistry* **34:**157–188.

Baron, J., D. M. McKnight, and A. S. Denning. 1991. Sources of dissolved and particulate organic material in Loch Vale Watershed, Rocky Mountain National Park, Colorado, USA. *Biogeochemistry* **15:**89–110.

Benner, R. H. 1998. Cycling of dissolved organic matter in the ocean. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 317–331. Springer-Verlag, Berlin.

Brown, A., D. M. McKnight, Y. Chin, M. Uhle, and E. C. Roberts (2002) The influence of mixotroph growth on DOM chemistry in Pony Lake, a eutrophic coastal pond in Antarctica. Manuscript in preparation.

Brüchert, V. 1998. Early diagenesis of sulfur in estuarine sediments: The role of sedimentary humic and fulvic acids. *Geochimica et Cosmochimica Acta* **62:**1567–1586.

Chin, Y., G. Aiken, and E. O'Loughlin. 1994. Molecular weight, polydispersivity, and spectroscopic properties of aquatic humic substances. *Environmental Science & Technology*. **28:**1853–1858.

Coble, P. G., S. A. Green, N. V. Blough, and R. B. Gagosian. 1990. Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. *Nature* **348:**432–435.

Cronan, C. S., and G. R. Aiken. 1985. Chemistry and transport of soluble humic substances in forested watersheds of the Adirondack Park, New York. *Geochimica et Cosmochimica Acta* **49:**1697–1705.

De Souza Sierra, M. M., O. F. X. Donard, M. Lamotte, C. Belin, and M. Ewald. 1994. Fluorescence spectroscopy of coastal and marine waters. *Marine Chemistry* **47:**127–144.

Easthouse, K. B., J. Mulder, N. Christophersen, and H. M. Seip. 1992. Dissolved organic carbon fractions in soil and stream water during variable hydrologic conditions at Birkenes, Southern Norway. *Water Resources Research* **28:**1585–1596.

Elder, J. F., N. B. Rybicki, V. Carter, and V. Weintraub. 2000. Sources and yields of dissolved carbon in northern Wisconsin stream catchments with differing amounts of peatland. *Wetlands* **20:**113–125.

Ertel, J. R., J. I. Hedges, and E. M. Perdue. 1984. Lignin signature of aquatic humic substances. *Science* **223:**485–487.

Ertel, J. R., J. I. Hedges, A. H. Devol, and J. E. Richey. 1986. Dissolved humic substances of the Amazon River system. *Limnology and Oceanography* **31:**739–754.

Ertel, J. R., J. M. Caine, and E. M. Thurman. 1993. Biomarker compounds as source indicators for dissolved fulvic-acids in a bog. *Biogeochemistry* **22:**195–212.

Fiebig, D. M., M. A. Lock, and C. Neal. 1990. Soil water in the riparian zone as a source of carbon for a headwater stream. *Journal of Hydrology* **116:**217–237.

Gu, B., T. L. Mehlhorn, L. Liang, and J. F. McCarthy. 1996. Competitive adsorption, displacement, and transport of organic matter on iron oxide. II. Displacement and transport. *Geochimica et Cosmochimica Acta* **60:**2977–2992.

Hedges, J. I., G. L. Cowie, J. E. Richey, and P. D. Quay. 1994. Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. *Limnology and Oceanography* **39:**743–761.

Hedin, L. O., J. J. Armesto, and A. H. Johnson. 1995. Patterns of nutrient loss from unpolluted, old-growth temperate forests: Evaluation of biogeochemical theory. *Ecology* **76:**493–509.

Hood, E. W., M. W. Williams, and N. Caine. (2002) Ecological controls on organic and inorganic nitrogen leaching across an alpine-subalpine ecotone, Green Lakes Valley, Colorado Front Range. *Ecosystems*, in press.

Ishiwatari, R. 1985. Geochemistry of humic substances in lake sediments. *In* "Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation, and Characterization" (G. R. Aiken, D. M. McKnight, R. I. Wershaw, and P. MacCarthy, Eds.), pp. 147–180.

Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. *In* "Isotope Tracers in Catchments Hydrology" (C. Kendall and J. J. McDonnell, Eds.), pp. 519–576. Elsevier, Amsterdam.

Kendall, C., S. R. Silva, and V. J. Kelly. 2001. Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrological Processes* **15:**1301–1346.

Klapper, L., D. M. McKnight, J. R. Fulton, E. L. Blunt-Harris, K. P. Nevin, D. R. Lovely, and P. G. Hatcher. 2002. Fulvic acid oxidation state detection using fluorescence spectroscopy. *Environmental Science & Technology*, in press.

Kouassi, A. M., and R. G. Zika. 1992. Light-induced destruction of the absorbency property of dissolved organic matter in seawater. *Toxicology and Environmental Chemistry* **35:**195–211.

Langford, C. H., and A. G. Bruccoleri. 2000. Photophysics and photochemistry of two well characterized fulvic acids. *IHSS* (*Toulouse, France*) **10:**69–71.

Leenheer, J. A., D. M. McKnight, E. M. Thurman, and P. MacCarthy. 1989. Structural components and proposed structural models of fulvic acid from the Suwannee River. *In* "Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures." U.S. Geological Survey Open-File Report 87–557, pp. 335–359.

McDowell, W. H., and G. E. Likens. 1984. Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook Valley. *Ecological Monographs* **58:**177–195.

McKnight, D. M., E. M. Thurman, R. L. Wershaw, and H. Hemond. 1985. Biogeochemistry of aquatic humic substances in Thoreau's Bog, Concord, Massachusetts. *Ecology* **66:**1339–1352.

McKnight, D. M., K. A. Thorn, and R. L. Wershaw. 1988. Rapid changes in dissolved humic substances in Spirit Lake and South Fork Castle Lake, Washington. *Limnology and Oceanography* **33:**1527–1541.

McKnight, D. M., K. E. Bencala, G. W. Zellweger, G. R. Aiken, G. L. Feder, and K. A. Thorn. 1992. Sorption of dissolved organic carbon by hydrous aluminum and iron oxides occurring at the confluence of Deer Creek with the Snake River, Summit County, Colorado. *Environmental Science & Technology* **26:**1388–1396.

McKnight, D. M., R. L. Smith, R. A. Harnish, C. L. Miller, and K. E. Bencala. 1993. Seasonal relationships between planktonic microorganisms and dissolved organic material in an alpine stream. *Biogeochemistry* **21:**39–59.

McKnight, D. M., E. D. Andrews, S. A. Spaulding, and G. R. Aiken. 1994. Aquatic fulvic acids in algal-rich Antarctic ponds. *Limnology and Oceanography* **39:**1972–1979.

McKnight, D. M., R. Harnish, R. L. Wershaw, J. S. Baron, and S. Schiff. 1997. Chemical characteristics of particulate, colloidal and dissolved organic material in Loch Vale Watershed, Rocky Mountain National Park. *Biogeochemistry* **36:**99–124.

McKnight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen. 2001. Spectrofluorometric characterization of aquatic fulvic acid for determination of

precursor organic material and general structural properties. *Limnology and Oceanography* **46:**38–48.

Mobed, J. J., S. L. Hemmingsen, J. L. Autry, and L. B. McGowan. 1996. Fluorescence characterization of IHSS humic substances: total luminescence spectra with absorbance correction. *Environmental Science & Technology* **30:**3061–3065.

Mopper, K., and C. A. Schultz. 1993. Fluorescence as a possible tool for studying the nature and water column distribution of DOC components. *Marine Chemistry* **41:**229–238.

Moran, M. A., and R. E. Hodson. 1990 Bacterial production on humic and nonhumic components of dissolved organic carbon. *Limnology and Oceanography* **35:**1744–1756.

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography* **42:**1307–1316.

Mulholland, P. J., and W. R. Hill. 1997. Seasonal patterns in streamwater nutrient and dissolved organic carbon concentrations: Separating catchment flow path and in-stream effects. *Water Resources Research* **33:**1297–1306.

Palmer, S. M., D. Hope, M. F. Billet, J. J. C. Dawson, and C. L. Bryant. 2001. Sources of organic and inorganic carbon in a headwater stream: Evidence from carbon isotopic studies. *Biogeochemistry* **52:**321–338.

Perdue, E. M. 1985. Acidic functional groups of humic substances. *In* "Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation, and Characterization," (G. R. Aiken, D. M. McKnight, R. L. Wershaw, and P. MacCarthy, Eds.), pp. 493–526.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowdon, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. *Science* **292:**86–90.

Pullin, M. J., and S. E. Cabaniss. 1997. Physiochemical variations in DOM-synchronous fluorescence: Implications for mixing studies. *Limnology and Oceanography* **42:**1766–1773.

Raymond, P. A., and J. E. Bauer. 2001. Use of ^{14}C and ^{13}C natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: A review and synthesis. *Organic Geochemistry*, **32:**469–485.

Schiff, S. L., R. Aravena, S. E. Trumbore, and P. J. Dillon. 1990. Dissolved organic carbon cycling in forested watersheds: A carbon isotope approach. *Water Resources Research* **26:**2949–2957.

Schiff, S. L., R. Aravena, S. E. Trumbore, M. J. Hinton, R. Elgood, and P. J. Dillon. 1997. Export of DOC from forested catchments on the Precambrian Shield of Central Ontario: Clues from ^{13}C and ^{14}C. *Biogeochemistry* **36:**43–65.

Scully, N. M., and D. R. S. Lean. 1994. The attenuation of ultraviolet light in temperate lakes. *Archiv für Hydrobiologie* **43:**135–144.

Steinberg, C., and U. Muenster. 1985. Geochemistry and ecological role of humic substances in lakewater. *In* "Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation, and Characterization" (G. R. Aiken, D. M. McKnight, R. L. Wershaw, and P. MacCarthy, Eds.), pp. 105–146. Wiley, New York.

Stevenson, F. J. 1985. Geochemistry of soil humic substances. *In* "Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation, and Characterization" (G. R. Aiken, D. M. McKnight, R. L. Wershaw, and P. MacCarthy, Eds.), pp. 105–146. Wiley, New York.

Thurman, E. M. 1985. "Organic Geochemistry of Natural Waters." Martinus Nijhoff/Dr. W. Junk, Dordrecht, the Netherlands.

Thurman, E. M., and R. I. Malcolm. 1981. Preparative isolation of aquatic humic substances. *Environmental Science & Technology* **15:**463–466.

Vairavamurthy, M. A., W. Zhou, T. Eglinton, and B. Manovitz. 1994. Sulfonates: A novel class or organic-sulfur compounds in marine sediments. *Geochimica et Cosmochimica Acta* **58:**4681–4687.

Vitousek, P. M., L. O. Hedin, P. A. Matson, J. H. Fownes, and J. Neff. 1998. Within-system element cycles, input-output budgets, and nutrient limitation. *In* "Successes, Limitations and Frontiers in Ecosystem Science" (M. L. Pace, and P. M. Groffman, Eds.), pp. 432–451. Springer-Verlag, Berlin.

Wallis, P. M. 1979. "Sources, Transportation, and Utilization of Dissolved Organic Matter in Groundwater and Streams." Scientific Series No. 100. Kananaskis Centre for Environmental Research, University of Calgary.

Wang, Y., T. G. Huntington, L. J. Osher, L. I. Wassenaar, S. E. Trumbore, R. G. Amundson, J. W. Harden, D. M. McKnight, S. L. Schiff, G. R. Aiken, W. B. Lyons, R. O. Aravena, and J. S. Baron. 1998. Carbon cycling in terrestrial environments. *In* "Isotope Tracers in Catchment Hydrology" (C. Kendall, and J. J. McDonnell, Eds.), pp. 577–602. Elsevier, Amsterdam.

Waters, S. B. 1999. Responses of algal communities to environmental change in an alpine lake, Green Lakes Valley, Colorado. Master's Thesis, University of Colorado, Boulder, CO.

Wetzel, R. G., P. G. Hatcher, and T. S. Bianchi. 1995. Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology and Oceanography* **40:**1369–1380.

Williams, M. W., J. S. Baron, N. Caine, R. Sommerfeld, and R. Sanford. 1996. Nitrogen saturation in the Rocky Mountains. *Environmental Science & Technology* **30:**640–646.

Zhou, Q. H., P. A. Maurice, and S. E. Cabaniss. 2001. Size fractionation upon adsorption of fulvic acid on goethite: Equilibrium and kinetic studies. *Geochimica et Cosmochimica Acta* **65:**803–812.

4

The Role of Monomers in Stream Ecosystem Metabolism

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I. INTRODUCTION

Dissolved organic matter (DOM) is the largest pool of organic matter in aquatic ecosystems (Wetzel, 1992), and its biogeochemistry influences the global carbon cycle. In streams and rivers, humic substances of terrestrial origin are the major constituents of the DOM pool (Thurman, 1985), and these polymeric molecules have been considered both chemically and biologically refractory (Cummins *et al*., 1972). Subsequent studies have shown that humic molecules are susceptible to photolytic (Geller, 1985; Wetzel, *et al*., 1995; Opsahl and Benner, 1998; see Chapter 10) and microbiological (Volk *et al*., 1997; Carlsson *et al*., 1999; Ellis *et al*., 2000) oxidation and may thus contribute to the energy flow in freshwater and coastal marine ecosystems. In contrast to humic substances and polymers, monomers are the simple, unpolymerized form of chemical compounds that are not easily degraded to simpler molecules of the same class. Monomers have a known structure and composition and are present in very low concentrations, and many of the biochemical pathways of monomers that contribute to bacterial metabolism have been elucidated (see Chapter 9).

Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

In this chapter we (1) present a rationale for distinguishing between the metabolism of monomeric and polymeric DOM; (2) review what is known about the sources, concentration, and composition of the monomeric organic carbon pool in aquatic environments; (3) discuss factors that contribute to the biodegradability of DOM; and (4) describe contrasting views of what DOM molecules support bacterial heterotrophy.

In aquatic systems generally, and for streams in particular, it is important to differentiate between the kinetics of monomeric and polymeric or humic degradation because of downstream transport. Downstream transport limits residence time, which in turn influences whether a molecule is utilized within a watershed or transported downstream (see Chapters 15 and 20). Viewed from the perspective of stream ecosystem metabolism, the time frame of monomer uptake or the time frame of the processes that transform polymeric DOM and transport some moiety into benthic bacterial cells, coupled with downstream transport, will determine the distances over which organic matter is utilized (spiraling length). The spiraling length of DOM, in turn, determines its contribution to both the energy budget and the longitudinal linkage of stream and river ecosystems.

This chapter focuses on the role of DOM in stream ecosystem metabolism, but similar questions regarding the biodegradability of monomeric and polymeric molecules and their roles in supporting heterotrophic metabolism exist for all aquatic environments from groundwaters to the oceans. Delivery of DOM to the oceans by rivers is sufficient to account for much of the oceans' DOM and may account for substantial heterotrophy within the oceans (Deuser, 1988). Surprisingly, terrigenous DOM accounts for less than 2.5% of the oceanic DOM (Opsahl and Benner, 1997) and is highly degraded, nitrogen-poor, and partially resistant to photolysis, resulting in a "mystery in the global carbon cycle": specifically, why is there so little terrigenous DOM in the pelagic oceans? (Hedges *et al.*, 1997; see Chapter 5). Despite the potential importance of extracellular DOM to global C-budgets, the turnover time for this carbon pool has not been determined for any ecosystem.

The major classes of DOM in natural waters, including stream and river water, have been reviewed with extensive references that cover the literature through 1984 (Thurman, 1985). The most common measure of organic matter is as organic carbon, and to facilitate a quantitative description of DOM we use units of carbon. The major classes of organic carbon in river water are fulvic acid, humic acid, hydrophilic acids, carbohydrates, carboxylic acids, and amino acids. Within these classes, only the carbohydrate, amino acid, and carboxylic acid categories contain a subset of monomers, of which the carboxylic acids perhaps constitute the largest though certainly the least investigated pool (Fig. 1). Our review of the monomer data for streams and rivers begins by identifying sources of monomers, describes the analytical methods used to quantify monomer concentrations,

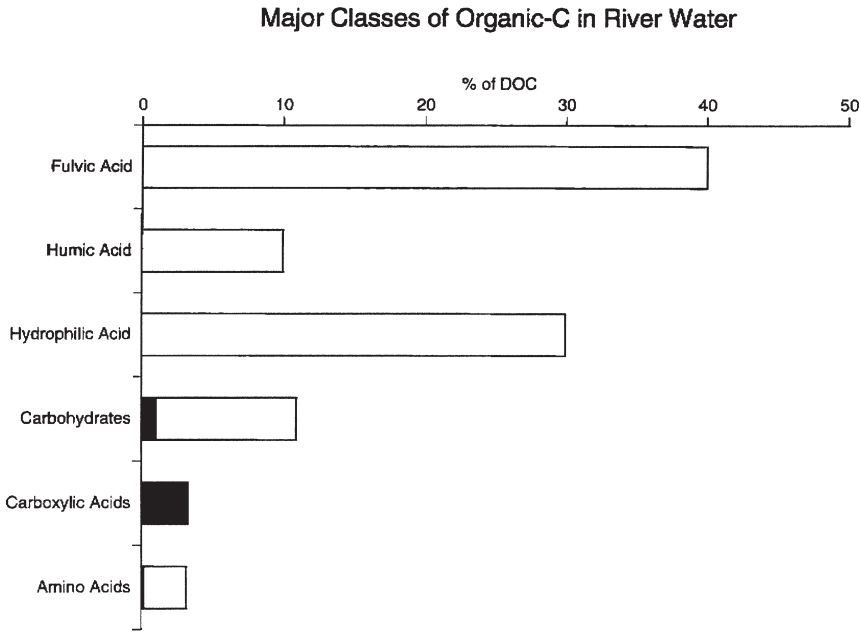

FIGURE 1 Major classes of organic carbon in river water. Monomeric-C is represented by black shading within the histograms. Modified with permission from E. M. Thurmen. 1985. "Organic Geochemistry of Natural Waters." Kluwer Academic Publishers Group.

summarizes the information from the Thurman text, and then provides an update from current references. We incorporate some information from other aquatic environments both for comparative purposes and to augment the sparse data sets that exist for lotic ecosystems.

II. MONOMER SOURCES AND CONCENTRATIONS

The ultimate sources of all organic compounds, including monomers, are autotrophic organisms, primarily photosynthetic plants. Biological, chemical, and physical processes play important roles in modifying the chemical structure and composition of organic matter and transporting DOM within ecosystems and across ecosystem boundaries in nature. Our list of monomer sources (Table I), with a focus on streams and rivers, is biased toward aquatic processes, though terrestrial processes contribute significantly to stream and river ecosystem functioning at the watershed scale (Hynes, 1975; see also Chapters 2 and 6). On a global scale, terrestrial plant production generates approximately twice as much organic matter as aquatic primary production, and most of the aquatic production is within

TABLE I Sources of Monomers in Aquatic Ecosystems

Monomer class	*Source*	*Reference*
Carbohydrate	Aquatic humic substances	Jahnel *et al.* (1998a, b)
Carbohydrate	Soil polysaccharides	Cheshire and Hayes (1990)
Carbohydrate	Aquatic humic substances	Watt *et al.* (1996)
Carbohydrate	Lake fulvic acid	Bertino *et al.* (1987)
Carbohydrate	Macrophytes	Boschker *et al.* (1995)
Carbohydrate	Macrophytes	Moers *et al.* (1990)
Carbohydrate	Higher plants	Benner *et al.* (1990)
Carbohydrate	Phytoplankton	Eberlein and Brockmann (1986)
Carbohydrate	Bacteria	Fox *et al.* (1998)
Amino acid	Higher plants	Stanley *et al.* (1987)
Amino acid	Macrophytes	Thomas and Eaton (1996)
Amino acid	Aquatic humic substances	Jahnel and Frimmel (1996)
Amino acid	Aquatic humic substances	Jahnel *et al.* (1988b)
Amino acid	Zooplankton feeding	Riemann *et al.* (1986)
Carboxylic acids	Wetland water (photolysis)	Wetzel *et al.* (1995)
Carboxylic acids	River water (photolysis)	Bertilsson *et al.* (1999)
Carboxylic acids	Phytoplankton	Leboulanger *et al.* (1994)
Carboxylic acids	Precipitation	Elbert *et al.* (1989)
Hydrocarbons	Conifers	Button and Jüttner (1989)
Hydrocarbons	Epilithic biofilm	Jüttner and Dürst (1997)
Phenolics	Eelgrass	Quackenbush *et al.* (1986)

the open oceans (Whittaker and Likens, 1973), whereas approximately 70% of the organic C in forest ecosystems is contained in soils or peat (Dixon *et al.*, 1994). An important aspect of monomer sources is that not all monomers are the result of polymer degradation by either enzymatic hydrolysis or photolysis. Numerous direct sources of monomers occur within streams, such as active excretion by algae (Nalewajko, 1966), bacteria (Allen, 1976; Namkung and Rittmann, 1986), and animals (Park *et al.*, 1997), plus death and lysis of stream organisms (see also Chapter 1). Although neither viral attack (Wilhelm and Suttle, 1999) nor sloppy feeding (Lampert, 1978; Riemann *et al.*, 1986; Hygum *et al.*, 1997) has been investigated in streams, they are probably important processes that deserve investigation.

Analytical methods used to identify monomers have improved significantly from those that quantify whole classes of compounds, such as amino acids, peptides, proteins, and primary amines (Undefriend *et al.*, 1972) or carbohydrate-like compounds (Johnson and Sieburth, 1977) to ones that are molecule-specific (Table II). Most of these methods are based on combining chromatography techniques that can separate complex mixtures of molecules with highly sensitive detectors that can approach the nanomolar or picomolar range. Monomers are usually present at low concentrations, so

TABLE II Analytical Methods for Monomer Determinations

Compound class	*Analytical approach*	*Reference*
Carbohydrates	Wet chemistry/colorimetric detection	Johnson and Sieburth (1977)
	High-performance liquid chromatography–pulsed amperometric detection	Mopper *et al.* (1992)
	Thermospray mass spectrometry	Meon and Jüttner (1999)
	Gas chromatography/mass spectrometry	Cowie and Hedges (1984)
Carboxylic acids	Ion chromatography	Peldszus *et al.* (1996)
	Gas chromatography	Bethge and Lindstrom (1974)
	Capillary zone electrophoresis	Dahlen *et al.* (1996)
Amino acids	High performance liquid chromatography/fluorescence detection	Lindroth and Mopper (1979)
Turpenes	Gas chromatography/mass spectrometry	Jüttner (1988)
Phenols	Gas chromatography/flame ionization detection	Blaschke (1979)

that sample handling prior to analysis is a critical step, with care needed to preserve samples from biological degradation (Wangersky, 1993; Karlsson *et al.*, 1999) or prevent contamination through filtration (Fuhrman and Bell, 1985; Karlsson *et al.*, 1999). Caveats or concerns specific to individual analytical methods are addressed below for each monomer class.

In an "average" stream or river unpolluted by anthropogenic sources, and with a DOC concentration of 420 μM, less than 7% of the carbon can be attributed to monomers (Fig. 2). The average concentration for each monomeric C class represents a broad range, and our conversions of data from percentage of DOC to molar concentrations are based on the following assumptions concerning the average number of carbon atoms within each molecule class: carbohydrate, 6; carboxylic acids, 5.6; amino acids, 5; phenols, 8; hydrocarbons, 17.

The monomeric carbohydrates are molecules that do not yield simpler compounds upon hydrolysis, and contain either an aldehyde or ketone functional group. Monosaccharides typically contain from three to eight C atoms and can be subdivided into classes of neutral sugars and sugar derivatives, such as sugar acids, sugar alcohols, amino sugars, and methylated sugars. All of these molecules are presumably present in streams and rivers in low concentrations (690 nM for total monomeric carbohydrates; equal to 0.9% of the DOC), with a roughly equal distribution of carbon among the three major classes, neutral sugars, sugar acids, and all other sugar

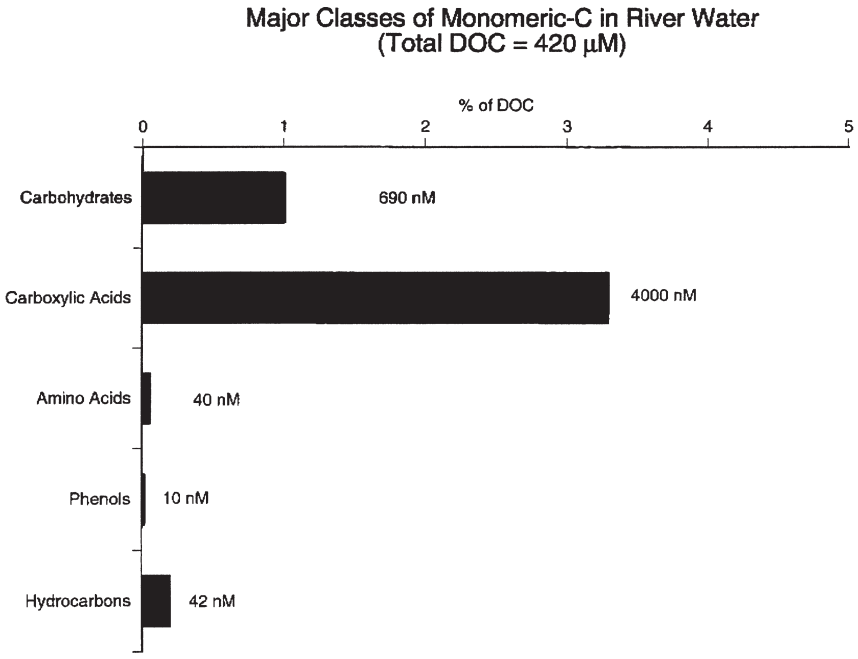

FIGURE 2 Monomeric organic carbon classes in an average stream.

derivatives. However, there is little information about the pool sizes for all but the neutral sugars in any aquatic environment.

The neutral sugars include glucose, the first organic product of photosynthesis and generally one of the most abundant monosaccharides in aquatic environments. Glucuronic acid and mannuronic acid are important sugar acids found in bacterial mucopolysaccharides and algae, respectively, and galacturonic acid is a constituent of plant cell walls. The amino sugar glucosamine is an important component of chitin, which is found in the cell wall structure in invertebrates, protozoa, fungi, and some algae, whereas the sugar alcohols mannitol and sorbitol are components of plant tissues and fungi. Another important group of sugar derivatives is the phosphate-containing compounds, such as the triose D-gylceraldehyde-3-phosphate, the tetrose D-erythrose-4-phosphate, the pentose ribulose-5-phosphate, and the hexose glucose-6-phosphate. Phosphorylation of monosaccharides is often required prior to transport into cells, involvement in biosynthesis, or participation as respiratory intermediates in oxidation reactions.

There are numerous reports of dissolved total saccharide concentrations and compositions in aquatic ecosystems, but few reports of monomeric carbohydrates, and those show broad concentration ranges within and between habitats (Table III). Some of the earliest estimates of dissolved free

TABLE III Dissolved Free Carbohydrates in Aquatic Ecosystems

Site	*Concentration (nM)*	*Reference*
River		
Piedmont stream	48–380	Gremm and Kaplan (1997)
	639–5125 (MBTH)	Volk *et al.* (1997)
Rocky Mountain stream	<11–333 (glucose only)	McKnight *et al.* (1993)
Forest stream	890 (MBTH)	McDowell and Likens (1988)
Swedish river (mildly polluted)	133 (glucose only)	Hobbie and Wright (1965)
Lake		
Eutrophic	4000	Münster (1984)
Eutrophic	2700–7000	Middleboe *et al.* (1995a)
Eutrophic	<1–48	Meon and Jüttner (1999)
Eutrophic	56–111 (glucose only)	Hobbie and Wright (1965)
Seawater		
Key Biscayne and others	<5–2300	Mopper *et al.* (1992)
Gulf of Mexico	2–15 (glucose only)	Skoog *et al.* (1999)
Equatorial Pacific	19–81	Rich *et al.* (1996)

glucose concentrations, accomplished with bioassays that used the kinetics of substrate uptake as the response factor (Hobbie and Wright, 1965), are similar to the HPLC-based estimates. In contrast, the highest concentrations of monosaccharides reported for streams and oceans involve data derived with the spectrophotometric 3-methyl-2-benzothiazolinone hydrochloride (MBTH) method (Johnson and Sieburth, 1977), as opposed to concentrations based on the identification of individual molecules, and thus may overestimate true concentrations. MBTH "carbohydrates" actually are carbohydrate-like substances (Senior and Chevolot, 1991). This method detects any substance with a terminal glycol function ($CHOH–CH_2OH$), so it will detect carbohydrates that have been modified and are perhaps no longer biologically labile or even complete molecules. Similarly, the NMR technique estimates the amount of C–O and O–C–O groups present in molecules or degradation products of molecules (Skoog and Benner, 1997).

The molar composition of the monosaccharide pool in the oceans is dominated by glucose (Rich *et al.*, 1996), especially at depth (Skoog and Benner, 1997), and whereas glucose is an important monomer in streams, rivers, and lakes, fructose and galactose have been reported at concentrations

similar to those of glucose (Jørgensen and Jensen, 1994; Gremm and Kaplan, 1997). Hydrolysis of water samples that is required prior to the analysis of total saccharide composition and concentration can destroy fructose, so although fructose is a major monosaccharide, it typically does not appear in descriptions of combined or polymeric carbohydrate compositions. Storm samples that include DOC transported to the stream without extensive terrestrial processing have a broader range of monosaccharides, including rhamnose, arabinose, mannose, galactose, xylose, arabinose, and ribose (Gremm and Kaplan, 1997).

The carboxylic acids can be subdivided into nonvolatile fatty acids, volatile fatty acids, hydroxy acids, dicarboxylic acids, and aromatic acids (Fig. 3). The nonvolatile fatty acids are molecules with more than five carbon atoms, such as stearic and palmitic acids, which are the degradation products of fats and triglycerides. Three different 18-C fatty acids that are important constituents of plants include oleic and linoleic acids that are abundant in plant seeds, and linolenic acid, which is abundant in plant leaves. Volatile fatty acids are short-chain molecules with one to five carbon atoms, such as acetic and valeric acid, associated with anaerobic metabolism. The hydroxy-acids are common intermediates in biochemical pathways, including the tricarboxylic acid cycle. The excretion of hydroxyacids by algae, such as the

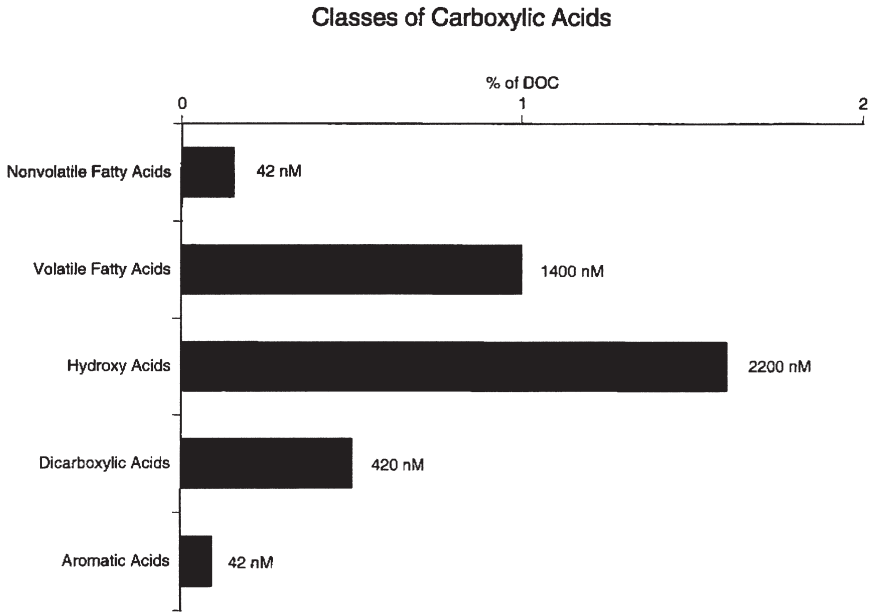

FIGURE 3 Monomeric carboxylic-C in stream water. Modified with permission from E. M. Thurman. 1985. "Organic Geochemistry of Natural Waters." Kluwer Academic Publishers Group.

release of glycolate produced during photorespiration (Leboulanger *et al.*, 1994), varies from species to species (Hellebust, 1974). Dicarboxylic acids are common degradation products within soils and include oxalic acid and succinic acid. The lignin degradation products ferulic, gallic, cinnamic, salicylic, gentisic, and syringic acids are aromatic acids, benzene rings with hydroxyl groups, that have been identified in water extracts of deciduous leaf or conifer needle litter (Larson, 1978; Blaschke, 1979).

The analysis of carboxylic acids has most often been performed with deriviatization followed by gas chromatography, which has an advantage of high chromatographic resolution and the possibility of analyte identification with mass spectroscopy. More recent studies have involved the use of HPLC (Wetzel *et al.*, 1995) or ion chromatography (Peldszus *et al.*, 1996; Brinkman *et al.*, 2000), which avoid a derivatization step and thus potential problems from highly polar or volatile derivatization products, and have shown detection limits in the submicromolar range. Of the monomeric compound classes in aquatic environments, the carboxylic acids are the least studied (see also Chapter 9), and this limits our present ability to estimate the metabolism that could be supported by monomers alone.

Amino acids are a potential carbon and nitrogen source for heterotrophs and have received considerable attention because of their importance to protein synthesis, bacterial metabolism, and algal/bacterial interactions, as well as the availability of sensitive analytical techniques that incorporate fluorescence detection (Lindroth and Mopper, 1979). Dissolved free amino acids (DFAAs) have been extensively studied in the oceans (see also Chapter 9), but only data from streams and rivers, lakes, and estuaries are presented here (Table IV). The range of concentrations within and between sites is extensive, and there are few studies that provide insight into the dynamics or mechanisms behind these variations. In streams, dramatic DFAA changes probably are associated with dramatic changes in hydrology. For example, in the Stridbacken, a stream in northern Sweden, concentrations of DFAA measured during a spring flood increased to a peak concentration of 900 nM in April, 2 weeks prior to the apparent beginning of flood flows, and then declined to 150 nM in May, at the end of the snow melt. These dynamics were interpreted as a change in flow paths to include shallow, organic rich soil layers as the thawing of frozen soils began (Stepanauskas *et al.*, 2000).

The composition of the DFAA pool in streams and rivers has been described for only a few sites, but these data show some consistency in their patterns. In White Clay Creek glycine and aspartate made up 40% of the DFAA, and when alanine, glutamate, serine, and threonine were included, these six amino acids accounted for 70% to 75% of the total DFAA (Volk *et al.*, 1997). In the Lillan and Stridbacken, two boreal rivers, glutamate, aspartate, serine, and alanine were the dominant DFAA (Stepanauskas *et al.*, submitted for publication). The White Clay Creek eventually flows into

TABLE IV Dissolved Free Amino Acids in Aquatic Ecosystems

Site	*Concentration (nM)*	*Reference*
River		
Arctic river	8–2000	Lock *et al.* (1989)
Boreal stream	130–900	Stepanauskas *et al.* (2000)
Piedmont stream	7–67	Volk *et al.* (1997)
Forest stream	133	McDowell and Likens (1988)
Coastal plain river	116	Hobbie (1969)
Lake		
Mesotrophic	<20–480	Simon (1998)
Mesotrophic	<100–455	Jørgensen and Jensen (1994)
Eutrophic	643–1330	Thomas (1997)
Eutrophic	23–1300	Münster and Chrøst (1990)
Eutrophic	78–1500	Jørgensen (1987)
Oligotrophic	200–3700	Jørgensen (1987)
Oligotrophic	411–691	Thomas (1997)
Estuary		
Delaware Bay	50–1400	Coffin (1989)
Delaware Bay	300–700	Middleboe *et al.* (1995b)
Santa Rosa Sound	190	Jørgensen *et al.* (1993)
Chesapeake Plume	6.5–23	Fuhrman (1990)

the Delaware River and then the estuary, and analyses of samples from the estuary showed that glycine, serine, glutamate, aspartate, and alanine accounted for 75% of the DFAA (Coffin, 1989). Interstitial waters from tropical mangrove sediments revealed glutamate, aspartate, serine, glycine, and alanine as the dominant DFAA (Stanley *et al.*, 1987). Alanine, aspartate, glycine, leucine, and serine constituted at least 60% of the DFAA pool in the Oosterschelde basin (Laanbroek *et al.*, 1985). In analyses of potential DFAA sources, diatom cell walls were enriched with glycine, serine, and threonine, diatom cell plasma was enriched with glutamic acid, tyrosine, and phenylalanine (Hecky *et al.*, 1973); and maple leaf leachate contained glutamate, valine, aspartate, and leucine as the dominant molecules in the DFAA pool (Armstrong and Bärlocher, 1989).

III. FACTORS AFFECTING BIOLOGICAL LABILITY

Biodegradability of DOM is often considered a function of molecular structure, including size, stoichiometry, spatial arrangement of atoms, and molecular constituents that alter electron density (Brezonik, 1990; Pitter and Chudoba, 1990; Boethling *et al.*, 1994). Aliphatic molecules generally are

more biologically labile than aromatic compounds, and amide functional groups make molecules more susceptible to electrophilic attack, whereas hydroxyl groups decrease that potential (Pitter and Chudoba, 1990). Others have used quantitative structure–activity relationships to show that the aliphatic carbon content of riverwater (Sun *et al*., 1997) and the oxidation state of DOM (Vallino *et al*., 1996) are related to bioavailability and rates of utilization. Enzymatic activities related to molecular structure include the recognition of organic molecules by surface-associated permease enzymes required to transport molecules across cell membranes against concentration gradients (Milner *et al*., 1987), the extracellular enzymatic hydrolysis of macromolecules (Chrøst *et al*., 1986) that often precedes permease transport, and subsequent intercellular enzymatic pathways such as aromatic ring cleavage (Dagley, 1971) or degradative pathways that supply substrates to catabolic and anabolic processes (Lehninger, 1975).

Biodegradability of DOM, from the ecosystem perspective, can involve other factors, such as hydrodynamics and stream size, that are independent of the intrinsic, structurally based biodegradability of a molecule, but influence ultimate utilization. For example, flow velocity can alter laminar boundary layers that limit access to biofilms (Carling, 1992) or influence mass transfer coefficients (Logan and Kirchman, 1991), and hydrologic exchange rates can affect streambed biofilm activity (Battin, 2000). DOM supplied from the stream must be transported to the streambed and hyporheic zone communities from the well-mixed water column, through the benthic boundary layer — a transitional region of turbulent flow in the uppermost layers of the porous streambed — to a deeper zone of laminar flow. As a result, DOM removed from the near-surface region of the stream bed is expected to be rapidly replenished by turbulent mixing, whereas DOM may become depleted in deeper regions because of limited transport from the stream (Packman and Bencala, 2000).

When mixed classes of DOM are considered, the interplay of hydrodynamics, transport, and varying uptake rates of different types of DOM may produce complex mixture dynamics. Net mixing is the product of simple hydrodynamic mixing and the concentration difference between the mixing water bodies. Rapid uptake of labile DOM from solution in the upper layers of the stream bed will produce a greater concentration difference and thus be more strongly influenced by turbulent mixing than will the uptake of less labile DOM. For the less labile DOM, the stream vs. subsurface concentration difference will be small, so that uptake will be more influenced by advective transport into the hyporheic zone than by turbulent mixing (Packman and Bencala, 2000). Similar dynamics may apply to DOM utilization by biofilms on the streambed surface. As biofilm theory suggests, uptake may be primarily limited by diffusion through a laminar sublayer, or by processes occurring within the biofilm (Gantzer *et al*., 1989; Grady *et al*., 1998). The uptake of more labile substances is likely

to be limited by the diffusion resistance of the laminar boundary layer, whereas less labile substances are likely to be limited by processes within the biofilm.

Data from glucose and arabinose injections to streams in the Pennsylvania Piedmont are consistent with a strong influence of hydrodynamics on relative uptake rates (Kaplan and Newbold, unpublished data). The uptake rate of glucose appears to be more strongly influenced by water velocity than the uptake rate of the less labile arabinose (Fig. 4a), so that the arabinose : glucose uptake ratio declined as velocity increased (Fig. 4b). The magnitude and trends of the glucose uptake are consistent with results from small experimental systems (Gantzer *et al*., 1988), and the effect of velocity can be interpreted as a reduction in diffusion resistance to uptake. Arabinose,

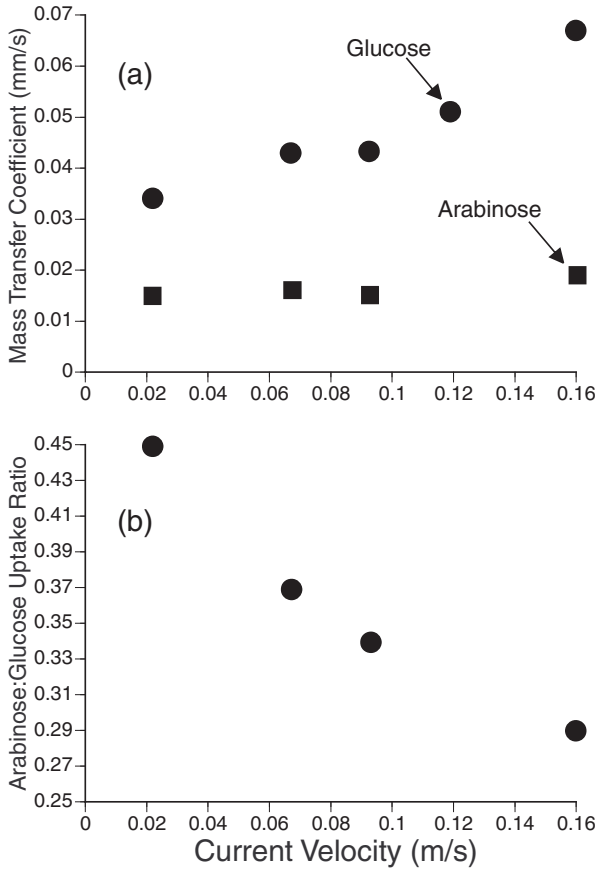

FIGURE 4 Uptake of glucose and arabinose in first- to fourth-order SE Pennsylvania woodland streams. (a) Mass transfer coefficients. (b) Ratios of mass transfer coefficients.

having a lower uptake rate within the biofilm, would develop lower concentration gradients across the laminar sublayer and therefore would be less influenced by velocity or turbulence-induced reductions in sublayer thickness.

The effects of stream size on solute dynamics and DOM lability are important, we think, because many forms of DOM that support stream ecosystem metabolism are probably utilized either without downstream transport (i.e., within the Aufwuchs or biofilm; Fletcher, 1992) or over spatial scales that extend beyond individual stream reaches. We have developed a conceptual model of DOM spiraling (Fig. 5) that incorporates refinements of the original spiraling concept (Newbold *et al*., 1982; Elwood *et al*., 1983; Minshall *et al*., 1985) and addresses both of these scenarios (Kaplan and Newbold, unpublished observations). In considering transport across stream orders, we suggest that the uptake length for a molecule of a particular biological lability within the water column will scale to stream order in a predictable manner, based on combined scaling rules derived from fluvial geomorphology and the modeling of solute dynamics. Specifically, we expect that uptake length increases in proportion to the downstream increase of channel length within a drainage basin. To the extent that the DOM pool is a mixture of molecules of different lability classes that are utilized over multiple stream reaches, this implies that there is a component of stream ecosystem metabolism that cannot be estimated solely from DOM uptake measurements within the water column. Similarly, we suggest that DOM molecules of intermediate lability provide an energy link or subsidy from upstream to downstream reaches. In the conclusion of this chapter, we describe a tracer-based approach to generating these estimates.

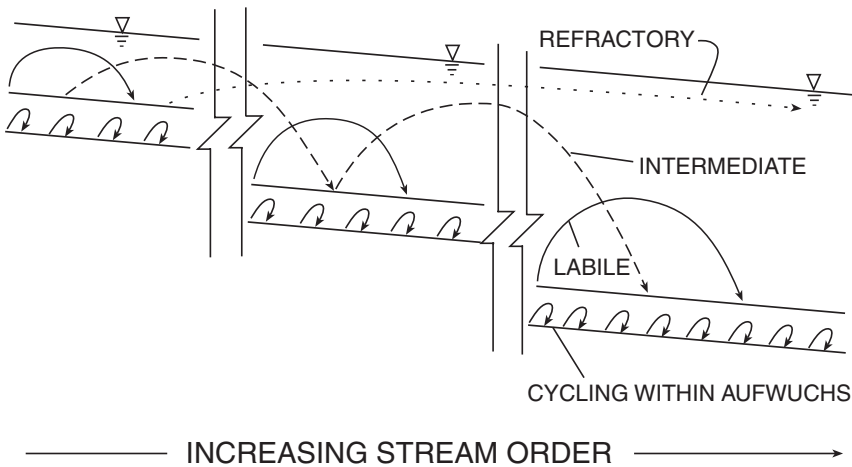

FIGURE 5 Schematic view of DOM cycling in a stream ecosystem, showing spatial scales of spiraling for different lability forms.

Not all DOM is biologically labile or even biodegradable. Kinetic, energetic, and concentration differences among DOM molecules provide the rationale for two contrasting, though not mutually exclusive, visions of DOM metabolism in aquatic environments. One is that a small pool of biologically labile molecules (Saunders and Storch, 1971; Allen, 1976) is cycled rapidly (Coffin *et al*., 1993). Although present in low concentrations, the rapid turnover of these labile molecules (Fuhrman and Ferguson, 1986) satisfies the energy and, in some cases, nutrient (Keil and Kirchman, 1991) requirements of aerobic heterotrophic bacteria (see also Chapter 9). Glucose concentration measurements, tracer-level uptake measurements, and bacterial carbon production estimates were combined in studies with marine and lacustrine bacterioplankton to estimate that either glucose alone or glucose as the dominant monosaccharide among others could contribute from 5% to 47% of the C required for bacterial production (Rich *et al*., 1996; Skoog *et al*., 1999; Bunte and Simon, 1999).

An alternative scenario is that a large pool of more refractory DOM, primarily humic substances, provides a significant supplement to the heterotrophic metabolism supported by monomers (Tranvik and Hofle, 1987; Moran and Hodson, 1994; Volk *et al*., 1997). Although biologically more recalcitrant and perhaps less energy yielding than monomers or complex polysaccharides (Amon and Benner, 1994), a portion of the humic substances is biologically degradable (Moran and Hodson, 1990; Carlsson *et al*., 1999). The high concentration of the humic substances compensates for slower rates of catabolism, and their supply and abundance may provide a degree of ecosystem stability (Wetzel, 1992; see also Chapter 19).

The notion that humic substances and other polymeric molecules contribute to bacterial metabolism in streams is supported by data from White Clay Creek showing that the biodegradable fraction of DOM was composed of 75% humic substances, 30% carbohydrates, 4% amino acids, and 39% DOM > 100 kDa (Volk *et al*., 1997; these categories are not mutually exclusive). The biodegradable carbohydrate fraction was primarily either polysaccharide or humic bound (Gremm and Kaplan, 1998), and the biodegradable amino acid fraction was overwhelmingly present in the combined form and was primarily humic bound (Volk *et al*., 1997). Knowledge of biodegradable DOM composition, lability, and concentration alone is not sufficient to estimate the contribution of the various molecular constituents to heterotrophic bacterial production. In situ uptake rates, rates of turnover or supply, and yields are essential, but little is known about these procedures.

Rates of supply may be controlled, in part, by the activity of hydrolytic enzymes that degrade combined molecules in dissolved or particulate organic matter to monomeric constituents prior to enzymatic transport into bacterial cells (Skoog *et al*., 1999; see also Chapter 13). At present, *in situ* rates of such exoenzymes have not been accurately measured, but enzyme potential has been estimated with the addition of artificial substrates in saturating

conditions (Chappell and Goulder, 1995; Sinsabaugh *et al*., 1997). An inhibitor-based method developed and applied to the decomposition of particulate organic matter from *Phragmites australis* (Boschker *et al*., 1995) addressed this issue, but this method has not been applied to hydrolysis of polymeric DOM, and it is unclear whether it would work in situations involving dilute substrate concentrations.

A conceptual model of DOM biodegradability, the size–reactivity continuum model, based on experimental studies with ultrafiltration of DOM, suggests that large molecules are biologically labile and susceptible to hydrolysis and uptake, whereas small molecules principally represent the cleaved moieties that were not incorporated into bacteria and have subsequently accumulated in the environment (Amon and Benner, 1996). We suggest that it is appropriate to explicitly include monomers in the model as they represent both the smallest and the most reactive DOM molecules. At the same time we also recognize that extracellular polymer degradation and monomer uptake are intimately connected, mixed substrate types contribute to bacterial metabolism, and the relative importance of polymeric versus monomeric compounds changes in space and time.

There has not been an assessment of the contribution of monomeric compounds to the support of bacterial metabolism in streams. While glucose and other monomeric DOM, including monomers in algal exudates, are rapidly utilized (Kaplan and Bott, 1982, 1989), studies of forested streams in New Hampshire (Findlay *et al*., 1993) and Ontario (Rosenfeld and Hudson, 1997) suggest that epilithic bacteria are not supported primarily by autochthonous production of epilithic algae. To assess whether it is plausible that monomeric compounds could support bacterial metabolism in an "average" stream, we can use the mass transfer coefficients from Figure 4a and an estimate of bacterial C demand to calculate the monomer concentration necessary to meet that demand.

We begin by assuming that heterotrophic respiration is dominated by bacterial respiration, and that total ecosystem respiration minus 50% of gross primary productivity equals heterotrophic respiration. Next, we assume that bacteria grow on C sources with 25% efficiency, so that 1.33 times heterotrophic respiration is equal to the C demand. Using components of the organic matter budget from White Clay Creek (Newbold *et al*., 1997), and assuming that organic matter is 45% C, we calculate that the bacterial C demand for White Clay Creek is 12 mg $C \cdot m^{-2} \cdot h^{-1}$. If V_F, the mass transfer coefficient for glucose, equals 4×10^{-5} m s^{-1}, and V_F times concentration equals the delivery of C to the streambed, then a concentration of 83 μg C/L of glucose-like monomers would satisfy 100% of the bacterial C demand.

This concentration is considerably lower than 330 μg C/L, the sum of all monomers in Figure 2. However, there are several caveats to consider. First, not all monomers can be expected to have the same lability as glucose, and, in fact, Figure 4 shows that at the velocities normal for White Clay

Creek, arabinose has a mass transfer coefficient nearly three-fold lower. In Lake Constance, galactose and mannose turnover times were three-fold longer than that of glucose, and turnover times for glucosamine and fructose were five- to 10-fold longer than that of glucose (Bunte and Simon, 1999). Similarly, in the equatorial Pacific, mannose turnover time exceeded that of glucose by four- to 25-fold (Rich *et al*., 1996). Second, the concentrations in Figure 2, based on data generated prior to the use of HPLC with pulsed amperometic detection for individual carbohydrate molecules, are high for the carbohydrate component, exceeding baseflow concentrations of dissolved free monosaccharides in White Clay Creek by nearly an order of magnitude. Last, the concentrations of carboxylic acids, the dominant form of monomer in Figure 2, are poorly understood. Nevertheless, the above calculations indicate that monomeric support for a substantial component of bacterial metabolism is plausible. Certainly it is reasonable to assume that both monomeric and polymeric DOM is important to stream ecosystem metabolism, though in reality we do not know the relative contributions from these DOM pools.

IV. CONCLUSIONS

The study of DOM biodegradation in aquatic ecosystems is intimately tied to the development of analytical techniques and the connection of chemical measurements of concentrations to biological measurements of utilitization. Monomers present in low concentrations present problems of analytical sensitivity, whereas analysis of polymers often requires an initial hydrolysis step, and thus structural information is lost. Separation and detection of the myriad organic molecules present in natural systems is an overwhelming task. Additionally, measuring the utilization of stream water DOM *in situ* is complicated, if not obscured, by processes that continually produce and transform DOM molecules even as they are degraded. Such complications can be reduced by measuring organic C as a surrogate for the individual molecules and performing studies in laboratoryscale systems such as plug-flow bioreactors in which a purely heterotrophic microbial community is supported by streamwater DOM, the concentration and composition of the biodegradable DOM that contributes to heterotrophic metabolism can be assayed (Kaplan and Newbold, 1995), and the kinetics of DOM biodegradation assessed (Gremm and Kaplan, 1998).

A distinct advantage of bioreactors is the ability to capture both the biological nature of the microbial flora (including bacteria that have different metabolic capabilities) and the complex geochemistry of the organic substrates in streamwater. However, as laboratory-scale systems, the degradation rates observed in bioreactors cannot be transferred to a stream without appropriate scaling considerations. One possible approach to such up-scaling

would be to use bioreactors to estimate rate constants for Monod-type uptake kinetics; these rate constants would be incorporated as parameters in a simulation model of the whole stream. A mechanistic model capable of such up-scaling, however, would require unprecedented detail in representing the hydrodynamics and microbial communities of the stream.

An alternative is a less mechanistic approach to up-scaling that employs tracers to map kinetics at the bioreactor level to those at the stream level. In this approach, the bulk of the scaling (e.g., of dimensions, hydrodynamics, and microbial populations) is accomplished by comparing the dynamics of the same tracers, such as ^{13}C-labeled DOM, in the two systems. Under this approach, the primary assumption regarding the fidelity of laboratory-scale systems as models of stream processes is that substances of similar lability at the bioreactor scale will also have similar lability in the stream, that is, that the rank ordering of lability is preserved. Given the complexity of up-scaling, which involves issues of both hydrodynamics and microbial processes, and the current lack of understanding of these issues, an empirical approach may be needed as a first step (Van den Heuvel, 1992). At present, this could offer a more robust method of describing whole-stream dynamics than would, for example, the construction of an ecosystem-level model parameterized from the dynamics of laboratory-scale measurements.

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REFERENCES

Allen, H. T. 1976. Dissolved organic matter in lakewater: Characteristics of molecular weight size-fractions and ecological implications. *Oikos* **27:**64–70.

Amon, R. M. W., and R. Benner. 1994. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* **369:**549–552.

Amon, R. M. W., and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography* **41:**41–51.

Armstrong, S. M., and F. Bärlocher. 1989. Adsorption and release of amino acids from epilithic biofilms in streams. *Freshwater Biology* **22:**153–159.

Battin, T. J. 2000. Hydrodynamics is a major determinant of streambed biofilm activity: From the sediment to the reach scale. *Limnology and Oceanography* **45:**1308–1319.

Benner, R., K. Weliky, and J. I. Hedges. 1990. Early diagenesis of mangrove leaves in a tropical estuary: Molecular-level analyses of neutral sugars and lignin-derived phenols. *Geochimica et Cosmochimica Acta* **54:**1991–2001.

Bertilsson, S., R. Stepanauskas, R. C. Hansson, W. Granéli, J. Wikner, and L. Tranvik. 1999. Photochemically induced changes in bioavailable carbon and nitrogen pools in a boreal watershed. *Aquatic Microbial Ecology* **19:**47–56.

Bertino, D. J., P. W. Albro, and J. R. Hass. 1987. Enzymatic hydrolysis of carbohydrates in aquatic fulvic acid. *Environmental Science & Technology* **21:**859–863.

Bethge, P. O., and K. Lindstrom. 1974. The determination of organic acids of low relative molecular mass (C1 to C4) in dilute aqueous solution. *Analyst* **99:**137–142.

Blaschke, H. 1979. Leaching of water-soluble organic substances from coniferous needle litter. *Soil Biology and Biochemistry* **11:**581–584.

Boethling, R. S., P. H. Howard, W. Meylan, W. Stiteler, J. Beauman, and N. Tirado. 1994. Group contribution method for predicting probability and rate of aerobic biodegradation. *Environmental Science & Technology* **28:**459–465.

Boschker, H. T. S., E. M. J. Dekkers, R. Pel, and Th. E. Cappenberg. 1995. Sources of organic carbon in the littoral of Lake Gooimeer as indicated by stable carbon isotope and carbohydrate compositions. *Biogeochemistry* **29:**89–105.

Brezonik, P. L. 1990. Principles of linear free-energy and structure-activity relationships and their applications to the fate of chemicals in aquatic systems. *In* "Aquatic Chemical Kinetics" (W. Stumm, Ed.), pp. 113–158, Wiley, New York.

Brinkman, T., C. Zwiener, and F. H. Frimmel. 2000. Trace level determination of low molecular weight organic acids in humic substances by ion exchange chromatography. *Vom Wasser* **94:**41–50.

Bunte, C., and M. Simon. 1999. Bacterioplankton turnover of dissolved free monosaccharides in a mesotrophic lake. *Limnology and Oceanography* **44:**1862–1870.

Button, D. K., and F. Jüttner. 1989. Terpenes in Alaskan waters: Concentrations, sources, and the microbial kinetics used in their prediction. *Marine Chemistry* **26:**57–66.

Carling, P. A. 1992. The nature of the fluid boundary layer and the selection of parameters for benthic ecology. *Freshwater Biology* **28:**273–284.

Carlsson, P., E. Graneli, and A. Z. Segatto. 1999. Cycling of biologically available nitrogen in riverine humic substances between marine bacteria, a heterotrophic nanoflagellate and a photosynthetic dinoflagellate. *Aquatic Microbial Ecology* **18:**23–36.

Chappell, K. R., and R. Goulder. 1995. A between-river comparison of extracellular-enzyme activity. *Microbial Ecology* **29:**1–17.

Cheshire, M. V., and M. H. B. Hayes. 1990. Composition, origins, structures, and reactivities of soil polysaccharides. *In* "Soil Colloids and Their Associations in Aggregates" (M. F. DeBoodt, M. H. Hayes, and A. Herbillon, Eds.), pp. 307–336. Plenum Press, New York.

Chrøst, R. J., R. Wcislo, and G. Z. Halemejko. 1986. Enzymatic decomposition of organic matter by bacteria in an eutrophic lake. *Archiv für Hydrobiologie* **107:**145–166.

Coffin, R B. 1989. Bacterial uptake of dissolved free and combined amino acids in estuarine waters. *Limnology and Oceanography* **34:**531–542.

Coffin, R. B., J. P. Connolly, and P. S. Harris. 1993. Availability of dissolved organic carbon to bacterioplankton examined by oxygen utilization. *Marine Ecology Progress Series* **101:**9–22.

Cowie, G. L., and J. I. Hedges. 1984. Carbohydrate sources in a coastal marine environment. *Geochimica et Cosmochimica Acta* **48:**2075–2087.

Cummins, K. W., J. J. Klug, R. G. Wetzel, R. C. Petersen, K. F. Suberkropp, B. A. Manny, J. C. Wuycheck, and F. O. Howard. 1972. Organic enrichment with leaf leachate in experimental lotic ecosystems. *BioScience* **22:**719–722.

Dagley, S. 1971. Catabolism of aromatic compounds by micro-organisms. *Advances in Microbial Physiology* **6:**1–46.

Dahlen, J., S. Bertilsson, and C. Pettersson. 1996. Effects of UV-A irradiation on dissolved organic matter in humic surface waters. *Environmental International* **22:**501–506.

Deuser, W. G. 1988. Whither organic carbon? *Nature* **332:**396–397.

Dixon, R. K., S. Brown, R. A. Houghton, A. M. Solomon, M. C. Trexler, and J. Wisniewski. 1994. Carbon pools and flux of global forest ecosystems. *Science* **263:**185–190.

Eberlein, K., and U. H. Brockmann. 1986. Development of particulate and dissolved

carbohydrates in parallel enclosure experiments with monocultures of *Thalassiosira rotula*. *Marine Ecology Progress Series* **32:**133–138.

Elbert, W., J. Hahn, and M. Lerch. 1989. A liquid chromatography system for measurement of organic acids in precipitation. *International Journal of Environmental Analytical Chemistry* **35:**149–159.

Ellis, B. D., P. Butterfield, W. L. Jones, G. A. McFeters, and A. K. Camper. 2000. Effects of carbon source, carbon concentration, and chlorination on growth related parameters of heterotrophic biofilm bacteria. *Microbial Ecology* **38:**330–347.

Elwood, J. W., J. D. Newbold, R. V. O'Neill, and W. Van Winkle. 1983. Resource spiralling: An operational paradigm for analyzing lotic ecosystems. *In* "Dynamics of Lotic Ecosystems" (T. D. Fontaine and S. M. Bartells, Eds.), pp. 3–27. Ann Arbor Science, Ann Arbor, MI.

Findlay, S., K. Howe, and D. Fontvieille. 1993. Bacterial-algal relationships in streams of the Hubbard Brook experimental forest. *Ecology* **74:**2326–2336.

Fletcher, M., 1992. Bacterial metabolism in biofilms. *In* "Biofilms — Science and Technology" (L. E. Melo *et al*., Eds.), pp. 113–124. Kluwer Academic, Dordrecht, the Netherlands.

Fox, K. F., D. S. Wunschel, A. Fox, and G. C. Stewart. 1998. Complementarity of GC-MS and LC-MS analyses for determination of carbohydrate profiles of vegetative cells and spores of bacilli. *Journal of Microbiological Methods* **33:**1–11.

Fuhrman, J. 1990. Dissolved free amino acid cycling in an estuarine outflow plume. *Marine Ecology Progress Series* **66:**197–203.

Fuhrman, J., and T. M. Bell. 1985. Biological considerations in the measurement of dissolved free amino acids in seawater and implications for chemical and microbiological studies. *Marine Ecology Progress Series* **25:**13–21.

Fuhrman, J. A., and R. L. Ferguson. 1986. Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements. *Marine Ecology Progress Series* 33:237-242.

Gantzer, C. J., B. Rittmann, and E. Herricks. 1988. Mass transport to streambed biofilms. *Water Research* **22:**709–722.

Gantzer, C. J., A. B. Cunningham, W. Gujer, B. Gutekunst, J. J. Heijnen, E. N. Lightfoot, G. Odham, B. E. Rittmann, E. Rosenberg, K. D. Stolzenbach, and A. J. B. Zehnder. 1989. *In* "Structure and Function of Biofilms" (W. G. Characklis and P. A. Wilderer, Eds.), pp. 73-89. Wiley, New York.

Geller, A. 1985. Light-induced conversion of refractory, high molecular weight lake water constituents. *Schweizerische Zeitschrift für Hydrologie* **47:**21–26.

Grady, C. P., X. Leslie, Jr., G. T. Daigger, and H. C. Lim. 1998. "Biological Wastewater Treatment." Dekker, New York.

Gremm, T. J., and L. A. Kaplan. 1997. Dissolved carbohydrates in streamwater determined by HPLC and pulsed amperometric detection. *Limnology and Oceanography* **42:**385–393.

Gremm, T. J., and L. A. Kaplan. 1998. Dissolved carbohydrate concentration, composition, and bioavailability to microbial heterotrophs in stream water. *Acta Hydrochimica Hydrobiologica* **26:**167–171.

Hecky, R. E., K. Mopper, P. Kilham, and E. T. Degens. 1973. The amino acid and sugar composition of diatom cell walls. *Marine Biology* **19:**323–331.

Hedges, J. I., R. G. Keil, and R. Benner. 1997. What happens to terrestrial organic matter in the ocean? *Organic Geochemistry* **27:**195–212.

Hellebust, J. A. 1974. Extracellular products. *In* "Algal Physiology and Biochemistry" (W. D. P. Stewart, Ed.), pp. 838–863. University of California Press, Berkeley, CA.

Hobbie, J. E. 1969. Heterotrophic bacteria in aquatic ecosystems: Some results of studies with organic radioisotopes. *In* "The Structure and Function of Fresh-Water Microbial Communities" (John Cairns, Jr., Ed.), American Microscopical Society Symposium, pp. 181–194. Blacksburg, VA.1

Hobbie, J. E. and R. T. Wright. 1965. Bioassay with bacterial uptake kinetics: glucose in freshwater. *Limnology and Oceanography* **10:**471–474.

Hygum, B. H., J. W. Petersen, and M. Søndergaard. 1997. Dissolved organic carbon released by zooplankton grazing activity — A high-quality substrate pool for bacteria. *Journal of Plankton Research* **19:**97–111.

Hynes, H. B. N. 1975. The stream and its valley: Edgardo Baldi Memorial Lecture. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **19:**1–15.

Jahnel, J. B., and F. H. Frimmel. 1996. Detection of glucosamine in the acid hydrolysis solution of humic substances. *Fresenius Journal of Analytical Chemistry* **354:**886–888.

Jahnel, J. B., P. Ilieva, and F. H. Frimmel. 1998a. HPAE-PAD — A sensitive method for the determination of carbohydrates. *Fresenius Journal of Analytical Chemistry* **360:**827–829.

Jahnel, J. B., P. Ilieva, G. A. Braun, and F. H. Frimmel. 1998b. Amino acids and carbohydrates in refractory organic acids. *Vom Wasser* **90:**205–216.

Johnson, K. M., and J. M. Sieburth. 1977. Dissolved carbohydrates in seawater. I. A precise spectrophotometric analysis for monosaccharides. *Marine Chemistry* **5:**1–13.

Jørgensen, N. O. G. 1987. Free amino acids in lakes: Concentrations and assimilation rates in relation to phytoplankton and bacterial production. *Limnology and Oceanography* **32:**97–111.

Jørgensen, N. O. G., and R. E. Jensen. 1994. Microbial fluxes of free monosaccharides and total carbohydrates in freshwater determined by PAD-HPLC. *FEMS Microbiology Ecology* **14:**79–94.

Jørgensen, N. O. G., N. Kroer, R. B. Coffin, X.-H. Yang, and C. Lee. 1993. Dissolved free amino acids, combined amino acids, and DNA as sources of carbon and nitrogen to marine bacteria. *Marine Ecology Progress Series* **98:**135–148.

Jüttner, F. 1988. Quantitative trance analysis of volatile organic compounds. *Methods in Enzymology* **167:**609–616.

Jüttner, F., and U. Dürst. 1997. High lipoxygenase activities in epilithic biofilms of diatoms. *Archiv für Hydrobiologie* **138:**451–463.

Kaplan, L. A., and T. L. Bott. 1982. Diel fluctuations of DOC generated by algae in a Piedmont stream. *Limnology and Oceanography* **27:**1091–1100.

Kaplan, L. A., and T. L. Bott. 1989. Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: Effects of temperature, water chemistry, and habitat. *Limnology and Oceanography* **34:**718-733.

Kaplan, L. A., and J. D. Newbold. 1995. Measurement of streamwater biodegradable dissolved organic carbon with a plug-flow bioreactor. *Water Research* **29:**2696–2706.

Karlsson, S., H. Wolrath, and J. Dahlen. 1999. Influence of filtration, preservation and storing on the analysis of low molecular weight organic acids in natural waters. *Water Research* **11:**2569–2578.

Keil, R. G., and D. L. Kirchman. 1991. Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. *Marine Ecology Progress Series* **73:**1–10.

Laanbroek, H. J., J. C. Verplanke, P. R. M. de Visscher, and R. de Vuyst. 1985. Distribution of phyto- and bacterioplankton growth and biomass parameters, dissolved inorganic nutrients and free amino acids during a spring bloom in the Oosterschelde basin, the Netherlands. *Marine Ecology Progress Series* **25:**1–11.

Lampert, W. 1978. Release of dissolved organic carbon by grazing zooplankton. *Limnology and Oceanography* **23:**831–834.

Larson, R. A. 1978. Dissolved organic matter of a low-colored stream. *Freshwater Biology* **8:**91–104.

Leboulanger, C., C. D. Gros, and H. Jupin. 1994. HPLC determination of glycolic acid in seawater. An estimation of phytoplankton photorespiration in the Gulf of Lions, western Mediterranean Sea. *Journal of Plankton Research* **16:**897–903.

Lehninger, A. L. 1975. "Biochemistry." Worth Publishers, New York.
Lindroth, P., and K. Mopper. 1979. High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivitization with o-phthaldialdehyde. *Analytical Chemistry* **51:**1667–1674.
Lock, M. A., T. E. Ford, D. M. Fiebig, M. C. Miller, M. Hullar, M. Kaufman, J. R. Vestal, B. J. Peterson, and J. E. Hobbie. 1989. A biogeochemical survey of rivers and streams in the mountains and foot-hills province of Arctic Alaska. *Archiv für Hydrobiologie* **115:**499–521.
Logan, B. E., and D. L. Kirchman. 1991. Uptake of dissolved organics by marine bacteria as a function of fluid motion. *Marine Biology* **111:**175–181.
McDowell, W. H., and G. E. Likens. 1988. Origin, composition and flux of dissolved organic carbon in the Hubbard Brook Valley. *Ecological Monographs* **58:**177–195.
McKnight, D. M., R. L. Smith, R. A. Harnish, C. L. Miller, and K. E. Bencala. 1993. Seasonal relationships between planktonic microorganisms and dissolved organic material in an alpine stream. *Biogeochemistry* **21:**39–59.
Meon, B., and F. Jüttner. 1999. Concentrations and dynamics of free mono- and oligosaccharides in a shallow eutrophic lake measured by thermospray mass spectrometry. *Aquatic Microbial Ecology* **16:**281–293.
Middleboe, M., M. Søndergaard, Y. Letarte, and N. H. Borch. 1995a. Attached and free-living bacteria: Production and polymer hydrolysis during a diatom bloom. *Microbial Ecology* **29:**231–248.
Middleboe, M., N. H. Borch, and D. L. Kirchman. 1995b. Bacterial utilization of dissolved free amino acids, dissolved combined amino acids and ammonium in the Delaware Bay estuary: Effects of carbon and nitrogen limitation. *Marine Ecology Progress Series* **128:**109–120.
Milner, J. L., B. Vink, and J. M. Wood. 1987. Transmembrane amino acid flux in bacterial cells. *CRC Critical Reviews in Biotechnology* **5:**1–47.
Minshall, G. W., K. W. Cummins, R. C. Petersen, C. E. Cushing, D. A. Burns, J. R. Sedell, and R. L. Vannote. 1985. Developments in stream ecosystem theory. *Canadian Journal of Fisheries and Aquatic Sciences* **42:**1045–1055.
Moers, M. E. C., M. Baas, J. W. DeLeeuw, J. J. Boon, and P. A. Schenek. 1990. Occurrence and origin of carbohydrates in peat samples from a red mangrove environment as reflected by abundances of neutral monosaccharides. *Journal Cosmochimica Acta* **54:**2463–2472.
Mopper, K., C. A. Schultz, L. Chevolot, C. Germain, R. Revuelta, and R. Dawson. 1992. Determination of sugars in unconcentrated seawater and other natural waters by liquid chromatography and pulsed amperometric detection. *Environmental. Science & Technology* **26:**133–138.
Moran, M. A., and R. E. Hodson. 1990. Bacterial production on humic and nonhumic components of dissolved organic carbon. *Limnology and Oceanography* **35:**1744–1756.
Moran, M. A., and R. E. Hodson. 1994. Support of bacterioplankton production by dissolved humic substances from three marine environments. *Marine Ecology Progress Series* **110:**241–247.
Münster, U. 1984. Distribution, dynamic and structure of free dissolved carbohydrates in the Plußsee, a north German eutrophic lake. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **22:**929–935.
Münster, U., and R. J. Chrøst. 1990. Origin, composition, and microbial utilization of dissolved organic matter. *In* "Aquatic Microbial Ecology" (J. Overbeck and R. J. Chrøst, Eds.), pp. 8–46. Springer-Verlag, Berlin.
Nalewajko, C. 1966. Photosynthesis and excretion in various planktonic algae. *Limnology and Oceanography* **11:**1–10.
Namkung, E., and B. E. Rittmann. 1986. Soluble microbial products (SMP) formation kinetics by biofilms. *Water Resources* **20:**795–806.
Newbold, J. D., P. J. Mulholland, J. W. Elwood, and R. V. O'Neill. 1982. Organic carbon spiralling in stream ecosystems. *Oikos* **38:**266–272.

Newbold, J. D., T. L. Bott, L. A. Kaplan, B. W. Sweeney, and R. L. Vannote. 1997. Organic matter dynamics in White Clay Creek, Pennsylvania, USA. *Journal of the North American Benthological Society* **16:**46–50.

Opsahl, S., and R. Benner. 1997. Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature* **386:**480–482.

Opsahl, S. and R. Benner. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. *Limnology and Oceanography* **43:**1297–1304.

Packman, A. I., and K. E. Bencala. 2000. Modeling methods in the study of surface-subsurface hydrologic interactions. *In* "Streams and Ground Waters" (J. B. Jones and P. J. Mulholland, Eds.), pp. 45–80. Academic Press, San Diego, CA.

Park, J. C., M. Aizaki, T. Fukushima, and A. Otsuki. 1997. Production of labile and refractory dissolved organic carbon by zooplankton excretion: An experimental study using large outdoor continuous flow-through ponds. *Canadian Journal of Fisheries and Aquatic Sciences* **54:**434–443.

Peldszus, S., P. M. Huck, and S. A. Andrews. 1996. Determination of short-chain aliphatic, oxo- and hydroxy-acids in drinking water at low μg/L concentrations. *Journal of Chromatography* **723:**27–43.

Pitter, P., and J. Chudoba. 1990. "Biodegradability of Organic Substances in the Aquatic Environment." CRC Press, Boca Raton, FL.

Quackenbush, R. C., D. Bunn, and W. Lingren. 1986. HPLC determination of phenolic acids in the water-soluble extract of *Zostera marina L.* (eelgrass). *Aquatic Botany* **24:**83–89.

Rich, J. H., H. W. Ducklow, and D. L. Kirchman. 1996. Concentrations and uptake of neutral monosaccharides along 140°W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the DOM flux. *Limnology and Oceanography* **41:**595–604.

Riemann, B., N. O. G. Jørgensen, W. Lampert, and J. A. Fuhrman. 1986. Zooplankton induced changes in dissolved free amino acids and in production rates of freshwater bacteria. *Microbial Ecology* **12:**247–258.

Rosenfeld, J. S., and J. J. Hudson. 1997. Primary production, bacterial production, and invertebrate biomass in pools and riffles in southern Ontario streams. *Archiv für Hydrobiologie* **130:**310–316.

Saunders, G. W., and T. A. Storch. 1971. Coupled oscillatory control mechanism in a planktonic system. *Nature* **203:**58–60.

Senior, W., and L. Chevolot. 1991. Studies of dissolved carbohydrates (or carbohydrate-like substances) in an estuarine environment. *Marine Chemistry* **32:**19–35.

Simon, M. 1998. Bacterioplankton dynamics in a large mesotrophic lake. II. concentrations and turnover of dissolved amino acids. *Archiv für Hydrobiologie* **144:**1–23.

Sinsabaugh, R. L., S. Findlay, P. Franchini, and D. Fischer. 1997. Enzymatic analysis of riverine bacterioplankton production. *Limnology and Oceanography* **42:**29–38.

Skoog, A., and R. Benner. 1997. Aldoses in various size fractions of marine organic matter: Implications for carbon cycling. *Limnology and Oceanography* **42:**1803–1813.

Skoog, A., B. Biddanda, and R. Benner. 1999. Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. *Limnology and Oceanography* **44:**1625–1633.

Stanley, S. O., K. G. Boto, D. M. Alongi, and F. T. Gillan. 1987. Composition and bacterial utilization of free amino acids in tropical mangrove sediments. *Marine Chemistry* **22:**13–30.

Stepanauskas, R., H. Laudon, and N. O. G. Jørgensen. 2000. High DON bioavailability in boreal streams during a spring flood. *Limnology and Oceanography* **45:**1298–1307.

Sun, L., E. M. Perdue, J. L. Meyer, and J. Weis. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. *Limnology and Oceanography* **42:**714–721.

Thomas, J. D. 1997. The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshwater Biology* **38:**1–36.

Thomas, J. D., and P. Eaton. 1996. The accumulation of amino-acids and humic substances in media conditioned by axenic and non-axenic duckweed (Lemna minor L.) and their possible ecological significance. *Hydrobiologia* **333:**121–128.

Thurman, E. M. 1985. "Developments in Biogeochemistry: Organic Geochemistry of Natural Waters." Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht, the Netherlands.

Tranvik, L. J., and M. G. Hofle. 1987. Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. *Applied and Environmental Microbiology* **53:**482–488.

Undefriend, S., S. Stein, P. Bohlen, W. Dairman, W. Leimgruber, and M. Weigele. 1972. Fluorescamine: A reagent for assay of amino acids, peptides, proteins, and primary amines in the picomole range. *Science* **24:**871–872.

Vallino, J. J., C. S. Hopkinson, and J. E. Hobbie. 1996. Modeling bacterial utilization of dissolved organic matter: Optimization replaces Monod growth kinetics. *Limnology and Oceanography* **41:**1591–1609.

Van den Heuvel, J. C. 1992. Mass transfer in and around biofilms. *In* "Biofilms — Science and Technology" (L. F. Melo, T. R. Bott, M. M. Fletcher, and B. Capdeville, Eds.), pp. 239–250 Kluwer Academic, Dordrecht, the Netherlands.

Volk, C. J., C. B. Volk, and L. A. Kaplan. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. *Limnology and Oceanography* **42:**39–44.

Wangersky, P. J. 1993. Dissolved organic carbon methods: A critical review. *Marine Chemistry* **41:**61–74.

Watt, B. E., T. M. Hayes, M. H. B. Hayes, R. T. Price, R. L. Malcolm, and P. Jakeman. 1996. Sugars and amino acids in humic substances isolated from British and Irish waters. *In* "Humic Substances and Organic Matter in Soil and Water Environments: Characterization, Transformations and Interactions" (C. E. Clapp, M. H. B. Hayes, N. Sensesi, and S. M. Griffith Eds.), pp. 81–91. International Humic Substances Society, St. Paul, MN.

Wetzel, R. G. 1992. Gradient-dominated ecosystems: Sources and regulatory functions of dissolved organic matter in freshwater ecosystems. *Hydrobiologia* **229:**181–198.

Wetzel, R. G., P. G. Hatcher, and T. S. Bianchi. 1995. Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology and Oceanography* **40:**1369–1380.

Whittaker, R. H., and G. E. Likens. 1973. Carbon in the biota. *In* "Carbon and the Biosphere" (G. M. Woodwell and E. V. Pecan, Eds.), pp. 281–302. National Technical Information Service, Springfield, VA.

Wilhelm, S. W., and C. A. Suttle. 1999. Viruses and nutrient cycles in the sea. *BioScience* **49:**781–788.

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5

Molecular Indicators of the Bioavailability of Dissolved Organic Matter

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I. INTRODUCTION

The biosynthesis and remineralization of organic matter are usually tightly coupled processes in the global carbon cycle. Comparisons of global rates of primary production and reservoirs of bioreactive detrital organic matter demonstrate that the cycling of reduced carbon is efficient, with only a small percentage of annual production escaping remineralization processes (Hedges, 1992). Thus, the preservation of reduced carbon is the exception, and the remineralization of reduced carbon is the rule. Given these large-scale observations, it could appear that the chemical composition of organic matter has little influence on its remineralization. All major classes of biochemicals must be reactive, because a very small fraction of biosynthesized organic matter is preserved in the biosphere. However, it has long been recognized that some chemical components of cells are more rapidly degraded and remineralized than others. The influence of chemical composition on

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the reactivity of organic matter is most apparent during early stages of decomposition (i.e., diagenesis), when rates of remineralization are maximal and organic matter is most susceptible to biodegradation (e.g., Opsahl and Benner, 1995). During early diagenesis, remineralization processes are relatively rapid, and the chemical composition of detrital material has its maximal influence on the rates and pathways of decomposition.

This chapter focuses on the influence of chemical composition on the bioreactivity of dissolved organic matter (DOM), but the relationships between composition and reactivity described here are also applicable to particulate material. In aquatic ecosystems, dissolved organic carbon (DOC) is typically the most abundant form of detrital organic matter. DOC is operationally defined as the organic carbon that passes through a filter with a pore size of 0.2–0.7 μm, and it includes colloidal as well as dissolved molecules. In rivers, DOC typically accounts for ~60% of the total organic carbon load, whereas in the ocean DOC accounts for >95% of the detrital carbon in the water column. In addition to being the most abundant form of reduced carbon in aquatic systems, DOM is the primary substrate supporting bacterial growth and respiration. The "microbial loop" is responsible for much of the carbon and energy flow in aquatic systems, and the factors that influence the rates and transformations of DOM play a central role in ecosystem function and structure.

II. CHEMICAL COMPOSITION OF DISSOLVED ORGANIC MATTER

This chapter focuses on the composition and bioreactivity of DOM in rivers, lakes, and the surface ocean. These aquatic systems have wide ranges of concentrations and sources of DOM, and it is unrealistic to present average characteristics of DOM for such a diverse group of ecosystems. Lakes, small rivers, estuaries, and the coastal ocean are particularly heterogeneous systems that often receive large and varying quantities of allochthonous and autochthonous DOM. DOM sources are less variable for large rivers and the open ocean, and this section on chemical composition will present "typical" characteristics of DOM in these systems. The reader is directed to Münster (1993), Riemann and Søndergaard (1986), and Hessen and Tranvik (1998) for more information on the composition of DOM in lakes. Autochthonous production of DOM is often minimal in major rivers due to light limitation caused by suspended sediments and high concentrations of colored DOM. Most of the DOM in large rivers is derived from soils and has a vascular plant origin (Hedges *et al.*, 1994, 2000; Depetris and Kempe, 1993). In contrast, most DOM in the surface ocean is produced *in situ* by plankton (Druffel *et al.*, 1992). Very little terrigenous DOM is found in the open ocean (Druffel *et al.*, 1992; Opsahl and Benner, 1997).

The concentrations of DOC in major rivers typically range from 250 to 750 μM, and concentrations in the surface ocean range from 60 to 90 μM (Table I). Most of the river data compiled in Table I are from the Amazon River system (Hedges *et al*., 1994, 2000), the Parana River system (Depetris and Kempe, 1993), and the Mississippi River (Benner and Opsahl, 2001). The seawater data are from surface water samples collected in the Pacific and Atlantic Oceans (see Table I for references). Total hydrolyzable neutral sugars (glucose, galactose, mannose, xylose, fucose, rhamnose, and arabinose) account for about 1–2% of river DOC and 2–6% of ocean DOC, indicating

TABLE I Typical Chemical Characteristics of DOM from Major Rivers (Amazon, Parana, and Mississippi) and Surface Waters of the Open Ocean (Pacific and Atlantic)

Chemical characteristics	*Major carbon functional groups*	*Major rivers*	*Surface ocean*	*Reference*[a]
DOC (μM)		250–750	60–90	1, 2, 3
THNS (% DOC)		1–2	2–6	1, 2, 4, 5, 6, 7
THAA (% DOC)		1–3	1–3	1, 2, 4, 5, 6, 7
HS (% DOC)		40–70	5–25	1, 2, 8, 9
HS (C/N atom)		40–80	25–55	1, 2,8, 9, 10
HS (% C−C)	Methylene, methyl	30	44	10
HS (% C−O, O−C−O)	Alcohols, ethers	12	19	10
HS (% C=C)	Aromatic, unsaturated	35	19	10
HS (% COO, CNO)	Carboxyl, ester, amide	17	15	10
HS (% C=O)	Aldehydes, ketones	6	3	10
UDOM (% DOC)		45–80	25–40	2, 4, 6, 11, 12
UDOM (C/N atom)		20–50	15–18	2, 4, 6, 11, 12
UDOM (% C−C)	Methylene, methyl	30	25	13, 14, 15
UDOM (% C−O, O−C−O)	Alcohols, ethers	29	54	13, 14, 15
UDOM (% C=C)	Aromatic, unsaturated	16	5	13, 14, 15
UDOM (% COO, CNO)	Carboxyl, ester, amide	22	13	13, 14, 15
UDOM (% C=O)	Aldehydes, ketones	3	3	13, 14, 15
THNS (% UDOC)		1–2	6–13	2, 7, 12, 16
THAA (% UDOC)		1–2	3–4	2, 16

Note: Humic substances (HS) are operationally defined as DOC that is retained on XAD-2 or XAD-8 resins. UDOM is operationally defined as DOC that is retained by a membrane with a 1 nm pore size and 1000 Da molecular weight cutoff. ^{13}C-NMR spectroscopy was used for analysis of carbon functional groups, which are presented as a percentage of the total organic carbon in the sample. THNS, total hydrolyzable neutral sugars; THAA, total hydrolyzable amino acids.

[a]References: 1, Thurman (1985); 2, Benner (2002); 3, Degens *et al*. (1991); 4, Hedges *et al*. (1994); 5, Depetris and Kempe (1993); 6, Hedges *et al*. (2000); 7, Skoog and Benner (1997); 8, Druffel *et al*. (1992); 9, Ertel *et al*. (1986); (10) Hedges *et al*. (1992); 11, Benner *et al*. (1997); 12, Benner and Opsahl (2001); 13, Benner *et al*. (1992); 14, McCarthy *et al*. (1993); 15, R. Benner, unpublished observations; 16, McCarthy *et al*. (1996).

that surface ocean DOM is enriched in carbohydrates compared with that in rivers (Table I). Vascular plant tissues are rich in neutral sugar polymers (e.g., cellulose and hemicellulose), but it appears that these components are largely degraded in soils and contribute minimally to the DOM in rivers. Total hydrolyzable amino acids account for a similar percentage (1–3%) of DOC in rivers and in the ocean (Table I). Concentrations of free sugars and amino acids are very low in these systems, so most of these biochemicals occur in combined form as oligomers and polymers.

It is somewhat surprising that these two major classes of biochemicals account for such a small percentage of the DOC in these environments. Neutral sugars and amino acids typically account for 30–60% of the carbon in herbaceous vascular plant tissues and phytoplankton biomass, whereas

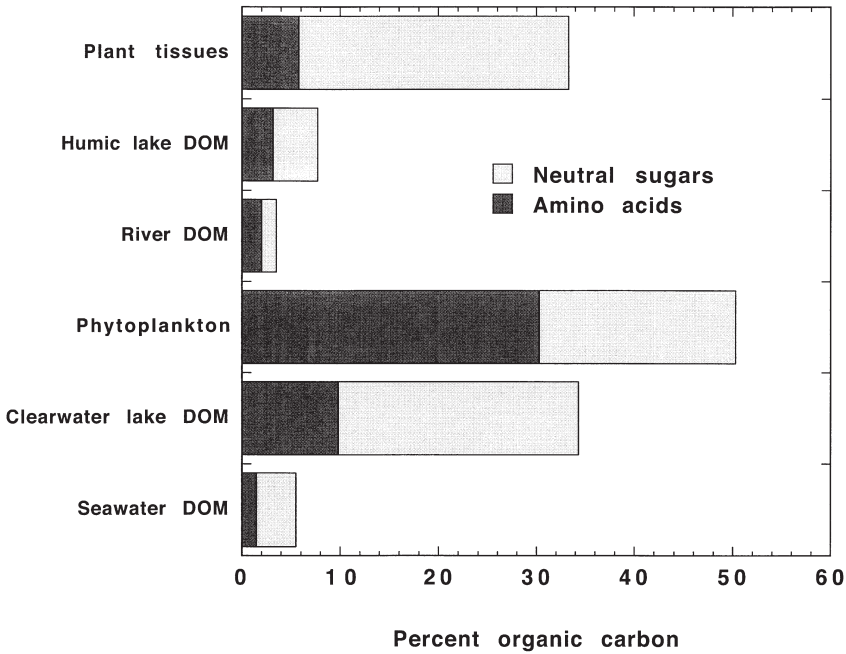

FIGURE 1 Percentages of organic carbon in plant tissues, lake DOM, river DOM, phytoplankton and seawater DOM identified as hydrolyzable neutral sugars and amino acids. Plant tissues include a wide variety of herbaceous tissues, including leaves, needles, and grasses [data from Cowie and Hedges (1992), Hedges *et al.* (1994, and Opsahl and Benner (1999)]. The humic and clearwater lake DOM data are from Tranvik and Jørgensen (1995). Values for river DOM and seawater DOM were taken from Table I and are representative of the Amazon, Parana, and Mississippi systems and surface waters of the open Pacific and Atlantic oceans. Average values of four major marine phytoplankton (*Phaeocystis* sp, *Emiliania huxleyi*, *Synechococcus bacillaris*, and *Skeletonema costatum*) are presented (data from A. Biersmith and R. Benner, unpublished observations, 1998).

they account for <10% of the DOC in major rivers and the surface ocean (Fig. 1). Hydrolyzable neutral sugars and amino acids are generally considered to be bioreactive components of organic matter (Ittekot *et al.*, 1988; Cowie and Hedges, 1994), and it appears that low concentrations of these biochemicals in DOM result from their selective removal during decomposition. As bioavailable components of DOM, these biochemicals can potentially serve as sensitive indicators of the microbial alteration of DOM. The term "labile" is commonly used by microbial ecologists to describe DOM that is biodegradable within hours to days. In this chapter the term "bioavailable" is used to describe DOM that is biodegradable within months.

The chemical compositions of DOM from several lakes for which similar data exist are presented for comparison to the river and ocean data. Total hydrolyzable neutral sugars accounted for 24.5% of the DOC in a clearwater lake (261 μM DOC) and 4.5% of the DOC in a humic lake (858 μM DOC) in Sweden (Tranvik and Jørgensen, 1995). Total hydrolyzable amino acids accounted for 9.8 and 3.2% of the DOC in these same two lakes, respectively. The relatively high yields of neutral sugars and amino acids in the clearwater lake are presumably derived from autochthonous sources, whereas DOM in the humic lake is largely derived from soils and aquatic vegetation. The concentration and composition of DOM in Lake Constance (Germany) were found to be seasonally variable, with DOC concentrations ranging from 137 to 192 μM and total hydrolyzable neutral sugars and amino acid concentrations ranging from 4 to 15% and 16 to 20%, respectively, of the DOC in May and June (Weiss and Simon, 1999). Lake Constance is a large mesotrophic lake, and most of the DOM is derived from planktonic organisms and processes.

Relatively few biochemicals can be measured directly in natural waters because concentrations of individual compounds are low (nanomolar) and salts and other components often interfere with these analyses. DOM can be concentrated and isolated from natural waters for more thorough chemical characterization, and two approaches for DOM isolation, adsorption onto solid phases and ultrafiltration are now widely used. The adsorption of DOM onto XAD resins is used to isolate a fraction of DOM that is operationally defined as "humic substances" (Thurman, 1985). More recently, tangential-flow ultrafiltration with 1000 Da cutoff membranes has been used to isolate the high-molecular-weight or colloidal fraction of DOM (Benner *et al.*, 1992, 1997).

Both of these methods have been used for DOM isolation from major rivers and the surface ocean, and the general characteristics of these fractions of DOM are presented in Table I. The major C functional groups of humic substances and ultrafiltered DOM (UDOM) have been characterized by solid-state, cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The samples of humic substances that were characterized by NMR spectroscopy were collected from the Amazon River

and surface Pacific Ocean (Hedges *et al.*, 1992). The UDOM samples that were characterized by NMR spectroscopy were collected from the Mississippi River and the surface Pacific Ocean (Benner *et al.*, 1992; R. Benner, unpublished observations). NMR spectroscopy is a particularly powerful approach for characterizing unknown structures, but due to limited availability of instruments and sample size (>10 mg of organic C) and purity (e.g., removal of paramagnetics) requirements, relatively few DOM isolates have been characterized.

Humic substances account for 40–70% of the DOC in rivers and 5–25% of the DOC in the ocean (Table I). It is important to note that recoveries of adsorbed humic substances from XAD resins are not quantitative, so the chemical characteristics of the recovered humic substances are not necessarily representative of all the humic substances retained by the resin. Tangential-flow ultrafiltration retains 45–80% of the DOC in rivers and 25–40% of the DOC in the surface ocean (Table I). Essentially all of the DOC retained during ultrafiltration is recovered for chemical characterization. In general, ultrafiltration recovers a larger fraction of the DOM from these systems. These methods also isolate DOM based on different mechanisms. Adsorption onto XAD resins at low pH chemically fractionates the DOM and isolates the more hydrophobic components, whereas ultrafiltration principally separates components of DOM on the basis of size and shape.

There are major differences in the chemical compositions of DOM isolated by XAD resins and ultrafiltration (Table I). In rivers and in the ocean, humic substances (XAD isolation) are depleted in N relative to UDOM. The C/N ratios of UDOM are more representative of bulk DOM than those of humic substances. Most of the functional groups identified by NMR are found in more than one class of compounds, so in most cases specific functional groups are not assigned to a particular group of biochemicals. However, in some circumstances it is possible to estimate the fraction of carbon associated with a biochemical class, such as carbohydrates. Carbohydrates are the most abundant polyalcohols in nature, and the ratio (4–5:1) of areas associated with NMR peaks at specific chemical shifts [e.g., ~72 ppm (C−O): ~102 ppm (O−C−O)] indicates that carbohydrates are their primary source (see Table I for references). In general, humic substances are depleted in carbohydrates (C−O and O−C−O) and enriched in aromatic and unsaturated C (C=C) relative to UDOM (Table I). As mentioned earlier, humic substances are relatively hydrophobic components of DOM, and it is consistent that they are depleted in N and carbohydrates and enriched in aromatic components. The UDOM fraction includes more hydrophilic components that are relatively enriched in N and carbohydrates. Humic substances from the ocean are enriched in aliphatic C (C−C) relative to UDOM, and this could reflect the more hydrophobic nature of the humic substances.

Chemical differences between these fractions of DOM are apparent, but there is considerable compositional overlap as well. Extraction of humic substances or ultrafiltration removes most of the colored DOM from water samples and most of the dissolved lignin (Ertel *et al*., 1986; Opsahl and Benner, 1998). This is consistent with the observation that many humic and fulvic acids have molecular masses greater than the cutoff (1000 Da) of the membranes used for DOM isolation by ultrafiltration (Thurman, 1985).

There are also structural differences between humic substances or UDOM collected from rivers and oceans (Table I). Humic substances and UDOM from rivers are enriched in aromatic components compared with their counterparts from the ocean. Terrestrial vegetation is relatively rich in aromatic components, such as lignins and tannins, and this is reflected in the greater aromatic nature of DOM in rivers. These biopolymers are relatively resistant to microbial degradation and are important components of river DOM. Humic substances and UDOM from the ocean are enriched in carbohydrates compared with their counterparts from rivers. This is consistent with observations of higher C-normalized yields of neutral sugars in bulk DOM from the ocean compared with rivers (Table I).

As mentioned earlier, the abundance of carbohydrates in ocean DOM relative to river DOM does not reflect the biochemical compositions of terrestrial plants and marine phytoplankton. It appears that carbohydrates in plant litter are largely degraded in soils, resulting in relatively low concentrations in river DOM. Carbohydrates are the major components of exudates from phytoplankton (Biddanda and Benner, 1997; Biersmith and Benner, 1998), and herbivorous grazing also releases carbohydrates to the DOM reservoir (Strom *et al*., 1997). The *in situ* production of dissolved carbohydrates in the surface ocean appears to result in a greater relative abundance of carbohydrates in the ocean relative to rivers.

The same methods used to analyze total hydrolyzable neutral sugars and amino acids in natural water samples were used to characterize these biomolecules in UDOM. The C-normalized yields of neutral sugars in bulk DOM and UDOM from rivers are similar (1–2%), whereas there is a minor difference in the size distribution of amino acids in river DOM, with a slight depletion of amino acids in UDOM or the high-molecular-weight fraction (Table I). A different picture emerges from comparison of C-normalized yields of neutral sugars and amino acids in bulk DOM and UDOM from the ocean. Yields of neutral sugars are substantially higher in UDOM (6–13%) compared with those in bulk DOM (2–6%). C-normalized yields of amino acids are also higher in UDOM (3–4%) compared with those in bulk DOM (1–3%). Overall, a substantially larger fraction of the high-molecular-weight component of marine DOM is identified at the molecular level compared with that from bulk marine DOM. These results indicate that low-molecular-weight DOM in the ocean has relatively low yields of recognizable biomolecules.

III. BIOREACTIVITY OF DISSOLVED ORGANIC MATTER

Given the central role of DOM in the microbial loop of aquatic systems, there is wide interest in measuring and comparing the bioreactivity of DOM from different sources. The most common approach for estimation of DOM bioreactivity is the use of natural water samples in bioassay experiments. There are many variations of bioassay experiments, as they are used for numerous purposes. The basic design of experiments to measure DOM bioreactivity is to incubate a filtered (0.6–1 μm pore size) water sample containing DOM and a natural microbial assemblage and to measure bacterial production and DOC consumption (DOC loss or respiration) to estimate the bioavailability of the DOM (e.g., Tranvik, 1988a, b). Bioassay experiments are typically incubated in the dark for periods of days to weeks, although the use of plug-flow bioreactors can dramatically reduce incubation times (Kaplan and Newbold, 1995). These experiments provide an estimate of the bioreactive fraction of the DOM, and they are compared to provide a relative index of the bioavailability of DOM in different environments (e.g., Søndergaard and Middleboe, 1995).

The strength of the bioassay approach is that it directly estimates the fraction of natural DOC that can be used by a natural microbial assemblage under defined conditions. However, there are numerous manipulations of water samples during bioassay incubations, and the effects of these manipulations on the measured parameters are not well known. For example, containment of water samples can rapidly alter microbial population structure. Nutrients, rather than carbon, can be limiting for microbial utilization of DOM. Moreover, there are no standard protocols for bioassay experiments. Different indicators of DOM utilization are measured by different investigators, and many of the measured parameters rely on conversion factors that are also quite variable. The extent of DOM utilization also depends upon the duration and temperature of the bioassay experiment. Despite these shortcomings, the bioassay experiment remains the best approach for estimating the bioavailability of DOM.

Søndergaard and Middleboe (1995) provided a synthesis of bioassay experiments that were conducted in lakes, rivers, and marine waters with diverse sources of DOM. On average, a similar fraction (14–19%) of DOC in these diverse environments was bioavailable or labile, although there was considerable variability about the mean for each of these aquatic systems. Recent studies investigating temporal variability in the bioavailable fraction of DOM in a large lake (Weiss and Simon, 1999) and small creek (Volk *et al*., 1997) noted as much variability in the fraction of bioavailable DOM within these systems as is noted among systems. Changes in the measured chemical composition of DOM were as large as changes in the fraction of bioavailable DOC. These observations indicate that DOM is very dynamic in these systems, and natural microbial populations respond rapidly to

changes in DOM composition. In their review, Søndergaard and Middleboe (1995) noted that a similar fraction of bioavailable DOC occurs in aquatic systems with very different concentrations of DOC. The buildup of higher concentrations of labile DOC in eutrophic environments is attributed to lower affinity enzyme systems in the bacterial populations inhabiting these organic-rich environments.

IV. RELATIONSHIPS BETWEEN THE CHEMICAL COMPOSITION AND BIOREACTIVITY OF DISSOLVED ORGANIC MATTER

Even though substrate quality (i.e., chemical composition) is widely believed to be an important factor influencing microbial utilization of DOM, there are relatively few studies relating the composition and bioavailability of DOM. Sensitive assays for the measurement of the relative activities of various extracellular enzymes can provide an indication of the chemical composition of the bioreactive components of DOM (Sinsabaugh and Findlay, 1995; Findlay *et al*., 1998). The enzymatic potential of bacterial populations appears to respond fairly rapidly to seasonal changes in DOM composition in the Hudson River system. These observations clearly indicate that the chemical composition of DOM influences the microbial processing of DOM.

The bulk composition of major bioelements (C, N, O, and H) of DOM has been related to the bioavailability of DOM (Kroer, 1993; Sun *et al*., 1997; Vallino *et al*., 1996; Hunt *et al*., 2000). Kroer (1993) and Hunt *et al*. (2000) found that bacterial production and growth efficiency increased with increasing N content of DOM from several rivers and estuaries, although del Giorgio and Cole (1998) argue that most N addition experiments have little or no effect on bacterial growth efficiency. Thus, it appears that there is sufficient bioavailable N (organic and inorganic) in many aquatic systems so that it plays a minor role regulating bacterial growth efficiency, whereas rates of bacterial production are often regulated by bioavailable DOC for which the N content of DOM is a good indicator. The N content of DOM could also be a good indicator of bioavailable P, because some biochemicals, such as nucleic acids, are rich in N and P. Bioavailable P appears to regulate bacterial production and growth efficiency in some environments [see references in del Giorgio and Cole (1998)].

Sun *et al*. (1997) used the H:C, O:C, and N:C of DOM from the Ogeechee River system to derive an empirical equation for prediction of bacterial production on DOM. These authors found that bacterial production was positively correlated with H:C and N:C ratios and negatively correlated with the O:C ratio. They also indicated that aliphatic components of river DOM were the primary C source supporting bacterial growth.

Vallino *et al.* (1996) also suggested that the degree of substrate oxidation is related to bacterial growth rates and yields. A consistent observation from these studies is that the N content of natural DOM was positively correlated with bacterial production. The negative correlation between bacterial production and O:C ratio observed by Sun *et al.* (1997) could indicate that carbohydrates are not important growth substrates for bacteria because carbohydrates are oxygen-rich molecules with relatively high O:C ratios. However, this does not indicate that carbohydrates are unreactive or resistant to bacterial utilization. Carbohydrates are known to be rapidly utilized by bacteria, and they probably function primarily as an energy source for bacteria. Relatively oxidized substrates, including glucose, are incorporated into bacteria with low efficiency (del Giorgio and Cole, 1998).

Amon and Benner (1994) observed very high rates of respiration in bioassay experiments with high-molecular-weight (HMW) DOM from a diatom bloom in marine waters. This HMW DOM was rich in carbohydrates (Benner and Opsahl, 2001) and supported high rates of bacterial metabolism. Low-molecular-weight (LMW) DOM supported much lower overall rates of bacterial metabolism, but it did support higher bacterial growth efficiencies (Amon and Benner, 1994). Additions of free amino acids to incubations with HMW and LMW DOM indicated that the amino acids principally supported bacterial growth in the presence of HMW DOM but were primarily used as an energy source in the presence of LMW DOM (Gardner *et al.*, 1996). Plankton respiration rates in surface waters of the Mississippi River plume do not appear to be strongly influenced by concentrations of DOC, but they are positively related to concentrations of combined neutral sugars in HMW DOM (Fig. 2). These observations indicate that studies of the relationship between the chemical composition and bioavailability of DOM should include measurements of DOC remineralization or respiration as well as bacterial production. Bacterial growth efficiencies in aquatic systems are highly variable (del Giorgio and Cole, 1998), and estimates of bacterial respiration based on measurements of bacterial production and estimates of growth efficiencies would provide only a crude estimate of bioavailable DOM.

Organic geochemists have long sought reliable indicators of the diagenetic state (i.e., extent of alteration) of natural organic matter. During early diagenesis of organic matter various biomolecules are preferentially degraded and other biomolecules are selectively preserved (e.g., Hatcher *et al.*, 1983; Benner *et al.*, 1987; Cowie and Hedges, 1994). Thus, even though the temporal scales of interest to geochemists and biologists are often quite different, biochemical indicators of the diagenetic state or bioavailability of organic matter are of great interest to both disciplines.

The bioreactive fraction of particulate organic matter (POM) in 13 major rivers was characterized based on hydrolyzable neutral sugar and amino acid yields (Ittekot, 1988), and the relationship between the yields of

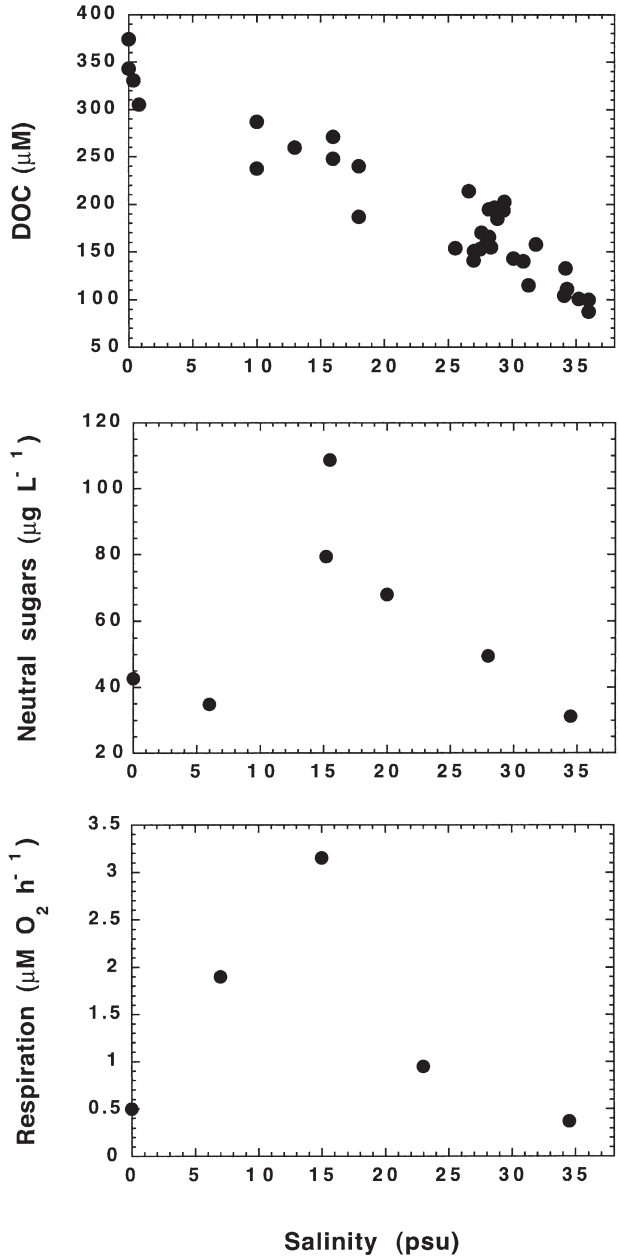

FIGURE 2 Concentrations of DOC and hydrolyzable neutral sugars in surface waters of the Mississippi River plume during July 1993 [data from Benner and Opsahl (2001)]. Neutral sugar concentrations are representative of the high-molecular-weight fraction of DOM isolated by tangential-flow ultrafiltration with 1000 Da molecular-weight-cutoff membranes. Surface water community respiration rates are from Pakulski *et al.* (2000).

these biochemicals and the diagenetic state of POM was further investigated by Cowie and Hedges (1994). They found that C-normalized yields (e.g., moles of neutral sugars or amino acids per mole of organic C) of these compounds are negatively related to the degree of oxidation and diagenetic state of organic matter. Hedges *et al.* (1994) extended the application of neutral sugar and amino acid yields as diagenetic indicators of POM and DOM in the Amazon River. Course particulate matter in the river had highez C-normalized yields of neutral sugars and amino acids than did DOM, indicating the fresher and more bioavailable nature of the particles (Hedges *et al.*, 1994). Dauwe and Middleburg (1998) used amino acids as diagenetic indicators in North Sea sediments, and Skoog and Benner (1997) used neutral sugars as diagenetic indicators of marine POM and DOM. Yields of combined neutral sugars in DOM from marine phytoplankton cultures are more than 3-fold higher than those in surface seawater DOM (Biersmith and Benner, 1998). These studies have established that phytoplankton biomass and freshly produced particulate and dissolved organic matter hve relatively high yields of neutral sugars and/or amino acids. During biogeochemical processing, there is a gradual progression of decreasing biochemical yields from particulate to dissolved organic matter and from water column to sedimentary organic matter, suggesting a diagenetic sequence or continuum. The yields of total hydrolyzable neutral sugars and amino acids in fresh biomass and DOM from various aquatic environments presented in Figure 1 reflect this diagenetic trend.

Total hydrolyzable neutral sugars and amino acids are clearly useful diagenetic indicators on seasonal, annual, and decadal time scales, but the utility of these biochemicals as molecular indicators of bioavailable DOM has only recently been addressed. Previous bioassay studies (see above) indicated that N-rich compounds are important components of bioavailable DOM. Amino acids are the major N-containing molecules in most organisms, so they are likely to be bioreactive components of DOM. Neutral sugars are major structural and energy storage components of most organisms, and there is ample evidence that neutral sugars are important carbon and energy sources for microorganisms. Likewise, radiotracer studies indicate that dissolved amino acids and neutral sugars can be rapidly utilized by natural populations of aquatic bacteria (e.g., Keil and Kirchman, 1991; Rich *et al.*, 1996; Skoog *et al.*, 1999; see Chapters 4 and 9).

Several recent studies investigated the bioavailability of DOM and total hydrolyzable neutral sugars and amino acids in lake (Weiss and Simon, 1999), creek (Volk *et al.*, 1997; Gremm and Kaplan, 1998), and marine (Amon *et al.*, 2001) waters. The DOM in the lake and marine environments was predominantly derived from plankton, whereas the DOM in the creek was predominantly derived from soils and decaying plant litter. Although limited in number, these systems represent a diverse array of aquatic environments. Each of these studies determined the percentages of DOC that were

utilized during bioassay experiments and the contribution of hydrolyzable neutral sugars and amino acids to the bioavailable DOC (Table II). A 2-fold variability was observed in the percentages of bioavailable DOC (BDOC) as well as in the contributions of neutral sugars and amino acids to BDOC. The temporal variability in Lake Constance was in large part caused by phytoplankton blooms, whereas temporal variability in White Clay Creek was mostly attributed to stormflow events.

In general, hydrolyzable neutral sugars and amino acids accounted for a higher percentage of BDOC in the plankton-dominated systems compared with the creek system. On average, 70% of the BDOC in Lake Constance was accounted for as neutral sugars and amino acids, and these biomolecules accounted for 58% of the BDOC derived from Arctic ice algae (Table II). The analysis of total hydrolyzable carbohydrates in DOM from White Clay Creek includes other carbohydrates besides neutral sugars and therefore overestimates the contribution of neutral sugars to BDOC (Volk *et al.*, 1997). This is evident when data from the Volk *et al.* (1997) study are compared with data from the Gremm and Kaplan (1998) study of the same environment (Table II). It appears that the spectrophotometric assay measures

TABLE II The Percentages of Bioavailable DOC (% BDOC) Accounted for as Total Hydrolyzable Neutral Sugars (THNS) and Total Hydrolyzable Amino Acids (THAA) in a Lake, Creek, and Marine Environment

Site	*Date*	*THNS (% BDOC)*	*THAA (% BDOC)*	*THNS + THAA (% BDOC)*
Lake Constance (Weiss and Simon, 1999)	May 3	4.9	51	56
	June 14	86	22	108
	July 5	21	27	48
	Sept. 13	57	13	70
	Average	**42**	**28**	**70**
White Clay Creek (Volk *et al.*, 1997)	Jan. 5	26[a]	3.2	29
	March 4	33[a]	4.2	37
	March 9	29[a]	5.0	34
	March 25	43[a]	3.9	47
	March 31	22[a]	2.4	25
	April 12	25[a]	6.1	31
	Average	**30**[a]	**4.2**	**34**
White Clay Creek (Gremm and Kaplan, 1998)	Seasonal range	10–18	—	—
Arctic Ocean (Amon *et al.*, 2001)	Sept.	39	19	58

Note: See the cited references for details about the bioassay incubation conditions.
[a]A spectrophotometric assay for dissolved carbohydrates was used rather than a chromatographic assay of individual neutral sugars.

about twice as much carbohydrate as the chromatographic assay of neutral sugars (Opsahl and Benner, 1999). Based on the higher carbohydrate yields, an average of 34% of BDOC was accounted for as carbohydrates and amino acids in White Clay Creek. Based on the neutral sugar yields of Gremm and Kaplan (1998), neutral sugars and amino acids accounted for ~20% of BDOC. These values are clearly lower than those for the lake and marine systems. In all of these systems, total hydrolyzable neutral sugars and amino acids account for a substantially higher percentage of BDOC (Table II) than of total DOC (Table I and Fig. I), indicating that these compounds are selectively utilized by microorganisms and therefore are key molecular indicators of BDOC.

Hydrolyzable neutral sugars and amino acids appear to play a greater role fueling the microbial loop in plankton-dominated systems than in systems receiving DOM derived from soils and decaying vegetation. These biochemicals also typically account for a greater percentage of the DOC in plankton-dominated systems. It appears that these fundamental ecosystem differences in the cycling of organic matter reflect the sources and diagenetic state of the DOM they receive. Systems with major autochthonous sources of DOM from planktonic organisms and processes directly receive freshly produced organic matter that is relatively rich in bioreactive components. In contrast, much of the DOM entering rivers and humic lakes has been previously subjected to decomposition processes in soils or during transport. The most bioavailable components of the organic matter may have been removed before it enters large rivers and lakes. It appears that hydrolyzable neutral sugars and amino acids account for <50% of the BDOC in systems with large contributions of highly degraded allochthonous DOM. Thus an intriguing question remains: What is the molecular identity of most BDOC in these systems?

The neutral sugar and amino acid components of organic matter are not uniformly bioavailable. A continuum of bioavailability exists for these biomolecules as it does for others, and this complicates applications of molecular indicators of the bioavailability of DOM. There are slowly cycling, background concentrations of these compounds in all systems, and these background concentrations likely vary among ecosystems. Perhaps if we could explore the "molecular architecture" of DOM as well as its "molecular pieces" we would be able to chemically discriminate the bioreactive neutral sugar and amino acid components from the biorefractory ones. Until we make more progress in this area, we need to recognize that biorefractory as well as bioavailable molecules are included in these chemical measurements. We are beginning to understand at the molecular level how the "quality" of organic matter affects its bioavailability. This is an area of research that has a bright future, as it is now apparent that the chemical composition of natures' most abundant organic matter reservoirs helps tailor the structure and function of ecosystems.

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REFERENCES

Amon, R. M. W., and R. Benner. 1994. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* **369:**549–552.

Amon, R. M. W., H.-P. Fitznar, and R. Benner. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnology and Oceanography*. **46:**287–297.

Benner, R. 2002. Chemical composition and reactivity. *In* "Biogeochemistry of Marine Dissolved Organic Matter" (D. Hansell and C. Carlson, Eds.), Academic Press, San Diego.

Benner, R., and S. Opsahl. 2001. Molecular indicators of the sources and transformations of dissolved organic matter in the Mississippi River plume. *Organic Geochemistry* **32:**597–611.

Benner, R., M. F. Fogel, E. K. Sprague, and R. E. Hodson. 1987. Depletion of 13C in lignin and its implications for stable carbon isotope studies. *Nature* **329:**708–710.

Benner, R., J. D. Pakulski, M. McCarthy, J. I. Hedges, and P. G. Hatcher. 1992. Bulk chemical characterization of dissolved organic matter in the ocean. *Science* **255:**1561–1564.

Benner, R., B. Biddanda, B. Black, and M. McCarthy. 1997. Abundance, distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Marine Chemistry* **57:**243–263.

Biddanda, B., and R. Benner. 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnology and Oceanography* **42:**506–518.

Biersmith, A. and R. Benner. 1998. Carbohydrates in phytoplankton and freshly-produced dissolved organic matter. *Marine Chemistry* **63:**131–144.

Cowie, G. L., and J. I. Hedges. 1992. Sources and reactivities of amino acids in a coastal marine environment. *Limnology and Oceanography* **37:**703–724.

Cowie, G. L., and J. I. Hedges. 1994. Biochemical indicators of diagenetic alteration in natural organic matter mixtures. *Nature* **369:**304–307.

Dauwe, B., and J. J. Middleburg. 1998. Amino acids and hexosamines as indicators of organic matter degradation state in North Sea sediments. *Limnology and Oceanography* **43:**782–798.

Degens, E. T., S. Kempe, and J. E. Richey, Eds. 1991. "Biogeochemistry of Major World Rivers." Wiley, New York.

del Giorgio, P. A. and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology Systematics* **29:**503–541.

Depetris, P. J., and S. Kempe. 1993. Carbon dynamics and sources in the Parana River. *Limnology and Oceanography* **38:**382–395.

Druffel, E. R. M., P. M. Williams, J. E. Bauer, and J. R. Ertel. 1992. Cycling of dissolved and particulate organic matter in the open ocean. *Journal of Geophysical Research* **97:**15639–15659.

Ertel, J. R., J. I. Hedges, A. H. Devol, J. E. Richey, and M. N. G. Ribeiro. 1986. Dissolved humic substances of the Amazon River system. *Limnology and Oceanography* **31:**739–754.

Findlay, S., R. L. Sinsabaugh, D. T. Fischer, and P. Franchini. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* **1:**227–239.

Gardner, W. S., R. Benner, R. Amon, J. B. Cotner, J. F. Cavaletto, and J. R. Johnson. 1996. Effects of high molecular weight dissolved organic matter on nitrogen dynamics in the Mississippi River plume. *Marine Ecology — Progress Series* **133:**287–297.

Gremm, Th. J., and L. A. Kaplan. 1998. Dissolved carbohydrate concentration, composition, and bioavailability to microbial heterotrophs in stream water. *Acta Hydrochimica et Hydrobiologica* **26:**167–171.

Hatcher, P. G., E. C. Spiker, N. M. Szeverenyi, and G. E. Maciel. 1983. Selective preservation and origin of petroleum-forming aquatic kerogen. *Nature* **305:**498–501.

Hedges, J. I. 1992. Global biogeochemical cycles: Progress and problems. *Marine Chemistry* **39:**67–93.

Hedges, J. I., P. G. Hatcher, J. R. Ertel, and K. J. Meyers-Sculte. 1992. A comparison of dissolved humic substances from seawater with Amazon River counterparts by 13C-NMR spectrometry. *Geochimica et Cosmochimica Acta* **56:**1753–1757.

Hedges, J. I., G. L. Cowie, J. E. Richey, P. D. Quay, R. Benner, and M. Strom. 1994. Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. *Limnology and Oceanography* **39:**743–761.

Hedges, J. I, E. Mayorga, E. Tsamakis, M. E. McClain, A. Aufdenkampe, P. Quay, J. Richey, R. Benner, S. Opsahl, B. Black, T. Pimental, J. Quintanilla, and L. Maurice. 2000. Organic matter in Bolivian tributaries of the Amazon River: A comparison to the lower mainstream. *Limnology and Oceanography* **45:**1449–1466.

Hessen, D. O., and L. J. Tranvik, Eds. 1998. "Aquatic Humic Substances: Ecology and Biogeochemistry." Springer-Verlag, Berlin.

Hunt, A. P., J. D. Parry, and J. Hamilton-Taylor. 2000. Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria. *Limnology and Oceanography* **45:**237–241.

Ittekot, V. 1988. Global trends in the nature of organic matter in river suspensions. *Nature* **332:**436–438.

Kaplan, L. A., and J. D. Newbold. 1995. Measurement of streamwater biodegradable dissolved organic carbon with a plug-flow bioreactor. *Water Research* **29:**2696–2706.

Keil, R. G., and D. L. Kirchman. 1991. Contributions of dissolved amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. *Marine Ecology — Progress Series* **73:**1–10.

Kroer, N. 1993. Bacterial growth efficiency on natural dissolved organic matter. *Limnology and Oceanography* **38:**1282–1290.

McCarthy, M. D., J. I. Hedges, and R. Benner. 1993. The chemical composition of dissolved organic matter in seawater. *Chemical Geology* **107:**503–507.

McCarthy, M., J. I. Hedges, and R. Benner. 1996. Major biochemical composition of dissolved high molecular weight organic matter in seawater. *Marine Chemistry* **55:**281–298.

Münster, U. 1993. Concentrations and fluxes of organic carbon in the aquatic environment. *Antonie Leeuwenhoek* **63:**243–274.

Opsahl, S., and R. Benner. 1995. Early diagenesis of vascular plant tissues: Lignin and cutin decomposition and biogeochemical implications. *Geochimica et Cosmochimica Acta* **59:**4889–4904.

Opsahl, S., and R. Benner. 1997. Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature* **386:**480–482.

Opsahl, S., and R. Benner. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. *Limnology and Oceanography* **43:**1297–1304.

Opsahl, S., and R. Benner. 1999. Characterization of carbohydrates during early diagenesis of five vascular plant tissues. *Organic Geochemistry* **30:**83–94.

Pakulski, J. D., R. Benner, T. Whitledge, R. Amon, B. Eadie, L. Cifuentes, J. Ammerman, and D. Stockwell. 2000. Microbial metabolism and nutrient cycling in the Mississippi and Atchafalaya River plumes. *Estuarine Coastal and Shelf Science* **50:**173–184.

Rich, J., H. W. Ducklow, and D. L. Kirchman. 1996. Concentrations and uptake of neutral monosaccharides along 140 W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the DOM flux. *Limnology and Oceanography* **41:**595–604.

Riemann, B., and M. Søndergaard, Eds. 1986. "Carbon Dynamics in Eutrophic, Temperate Lakes." Elsevier, Amsterdam.

Sinsabaugh. R. L., and S. Findlay. 1995. Microbial production, enzyme activity and carbon turnover in surface sediments of the Hudson River Estuary. *Microbial Ecology* **30:**127–141.

Søndergaard, M., and M. Middleboe. 1995. A cross-system analysis of labile dissolved organic carbon. Marine *Ecology — Progress Series* **118:**283–294.

Skoog, A., and R. Benner. 1997. Aldoses in various size fractions of marine organic matter: Implications for carbon cycling. *Limnology and Oceanography* **42:**1803–1813.

Skoog, A., B. Biddanda, and R. Benner. 1999. Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. *Limnology and Oceanography* **44:**1625–1633.

Strom, S., R. Benner, S. Ziegler, and M. Dagg. 1997. Planktonic grazers are a potentially important source of marine dissolved organic carbon. *Limnology and Oceanography* **42:**1364–1374.

Sun, L., E. M. Perdue, J. L. Meyer, and J. Weis. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. *Limnology and Oceanography* **42:**714–721.

Thurman, E. M. 1985. "Organic Geochemistry of Natural Water." Nijhoff, Dordrecht.

Tranvik, L. J. 1988a. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. *Microbial Ecology* **16:**311–322.

Tranvik, L. J. 1998b. Degradation of dissolved organic matter in humic waters by bacteria. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 259–283. Springer-Verlag, Berlin.

Tranvik, L. J., and N. O. G. Jørgensen. 1995. Colloidal and dissolved organic matter in lake water: Carbohydrate and amino acid composition, and ability to support bacterial growth. *Biogeochemistry* **30:**77–97.

Vallino, J., J., C. S. Hopkinson, and J. E. Hobbie. 1996. Modeling bacterial utilization of dissolved organic matter: optimization replaces Monod growth kinetics. *Limnology and Oceanography* **41:**1591–1609.

Volk, C. J., C. B. Volk, and L. A. Kaplan. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. *Limnology and Oceanography* **42:**39–44.

Weiss, M., and M. Simon. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: the relative significance of amino acids and carbohydrates. *Aquatic Microbiol Ecology*. **17:**1–12.

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6

Large-Scale Patterns in Dissolved Organic Carbon Concentration, Flux, and Sources

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I. INTRODUCTION

The concentration and flux of dissolved organic carbon (DOC) are important characteristics of aquatic ecosystems, with important effects on light and ultraviolet (UV)-B attenuation, ecosystem respiration, nutrient

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availability, metal toxicity, and the global carbon balance. In this chapter I present a summary of data and analyses of DOC concentrations in rivers, lakes, and the ocean, DOC fluxes in rivers, and DOC sources in rivers and lakes. The focus is primarily on studies covering relatively large spatial scales (regional, continental, and global) rather than on studies of single ecosystems or those with a limited spatial extent. What are the spatial patterns in DOC concentration and flux and are these predictable from ecosystem, landscape, or climatological characteristics? These are the questions addressed here. The results of this synthesis generally show that the concentration and flux of DOC are related more strongly to climate and topography than to the internal properties of aquatic ecosystems.

II. DISSOLVED ORGANIC CARBON CONCENTRATIONS

A. Streams and Rivers

The concentration of DOC in streams and rivers varies from about 0.5 to 50 mg L^{-1} for most systems. DOC concentration appears to be influenced primarily by climate (precipitation) and landform (presence of wetlands). In addition, in upland catchments, the flowpath of water through soil is an important determinant of DOC concentration. DOC concentrations are higher during periods when the dominant flowpaths through the catchment are near the surface through the organic-rich upper soil horizon rather than through the lower soil horizons that often have high DOC sorption capacity (McDowell and Wood, 1984; see Chapter 2).

At the largest spatial scale, precipitation appears to be an important determinant of river DOC concentration. In a global analysis of river DOC, Meybeck (1988) reported that DOC concentrations typically range from lows of 1–3 mg L^{-1} in rivers of arid and semiarid regions to highs of 7–8 mg L^{-1} typical of rivers from the taiga and wet tropics (Table I). Meybeck reported a global discharge-weighted mean DOC concentration of 6.3 mg L^{-1}. In an analysis of 20 of the world's largest rivers, Spitzy and Leenheer (1991) reported discharge-weighted mean annual DOC concentrations of 2.5–16.1 mg L^{-1}, with both mean and median values of approximately 6 mg L^{-1}. In contrast to Meybeck's analysis, the Spitzy and Leenheer study did not suggest a tight link between DOC concentration and precipitation. Four of the five rivers with the highest runoff values had discharge-weighted mean concentrations <4 mg L^{-1} whereas three of the five rivers with the lowest runoff values had discharge-weighted mean concentrations >5 mg L^{-1}.

There have been a number of regional analyses showing that stream and river DOC concentrations appear to be highly influenced by the flowpath of water across the landscape. Streams draining landscapes dominated by water flowpaths at the land surface in contact with organic-rich soil horizons

TABLE I Typical DOC Concentrations in Rivers of Different Biomes from High Latitudes to Low Latitudes

Climate zone	*Typical DOC concentration (mg L^{-1})*
Tundra	2
Taiga	7
Temperate	4
Semi-arid regions	1
Wet tropics	8
Dry tropics	3
Discharge-weighted global mean	6.3

From Meybeck 1988

TABLE II Regional Analyses of Stream and River DOC Concentrations and Significant Predictors

Region	*DOC concentration range (mg L^{-1})*	*Predictors*	*Reference*
Scotland ($n = 32$)	0.8–10.6 (12-wk means)	Soil C, peatlands ($r^2 = 0.87$)	Aitkenhead *et al.* (1999)
Nova Scotia ($n = 42$)	2.5–63	% wetlands ($r^2 = 0.49$)	Gorham *et al.* (1998)
Quebec ($n = 42$)	3.5–40 (8-mo means)	% wetlands ($r^2 = 0.26$–0.67)	Eckhardt and Moore (1990)
Wisconsin ($n = 24$)	<1–45	% wetlands ($r^2 = 0.57$)	Gergel *et al.* (1999)
North America ($n = 31$)	0.7–30.6 (annual means)	channel slope ($r^2 = 0.44$)	Mulholland (1997)

have higher DOC concentrations than those receiving drainage along deeper flowpaths. A relatively large amount of variability in stream DOC concentrations can be explained by either the percentage of wetlands in the catchment or average channel slope, a parameter that tends to vary inversely with the importance of wetlands in the catchment (Table II). In a study of 32 streams in Scotland, Aitkenhead *et al.* (1999) reported that soil carbon storage and percentage of peatland in the catchment each explained >85% of the variation in DOC concentration. For a given peatland percentage, DOC concentrations were about 1.5 times greater for lowland catchments than for upland catchments, possibly indicating an additional effect of greater near-surface water flowpaths in the lowlands. Similarly, in studies of 42 streams in Nova Scotia (Gorham *et al.*, 1998), 42 streams in Quebec (Eckhardt and Moore, 1990), and 24 rivers in Wisconsin (Gergel *et al.*, 1999), wetland percentage was the best predictor of DOC concentration,

generally explaining at least 50% of the variation in concentration. In a study of small streams primarily in the United States, Mulholland (1997) did not have data on wetland percentage, but reported that average channel slope explained 44% of the variation in DOC concentration. The effect of wetlands on DOC may vary seasonally. In the Nova Scotia study of Gorham *et al.* (1998) there was a much steeper relationship between DOC concentration and wetland percentage in summer and early autumn than in winter and early spring (Fig. 1).

Water flowpath through soil is also an important determinant of variations in DOC concentration over time within individual streams. In well-drained catchments numerous studies have shown that stream DOC

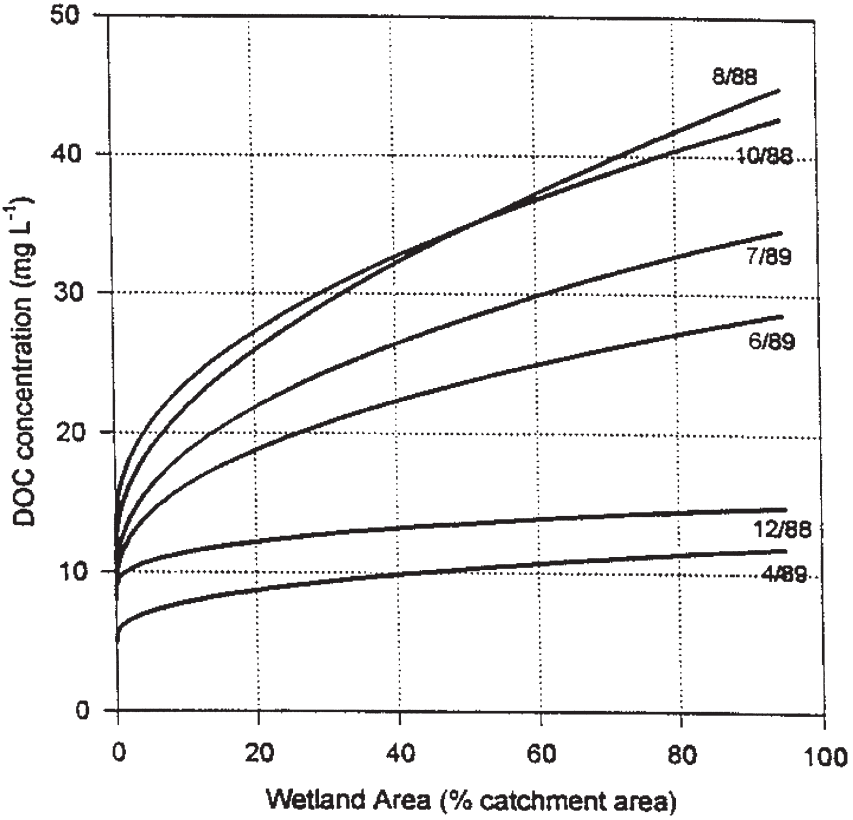

FIGURE 1 Relationship between DOC concentration in streams on six dates to the percentage of wetlands in the catchment based on nonlinear regression models. (From Gorham, E. *et al.*, 1998. The chemistry of streams in southwestern and central Nova Scotia, with particular reference to catchment vegetation and the influence of dissolved organic carbon primarily from wetlands. *Wetlands* **18:**115–132. Copyright 1998 American Geophysical Union. Reproduced by permission of American Geophysical Union.)

concentrations increase sharply during high discharge events, often peaking with peak discharge (Eckhardt and Moore, 1990: Mulholland *et al*., 1990: Hornberger *et al*., 1994; Hinton *et al*., 1997; Sakamoto *et al*., 1999; Butturini and Sabater, 2000). It appears that both flushing of DOC from near-stream riparian soils (Hemond, 1990; Hinton *et al*., 1998) and from surface soils in upslope areas (Cronin, 1990; Hornberger *et al*., 1994) are responsible for the increases in stream DOC concentrations during storms. Regardless of whether the DOC source is riparian or upslope soils, it seems clear that a shift toward increasing importance of shallow flowpaths relative to deeper flowpaths during higher flows is the dominant mechanism. In contrast, for streams draining wetlands, relationships between DOC concentration and discharge are poor and can even be negative (Mulholland *et al*., 1981; Hinton *et al*., 1997; Schiff *et al*., 1998). This is probably because in wetlands surface flowpaths predominate at all times and increased discharge can result in a depletion of available DOC, thus diluting DOC concentrations.

Seasonal variations in readily leachable organic carbon in soil also can produce seasonal changes in stream DOC concentrations. Higher DOC concentrations after autumn leaf-fall have been reported for streams draining deciduous forest catchments in several temperate zone locations. Stream DOC concentrations during late autumn and winter were about 1–3 mg L^{-1} higher than during other periods in streams in Tennessee (Mulholland and Hill, 1997), Pennsylvania, Michigan (Moeller *et al*., 1979), and southern Quebec (Eckhardt and Moore, 1990). However, it is unclear whether this seasonal peak in DOC concentration is a consistent regional pattern for streams and rivers draining areas of the temperate zone that are dominated by deciduous forests.

Spatial variation in DOC concentrations along river continua appears to be less consistent and dramatic than variation due to hydrologic changes. Results from the river continuum project in the United States suggest that there is little variation in DOC concentrations from first order to fourth order streams in 4 widely separated geographic regions (Moeller *et al*., 1979). However, in a study of the Amazon River basin, Hedges *et al*. (2000) reported that DOC concentrations increased from about 1 mg L^{-1} in the headwater tributaries of Bolivia to about 4 mg L^{-1} in the lower Amazon River. This longitudinal increase in DOC concentration probably reflects changes in basin morphometry, with lower DOC inputs in the steeper headwater drainages and higher DOC inputs contributed by the flatter drainages with larger riparian wetlands in lower portions of the basin. Longitudinal increases in DOC concentration might be expected in many large river basins that originate in more mountainous areas because the relative importance of wetlands should increase in the lower, flatter portions of the basin. However, spatial patterns in DOC may be altered in many river basins by large inputs of sewage or other anthropogenic DOC sources and by changes in the morphology (e.g., channelization on impoundment)

of higher-order streams that alter flowpaths and hydrologic connections with riparian wetlands.

B. Lakes

There have been a number of regional studies of DOC in lakes, mostly from North America, and these indicate that concentrations can vary by over 2 orders of magnitude (Table III). The range in lake DOC concentrations in humid temperate climates, however, is considerably lower than the range in lakes in arid climates.

In one of the first and largest of the regional studies, Rasmussen *et al.* (1989) examined 337 lakes in the northern United States and Canada and reported that lake color (a surrogate for humic DOC) was positively related to the lake drainage/lake area ratio and negatively related to watershed slope, mean lake depth, and lake area. These results suggested the importance of allochthonous organic carbon inputs, wetlands, and water residence time as controls on the concentration of humic DOC. In a study of 59 lakes in southwestern Quebec, Houle *et al.* (1995) reported that DOC concentrations ranged from 0.7 to 28.2 mg L^{-1}. A multiple regression analysis indicated that flowpath factor (a function of drainage area/lake perimeter ratio and catchment slope) and the inverse of maximum lake depth (a proxy for water residence time) were significant predictors of DOC concentration. These results suggested that DOC concentrations depended primarily on DOC inputs from the catchment and in-lake losses of DOC. In a study of 30 Canadian Shield lakes of southeastern Quebec, D'Arcy and Carignan (1997) reported a somewhat smaller range in DOC concentration (1.3–7.6 mg L^{-1}), but also found a significant relationship between average catchment slope and DOC concentration. These investigators suggested that the slope effect was the result of waterlogging of gentle slopes during periods of high runoff, thereby promoting shallow lateral flow and transport of DOC from surface soils. They also reported a weak but significant relationship between DOC concentration and conifer abundance in the catchment. In a study at the Experimental Lakes Area of southwestern Ontario, Curtis and Schindler (1997) found that DOC concentration ranged from 1 to 13.7 mg L^{-1} and was positively correlated with the size of the catchment relative to that of the lake (an indicator of allochthonous inputs) and negatively correlated with water residence time (an indicator of the potential for in-lake losses of DOC). Gergel *et al.* (1999) reported a DOC concentration range of 2–23 mg L^{-1} for 119 lakes in Wisconsin, United States, with a higher mean concentration for drainage lakes (7.2 mg L^{-1}) than for seepage lakes (5.5 mg L^{-1}). Percentage of wetlands in the drainage was the strongest predictor of lake DOC concentration in this study, although it accounted for only 26% of the variation in DOC, substantially lower than the variation in river DOC concentration explained by wetlands in the same

TABLE III Regional Analyses of Lake DOC Concentrations and Significant Predictors

Region	*DOC concentration range (mg L^{-1})*	*Predictors*	*Reference*
Humid climates			
Northern United States and Canada (337 lakes)	1–25*	Drainage area: lake area (+) Watershed slope (–) Mean lake depth (–) Lake area (–)	Rasmussen *et al*. (1989)
SW Quebec (59 lakes)	0.7–28.2 (median 6.9)	Flowpath factor (+), 1/max. depth (+)	Houle *et al*. (1995)
SE Quebec (30 lakes)	1.3–7.6 (median 4.2)	Catchment slope (–) Drainage area: lake area (+) % conifers in drainage (+)	D'Arcy and Carignan (1997)
SW Ontario (12 lakes)	1.0–13.7	Area catchment/lake (+) Water residence time (–)	Curtis and Schindler (1997)
Wisconsin (119 lakes)	2–23	% wetlands in drainage (+)	Gergel *et al*. (1999)
Arid climates			
Alberta (23 saline lakes)	20–330	Salinity (+) Water residence time (+)	Curtis and Adams (1995)

*Humic DOC only, derived from water color measurements.

region. Finally, the importance of in-lake DOC losses was also shown in an 8-year study of seven unproductive lakes in central Ontario by Dillon and Molot (1997a). These authors reported a somewhat lower range for mean DOC concentrations (1.8–5 mg L^{-1}), and using mass balance analysis, they determined that in-lake retention of DOC was inversely related to areal water loading rate

Collectively, the regional studies of humid-zone lakes show that catchment and lake morphometry are the most important determinants of DOC concentration via their influence on allochthonous inputs and in-lake losses. Lakes draining relatively large and flat catchments tend to have higher allochthonous DOC inputs as a result of greater importance of shallow flowpaths through soils and greater percentages of wetlands. Shallow lakes or those with high drainage area to surface area ratios tend to have shorter water residence times and thus lower in-lake DOC losses via biological or UV oxidation and flocculation and settling. Shallow lakes also are more likely to have larger littoral zones that may also contribute greater amounts of DOC (see Chapter 1). Nonetheless, it appears that variation in allochthonous inputs is more important than variation in in-lake production or loss processes as a control on large-scale patterns of lake DOC (see Chapter 2 for additional discussion of allochthonous sources of DOC in surface waters).

Controls on lake DOC concentrations in arid lands may be somewhat different from the controls in humid climates. In a study of 23 lakes in Alberta, Canada, over a large gradient in salinity, Curtis and Adams (1995) reported DOC concentrations of 20–330 mg L^{-1} (Table III). In this study, DOC was positively related to salinity, a proxy for water residence time, and a pattern opposite that of lakes in humid regions. The authors suggest that this pattern is the result of increasing evaporative concentration of refractory DOC as well as other solutes as water residence time increases. Thus, it appears that the relationship between DOC concentration and water residence time in lakes is the net effect of two opposing processes: (1) increasing DOC losses (declining concentrations) with increasing water residence time dominating at low to moderate residence times (freshwater lakes) and (2) evaporative concentration of refractory DOC dominating at very long water residence times (saline lakes).

Landscape disturbances that alter the amount or characteristics of soil organic carbon can have a small but significant impact on the DOC concentrations of boreal lakes, at least for several years after the disturbance. In a study of 38 boreal lakes in Quebec, Canada, Carignan *et al.* (2000) reported that lake DOC concentrations were significantly higher (up to 3 times) in catchments that had been logged within 3 years of the study compared with reference lakes. These authors also evaluated the effect of wildfire, and although differences in DOC concentrations between burnt and reference lakes were not significant, DOC concentrations were somewhat higher in the burnt catchments and increased significantly with time after wildfire. In

a similar study of logging and fire effects on 116 boreal lakes in Ontario, Canada, France *et al.* (2000) reported that DOC concentrations increased in relation to the severity of previous (4–13 years) forest clearance, with the greatest increases in DOC associated with whole-catchment clearcut. Removing the landscape morphometry influence by normalizing for the catchment drainage ratio, France and colleagues showed that lake DOC concentrations increased by 2–3 mg L^{-1} in clear-cut or burnt watersheds relative to reference watersheds. In both of these studies, however, it is unclear whether the DOC increases resulting from these forest disturbances were primarily the result of hydrologic changes after forest removal (greater lateral flow through surface soils produced by reduced transpiration and increases in soil moisture) or of increases in soil DOC sources after disturbance.

Climate changes may also have significant effects on lake DOC concentrations. In a 20-year study of boreal lakes in the Experimental Lakes Area of northwestern Ontario, Schindler *et al.* (1997) reported that lake DOC concentrations declined by 15–25% as mean annual temperatures increased by 1.6°C, precipitation declined by 40%, and runoff declined by 70% due to increased evaporation and decreased precipitation. The primary reason for the decline in lake DOC was reduced inputs of DOC from terrestrial catchments, although in-lake removal of DOC also increased slightly via either increased acidification, UV light penetration, or microbial degradation.

C. Oceans

The range of DOC concentrations in the ocean (about 0.5–3.0 mg L^{-1}) is very small compared with the range in rivers and lakes. This is primarily because of the isolation of much of the ocean from terrestrial organic carbon sources and long water residence times that enhance DOC loss. In a survey of the Atlantic, Southern, and Pacific Oceans, Martin and Fitzwater (1992) reported that surface DOC concentrations (about 1.5 mg L^{-1}) are higher than deep-water concentrations, presumably because of higher autochthonous sources of DOC to surface waters. In a survey of deep water DOC along a water age gradient, Hansell and Carlson (1998) found a one-third decline in DOC concentration from the younger north Atlantic (0.6 mg L^{-1}) to the much older waters of the north Pacific (0.4 mg L^{-1}), indicating net consumption of DOC within the deep ocean.

III. DISSOLVED ORGANIC CARBON EXPORTS IN RIVERS AND STREAMS

A. Global and Continental-Scale Studies

Several global and continental-scale studies of total organic carbon (TOC) export in large rivers have focused on the question of whether the oceans are an important sink for atmospheric C via river transport of

terrigenous organic matter. These studies have shown that the export of TOC (most of which is DOC) in rivers is primarily a function of runoff (positive relationship) because runoff varies to a much greater extent than does TOC concentration and export is simply a product of discharge-weighted mean annual concentration and runoff. In one of the first global studies, Schlesinger and Melack (1981) reported that TOC exports were largely a function of climate (runoff) with exports generally ≤ 1 gC m^{-2} y^{-1} for arid lands, grasslands, and tundra and generally 2–10 gC m^{-2} y^{-1} for boreal, temperate, and tropical forests. For temperate forests, the relationship between TOC export and runoff was nonlinear, with an asymptote in export of about 6 gC m^{-2} y^{-1} at high values of runoff (>200 cm), suggesting limitations on the supply of carbon available for transport. Mulholland and Watts (1982) compiled TOC export data for rivers of North America and found the highest exports from the wet boreal areas of northeastern Canada and New England (annual regional means of 5–6 gC m^{-2} y^{-1}) and from the mid-Atlantic and southeastern United States. (annual regional means of 3–4 gC m^{-2} y^{-1}). These authors report a linear relationship between annual TOC export and runoff (no asymptote in export at high runoff), although their dataset included runoff values generally <100 cm. A more recent global-scale study by Meybeck (1993) using data from 40 of the world's largest rivers showed a bimodal pattern of TOC export with latitude (reflecting a bimodal pattern in runoff), with peaks of 5–6 gC m^{-2} y^{-1} for wet boreal and temperate forests and 9–10 gC m^{-2} y^{-1} for wet tropical forests. Minima of ≤ 1 gC m^{-2} y^{-1} were found for high latitude tundra and mid–low latitude arid regions. Finally, in a review of DOC exports in streams and rivers of varying size in North America and Europe, Hope *et al.* (1994) reported a range of exports from 1 to 10 gC m^{-2} y^{-1}, with somewhat higher exports from boreal forests than from temperate forests.

The relationship between TOC export and latitude/climate observed in the global and continental-scale studies appears to be a reflection of several factors involving sources (primarily organic matter in soils, see Chapter 2) and hydrologic processes. Soil organic matter storage is positively influenced by primary production (highest in warm, wet climates) and negatively influenced by oxidation rates in soils (lowest in cool, wet climates). The mobilization and transport of soil organic carbon to aquatic ecosystems is positively related to the flux of water across the landscape, driven by the balance between precipitation and evapotranspiration. Thus, water flux (runoff) exerts primary control on organic matter export, with the effects of temperature somewhat diminished because temperature has positive effects on both sources (production) and sinks (oxidation) of soil organic matter.

B. Regional Studies and Predictors of Dissolved Organic Carbon Export

Regional studies of TOC or DOC export, which include much smaller streams and rivers than those considered in global studies, have shown

TABLE IV Significant Predictors of River DOC (or TOC) Exports from Regional Studies

Region	*DOC export* ($gC\ m^{-2}\ y^{-1}$)	*Predictors*	*Reference*
Finland (n = 22)	2.6–8.8 (TOC)	% peatlands (+) Precipitation (+) pH (–)	Kortelainen *et al.* (1997)
Great Britain (n = 85)	0.8–10.4	Soil C storage (+) Precipitation (+) Catchment slope (–)	Hope *et al.* (1997)
Canada's Atlantic Provinces (n = 26)	1.6–12.4 (TOC)	Precipitation (+) Basin slope (–)	Claire *et al.* (1994)
Central Ontario (n = 20)	1–10	% peatlands (+)	Dillon and Molot (1997b)
North America (n = 31)	0.3–25	Precipitation (+) Channel slope (–)	Mulholland (1997)

similar to somewhat greater ranges in export rates (Table IV). Studies of TOC exports in rivers in Finland (Kortelainen *et al.*, 1997) and in the Atlantic provinces of Canada (Claire *et al.*, 1994) have reported a range of exports of 2.6–8.8 and 1.6–12.4 $gC\ m^{-2}\ y^{-1}$, respectively. Similar ranges in DOC exports were reported by Dillon and Molot (1997b) for 20 streams in central Ontario (1–10 $gC\ m^{-2}\ y^{-1}$) and by Hope *et al.* (1997) for 85 rivers in Great Britain (0.8–10.4 $gC\ m^{-2}\ y^{-1}$). Mulholland (1997) reported a wider range in DOC exports (0.3 to 25 $gC\ m^{-2}\ y^{-1}$) in a study of 31 small streams, mostly in the United States and Quebec, Canada.

There was considerable agreement as to the primary determinants of DOC export in these studies. Annual precipitation was a significant predictor in all but the central Ontario study, and measures of the importance of wetlands (percentage of peatlands and soil carbon storage) or the potential for shallow water flowpaths (catchment slope and channel slope) were significant predictors of DOC export in all of the studies (Table IV). In the central Ontario study of Dillon and Molot (1997b) the effect of peatlands was particularly strong, explaining 78% of the variance in long-term DOC export (Fig. 2). In a recent study and the most spatially comprehensive to date, Aitkenhead and McDowell (2000) analyzed DOC export data for 164 catchments from around the world and reported values ranging from 0.1 to 13.9 $gC\ m^{-2}\ y^{-1}$. When they grouped the data into 15 different biome types, they found that the soil C/N ratio was a strong predictor of export with the mean soil C/N ratio explaining 99% of the variation in mean biome DOC export. This biome-scale relationship between soil C/N and DOC export may be another indication of the importance of wetlands (which generally have higher C/N ratios) or may be a reflection of the balance between metabolic demand for soil organic carbon by microbial communities (higher at low

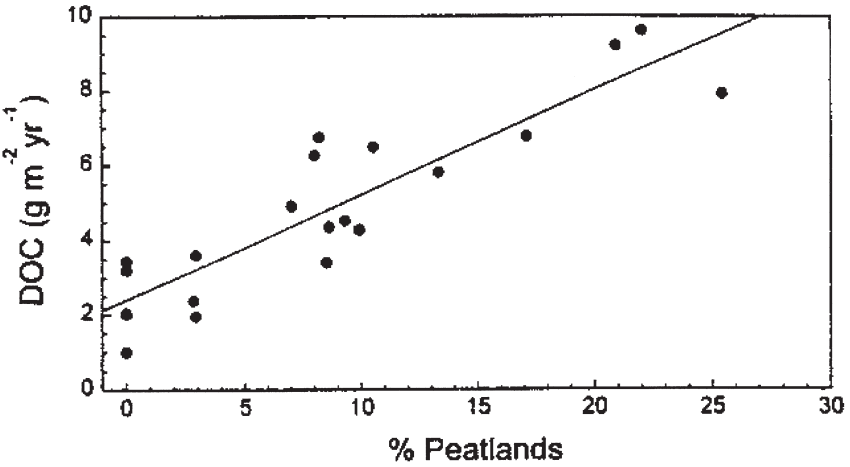

FIGURE 2 Relationship between mean annual DOC export during 1980 through 1992 and percentage of peat cover in catchments from central Ontario. (From Dillon, P. J., and L. A. Molot. 1997b. Effect of landscape form on export of dissolved organic carbon, iron, and phosphorus from forested stream catchments. *Water Resources Research* 33:2591–2600. Copyright 1997 American Geophysical Union. Reproduced by permission of American Geophysical Union.

C/N ratios) versus that available for leaching and transport (greater at high C/N ratios).

Most studies of DOC export probably underestimate annual exports because sampling frequencies are often inadequate to completely account for the higher discharge events when DOC concentrations and transport increase sharply. This is particularly true for smaller rivers and streams in which daily sampling frequencies may be required for good estimates of annual export. Even for large rivers, sampling frequencies of at least monthly (and usually more often) are a minimum requirement for good estimates of export. Very few studies have used such sampling frequencies.

C. Export/Runoff Relationships

With the exception of the study of Schlesinger and Melack (1981), who considered particulate as well as dissolved organic carbon, relationships between DOC export and runoff that have been reported are generally linear. This suggests that differences in mean annual DOC concentrations are small compared with differences in runoff across systems and therefore, differences in DOC export are primarily the result of differences in runoff. There appears to be considerable variation in the export–runoff slopes determined in different studies, however, with values ranging from 2.2 to 13 mg L^{-1} (Fig. 3). Thus, despite generally linear export–runoff relationships, there is considerable variation in discharge-weighted mean annual DOC

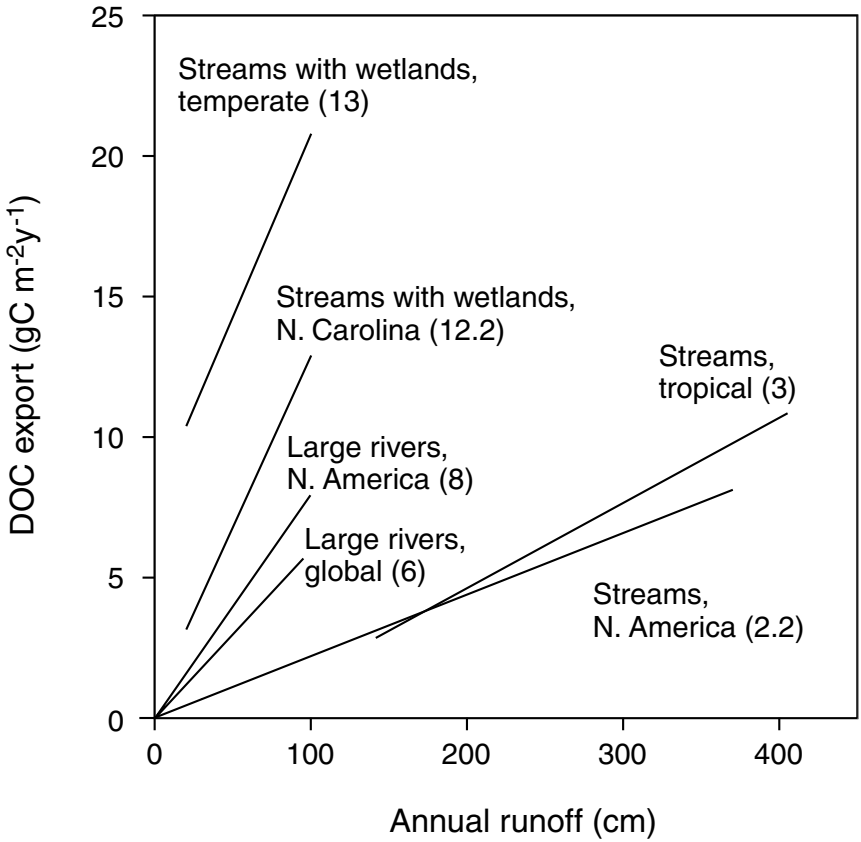

FIGURE 3 Relationships between annual runoff and watershed export of DOC in streams and rivers reported in the literature. The respective lines extend only over the range of runoff values included in the dataset. The slopes of each line are approximately equivalent to the mean annual DOC concentration for that group (in parentheses). Sources for each relationship are as follows: streams with wetlands, temperate (Mulholland, 1997); streams with wetlands, N. Carolina (Mulholland and Kuenzler, 1979); large rivers, global (Spitzy and Leenheer, 1991); large rivers, N. America (Mulholland and Watts, 1992); streams, tropical (McDowell and Asbury, 1994); streams, N. America (Mulholland, 1997).

concentrations across groups of catchments. This variation appears to be related more to system size and the influence of wetlands than to latitude. Small streams in well-drained catchments have the lowest mean DOC concentrations, and there is little difference in mean DOC concentrations between temperate (2.2 mg L^{-1}) and tropical streams (3 mg L^{-1}). Mean DOC concentrations in streams draining catchments with sizeable wetlands are particularly large (12.2 and 13 mg L^{-1}). Large rivers appear to have intermediate mean DOC concentrations, perhaps because they integrate exports from well-drained upland areas and from wetlands across the landscape.

An important implication of linearity in relationships between DOC export and runoff over very large ranges in runoff is that the DOC pool available for mobilization and export by water is very large and not easily depleted by high fluxes of water. As noted earlier in this chapter, this may be a reflection of the strong positive effect of water on soil organic matter storage (stimulation of production and inhibition of oxidative losses) as well as mobilization and transport from soils to aquatic ecosystems.

The strong relationship between DOC export and runoff also suggests that climate changes may have a significant effect on DOC export. As described in Section II.B, Schindler *et al.* (1996, 1997) have shown that a long-term decline in runoff in the Experimental Lakes area of northwestern Ontario has resulted in substantial declines in DOC export in streams to lakes. Stream DOC export from one catchment has declined by about 50% over the past 20 years in response to a 60–70% decline in runoff (Schindler *et al.*, 1996). Clair *et al.* (1999) developed a model of DOC exports in Canadian rivers and, using a climate change scenario showing increases in runoff for many areas of Canada, predicted that DOC exports would increase by about 14% under a doubled CO_2 atmosphere.

IV. SOURCES OF DISSOLVED ORGANIC CARBON

Excellent overviews of the sources and composition of DOC in aquatic ecosystems are provided in Chapters 1, 2, and 4. In this chapter, I focus on sources of DOC at large spatial scales.

A. Streams

I have found no broad-scale studies of sources of DOC in streams, specifically the relative contributions of autochthonous and allochthonous sources. There are several approaches for separating allochthonous from autochthonous sources that have been used in studies of single streams. Diel fluctuations in DOC concentrations can be used to determine minimum contributions of algal DOC. In a study of White Clay Creek, Pennsylvania, Kaplan and Bott (1982) showed that 20% of the DOC in streamwater during spring peak in GPP was derived from algal excretion. Similarly, Mulholland (1992) showed that 5–10% of the DOC in Walker Branch, Tennessee was from algae during spring.

Longitudinal patterns in DOC in small streams arising as spring seeps can be used to identify in-stream DOC sources, although DOC increases could be from leaching of stored terrigenous material as well as from algae. Kaplan *et al.* (1980) reported DOC increases of 0.5–1 mg L^{-1} over the first 20–100 m in several first-order streams in the White Clay Creek drainage

basin (representing a doubling of concentrations). Wallace *et al.* (1982) reported that DOC increased by approximately 0.5 mg L^{-1} above the value for spring seeps along a 2-km reach of stream (a 300% increase).

Carbon isotope composition can often be used to separate algal sources from terrestrial plant sources of DOC. DOC produced by algae can be more depleted in ^{13}C relative to DOC leached from terrestrial plant detritus because of algal use of highly ^{13}C-depleted dissolved CO_2 derived from respiration in streams. Although there have been a number of ^{13}C studies in streams to identify autochthonous and allochthonous sources of particulate organic matter and invertebrate food resources (e.g., Rounick *et al.*, 1982; Rosenfeld and Roff, 1992), fewer studies have used this approach for determining DOC sources in streams. As discussed above, Hedges *et al.* (2000) used ^{13}C content as a DOC source indicator in their study of the Amazon River and its tributaries and found that most of the DOC throughout the basin appeared to be from terrestrial C3 plants. Schiff *et al.* (1997) used ^{13}C measurements to identify sources of DOC in streams draining into Harp Lake in southeastern Ontario and reported that it was predominantly from surface soils. Schiff and colleagues also measured the ^{14}C content of stream DOC and found that stream DOC was composed of both older (prior to nuclear weapons testing), highly processed DOC and younger (after weapons testing) DOC from surface soils with the relative contributions of these two DOC pools changing as a function of hydrology and season. Similarly, Raymond and Bauer (2001) used ^{13}C and ^{14}C measurements to determine that the DOC in four large rivers that discharge to the western North Atlantic Ocean was composed of both old and young carbon, primarily of terrestrial origin. They also found that the younger DOC was preferentially oxidized by bacteria, suggesting that primarily old carbon is exported across the continental shelf to the open ocean. Additional synoptic studies of ^{13}C and ^{14}C in DOC are needed to provide important information on broad spatial patterns and controls on the sources of DOC in rivers and streams.

B. Lakes

For lakes, relationships between DOC concentration and some measure of the relative size of the drainage area (e.g., drainage area/lake area ratio) and between DOC concentration and water residence time can be used to estimate the importance of autochthonous DOC by extrapolation. In the study of Experimental Lakes Area lakes by Curtis and Schindler (1997), the *y* intercept of the DOC concentration versus catchment/lake area ratio (the DOC value at minimal catchment area) is approximately 1–2 mg L^{-1}. In the same study, the asymptote of the DOC concentration versus water residence time relationship at infinitely long residence times is roughly 2–3 mg L^{-1}. This would suggest that for lakes in this region, autochthonous sources account for perhaps as much as 3 mg L^{-1} of the DOC present.

Stable carbon isotope ratios have also been used to determine the sources of lake DOC. Baron *et al.* (1991) used ^{13}C analysis to show that autochthonous sources dominated the DOC of an alpine lake during most periods while allochthonous sources dominated the DOC in a subalpine lake. The high DOC concentrations observed during spring snowmelt and early summer in both lakes, however, were mostly derived from allochthonous sources. As with streams and rivers, synoptic regional studies of ^{13}C and ^{14}C would provide important new information on broad spatial patterns and controls on the sources of DOC in lakes.

C. Oceans

In contrast to rivers and lakes, autochthonous processes appear to be the overwhelmingly dominant source of DOC in the oceans. In a survey of shallow and deep waters of the Atlantic and Pacific Oceans, Opsahl and Benner (1997) used concentrations of lignin in DOM to determine that terrigenous matter accounted for only 0.7–2.4% of the DOC present. Terrigenous DOC concentrations were about 2.6 times higher in the Atlantic than in the Pacific Ocean, presumably because of the 3.6 times greater riverine water discharge to the Atlantic.

A recent study of rainwater DOC suggested that rain also may be a significant source of DOC to the open ocean. The DOC concentration of marine rain was reported to be 0.3 mg L^{-1} and the global rainwater DOC flux to the oceans was estimated to be 0.43×10^{12} gC yr^{-1}, about the same magnitude as the river flux of DOC (Willey *et al.*, 2000).

V. SYNTHESIS AND FUTURE RESEARCH NEEDS

The concentration and flux of DOC in aquatic ecosystems appears to be related more to climate and landscape topography than to internal properties and processes of aquatic ecosystems (Fig. 4). Variability in the input of DOC originating from terrestrial plants generally establishes the variability in aquatic DOC over both time and space. This is probably because a much higher proportion of land plant production is in the form of lignin and other organic compounds that are more resistant to biological oxidation than the organic matter produced by aquatic plants. Thus, a much higher proportion of land plant production accumulates as detrital organic carbon and is available for export to adjacent aquatic ecosystems as DOC. Aquatic ecosystems that receive drainage from areas with large storage of organic carbon in surface soils and that have topographic characteristics promoting surface or shallow subsurface flow of water (e.g., wetlands)

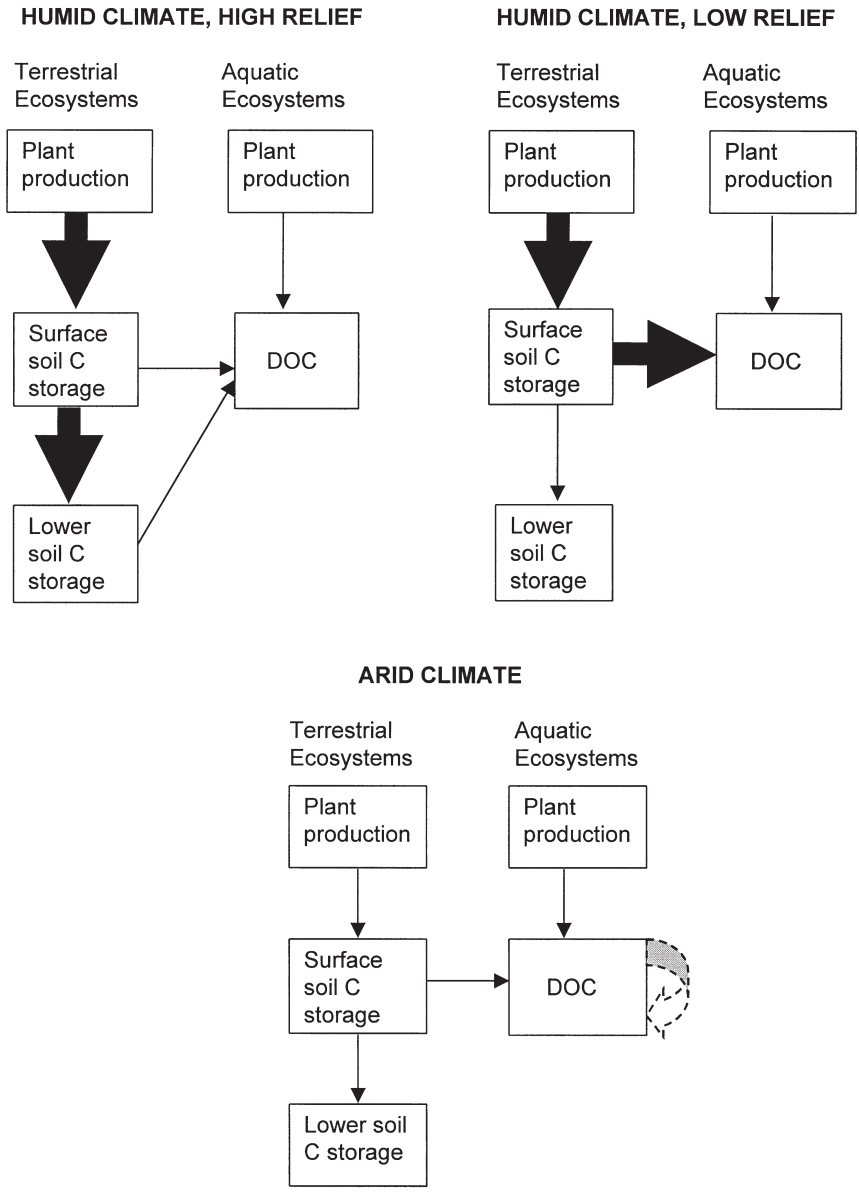

FIGURE 4 Effects of climate and landscape topography on the concentrations and fluxes of DOC in aquatic ecosystems. Differences in the widths of the arrows indicate differences in relative fluxes of organic carbon within and between terrestrial and aquatic ecosystems. The dashed and curved arrow in the arid climate diagram indicates the potential for large evaporative concentration leading to high DOC concentrations despite relatively low input fluxes.

generally have higher concentrations and fluxes of DOC. Because of the effect of climate on the preservation of soil organic matter, aquatic ecosystems in cool, wet climates often have higher concentrations of DOC. In addition, more humid climates generate greater runoff from land to water and thus greater fluxes of DOC into and through aquatic ecosystems. Finally, several lines of evidence suggest that, in many landscapes, the DOC pool available for export is very large and not easily depleted by high fluxes of water. This may be a reflection of the strong positive effect of water on soil organic matter storage (stimulation of production and inhibition of oxidative losses) as well as on the mobilization and transport of DOC from soils to aquatic ecosystems.

Two areas in need of future research stand out. First, we need a better understanding of differences in the sources of DOC in aquatic ecosystems under different hydrologic conditions or in different biomes. In particular, the relative importance of recently fixed carbon versus old carbon in DOC flux to the oceans is an important question with regard to understanding the global carbon cycle. Second, the impacts of human activities on large-scale patterns of DOC concentration and flux also are not very well understood. In particular, the effects of changes in catchment land use (e.g., conversion of unmanaged lands to agriculture or urbanization), drainage network morphology (e.g., channelization and impoundment), and climate (temperature and precipitation patterns) on DOC concentration and flux are not well understood. We might expect that conversion of unmanaged lands to agriculture or urban uses and channelization of streams and rivers would tend to reduce DOC fluxes. A warmer, drier climate also might be expected to reduce DOC fluxes. Although increases in precipitation might increase DOC fluxes, increasing evaporative demand under a warmer climate might offset the effect of increasing precipitation. Because DOC has such an important role in the structure and functioning of aquatic ecosystems, it is important that we gain a better understanding of how current and future human activities might affect large-scale dynamics of DOC.

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REFERENCES

Aitkenhead, J. A., and W. H McDowell. 2000. Soil C : N ratio as a predictor of annual DOC flux at local and global scales. *Global Biogeochemical Cycles* **14:**127–138.

Aitkenhead, J. A., D. Hope, and M. F. Billett. 1999. The relationship between dissolved organic carbon in stream water and soil organic carbon pools at different spatial scales. *Hydrological Processes* **13:**1289–1302.

Baron, J., D. McKnight, and A. S. Denning. 1991. Sources of dissolved and particulate organic material in Lock Vale Watershed, Rocky Mountain National Park, Colorado, USA. *Biogeochemistry* **15:**89–110.

Butturini, A., and F. Sabater. 2000. Seasonal variability of dissolved organic carbon in a Mediterranean stream. *Biogeochemistry* **51:**303-321.

Carignan, R., P. D'Arcy, and S. Lamontagne. 2000. Comparative impacts of fire and forest harvesting on water quality in Boreal Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **57** (Suppl. 2):105–117.

Claire, T. A., T. L. Pollock, and J. M. Ehrman. 1994. Exports of carbon and nitrogen from river basins in Canada's Atlantic Provinces. *Global Biogeochemical Cycles* **8:**441–450.

Claire, T. A., J. M. Ehrman, and K. Higuchi. 1999. Changes in freshwater carbon exports from Canadian terrestrial basins to lakes and estuaries under a $2 \times CO_2$ atmospheric scenario. *Global Biogeochemical Cycles* **13:**1091–1097.

Cronin, C. S. 1990. Patterns of organic acid transport from forested watersheds to aquatic ecosystems. *In* "Organic Acids in Aquatic Ecosystems" (E. M. Perdue and E. T. Gjessing, Eds.) pp. 245–260. Wiley, New York, NY.

Curtis, P. J., and H. E. Adams. 1995. Dissolved organic matter quantity and quality from freshwater and saltwater lakes in east-central Alberta. *Biogeochemistry* **30:**59–76.

Curtis, P. J., and D. W. Schindler. 1997. Hydrologic control of dissolved organic matter in low-order Precambrian Shield lakes. *Biogeochemistry* **36:**125–138.

D'Arcy, P., and R. Carignan. 1997. Influence of catchment topography on water chemistry in southeastern Quebec Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **54:**2215–2227.

Dillon, P. J., and L. A. Molot. 1997a. Dissolved organic and inorganic carbon mass balances in central Ontario lakes. *Biogeochemistry* **36:**29–42.

Dillon, P. J., and L. A. Molot. 1997b. Effect of landscape form on export of dissolved organic carbon, iron, and phosphorus from forested stream catchments. *Water Resources Research* **33:**2591–2600.

Eckhardt, B. W., and T. R. Moore. 1990. Controls on dissolved organic carbon concentrations in streams, southern Quebec. *Canadian Journal of Fisheries and Aquatic Sciences* **47:**1537–1544.

France, R., R. Steedman, R. Lehmann, and R. Peters. 2000. Landscape modification of DOC concentration in boreal lakes: implications for UV-B sensitivity. *Water, Air, and Soil Pollution* **122:**153–162.

Gergel, S. E., M. G. Turner, and T. K. Kratz. 1999. Dissolved organic carbon as an indictor of the scale of watershed influence on lakes and rivers. *Ecological Applications* **9:**1377–1390.

Gorham, E., J. K. Underwood, J. A. Janssens, B. Freedman, W. Maass, D. H. Waller, and J. G. Ogden. 1998. The chemistry of streams in southwestern and central Nova Scotia, with particular reference to catchment vegetation and the influence of dissolved organic carbon primarily from wetlands. *Wetlands* **18:**115–132.

Hansell, D. A., and C. A. Carlson. 1998. Deep-ocean gradients in the concentration of dissolved organic carbon. *Nature* **395:**263–266.

Hedges, J. I., E. Mayorga, E. Tsamakis, M. E. McClain, A. Aufdenkampe, P. Quay, and J. E. Richey. 2000. Organic matter in Bolivian tributaries of the Amazon River: A comparison to the lower mainstream. *Limnology and Oceanography* **45:**1449–1466.

Hemond, H. F. 1990. Wetlands as the source of dissolved organic carbon to surface waters. *In* "Organic Acids in Aquatic Ecosystems" (E. M. Perdue and E. T. Gjessing, Eds.), pp. 301–313. Wiley, New York, NY.

Hinton, M. J., S. L. Schiff, and M. C. English. 1997. The significance of storms for the concentration and export of dissolved organic carbon from two Precambrian Shield catchments. *Biogeochemistry* **36:**67–88.

Hinton, M. J., S. L. Schiff, and M. C. English. 1998. Sources and flowpaths of dissolved organic carbon during storms in two forested watersheds of the Precambrian Shield. *Biogeochemistry* **41:**175–197.

Hope, D., M. F. Billett, and M. S. Cresser. 1994. A review of the export of carbon in river water: Fluxes and processes. *Environmental Pollution* **84:**301–324.

Hope, D, M. F. Billett, R. Milne, and T. A. W. Brown. 1997. Exports of organic carbon in British rivers. *Hydrological Processes* **11:**325–344.

Hornberger, G. M., K. E. Bencala, and D. M. McKnight. 1994. Hydrological controls on dissolved organic carbon during snowmelt in the Snake River near Montezuma, Colorado. *Biogeochemistry* **25:**147–165.

Houle, D., R. Carignan, and M. Lachance. 1995. Dissolved organic carbon and sulfur in southwestern Quebec lakes: Relationships with catchment and lake properties. *Limnology and Oceanography* **40:**710–717.

Kaplan, L. A., R. A. Larson, and T. L. Bott. 1980. Patterns of dissolved organic carbon in transport. *Limnology and Oceanography* **25:**1034–1043.

Kaplan, L. A., and T. L. Bott. 1982. Diel fluctuations of DOC generated by algae in a piedmont stream. *Limnology and Oceanography* **27:**1091–1100.

Kortelainen, P., S. Saukkonen, and T. Mattson. 1997. Leaching of nitrogen from forested catchments in Finland. *Global Biogeochemical Cycles* **11:**627–638.

Martin, J. H., and S. E. Fitzwater. 1992. Dissolved organic carbon in the Atlantic, Southern, and Pacific oceans. *Nature* **356:**699–700.

McDowell, W. H., and C. E. Asbury. 1994. Export of carbon, nitrogen, and major ions from three tropical montane watersheds. *Limnology and Oceanography* **39:**111–125.

McDowell, W. H., and T. Wood. 1984. Podzolization: Soil processes control dissolved organic carbon concentrations in stream water. *Soil Science* **137:**23–32.

Meybeck, M. 1988. How to establish and use world budgets of riverine materials. *In* "Physical and Chemical Weathering in Geochemical Cycles" (A. Lerman and M. Meybeck, Eds.), pp. 247–272. Kluwer Academic, Dordrecht.

Meybeck, M. 1993. Riverine transport of atmospheric carbon: sources, global typology and budget. *Water, Air, and Soil Pollution* **70:**443–463.

Moeller, J. R., G. W. Minshall, K. W. Cummins, R. C. Petersen, C. E. Cushing, J. R. Sedell, R. A. Larson, and R. L. Vannote. 1979. Transport of dissolved organic carbon in streams of differing physiographic characteristics. *Organic Geochemistry* **1:**139–150.

Mulholland, P. J. 1992. Regulation of nutrient concentrations in a temperate forest stream: Roles of upland, riparian, and instream processes. *Limnology and Oceanography* **37:**1512–1526.

Mulholland, P. J. 1997. Dissolved organic matter concentration and flux in streams. *Journal of the North American Benthological Society* **16:**131–141.

Mulholland, P. J. and W. R. Hill. 1997. Seasonal patterns in streamwater nutrient and dissolved organic carbon concentrations: Separating catchment flow path and in-stream effects. *Water Resources Research* **33:**1297–1306.

Mulholland, P. J., and E. J. Kuenzler. 1979. Organic carbon export from upland and forested wetland watersheds. *Limnology and Oceanography* **24:**960–966.

Mulholland, P. J. and J. A. Watts. 1982. Transport of organic carbon to the oceans by rivers of North America: a synthesis of existing data. *Tellus* **34:**176–186.

Mulholland, P. J., L. A. Yarbro, R. P. Sniffen, and E. J. Kuenzler. 1981. Effect of floods on nutrient and metal concentrations in a Coastal Plain stream. *Water Resources Research* **17:**758–764.

Mulholland, P. J., G. V. Wilson, and P. M. Jardine. 1990. Hydrogeochemical response of a forested watershed to storms: effects of preferential flow along shallow and deep pathways. *Water Resources Research* **26:**3021–3036.

Opsahl, S., and R. Benner. 1997. Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature* **386:**480–482.

Rasmussen, J. B., L Godbout, and M. Schallenberg. 1989. The humic content of lake water and its relationship to watershed and lake morphometry. *Limnology and Oceanography* **34:**1336–1343.

Raymond, P. A., and J. E. Bauer. 2001. Riverine export of aged terrestrial organic matter to the North Atlantic Ocean. *Nature* **409:**497–500.

Rosenfeld, J. S., and J. C. Roff. 1992. Examination of the carbon base in southern Ontario streams using stable isotopes. *Journal of the North American Benthological Society* **11:**1–10.

Rounick, J. S., M. J. Winterbourn, and G. L. Lyon. 1982. Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: A stable carbon isotope study. *Oikos* **39:**191–198.

Sakamoto, T., M. Takahashi, T. Terajima, Y. Nakai, and Y. Matsuura. 1999. Comparison of the effects of rainfall and snowmelt on the carbon discharge of a small, steep, forested watershed in Hokkaido, northern Japan. *Hydrological Processes* **13:**2301–2314.

Schiff, S., R. Aravena, E. Mewhinney, R. Elgood, B. Warner, P. Dillon, and S. Trumbore. 1998. Precambrian Shield wetlands: Hydrologic control of the sources and export of dissolved organic carbon. *Climatic Change* **40:**167–188.

Schiff, S. L., R. Aravena, S. E. Trumbore, M. J. Hinton, R. Elgood, and P. J. Dillon. 1997. Export of DOC from forested catchments on the Precambrian Shield of Central Ontario: Clues from ^{13}C and ^{14}C. *Biogeochemistry* **36:**43–65.

Schindler, D. W., S. E. Bayley, B. R. Parker, K. G. Beaty, D. R. Cruikshank, E. J. Fee, E. U. Schindler, and M. P. Stainton. 1996. The effects of climatic warming on the properties of boreal lakes and streams at the Experimental Lakes Area, northwestern Ontario. *Limnology and Oceanography* **41:**1004–1017.

Schindler, D. W., P. J. Curtis, S. E. Bayley, B. R. Parker, K. G. Beaty, and M. P. Stainton. 1997. Climate-induced changes in the dissolved organic carbon budgets of boreal lakes. *Biogeochemistry* **36:**9–28.

Schlesinger, W. H., and J. M. Melack. 1981. Transport of organic carbon in the world's rivers. *Tellus* **33:**172–187.

Spitzy, A. and J. Leenheer. 1991. Dissolved organic carbon in rivers. *In* "Biogeochemistry of Major World Rivers" (E. T. Degens, S. Kempe, and J. E. Richey, Eds.), pp. 213–232, Wiley, Chichester, UK.

Wallace, J. B., D. H. Ross, and J. L. Meyer. 1982. Seston and dissolved organic carbon dynamics in a southern Appalachian stream. *Ecology* **63:**824–838.

Willey, J. D., R. J. Kieber, M. S. Eyman, and G. B. Avery, Jr. 2000. Rainwater dissolved organic carbon: Concentrations and global flux. *Global Biogeochemical Cycles* **14:**139–148.

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7

The Speciation of Hydrophobic Organic Compounds by Dissolved Organic Matter

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I. INTRODUCTION

Dissolved organic matter (DOM) is ubiquitous to natural waters and is known to undergo a number of reactions of environmental interest. Among these reactions neutral hydrophobic organic contaminants (HOCs) can partition into or be bound to DOM. This process can significantly alter the

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fate of HOCs by rendering them mobile and changing their bioavailability. The degree to which this occurs depends on the type and amount of DOM present. In many aquatic systems in which DOM levels are low in the water column (≤0.5 mM carbon), the impact of HOC–DOM complexes on ecosystems is expected to be minimal. Conversely, other environments may have high levels of DOM, which could affect the fate of HOC. In sediments, for example, porewater concentrations of DOM can exceed 3–4 mM carbon (Brownawell and Farrington, 1986; Chin and Gschwend, 1991; McGroddy *et al*., 1995; Chin *et al*., 1998), and the amount of DOM present may play a significant role in the bioavailability of HOCs to benthic organisms and the ability of microorganisms to degrade them.

In the early to mid 1980s it was widely believed that DOM played an important role in the mobilization of HOCs in surface and subsurface waters (Carter and Suffet, 1982; Hassett and Anderson, 1982). These beliefs were, in part, based upon the perceived existence of "super colloids" comprising of DOM or DOM adsorbed onto submicron particles capable of very strongly binding even weakly hydrophobic HOCs (Voice and Weber, 1985; Means and Wijayaratne, 1982, 1984). Research conducted in the later 1980s, however, have shown that many of the unusually high binding constants reported in the literature may have been the result of analytical artifacts (Gschwend and Wu, 1985; Morel and Gschwend, 1987; Chin and Weber, 1989; Chin *et al*., 1990). To date there have been no field studies showing DOM-enhanced mobilization of HOCs, but substantial circumstantial evidence exists from laborating studies, and it is plausible that DOM — mediated transport of HOCs can occur under the proper conditions (McCarthy and Zachara, 1989; Backhus and Gschwend, 1990). As stated previously these include environments in which DOM concentrations are high and the type of HOCs present will readily form strong complexes with the DOM substrate. Moreover, the composition of the DOM also plays an important role as will be demonstrated elsewhere in this chapter. Examples of such aquatic systems include wetlands (Chin *et al*., 1997, 1998), "blackwater" rivers, and sediment porefluids (Brownawell and Farrington, 1986; Chin and Gschwend, 1992; McGroddy *et al*., 1995; Burgess *et al*., 1996a, b). Finally, evidence in the literature suggests that the binding of DOM can influence the bioaccumulation of HOCs. Under most circumstances DOM renders a fraction of the HOC less bioavailable. Conversely, a smaller number of studies reported an increase in contaminant uptake at surprisingly low DOM concentrations (Haitzer *et al*., 1998). Thus, whereas understanding the nature of the DOM–HOC complex is necessary to assess the effect of DOM on bioavailability, other processes may also play a role.

Recent work involving the binding of HOCs to DOM has also centered on how the binding phenomenon may enhance the degradation of the contaminant. A number of studies have suggested that this can occur in sunlight through photoreduction processes (Burns *et al*., 1996, 1997; Friesen *et al*.,

1996), whereby the formation of the DOM–HOC complex is a precursor to the subsequent transformation of the pollutant by photoreduction. Finally, several investigators (Weber, 1996; Lindsey and Tarr, 2000) have shown that the presence of DOM can inhibit the transformation of HOCs by other pathways, for example, chemical reduction. Although a specific mechanism has not been identified for this phenomenon, the formation of DOM–HOC complexes may remove the target contaminant from the reactive species.

In this chapter, the speciation of HOC in the presence of DOM is critically reviewed. Specifically, models will be examined that describe the equilibrium partitioning of HOCs between water and the DOM phase, current analytical techniques for measuring DOM–HOC binding will be critically evaluated, and the importance of DOM's structure in influencing the speciation of HOCs in aquatic systems will be discussed. Finally, the degree to which DOM can influence the bioavailability of organic contaminants to organisms is examined.

II. THEORETICAL CONSIDERATIONS

The partitioning phenomenon is the most elementary model used to describe DOM–HOC interactions; it can be quantified using a simple equilibrium type relationship

$$K_{dom} = C_{dom}/C_w, \tag{1}$$

where K_{dom} is the HOC–DOM binding constant and C_{dom} and C_w are the masses of HOC in the DOM and water phases, respectively. In terms of solute activity coefficients in the respective phases (γ_w and γ_{dom}), we can rewrite Equation 1 as

$$\ln K_{dom} = \ln \gamma_w - \ln \gamma_{dom} + \ln V_w/V_{dom} \tag{2}$$

where V_w and V_{dom} are the molar volumes for water and the DOM phase, respectively. Equation 2 is only valid for cases in which the entropy of mixing is ideal, that is, when the sizes of the molecules in the organic phase and the solutes are approximately the same. DOM components, however, can be substantially larger than many HOCs (greater than 1 order of magnitude in some cases), and the entropy of mixing deviates from ideal behavior. Assuming that the DOM phase comprise large humic substances, the Flory–Huggins equation, originally developed to describe the interaction between polymers and an organic solvent, can be applied. To account for the nonideal entropy of the mixing term, the solute and solvent volume fractions (ϕ_i and ϕ_p) are used in lieu of the mole fraction (X_i). As a result Equation 2 becomes

$$\ln K_{dom} = \ln \gamma_w + \ln (V_w/V_i) - \ln \rho - (\phi_p(1 - V_i/V_{om}) + \chi)\phi_p^2, \tag{3}$$

where ρ is the density of the humic phase, V_i the solute molar volume, and χ is the Flory parameter. Details regarding the derivation of Equation 3 can be found in Chiou *et al.* (1983); Chin and Weber (1989), and Spurlock and Biggar (1994).

One of the simplifying assumptions of this model is that the ratio of the solute and humic molar volumes (i.e., V_i/V_{om}) is small and can be ignored. For soil organic matter this assumption is probably valid (Chiou *et al.*, 1983; Spurlock and Biggar, 1994); however, for aquatic humic substances the molar volumes are significantly smaller based upon newly reported molecular weights for these substances (from <1000 to about 4000 amu) (Chin *et al.*, 1994; Pelekani *et al.*, 1999; Specht and Frimmel, 2000) and the assumed density of 1.2 g/mL (Chiou *et al.*, 1983), which results in DOM molar volumes of approximately 800–3000 mL/mol). Given that the molar volumes of many HOCs range from less than 100 to 200–300 mL/mol, this ratio can become significant for the binding of large HOCs to small DOM molecules. With respect to the earlier literature, many of the modeling efforts examined the large database measured for the speciation of HOCs by commercial humic acids (e.g., those supplied by Aldrich or Fluka). Because the molecular weights of these substances are significantly larger than those of aquatic fulvic acids (weight-average molecular weight of about 4500; Chin *et al.*, 1994), the molar volume ratio is small enough to be neglected. In reassessing binding to smaller aquatic DOM substrates, the molar volume ratio must be taken into consideration if such values can be obtained from the literature or measured.

The Flory parameter is in essence analogous to the activity coefficient of the HOC in the DOM phase and consists of two components χ_h and χ_s, which are the enthalpic and entropic contributions, respectively:

$$\chi = \chi_h + \chi_s. \tag{4}$$

The χ_h term quantifies the nonideal heat of mixing between the HOC and DOM phase and is typically quantified using the one-solubility parameter Scatchard–Hildebrand equation

$$\chi_h = V_i(\delta_i - \delta_{om})^2/RT, \tag{5}$$

where δ_i and δ_{om} are the solubility parameters (in $(cal/ml)^{0.5}$) for the HOC and DOM, respectively. Chiou and co-workers (1983) elucidated δ_{om} for soil organic matter (SOM) using experimental HOC sorption equilibrium constants. They reported an average value of 13 whereas Karickhoff (1984) estimated a value of 10 based upon dichloromethane's interaction with SOM. More recently, Kopinke and co-workers (1995) elucidated the solubility parameters for humic and fulvic acids derived from partition data obtained for toluene and trichloroethene. The range of values determined from these two HOCs are remarkably similar with humic acids, but are smaller (12.65 and 12.8) than those reported for fulvic acids (13.75–14.6). Again these

values are consistent with what we know about humic and fulvic acids; that is, humic acids are more nonpolar and better phases for HOC partitioning processes than fulvic acids.

One limitation of the one-solubility parameter model is that it assumes that the solute can only interact with the organic matter through London forces. Although this assumption may be reasonable for SOM, DOM is typically more polar and can participate in other types of van der Waals interactions. These include permanent dipole-induced dipole (Debye) and permanent dipole-permanent dipole (Keesom) interactions in which the degree of binding that occurs depends on the polarizability of the DOM (Gauthier *et al*., 1987; Uhle *et al*., 1999). To account for these types of interactions Chin and Weber (1989) segregated the solubility parameter terms into three components to account for all these different types of molecular interactions to χ_h,

$$\chi_h = V_i[(\delta_i - \lambda_p) + \tau_p^2 - 2\psi]/RT, \quad (6)$$

where λ, τ, and ψ are the London, Keesom, and Debye solubility parameters for the DOM phase, respectively, and cumulatively represent all components of a van der Waals force (Chin and Weber, 1989; Spurlock and Biggar, 1994). To use this approach, some structural aspects of the DOM phase need to be considered. Chin and Weber (1989) used two analogs, methylsalicylic acid ($\lambda = 7.8$, $\tau = 7.16$) and polymaleic acid (PMA) ($\lambda = 8.09$, $\tau = 10.9$) to describe the interactions of HOC between a commercial humic acid (Aldrich) and aquatic DOM, respectively. The Debye solubility parameter is determined empirically from the product of λ and τ (Hildebrand *et al*., 1970). By using the available data, predictions of K_{dom} using methyl salicylate were in good agreement with experimentally determined values for all the nonpolar HOCs and Aldrich humic acid (Fig. 1). Because of its high aromatic composition, methyl salicylic acid was presumably a good quasi-monomeric analog for Aldrich humic acid. For the two substituted phenols, however, the model significantly underpredicted the measured values. Given the ability for these compounds to participate in hydrogen bonds with water, their aqueous activity coefficients may not be constant as a function of concentration. Moreover, given the very narrow range of K_{dom} values reported by Ohlenbusch and co-workers (2000) for a series of substituted phenols that span K_{ow} values of 4 orders of magnitude, interactions between the phenols and humic materials may be more complex than simple van der Waals type interactions.

PMA was selected based upon structural similarities between it and some types of DOM (Spiteller and Schnitzer, 1983). Recently, however, several investigators have demonstrated that PMA may not have as many structural similarities to DOM (specifically fulvic acids) as once believed (Hess and Chin, 1996; Kilduff *et al*., 1996). These new data may explain, in part, the poorer estimates of K_{dom} reported by Chin and Weber (1989)

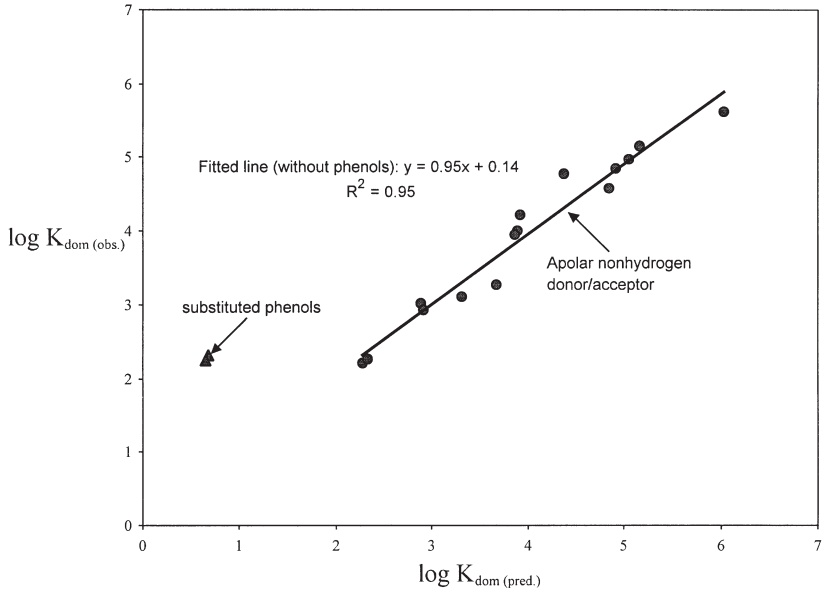

FIGURE 1 Predicted and observed DOM binding constants using the Flory–Huggins equation and methylsalicylic acid as a DOM analog.

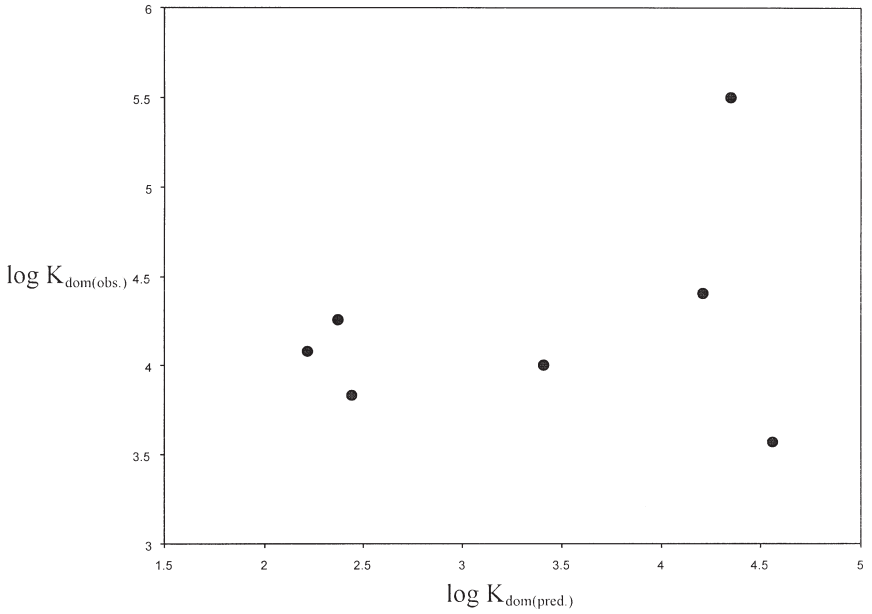

FIGURE 2 Predicted and observed DOM binding constants using the Flory–Huggins equation and polymaleic acid as a DOM analog.

for aquatic fulvic acids. Moreover, it is highly likely that lack of information regarding the HOC/DOM molar volume ratio may also influenced the model's ability to accurately predict K_{dom}. To test the suitability of this humic analog, predicted K_{dom} values for a variety of compounds reveal much poorer correlation to measured values for a variety of DOMs reported in the literature (Fig. 2). To date there exists no suitable synthetic polymer that accurately mimics the behavior of DOM. Until such a substance can be identified, estimation of K_{dom} is limited to studying the binding of HOCs to either SOM (using the one solubility parameter approach) or to commercial humic acids, which appear to be more uniform in composition. This approach is still useful, however, in that the vast majority of organic pollutant bioaccumulation studies conducted in the presence of DOM have been done using commercial humic acids. Although such an approach may not represent the actual effects of DOM on organic contaminant bioavailability in natural waters, it does provide a reference point for other studies that use aquatic DOM sources (Haitzer *et al.*, 1998). In a subsequent section of this chapter, recent evidence suggesting that steric and polarization effects (beyond what can attributed to partitioning) may influence binding is presented.

III. ANALYTICAL METHODS FOR MEASURING K_{dom}

To date a myriad of techniques exist for measuring the equilibrium binding between hydrophobic organic contaminants and DOM. Each method has its strengths and weaknesses, and some have serious flaws, thereby making the reported binding constants suspect. Typically the measurement of K_{dom} is based upon (1) static systems in which the analyte is measured in an unperturbed state of equilibrium between the DOM and aqueous phase and (2) dynamic systems whereby the analyte–DOM complex is separated from the free analyte in solution. Techniques using the latter approach are prone to disequilibrium artifacts because one assumes that the complex will remain stable over the time period required for the separation. The next sections discuss a range of more commonly used methods in detail and highlight their respective strengths and weaknesses.

A. Solubility Enhancement

The solubility enhancement method is the most straightforward "static" technique in which the "apparent" solubility (S^*_w) of a HOC is measured in the presence of increasing amounts of DOM (Chiou *et al.*, 1986, 1987; Chin *et al.*, 1997; Uhle *et al.*, 1999). Briefly, the analyte is added in excess (10–100 times the reported aqueous solubility) to glass reactors by plating the solute out from a stock solution. Buffered DOM solutions at various concentrations are added to each tube along with a control reactor containing

no DOM. After a sufficient amount of time has elapsed to ensure that equilibrium is attained (determined by kinetic studies), the solutions are assayed for the compound by high-pressure liquid chromatography, gas chromatography, or scintillation counting (if radiolabeled compounds are used). In many cases a separate extraction step is needed to concentrate the analyte, and care must be taken to ensure that both the bound and free compounds are efficiently extracted. The apparent solubility is defined as the true aqueous solubility (S_w) plus the amount of analyte complexed to the DOM substrate:

$$S^*_w = S_w + S_w[\text{DOM}]K_{dom}. \tag{7}$$

Rearranging Equation 7 yields

$$S^*_w/S_w = 1 + K_{dom}[\text{DOM}], \tag{8}$$

where [DOM] is in kilograms per liter. The binding constant is then determined from a plot of DOM versus the ratio of the apparent and true aqueous solubilities. This method provides unambiguous values for K_{dom} because it involves no separation of the DOM from the aqueous phase and it measures both free and bound analyte.

The solubility enhancement approach has obvious advantages, but it also has limitations. The method is time consuming and difficult to execute despite the rather simplistic experimental design. For example, insoluble analyte crystals may be inadvertently be extracted, which results in higher than expected values of S^*_w. For those analytes that are slightly volatile, excessive losses of the analyte could occur during the plating process (Uhle *et al*., 1999). Moreover, a large amount of DOM is needed for each experiment and because of the difficult nature of obtaining these materials, such an approach may not be feasible for many investigators. Finally, the solubility enhancement technique cannot be used to determine the K_{dom} values for the less hydrophobic HOCs possessing *n*-octanol/water (K_{ow}) partition coefficients of $10^{4.5}$ or less. Chiou and co-workers (1986) reported no solubility enhancement for trichlorobenzene (log K_{ow} ~ 4.0) even at high DOM concentrations (>60 mg/L). Although it is certainly possible to use very high concentrations of DOM (hundreds of milligrams per liter) the scarcity of the material coupled with processes such as DOM precipitation at such high concentrations would render this approach impractical. Nonetheless, reliable measurements of K_{dom} can be achieved using this method for many HOCs if the amount of DOM available is not limiting.

B. Dialysis Techniques

The dialysis method is another technique that measures the distribution of a HOC in static equilibrium with the DOM substrate (Carter and Suffet, 1982; Chin and Weber, 1989; Arnold *et al*., 1999; O'Loughlin *et al*., 2000).

Unlike the solubility enhancement approach, the dialysis membrane physically separates the DOM substrate from the aqueous phase. This is accomplished by using a membrane pore with a size small enough to retain the DOM phase but large enough to allow the analyte to pass through freely. After equilibrium is achieved the free analyte (C_w) is assayed outside the membrane while both the free and bound HOCs will be present inside the membrane. Subtraction of the free analyte concentration from its concentration within the membrane yields the bound HOC (C_{dom}). Unlike the solubility enhancement method, the use of dialysis bags allows one to conduct the experiments at solute concentrations below their aqueous solubility. Moreover, by varying the initial HOC concentration in each reactor containing the dialysis bag, one can conduct the experiment at one DOM concentration. The bound and free analytes can then be fitted to a linear isotherm, that is, Equation 1.

The dialysis technique has several advantages over the solubility enhancement method including the use of significantly less DOM for each experiment (provided that small volume bags are used), and the addition of the analyte at levels below their aqueous solubility concentration (which circumvents the measurement of errant undissolved solute crystals). Moreover, higher concentrations of DOM can be used, and it becomes possible to measure the K_{dom} values for weaker binding HOCs (Chin and Weber, 1989). Even with these advantages this method is prone to analytical problems. First and foremost is leakage of the DOM from the dialysis bag. This can occur at the points where the bag is secured or across the membrane itself. Because molecular weights of most DOMs are on the order of several hundred to several thousand daltons (Aiken and Malcolm, 1987; Chin *et al*., 1994; Pelekani *et al*., 1999; Specht and Frimmel, 2000), no ideal dialysis membrane exists. Membrane sizes on the order of several hundred daltons have pore sizes that are insufficiently large to allow the largest HOCs to pass through freely. Indeed, even for small analytes (100 Da or less) the times to equilibrium may range from weeks to months. Conversely, larger membrane pore sizes (up to 1000 Da) may result in excessive loss of the smaller DOM components. To date leakage of DOM has not been thoroughly evaluated, but it may impact the quality of the data generated, resulting in lower than expected partition coefficients. The limited binding data measured using this method do, however, appear to agree with values determined by solubility enhancement for identical HOCs bound to similar substrates, for example, commercial humic acids (Chiou *et al*., 1987; Chin and Weber, 1989; Chin *et al*., 1997).

C. Fluorescence Quenching

Fluorescence quenching is a popular technique for measuring K_{dom} values for polycyclic aromatic hydrocarbons (PAHs) (Gauthier *et al*., 1986, 1987;

Backhus and Gschwend, 1990; Chin and Gschwend, 1992; Herbert *et al.*, 1993; Schlautman and Morgan, 1993). This method is fast and requires only small quantities of sample, but can only be used to measure K_{dom} values for fluorescing organic compounds. Briefly, fluorescence is the process through which photoexcited state molecules are able to return to the ground state through the emission of light at wavelengths longer than the excitation wavelength. If the fluorescing analyte forms a complex with DOM, its fluorescence can be quenched, a process known as static quenching. Typically, this is assumed to be an efficient process; that is, all PAH–DOM complexes do not fluoresce. Data from Backhus and Gschwend (1992), however, have shown that static quenching may not be 100% efficient for certain organic substrates, but appeared to be so for humic acids. If one assumes a high efficiency of quenching by DOM, then the ratio of the PAH fluorescence emission intensity in the absence and presence of DOM reflects the degree of binding that can occur between the PAH and DOM phases. Fluorescence quenching data can be fitted to the static version of the Stern–Volmer equation to elucidate the binding constant (Lackowicz, 1983)

$$\frac{F^0}{F} = 1 + K_{dom}[\text{DOM}], \tag{9}$$

where F^0 and F are probe fluorescence values in the absence and presence of a static quencher, respectively. In using this approach, one assumes that all quenching by DOM is static in nature. Typically, however, values obtained by this method are higher than K_{dom} values measured by other static equilibrium methods for identical PAHs and DOM substrates (Danielsen *et al.*, 1995; Doll *et al.*, 1999; Kopinke *et al.*, 2000). Thus, this method may be subject to artifacts because it only measures the fluorescence of the analyte and not the analyte itself.

Quenching of PAHs by chemical species can also occur through collisional processes (i.e., dynamic quenching). Molecular oxygen is a ubiquitous collisional quencher capable of quenching nearly any type of fluorescing molecule (Lackowicz, 1983). Many PAH–DOM studies using fluorescence quenching did not appear to take into account the effects of molecular oxygen as a potential PAH quencher (Gauthier *et al.*, 1986, 1987; Backhus and Gschwend, 1990; Chin and Gschwend, 1992; Schlautman and Morgan, 1993). Presumably, the effects of dynamic quenching by molecular oxygen or other species were assumed to be small (given its low solubility in water) and/or were accounted for in separate control experiments. This later assumption was reasonable if dynamic quenching of the probe by molecular oxygen was believed to be independent of static quenching processes by the humic materials. Moreover, deviations from the Stern–Volmer equation (i.e., nonlinear quenching) are indicative of both dynamic and static quenching occurring concurrently. In the event that dynamic and static quenching can be attributed to one quencher (i.e., DOM), both the static quenching constant

(equivalent to K_{dom} for purposes of this work) and the collisional quenching constant K_d can be described using the equation

$$(F_o/F - 1)/[\text{DOM}] = (K_d + K_{dom}) + K_d K_{dom}[\text{DOM}]. \quad (10)$$

The values for K_d and K_{dom} can be determined using the equation

$$K_{dom}^2 - K_{dom} I + S, \quad (11)$$

where I is equal to $(K_d + K_{dom})$ and S is equivalent to $K_d K_{dom}$ (Lackowicz, 1983). At very high humic substance concentrations ($>3 \times 10^{-5}$ kg/L), Danielsen and co-workers (1995) observed deviations from Equation 9, but were unable to elucidate K_{dom} using Equation 11, because values of I and S were unsolvable. These results suggest that dynamic quenching by DOM itself is not important and that the presence of a second collisional quencher may be responsible for our observations. PAH lifetime measurements made by other investigators (Morra *et al.*, 1990; Puchalski *et al.*, 1992) have also shown that collisional quenching by humic and fulvic acids are not significant.

In the presence of multiple quenchers the collisional quenching processes can only be quantified by measuring changes in a fluorescent probe's lifetime in the presence of a quencher. As stated previously, fluorescence lifetime measurement of PAH compounds from other investigators (Morra *et al.*, 1990; Puchalski *et al.*, 1992) exhibited no change in the presence of increasing concentrations of humic substances. In an effort to minimize the effects of dissolved oxygen as a quencher, fluorescent lifetime experiments are generally conducted in the presence of an inert gas (argon) (Morra *et al.*, 1990). From our observations of the literature it *appears* that most fluorescence quenching experiments are conducted without such precautions for the reasons stated previously. Danielsen and co-workers (1995) observed significant changes in the lifetime of pyrene in the presence of DOM and molecular oxygen at equilibrium with the overlying air over controls containing only pyrene and molecular oxygen. Indeed, they reported no dynamic quenching of pyrene by DOM in the absence of oxygen, which corroborates the findings of Morra and co-workers (1990).

Work conducted by Tiller and Jones (1997) demonstrated that the fluorescence of PAHs decayed over time under both under anoxic and oxic conditions. Typically, however, the presence of dissolved oxygen had a more pronounced influence on baseline fluorescence decay for all the PAHs studied. Moreover, certain PAHs (pyrene and anthracene) were more susceptible to this phenomenon than others. To date a mechanism to explain this phenomenon has not been identified, but it is probably a combination of complex pathways including the reaction of the analyte with reactive oxygen species formed from the excited triplet state DOM and the direct photolysis of the analyte by the excitation light source. Thus, the application of fluorescence quenching for measuring K_{dom} is probably limited to systems, which can be analyzed under anoxic conditions.

D. Other Static Methods

Several other static methods have been developed recently. Two of these approaches, solid phase microextraction (SPME) and complexation–flocculation, show particular promise (Poerschman *et al*., 1997; Laor and Rebhun, 1997). The SPME method uses a fused silica fiber coated with an organic matrix (polyacrylate or polydimethylsiloxane) inserted in a needle. The needle is inserted pass a septum into the solution phase containing DOM (or no DOM for the controls) and the analyte. After equilibration the fiber is withdrawn and directly injected into a gas chromatograph where the analyte is thermally desorbed from the SPME. For volatile compounds the headspace can be analyzed provided that the Henry's law constant is known. Based upon the DOM concentration in the aqueous phase the amount of compound extracted by SPME can be compared with concentrations in the control samples (no DOM) and with Equation 8 K_{dom} can be determined (Poerschman *et al*., 1997; Doll *et al*., 1999). One advantage of this method is that K_{dom} values can be measured for those HOCs that are more water-soluble, that is, those with smaller octanol/water partition coefficients. Indeed Ohlenbusch and co-workers (2000) were able to measure the K_{dom} values for a number of fairly water-soluble substituted phenols to Aldrich humic acid. Surprisingly, there appeared to be little variability in the range of K_{dom} values measured (less than 1 order of magnitude from 3-methoxyphenol, the most polar, to pentachlorophenol, the most hydrophobic) for the neutral species, while their K_{ow} values ranged over more than 3 orders of magnitude (from log K_{ow} of 1.57 to 5.01). Other potential problems exist such as cases where the measured K_{dom} values of the analytes exceeded their K_{ow} values, implying a DOM phase that is more hydrophobic than that of octanol. Finally, it is unclear whether the DOM phase can interfere with the analyte mass transfer from solution to the solid phase. Thus, although this method shows promise, its universal applicability for measuring organic compounds–DOM speciation is still under investigation.

The coagulation–flocculation (CF) method relies on the removal of the DOM phase by the addition of alum to form Al(III)–DOM complexes that coagulate and settle out of the water column. Although there is some controversy about whether this method is static or dynamic (because the coagulation process could alter the sorptive properties of the DOM and hence perturb equilibrium) (Giessl, 1999), the proponents of this method suggest that it is static (because equilibrium could be rapidly reestablished after the CF step). Laor and Rebhun (1997) reported PAH binding constants to Aldrich humic acid that were lower than those determined by fluorescence quenching. Indeed values measured using the CF technique were used to model the breakthrough of phenanthrene in the presence of 30 mg/L Aldrich humic acid in a soil column. The model fits to the data were statistically significant when K_{dom} values determined by the CF method were used.

Conversely, K_{dom} values modeled using fluorescence quenching binding constants significantly underestimated the breakthrough curves.

E. Reverse-Phase Chromatography

The reverse-phase chromatography method is a dynamic process whereby the free analyte is separated from the bound fraction after an initial equilibrium is achieved. The process uses a hydrophobic medium packed in either a small extraction column (e.g., Sep-Pak) or an equivalent product (Landrum *et al*., 1984, 1985, 1987; Evans, 1988; Eadie *et al*., 1990, 1992) packed in a larger glass column (Burgess *et al*., 1996a, 1996b; Burgess and Ryba, 1998). The principle of this method relies upon the separation of the bound phase from the free analyte. After a sufficient amount of time has been allowed for equilibrium to be attained between the HOC and the DOM phase, the solution is passed across the column. The free analyte is presumably sorbed by the reverse-phase medium while the HOC–DOM complex passes through the column. This method is predicated on the assumptions that (1) the HOC–DOM complexes are nonlabile and/or the kinetics of HOC–DOM uncoupling is significantly slower than its retention time in the column and (2) the sorption of DOM by the stationary phase is insignificant. If these conditions are satisfied the technique is simple and fast.

Recent evidence has suggested that the reverse-phase method may be subject to artifacts. Typically, DOM–HOC complexes are weak and may be subject to uncoupling from the DOM substrate under nonequilbrium conditions. Because the kinetics of HOC formation is relatively fast (seconds to over a minute) (Poerschman *et al*., 1997), the reverse reaction may be equally fast. Indeed Landrum and co-workers (1984) observed flow rate dependencies that suggests uncoupling of the DOM–HOC complex.

One other complicating factor is the formation of complexes of different strengths. Thus, desorption kinetics from the weaker complexes (i.e., London forces) maybe more rapid than those from the stronger sites (Debye, Keesom, or even hydrogen bonds). This phenomenon would lead to smaller than expected K_{dom} values because the stationary phase would capture both the free and labile phases. Indeed when K_{dom} values measured by the reverse-phase approach are compared with those determined by other static methods for the same HOC and DOM substrate the former results in lower values (Raber *et al*., 1998). Indeed, in some instances when fulvic acids are used as the substrate, the variability in K_{dom} values may be very small for a series of compounds with a large range of hydrophobicities. For example, Burgess and Ryba (1998) reported the binding of a number of polychlorinated biphenyl (PCB) congeners to a soil fulvic acid that did not vary by more than 1 order of magnitude, but whose octanol/water partition coefficients span 3 orders of magnitude. Even more unusual were the differences observed

between results obtained when different stationary phase bead sizes are used. The larger 3 mm diameter C_{18} beads resulted in K_{dom} values that were consistently 1 order of magnitude larger than values obtained with the smaller 0.075 mm beads. Experiments conducted with humic acids, however, resulted in K_{dom} values that were consistent with literature values determined by other methods (Burgess and Ryba, 1998). Other anomalous results were also observed by Eadie and co-workers (1990), where they observed little differences in K_{dom} values for a variety of different types of DOMs. Thus, whereas this method has advantages in speed and convenience, the K_{dom} values obtained are typically smaller and less consistent than those determined by other means.

Recent work has shown that the C_{18} columns used can adsorb significant amounts of DOM. For example Roubeuf and co-workers (2000) have demonstrated that C_{18} preparative columns similar to those used in the reverse-phase binding studies can be used to isolate DOM from natural waters in a manner similar to the XAD chromatography method. The effect of DOM sorption to the C_{18} media has been reported to be as high as 25% and has been shown by Ozretich and co-workers (1995) to have a significant impact on the K_{dom} values. These investigators also reported that this phenomenon was responsible for the observed decrease in K_{dom} as DOM levels increased as observed by Landrum and co-workers (1994). By accounting for the fraction sorbed by the cartridges this artifact can be eliminated.

IV. THE EFFECT OF DISSOLVED ORGANIC MATTER COMPOSITION ON HYDROPHOBIC ORGANIC CONTAMINANT SPECIATION

Recent advances in the characterization of the physicochemical properties of humic substances have yielded insights into their ability to interact with HOCs. For example, the application of high-pressure size exclusion chromatography has allowed us to determine, with reasonable accuracy, the molecular weight distributions of aquatic humic and fulvic acids (i.e., relative to size determinations made by vapor pressure osmometry) (Chin *et al*., 1994; Pelekani *et al*., 1999; Specht and Frimmel, 2000). Moreover, it has been demonstrated that humic and fulvic isolates have low polydispersities (i.e., they reside in a narrow range of molecular weights) and that the larger materials have more aromatic moieties (Chin *et al*., 1994). The use of quantitative cross-polarization magic angle spin ^{13}C-nuclear magnetic resonance (NMR) along with the application of novel fluorescence spectroscopy methods as well as conventional ultraviolet/visible spectroscopy has revolutionized the way DOM is characterized (Gauthier *et al*., 1987; Traina *et al*., 1990; Chin *et al*., 1994; Orem and Hatcher, 1987; Chefetz *et al*., 2000; Cabaniss, 1992; Coble, 1996; McKnight *et al*., 2001).

To date many HOC binding studies have used DOM isolates such as fulvic or humic acid fractions. Because they have undergone extensive isolation by column chromatography, the further fractionation of these materials would yield little new information with respect to their binding propensity. A more productive approach would be to study the HOC binding properties to DOM as sorbents unique to their environment. Work by a number of investigators (Gauthier *et al*., 1987; Chiou *et al*., 1987; Chin *et al*., 1997; Tanaka *et al*., 1997) demonstrated that aquatic fulvic acids from higher plant sources are more capable of binding HOCs than those materials extracted from waters that receive little inputs of terrestrially derived organic carbon. More recent work by Raber and co-workers (1998) has demonstrated differences in PAH binding between humic materials derived from mineral soil and those isolated from forest floor materials. In all cases it appears that the degree to which HOC binding occurs is closely linked to the structural characteristics of the DOM substrate. Thus, one would expect to observe a relationship between K_{dom} and some unique quantitative property of the target DOM.

Prior to the widespread use of spectroscopic parameters for elucidating the structure of DOM, elemental analysis provided a rough estimate of some DOM properties such as polarity and aromaticity. Chiou and co-workers (1987) used the oxygen to carbon (O/C) ratio to demonstrate that the binding of HOCs to DOM was largely a function of the substrate polarity, where K_{dom} increased when the O/C value decreased. More recently Chin and co-workers (1997) observed an inverse trend between the O/C ratio and K_{dom} and attributed their findings to artifacts. They argued that polarity is not a valid parameter to describe fulvic acids isolated by XAD column chromatography because the isolation method fractionates the fulvic acid from the rest of the DOM phase based upon polarity. Thus, any DOM components isolated in this manner should possess similar polarity irrespective of its origin (terrestrial versus aquatic). Indeed the actual oxygen content for the wide range of fulvic acids used in the study did not differ by more than 4.1%. Perminova and co-workers (1999) corroborated this finding and observed a better correlation between H/C ratios and PAH K_{dom} values.

Work conducted by a number of investigators (Chin *et al*., 1997; Tanaka *et al*., 1997; Perminova *et al*., 1999) independently demonstrated that pyrene binding to DOM was positively correlated to the aromaticity of the DOM substrate (as quantified by both ^{13}C and proton NMR) using the solubility enhancement method (Fig. 3). Gauthier and co-workers (1987) also observed this effect for pyrene binding to humic materials as determined by fluorescence quenching. Moreover, all these investigators observed a similar relationship between K_{dom} and the DOM molar extinction coefficient measured at 280 or 254 nm (Fig. 4). The latter parameter has been shown by others (Gauthier *et al*., 1987; Traina *et al*., 1990; Chin *et al*., 1994) to be a reliable estimate of the substrate aromaticity in lieu of NMR

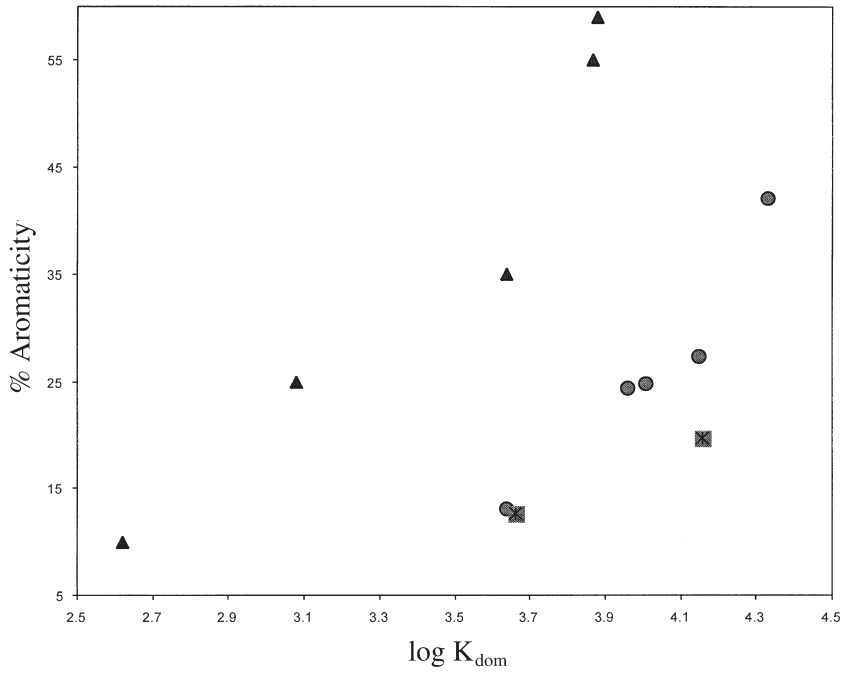

FIGURE 3 Relationship between the binding coefficient for various organic contaminants and DOM aromaticity (as quantified by NMR). ●, Chin *et al.* (1997); ▲, Tanaka *et al.* (1997); ■, Uhle *et al.* (1999).

data. Permoniova and co-workers (1999), however, argued that aromaticity measured by NMR was a better predictor of K_{dom} than the extinction coefficient. Indeed, recent work conducted by Uhle and co-workers (1999) has shown a dramatic difference in the PCB binding ability of a fulvic acid derived from a eutrophic Antarctic lake (which receives no higher plant inputs) to that isolated from a blackwater swamp. The blackwater swamp fulvic acid resulted in consistently higher binding for three PCB congeners relative to those measured for the Antarctic lake fulvic acid. The latter material has a much lower aromaticity (12.7%) than the swamp fulvic acid (19.7%) as determined by ^{13}C-NMR.

Several investigators (Liu and Amy, 1993; Johnson and Amy, 1995; Chin *et al.*, 1997) observed that the binding of HOCs to DOM depends on the molecular weight of the substrate. For example, Liu and Amy (1993) reported that phenanthrene binding was greater for the larger of two DOMs sampled from an aquifer in South Carolina. Chin and co-workers (1997) in a study using a variety of fulvic acids that spanned a large range of molecular weights (from <1000 to >4000 Da) observed similar results

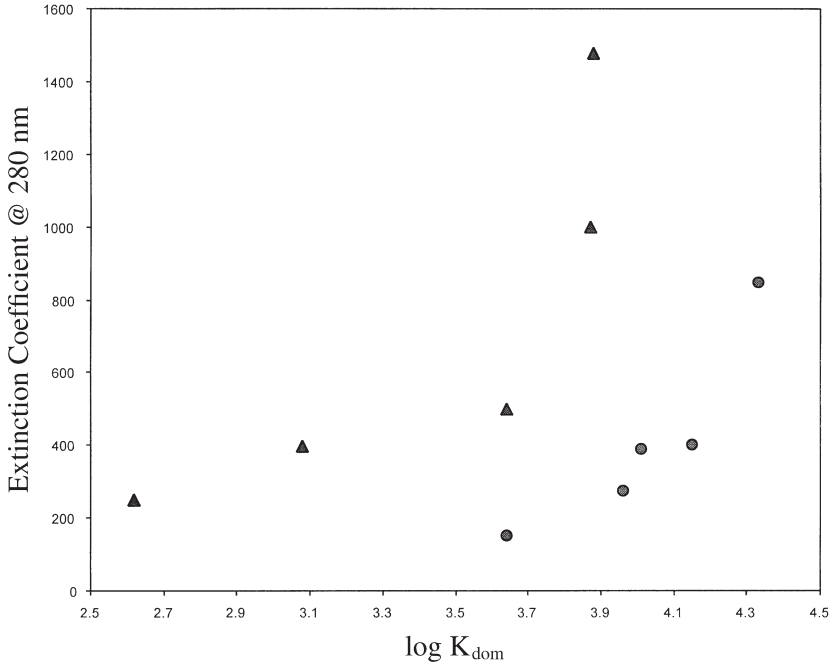

FIGURE 4 Relationship between the binding coefficient for various organic contaminants and the DOM extinction coefficient at 280 nm. ●, Chin *et al.* (1997); ▲, Tanaka *et al.* (1997); ■, Uhle *et al.* (1999).

for the speciation of pyrene and were able to develop a strong correlation to weight-average molecular weight. Unfortunately, weight-average molecular weights of many DOM isolates also correlate positively to aromaticity; that is, larger DOM components typically are enriched with aromatic moieties (Chin *et al.*, 1994; Peuravuori and Pihlaja, 1997), and one cannot delineate between contributions from these two properties.

In addition to the composition of the DOM phase, other properties of a HOC molecule beyond simple hydrophobic effects can influence binding. Gauthier and co-workers (1987) reported that an increase in the polarizability of aromatic rich dissolved organic matter could increase the van der Waals interactions between the substrate and HOC. This may in part explain the increased K_{dom} values reported for the more aromatic DOM phases. Uhle and co-workers (1999) also evoked such a process coupled with effects associated with the steric configuration of the analyte. For example, they observed differences in K_{dom} between two tetrachlorobiphenyl congeners. The co-planar congener (3, 3′, 4, 4′-PCB) was more strongly bound by both DOM substrates used in the study, whereas the ortho substituted compound

(2, 2′, 5, 5′-PCB) possessed a significantly lower K_{dom} value. They hypothesized that the lack of free rotation around the 1,1′ carbon bond in the later PCB congener (due to the presence of the chlorines in the ortho positions) resulted in a nonplanar rigid molecule that would be less susceptible to DOM polarization effects. Thus, it is apparent that both the structures of the DOM phase and the analyte play important roles in binding processes.

V. EFFECTS OF DISSOLVED ORGANIC MATTER ON HYDROPHOBIC ORGANIC CONTAMINANT BIOAVAILABILITY TO AQUATIC ORGANISMS

A number of studies have demonstrated that DOM plays a role in altering the bioavailability of HOCs to a number of organisms including amphipods (Landrum *et al*., 1985, 1987) fish (Spacie *et al*., 1983; McCarthy and Jimenez, 1985; Muir *et al*., 1994; Lorenz *et al*., 1996), and water fleas (Leversee *et al*., 1983; Kukkonen *et al*., 1989; Oikari and Kukkonen, 1990). In most of the studies the presence of DOM resulted in a beneficial effect with a decrease in uptake of the target contaminant. The degree to which this occurs depends upon both the properties of the DOM and organic contaminant, but data obtained for these studies are difficult to interpret. For example, the role of organic waste products from the organisms in the bioaccumulation process is unknown. Nonetheless, many investigators reported a reduction in the bioconcentration factor for those contaminants with the highest octanol/water partition coefficients in a specific system. Structural variability between contaminants with similar octanol/water partition coefficients (i.e., molecules with planar versus nonplanar configurations), however, have not been investigated to date. Because differences in molecular configuration played an important role in the relative binding of pollutants with similar K_{ow} values, one would expect to see a similar effect for bioavailability.

Many of the bioavailability studies conducted to date have relied upon the use of commercial humic acids. Because of their availability, cost, and ease of preparation these substances are ideally suited for these experiments that may (depending upon the organism) require large volumes of water. Nonetheless, Haitzer and co-workers (1998) reported that studies which use commercially available humic acids found consistently larger reductions in the bioavailability than similar studies which used either unaltered surface water or DOM extracted from natural sources (soil or water). These results appear consistent with the greater binding capacity of the commercial humic acids relative to naturally derived DOM. Differences in the ability for naturally derived DOM to reduce the bioavailability of organic pollutants to organisms have yielded mixed results. Landrum and co-workers (1987) found large DOM variability in the bioconcentration of PAHs to

benthic amphipods. Kukkonen and co-workers (1989), however, observed little variability in the bioavailability of benzo(*a*)pyrene to *Daphnia magna* in the presence of four different types of natural DOM. To date, however, there has been no comprehensive study that correlates the reduction in the bioaccumulation of organic contaminants to a specific organism to the structural characteristics of the DOM substrate (as determined by spectroscopy).

A few studies (Kukkonen *et al*., 1989; Kukkonen and Oikari, 1991; Muir *et al*., 1994) have reported an enhancement of organic contaminant bioavailability to various organisms. In all cases this occurred at low DOM concentrations (< 10 mg/L carbon). Currently, a specific mechanism has not been determined, but Muir and co-workers (1994) have suggested that organic mucus in fish can act in a manner similar to that of DOM and bind contaminants. They observed "lower than expected" bioaccumulation of pyretheroids in control studies followed by an enhancement with the addition of small amounts of DOM. Nonetheless, this does not explain the enhanced bioaccumulation observed for other aquatic organisms.

VI. CONCLUSIONS

Dissolved organic matter is capable of influencing the speciation of hydrophobic organic compounds in natural waters. Based upon the previous discussion, the degree to which this occurs is highly variable, but depending upon the general structure of DOM (as determined by spectroscopy and other analytical methods) and the properties of the analyte, rough estimates of K_{dom} can be determined using both empirical and semiempirical methods. The importance of this process, however, depends largely on the system of interest. To illustrate this point, the percentage of HOC bound to DOM can be estimated by

$$\% \text{ bound} = [\text{DOM}]K_{dom}/(1 + [\text{DOM}]K_{dom}) \times 100, \qquad (12)$$

where DOM is quantified as kilograms per liter of organic carbon. By using K_{dom} values measured for an algal derived DOM, a blackwater swamp fulvic acid, and sediment porewater DOM for pyrene and a PCB congener, the amount of each HOC bound can be determined for its respective environment (Fig. 5). For the groundwater case, the K_{dom} for the algal DOM is used because the properties of groundwater DOM are similar to those of material isolated from eutrophic environments. In aquatic environments in which the DOM concentration is low coupled with small binding constants the effect of the DOM phase is minimal on HOC speciation. In unique environments with high DOM the amount of HOC bound can be significant. Thus, the degree to which facilitated DOM-mediated transport or transformation can occur is highly specific to the HOC and DOM substrate properties.

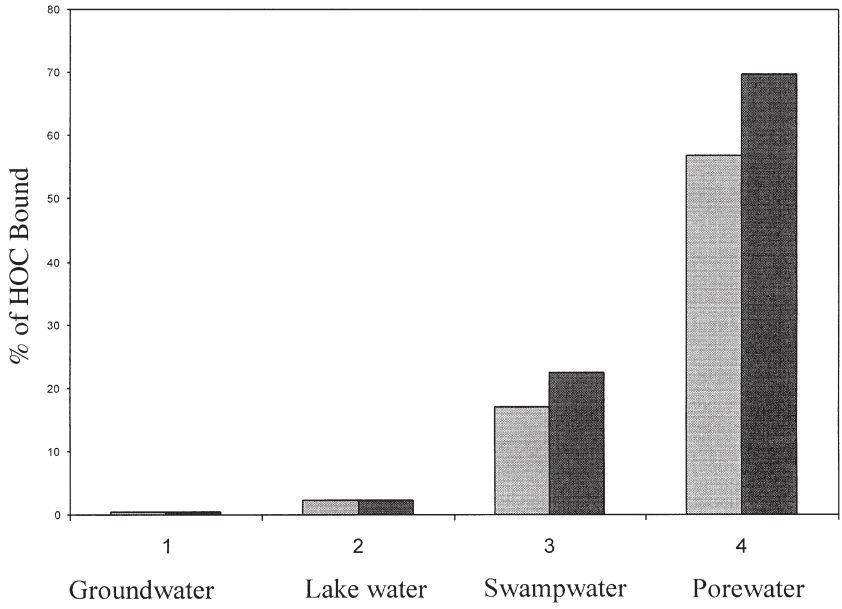

FIGURE 5 Relative fraction of bound pyrene (□) or 2,2′,5-PCB (■) to DOM isolated from various aquatic environments.

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REFERENCES

Aiken, G. R., and R. L. Malcolm. 1987. Molecular weight of aquatic fulvic acids by vapor pressure osmometry. *Geochimica et Cosmochimica Acta* **51:**2177.

Arnold, C. G., A. Ciani, S. R. Mueller, A. Amirbahman, and R. Schwarzenbac. 1998. Association of triorganotin compounds with dissolved humic acids. *Environmental Science and Technology* **32:**2976.

Backhus, D. A., and P. M. Gschwend. 1990. Fluorescent polycyclic aromatic hydrocarbons as probes for studying the impact of colloids on pollutant transport in groundwater. *Environmental Science and Technology* **25:**1214.

Brownawell B. J., and J. W. Farrington. 1986. Biogeochemistry of PCBs in interstitial waters of a coastal marine sediment. *Geochimica et Cosmochimica Acta* **50:**157.

Burgess, R. M., and S. A. Ryba. 1998. A comparison of colloid-contaminant C_{18} based isolation techniques using PCB contaminated humic substances and interstitial water. *Chemosphere* **36:**2549.

Burgess, R. M., R. A. McKinney, W. A. Brown, J. G. Quinn. 1996. Isolation of marine sediment colloids and associated polychlorinated biphenyls: A comparison of ultrafiltration and reverse phase chromatography. *Environmental Science and Technology* **30:**1923.

Burgess, R. M., R. A. McKinney, and W. A. Brown. 1996a. Enrichment of marine sediment colloids with polychlorinated biphenyls: Trends resulting from PCB solubility and chlorination. *Environmental Science and Technology* **30:**2556.

Burns, S. E., J. P. Hassett, and M. V. Rossi. 1996. Binding effects on humic-mediated photoreaction: Intrahumic dechlorination of Mirex in water. *Environmental Science and Technology* **30:**2934.

Burns, S. E., J. P. Hassett, and M. V. Rossi. 1997. Mechanistic implications of the intrahumic dechlorination of mirex. *Environmental Science and Technology* **31:**1365.

Cabaniss, S. E. 1992. Synchronous fluorescence spectra of metal-fulvic acid complexes. *Environmental Science and Technology* **26:**1133.

Carter C. W., and I. H. Suffet. 1982. Binding of DDT to dissolved humic material. *Environmental Science and Technology* **16:**735.

Chefetz, B., A. P. Deshmukh, P. G. Hatcher, and E. Guthrie. 2000. Pyrene sorption by natural organic matter. *Environmental Science and Technology* **34:**2925.

Chin, Y. P., and P. M. Gschwend. 1991. The size, abundance, and configuration of porewater organic colloids from recent sediments. *Geochimica et Cosmochimica Acta* **55:**1309.

Chin, Y. P., and P. M. Gschwend. 1992. Partitioning of PAHs between porewater colloidal organic matter and marine interstitial waters. *Environmental Science and Technology* **26:**1621.

Chin, Y. P., and W. J. Weber. 1989. Estimating the effects of dispersed organic polymers on the sorption of contaminants by natural solids. 1: A predictive thermodynamic humic substance-organic solute interaction model. *Environmental Science and Technology* **23:**978.

Chin, Y. P., W. J,. Weber, Jr., and B. J. Eadie. 1990. Estimating the effects of dispersed organic polymers on the sorption of contaminants by natural solids. II: Sorption studies in the presence of humic substances. *Environmental Science and Technology* **24:**837.

Chin, Y. P., G. R. Aiken, and E. O'Loughlin. 1994. The molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environmental Science and Technology* **28:**1853.

Chin, Y. P., G. R. Aiken, and K. M. Danielsen. 1997. Binding of pyrene to aquatic and commercial humic substances: The role of molecular weight and aromaticity. *Environmental Science and Technology* **31:**1630.

Chin, Y. P., C. R. Swank, S. J. Traina, and D. Backhus. 1998. Abundance and properties of natural organic matter in the pore waters of a freshwater wetland. *Limnology and Oceanography* **43:**1287.

Chiou, C. T., P. E. Porter, and D. W. Schmedding. 1983. Partitioning equilibria of nonionic compounds between soil organic matter and water. *Environmental Science and Technology* **17:**227.

Chiou, C. T., R. L. Malcolm, T. I. Brinton, and D. E. Kile. 1986. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids, *Environmental Science and Technology* **20:**502.

Chiou C. T., D. E. Kile, T. I. Brinton, R. L Malcolm, J. A. Leenheer, and P. MacCarthy. 1987. A comparison of water solubility enhancement of organic solutes by aquatic humic materials and commercial humic acids. *Environmental Science and Technology* **21:**1231.

Coble P. G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry* **51:**325.

Danielsen, K. M., Y. P. Chin, J. S. Buterbaugh, T. L. Gustafson, and S. J. Traina. 1995. The solubility enhancement and fluorescence quenching of pyrene by humic substances. *Environmental Science and Technology* **29:**2162.

Doll, T. E., F. H. Frimmel, M. U. Kumke, and G. Ohlenbusch. 1999. Interaction between NOM and polycyclic aromatic compounds (PAC)-comparison of fluorescence quenching and solid phase microextraction. Fresenius *Journal of Analytical Chemistry* **364:**313.

Eadie, B. J., N. R. Morehead, P. F. Landrum. 1990. Three phase partitioning of hydrophobic organic compounds in Great Lakes waters. *Chemosphere* **20:**161.

Eadie, B. J., N. R. Morehead, J. V. Klump, and P. F. Landrum. 1992. Distribution of hydrophobic organic compounds between dissolved and particulate organic matter in Green Bay waters. *Journal of Great Lakes Research* **18:**91.

Evans, H. E. 1988. The binding of three PCB congeners to dissolved organic carbon in freshwaters. *Chemosphere* **17:**2325.

Friesen, K. J., M. M. Foga, and M. D. Loewen. 1996. Aquatic photodegradation of polychlorinated dibenzofurans: Rates and photoproduct analysis. *Environmental Science and Technology* **30:**2504.

Gauthier, T. D., E. C. Shane, W. F. Guerin, Seitz W. R., and C. L. Grant. 1986. Fluorescence quenching method for determining equilibrium constants for polycyclic aromatic hydrocarbon bonding to dissolved humic materials. *Environmental Science and Technology* **20:**1162.

Gauthier, T. D., W. R. Seitz, and C. L. Grant. 1987. Effects of structural and compositional variations of dissolved humic materials on pyrene Koc values. *Environmental Science and Technology* **21:**243.

Giessl, H. 1999. Dom-flocculation: A suitable approach for separating free and DOM bound herbicides? *Environmental Science and Pollution Research* **6:**77.

Gschwend, P. M., and S. Wu. 1985. On the constancy of sediment-water partition coefficient of hydrophobic organic pollutants. *Environmental Science and Technology* **19:**90.

Haitzer, M., S. Hoss, W. Traunspurger, and C. Steinberg. 1998. Effects of DOM on the bioconcentration of organic chemicals in aquatic organisms. *Chemosphere* **37:**1335.

Hess, A., and Y. P. Chin. 1996. Physical and chemical characteristics of polymaleic acid, a synthetic organic colloid analog. *Colloids and Surfaces A* **107:**141–154.

Hassett, J. P., and M. A. Anderson. 1982. Effects of dissolved organic matter on the adsorption of hydrophobic compounds by river and sewage borne particles. *Water Research* **16:**681.

Herbert, B. E., P. M. Bertsch, and J. M. Novak. 1993. Pyrene sorption by water-soluble organic carbon. *Environmental Science and Technology* **27:**398.

Hildebrand, J. H., J. M. Prausnitz, and R. L. Scott. 1970. "Regular and Related Solutions". van Nostrand Reinhold, New York.

Johnson, W. P., and G. L. Amy. 1995. Facilitated transport and enhanced desorption of polycylic aromatic hydrocarbons by natural organic matter in aquifer sediments. *Environmental Science and Technology* **29:**807.

Karickhoff, S. W. 1984. Pollutant sorption in aquatic systems. *Journal of Hydraulic Engineering* **10:**707.

Kilduff, J. E., T. Karanfil, Y. P. Chin, and W. J. Weber. 1996. Adsorption of natural organic polyelectrolytes by activated carbon: A size-exclusion chromatography study. *Environmental Science and Technology* **30:**1336.

Kopinke, F. D., J. Poerschmann, and U. Stottmister. 1995. Sorption of organic pollutants on anthropogenic humic matter. *Environmental Science and Technology* **29:**901.

Kopinke, F. D., A. Georgi, K. MacKenzie. 2000. Sorption and chemical reactions of PAHs with dissolved humic substances. *Refractory Organic Substances in the Environment* (*ROSE*) **II:**35.

Kukkonen, J., and A. Oikari. 1991. Bioavailability of organic pollutants in boreal waters with varying levels of organic matter. *Water Research* **25:**455.

Kukkonen, J., A. Oikari, S. Johnsen, and E. T. Gjessing. 1989. Effect of humus concentration on benzo(*a*)pyrene accumulation from water to *Daphnia magna*. *Science of the Total Environment* **79:**197.

Lakowicz, J. 1983. "Principles of Fluorescence Spectroscopy." Plenum, New York.

Landrum, P. F., S. R. Nihart, B. J. Eadie, and W. S. Gardner. 1984. Reverse-phase separation method for determining pollutant binding to Aldrich humic acid and dissolved organic carbon in natural waters. *Environmental Science Technology* **18:**187.

Landrum, P. F., M. D. Reinhold, S. R. Nihart, and B. J. Eadie. 1985. Predicting the bioavailability of *P. hoyi* in the presence of humic and fulvic materials, and natural dissolved organic matter. *Environmental Toxicology and Chemistry* **4:**459.

Landrum, P. F., S. R. Nihart, B. J. Eadie, and L. R. Herche. 1987. Reduction in the bioavailability of organic contaminants to the amphipod *P. hoyi* by dissolved organic matter in sediment interstitial waters. *Environmental Toxicology and Chemistry* **6:**11.

Laor, Y., and M. Rebhun. 1997. Compexation–flocculation: A new method to determine binding coefficients of organic contaminants to dissolved humic substances. *Environmental Science and Technology* **31:**3558.

Leversee, G. J., P. F. Landrum, J. P. Giesy, and T. Fannin. 1983. Humic acids reduce the bioaccumulation of some polycyclic aromatic hydrocarbons. *Canadian Journal of Fisheries and Aquatic Science* **40:**63.

Lindsey, M. E., and M. A. Tarr. 2000. Inhibition of hydroxyl radical reaction with aromatics by dissolved natural organic matter. *Environmental Science and Technology* **34:**444.

Liu, H., and G. Amy. 1993. Modeling partition and transport interactions between natural organic matter and PAHs in groundwater. *Environmental Science and Technology* **27:**1553.

Lorenz, R., R. Brueggemann, C. E. W. Steinburg, and O.H. Spieser. 1996. Humic material changes effect of terbutylazine on behavior of zebrafish. *Chemosphere* **33:**2145.

McCarthy, J. F., and J. M. Zachara. 1989. Subsurface transport of contaminants. *Environmental Science and Technology* **23:**496.

McCarthy, J. F., and B. D. Jimenez. 1985. Reduction in bioavailability in bluegills to polycyclic aromatic hydrocarbons bound to dissolved humic materials. *Environmental Toxicology and Chemistry* **4:**511.

McGroddy, S. E., J. W. Farrington, and P. M. Gschwend. 1996. Comparison of the *in situ* and desorption sediment and water partitioning of polycyclic aromatic hydrocarbons and polychlorinated biphenyls. *Environmental Science and Technology* **30:**172.

McKnight, D. M., E. W. Boyer, P. Doran, P. K. Westerhoff, T. Kulbe, and D. T. Andersen. 2001. Spectrofluorometric characterization of aquatic fulvic acid for determination of precursor organic material and general structural properties. *Limology and Oceanography* **46:**38.

Means, J. C., and R. D. Wijayaratne. 1982. Role of natural colloids in the transport of hydrophobic pollutants. *Science* **215:**968.

Means, J. C., and R. D. Wijayaratne. 1984. Chemical characterization of estuarine colloidal matter implications for adsorptive processes. *Bulletin of Marine Science* **35:**449.

Morel, F. M. M., and P. M. Gschwend. 1987. The role of colloids in the partitioning of solutes in natural water. *In* "Aquatic Surface Chemistry," (W. Tumm, Ed.), p. 405. Wiley, New York.

Morra, M., M. O. Corapcioglu, R. von Wandruzka, D. Marshall, and K. Topper. 1990. Fluorescence quenching and polarization studies of naphthalene and 1-naphthol interaction with humic acid. *Journal of the Soil Science Society* **54:**1283.

Muir, D. G., B. R. Hobden, and M. R. Servos. 1994. Bioconcentration of pyrethroid insecticides and DDT by rainbow trout, uptake, depuration, and effect of dissolved organic carbon. *Aquatic Toxicology* **29:**230.

Ohlenbusch, G., M. Kumke, and F. H. Frimmel. 2000. Sorption of phenols to dissolved organic matter investigated by solid phase extraction. *Science of the Total Environment* **253:**63.

Oikari, A, and J. Kukkonen. 1990. Bioavailability of benzo(*a*)pyrene and dehydroabietic acid from a few lake waters containing varying amounts of dissolved organic carbon concentrations to *Daphnia magna*. *Bulletin of Environmental Contamination and Toxicology* **45:**54.

O'Loughlin, E. J., S. J. Traina, and Y. P. Chin. 2000. Association of organotin compounds with aquatic and terrestrial humic ltface substances. *Environmental Toxicology and Chemistry* **19:**2015.

Orem, W. H., and P. G Hatcher. 1987. Solid-state ^{13}C-NMR studies of dissolved organic matter in porewaters from different depositional environments. *Organic Geochemistry* **2:**73.

Ozretich, R. J., L. M. Smith, F. A. Roberts. 1995. Reverse phase separation of estuarine interstitial water fractions and the consequences of C_{18} retention of organic matter. *Environmental Toxicology and Chemistry* **14:**1261.

Pelekani, C., G. Newcombe, V. L. Snoeyink, C. Hepplewhite, S. Assemi, and R. Beckett. 1999. Characterization of natural organic matter using high performance size exclusion chromatography. *Environmental Science and Technology* **33:**2807.

Perminova, I. V., N. Grechischcheva, and V. S. Petrosyan. 1999. Relationships between structure and binding affinity of humic substances for PAHs: Relevance of molecular descriptors. *Environmental Science and Technology* **33:**3781.

Peuravuori, J., and K. Pihlaja. 1997. Molecular size distribution and spectroscopic properties of aquatic humic substances. *Analytica Chimica Acta* **337:**133.

Poerschman, J., Z. Zhang, F. D. Kopinke, and J. Pawliszyn. 1997. Solid phase microextraction for determining the distribution of chemicals in aqueous matrices. *Analytical Chemistry* **69:**597.

Puchalski, M. M., M. J. Morra, and R. von Wandruszka. 1992. Fluorescence quenching of synthetic organic compounds by humic materials. *Environmental Science and Technology* **26:**1787.

Raber, B., I. Kogel-Knauber, C. Stein, and D. Klem. 1998. Partitioning of polycyclic aromatic hydrocarbons to dissolved organic matter from different soils. *Chemosphere* **36:**79.

Roubeuf, V., S. Mounier, J. Y. Benaim. 2000. Solid phase extraction applied to natural waters: Efficiency and selectivity. *Organic Geochemistry* **31:**127.

Schlautman, M. A., and J. J. Morgan. 1993. Effects of aqueous chemistry on the binding of PAHs by dissolved humic materials. *Environmental Science and Technology* **27:**961.

Spacie, A., P. F. Landrum, and G. J. Leversee. 1983. Uptake, depuration, and transformation of anthracene and benzo(*a*)pyrene in bluegill sunfish. *Ecotoxicology Environmental Safety* **7:**330.

Specht, C. H., and F. H. Frimmel. 2000. Specific interactions of organic substances in size-exclusion chromatography. *Environmental Science and Technology* **34:**2361.

Spiteller, M., and M. Schnitzer. 1983. A comparison of the structure of polymaleic acid and a soil fulvic acid. *Journal of Soil Science* **34:**525.

Spurlock, F. C., and J. W. Biggar. 1994. Thermodynamics of organic chemical partition in soils. *Environmental Science and Technology* **28:**959.

Tanaka, S., K. Oba, M.Fukushima, K. Nakayasu, and K. Hasebe. 1997. Water solubility of pyrene in the presence of humic substances. *Analytica Chimica Acta* **337:**351.

Tiller, C. L., and K. D. Jones. 1997. Effects of dissolved oxygen and light on determination of Koc values for PAHs using fluorescence quenching. *Environmental Science and Technology* **31:**424.

Traina, S. J., J. Novak, and N. E. Smeck. 1990. An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids. *Journal of Environmental Quality* **19:**151

Uhle, M. E., Y. P. Chin, G. R. Aiken, and D. M. McKnight. 1999. Binding of polychlorinated biphenyls to aquatic humic substances. *Environmental Science and Technology* **33:**2715.

Voice, T. C., and W. J. Weber. 1985. Solids effect in liquid/solid partitioning. *Environmental Science and Technology* **19:**789.

Weber, E. J. 1996. Iron-mediated reductive transformations: Investigation of reaction mechanism. *Environmental Science and Technology* **30:**716.

8

Elemental Complexation by Dissolved Organic Matter in Lakes: Implications for Fe Speciation and the Bioavailability of Fe and P

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Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

I. INTRODUCTION

Dissolved organic matter (DOM) represents an important carbon reservoir in many freshwater aquatic ecosystems, especially in humic rich lakes that receive large allochthonous DOM inputs from their watersheds (Dillon and Molot, 1997). Developing a better understanding of the chemical composition of that DOM and its availability to a diverse group of microbes is currently the focus of an immense research effort, as evidenced by the many chapters in this book. DOM, however, serves as more than a microbial substrate (see Chapters 7 and 19). Humic and fulvic acids, which make up the majority of the DOM pool (see Chapter 3), have highly reactive aliphatic and aromatic carboxyl and hydroxyl groups that complex with other elements in the water column. Therefore DOM as a chelating agent of biologically important or toxic (Driscoll *et al*., 1994; see Chapter 7) elements may affect the availability of those elements to microorganisms and as a consequence alter system productivity and metabolism. This role of DOM at the ecosystem level is poorly understood.

The main focus of this chapter will be to examine the effect of DOM as a chelating agent for the essential trace metal iron (Fe) in aquatic systems and to determine how that complexation may affect the bioavailability of both Fe and phosphorus (P) in lakes. It is also important to note that when we refer to the binding of DOM we are referring largely to the dissolved humic and fulvic substances, which comprise the greatest proportion of the DOM pool. Freshwater lakes range from clear blue, to green, to brown in color. Humic and fulvic organic acids are the substances that give some lakes their characteristic brown color. These substances are allochthonous in origin, being the degradation products of terrestrial organic matter, and are believed to be the main organic chelates of Fe and other metals in freshwater aquatic systems.

There are two general but opposing views of DOM as a chelator of Fe and P in lakes. The first one is that the binding of Fe and P by the DOM enhances the availability of these elements to planktonic organisms by keeping them in a dissolved state (Koenings and Hooper, 1976; Sunda, 1995). Dissolved Fe(III) is highly reactive in oxygenated waters and will quickly form inert particles capable of sorbing free PO_4, and this complex will precipitate out of the water column (Stumm and Morgan, 1996). In contrast, organically bound Fe and P will remain suspended for longer periods of time and may eventually be taken up by organisms after release from the organic component. The second point of view is that the chelation of Fe and P by DOM actually reduces the availability of these elements to organisms (Hutchinson, 1957; Hessen, 1998; Wetzel, 2001). Reduced availability may or may not mean that these elements limit system biomass or

production, but the added energetic expenditure in acquiring the element may reduce the metabolic efficiency of the system.

As compared with the ocean, the concentration of Fe in lakes is quite high (Wetzel, 2001), and it is generally thought that the productivity in lake systems is not limited by the availability of Fe. However, there are some exceptions, including hardwater marl lakes (Schelske, 1962; Wetzel, 1965), saline lakes (Evans and Prepas, 1997), ultraoligotrophic lakes (Chang *et al*., 1992), and some of the Great Lakes (Twiss *et al*., 2000). Humic lakes are not traditionally thought to be Fe limited (although see Jackson and Hecky, 1980), but the role of DOM in the chelation of Fe may make that Fe difficult to acquire, and in some cases organisms in these systems may be Fe-stressed. The importance of Fe speciation and the availability of those Fe species to organisms in freshwaters remain untested. Fe speciation is influenced by a myriad of factors, including system pH, ionic strength, temperature, oxygen availability, and the presence of organic chelators, including allochthonous DOM. Given that these chemical characteristics vary greatly among lakes, speciation and thus the availability of Fe should also vary substantially.

The influence of DOM on the chelation and bioavailability of Fe to freshwater microorganisms in the oxygenated zones of the water column are poorly understood and are controlled by a complex set of interacting processes. Figure 1 is a general schematic representation of how the Fe and P bound to DOM may become available to organisms via various reduction pathways, such as photochemical, dark, and biological reduction or biologically mediated pathways, such as siderophore production or enzyme hydrolysis. Some phytoplankton species, so-called mixotrophs, and other consumers can acquire these essential elements via grazing.

The main objectives of this chapter are to elucidate the role of DOM as a complexing agent of Fe and P and how the availability of those bound essential elements might influence primary and bacterial production in lakes. The first section reviews the biological importance of Fe and how organisms acquire this element. Examples are drawn from marine studies carried out under Fe limitation. We go on to argue that the biological demand for Fe should be higher in humic lakes relative to their clear-water counterparts. The second section describes Fe chemistry in freshwaters and the role of allochthonous DOM in altering that chemistry. We then summarize what is known about the DOM-Fe-P complex and how this complex may influence P availability to lake phytoplankton. Assuming that the elements bound in the DOM-Fe-P complex are not directly available to organisms, we outline several mechanisms that should enhance the availability of both Fe and P. Finally we provide a conceptual framework to describe how DOM influences the cycling of Fe and P in humic-rich lakes.

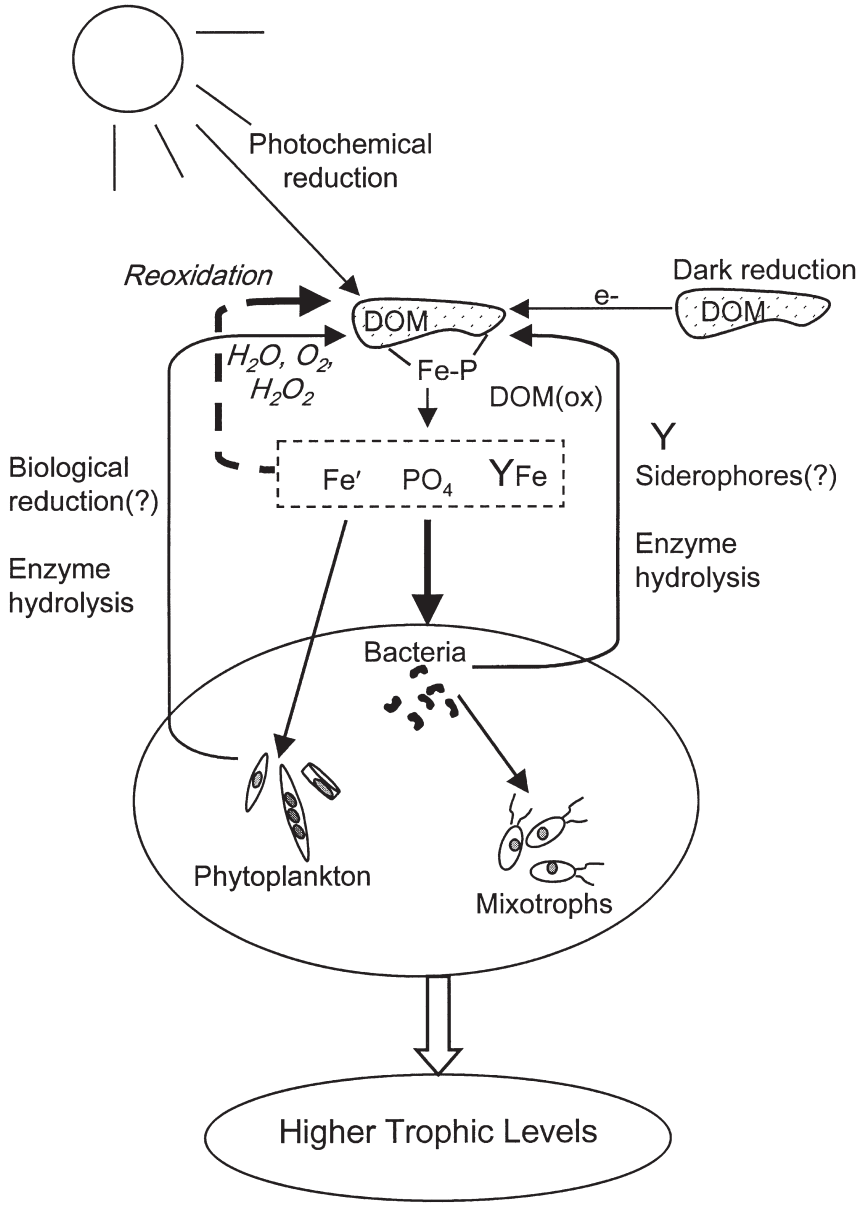

FIGURE 1 Schematic representation of the cycling Fe and P in humic-rich systems: DOM complexes both essential elements and renders them unavailable to organisms without transformation. Y represents siderophore ligand, and Fe′ is iron in free ionic form.

II. BIOLOGICAL IMPORTANCE OF Fe

Iron is an essential micronutrient for all organisms. It is a required element in cytochromes and the Fe-S centers of redox proteins involved in key metabolic processes such as photosynthesis, respiration, and the reduction of nitrate. Given the importance of Fe in these major metabolic enzymes, microorganisms under Fe-deficient conditions reduce their cellular Fe quotas (Fe : C content) (Sunda *et al*., 1991) and have reduced photosynthetic (Raven, 1988; Green *et al*., 1994) and bacterial growth (Tortell *et al*., 1996) efficiencies. Thus ecosystem C metabolism is regulated in part by the availability of Fe.

Understanding the Fe cycle in aquatic systems and how the availability and speciation of that Fe affects system metabolism has advanced over the last decade because of research carried out in the Fe-limited regions of the sea (Martin *et al*., 1994; Coale *et al*., 1996; Boyd *et al*., 2000). The availability of iron (or any metal) to organisms is dependent on (1) total concentration of the metal, (2) its chemical speciation, and (3) how the physical-chemical properties of a system alter that speciation (Buffle, 1988). In Fe-limited regions of the ocean, ultra-trace concentrations of Fe (Johnson *et al*., 1997) together with the binding of Fe to insoluble colloids, detritus (Price and Morel, 1998), and unidentified dissolved organic ligands (Gledhill and van den Berg, 1994; Rue and Bruland, 1995, 1997), results in a vanishingly low concentration of labile dissolved inorganic Fe. Until recently, algal Fe acquisition was thought to be restricted to the uptake of dissolved inorganic iron species via a saturable cell surface transport system, whereby Fe binds to a surface receptor and is subsequently transported across the cell membrane (Hudson and Morel, 1990). Diffusion limits the rate of inorganic Fe uptake, and, given the low oceanic levels of dissolved inorganic Fe, this mode of Fe acquisition alone cannot account for the relatively high growth rates of the phytoplankton community observed in Fe-limited regions (Price and Morel, 1998). Recent studies indicate that eukaryotic algae are able to reduce Fe bound in the dissolved organic complexes (Hutchins *et al*., 1999; Maldonado and Price, 1999). Mixotrophic algae, which are abundant in the Fe-limited oceanic regions, can acquire iron from grazing bacteria (Maranger *et al*., 1998) and Fe colloids (Nodwell and Price, 2001). Mixotrophic species have also been shown to dominate under Fe-limiting situations in lakes (Auclair, 1995), and iron acquisition via the consumption of bacteria and cyanobacteria was hypothesized.

Bacteria have high Fe requirements and account for up to 50% of the biological iron pool in Fe-limited regions (Tortell *et al*., 1996). Bacterial production is stimulated with the addition of Fe (Pakulski *et al*., 1996). Under conditions of Fe deprivation, microorganisms release small molecules (MW <1000 Da) called siderophores that chelate Fe(III) with a strong affinity (Neilands, 1981, 1995). In controlled laboratory experiments, both

marine and freshwater bacteria and cyanobacteria have been demonstrated to produce and take up siderophores (Wilhelm and Trick, 1994; Wilhelm, 1995; Granger and Price, 1999). Although there is no direct evidence of siderophore-bound Fe *in situ*, the conditional stability constants of these isolated siderophores are within the same range as the dissolved, organically complexed Fe in Fe-limited regions of the ocean (Rue and Bruland, 1995). The stability constant, *K*, is the measured strength of the metal-ligand complex relative to the ligand and metal. A high *K* value would imply that the formed complex is very stable, and that the dissociation rate of the metal from its ligand would be quite low.

Plankton in humic lakes are unlikely to be Fe-limited because total Fe is well-correlated with both DOC and color (Figs. 2A and 2B) and has long been associated with humic substances (Shapiro, 1957, 1966). Indeed, the strong relationship between total Fe and color, a surrogate for humic substances, is not significantly different from unity (Fig. 2B), suggesting that these elements are strongly linked. Hence the chelating properties of DOM and the formation of DOM-Fe and DOM-Fe-P complexes may make the acquisition of Fe and P difficult for microorganisms. Hutchins *et al*. (1998) presented a classification scheme of Fe condition for coastal plankton from Fe-replete to Fe-stressed to severely Fe-stressed to Fe-limited, whereby under stressed conditions, organisms have adapted to low levels of Fe at the expense of lower growth and photosynthetic efficiency. Given what is known about the acquisition of Fe, the need for Fe in system metabolism, and the complex chemistry of Fe in natural waters, it is possible that the planktonic community in brown-water lakes is Fe-stressed, and Fe availability may affect the metabolic efficiency of the system.

III. BIOLOGICAL Fe DEMAND IN BROWN- VERSUS CLEAR-WATER SYSTEMS

Although untested, there are two reasons to suspect that the biological demand for Fe in humic lakes would be greater than the demands in clear-water systems. Humic substances compete with the light-harvesting pigments of phytoplankton for photons that are required for photosynthesis. Therefore the phytoplankton growing in light-limited humic lakes would likely have a higher iron requirement to support the synthesis of additional light-harvesting pigments and Fe-containing redox proteins for photosynthesis (Raven, 1990; Sunda and Huntsman, 1997).

Second, in humic systems, there is an increase in the production of biologically harmful oxygen radicals such as O_2^-, H_2O_2, and OH•, all of which are toxic by-products of the photo-oxidation of DOM (Zarifou *et al*., 1984). Even low concentrations of added H_2O_2 (<100 nM) have been demonstrated to have negative effects on aquatic bacteria (Xenopoulos and Bird, 1997)

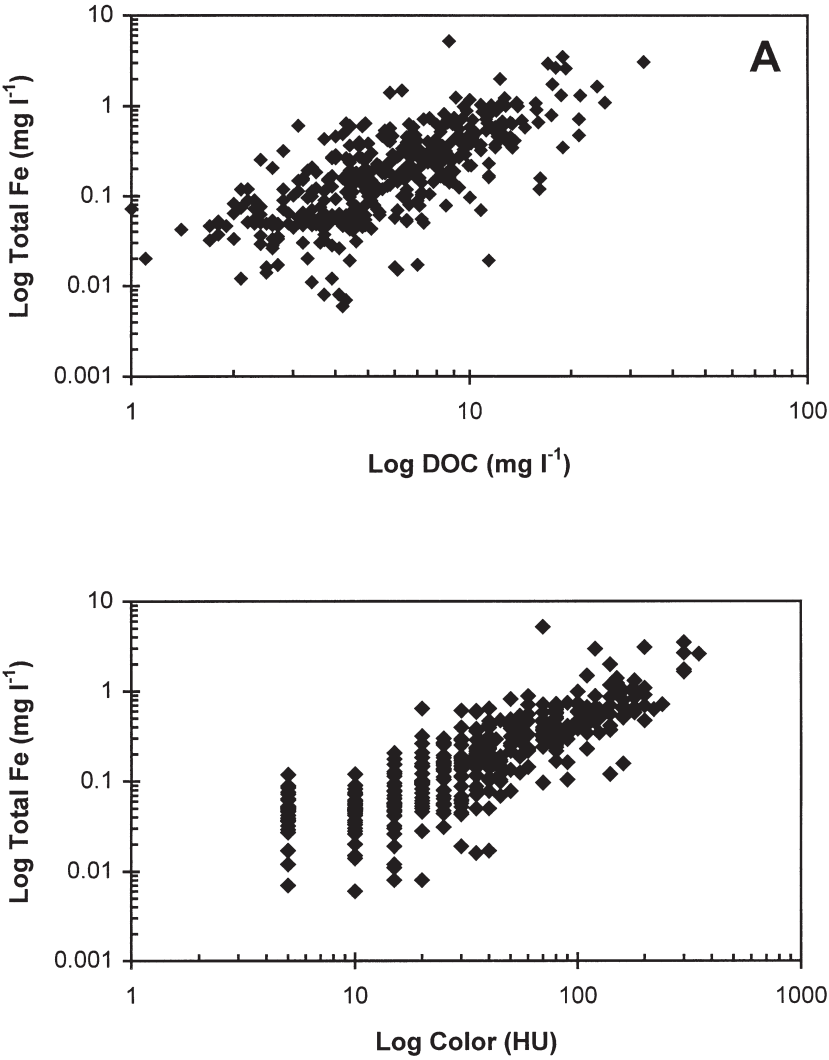

FIGURE 2 The log-log relationship between (A) total Fe and DOC concentration (TFe = 0.34 × DOC + 1.02, r^2= 0.49, $p < 0.0001$, $n = 405$) and (B) total Fe and color (TFe = 0.97 × Color – 2.27, r^2= 0.66, $p < 0.0001$, $n = 402$) in New York lakes. Data are from the Adirondack Lake Survey, Oswegatchie-Black region, 1985–87.

and phytoplankton. Brown waters can accumulate particularly high concentrations of H_2O_2 in epilimnetic surface waters (Cooper *et al.*, 1988). To defend themselves against active oxygen species, microorganisms produce a series of enzymes, including catalases, peroxidases, and several classes of superoxide dismutases. With the exception of certain superoxide dismutases,

all of these enzymes require Fe (Miessler and Tarr, 1999) so microorganisms living in humic systems may have higher Fe requirements.

IV. DOM AND Fe CHEMISTRY IN FRESHWATER

Even in the absence of DOM and/or biological activity, Fe chemistry is complex. In oxygenated water at pH <1.0, iron exists primarily as dissolved Fe^{3+} (or $Fe(H_2O)_6^{3+}$). However, as the pH increases toward circumneutral, Fe(III) undergoes stepwise hydrolysis:

$$Fe(H_2O)_6^{3+} + OH^- \leftrightarrow Fe(H_2O)_5(OH)^{2+} + H_2O.$$

Eventually this process forms the neutral species $Fe(H_2O)_3(OH)_3^0$, which precipitates as amorphous iron hydroxide, which may settles out of the water column. Figure 3 shows the predicted effect of pH on the relative concentrations of the various iron hydrolysis species with and without considering the iron hydroxide solid, which dominates the speciation above pH 3.0 at 1 μM total iron. The log of the solubility product of this solid is –38.8, indicating that iron is very insoluble at natural pH values. Over time, this metastable amorphous material converts to more thermodynamically

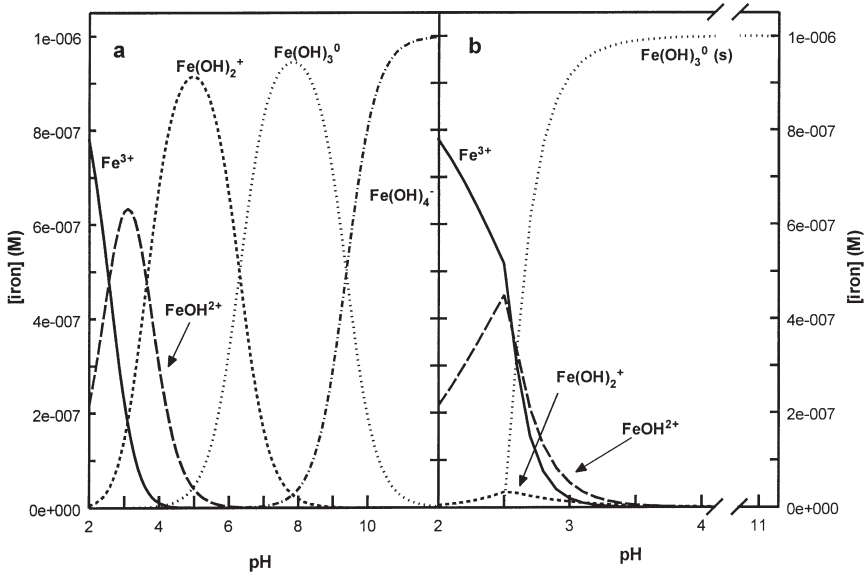

FIGURE 3 The predicted speciation of iron in aqueous solution without the effect of organic ligand complexation. The predicted concentrations of the species were calculated using the computer program Titrator (Cabaniss, 1987) and complexation constants from the NIST database (Smith *et al.*, 1993). In (a) the formation of the $Fe(OH)_3$ solid is ignored, whereas in (b) it is considered. In the absence of complexing ligands the speciation of iron at circumneutral pH is dominated by iron colloids.

stable (and even less soluble) crystalline phases, such as goethite or lepidocrocite. These precipitated and coagulated forms of Fe generally predominate in aquatic systems and are considered to be biologically inert.

Despite its inherent insolubility in the pH range typically found in natural waters (6–8), dissolved iron can be found at micromolar concentrations in fresh surface waters (Pullin and Cabaniss, 2001). This is due to the interaction of Fe with DOM, whereby Fe (and other metals) bind to the aliphatic and aromatic carboxyl and hydroxyl functional groups of DOM to form dissolved complexes (Fig. 4). DOM also modifies the surface charge and reactivity of iron solids so that precipitation, coagulation, and settling are slowed or prevented. This allows the complexes to remain suspended in the water column and pass through filters traditionally used to separate dissolved and particulate materials (0.2 or 0.45 μm). Recent evidence indicates that iron-containing colloids present in natural waters can have particle sizes as small as 0.05 μm (Pullin and Cabaniss, 2001), in agreement with particle size distributions measured in seawater (Vaillancourt and Balch, 2000). The DOM-particle interaction may be a surface coating (Tipping, 1981) or even a fiber-like network connecting smaller individual particles (Cornell and Schwertmann, 1979; Taillefert *et al.*, 2000). The presence of DOM can also slow or completely inhibit the transformation of amorphous iron hydroxide to more crystalline and less reactive phases (Schwertmann, 1966; Cornell and Schwertmann, 1979).

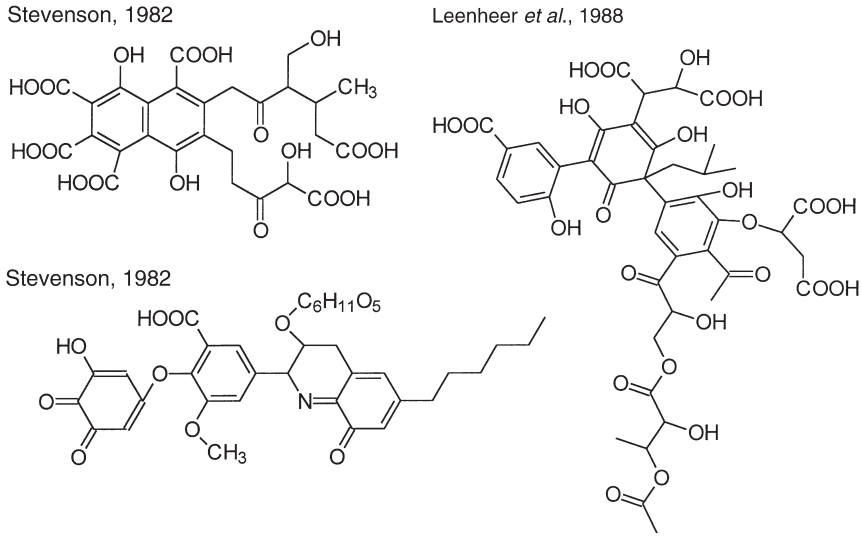

FIGURE 4 Three proposed DOM model structures, showing a variety of carboxyl (COOH) and hydroxyl (COH) metal (and H^+) binding sites. Reprinted with permission from Stevenson, F. J. 1982. "Humus Chemistry: Genesis, Composition, Reactions." Copyright 1982 Wiley, New York. Reprinted in part with permission from Leenheer *et al.*, 1998. *Environmental Science & Technology* **32**:2410–2416. Copyright 1998 American Chemical Society.

Phosphorus, and particularly the availability of PO_4, is well known to limit the productivity in most lakes (Schindler, 1977). Important abiotic interactions exist between the DOM-Fe complex and P. These interactions may reduce the availability of phosphate, thus resulting in the exacerbation of P limitation in humic-rich lakes. In oxygenated waters and in the absence of DOM, Fe hydroxide particles react with PO_4 to form a complex that is quick to settle to bottom sediments. Indeed, this coprecipitation can be a primary sink for phosphates in lakes (Stumm and Morgan, 1996). Given the important role of Fe hydroxides in binding PO_4, Fe hydroxides associated with DOM are suspected of binding P. No clear evidence exists to support the direct binding of PO_4 to DOM (Stewart and Wetzel, 1981), and it is believed that the PO_4 must be associated with a metal oxide bound to the DOM.

V. THE DOM-Fe-P COMPLEX IN LAKES

Several studies support the idea of a DOM-Fe-P complex (Table I), which results in the maintenance of dissolved forms of both Fe and P in the water column, thus reducing their loss to the sediments. However, the

TABLE I Information Summary of Some Studies Looking at the Interaction of DOC, Fe, and P Interactions in Lakes

Authors	*Questions*	*Conclusions*
Jackson and Schindler (1975)	Do humic-Fe complexes adsorb P?	P absorbs to the humic-Fe complex, and this abiotic interation may influence P cycling.
Koenings and Hooper (1976)	Do interactions between colloidal organic matter (COM) and Fe affect P speciation?	COM chelates Fe reducing hydroxide formation and associated P sedimentation; PO_4 is left free in solution.
Lijklema (1980)	What are the effects of pH and aging on the PO_4 adsorption properties of Fe hydroxides?	P adsorbs to amorphous Fe hydroxides; there is greater P adsorption to newly formed Fe hydroxides.
Jackson and Hecky (1980)	Do the chelating properties of DOM affect primary production (PP)?	PP is depressed by the complexation of Fe by DOM.
Francko and Heath (1979)	How are complex P compounds released in a clear lake?	Variety of P compounds; PO_4 is released by APA, but not by UV exposure.
Francko and Heath (1979)	How are complex P compounds released in a brown-water lake?	DOC-Fe-P complex; PO_4 is released upon UV exposure, but not by APA.

(*continued*)

TABLE I (*continued*)

Authors	*Questions*	*Conclusions*
Francko and Heath (1982)	Is the DOC-Fe-P complex photosensitive?	Yes: with UV exposure, PO_4 is released from the DOC-Fe-P complex.
Francko (1986)	How does the addition of Fe and DOC to a clear eutrophic lake affect P dynamics?	↑P-uptake, ↑APA and ↑free PO_4 with UV exposure proposed: ↑productivity (untested)
Francko (1986)	How does the addition of Fe and DOC to a clear oligotrophic lake affect P dynamics?	↓P-uptake, ↑APA and ↑free PO_4^- with UV exposure proposed: ↓productivity (untested)
Cotner and Heath (1990)	Is the photoreduction of Fe responsible for PO_4 release in the DOC-Fe-P complex?	Fe and UV treatments: Fe^{3+} reduced to Fe^{2+} releasing (small amounts of) P
De Haan and de Boer (1986)	What are the interactions between Fe, P, and DOC in a highly alkaline and humic-rich lake?	Small amount of Fe bound to DOC (mainly FA) because of completion with Cu; FA-Fe-P complex formed as colloidal aggregate.
Jones *et al.* (1988)	How does P move among humic fractions, and is that movement stimulated by Fe additions?	At low [DOC], ↑Fe-P precipitation; Fe additions stimulated P binding; P binds less strongly in some humic fractions.
De Hann *et al.* (1990)	How do varying concentrations of DOC affect the abiotic movement of Fe and P?	Fe and P bind to humics; small amount of P recovered on DOC; Fe binding apparently stronger and faster than P binding.
Jones *et al.* (1993)	What are the effects of pH and ionic strength on speciation of Fe and P with DOC?	DOC-Fe-P complex under high [DOC]; ↓complexation with ↓pH; Fe binds more with ↑ionic strength; at low DOC ↑P bound to Fe-hydroxides
Shaw (1994)	Under what conditions of varying [DOC], ionic strength, and pH do Fe and P go into particulate state?	DOC kept Fe and P in dissolved state independent of ionic strength.
Tate *et al.* (1995)	What regulates the bioavailability of PO_4 in a low-PO_4, high-Fe oxide stream?	Newly formed Fe hydroxides (from photoreduction) resulted in ↑P adsorption and bioavailability.
Shaw *et al.* (2000)	How do Fe and P associate with natural DOC of different molecular weight fractions?	HMW DOC acts as peptizing agents for inorganic colloids of Fe and P.

APA; alkaline phosphatase activity; FA; fulvic acids.

bioavailability of the Fe and P bound in that complex remains an important open question. Two mechanisms have been proposed to explain the formation of this complex. Shapiro (1967) suggested that small inorganic colloids containing Fe and P are peptized (dispersed in a colloidal form) by the surface adsorption of DOM. The alternative is that Fe hydroxides adsorbed to the surface of humic materials form a bridge between the DOM and the PO_4 (Schnitzer and Khan, 1972). These descriptions of the DOM-Fe-P complexes are not unlike the complex formation described for DOM and Fe (Tipping, 1981; Taillefert *et al*., 2000) and are schematically represented in Figure 5. Both types of complexes probably exist in nature.

Using gel chromatography and radiotracers, several studies have looked at the abiotic movement of PO_4 and Fe in prefiltered lake water samples of varying DOM concentrations (Jones *et al*., 1988, 1993; de Haan *et al*., 1990). Jones *et al*., (1988) added labeled $^{33}PO_4$ and varying concentrations of Fe in lake water with different humic content. After an incubation period, this water was fractionated with the use of a gel chromatography column filled with Sephadex G-100. Three peaks of radioactivity were identified from the eluates and were associated with the following nominal molecular weights: Peak I, >100,000 Da; Peak II, 10,000–20,000 Da; and Peak III represented free ionic PO_4. In general, a greater proportion of the PO_4 in this study was

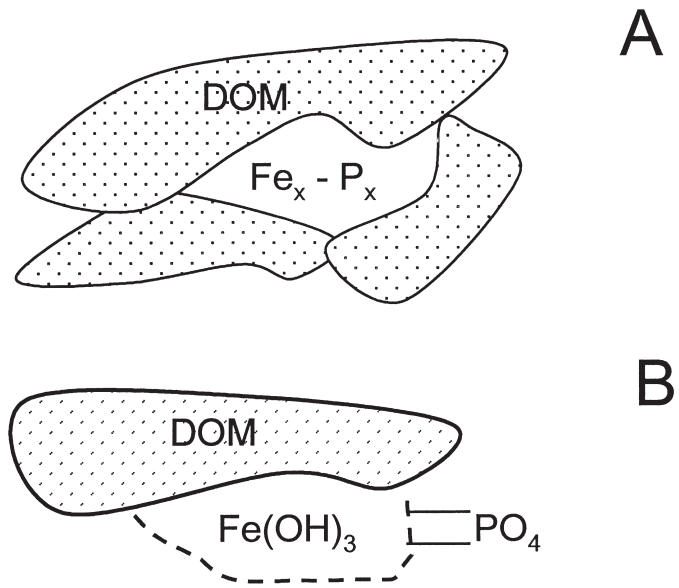

FIGURE 5 Schematic representation of DOM-Fe-P complexes. (A) DOM as a peptizing agent surrounding an inorganic Fe colloid with associated P. (B) Associated Fe hydroxide acting as a "metal bridge" to bind PO_4. The Fe may be mono- or polynuclear in nature, as denoted by the X subscript.

bound in the Peak II fraction, and the concentration of P in this fraction increased linearly, with a slope of 1, with higher concentrations of Fe ($r^2 = 0.88$) and DOM ($r^2 = 0.85$). It should be pointed out that the molecular weights associated with the peaks are calibrated with proteins of known molecular weights and not with organic acids. Therefore the nominal molecular weights associated with the peaks by this technique are likely not representative of the actual molecular weights of the DOM. Regardless, the elution patterns and the relative comparisons among peaks remain robust.

In some lakes the peak in PO_4 of the elution pattern found with the gel filtration technique is very different. When the same procedure was carried out with water from Lake Tjeukemeer, a eutrophic, high-DOM, and alkaline lake in the Netherlands, a relatively higher proportion of ^{33}P was found in the high-molecular-weight (HMW) fraction (Jones *et al*., 1988). Another study demonstrated that Fe binding to the fulvic acid fraction was unimportant in Lake Tjeukemeer, and it was concluded that the high concentration of Cu outcompeted the Fe for those binding sites (de Haan and de Boer, 1986). Some lakes appear to have a higher proportion of humic to fulvic acids (Jackson and Schindler, 1975). This could potentially influence how P and Fe are bound to DOM, resulting in differences in bioavailability (Buffle, 1988).

In a time course, it was observed that Fe bound much more rapidly than did PO_4 (de Haan *et al*., 1990). Indeed, 20% of the added Fe bound to the DOM in less than a minute, whereas it took 24 h for the equivalent proportion of PO_4 to bind. De Haan *et al*. (1990) also found that a greater proportion of the Fe and PO_4 bound in the lower molecular weight fraction. Upon further refractionation of this peak, they found that the Fe eluted in a pattern identical to its original one, whereas PO_4 formed two distinct peaks. The first peak corresponded to the original, whereas the second one coincided with the elution volume of free ionic PO_4. From these results it was concluded that Fe was bound more tightly to the DOM and that the binding of PO_4 was reversible. Jones *et al*. (1988) also observed the displacement of $^{33}PO_4$ from the HMW complexes by the addition of excess free $^{31}PO_4$. However, the displacement was incomplete, suggesting that at least part of the PO_4 was more tightly bound and potentially less bioavailable. This may be due in part to the different PO_4 associations whereby PO_4 bound by metal bridge exchanges with free ionic PO_4 to a greater extent than PO_4 bound in the organically coated Fe colloids. Interestingly, the proportion of the added labeled PO_4 that binds to the DOM-Fe complex is small relative to the amount of Fe that binds to the DOM. Up to 85% of the added ^{55}Fe was associated with the DOM, but only 15% of the added radiolabeled P was bound (De Haan *et al*., 1990; Jones *et al*., 1993).

Some studies have suggested that the strength of the DOM-Fe-P complex is influenced by both the pH and the ionic strength of the water. Jones *et al*. (1993) found that there was a decrease in the amount of Fe and PO_4

associated with the humic fractions at lower pH values. The reduction in the Fe : C and the P : C molar ratios with decreasing pH was suggestive of decreased binding of Fe by the DOM and/or of P by the DOM-Fe. The strength of the binding of dissolved and particulate iron to DOM is reduced at a lower pH (Tipping, 1981), as the higher concentration of H^+ competes with the Fe for the ligand-binding sites on the organic acids. This effect is apparently strong enough to outweigh the decreased tendency for Fe(III) to precipitate at lower pH values. The relatively weak association of Fe(II) with DOM and phosphate and the decreased rate of Fe(II) oxidation at lower pH values may also cause a net decrease in DOM-Fe-P at low pH when Fe(III)-DOM reduction mechanisms are active (discussed below). The effect of pH on the Fe-P association is less clear. Normally, the release of OH^- during the ligand-exchange process that accompanies surface binding of anions to oxides would favor the Fe-P association at decreased pH (Dzombak and Morel, 1987; Stumm and Morgan, 1996). However, the decreased stability of the iron oxide particles and the Fe-DOM complex at low pH may be more important overall. Additionally, the protonation of the phosphate anion itself at lower pH values may also decrease its ability to bind to any DOM-Fe present.

Shaw (1994) looked at the effect of pH on the formation of particulate Fe and PO_4 in samples with and without humics. In the presence of humics, practically all of the added Fe and PO_4 across the pH range remained in the dissolved state. In clear waters, particulates formed at pH ≥5 at low ionic strength and at pH ≥7 at high ionic strength. Humics therefore maintain both elements in dissolved form at circumneutral pH, but at more acidic pH, the association with the DOM may not be necessary to maintain Fe and PO_4 in dissolved form. However, the speciation of the dissolved forms of Fe and PO_4 in these two scenarios would differ substantially.

The ionic strength and composition of the lake water are also suspected to play an important role in the formation of the DOM-Fe-P complex (Reuter and Perdue, 1977; Jones *et al*., 1993). At reduced ionic strength fewer ions would be competing for the ligand-binding sites of the DOM, and the activity of the charged iron species would be increased; thus in brown water lakes of lower ionic strength, the DOM-Fe-P complex is more likely to form. Indeed, the combined concentration of Al and Fe appears to be highly correlated with DOM in natural waters (Perdue *et al*., 1976), more so than the elements taken separately, suggesting that there is a competition among them for the same DOM chelating sites.

However, direct evidence of the importance of ionic strength in limiting the formation of the DOM-Fe-P complex in freshwaters remains scant. In soils, decreased metal-fulvic binding at higher ionic strength has been noted (Schnitzer and Hansen, 1970). A reduction in the binding of Fe to humics was observed in lakes (Stewart and Wetzel, 1981) and in soils (Kretzschmar and Sticher, 1997) with high Ca concentrations. Indeed, because of the

effects on Fe speciation, terrestrial and aquatic plants growing in calcareous soils and sediments are often observed to be Fe stressed (Loeppert, 1986; Duarte *et al*., 1996). In contrast, De Haan *et al*. (1990) observed no effect on the proportion of radiolabeled Fe and P eluted by gel filtration when they artificially increased the ionic strength of natural high-DOC lake samples. However, in a different study, natural systems of similar DOM concentrations but contrasting ionic strength had different elution patterns where Fe and P were associated with different peaks (Jones *et al*., 1993; Shaw *et al*., 2000).

Much of the Fe and P associated with DOM entering lakes originates from the soils of the surrounding watershed (Driscoll *et al*., 1988; Dillon and Molot, 1997). Dolfing *et al*. (1999) found that most of the P in their soil extracts was in the form of P associated with organically coated Fe colloids. Thus transformation within the lake is likely required to enhance the availability of these elements. Recently Shaw *et al*. (2000) suggested that humics promote the association of Fe and PO_4 with higher molecular weight fractions by primarily acting as peptizing agents for inorganic colloids containing Fe and P, as described by Shapiro (1967) and Tipping (1981). Their results also demonstrated that under certain conditions increasing the concentration of humic substances of a particular molecular weight size fraction enhanced Fe and PO_4 binding. They suggested that the latter was the result of the DOM-Fe-P complex formation via the metal bridge as described by Schnitzer and Khan (1972).For example, newly formed Fe hydroxides associated with DOM tend to be more bioavailable (Finden *et al*., 1984; Wells *et al*., 1991) and tend to bind PO_4 with greater affinity than older hydroxides (Lijklema, 1980; Tate *et al*., 1995). Newer Fe hydroxides are likely bound to the surface of humics rather than organically coated. Therefore the Fe of newly formed hydroxides would likely be more available via biological reduction, and the PO_4 associated with this Fe appears to be more easily dissociated from the complex.

VI. FACTORS INFLUENCING THE BIOAVAILABILITY OF Fe AND P BOUND TO DOM

Given that the Fe and P bound to the DOM in humic lakes are probably not directly available to organisms, the DOM-Fe-P complex would have to undergo some form of transformation to yield bioavailable Fe and P species. In terms of biological uptake, in general it can be said that Fe is preferred in the dissolved free ionic form and P as orthophosphate (PO_4). Several known processes result in the transformation of Fe and P from the DOM-Fe-P complex into biologically available species. Two of the mechanisms are physical-chemical processes: (1) UV-mediated photoreduction and (2) DOM-mediated chemical reduction or “dark” reduction. Other mechanisms are

biologically mediated and include (1) biological reduction, (2) siderophore production (Fe only), (3) enzyme-mediated hydrolysis (P only), and (4) grazer regeneration.

Of the mechanisms that increase the bioavailability of Fe and/or P, three of them induce the redox cycling of Fe. There are two common redox states of iron in natural waters, Fe(III) and Fe(II). Fe(III) is thermodynamically favored in oxygenated surface waters of circumneutral pH, and generally that form of Fe is bound in Fe-DOM complex. Photochemical, dark, and biological reduction all involve the reduction of Fe(III) bound in the DOM complex, which results in the creation of either free ionic Fe(II) or an Fe(II)-DOM complex (Fig. 6). The Fe(II)-DOM complex is considered weaker and/or more kinetically labile than Fe(III) complexes (Langford and Gray, 1965; Langford *et al.*, 1981; Stumm and Morgan, 1996), and the Fe dissociates more readily from the complex to form free Fe(II), thus enhancing Fe bioavailability. Given that PO_4 binds preferentially with Fe(III) (Lijklema, 1980), the photochemical, dark, and biological reduction of Fe bound to the DOM should also cause the release of the PO_4 associated with that Fe.

Of all the mechanisms that should enhance the bioavailability of complexed Fe and P, the photochemical reduction of Fe and the subsequent release of P from the DOM-Fe-P complex have received the most attention

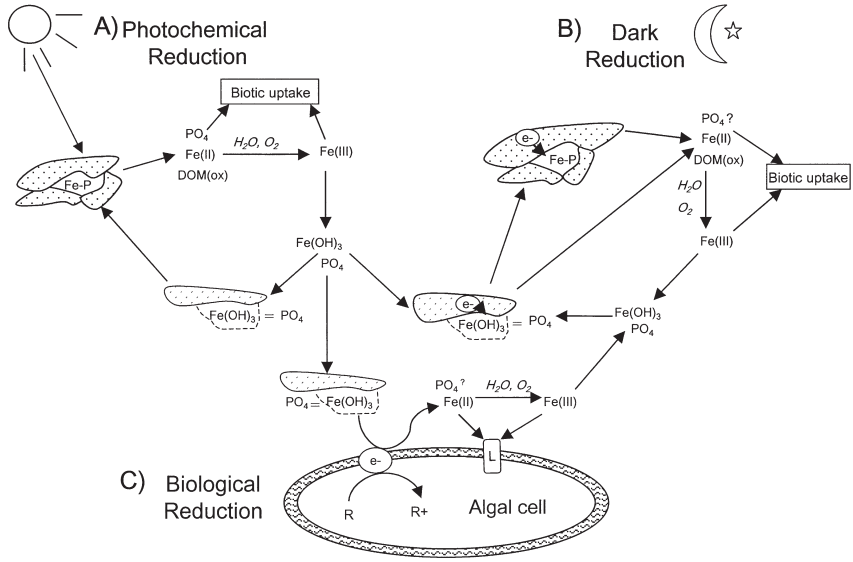

FIGURE 6 The REDOX cycling of Fe bound to DOM via (A) photochemical and (B) dark and (C) biological reduction. Oxidation of Fe is considered immediate, given that the reactions are taking place in oxygenated water and in the presence of H_2O_2. Associated PO_4 release is depicted; however, where this release has not been observed, but is suspected, it is denoted with a question mark.

(Fig. 6A). DOM-Fe complexes absorb UV and visible light, which results in the direct photochemical reduction of the Fe (Waite and Morel, 1984a; Voelker *et al*., 1997). Additionally, Fe(III) can also be reduced by superoxide radicals (Voelker and Sedlak, 1995), a by-product of the reaction of DOM with sunlight (Zarifou *et al*., 1984). A number of researchers have reported a diurnal cycle of Fe(II) in the surface waters of lakes (McMahon, 1969; Collienne, 1983; McKnight *et al*., 1988; Sulzberger *et al*., 1990; Sivan *et al*., 1998; Emmenegger *et al*., 2001). This is hypothesized to be the result of daytime photochemical or biochemical reduction of Fe, followed by re-oxidation at night.

In a comparison between clear and colored lakes, Francko and Heath (1979) found that PO_4 was released with UVR exposure in a colored lake, but that UVR had no effect on PO_4 release in the clear system. Upon further investigation, the release of PO_4 was indeed linked to the reduction of Fe from the complex (Francko and Heath, 1982). Cotner and Heath (1990) examined the effects of added Fe and UVR on the release of PO_4 in a temperate acid bog lake over the course of the growing season. They found that the amount of PO_4 released increased with added Fe and UVR exposure.

The Fe(II) formed by this and other processes can be reoxidized by dissolved O_2 or H_2O to form Fe(III) again. It has been reported that the Fe(II)-DOM complex can be oxidized at a faster or slower rate than free Fe(II), depending on conditions (Theis and Singer, 1974; Miles and Brezonik, 1981; Liang *et al*., 1993; Voelker and Sulzberger, 1996; Emmenegger *et al*., 1998; Pullin 1999). If the rate of reduction is slightly faster than oxidation, steady-state concentrations of free Fe(II) will persist (Voelker *et al*., 1997; Pullin 1999). This is favored in waters of lower pH and at anoxic interfaces (Buffle *et al*., 1989), where the reoxidation of Fe(II) is slowed. Interestingly, and probably as a function of the acidic pH of Triangle Bog Lake (pH <5), the rates of reoxidation of Fe(II) to Fe(III) were reduced relative to the rates of photoreduction which resulted in a net accumulation of Fe(II) (Cotner and Heath, 1990). Although Fe(II) was always produced with UVR exposure, PO_4 was not always released. It was suggested that the depletion of Fe by the algae during a bloom period (no Fe accumulation observed) could have limited the UV-sensitive P release in this lake.

Fe(III) can react chemically with DOM to form Fe(II) in what is known as "dark" or nonphotochemical Fe reduction (Stumm and Morgan, 1996; Waite and Morel, 1984b; Voelker *et al*., 1997; Pullin, 1999) (Fig. 6B). This process probably involves the binding of Fe(III) by catechol-type ligands, followed by an electron transfer to form Fe(II) (Pullin, 1999). Electron transfer from nonbinding humic quinone moieties has also been suggested (Scott *et al*., 1998). Photochemical Fe reduction is directly linked to light intensity (Emmenegger *et al*., 2001), and, given the rapid attenuation of sunlight and ultraviolet radiation (UVR) in brown water lakes (Scully and Lean, 1994), dark reduction may be an important process by which chelated

Fe is made more bioavailable at depth and at night. Whether P is released via this dark reduction remains unknown, but this could also be an important mechanism of P release, particularly at depth.

Finally, Fe(III)-DOM can undergo biologically mediated reduction at the surface of algal cells where nonspecific redox proteins that span the plasmalemma can reduce the Fe(III) bound to a variety of organic ligands (Anderson and Morel, 1980; Jones *et al*., 1987; Hutchins *et al*., 1999; Maldonado and Price, 1999). The Fe(II) dissociates from the ligand and is either taken up directly or is reoxidized before internalization (Fig. 6C). In one study, organically complexed Fe was provided to the phytoplankton both as siderophore-bound Fe and in the form of tetrapyrroles (Hutchins *et al*., 1999). Interestingly, eukaryotic phytoplankton preferred Fe bound to porphyrins (cellular products) as compared with the prokaryotic organisms that preferentially took up Fe bound to siderophores. The conditional stability constants of these complexes are similar ($\log K_{cond}$ = 19–22) (Butler, 1998; Granger and Price, 1999; Hutchins *et al*., 1999), but their structures were different. Both complex Fe in a mononuclear fashion, but the siderophore ligands were hexadentate in structure, encasing the Fe atom, whereas the porphyrin was tetradentate and planar in shape (Fig. 7). The stereochemical shielding of the Fe in the hexadentate siderophore Fe complex may restrict its reduction by the nonspecific algal reductase.

Peaks in Fe(II) concentrations have been observed at the chlorophyll maximum of at least one lake (Emmenegger *et al*., 2001). They suggested that this was a function of biological reduction by the algae, as there was not enough light reaching that depth to account for the Fe(II) accumulation. However, the biological reduction of Fe bound to humics and fulvic acids is not known. If steric hindrance impedes the rate of biological reduction, Fe adsorbed to the surface of the DOM would be more accessible to organisms relative to the physically inaccessible Fe surrounded by large humic acids. There are some reports that the addition of Fe associated with humics and fulvics stimulates phytoplankton growth in culture (Prakash *et al*., 1973; Finden *et al*., 1984), but this response appears to be species dependent (Shapiro, 1967). Prakash *et al*. (1973) found that the addition of low-molecular-weight humic and fulvic fractions enhanced phytoplankton growth in cultures, but elevated concentrations or the addition of high-molecular-weight organic acids inhibited growth. They concluded that this was due mainly to the excessive metal binding of the humics and would be consistent with the steric hindrance argument.

If the Fe bound in Fe-DOM complex is not directly available for biological reduction, it could become available in highly humic lakes through the biological synthesis and release of siderophores by prokaryotic organisms. The very high binding constants of siderophores for Fe(III) (as discussed above) would result in the removal of iron from Fe-DOM complexes that have a lower binding affinity (Urban *et al*., 1990). Thus the availability of Fe

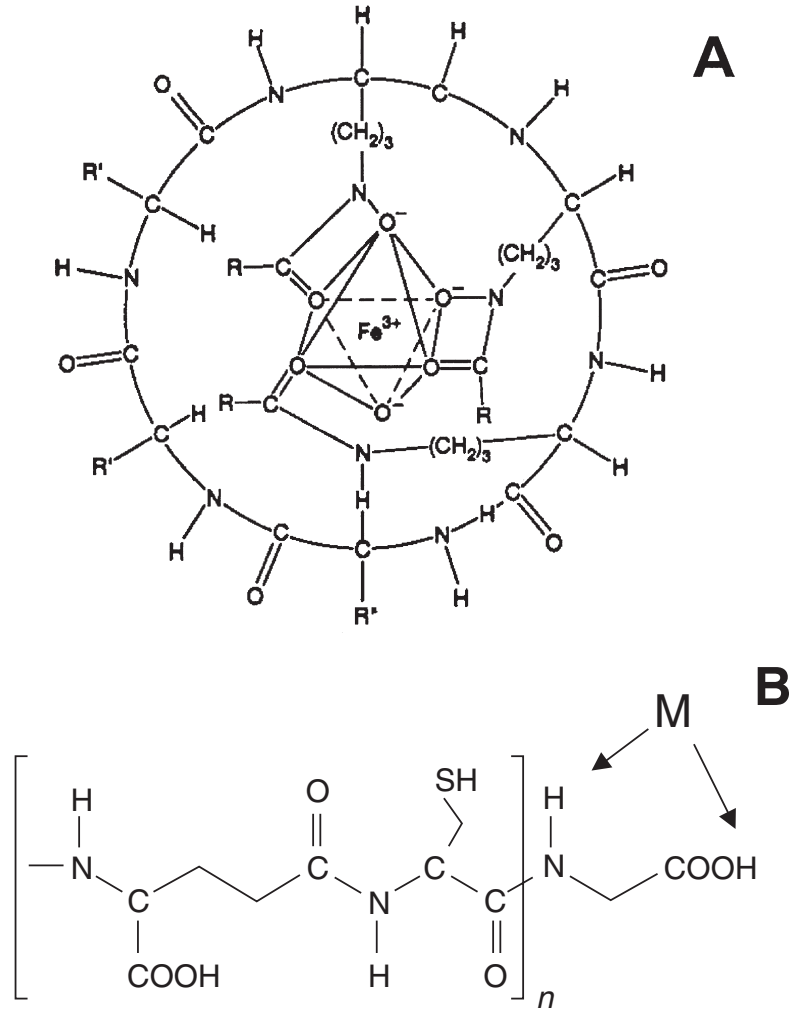

FIGURE 7 Examples of Fe ligands. (A) The bacterial hexadentate siderophore enterobactin. (B) The planar porphyrin phytochelatin. M denotes the metal binding site. Reprinted with permission from Stumm W., and J. J. Morgan. 1996. "Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters." Copyright 1996 Wiley, New York.

for biological reduction may be a two-step process whereby the prokaryotes secrete compounds that form Fe-ligand complexes that are in turn available to them and to certain algal species by a specific reduction or internalization pathway (Fig. 8).

Microorganisms also play an important role in the regulation of the chemical environment by synthesizing and excreting ectoenzymes. Enzyme-

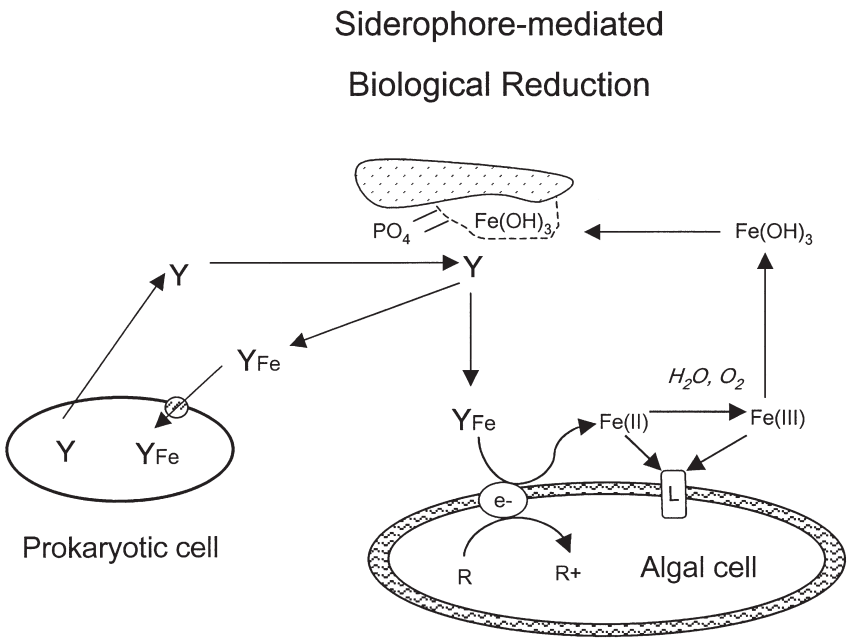

FIGURE 8 Siderophore-mediated biological reduction schematically presented, whereby prokaryotes synthesize Fe binding ligands (Y) that have stronger affinity for Fe than the humic ligands. The Fe is either internalized directly or reduced off the siderophore complex by an electron donor surface cell protein and subsequently internalized by a cell surface ligand (L).

mediated hydrolysis (see Chapter 13) is an important process by which phytoplankton and bacteria can access HMW dissolved organic phosphorus. Alkaline phosphatase activity (APA) as an enzymatic mode of P acquisition under P-limited conditions has received the most attention (Chrøst, 1990). However, the reports of APA in brown versus clear-water lakes are inconclusive. Francko and Heath (1979) measured APA in a clear-water lake, but no APA was observed in a humic lake. They concluded that P was cycled differently in brown and clear-water lakes: UV-mediated P release from DOM-Fe-P complexes was the most important mechanism by which P was made available to organisms in humic lakes, whereas enzyme-mediated hydrolysis of dissolved organic phosphorus (DOP) was most important in clear water systems. Other studies have reported stimulated APA upon the addition of humic substances to lake samples (Auclair *et al*., 1985; Francko, 1986). However, in both of those studies, humic acid additions were made to very clear-water systems to study the effects of increased DOM loading on the P cycling of these lakes. Enhanced APA was observed in both the eutrophic and oligotrophic systems with humic addition, but increased PO_4 uptake rates were observed only in the eutrophic system. It is possible that

the organisms in both of these systems were primed to release the alkaline phosphatase enzyme and that APA observed with and without subsequent PO_4 uptake was perhaps a function of different P species among the systems.

VII. ELEMENTAL ACQUISITION IN HUMIC LAKES: IMPLICATIONS FOR ECOSYSTEM STRUCTURE AND FUNCTION

Bacteria are known to outcompete algae for the uptake of available P in P-limited systems (Currie and Kalff, 1984). The greater ability of the bacterial community to acquire P has also been demonstrated in a small humic lake (Salonen *et al*., 1994). Bacteria tend to dominate in terms of both biomass and production in humic lakes (Tranvik, 1989; Hessen, 1992) and are likely the most important mobilizers of both Fe and P in these systems.

Although primary production is predominantly light limited in humic lakes, it is quite possible that the structure of the microbial food web in these aquatic ecosystems is affected by the difficulty of acquiring Fe and P bound in the DOM-Fe-P complex. These required elements move to higher trophic levels primarily via the initial mobilization by bacteria and then by a series of grazing organisms, including mixotrophic phytoplankton. Mixotrophic nanoflagellate and dinoflagellate species that simultaneously graze bacteria and perform photosynthesis (Raven, 1997; Jones, 2000) tend to dominate the phytoplankton in humic systems (Jansson, 1998; Jones, 1998). Indeed, in some humic lakes mixotrophic nanoflagellates are simultaneously the most abundant primary producer and the most important grazer of bacteria (Jansson *et al*., 1999; Bergström and Issakson, 2001). These organisms can acquire P (Nygaard and Tobiesen, 1993) and Fe (Maranger *et al*., 1998) via phagotrophy. Nanoflagellates, both heterotrophic and mixotrophic, graze on bacteria and acquire P and Fe with a relatively high assimilation efficiency, around 30% or greater (Caron *et al*., 1993; Rothhaupt, 1996; Chase and Price, 1997; Maranger *et al*., 1998). However, important feedbacks also exist within these grazing assemblages. The Fe and P regenerated by nanoflagellates and other zooplankton grazers may be more bioavailable than the initial DOM-Fe-P complex and could become an important source of dissolved nutrients for both bacteria and nonmixotrophic eukaryotic phytoplankton (Hutchins *et al*., 1994; Salonen *et al*., 1994; Barbeau *et al*., 1996).

Given how the DOM-Fe-P complex influences ecosystem structure, it is also likely that the DOM-Fe-P complex influences humic lake ecosystem function in terms of C metabolism. First, the bacterial conversion of dissolved C into biomass it is quite low, with an average of 25% across a trophic gradient (del Giorgio *et al*., 1997). And despite the evidence that bacteria may be better equipped to acquire the Fe and P bound to DOM, the added energetic expenditure in acquiring these elements may result in a significant

reduction in bacterial C growth efficiency (Tortell *et al.*, 1996, Cimbleris and Kalff, 1998) in humic-rich systems. Second, the role of mixotrophic phytoplankton add another level of complexity to the C-energetics of humic lakes. The C-transfer from bacteria to photosynthetic mixotrophs could range widely in efficiency, given that most species rely on dissolved inorganic carbon for their photosynthetic needs (Jones, 2000). It is also hypothesized that the C from bacteria ingested by the mixotrophs is initially respired and then fixed photosynthetically (Caron e*t al.*, 1993). Indeed, mixotrophic phytoplankton add another level of trophic transfer that results in additional C loss from these ecosystems. Thus we speculate that the complexation of Fe and P by DOM plays an important role in determining the structure and the function of humic lake ecosystems.

VIII. CONCLUSIONS

Given that a large proportion of lakes in temperate and boreal regions are stained brown, the influence of dissolved humic substances on the structure, function, and composition of these aquatic ecosystems are important ecological question. When comparing clear-water versus humic lakes, it is generally thought that per unit P, brown-water lakes tend to have lower system primary productivity (Hessen, 1998; Wetzel, 2001). Nürnberg and Shaw (1998), in a large-scale comparative study, reported that lakes with high DOC tended to have higher algal biomass, measured as chlorophyll *a*, than lakes with lower concentrations of DOC. However, this overall relationship explained very little variance ($r^2 = 0.17$) and did not make the important and necessary distinction between highly colored humic lakes and DOC-rich hypereutrophic lakes. The high DOC concentrations and associated color of these hypereutrophic lakes are largely algal in origin and would differ greatly from the humic and fulvic content of the DOC in brown lakes. Interestingly, a subset of data from this same study reported negative relationships between annual areal primary production (APP) versus lake color and light extinction, both surrogates for humic acid concentration. Light limitation is likely to be the main reason for this reduction in primary productivity because these dissolved humic materials efficiently absorb light necessary for photosynthesis (Jones, 1998; Carpenter *et al.*, 1998). However, APP should account, at least in part, for light limitation, given that the primary production values are considered in the epilimnion only (Nürnberg and Shaw, 1998). Another study, albeit with a small sample size, also observed a negative relationship in epilimnetic primary production across a DOC-color gradient (Jansson *et al.*, 2000). Although we agree that shading by colored DOM is important in inhibiting phytoplankton production, we hypothesize that the chelation of essential elements by DOM is a significant mechanism that reduces the bioavailability of Fe and P and contributing significantly to the lower primary productivity in these systems.

This chapter summarizes the importance of Fe to aquatic organisms and how the binding of both Fe and P by DOM may impinge on plankton Fe, P acquisition and describes the various pathways that may enhance the bioavailability of these elements. Certain organisms appear to have developed superior acquisition strategies. As a consequence, bacteria and mixotrophic flagellates tend to dominate the plankton in humic lakes and likely act as an important Fe and P link to higher organisms. Although light limitation may be the most important factor in suppressing total primary productivity in humic-rich lakes, we speculate that the chelation of essential elements by humic substances has a marked impact on both the structure and functioning of these ecosystems.

Although the effects of DOM on the speciation of Fe and P are certainly complicated, a number of generalities can be drawn. DOM slows the removal of Fe and P from the water column through complexation. However, this complexation also creates a slow-reacting pool of Fe and P, making these essential elements difficult to acquire by organisms. The biological availability of the Fe bound to DOM remains untested in lake waters. A number of processes, including enzyme hydrolysis; photochemical, dark, and biological reduction; and possibly siderophore production, may provide a mechanism for releasing this bound Fe and P slowly into the aqueous matrix, when it can be used by biota. Given the importance of Fe for the metabolic function of bacteria and phytoplankton, this idea hints that complexation of Fe may influence the energetics of an ecosystem even if overall primary productivity is controlled by another element (such as P or N) and/or light availability.

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REFERENCES

Anderson, M. A., and F. M. M. Morel. 1980. Uptake of Fe (II) by a diatom in oxic culture medium. *Marine Biology Letters* **1:**263–268.

Auclair, J. C. 1995. Implications of increased UV-B induced photoreduction: Iron(II) enrichment stimulated picocyanobacterial growth and the microbial food web in clear-water acidic Canadian Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **52:**1782–1788.

Auclair, J. C., P. Brassard, and P. Couture. 1985. Total dissolved phosphorus: Effects of two molecular weight fractions on phosphorus cycling in natural phytoplankton communities. *Water Research* **19:**1447–1453.

Barbeau, K., J. W. Moffett, D. A. Caron, P. L. Croot, and D. L. Erdner. 1996. Role of protozoan grazing in relieving iron limitation of phytoplankton. *Nature* **380:**61–64.

Bergström, A., and A. Isaksson. 2001. The importance of mixotrophic flagellates as bacterial grazers in a large humic lake — results from a three-year study in northern Sweden. *In* "American Society of Limnology and Oceanography Meeting," Albuquerque, NM, Abstract, p. 23.

Boyd, P. W., A. J. Watson, C. S. Law, E. R. Abraham, T. Trull, R. Murdoch, D. C. E. Bakker, A. R. Bowie, M. Charette, P. Croot, K. Downing, R. Frew, M. Gall, M. Hadfield, J. Hall, M. Harvey, G. Jameson, J. La Roche, M. Liddicoat, R. Ling, M. Maldonado, R. M. McKay, S. Nodder, S. Pickmere, R. Pridmore, S. Rintoul, K. Safi, P. Sutton, R. Strzepek, K. Tanneberger, S. Turner, A. Waite, and J. Zeldis. 2000. A mesoscale phytoplankton bloom in the Polar Southern Ocean stimulated by iron fertilization. *Nature* **407:**695–702.

Buffle, J. 1988. "Complexation Reactions in Aquatic Systems: An Analytical Approach," p. 692. Wiley, New York.

Buffle, J., R. R. De Vitre, D. Perret, and G. G. Leppard. 1989. Physico-chemical characteristics of a colloidal iron phosphate species formed at an oxic-anoxic interface of a eutrophic lake. *Geochimica et Cosmochimica Acta* **53:**399–408.

Butler, A. 1998. Acquisition and utilization of transition metal ions by marine organisms. *Science* **281:**207–210.

Cabaniss, S. E. 1987. TITRATOR: An interactive program for aquatic equilibrium calculations. *Environmental Science and Technology* **21:**209–210.

Caron, D. A., R. W. Sanders, E. L. Lim, C. Marrase, L .A. Amaral, S. Whitney, R. B. Aoki, and K. G. Porter. 1993. Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. *Microbial Ecology* **25:**93–111.

Carpenter, S. R., J. J. Cole, J. F. Kitchell, and M. L. Pace. 1998. Impact of dissolved organic carbon, phosphorus, and grazing on phytoplankton biomass and production in experimental lakes. *Limnology and Oceanography* **43:**73–80.

Chang, C. C. Y., J. S. Kuwabara, and S. P. Pasilis. 1992. Phosphate and iron limitation of phytoplankton biomass in Lake Tahoe. *Canadian Journal of Fisheries and Aquatic Sciences* **49:**1206–1215.

Chase, Z., and N. M. Price. 1997. Metabolic and oceanographic consequences of Fe deficiency in heterotrophic marine protozoa. *Limnology and Oceanography* **42:**1673–1684.

Chrøst, R. J. 1990. Ectoenzymes in aquatic environments: Origin, activity and ecological significance. *In* "Advanced Biochemical and Molecular Approaches to Aquatic Microbial Ecology" (J. Overbeck and R. J. Chrøst, Eds.), pp. 47–78. Springer, New York.

Cimbleris, A. C. P., and J. Kalff. 1998. Planktonic bacterial respiration as a function of C : N : P ratios across temperate lakes. *Hydrobiologia* **384:**89–100.

Coale, K. H., K. S. Johnson, S. E. Fitzwater, R. M. Gordon, S. Tanner, F. P. Chavez, L. Ferioli, C. Sakamoto, P. Rogers, F. Millero, P. Steinberg, P. Nightingale, D. Cooper, W. P. Cochlan, M. R. Landry, J. Constantinou, G. Rollwagen, A. Trasvina, and R. Kudela. 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature* **383:**495–501

Collienne, R. H. 1983. Photoreduction of iron in the epilimnion of acidic lakes. *Limnology and Oceanography* **28:**83–100.

Cooper, W. J., R. G. Zika, R. G. Petasne, and J. M. C. Plane. 1988. Photochemical formation of H_2O_2 in natural waters exposed to sunlight. *Environmental Science and Technology* **22:**1156–1169.

Cornell, R. M., and U. Schwertmann. 1979. Influence of organic anions on the crystallization of ferrihydrite. *Clays and Clay Minerals* **27:**402–410.

Cotner, J. B., and R. T. Heath. 1990. Iron redox effects on photosensitive phosphorus release from dissolved humic materials. *Limnology and Oceanography* **35:**1175–1181.

Currie, D., and J. Kalff. 1984. The relative importance of phytoplankton and bacterioplankton in phosphorus uptake in freshwater. *Limnology and Oceanography* **29:**298–310.

De Haan, H., and T. de Boer. 1986. Geochemical aspects of aqueous iron, phosphorus and dissolved organic carbon in the humic Lake Tjeukemeer, the Netherlands. *Freshwater Biology* **16:**661–672.

De Haan, H., R. I. Jones, and K. Salonen. 1990. Abiotic transformations of iron and phosphate in humic lake water revealed by double-isotope labeling and gel filtration. *Limnology and Oceanography* 35:491–497.

del Giorgio, P. A., J. J. Cole, and A. Cimbleris. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* **385:**148–150.

Dillon, P. J., and L. A. Molot. 1997. Effect of landscape form on export of dissolved organic carbon, iron, and phosphorus from forested stream catchments. *Water Research* **33:**2591–2600.

Dolfing, J., W. J. Chardon, and J. Japenga. 1999. Association between colloidal iron, aluminum, phosphorus, and humic acids. *Soil Science* **164:**171–179.

Driscoll, C. T., R. D. Fuller, and D. M. Simone. 1988. Longitudinal variations in trace metal concentrations in a northern forested ecosystem. *Journal of Environmental Quality* **17:**101–107.

Driscoll, C. T., C. Yan, C. L. Schofield, R. Munson, and J. Holapple. 1994. The mercury cycle and fish in the Adirondack lakes. *Environmental Science and Technology* **28:**136–143.

Duarte, C. M., M. Merino, and M. Gallegos. 1995. Evidence of iron deficiency in seagrasses growing above carbonate sediments. *Limnology and Oceanography* **40:**1153–1158.

Dzombak, D. A., and F. M. M. Morel. 1987. Adsorption of inorganic pollutants in aquatic systems. *Journal of Hydraulic Engineering ASCE*. **113:**430–475.

Emmenegger, L., D. W. King, L. Sigg, and B. Sulzberger. 1998. Oxidation kinetics of Fe(II) in a eutrophic Swiss lake. *Environmental Science and Technology* **32:**2990–2996.

Emmengger, L., R. Schönenberger, L. Sigg, and B. Sulzberger. 2001. Light-induced redox cycling of iron in circumneutral lakes. *Limnology and Oceanography* **46:**49–61.

Evans, J. C., and E. E. Prepas. 1997. Relative importance of iron and molybdenum in restricting phytoplankton biomass in high phosphorus saline lakes. *Limnology and Oceanography* **42:**461–472.

Finden, D. A. S., E. Tipping, G. H. M. Jaworski, and C. S. Reynolds. 1984. Light-induced reduction of natural iron(III). oxide and its relevance to phytoplankton. *Nature* **309:**783–784.

Francko, D. A. 1986. Epilimnetic phosphorus cycling: Influence of humic materials and iron on coexisting major mechanisms. *Canadian Journal of Fisheries and Aquatic Sciences* **43:**302–310.

Francko, D. A., and R. T. Heath. 1979. Functionally distinct classes of complex phosphorus compounds in lake water. *Limnology and Oceanography* **24:**463–473.

Francko, D. A., and R. T. Heath. 1982. UV-sensitive complex phosphorus: Association with dissolved humic material and iron in a bog lake. *Limnology and Oceanography* **27:**564–569.

Gledhill, M., and C. M. G. Van den Berg. 1994. Determination of complexation of iron(III) with natural organic complexing ligands in seawater using cathodic stripping voltammetry. *Marine Chemistry* **47:**41–54.

Granger, J., and N. M. Price. 1999. The importance of siderophores in iron nutrition of heterotrophic marine bacteria. *Limnology and Oceanography* **45:**541-555.

Green, R. M., Z. S. Kolber, D. G. Swift, N. W. Tindale, and P. G. Falkowski. 1994. Physiological limitation of phytoplankton photosynthesis in the eastern equatorial Pacific determined from variability in the quantum yield of fluorescence. *Limnology and Oceanography* **39:**1061–1074.

Hessen, D. O. 1992. Dissolved organic carbon in a humic lake; effects on bacterial production and respiration. *Hydrobiologia* **229:**115–123.

Hessen, D. O. 1998. Food webs and carbon cycling in humic lakes. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 285–315. Springer-Verlag, Berlin.

Hudson, R. J. M., and F. M. M. Morel. 1990. Iron transport in marine phytoplankton: Kinetics of cellular and medium coordination reactions. *Limnology and Oceanography* **35:**1002–1020.

Hutchins, D. A., and K. W. Bruland. 1994. Grazer-mediated regeneration and assimilation of Fe, Zn, and Mn from planktonic prey. *Marine Ecology Progress Series* **110:**259–269.

Hutchins, D. A., G. R. DiTullio, Y. Zhang, and K. W. Bruland. 1998. An iron limitation mosaic in the California upwelling regime. *Limnology and Oceanography* **43:**1037–1054.

Hutchins, D. A., A. E. Witter, A. Butler, and G. W. Luther. 1999. Differential utilization of chelated iron species by marine phytoplankton taxa. *Nature* **400:**858–861.

Hutchinson, G. E. 1957. "A Treatise on Limnology. Geography, Physics, and Chemistry" (Y. H. Edmonson, Ed.), Vol. I. Wiley, New York.

Jackson, T. A., and R. E. Hecky. 1980. Depression of primary productivity by humic matter in lake and reservoir waters of the boreal forest zone. *Canadian Journal of Fisheries and Aquatic Sciences* **37:**2300–2316.

Jackson, T. A., and D. W. Schindler. 1975. The biogeochemistry of phosphorus in an experimental lake environment: Evidence for the formation of humic-metal-phosphate complexes. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **19:**211–221.

Jansson, M. 1998. Nutrient limitation and bacteria-phytoplankton interactions in humic lakes. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 177–195. Springer-Verlag, Berlin.

Jansson, M., A. K. Bergstrom, P. Blomqvist, A. Isaksson, and A. Jonsson. 1999. Impact of allochthonous organic carbon on microbial food web carbon dynamics and structure in Lake Ortrasket. *Archive für Hydrobiologia* 144:409–428.

Jansson, M., A. K. Bergstrom, P. Blomqvist, and S. Drakare. 2000. Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology* **8:**3250–3255.

Johnson, K. S., R. M. Gordon, and K. H. Coale. 1997. What controls dissolved iron concentrations in the world's oceans? *Marine Chemistry* **57:**137–161.

Jones, G. J., B. P. Palenik, and F. M. M. Morel. 1987. Trace metal reduction by phytoplankton: The role of plasmalemma redox enzymes. *Journal of Phycology* **23:**237–244.

Jones, R. I. 1998. Phytoplankton, primary production and nutrient cycling. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 146–175. Springer-Verlag, Berlin.

Jones, R. I. 2000. Mixotrophy in planktonic protists: An overview. *Freshwater Biology* **45:**219–226.

Jones, R. I., K. Salonen, and H. De Haan. 1988. Phosphorus transformations in the epilimnion of humic lakes: Abiotic interactions between dissolved humic materials and phosphate. *Freshwater Biology* **19:**357–369.

Jones, R. I., P. J. Shaw, and H. De Haan. 1993. Effects of dissolved humic substances on the speciation of iron and phosphate at different pH and ionic strength. *Environmental Science & Technology* **27:**1052–1059.

Koenings, J. P., and F. F. Hooper. 1976. The influence of colloidal organic matter on iron and iron-phosphorus cycling in an acid bog lake. *Limnology and Oceanography* **21:**684–696.

Kretzschmar R., and H. Sticher. 1997. Transport of humic-coated iron oxide colloids in a sandy soil: Influence of Ca^{2+} and trace metals. *Environmental Science & Technology* **31:**3497–3504.

Langford, C. H., and H. B. Gray. 1965. "Ligand Substitution Processes." W. A. Benjamin, New York.

Langford, C. H., S. M. Wong, and A. W. Underdown. 1981. The interaction of a soil fulvic acid with precipitating hydrous ferric oxide at pH = 6. *Canadian Journal of Chemistry* **59:**181–186.

Leenheer, J. A., G. K. Brown, P. MacCarthy, and S. E. Cabaniss. 1998. Models of metal binding structures in fulvic acids from the Suwannee River, Georgia. *Environmental Science & Technology* **32:**2410–2416.

Liang, L., J. A. McNabb, J. M. Paulk, B. Gu, and J. F. McCarthy. 1993. Kinetics of Fe (II) oxidation at low partial pressures of oxygen in the presence of natural organic matter. *Environmental Science & Technology* **27:**1864–1870.

Lijklema, L. 1980. Interaction of orthophosphate with iron (III) and aluminum hydroxides. *Environmental Science and Technology* **14:**537–541

Loeppert, R. H. 1986. Reactions of iron and carbonates in calcareous soils. *Journal of Plant Nutrition* **9:**195–214

Maldonado, M. T., and N. M. Price. 1999. Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. *Deep-Sea Research II* **46:**2447–2473.

Maranger, R., D. F. Bird, and N. M. Price. 1998. Iron acquisition by photosynthetic marine phytoplankton from ingested bacteria. *Nature* **396:**248–251.

Martin, J. H., and others. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. *Nature* **371:**123–129.

McKnight, D. M., B. A. Kimball, and K. E. Bencala. 1988. Iron photoreduction and oxidation in an acidic mountain stream. *Science* **240:**637–640.

McMahon, J. W. 1969. The annual and diurnal variation in the vertical distribution of acid soluble ferrous and total iron in a small dimictic lake. *Limnology and Oceanography* **14:**357–367.

Miessler, G. L., and D. A. Tarr. 1999. "Inorganic Chemistry," 2nd Ed., p. 642. Prentice Hall, Upper Saddle River, NJ.

Miles, C. J., and P. L. Brezonik. 1981. Oxygen consumption in humic-colored waters by a photochemical ferrous-ferric catalytic cycle. *Environmental Science & Technology* **15:**1088–1095.

Neilands, J. B. 1981. Iron adsorption and transport in microorganisms. *Annual Reviews in Nutrition* **1:**27–46.

Neilands, J. B. 1995. Siderophores: Structure and function of microbial iron transport compounds. *The Journal of Biological Chemistry* **270:**26723–26726.

Nodwell, L. M., and N. M. Price. 2001. Direct utilization of inorganic iron by marine mixotrophic phytoplankton. *Limnology and Oceanography* **46:**765–777.

Nürnberg, G. K., and M. Shaw. 1998. Productivity of clear and humic lakes: Nutrients, phytoplankton, and bacteria. Hydrobiologia **382:**97–112.

Nygaard, K., and A. Tobiessen. 1993. Bacterivory in algae: A survival strategy during nutrient limitation. *Limnology and Oceanography* **38:**273–279.

Pakulski, J. D., R. B. Coffin, C. A. Kelley, S. L. Holder, R. Downer, P. Aas, M. M. Lyons, and W. H. Jeffrey. 1996. Iron stimulation of Antarctic bacteria. *Nature* **383:**133–143.

Perdue, E. M., K. C. Beck, and J. H. Reuter. 1976. Organic complexes of iron and aluminum in natural waters. *Nature* **260:**418–420.

Prakash, A., M. A. Rashid, and A. Jensen. 1973. Influence of humic substances of the growth of marine phytoplankton: Diatoms. *Limnology and Oceanography* 18:516–524.

Price, N. M., and F. M. M. Morel. 1998. Biological cycling of iron in the ocean. *In* "Iron Transport and Storage in Microorganisms Plants and Animals: Metal Ions in Biological Systems" (A. Sigel and H. Sigel, Eds.), pp. 1–36. Dekker, New York.

Pullin, M. J. 1999. The thermodynamics and kinetics of iron-dissolved organic matter interactions in model fresh waters. Ph.D. Dissertation, Kent State University.

Pullin, M. J., and S. E. Cabaniss. 2001. Colorimetric flow-injection analysis of dissolved iron (II) and total iron in natural waters containing dissolved organic matter. *Water Research* **35:**363–372.

Raven, J. A. 1988. The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources. *New Phytologist* **116:**1–18.

Raven, J. A. 1990. Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and C assimilation pathway. *New Phytologist* **116:**1–17.

Raven, J. A. 1997. Phagotrophy in phototrophs. *Limnology and Oceanography* **42:**198–205.

Reuter, J. H., and E. M. Perdue. 1977. Importance of heavy metal-organic matter interactions in natural waters. *Geochimica et Cosmochimica Acta* **45:**325-334.

Rich, H. W., and F. M. M. Morel. 1990. Availability of well-defined iron colloids to the marine diatom *Thalassiosira weissflogii*. *Limnology and Oceanography* **35:**652–662.

Rothhaupt, K. O. 1996. Utilization of substitutable carbon and phosphorus sources by the mixotrophic chrysophyte *Ochromonas* sp. *Ecology* **77:**706–715.

Rue, E. L., and K. W. Bruland. 1995. Complexation of iron(III) by natural organic ligands in the central north Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. *Marine Chemistry* **50:**117–138.

Rue, E. L., and K. W. Bruland. 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. *Limnology and Oceanography* **42:**901–910.

Salonen, K., R. I. Jones, H. De Haan, and M. James. 1994. Radiotracer study of phosphorus uptake by plankton and redistribution in the water column of a small humic lake. *Limnology and Oceanography* **39:**69–83.

Schelske, C. L. 1962. Iron, organic matter, and other factors limiting primary productivity in a marl lake. *Science* **136:**45.

Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. *Science* **195:**260–262.

Schnitzer, M., and E. H. Hansen. 1970. Organo-metallic interactions in soils. 8. An evaluation of methods for the determination of stability constants of metal-fulvic acid complexes. *Soil Science* **109:**333–340.

Schnitzer, M., and S. U. Khan. 1972. "Humic Substances in the Environment." Dekker, New York.

Schwertmann, U. 1966. Inhibitory effect of soil organic matter on the crystallization of amorphous ferric hydroxide. *Nature* **212:**645–646.

Scott, D. T., D. M. McKnight, E. L. Blunt-Harris, S. E. Kolesar, and D. R. Lovley. 1998. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. *Environmental Science & Technology* **32:**2984–2989.

Scully, N. M., and D. R. S. Lean. 1994. The attenuation of ultraviolet radiation in temperate lakes. *Archiv für Hydrobiologie, Ergebnisse der Limnologie* **43:**135–144

Shapiro, J. 1957. Chemical and biological studies on the yellow organic acids of lake water. *Limnology and Oceanography* **2:**161–179.

Shapiro, J. 1966. The relation of humic color to iron in natural waters. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **16:**477–484.

Shapiro, J. 1967. Yellow organic acids of lake water: Differences in their composition and behavior. *In* "Chemical Environment in the Aquatic Habitat" (H. L. Golterman and R. S. Clymo, Eds.), pp. 202–216. North-Holland, Amsterdam.

Shaw, P. J. 1994. The effect of pH, dissolved humic substances, and ionic composition on the transfer of iron and phosphate to particulate size fractions in epilimnetic lake water. *Limnology and Oceanography* **39:**1734–1743.

Shaw, P. J., R. I. Jones, and H. De Haan. 2000. The influence of humic substances on the molecular weight distributions of phosphate and iron in epilimnetic lake waters. *Freshwater Biology* **45:**383–393.

Sivan, O., Y. Erel, D. Mandler, and A. Mishri. 1998. The dynamic redox chemistry of iron in the epilimnion of Lake Kinneret. Sea of Galilee. *Geochimica et Cosmochimica Acta* **62:**565–576.

Stevenson, F. J. 1982. "Humus Chemistry: Genesis, Composition, Reactions," p. 261. Wiley, New York.

Stewart A. J., and R. G. Wetzel. 1981. Dissolved humic materials: Photodegradation, sediment effects, and reactivity with phosphate and calcium carbonate precipitation. *Archiv für Hydrobiologie* **92:**265–286.

Stumm, W., and J. J. Morgan. 1996. "Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters." Wiley, New York.

Sulzberger, B., J. L. Schnorr, R. Giovanoli, J. G. Hering, and J. Zobrist. 1990. Biogeochemistry of iron in an acidic lake. *Aquatic Sciences* **52:**56–74.

Sunda, W. G. 1995. The influence of nonliving organic matter on the availability and cycling of plant nutrients in seawater. *In* "Role of Non-Living Organic Matter in the Earth's Carbon Cycle" (R. G. Zepp and C. H. Sonntag, Eds.), pp. 191–207. Wiley, New York.

Sunda, W. G., and S. A. Huntsman. 1997. Interrelated influence of iron, light and cell size on marine phytoplankton growth. *Nature* **390:**389–392.

Sunda, W. G., D. G. Swift, and S. A. Huntsman. 1991. Low iron requirement for growth in oceanic phytoplankton. *Nature* **351:**55–57.

Taillefert, M., C. P. Lienemann, J. F. Gaillard, and D. Perret. 2000. Speciation, reactivity, and cycling of Fe and Pb in a meromictic lake. *Geochimica et Cosmochimica Acta* **64:**169–183.

Tate, C. M., R. E. Broshears, and D. M. McKnight. 1995. Phosphate dynamics in an acidic mountain stream: Interactions involving algal uptake, sorption by iron oxide, and photoreduction. *Limnology and Oceanography* **40:**938–946.

Theis, T. L., and P. C. Singer. 1974. Complexation of iron (II) by organic matter and its effect on iron (II) oxygenation. *Environmental Science & Technology* **8:**569–573.

Tipping, E. 1981. The adsorption of aquatic humic substances by iron oxides. *Geochimica et Cosmochimica Acta* **45:**191–199.

Tortell, P. D., M. T. Maldonado, and N. M. Price. 1996. The role of heterotrophic bacteria in iron-limited ocean ecosystems. *Nature* **383:**330–332.

Tranvik, L. J. 1989. Bacterioplankton growth, grazing mortality and quantitative relationship to primary production in a humic and a clear water lake. *Journal of Phytoplankton Research* **11:**985–1000.

Twiss, M. R., J. C. Auclair, and M. N. Charlton. 2000. An investigation into iron-stimulated phytoplankton productivity in epipelagic Lake Erie during thermal stratification using trace metal clean techniques. *Canadian Journal of Fisheries and Aquatic Sciences* **57:**86–95.

Urban, N. R., E. Gorham, J. K. Underwood, F. B. Martin, and J. G. Ogden, III. 1990. Geochemical processes controlling concentrations of Al, Fe, and Mn in Nova Scotia lakes. *Limnology and Oceanography* **35:**1516–1534.

Vaillancourt, R. D., and W. M. Balch. 2000. Size distribution of marine submicron particles determined by flow field-flow fractionation. *Limnology and Oceanography* **45:**485–492.

Voelker, B. M., and D. L. Sedlak. 1995. Iron reduction by photoproduced superoxide in seawater. *Marine Chemistry*. **50:**93–103.

Voelker, B. M., and B. Sulzberger. 1996. Effects of fulvic acid on Fe (II) oxidation by hydrogen peroxide. *Environmental Science & Technology* **30:**1106–1114.

Voelker, B. M., F. M. M. Morel, and B. Sulzberger. 1997. Iron redox cycling in surface waters: Effects of humic substances and light. *Environmental Science & Technology* **31:**1004–1011.

Waite, T. D., and F. M. M. Morel. 1984a. Photoreductive dissolution of colloidal iron oxides in natural waters. *Environmental Science & Technology* **18:**860–868.

Waite, T. D., and F. M. M. Morel. 1984b. Ligand exchange and fluorescence quenching studies of the fulvic acid-iron interaction. *Analytica Chemica Acta* **162:**263–274.

Wells, M. L., L. M. Mayer, and R. R. L. Guillard. 1991. A chemical method for estimating the availability of iron to phytoplankton in seawater. *Marine Chemistry* **33:**23–40.

Wetzel, R. G. 1966. Productivity and nutrient relationships in marl lakes of northern Indiana. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **16:**321–332.

Wetzel, R. G. 2001. "Limnology: Lake and River Ecosystems," 3rd Ed. Academic Press, San Diego.

Wilhelm, S. W. 1995. The ecology of cyanobacteria in iron-limited environments: A review of physiology and implications for aquatic environments. *Aquatic Microbial Ecology* **9:**295–303.

Wilhelm, S. W., and C. G. Trick. 1994. Iron-limited growth of cyanobacteria: Multiple siderophore production is a common response. *Limnology and Oceanography* **39:**1979–1984.

Xenopoulos, M. A., and D. F. Bird. 1997. Effect of acute exposure to hydrogen peroxide on the production of phytoplankton and bacterioplankton in a mesohumic lake. *Photochemistry and Photobiology* **66:**471–478.

Zarifou, O. C., J. Joussot-Dubien, R. G. Zepp, and R. G. Zika. 1984. Photochemistry of natural waters. *Environmental Science & Technology* **18:**356–371.

SECTION TWO

TRANSFORMATION AND REGULATION

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9

The Contribution of Monomers and Other Low-Molecular Weight Compounds to the Flux of Dissolved Organic Material in Aquatic Ecosystems

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Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

I. INTRODUCTION

Some of the first studies on fluxes of dissolved organic material (DOM) examined the uptake of compounds such as glucose and amino acids (Banoub and Williams, 1972; Hobbie et al., 1972; Parsons and Strickland, 1962; Wright and Hobbie, 1965), that is, monomers that make up the building blocks of biopolymers and macromolecules found in organisms. These studies contributed to the recognition of the importance of DOM and bacteria in aquatic ecosystems (Williams, 2000), although many more studies and years of work were needed before both were fully appreciated. Aside from historical reasons, no review of DOM would be complete without considering monomers, because we probably know more about the uptake of labile low-molecular-weight (LMW) DOM than about the use of other DOM components. This extensive work has shown that monomers are important in cycles of carbon and other elements. Although concentrations are low, turnover of monomers can be fast enough such that these compounds can support a large proportion of bacterial growth and comprise a large fraction of the labile DOM flux (see also Chapter 4).

The first goal of this chapter is to review the data supporting the previous statement, that monomer uptake can support substantial heterotrophic bacterial growth in freshwaters and the oceans. Although estimates of sugar and organic acid fluxes are available, past work has mainly concentrated on amino acids, and perhaps for good reason. Amino acids (in the form of protein) make up about 50% of the dry weight of planktonic organisms and thus potentially are a large fraction of the DOM derived from these organisms. The free amino acid studies then lead us to consider the degradation of protein and its relationship to free amino acid fluxes. In addition to being important per se, protein degradation serves to illustrate several issues regarding the coupling of monomer and polymer utilization by bacteria. The final goal of this review is to examine whether different types of bacteria use amino acids and protein. Data from a new approach will be presented to argue that free amino acids and protein are used to varying degrees by different bacterial groups. These data can be used to address more general questions concerning functional groups of heterotrophic bacteria in aquatic ecosystems.

II. UPTAKE OF AMINO ACIDS AND GLUCOSE

Concentrations, turnover, and uptake of free amino acids probably have been examined more extensively than any other DOM component. For several decades it has been possible to measure turnover of dissolved free amino acids (DFAA) from the uptake of ^{14}C- or ^{3}H-labeled amino acids (e.g., Azam and Holm-Hansen, 1973). Equally important was the development

of high-performance liquid chromatographic (HPLC) methods for measuring DFAA concentrations without preconcentration of the sample (Lindroth and Mopper, 1979). These methods allow investigators to measure the very low DFAA concentrations often present in aquatic systems and thus to estimate uptake of these compounds, that is, concentrations multiplied by a turnover rate constant obtained with radiolabeled tracers.

Several studies have measured DFAA concentrations and turnover (see Chapter 4 and Munster, 1993), but here we concentrate on those that compare DFAA uptake with bacterial production. The fraction of bacterial production supported by DFAA is one index for the relative importance of amino acids, not only in supporting bacterial growth but also in the overall flux of DOM. ("Flux" is used here to indicate both production and uptake in a quasi-steady state.) If DOM concentrations are constant, DOM production will equal total uptake rates by microbes; there is no evidence of photo-oxidation of amino acids and of the other compounds discussed here (see Chapter 10). Total uptake includes respiration and assimilation into biomass. Here "assimilation" is defined as the appearance of a radioactive compound in cells (both cellular LMW and HMW pools); respiration is excluded.

A common observation about DFAA and other labile LMW DOM components is that concentrations are low (usually nanomolar level), but turnover is fast, such that the flux of these compounds can be large. One indication of this high flux is the large fraction of bacterial production that can be supported by the DFAA pool in a wide range of environments (Table I). Generally over 20% of bacterial production can be accounted for by DFAA assimilation. (Respiration is ignored in these studies, but it will be discussed below.) The highest rates of DFAA assimilation relative to bacterial production have been found in estuaries, where the overall average approaches 100% (Table II). We may expect that DFAA fluxes would be more important in oceanic systems and other habitats dominated by input of DOM from protein-rich plankton. In contrast to the oceans, the supply of DOM to freshwaters and perhaps some coastal marine systems can be augmented by organic carbon from terrestrial sources, which would be poor in protein but rich in structural polysaccharides such as lignin and cellulose. The hypothesis that amino acid use (relative to bacterial production) is highest in the oceans is not supported by the available data. The lowest estimate of DFAA assimilation as a fraction of bacterial production (4%) is from the Sargasso Sea (Keil and Kirchman, 1999; but see Suttle *et al.*, 1991), and generally DFAA seem less important in oceanic regimes than elsewhere (Table I).

An HPLC method suitable for measuring low concentrations of glucose and other monosaccharides in natural waters was introduced to aquatic ecologists more than 10 years after the HPLC method for DFAA (Mopper *et al.*, 1992). Like DFAA, monosaccharide concentrations are quite low, but

TABLE I Summary of Aquatic Studies That Compared Free Amino Acid Uptake and Bacterial Production

Regime	*Location*	*Percentage of bacterial production*	*Comments*[a]	*Reference*
Oceanic	Subarctic Pacific	41	Four-month average	Kirchman and Wheeler (1998)
	Subarctic Pacific	24	Batch	Keil and Kirchman (1991)
	North Atlantic	21		Kirchman *et al.* (1994)
	Sargasso Sea	4		Keil and Kirchman (1999)
	Sargasso Sea	20	Individual amino acids	Suttle *et al.* (1991)
	Gulf of Mexico	14	Batch	Kroer *et al.* (1994)
Coastal Marine	Delaware	5–100	Seasonal study	Hoch and Kirchman (1995)
	Santa Rosa Sound (Florida)	77	Batch	Jørgensen *et al.* (1993)
	Flax Pond (New York)	120	Batch	Jørgensen *et al.* (1993)
	Chesapeake plume	15–64	4 DFAA	Fuhrman (1990)
Estuarine	Delaware	5–1000		Hoch and Kirchman (1995)
	Delaware	88	Batch	Keil and Kirchman (1991)
	Delaware	44	Batch	Middelboe *et al.* (1995)
Lakes	Constance (Germany)	30–48	Seasonal	Rosenstock and Simon (1993)
	Constance (Germany)	13		Rosenstock and Simon (2001)
	Almind (Denmark)	93 (44–172)	May–June	Jørgensen (1987)
	Hylke (Denmark)	28 (9–77)	May–June	Jørgensen (1987)
	Slotssø (Denmark)	37 (6–75)	May–June	Jørgensen (1987)
	Furesø (Denmark)	53	Diel study; dark incubation	Jørgensen and Jensen (1994)
	Klintsjön (Sweden)	17	Batch; clear water	Tranvik and Jørgensen (1995)
	Skärshultsjön (Sweden)	30	Batch; humic	Tranvik and Jørgensen (1995)

[a] "Batch" indicates that rates were calculated from the decrease over time in concentrations in "bacteria-only" incubations, that is, water filtered through ca. 1-μm-pore-size filters. Otherwise, uptake rates were estimated from DFAA concentrations and uptake of radiolabeled amino acids.

TABLE II Summary of Studies That Compared Glucose Uptake with Bacterial Production

Regime	*Location*	*Percentage of bacterial production*	*Comments*[a]	*Reference*
Ocean	Gulf of Mexico	1–10		Skoog *et al.* (1999)
	Equatorial Pacific	30	Feb–Mar (El Niño)	Rich *et al.* (1996)
	Equatorial Pacific	15–30	Aug–Oct	Rich *et al.* (1996)
	Central Arctic	10–97		Rich *et al.* (1997)
	Ross Sea	0–6		Kirchman *et al.* (2001)
	Antarctic Polar Front Zone	0-–10		Kirchman *et al.* (2001)
Lakes	Furesø (Denmark)	21	Diel	Jørgensen and Jensen (1994)
	Klintsjön (Sweden)	1	Batch; clear water	Tranvik and Jørgensen (1995)
	Skärshultsjön (Sweden)	1	Batch; humic	Tranvik and Jørgensen (1995)

[a] "Batch" means that uptake was measured by the decrease in concentrations in water filtered through small pore size filters that remove sources of glucose but not bacteria that take up glucose. Otherwise, uptake rates were estimated from glucose concentrations and uptake of radiolabeled glucose.

fluxes can be high; glucose alone can support 20–30% of bacterial production in some oceanic regimes and in one Danish lake (Table II). In the Gulf of Mexico, Antarctic seas, and in two Swedish lakes, glucose accounted for <10% of bacterial growth. A few studies have measured glucose uptake compared with DFAA uptake.

Nearly all studies concentrate on glucose and ignore the uptake of other monosaccharides, but this may not lead to substantial underestimates of monosaccharide fluxes. In many environments, glucose is the dominant monosaccharide, sometimes even the only monomer that can be measured, especially when total concentrations are less than 20 nM; some examples include the Gulf of Mexico, the equatorial Pacific, and the Antarctic seas (see Table II for references). Also, the turnover of glucose seems to be higher than that of other monosaccharides, although few studies have examined this issue.

The uptake of various sugars has been examined more often in freshwaters than in the oceans. Glucose turnover and assimilation were somewhat higher than that of fructose in a Danish lake (Jørgensen and Jensen, 1994). In the most extensive study of this issue, Bunte and Simon (1999) found that turnover rate constants for glucose were much higher than for galactose, fucose, mannose, and glucosamine over a year in Lake Constance.

(The turnover rate constant has units of per time and is the inverse of the turnover time.) Glucose also appears to be used more quickly than other sugars in streams (see Chapter 4).

Glucose was also taken up more quickly than other sugars in the one marine system where this issue has been examined so far. Rich *et al.* (1996) found glucose turnover to be much faster than mannose turnover in the equatorial Pacific. Instead of using radiolabeled tracers, Rich (1993) used seawater cultures to compare use of various monosaccharides in the equatorial Pacific. He added a mixture of sugars to "bacteria-only" surface water (i.e., 0.6 μm filtrate) and followed concentrations over time. The rates estimated by this approach cannot be applied directly to natural systems because of possible artifacts from using high sugar concentrations and bacteria-only incubations. Also, depletion of individual sugars over time was complicated (Fig. 1), making it difficult to use simple rate constants (here, slope of log(concentration) versus time) to examine use of different sugars. Nonetheless, the results are instructive, and we have few other data on this question. The data from Rich (1993) indicate that glucose and perhaps fructose are used more quickly than other sugars.

In analyzing Rich's data, I examined the total amount of sugars used over the first two or three time points in the two experiments given in Figure 1. In both experiments, depletion of glucose and fructose was higher than that of the other sugars (Table III), and in the second experiment glucose uptake was clearly greater than even fructose use (Table III). Also, glucose was drawn down to below 50 nM and approached unmeasurable levels faster than the other sugars, except for fructose in one experiment (Table III), which also was used rapidly (Fig. 1). These data suggest that

TABLE III Depletion of Selected Monosaccharides by Natural Bacterial Assemblages in the Equatorial Pacific

	Uptake (nM)		*Time to depletion (h)*	
Sugar	*Exp 1*[a]	*Exp 2*	*Exp 1*	*Exp 2*
Glucose	98	181	30	11
Fructose	124	58	30	32
Arabinose	29	7	51	39
Rhamnose	70	18	42	32
Fucose	8	10	42	11
Galactose	27	16	42	32
Mannose	27	11	51	39

Sugar uptake was the total amount of each sugar used over the first three to four time points of the incubation. Data are presented in Figure 1. From Rich (1993).

[a] Experiment 1 (Exp 1) and 2 (Exp 2) refer to A and B, respectively, in Figure 1.

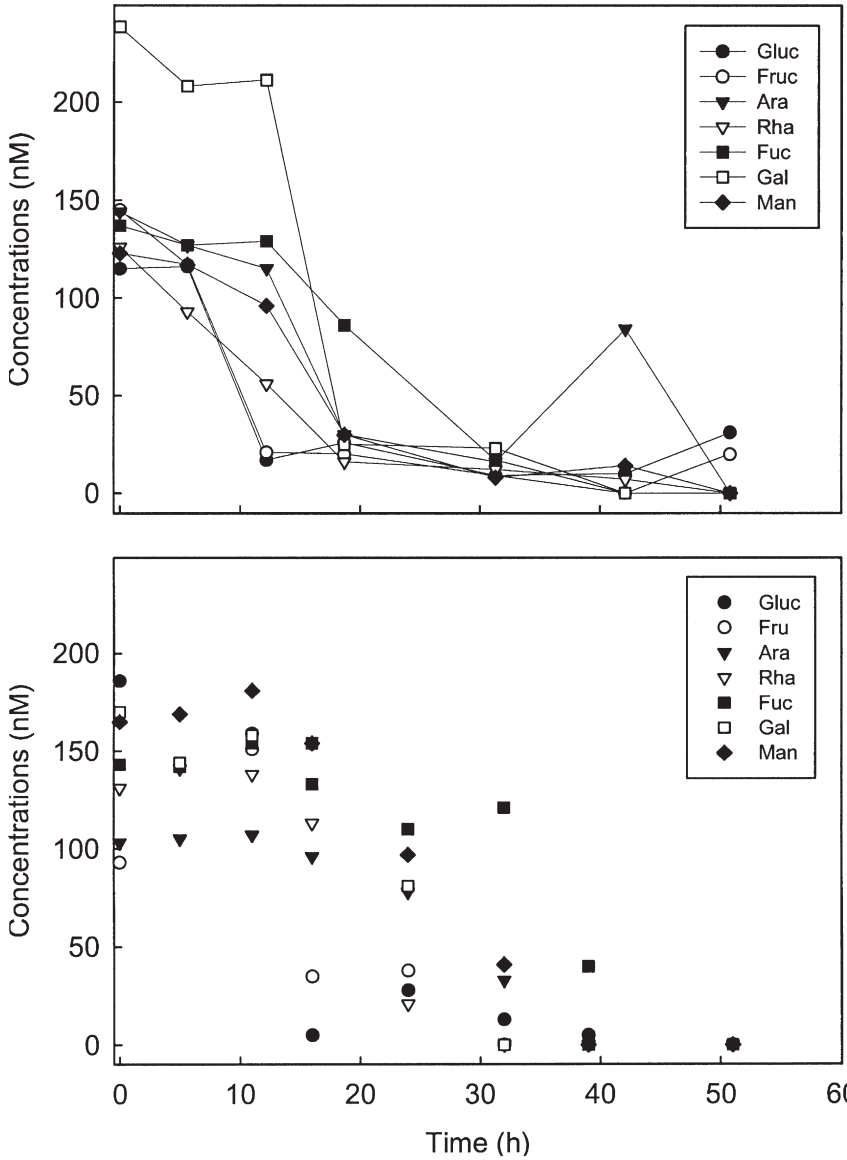

FIGURE 1 Concentrations of neutral sugars over time in "bacteria-only" dark seawater incubations (0.6 μm filtrate) from the equatorial Pacific. Concentrations were measured by pulsed amperometric detection HPLC as originally described by Mopper *et al.* (1992). The top panel is from an experiment conducted in September 1992 at 12°S, 135°W, whereas the experiment illustrated in the bottom panel was conducted in August 1992 at the equator, 140°W. For more experimental details, see Rich *et al.* (1996). Data taken from Rich (1993).

glucose and perhaps fructose are used more quickly than other neutral monosaccharides.

The importance of glucose and other free sugars in DOM fluxes is consistent with other data. Concentrations and fluxes of polysaccharides are thought to be high in oceanic systems (e.g., Biddanda and Benner, 1997; Burney, 1986), so equally high fluxes of glucose may be expected. Similarly, glucose fluxes may high in lakes receiving allochthonous inputs of DOM from terrestrial plants with large amounts of structural, glucose-containing polysaccharides, such as cellulose (a β1–4 glucose polymer). However, the few available data indicate that glucose fluxes can be equally high and low in both oceans and lakes (Table II) and that there is, at best, a complex relationship between presumed polysaccharide availability and glucose uptake. For example, phytoplankton blooms of the Ross Sea consist of algae known to produce copious polysaccharides, yet glucose turnover and fluxes in this Antarctic sea are low (Table II; Kirchman *et al*., 2001). The relationship between monomer and polymer utilization will be discussed in more detail below.

A few studies have examined both DFAA and glucose. When averaged over all environments, assimilation of DFAA seems to support more bacterial production than glucose assimilation (Tables II and III). In two Swedish lakes, DFAA uptake was 10- to 30-fold higher than glucose uptake (Tranvik and Jørgensen, 1995). The difference was about 3-fold in the central Arctic (Rich *et al*., 1997). DFAA fluxes were about 1.7-fold higher than glucose fluxes in a Danish lake, although the combined uptake of both glucose and fructose was about equal to DFAA uptake (Jørgensen and Jensen, 1994); the problem of comparing one compound (glucose) with a mixture (DFAA) is discussed below. Uptake of DFAA appears higher than that of glucose because both concentrations and rate constants are higher for DFAA. There are more data on turnover rate constants than on concentrations for both DFAA and glucose. In the Delaware Estuary, turnover rate constants for the DFAA pool are about 50% higher than those for glucose, especially when activity is high (Fig. 2). Rate constants for amino acids were also higher than those for glucose in the central Arctic (Rich *et al*., 1997), Lake Constance (Weiss and Simon, 1999), the Gulf of Mexico (Skoog *et al*., 1999), and the equatorial Pacific (Kirchman and Borch, manuscript in preparation). One of the largest differences was observed in the Ross Sea and Antarctic Polar Front, where DFAA turnover was about 7-fold higher than glucose turnover (Kirchman *et al*., 2001).

Since the DFAA pool consists of more than a single compound, it would be more appropriate to compare glucose fluxes with the fluxes of an individual free amino acid occurring in high concentration (e.g., serine or glycine). However, turnover of these individual free amino acids is, if anything, faster than the turnover of amino acid mixes (e.g., Fuhrman and Ferguson, 1986; Suttle *et al*., 1991). Alternatively, we could compare uptake

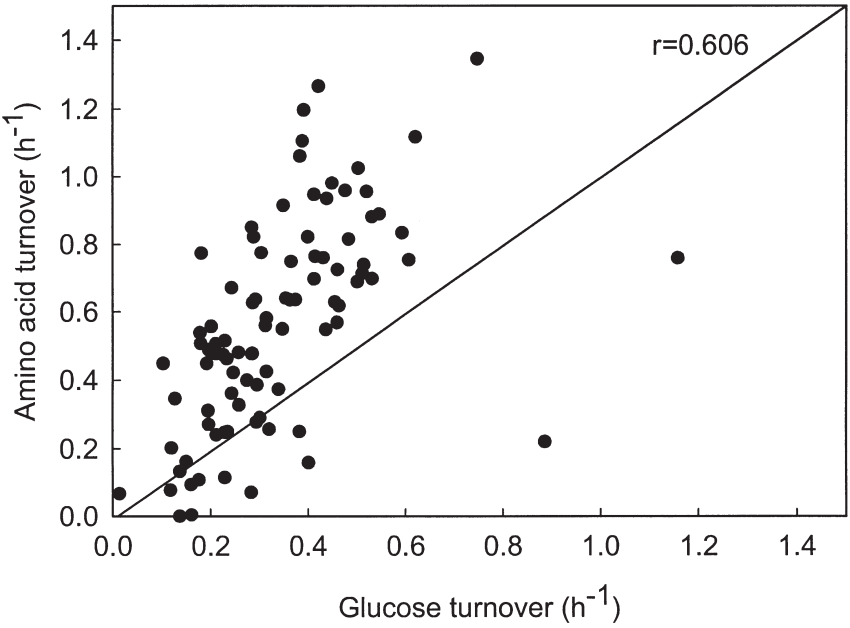

FIGURE 2 Amino acid turnover versus glucose turnover in the Delaware Estuary. Surface water was sampled throughout the salinity gradient (approximately 12 stations; see Hoch and Kirchman, 1993) during a single transect each month in July, September, and October 1995 and again in March, April, May, June, and September 1996. The added concentration was 0.5 nM and the incubation time was 0.25 to 1 h, depending on the rates. The incubation was stopped by filtering through a 0.45-μm cellulose acetate filter, which was then rinsed three times with filtered estuarine water. Each point is a mean of triplicates with a killed blank subtracted out. The diagonal is the 1:1 line.

of the entire DFAA pool with uptake of all monosaccharides, but again the implication of the DFAA versus glucose comparison does not change. What little we know about sugar uptake indicates that glucose dominates the total monosaccharide flux. As reviewed above, concentrations of free glucose are usually higher than those for other monosaccharides, and turnover of glucose is faster, with the possible exception of fructose.

So, it seems that DFAA fluxes are higher than sugar fluxes. Some studies indicate that bacteria prefer amino acids over using glucose and an inorganic nitrogen source such as ammonium (e.g., Church *et al.*, 2000; Kirchman, 1990), but this preference is not always observed (Goldman and Dennett, 2000; Kirchman and Rich, 1997). Uptake of DFAA may be higher than glucose uptake because amino acids can be used in biosynthesis or in catabolism for energy production. Another, perhaps more likely explanation is that the fluxes reflect the composition of DOM that is being produced by various mechanisms within the ecosystem. If this is so, the

difference in uptake rates would reflect adaptation by heterotrophic bacteria to production rates of these two classes of organic material. Consistent with this hypothesis is the observation that peptidase activity is higher than that of carboxylases (see Chapters 13 and 15).

III. UPTAKE OF OTHER MONOMERS AND LMW COMPOUNDS: ORGANIC ACIDS

There have been few studies on the uptake of monomers other than free amino acids and monosaccharides. One could argue that this lack of data is not hindering our understanding of DOM fluxes for systems where we expect plankton to be the major source of DOM. Because proteins and polysaccharides dominate plankton biomass, amino acids and monosaccharides would dominate fluxes of LMW DOM, and other monomers can be safely ignored. But this viewpoint would miss several potentially important compounds, such as the purine and pyrimidine bases and pentose sugars that make up DNA and RNA. These nucleic acids are a substantial fraction (ca. 20%) of cell biomass, especially for picoplankton (Fuhrman and Azam, 1980; Kirchman, 2000), and thus they potentially contribute substantially to DOM fluxes. We know only a little about ATP turnover (e.g., Azam and Hodson, 1977; Ammerman and Azam, 1985). Free pentoses (ribose and deoxyribose) are measurable by available HPLC methods (Mopper *et al*., 1992), but they have not been observed to date in the free monomer pool (see references in Table II). Fluxes of amino sugars are also potentially large, because these compounds are found in cell wall material (chitin and peptidoglycan) of several common aquatic organisms (crustaceans and bacteria). The preliminary data indicate, however, that concentrations of amino sugars are not as high as expected (Kaiser and Benner, 2000).

Other compounds that we should consider are those derived from ligno-cellulose. Production of LMW components during oxidation of lignin, a biopolymer found only in terrestrial plants, may be quite high in freshwaters where the input of ligno-cellulose and other allochthonous DOM is substantial (see Chapter 2). But we know little about LMW byproducts from ligno-cellulose degradation (Benner and Hodson, 1985; Moran and Hodson, 1989).

A focus on compounds found only in cellular macromolecules would overlook labile LMW compounds produced by photochemical reactions. Although many organic compounds produced by photochemistry can also be produced by biotic processes, the contribution of photochemically-derived organic compounds to the DOM flux may be much larger than indicated by the occurrence of these compounds in cellular macromolecules or metabolic processes. Technically, most organic compounds produced by photochemical

reactions are not monomers, that is, they are not the building blocks for cellular macromolecules. Many LMW compounds are potentially produced by photochemistry (see Chapter 10), but the fluxes of organic acids other than amino acids seem to be quite high and are of particular interest here. Unfortunately, few studies have simultaneously measured production and uptake of organic acids and bacterial production (Table IV). Pyruvate fluxes appear to be low relative to bacterial production (Kieber *et al*., 1989; Obernosterer *et al*., 1999), but the other organic acids examined to date potentially support considerable bacterial growth (Bertilsson and Tranvik, 1998).

One organic acid, glycolate, should be examined more closely. This two-carbon organic acid was among the first compounds to be examined by microbial ecologists (Wright and Shah, 1977), because it was known to be excreted by phytoplankton during photorespiration. More recently, Leboulanger *et al*. (1997) reported that glycolate concentrations ranged from 3 to 140 μg-C L^{-1} in tropical Atlantic surface waters (<0.02 to 1 μM), much higher than the highest concentrations reported so far for DFAA and free neutral sugars. These authors used diel changes in glycolate concentrations to estimate that up to 50% of all primary production was routed through glycolate. Leboulanger *et al*. (1998) examined glycolate production and consumption by the green alga *Dunaliella tertiolecta*. Unlike several

TABLE IV Summary of Studies Comparing Uptake of Organic Acids to Bacterial Production

Compound	*Location*	*Percentage of Bacterial Production*	*Comments*	*Reference*
Pyruvate	East US coast and Sargasso	2–4	Bacterial production estimated indirectly	Kieber *et al*. (1989)
Pyruvate	Mediterranean Sea	7–14	Different locations	Obernosterer *et al*. (1999)
Acetate	Skärshultsjön (Sweden)	>100		Bertilsson and Tranvik (1998)
Acetate	Fiolen (Sweden)	20		Bertilsson and Tranvik (1998)
Formate	Skärshultsjön	>100		Bertilsson and Tranvik (1998)
Formate	Fiolen	>100		Bertilsson and Tranvik (1998)
Malonate	Skärshultsjön	>100		Bertilsson and Tranvik (1998)
Malonate	Fiolen	0		Bertilsson and Tranvik (1998)

other organic acids, glycolate is probably not involved in photochemical reactions because its absorbance of visible light is low (K. Mopper, personal communication).

Finally, some sulfur-containing organic compounds constitute another class of LMW organic material worth mentioning here. Kiene *et al.* (1999) suggested that marine heterotrophic bacteria obtain much of their sulfur from two non-amino-acid sulfur compounds, dimethylsulfoniopropionate (DMSP) and methanethiol. In the Gulf of Mexico, DMSP provides nearly all of the sulfur required by heterotrophic bacteria but <5% of their carbon (Kiene and Linn, 2000). In addition to supplying sulfur to heterotrophic bacteria with sulfur, DMSP use is important because marine bacteria release dimethylsulfide (DMS) during DMSP degradation (Gonzalez *et al.*, 1999). Oceanic DMS is a major source of reduced sulfur in the atmosphere and acts as cloud nucleation sites (Charlson *et al.*, 1987).

IV. RESPIRATION OF MONOMERS

Nearly all of the studies mentioned above compared assimilation of radiolabeled monomers with bacterial biomass production as estimated by leucine or thymidine incorporation. To examine the total flux of monomers and DOM we must consider respiration.

The study of Weiss and Simon (1999) provides one of the more detailed data sets on respiration of amino acids and glucose. Respiration of amino acids was slightly higher than that for glucose (34% vs. 23%) when averaged over the entire season in Lake Constance, but this difference was small compared with the large temporal and spatial variation in both glucose and amino acid respiration (Weiss and Simon, 1999). The estimate for the DFAA pool is a composite of the respiration of individual amino acids, which ranges from about 10% for leucine to 50% or higher for aspartate and glutamate (Crawford *et al.*, 1974). Less information is available on respiration of sugars other than glucose. Respiration of glucose appears to be lower than respiration of mannose by 2-fold in the equatorial Pacific (Rich *et al.*, 1996), but respiration of fructose is about equal to glucose respiration in Danish lakes (Jørgensen and Jensen, 1994).

There are few studies of respiration of organic acids other than amino acids. Percentage respiration of malonic and acetic acids seems similar (ca. 20%) to that of the other compounds mentioned so far, according to experiments in a Swedish lake (Bertilsson and Tranvik, 1998). In contrast, nearly all of the formic acid (98%) taken up by bacteria is mineralized to CO_2 (Bertilsson and Tranvik, 1998).

The efficiency with which bacteria use LMW compounds for growth seems much higher (with the exception of formic acid) than current estimates of overall growth efficiency. To address this issue, we can calculate a

"percentage retention efficiency" from data on uptake and respiration of radiolabeled (usually ^{14}C) organic compounds measured in short-term incubations (hour time scale). The percentage retention efficiency can be defined as Rb/(Rr + Rb)*100, where Rb is the radioactivity retained in biomass and Rr is the radioactivity respired either as $^{14}CO_2$ or $^{3}H_2O$. This retention efficiency is equivalent to [100-% respiration], and it may be related to the bacterial growth efficiency (but see below). With the exception of formic acid, the retention efficiencies for the monomers examined to date are much higher than current estimates of the growth efficiency (see recent review by del Giorgio and Cole, 2000). Retention efficiencies for monomers generally exceed 50%, whereas the overall growth efficiency of heterotrophic bacteria is generally much less than 50%, varying from 15% in the oceans to nearly 40% in estuaries (del Giorgio and Cole, 2000).

Microbial ecologists no longer attempt to estimate overall growth efficiencies of heterotrophic bacterial assemblages from experiments with radiolabeled monomers (del Giorgio and Cole, 2000), because of problems in extrapolating from retention efficiencies of selected compounds to the growth efficiency (e.g., King and Berman, 1984). These problems include isotope dilution and the brevity of uptake experiments (hours) compared with the bacterial generation time (a day or more) in natural habitats. Nevertheless, if net uptake of monomers appears to support a large fraction of bacterial growth, then the retention efficiency of these monomers should be indicating something important about bacterial metabolism and perhaps the growth efficiency.

V. COUPLING POLYMER HYDROLYSIS AND MONOMER UPTAKE

It is beyond the scope of this review to discuss in great detail the use of macromolecules by heterotrophic bacteria in aquatic systems (see Chapter 13). Suffice it to say that both protein and polysaccharides are important components of the DOM flux and can support substantial bacterial growth (Jørgensen *et al.*, 1993; Keil and Kirchman, 1999; Skoog *et al.*, 1999; Rosenstock and Simon, 2001). We probably know the most about use of protein and dissolved combined amino acids, as these pools make up the largest identified components of the dissolved organic nitrogen (DON) pool (e.g., McCarthy *et al.*, 1997). Although a complete review is not possible, it is appropriate to consider the coupling between monomer uptake and polymer degradation.

These two processes may be coupled because hydrolysis of biopolymers may contribute to the production of various free monomers such as DFAA and monosaccharides. Polymers and other macromolecules must be hydrolyzed first to LMW compounds (approximately 500 Da) before transport by

bacteria is possible (see Chapter 13). Since this hydrolysis step must be extracellular, some hydrolysis by-products may diffuse away and mix into the bulk pool of monomers and other LMW compounds. If this is so, uptake of monomers could be considered as simply a step in the degradation of macromolecules.

It has been argued that a large fraction of the production and uptake of monosaccharides is coupled to, and depends on, the hydrolysis of polysaccharides. Skoog *et al.* (1999) observed that addition of high-molecular-weight DOM (HMW DOM) had no effect on glucose uptake in surface waters, but addition of polymers substantially inhibited glucose uptake in deeper waters. These data indicate that high-molecular-weight DOM is a source of free glucose at least in the deep sample, but these results cannot be used to argue for or against HMW DOM being a source of glucose in the surface waters.

The molecular composition of the free sugar pool is not consistent with HMW DOM being a source of free glucose in all aquatic habitats. If hydrolysis of HMW DOM were the main source, we would expect the composition of the free pool to be similar to that of the HMW pool. The HMW DOM and total "combined" sugars (those released by acid hydrolysis) are composed of several neutral sugars, each occurring in roughly the same proportion, whereas the free neutral monosaccharide pool consists of only glucose at low concentrations (e.g., Skoog *et al.*, 1999). However, free neutral sugars in addition to glucose are observed when total concentrations of free sugars are high (>50 nM) (Rich *et al.*, 1996), suggesting that hydrolysis of polysaccharides and other HMW DOM contributed to those high concentrations.

Several amino acids are observed in the DFAA pool at all DFAA concentrations (e.g., Suttle *et al.*, 1991), consistent with degradation of HMW DOM and, specifically, protein contributing to the DFAA flux. However, this conclusion is not supported by an experiment directly examining the coupling between protein hydrolysis and DFAA fluxes. Keil and Kirchman (1993) followed the fate of a uniformly ^{14}C-labeled algal protein introduced into estuarine water (Fig. 3). Sixty-four percent of the ^{14}C-protein taken up by bacteria either was assimilated into cells or was respired during the incubation, but the remaining 36% of the ^{14}C protein was released into two different DOM pools, one of which was less than 2 kDa; this LMW pool would include free amino acids. Although amino acid production was not examined directly, the release rate into the LMW DOM pool sets an upper limit to the release rate of free amino acids during protein degradation. It is an upper limit because the LMW DOM flux measured by Keil and Kirchman (1993) would include oligopeptides in addition to free amino acids.

Figure 3 illustrates that the release rate into the LMW DOM pool was only 10% (33 versus 330 ng L^{-1} h^{-1}) of the uptake rate of free amino acids. These data imply that most of the free amino acids were not originating from

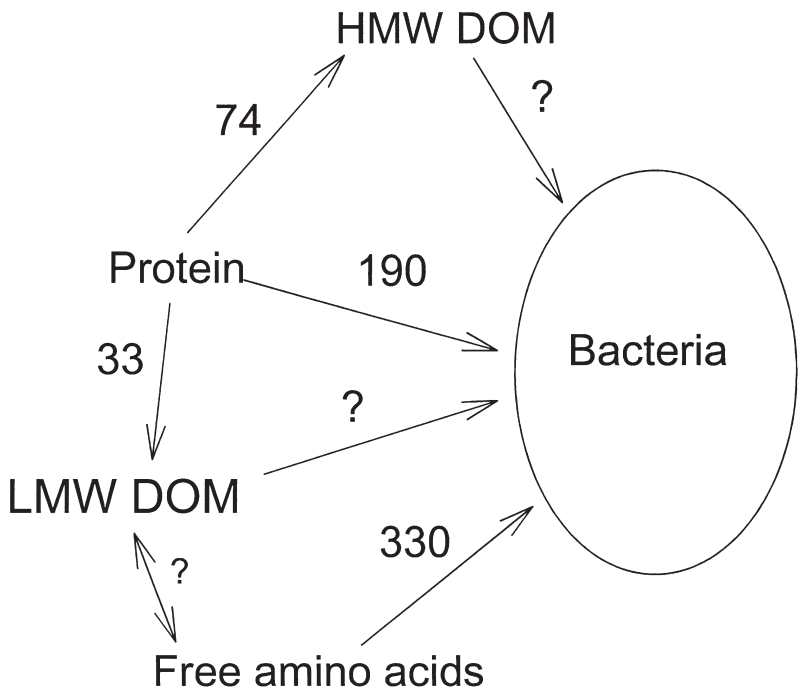

FIGURE 3 Fate of ^{14}C-protein added to estuarine water. The numbers are rates in ng L^{-1} h^{-1}. The initial protein was >100 kDa. HMW DOM is DOM less than 30 kDa but greater than 2 kDa, whereas LMW DOM is DOM less than 2 kDa. No estimates are available for the processes with question marks. The uptake of free amino acids was measured with ^{3}H-amino acids in separate incubations, and DFAA concentrations were determined by HPLC. Data taken from Keil and Kirchman (1993).

protein degradation, but rather were produced by some other mechanism. A counter-argument is that the observed radioactivity in the LMW DOM pool was associated with compounds *not* assimilated into cells and that the actual release and uptake rates were much higher. To examine this possibility, we can use the estimate of "direct" protein assimilation into bacterial biomass as another upper limit to the production rate of free amino acids. It is an upper limit because it includes uptake of oligopeptides and even amino acids that have not mixed with the DFAA pool. This direct assimilation rate was 190 ng L^{-1} h^{-1}, substantially lower than the free amino acid uptake rate, again indicating that free amino acids could not have come from protein degradation and must have been produced by another mechanism. One alternative is direct excretion or release of DFAA by organisms other than bacteria. Many organisms are known to release free amino acids, including protozoa, zooplankton, and phytoplankton (see Nagata, 2000, for a recent review).

Even if free amino acids are produced mainly by these organisms, release of various organic compounds during protein degradation does appear to be substantial relative to total hydrolysis and direct uptake by bacteria (Fig. 3). Other work has demonstrated that heterotrophic bacteria also release DOM during the degradation of the macromolecules making up marine snow (Smith *et al*., 1992) and the common biopolymer chitin (Kirchman and White, 1999). This DOM release has many implications for examining degradation of HMW organic material and particulate detritus and the functioning of extracellular enzymes (see Chapter 13).

VI. SPECIFIC BACTERIA USING AMINO ACIDS AND PROTEIN: FUNCTIONAL GROUPS OF HETEROTROPHIC BACTERIA

The implicit assumption in comparing DOM uptake with bacterial production is that all bacteria participate equally in using the specified DOM component. However, the diversity of bacterial communities in aquatic systems is now well known to be quite high (e.g., see reviews by Giovannoni and Rappé, 2000; Hugenholtz *et al*., 1998), raising the possibility that different bacterial groups vary in their contribution to biomass production and DOM uptake. One approach to this problem is a combination of microautoradiography to examine DOM uptake and fluorescent *in situ* hybridization (FISH) to identify bacteria, specifically those using ^{3}H-labeled DOM components. Three different versions of this combined microautoradiography-FISH method have been published (Cottrell and Kirchman, 2000b; Lee *et al*., 1999; Ouverney and Fuhrman, 1999), but the micro-FISH version has some technical advantages (Cottrell and Kirchman, 2000b). The specific application of micro-FISH relevant to this review is relative use of amino acids and proteins.

Cottrell and Kirchman (2000b) found that the capacity to assimilate protein and amino acids was not uniformly distributed among the four bacterial groups the authors examined in Delaware coastal waters (Fig. 4). Specifically, bacteria in the *Cytophaga-Flavobacter* cluster dominated protein use, whereas members of the α-subdivision of proteobacteria were the least successful in using protein. The opposite pattern was observed for free amino acid uptake; many of the microbes taking up amino acids were α-proteobacteria, but a few were from the *Cytophaga-Flavobacter* cluster. Similar results were obtained for another experiment with a sample from the Delaware Estuary (Cottrell and Kirchman, 2000b). In contrast, the *Cytophaga-Flavobacter* cluster and α-proteobacteria appeared to be equally important in using amino acids in California coastal waters (Ouverney and Fuhrman, 1999). Consistent with the results of Cottrell and Kirchman (2000b), Schweitzer *et al*. (2001) found net amino acid uptake when lake

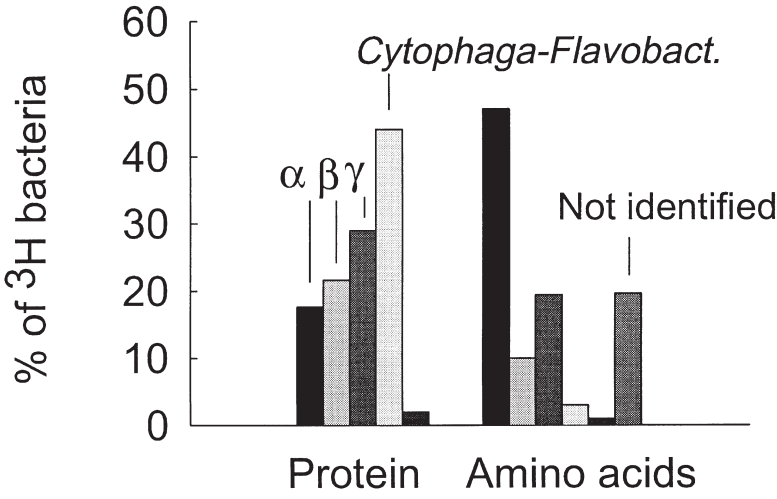

FIGURE IV Relative number of bacteria in each phylogenetic group that were actively assimilating either amino acids or protein. The Greek letters (α, β, γ) refer to subdivisions of proteobacteria. The high GC Gram-positive bacteria were <2%. Data taken from Cottrell and Kirchman (2000b).

snow aggregates were dominated by α-proteobacteria, but net release of amino acids when β-proteobacteria declined and the *Cytophaga-Flavobacter* cluster and α-proteobacteria increased. Uptake of *N*-acetylglucosamine and chitin also varied among phylogenetic groups (Cottrell and Kirchman, 2000b). Bacteria in the *Cytophaga-Flavobacter* cluster and γ-subdivision of proteobacteria were most numerous among the cells using chitin and *N*-acetylglucosamine.

Strictly speaking, the micro-FISH assay accounts for the number of active cells in a particular phylogenetic group, not their relative uptake of a compound. However, it seems likely that the contribution of a phylogenetic group to DOM use will follow the number of active bacteria in that group, and it is unlikely that a low number of active cells in one group would dominate uptake when a large number of cells in another group are scored positive in the micro-FISH assay. Regardless of the precise interpretation of the micro-FISH results, the data do indicate that not all bacteria are equal in their use of DOM. Clearly, the participation of various phylogenetic groups in the uptake of a compound does not simply follow the relative abundance of these groups in the bacterial community (Cottrell and Kirchman, 2000b).

The focus of Cottrell and Kirchman (2000b) and Ouverney and Fuhrman (1999) on the division level (e.g., *Cytophaga-Flavobacter* cluster) and subdivisions of proteobacteria is due in part to the availability of FISH probes. With some exceptions (Eilers *et al*., 2000), probes are not available for finer phylogenetic levels of aquatic bacteria (e.g., clades within the

Cytophaga-Flavobacter cluster), even though we know that each of the bacterial groups depicted in Figure 4 include many diverse ribotypes (akin to species). If we knew which (if any) bacterial ribotypes dominate aquatic systems, new probes could be developed and used with micro-FISH to examine their participation in DOM uptake. But little is known about the relative abundance of specific bacterial ribotypes and groups at taxonomic levels finer than division or subdivision. Clone libraries of 16S rRNA genes can be used to design new FISH probes, even though libraries and other PCR-based approaches appear to be biased (see Chapter 14; Cottrell and Kirchman, 2000a; Glöckner *et al*., 1999). In spite of the large number of studies on bacterial diversity, many basic questions remain unanswered.

The diversity of bacteria within the major phylogenetic groups examined by micro-FISH to date may lead to complicated and even inconsistent patterns in DOM use. We already see differences in amino acid use by the *Cytophaga-Flavobacter* cluster and α-proteobacteria in coastal waters of California and Delaware (Cottrell and Kirchman, 2000b; Ouverney and Fuhrman, 1999). These apparent inconsistencies may be due to the same bacterial groups responding to different environmental conditions; after all, California and Delaware waters differ in several respects. These waters also probably differ in the composition within the bacterial groups, within the α-proteobacteria, for example. Variation within a particular bacteria group would complicate our search for generalizations about DOM uptake if this variation were also accompanied by variation in the capacity to use specific DOM components. Not all *Cytophaga* ribotypes, for example, may be equally adept at using chitin and protein. If so, then uptake of a specific DOM component may be best understood by examining subgroups of the *Cytophaga-Flavobacter* cluster and the proteobacterial subdivisions.

Stated in another way, we are trying to identify which bacteria make up the functional group using a selected DOM component. Use of the term "functional group" may seem odd here because it is usually used to describe more diverse metabolic types, such as nitrifiers and denitrifies. But it is an appropriate term because we wish to define a particular collection of bacteria by their ecological role (use of a specific DOM component), rather than by phylogenetic relationships, which potentially are quite distant. The value of having distinct functional groups for DOM uptake is discussed below.

Although functional groups are defined independently of bacterial taxonomy and phylogeny, tying function and phylogeny together is interesting per se and is potentially a powerful approach to examining DOM uptake. If we can identify any relationships between DOM uptake and the phylogenetic makeup of bacterial communities, we then can take advantage of the large data sets and numerous molecular tools for examining ribosomal RNA and genes. It is often stated that there is no relationship between function and microbial phylogeny, but a more optimistic and perhaps accurate observation is that the relationship is unknown.

VII. HETEROTROPHIC BACTERIA AS MORE THAN ONE FUNCTIONAL GROUP: DOES IT MATTER?

Let us assume that eventually we will be able to determine the role of various bacterial groups and ribotypes in DOM uptake. Establishing these roles seems a worthy goal per se, given the importance of both DOM and bacteria in aquatic ecosystems. After all, heterotrophic bacteria are among the most numerous organisms in the biosphere (Whitman *et al*., 1998). However, it is still reasonable to ask if this information will help us better understand DOM dynamics and fluxes. If heterotrophic bacteria do in fact consist of several consistent functional groups, will this information improve models that involve DOM and bacteria? Current models usually have only a single compartment for DOM and another for bacteria (e.g., Fasham *et al*., 1999; see Chapter 18), that is, all aerobic heterotrophic bacteria are in a single functional group. The "function" of microbial diversity has been examined in soils (de Ruiter *et al*., 1998; Setälä *et al*., 1998), although bacteria are still depicted as a single compartment in those models.

Actually, microbial ecologists have long appreciated the impact of metabolic diversity on DOM fluxes, even the uptake of simple monomers like amino acids. For example, Williams (1973) examined how the Michaelis–Menten kinetic model of DOM uptake is affected when DOM is used by a diverse bacterial assemblage with different half-saturation constants. Similarly, to explain "multi-phasic kinetics," Azam and Hodson (1981) evoked the presence of different bacteria with uptake systems that vary in substrate affinity. Since the first studies of bacterial growth rates from bulk measurements of bacterial production and numbers, microbial ecologists have recognized that different groups of heterotrophic bacteria probably grow at different rates.

The utility of having more than one compartment for bacteria and DOM will probably depend on the model and the question being addressed. There is no need to think about the number of bacterial compartments for large-scale carbon cycle models, because these have at most a single parameter for all aquatic biology. It is a more relevant question for biogeochemical models in which bacterial parameters are already embedded. These parameters include bacterial biomass, C : N ratio of that biomass, growth rate, and growth efficiency.

Each of these bacterial parameters is likely to vary, perhaps greatly, among various bacterial groups and is likely to be influenced by what DOM these bacterial groups are using. Growth rates of the entire bacterial assemblages are often limited and set by the supply of labile DOM (see review by Williams, 2000), so it is likely that growth of individual bacterial groups will also be set by DOM. Of course, growth rates contribute to the biomass level of a bacterial group, but standing stocks are also affected by mortality. Both viral lysis and grazing are well known to vary with the type of bacteria

(reviewed by Fuhrman, 2000, and Strom, 2000). Growth efficiency can vary greatly among different ecosystems (del Giorgio and Cole, 2000), and perhaps it varies as much among different bacterial groups, especially if they use different DOM components with different C : N ratios. The C : N ratio of bacterial biomass seems fairly invariant for heterotrophic bacteria (e.g., Fukuda *et al*., 1998), but the recent study of Goldman and Dennett (2000) showed that this ratio too may vary with growth conditions. In short, many key bacterial parameters are likely to vary greatly among bacterial groups. We probably need to gather data on how these key bacterial parameters actually vary among bacterial groups before modelers will be convinced that it is necessary to subdivide the bacterial compartment in their models.

VIII. UNKNOWNS, UNRESOLVED ISSUES, AND CONCLUSIONS

Microbial ecologists have examined the uptake of amino acids and glucose for over 30 years, almost to the exclusion of work on other monomers and LMW compounds. Further measurements of amino acids and glucose uptake may reveal new surprises, especially with respect to the relationship between retention efficiency and growth efficiency. A more pressing need is to understand better the relationship between uptake of monomers like amino acids and the hydrolysis and mineralization of HMW DOM. More data on fluxes of sugars other than glucose may give us new insights into that relationship. It is surprising how little we know about the uptake of other monomers making up the major macromolecules found in aquatic organisms. In particular, we need more information about the fluxes of nucleic acid constituents (the bases and sugars) and amino sugars, because nucleic acids and biopolymers of amino sugars (peptidoglycan and chitin) are major components of aquatic organisms.

Organic acids are another class of compounds largely neglected by microbial ecologists, at least those working in oxic habitats. The production of organic acids by photochemical reactions and other impacts of photochemistry on DOM have been examined rather extensively (Mopper and Kieber, 2002), but we still need more information. We cannot begin to answer the question; how much of the LMW DOM used by heterotrophic bacteria is from direct release by organisms, from hydrolysis of HMW DOM, or from photochemical reactions? There probably is not a single, simple answer, but we should be able to discuss this question more intelligently than is now possible.

Understanding DOM turnover should remain a major goal of microbial ecologists working in aquatic systems, but we also now have the tools for examining the taxonomic makeup of natural bacterial assemblages and for determining their ecological function. We are only beginning to examine uptake of DOM by specific bacterial groups, and much more work needs to

be done, but the preliminary studies are interesting and potentially important. It is quite surprising that uptake of even free amino acids does not seem to be equal among diverse phylogenetic groups of bacteria. While the value of these data in modeling C and N cycles may be unclear at this time, it still seems prudent to know more about the organisms (aerobic heterotrophic bacteria) mediating a process (DOM uptake) that accounts for 50% or more of the fate of primary production in aquatic ecosystems.

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REFERENCES

Ammerman, J. W., and F. Azam. 1985. Bacterial 5′-nucleotidase in aquatic ecosystems: A novel mechanism of phosphorus regeneration. *Science* **227:**1338–1340.

Azam, F., and R. E. Hodson. 1977. Dissolved ATP in the sea and its utilization by marine bacteria. *Nature* **267:**696–698.

Azam, F., and R. E. Hodson. 1981. Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. *Marine Ecology Progress Series* **6:**213–222.

Azam, F., and O. Holm-Hansen. 1973. Use of tritiated substrates in the study of heterotrophy in seawater. *Limnology and Oceanography* **23:**191–196.

Banoub, M. W., and P. J. L. Williams. 1972. Measurements of microbial activity and organic material in the western Mediterranean Sea. *Deep-Sea Research I* **19:**433–443.

Benner, R., and R. E. Hodson. 1985. Microbial degradation of the leachable and lignocellulosic components of leaves and wood from *Rhizophora mangle* in a tropical mangrove swamp. *Marine Ecology Progress Series* **23:**221–230.

Bertilsson, S., and L. J. Tranvik. 1998. Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. *Limnology and Oceanography* **43:**885-–895.

Biddanda, B., and R. Benner. 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnology and Oceanography* **42:**506–518.

Bunte, C., and M. Simon. 1999. Bacterioplankton turnover of dissolved free monosaccharides in a mesotrophic lake. *Limnology and Oceanography* **44:**1862–1870.

Burney, C. M. 1986. Bacterial utilization of total *in situ* dissolved carbohydrate in offshore waters. *Limnology and Oceanography* **31:**427–431.

Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren. 1987. Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. *Nature* **326:**655–661.

Church, M. J., D. A. Hutchins, and H. W. Ducklow. 2000. Limitation of bacterial growth by dissolved organic matter and iron in the southern ocean. *Applied and Environmental Microbiology* **66:**455–466.

Cottrell, M. T., and D. L. Kirchman. 2000a. Community composition of marine bacterioplankton determined by 16S rDNA clone libraries and fluorescence *in situ* hybridization. *Applied and Environmental Microbiology* **66:**5116–5122.

Cottrell, M. T., and D. L. Kirchman. 2000b. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Applied and Environmental Microbiology* **66:**1692–1697.

Crawford, C. C., J. E. Hobbie, and K. L. Webb. 1974. The utilization of dissolved free amino acids by estuarine microorganisms. *Ecology* **55:**551–563.

del Giorgio, P. A., and J. J. Cole. 2000. Bacterial energetics and growth efficiency. *In* "Microbial Ecology of the Ocean" (D. L. Kirchman, Ed.), pp 289–325. Wiley-Liss, New York.

de Ruiter, P. C., A. M. Neutel, and J. C. Moore. 1998. Biodiversity in soil ecosystems: The role of energy flow and community stability. *Applied Soil Ecology* **10:**217–228.

Eilers, H., J. Pernthaler, F. O. Glöckner, and R. Amann. 2000. Culturability and in situ abundance of pelagic bacteria from the North Sea. *Applied and Environmental Microbiology* **66:**3044–3051.

Fasham, M. J. R., P. W. Boyd, and G. Savidge. 1999. Modeling the relative contributions of autotrophs and heterotrophs to carbon flow at a Lagrangian JGOFS station in the northeast Atlantic: The importance of DOC. *Limnology and Oceanography* **44:**80–94.

Fuhrman, J. A. 1990. Dissolved free amino acid cycling in an estuarine outflow plume. *Marine Ecology Progress Series* **66:**197–203.

Fuhrman, J. A. 2000. Impact of viruses on bacterial processes. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 327–350. Wiley-Liss, New York.

Fuhrman, J. A., and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Applied and Environmental Microbiology* **39:**1085–1095.

Fuhrman, J. A., and R. L. Ferguson. 1986. Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: Agreement between chemical and microbiological measurements. *Marine Ecology Progress Series* **33:**237–242.

Fukuda, R., H. Ogawa, T. Nagata, and I. Koike. 1998. Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. *Applied and Environmental Microbiology* **64:**3352–3358.

Giovannoni, S. J., and M. S. Rappé. 2000. Evolution, diversity and molecular ecology of marine prokaryotes. *In* "Microbial Ecology of the Ocean" (D. L. Kirchman, Ed.), pp. 47–84. Wiley-Liss, New York.

Glöckner, F. O., B. M. Fuchs, and R. Amann. 1999. Bacterioplankton compositions of lakes and oceans: A first comparison based on fluorescence *in situ* hybridization. *Applied and Environmental Microbiology* **65:**3721–3726.

Goldman, J. C., and M. R. Dennett. 2000. Growth of marine bacteria in batch and continuous culture under carbon and nitrogen limitation. *Limnology and Oceanography* **45:**789–800.

Gonzalez, J. M., R. P. Kiene, and M. A. Moran. 1999. Transformation of sulfur compounds by an abundant lineage of marine bacteria in the alpha-subclass of the class Proteobacteria. *Applied and Environmental Microbiology* **65:**3810–3819.

Hobbie, J. E., O. Holm-Hansen, T. T. Packard, L. R. Pomeroy, R. W. Sheldon, J. P. Thomas, and W. J. Wiebe. 1972. A study of the distribution and activity of microorganisms in ocean water. *Limnology and Oceanography* **17:**544–555.

Hoch, M. P., and D. L. Kirchman. 1993. Seasonal and interannual variability in bacterial production and biomass in a temperate estuary. *Marine Ecology Progress Series* **98:**283–295.

Hoch, M. P., and D. L. Kirchman. 1995. Ammonium uptake by heterotrophic bacteria in the Delaware Estuary and adjacent coastal waters. *Limnology and Oceanography* **40:**886–897.

Hugenholtz, P., B. M. Goebel, and N. R. Pace. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology* **180:**4765–4774.

Jørgensen, N. O. G. 1987. Free amino acids in lakes: Concentrations and assimilation rates in relation to phytoplankton and bacterial production. *Limnology and Oceanography* **32:**97–111.

Jørgensen, N. O. G., and R. E. Jensen. 1994. Microbial fluxes of free monosaccharides and total carbohydrates in fresh-water determined by PAD-HPLC. *FEMS Microbiology Ecology* **14:**79–93.

Jørgensen, N. O. G., N. Kroer, R. B. Coffin, X. H. Yang, and C. Lee. 1993. Dissolved free amino acids, combined amino acids, and DNA as sources of carbon and nitrogen to marine bacteria. *Marine Ecology Progress Series* **98:**135–148.

Kaiser, K., and R. Benner. 2000. Determination of amino sugars in environmental samples with high salt content by high performance anion exchange chromatography and pulsed amperometric detection. *Analytical Chemistry* **72:**2566–2572.

Keil, R., and D. Kirchman. 1991. Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. *Marine Ecology Progress Series* **72:**1–10.

Keil, R. G., and D. L. Kirchman. 1993. Dissolved combined amino acids: Chemical form and utilization by marine bacteria. *Limnology and Oceanography* **38:**1256–1270.

Keil, R. G., and D. L. Kirchman. 1999. Utilization of dissolved protein and amino acids in the northern Sargasso Sea. *Aquatic Microbial Ecology* **18:**293–300.

Kieber, D. J., J. McDaniel, and K. Mopper. 1989. Photochemical source of biological substrates in seawater: Implications for carbon cycling. *Nature* **341:**637–639.

Kiene, R. P., and L. J. Linn. 2000. Distribution and turnover of dissolved DMSP and its relationship with bacterial production and dimethylsulfide in the Gulf of Mexico. *Limnology and Oceanography* **45:**849–861.

Kiene, R. P., L. J. Linn, J. Gonzalez, M. A. Moran, and J. A. Bruton. 1999. Dimethylsulphoniopropoinate and methanethiol are important precursors of methionine and protein-sulfur in marine bacterioplankton. *Applied and Environmental Microbiology* **65:**4549–4558.

King, G. M., and T. Berman. 1984. Potential effects of isotopic dilution on apparent respiration in ^{14}C heterotrophy experiments. *Marine Ecology Progress Series* **19:**175–180.

Kirchman, D. L. 1990. Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. *Marine Ecology Progress Series* **62:**47–54.

Kirchman, D. L. 2000. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria. In "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 261–288. Wiley-Liss, New York.

Kirchman, D. L., and J. H. Rich. 1997. Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean. *Microbial Ecology* **33:**22–30.

Kirchman, D. L., and P. A. Wheeler. 1998. Uptake of ammonium and nitrate by heterotrophic bacteria and phytoplankton in the sub-Arctic Pacific. *Deep-Sea Research I* **45:**347–365.

Kirchman, D. L., and J. White. 1999. Hydrolysis and mineralization of chitin in the Delaware Estuary. *Aquatic Microbial Ecology* **18:**187–196.

Kirchman, D. L., H. W. Ducklow, J. J. McCarthy, and C. Garside. 1994. Biomass and nitrogen uptake by heterotrophic bacteria during the spring phytoplankton bloom in the north Atlantic Ocean. *Deep-Sea Research I* **41:**879–895.

Kirchman, D. L., B. Meon, H. W. Ducklow, C. A. Carlson, D. A. Hansell, and G. Steward. 2001. Glucose fluxes and concentrations of dissolved combined sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. *Deep-Sea Research II*, **48:**4179–4197.

Kroer, N., N. O. G. Jorgensen, and R. B. Coffin. 1994. Utilization of dissolved nitrogen by heterotrophic bacterioplankton — a comparison of three ecosystems. *Applied and Environmental Microbiology* **60:**4116–4123.

Leboulanger, C., L. Oriol, H. Jupin, and C. Descolas-Gros. 1997. Diel variability of glycolate in the eastern tropical Atlantic Ocean. *Deep-Sea Research I* **44:**2131–2139.

Leboulanger, C., V. Martin-Jezequel, C. Descolas-Gros, A. Sciandra, and H. J. Jupin. 1998. Photorespiration in continuous culture of *Dunaliella tertiolecta* (Chlorophyta): Relationships between serine, glycine, and extracellular glycolate. *Journal of Phycology* **34:**651–654.

Lee, N., P. H. Nielsen, K. H. Andreasen, S. Juretschko, J. L. Nielsen, K. H. Schleifer, and M. Wagner. 1999. Combination of fluorescent in situ hybridization and microautoradiography — a new tool for structure-function analyses in microbial ecology. *Applied and Environmental Microbiology* **65:**1289–1297.

Lindroth, P., and K. Mopper. 1979. High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivitization with *o*- phthaldialdehyde. *Analytical Chemistrry* **51:**1667–1674.

McCarthy, M., T. Pratum, J. Hedges, and R. Benner. 1997. Chemical composition of dissolved organic nitrogen in the ocean. *Nature* **390:**150–154.

Middelboe, M., N. H. Borch, and D. L. Kirchman. 1995. Bacterial utilization of dissolved free amino acids, dissolved combined amino acids and ammonium in the Delaware Bay estuary: Effects of carbon and nitrogen limitation. *Marine Ecology Progress Series* **128:**109–120.

Mopper, K., and D. J. Kieber. 2002. Impact of DOM photochemistry on the biogeochemical cycling of carbon, nitrogen, sulfur and phosphorus in the sea. *In* "Biogeochemistry of Marine Dissolved Organic Matter" (D. Hansell and C. A. Carlson, Eds.), pp. 455–489 Academic Press, New York.

Mopper, K., C. A. Schultz, L. Chevolot, C. Germain, R. Revuelta, and R. Dawson. 1992. Determination of sugars in unconcentrated seawater and other natural waters by liquid chromatography and pulsed amperometric detection. *Environmental Science & Technology* **26:**133–138.

Moran, M. A., and R. E. Hodson. 1989. Formation and bacterial utilization of dissolved organic carbon derived from detrital lignocellulose. *Limnology and Oceanography* **34:**1034–1047.

Munster, U. 1993. Concentrations and fluxes of organic carbon substrates in the aquatic environment. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* **63:**243–274.

Nagata, T. 2000. Production mechanisms of dissolved organic matter. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 121–152. Wiley-Liss, New York.

Obernosterer, I., G. Kraay, E. de Ranitz, and G. J. Herndl. 1999. Concentrations of low molecular weight carboxylic acids and carbonyl compounds in the Aegean Sea (eastern Mediterranean) and the turnover of pyruvate. *Aquatic Microbial Ecology* **20:**147–156.

Ouverney, C. C., and J. A. Fuhrman. 1999. Combined microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types *in situ*. *Applied and Environmental Microbiology* **65:**1746–1752.

Parsons, T. R., and J. D. H. Strickland. 1962. On the production of particulate organic carbon by heterotrophic processes in seawater. *Deep-Sea Research* **8:**211–222.

Rich, J. H. 1993. "Monosaccharides as a Carbon Source for Bacterial Production in the Equatorial Pacific," p. 85. College of Marine Studies, University of Delaware, Newark, DE.

Rich, J. H., H. W. Ducklow, and D. L. Kirchman. 1996. Concentrations and uptake of neutral monosaccharides along 140 degrees W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the DOM flux. *Limnology and Oceanography* **41:**595–604.

Rich, J., M. Gosselin, E. Sherr, B. Sherr, and D. L. Kirchman. 1997. High bacterial production, uptake and concentrations of dissolved organic matter in the central Arctic Ocean. *Deep-Sea Research II* **44:**1645–1663.

Rosenstock, B., and M. Simon. 1993. Use of dissolved combined and free amino acids by planktonic bacteria in Lake Constance. *Limnology and Oceanography* **38:**1521–1531.

Rosenstock, B., and M. Simon. 2002. Sources and sinks of dissolved free amino acids and protein in a large and deep mesotrophic lake. *Limnology and Oceanography* **46:**644–654.

Schweitzer, B., I. Huber, R. Amann, W. Ludwig, and M. Simon. 2001. Alpha- and beta-Proteobacteria control the consumption and release of amino acids on lake snow aggregates. *Applied and Environmental Microbiology* **67:**632–645.

Setälä, H., J. Laakso, J. Mikola, and V. Huhta. 1998. Functional diversity of decomposer organisms in relation to primary production. *Applied Soil Ecology* **9:**25–31.

Skoog, A., B. Biddanda, and R. Benner. 1999. Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. *Limnology and Oceanography* **44:**1625–1633.

Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359:**139–142.

Strom, S. L. 2000. Bacterivory: Interactions between bacteria and their grazers. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 351–386. Wiley-Liss, New York.

Suttle, C. A., A. M. Chan, and J. A. Fuhrman. 1991. Dissolved free amino acids in the Sargasso Sea: Uptake and respiration rates, turnover times, and concentrations. *Marine Ecology Progress Series* **70:**189–199.

Tranvik, L. J., and N. O. G. Jørgensen. 1995. Colloidal and dissolved organic-matter in lake water — carbohydrate and amino acid composition, and ability to support bacterial growth. *Biogeochemistry* **30:**77–97.

Weiss, M., and M. Simon. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: The relative significance of amino acids and carbohydrates. *Aquatic Microbial Ecology* **17:**1–12.

Whitman, W. B., D. C. Coleman, and W. J. Wiebe. 1998. Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences of the United States of America* **95:**6578–6583.

Williams, P. J. l. 1973. The validity of the application of simple kinetic analysis to heterogeneous microbial populations. *Limnology and Oceanography* **18:**159–165.

Williams, P. J. l. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 153–200. Wiley-Liss, New York.

Wright, R. T., and J. E. Hobbie. 1965. The uptake of organic solutes in lake water. *Limnology and Oceanography* **10:**22–28.

Wright, R. T., and N. M. Shah. 1977. The trophic role of glycolic acid in coastal seawater. II. Seasonal changes in concentration and heterotrophic use in Ipswich Bay, Massachusetts, USA. *Marine Biology* **43:**257–263.

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Photochemically Mediated Linkages between Dissolved Organic Matter and Bacterioplankton

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Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

I. INTRODUCTION

One of the fundamental roles that dissolved organic matter (DOM) plays in aquatic environments is to serve as a source of carbon and energy to microbial food webs (Pomeroy, 1974; Azam *et al*., 1983). Although this role is widely recognized, efforts to understand it have been hampered by the chemical heterogeneity of the DOM pool. The thousands of different compounds that comprise DOM make studying bulk properties of this material a frustrating exercise and suggest it may not be the best approach to fully understand DOM–microbial linkages. Yet no adequate tools exist to individually probe each of the chemical components of the DOM pool at the necessary level of detail and on the appropriate time scales.

Despite these challenges, some important strides have been made during the last decade in understanding the routes by which DOM enters the microbial food web. One of the most important of these findings has been the recognition that macromolecules in the DOM pool can be photodegraded to more biologically labile molecules and then directly assimilated by bacterioplankton. The absorption of sunlight by DOM results in the reduction of the average molecular weight (Opsahl and Benner, 1998; Zepp *et al*., 1998), the modification of light-absorbing capabilities through "bleaching" in the ultraviolet (UV) and visible spectral regions (Vodacek *et al*., 1997; Moran *et al*., 2000), and the formation of a range of photoproducts (Mopper *et al*., 1991; Bertilsson and Tranvik, 1998; Moran and Zepp, 2000). Some DOM photoproducts are inorganic compounds (e.g., carbon monoxide, carbon dioxide, carbonyl sulfide; Andreae and Ferek, 1992; Valentine and Zepp, 1993; Salonen and Vähätalo, 1994; Miller and Zepp, 1995; Granéli *et al*., 1996) that represent direct photochemical mineralization of carbon but generally have no direct effect on the microbial food web. Other DOM photoproducts are organic molecules that remain part of the DOM pool and have altered susceptibility to biological degradation (Fig. 1).

Strome and Miller (1978) first suggested a link between photochemical alteration of DOM and biological activity by describing a "priming effect" of sunlight on the decomposition of DOM by bacteria. A decade later, the idea of photochemically mediated linkages between DOM and microorganisms was reintroduced by Geller (1986), and the potential role of photochemistry in affecting the supply of biological substrates became more widely recognized (Moran and Zepp, 1997). In this chapter, we summarize what we have learned recently about photochemically-mediated trophic linkages between DOM and microorganisms by focusing on two important issues: (1) Is DOM photoproduct formation in aquatic environments a *predictable* process with regard to compounds formed and effect on microbial activity? (2) Is DOM photoproduct formation in aquatic environments a *significant* process with regard to the other major pathways that link DOM and the microbial food web?

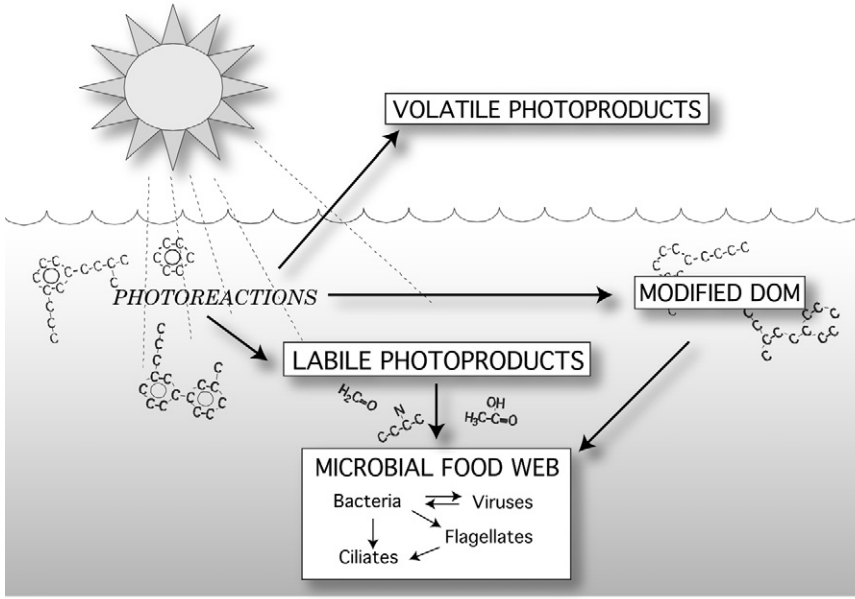

FIGURE 1 Photodegradation of DOM in surface waters of aquatic environments provides organic matter to the microbial food web.

II. IS DISSOLVED ORGANIC MATTER PHOTOPRODUCT FORMATION PREDICTABLE?

A number of studies have investigated the specific photoproducts formed when natural DOM from lakes, rivers, estuaries, and oceans is exposed to sunlight. We asked whether patterns emerge from a survey of these studies, looking specifically for evidence that some compounds are ubiquitous components of the photoproduct pool across all environments or for fundamental differences between marine and freshwater environments in terms of the suite of DOM photoproducts produced.

A. Predicting Specific Photoproducts

Nineteen different compounds (or compound classes) that are known to be rapidly assimilated by bacterioplankton have been identified as DOM photoproducts (Table I). Five of these photoproducts are formed with source DOM from both freshwater and marine environments: acetaldehyde, formaldehyde, glyoxylate, pyruvate, and amino acids. Nine others have been reported only from freshwater systems (acetate, butyrate, citrate, formate, levulinate, malonate, oxalate, succinate, and dissolved carbohydrates), whereas five have been reported only from marine systems (acetone, butanal,

TABLE I Biologically Labile Photoproducts Formed from Freshwater and Marine DOM

Compound	*Reference*	*System*	*Light source*	*Analytical method*
		Freshwater ecosystems		
Acetaldehyde	Kieber *et al*. (1990)	Wetland and river	Natural	HPLC
	Backlund (1992)	Lake	Artificial (UV-C)	GC
	Allard *et al*. (1994)	Lake	Artificial (UV-C)	Capillary ion electrophoresis
Acetate	Wetzel *et al*. (1995)	Wetland	Nnatural and artificial	HPLC
	Dahlén *et al*. (1996)	Lake	Artificial	Capillary ion electrophoresis
	Bertilsson and Allard (1996)	Lake	Artificial	Capillary ion electrophoresis
	Bertilsson and Tranvik (1998)	Lakes	Natural and artificial	Capillary ion electrophoresis
	Bertilsson and Tranvik (2000)	Lakes	Artificial	Capillary ion electrophoresis
	Bertilsson *et al*. (1999)	Rivers and groundwater	Artificial	Capillary ion electrophoresis
	Lindell *et al*. (1996)	Lakes	Artificial	Capillary ion electrophoresis
	Tranvik and Bertilsson (2001)	Lakes	Artificial	Capillary ion electrophoresis
Butyrate	Bertilsson and Allard (1996)	Lake	Artificial	Capillary ion electrophoresis
Citrate	Wetzel *et al*. (1995)	Wetland	Natural and artificial	HPLC
Formaldehyde	Kieber *et al*. (1990)	Wetland and river	Natural	HPLC
Formate	Allard *et al*. (1994)	Lake	Artificial (UV-C)	Capillary ion electrophoresis
	Wetzel *et al*. (1995)	Wetland	Natural and artificial	HPLC
	Dahlén *et al*. (1996)	Lake	Artificial	Capillary ion electrophoresis
	Bertilsson and Allard (1996)	Lake	Artificial	Capillary ion electrophoresis
	Bertilsson and Tranvik (1998)	Lakes	Natural and artificial	Capillary ion electrophoresis
	Bertilsson and Tranvik (2000)	Lakes	Artificial	Capillary ion electrophoresis
	Bertilsson *et al*. (1999)	Rivers and groundwater	Artificial	Capillary ion electrophoresis
	Lindell *et al*. (1996)	Lakes	Artificial	Capillary ion electrophoresis
	Tranvik and Bertilsson (2001)	Lakes	Artificial	Capillary ion electrophoresis
Glyoxylate	Kieber *et al*. (1990)	Wetland and river	Natural	HPLC
Levulinate	Wetzel *et al*. (1995)	Wetland	Natural and artificial	HPLC
Malonate	Backlund (1992)	Lake	Artificial (UV-C)	GC

	Dahlén *et al*. (1996)	Lake	Artificial	Capillary ion electrophoresis
	Bertilsson and Allard (1996)	Lake	Artificial	Capillary ion electrophoresis
	Bertilsson and Tranvik (1998)	Lakes	Natural and artificial	Capillary ion electrophoresis
	Bertilsson and Tranvik (2000)	Lakes	Artificial	Capillary ion electrophoresis
	Bertilsson *et al*. (1999)	Rivers and groundwater	Artificial	Capillary ion electrophoresis
	Lindell *et al*. (1996)	Lakes	Artificial	Capillary ion electrophoresis
	Tranvik and Bertilsson (2001)	Lakes	Artificial	Capillary ion electrophoresis
Oxalate	Backlund (1992)	Lake	Artificial (UV-C)	GC
	Allard *et al*. (1994)	Lake	Artificial (UV-C)	Capillary ion electrophoresis
	Dahlén *et al*. (1996)	Lake	Artificial	Capillary ion electrophoresis
	Bertilsson and Allard (1996)	Lake	Artificial	Capillary ion electrophoresis
	Bertilsson and Tranvik (1998)	Lakes	Natural and artificial	Capillary ion electrophoresis
	Bertilsson and Tranvik (2000)	Lakes	Artificial	Capillary ion electrophoresis
	Bertilsson *et al*. (1999)	Rivers and groundwater	Artificial	Capillary ion electrophoresis
	Lindell *et al*. (1996)	Lakes	Artificial	Capillary ion electrophoresis
	Tranvik and Bertilsson (1999)	Lakes	Artificial	Capillary ion electrophoresis
Pyruvate	Wetzel *et al*. (1995)	Wetland	Natural and artificial	HPLC
Succinate	Allard *et al*. 1994)	Lake	Artificial (UV-C)	Capillary ion electrophoresis
	Bertilsson and Allard (1996)	Lake	Artificial	Capillary ion electrophoresis
DFAA and DCAA	Jørgensen *et al*. (1998)	Lake	Natural	HPLC
TDCHO	Jørgensen *et al*. (1998)	Lake	Natural	HPLC
		Marine ecosystems		
Acetaldehyde	Mopper and Stahovec (1986)	Coastal seawater	Natural and artificial (UV-C)	HPLC
	Kieber *et al*. (1990)	Seawater	Natural	HPLC
	Mopper *et al*. (1991)	Open-ocean seawater	Natural	HPLC
	Obernosterer *et al*. (1999)	Seawater	Natural	HPLC
Acetone	Mopper and Stahovec (1986)	Coastal seawater	Artificial (UV-C)	HPLC
	Obernosterer *et al*. (1999)	Seawater	Natural	HPLC
Butanal	Mopper and Stahovec (1986)	Coastal seawater	Artificial (UV-C)	HPLC

(*continues*)

TABLE I (*continued*)

Compound	*Reference*	*System*	*Light source*	*Analytical method*
		Freshwater ecosystems		
Formaldehyde	Mopper and Stahovec (1986)	Coastal seawater	Natural & artificial (UV-C)	HPLC
	Kieber *et al.* (1990)	Seawater	Natural	HPLC
	Mopper *et al.* (1991)	Open-ocean seawater	Natural	HPLC
Glyoxal	Mopper and Stahovec (1986)	Coastal seawater	Natural & artificial (UV-C)	HPLC
	Mopper *et al.* (1991)	Open ocean seawater	Natural	HPLC
Glyoxylate	Kieber and Mopper (1987)	Seawater	Natural	HPLC
	Kieber *et al.* (1990)	Seawater	Natural	HPLC
	Mopper *et al.* (1991)	Open-ocean seawater	Natural	HPLC
Methylglyoxal	Mopper and Stahovec (1986)	Coastal seawater	Artificial (UV-C)	HPLC
Propanal	Mopper and Stahovec (1986)	Coastal seawater	Natural & artificial (UV-C)	HPLC
Pyruvate	Mopper and Stahovec (1986)	Coastal seawater	Artificial (UV-C)	HPLC
	Kieber and Mopper (1987)	Seawater	Natural	HPLC
	Kieber *et al.* (1989)	Seawater	Natural	HPLC
	Mopper *et al.* (1991)	Open-ocean seawater	Natural	HPLC
	Obernosterer *et al.* (1999)	Seawater	Natural	HPLC
DPA	Bushaw-Newton and Moran (1999)	Coastal seawater	Natural	Wet chemistry

DFAA, dissolved free amino acids; DCAA, dissolved combined amino acids; TDCHO, total dissolved combined carbohydrates; DPA, dissolved primary amines.

glyoxal, methylglyoxal, and propanal). With one exception (butanal), all photoproducts reported when UV-C light was used during irradiation (i.e., radiation in the 200- to 280-nm range, which is naturally removed by the earth's atmosphere and therefore of questionable ecological relevance) were also found when natural solar wavelengths were used (Table I).

The apparent differences in identified photoproducts between marine and freshwater systems may be due to fundamental differences in DOM composition (see Chapters 3 and 5) or to differences in analytical approaches (e.g., capillary electrophoresis has often been the method of choice to identify DOM photoproducts in freshwater systems, but this method is not appropriate for high-salinity marine samples; Table I). The fact that most of the labile photoproducts found only in freshwater environments have been identified by more than one analytical approach, however, suggests that methods alone cannot explain the 14 nonoverlapping photoproducts. On the other hand, studies conducted by the same researcher(s) tend to report the same suite of compounds, even across marine/freshwater boundaries (e.g., Kieber *et al*., 1990), suggesting that optimization of the analytical approach and/or researcher focus may be influencing the data. More studies identifying DOM photoproducts have been conducted in freshwater environments than in marine environments (12 vs. 6), a factor that is also likely to influence the number of reported photoproducts. At this point, evidence is insufficient to determine whether DOM photoproducts that are currently unique to either marine or freshwater environments can be attributed to inherent DOM compositional differences or to analytical approach.

B. Compound-Independent Perspective

An alternative way to examine whether the trophic linkages between DOM and the microbial community can be predicted in aquatic ecosystems is to take a compound-independent perspective, that is, to examine changes in microbial activity rather than measure the photoproducts themselves. The advantage of this approach is that it integrates positive and negative effects of photochemical processes, rather than just determines the formation rates of a specific photoproduct. For example, some studies have demonstrated that measured labile photoproducts alone cannot account for the observed increases in microbial activity following irradiation of DOM (Miller and Moran, 1997; Bertilsson *et al*.. 1999). Others demonstrated net negative effects on the transfer of carbon from DOC to the bacterial community, even though biologically labile photoproducts were being produced (Tranvik and Bertilsson, 2001). Thus, identifiable labile photoproducts alone cannot account for all effects of irradiation on DOM quality.

Net effects of irradiation on bacterial utilization of bulk DOM have been reported in 14 studies from freshwater ecosystems and 6 studies from marine environments (Table II). The challenge of synthesizing these studies

TABLE II Net Effects of Irradiation on the Biodegradability of DOM in Freshwater and Marine Ecosystems

Reference	*System*	*Light source*	*Measure*	*Measured effect*
		Freshwater ecosystems		
Geller (1986)	Lake	Glass-filtered natural	ΔDOC	+5%–+10%
Lindell *et al.* (1995)	Lake	Artificial	Bacterial biomass	+580%
Bertilsson and Allard (1996)	Lake	Artificial	Bacterial cell number	+300%
Lindell *et al.* (1996)	Lakes	Natural	Bacterial biomass	+83%–+175%
Amon and Benner (1996)	River	Pyrex filtered Natural	Bacterial respiration and protein production	0%
Reitner *et al.* (1997)	Lake	Natural	Bacterial cell number	+6%–+66% (45% avg.)
Bano *et al.* (1998)	Wetland	Natural and artificial (solar simulator)	Bacterial biomass	+104%–+172%
Reche *et al.* (1998)	Lake	Glass-filtered natural	Bacterial cell number	–36%–+162% (54% avg.)
Jørgensen *et al.* (1998)	Lake	Natural	Bacterial protein production	+35%–+80%
Moran *et al.* (1999)	River	Natural	Bacterial respiration	0%–+250%
Moran *et al.* (2000)	River	Artificial	Bacterial respiration	+340%
Bertilsson *et al.* (1999)	Lakes	Artificial	Bacterial cell number	+70%
Tranvik and Bertilsson (1999)	Lakes	Artificial	Bacterial cell number	–45%–+139%
Findlay *et al.* (2001)	Groundwater	Natural	Bacterial respiration	0%–+60%
		Marine ecosystems		
Miller and Moran (1997)	Coastal seawater	Artificial (solar simulator)	Bacterial protein production	+143%
Benner and Biddanda (1998)	Seawater	Natural	Bacterial protein production	surface: –75% deep: +40%
Chróst and Faust (1999)	Coastal seawater	Polystyrene filtered natural	Bacterial DNA production	+200%
Obernosterer *et al.* (1999)	Seawater	Natural	Bacterial DNA production	surface: –50% deep: +100%
Ziegler and Benner (2000)	Coastal seawater	Natural	Bacterial protein production	0%
Obernosterer and Herndl (2000)	Coastal seawater	Natural	Bacterial cell number	+60% and 70% (two sites)

is significant because of the wide variety of methodological approaches used. Every study exposed DOM to a different dose of radiation. Both natural and artificial light sources were used, with some artificial sources closely reproducing the natural solar spectrum and others providing only UV-A radiation. Incubations were carried out in both quartz and glass vessels, with differing absorptivity of the samples and optical thickness during irradiation. Many of these differences will affect the amount of light present as well as what proportion of the light is absorbed by the DOM, two parameters that are fundamental to photochemical reaction rates (Miller, 2000). In addition, a variety of bacterial activity measures were used to assess biological effects, with evidence that, even within the same experiment, results based on different metrics for bacterial activity varied considerably (Lindell *et al*., 1995, 1996; Bano *et al*., 1998; Obernosterer *et al*., 1999). For example, Bano *et al*. (1998) measured a 104% enhancement of bacterial biomass accumulation (cell number × average cell size) but a 172% increase in bacterial incorporation of ^{3}H-leucine following irradiation of wetland DOM, whereas Lindell *et al*. (1995) measured a 580% enhancement of bacterial biomass accumulation but only a 60% enhancement of cell number following irradiation of lake DOM. Finally, in some studies the calculations of irradiation effects accounted for the reduction of the DOC pool by the photochemical formation of carbon gases (i.e., the calculations considered the fact that the DOC pool was smaller in the irradiated samples than in the nonirradiated samples), whereas other studies did not.

Despite the difficulties of comparing these studies quantitatively, two patterns emerge from the data. First, irradiation of DOM from marine surface waters does not always stimulate bacterial uptake of DOM. In three of the six marine studies, irradiation of DOM showed no net effect or a negative effect on bacterial activity for at least some samples (Benner and Biddanda, 1998; Obernosterer *et al*., 1999; Ziegler and Benner, 2000). Absence of stimulation of bacterial growth on DOM has also been reported in freshwater systems, but with a somewhat lower frequency; out of the 14 freshwater samples, this result was reported five times (36%). Although sample sizes are too small for a robust analysis, and nonreporting of negative data may influence the literature, this pattern suggests that DOM in marine surface waters may be less photochemically reactive than freshwater DOM, or that the effect of the biologically labile photoproducts is more often outweighed by co-occurring negative effects on lability of marine DOM (Benner and Biddanda, 1998).

The second pattern evident in Table II is that the photochemically mediated transfer of carbon into the microbial food web is more pronounced for deep-water marine DOM than for surface water marine DOM. In two studies that explicitly compared relative photoreactivity of surface and deep samples, the biological lability of deep-water DOM was consistently greater after exposure to sunlight, whereas surface waters typically

showed a net negative effect of irradiation (Benner and Biddanda, 1998; Obernosterer *et al*., 1999). Direct chemical measures of DOM photoproducts from several depths in the Sargasso Sea (Mopper *et al*., 1991) further supports the pattern of increased formation of biologically labile photoproducts from DOM collected deeper in the water column. These depth-related patterns may be driven by previous history of photobleaching or by inherent differences in DOM composition, or both. Deep-ocean DOM has greater aromaticity and lower concentrations of carbohydrates than DOM from surface water (Benner *et al*., 1992; see also Chapter 5), factors likely to influence the proportion of chromophoric compounds present.

C. Enhancement versus Inhibition

Although earlier research viewed the photochemical degradation of DOM as a mechanism for enhancing carbon transfer to the microbial food web (Moran and Zepp, 1997), new hypotheses are emerging to explain the contrasting positive and negative effects on biological availability evident in Table II. These hypotheses have largely focused on the source and/or age of the DOM. DOM of recent origin derived primarily from phytoplankton or zooplankton grazing activity may undergo sunlight-mediated reactions that render it, on average, less biologically reactive (Benner and Biddanda, 1998; Tranvik and Kokalj, 1998; Obernosterer *et al*., 1999). One example of a possible mechanism for a reduction in bioavailability is photochemical condensation into refractory macromolecules, such as the formation of marine humic substances from fatty acids (Harvey *et al*., 1983). In contrast, older DOM or DOM of vascular plant origin is hypothesized to undergo photoreactions that render it more biologically labile, likely related in part to the breakdown of aromatic material into more readily degraded structures.

Whether the net effect of irradiation on bacterioplankton processing of DOM is positive or negative might be predictable from indices of DOM source or lability. Obernosterer *et al*. (1999) suggested that the balance between enhancement and inhibition of DOM uptake in seawater was related to initial biological lability, expressed as the ratio of bacterial activity : DOC concentration; the regression of this parameter against percentage change in bacterial production explained 62% of the variance in bacterial response to irradiated DOM. Tranvik and Bertilsson (2001) demonstrated that a regression model that includes parameters for the contribution of humic substances (based on absorbance at 250 nm) and algal-derived organic matter (based on chlorophyll concentrations) could account for 80% of the observed variability in irradiation-induced changes in DOM lability. Similarly, Reche *et al*. (1998) demonstrated that a regression model based on DOC-specific absorbance successfully accounted for 37% of the variance in irradiation-induced changes in DOM lability. Although the parameters in the regression models differed, these studies have converged

on the hypothesis that older, more aromatic, terrestrially derived DOM will generally have enhanced lability following irradiation, whereas recent, algal-derived DOM will generally become more refractory (Fig. 2).

Studies that focus on specific fractions of DOM, rather than the bulk pool, provide a dataset for evaluation of the hypothesis that source and age of DOM determine the biological outcome of photodegradative processes. DOM photodegradation studies have been conducted on algal leachates (Tranvik and Kokalj, 1998), vascular plant leachates (Wetzel *et al.*, 1995), and humic substances of DOM from freshwater and marine ecosystems (Miller and Moran, 1997; Reitner *et al.*, 1997; Bano *et al.*, 1998; Bushaw-Newton and Moran, 1999; Obernosterer and Herndl, 2000) (Table III). Indeed, all of the DOM components representative of recently produced and/or biologically labile organic matter (e.g., proteins, algal leachate) showed net negative effects of irradiation on biological lability. In contrast, the vascular plant–derived components and humic substances fractions generally showed net positive effects (Table III).

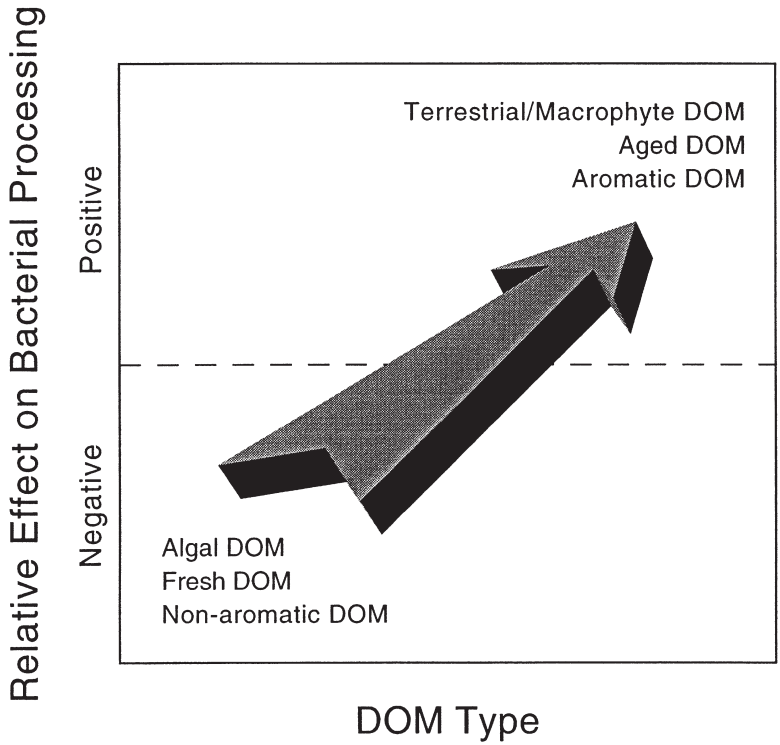

FIGURE 2 Hypothesized effect of age and/or source material of DOM (i.e., DOM quality) as a regulator of the impact of photochemical alterations on bacterial processing.

TABLE III Net Effects of Irradiation on the Biodegradability of Specific Components of DOM

Reference	*DOM component*	*Light source*	*Measure*	*Measured effect*
		Freshwater ecosystems		
Wetzel *et al.* (1995)	Vascular plant leachate (wetland)	Artificial	Bacterial protein production	+50%–+100%
Bushaw *et al.* (1996)	Humic substances (lake)	Natural	Bacterial protein production	+200%
Naganuma *et al.* (1996)	Complex proteins	Artificial	Bacterial cell density	–60%
Reitner *et al.* (1997)	Humic substances (lake)	Natural	Bacterial cell number	+32%
Reitner *et al.* (1997)	Nonhumic compounds (lake)	Natural	Bacterial cell number	+116%
Bano *et al.* (1998)	Humic substances (wetland)	Artificial (solar simulator)	Bacterial biomass	+242%
Bano *et al.* (1998)	Nonhumic substances (wetland)	Artificial (solar simulator)	Bacterial biomass	+143%
Tranvik and Kokalj (1998)	Algal cell lysate	Artificial	Bacterial respiration	–15%–20%
Tranvik *et al.* (2000)	>1000 MW	Artificial	Bacterial cell number	+35%–+55%
		Marine ecosystems		
Keil and Kirchman (1994)	Protein	Natural	Bacterial uptake	–40%
Miller and Moran (1997)	Humic substances (coastal marsh)	Artificial (solar simulator)	Bacterial protein production	+121%
Pausz and Herndl (1999)	Algal extracellular release	Artificial	Bacterial uptake	–25%
Bushaw-Newton and Moran (1999)	Humic substances (estuary)	Natural	Bacterial cell number	0% and +41% (two samples)
Obernosterer and Herndl (2000)	Humic substances (coastal seawater)	Natural	Bacterial cell number	40% and 50% (two sites)
Obernosterer and Herndl (2000)	Nonhumic substances (coastal seawater)	Natural	Bacterial cell number	50% and 0% (two sites)

III. IS DISSOLVED ORGANIC MATTER PHOTOPRODUCT FORMATION ECOLOGICALLY SIGNIFICANT?

The ecological importance of DOM photochemistry to the microbial loop can be evaluated from two perspectives. The first is a "system-level" perspective: At a given time in a given system, do DOM photoreactions significantly influence *in situ* microbial activity? The second is a "long-term" perspective: During the irradiation of a given pool of DOM, do photochemical processes continue to operate over ecologically and geochemically important time scales?

A. System-Level Perspective

We assembled studies that attempted to quantify the importance of labile DOM photoproducts in the carbon budget of aquatic bacterioplankton. The first such calculation of this type was by Kieber *et al.* (1989), who concluded that photochemically produced pyruvate could account for 2–4% of the carbon flux to bacteria in the upper 10 m of the open ocean (Table IV). Moran and Zepp (1997) made a similar calculation for shelf waters, but included a suite of nine DOM photoproducts; in this case, labile photoproducts were calculated to account for over 20% of the bacterial carbon demand. A more recent calculation by Mopper and Kieber (2000) that included all labile DOM photoproducts (i.e., both characterized and uncharacterized compounds) suggests that labile photoproducts could more than account for the bacterial carbon demand in open ocean waters. In a freshwater lake, Bertilsson and Tranvik (1998) calculated that photoproduction of a suite of four biologically labile photoproducts was equal to or greater than the bacterial carbon demand in surface waters on sunny days. This collection of studies may be biased toward measuring only positive effects on microbial activity because only identifiable photoproducts were considered (regardless of other, potentially negative, chemical changes that may be occurring in the DOM) and because terrestrially influenced DOM was used in most of these studies. Nonetheless, all studies (three from marine environments and one from a freshwater environment) agree that photoreactions can play a substantial ecological role in the microbial uptake of DOM in sunlit surface waters (Table IV).

Because the fraction of the water column that experiences full sunlight conditions is generally small, it may be more appropriate to calculate the ecological relevance of DOM photoreactions on a depth-integrated basis. Calculating depth-integrated effects requires information on wavelength-specific light attenuation with depth (which varies considerably across systems) as well as wavelength-specific photoreaction rates (i.e., quantum yields). Moran and Zepp (1997) estimated the depth-integrated formation of biologically labile photoproducts in an average square meter of the ocean

TABLE IV Ecological Significance of Biologically Labile Photoproducts in Surface Waters (A) and on a Depth-Integrated Basis (B)

A. Surface

Reference	*Photoproduct*	*Rate*	*Significance*
Freshwater ecosystems			
Bertilsson and Tranvik (1998)	Acetate, formate, malonate, oxalate	12 μM C day^{-1}[a]	>100% of BCD[b]
Marine ecosystems			
Kieber *et al.* (1989)	Pyruvate	0.14 μM C day^{-1}	2–4% of BCD
Moran and Zepp (1997)	Acetaldehyde, acetate, formate, formaldehyde, glyoxal, pyruvate, glyoxylate, levulinate, propanal	0.9 μM C day^{-1}	20% of BCD
Mopper and Kieber (2000)	All labile photoproducts	2.3 μM C day^{-1}	>100% of BCD

B. Depth integrated

Reference	*Photoproduct*	*Rate*	*Significance*
Freshwater ecosystems			
Bertilsson and Tranvik (1998)	Acetate, formate, malonate, oxalate	46 mg C m^{-2} day^{-1}	10% of BCD
Marine Ecosystems			
Moran and Zepp (1997)	Acetaldehyde, acetate, formate, formaldehyde, glyoxal, pyruvate, glyoxylate, levulinate, propanal	2.5 g C m^{-2} yr^{-1}	4% of BCD

[a] Calculated from data in Bertilsson and Tranvik (1998) by assuming 7.6 h or noontime sun per day.
[b] BCD, bacterial carbon demand.

as 2.5 g C m^{-2} yr^{-1}, a value that is only about 4% of the annual bacterial carbon demand (Table IV). Bertilsson and Tranvik (1998) made a similar calculation in a freshwater lake for four identified low-molecular-weight photoproducts, estimating the depth-integrated production as 46 mg C m^{-2} day^{-1} in midsummer, or about 10% of the depth-integrated microbial respiratory carbon demand (460 mg C m^{-2} day^{-1}). Both of these calculations are based only on identified low-molecular-weight DOM photoproducts, and thus do not include additional labile photoproducts in the form of uncharacterized organic matter (Miller and Moran, 1997). Conversely, these studies do not consider potentially negative effects on biological lability that can occur at the same time the identified labile photoproducts are formed (Bertilsson and Tranvik, 2000). Recognizing these unresolved issues, we nonetheless hypothesize that DOM photoproducts are not likely to account for more than a few percent of the annual depth-integrated bacterial carbon demand in most systems.

B. Long-Term Perspective

A number of studies have attempted to quantify the long-term effects of photoreactions on DOM, concentrating largely on DOM exported from terrestrial environments to the ocean. Although the primary focus of many of these studies has been carbon mineralization via volatile photoproducts that do not directly affect bacterioplankton activity (Mopper *et al.*, 1991; Miller and Zepp, 1995; Amon and Benner, 1996), a few studies have specifically included biologically labile photoproducts in their models. Mopper *et al.* (1991) estimated that photodegradation of seawater DOM could result in degradation of 12–48% of the DOM pool during an oceanic mixing cycle, with approximately one third of this loss occurring via the formation (and subsequent bacterial utilization) of labile photoproducts. Kieber *et al.* (1990) estimated a half-life for riverine DOM in the ocean of only 15 yr based on rates of formation of six biologically available photoproducts. Moran *et al.* (1999) estimated that photoproduct formation could, in some cases, double the losses of riverine DOM on continental shelves, although the extent of photochemical stimulation was highly source dependent.

As DOM in surface waters is exposed to solar radiation for increasing periods of time, the formation of labile photoproducts either may continue at ecologically relevant rates or may slow (or stop completely) as the DOM becomes progressively photobleached. Which of these two scenarios better describes DOM photodegradation kinetics has important consequences for the validity of models addressing long-term yields of DOM photoproducts, because most of the models assume that photoproduct formation (which has been measured primarily over time frames from hours to days) occurs as a first-order reaction over time frames of months to years. The fact that

labile DOM photoproducts can be produced even from highly photodegraded material has recently been demonstrated for vascular plant–influenced DOM (Moran *et al*., 2000). Yet specific rates of photoproduct formation were found to slow over time, and there was evidence for the existence of a non-photoreactive component in the DOM pool (Moran *et al*., 2000). Thus, models that assume first-order formation kinetics over long time frames are likely overestimating the biogeochemical importance of labile DOM photoproducts.

IV. PHOTOCHEMICAL MODIFICATIONS OF DISSOLVED ORGANIC NITROGEN AND DISSOLVED ORGANIC PHOSPHORUS

Labile nitrogen- and phosphorus-rich photoproducts can also be formed during irradiation of DOM in freshwater and marine environments, although there is less information and more variability in the data than for carbon photoproduct formation. Although it is premature to draw any conclusions about the rate and predictability of nitrogen photoproduct formation, ammonium (Bushaw *et al*., 1996; Gardner *et al*., 1998; Bushaw-Newton and Moran, 1999; Tranvik *et al*., 2000), amino acids (Jørgensen *et al*., 1998; Bushaw-Newton and Moran, 1999), and nitrite (Kieber *et al*., 1999) have been reported for both marine and freshwater DOM. However, in other cases, evidence for photochemical formation of labile nitrogen could not be found (Bertilsson *et al*., 1999; Bushaw-Newton and Moran, 1999). Evidence for phosphate or other phosphorus-rich photoproducts has been suggested (Francko and Heath, 1982; Francko, 1986; Cotner and Heath, 1990; Tranvik *et al*., 2000) but not consistently demonstrated.

V. CONCLUSIONS

Is the transfer of DOM to microorganisms via photochemical changes to the DOM pool a predictable and ecologically significant process in aquatic ecosystems? At least five DOM photoproducts are reported from both marine and freshwater environments (acetaldehyde, formaldehyde, glyoxylate, pyruvate, and amino acids). However, differences in analytical approach make it difficult to assess the significance of the additional 14 compounds found to be specific to either one or the other ecosystem type. It is fair to say that we still have a limited understanding of the composition and variation of the DOM photoproduct pool across aquatic environments and cannot yet draw conclusions about inherent differences between marine and freshwater ecosystems. Predicting the net effect on bacterial activity of DOM photoreactions (as opposed to predicting specific photoproducts) is a much more tractable problem, and several regression

models are already demonstrating that the composition/source of the DOM (i.e., recent/algal contributions vs. older/vascular plant contributions) may have significant predictive power for understanding the transfer of photodegraded carbon to the microbial food web (Obernosterer *et al*., 1999; Tranvik and Bertilsson, 2001).

The ecological effects of labile photoproducts on marine microbial food webs is generally agreed to be significant for sunlit surface waters. However, depth-integrated approaches that include the portion of the water column below the light level needed to carry out photoreactions indicate a relatively minor system-wide role relative to other sources of labile carbon to the microbial food web. The long-term rates and kinetics of photoproduct formation, topics particularly relevant for systems such as hydrologically isolated lakes (Waiser and Robarts, 2000) and coastal ocean environments (Moran *et al*., 2000), are not well understood.

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REFERENCES

Allard, B., H. Borén, C. Pattersson, and G. Zhang. 1994. Degradation of humic substances by UV irradiation. *Environment International* **20:**97–101.

Amon, R. M. W., and R. Benner. 1996. Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system. *Geochimica et Cosmochimica Acta* **60:**1783–1792.

Andreae, M. O., and R. J. Ferek. 1992. Photochemical production of carbonyl sulfide in seawater and its emission to the atmosphere. *Global Biogeochemical Cycles* **6:**175–183.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L.-A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* **10:**257–263.

Backlund, P. 1992. Degradation of aquatic humic material by ultraviolet light. *Chemosphere* **25:**1869–1878.

Bano, N., M. A. Moran, and R. E. Hodson. 1998. Photochemical formation of labile organic matter from two components of dissolved organic carbon in a freshwater wetland. *Aquatic Microbial Ecology* **16:**95–102.

Benner, R., and B. Biddanda. 1998. Photochemical transformations of surface and deep marine dissolved organic matter: Effects on bacterial growth. *Limnology and Oceanography* **43:**1373–1378.

Benner, R., J. D. Palkulski, M. McCarthy, J. I. Hedges, and P. G. Hatcher. 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* **255:**1561–1564.

Bertilsson, S., and B. Allard. 1996. Sequential photochemical and microbial degradation of refractory dissolved organic matter in a humic freshwater system. *Archiv für Hydrobiologie* **48:**133–141.

Bertilsson, S., and L. J. Tranvik. 1998. Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. *Limnology and Oceanography* **43:**885–895.

Bertilsson, S., and L. J. Tranvik. 2000. Photochemical transformation of dissolved organic matter in lakes. *Limnology and Oceanography* **45:**753–762.

Bertilsson, S., R. Stepanauskas, R. Cuadros-Hansson, W. Granéli, J. Wikner, and L. Tranvik. 1999. Photochemically induced changes in bioavailable carbon and nitrogen pools in a boreal watershed. *Aquatic Microbial Ecology* **19:**47–56.

Bushaw-Newton, K. L., and M. A. Moran. 1999. Photochemical formation of biologically available nitrogen from dissolved humic substances in coastal marine systems. *Aquatic Microbial Ecology* **18:**285–292.

Bushaw, K. L., R. G. Zepp, M. A. Tarr, D. Schulz-Jander, R. A. Bourbonniere, R. E. Hodson, W. L. Miller, D. A. Bronk, and M. A. Moran. 1996. Photochemical release of biologically available nitrogen from dissolved organic matter. *Nature* **381:**404–407.

Chróst, R. J. and M. A. Faust. 1999. Consequences of solar radiation on bacterial secondary production and growth rates in subtropical coastal water (Atlantic Coral Reef off Belize, Central America). *Aquatic Microbial Ecology* **20:**39–48.

Cotner, J. B., and R. T. Heath. 1990. Iron redox effects on photosensitive phosphorus release from dissolved humic materials. *Limnology and Oceanography* **35:**1175–1181.

Dahlén, J., S. Bertilsson, and C. Pettersson. 1996. Effects of UV-A irradiation on dissolved organic matter in humic surface waters. *Environment International* **22:**501–506.

Findlay, S., J. M. Quinn, C. W. Hickey, G. Burrell, and M. Downes. 2001. Effects of land-use and riparian flowpath on delivery of dissolved organic carbon to streams. *Limnology and Oceanography* **46:**345–355.

Francko, D. A. 1986. Epilimnetic phosphorus cycling: Influence of humic material and iron on coexisting major mechanisms. *Canadian Journal of Fisheries and Aquatic Sciences* **43:**302–310.

Francko, D. A., and R. T. Heath. 1982. UV-sensitive complex phosphorus: Association with dissolved humic material and iron in a bog lake. *Limnology and Oceanography* **27:**564–569.

Gardner, W. S., J. F. Cavaletto, H. A. Bootsma, P. J. Lavrentyev, and F. Troncone. 1998. Nitrogen cycling rates and light effects in tropical Lake Maracaibo, Venezuela. *Limnology and Oceanography* **43:**1814–1825.

Geller, A. 1986. Comparison of mechanisms enhancing biodegradability of refractory lake water constituents. *Limnology and Oceanography* **31:**755–764.

Granéli, W., M. Lindell, and L. Tranvik. 1996. Photooxidative production of dissolved inorganic carbon in lakes of different humic content. *Limnology and Oceanography* **41:**698–706.

Harvey, G. R., D. A. Boran, L. A. Chesal, and J. M. Tokar. 1983. The structure of marine fulvic and humic acids. *Marine Chemistry* **12:**119–132.

Jørgensen, N. O. G., L. Tranvik, H. Edling, W. Granelí, and M. Lindell. 1998. Effects of sunlight on occurrence and bacterial turnover of specific carbon and nitrogen compounds in lake water. *FEMS Microbiology Ecology* **25:**217–227.

Keil, R. G., and D. L. Kirchman. 1994. Abiotic transformation of labile protein to refractory protein in sea water. *Marine Chemistry* **45:**187–196.

Kieber, D. J., and K. Mopper. 1987. Photochemical formation of glyoxylic and pyruvic acids in seawater. *Marine Chemistry* **21:**135–149.

Kieber, D. J., J. McDaniel, and K. Mopper. 1989. Photochemical source of biological substrates in sea water: Implications for carbon cycling. *Nature* **341:**637–639.

Kieber, R. J., X. Zhou, and K. Mopper. 1990. Formation of carbonyl compounds from UV-induced photodegradation of humic substances in natural waters: Fate of riverine carbon in the sea. *Limnology and Oceanography* **35:**1503–1515.

Kieber, R. J., A. Li, and P. J. Seaton. 1999. Production of nitrite from the photodegradation of dissolved organic matter in natural waters. *Environmental Science and Technology* **33:**993–998.

Lindell, M. J., W. Granéli, and L. J. Tranvik. 1995. Enhanced bacterial growth in response to

photochemical transformation of dissolved organic matter. *Limnology and Oceanography* **40:**195–199.

Lindell, M. J., W. Granéli, and L. J. Tranvik. 1996. Effects of sunlight on bacterial growth in lakes of different humic content. *Aquatic Microbial Ecology* **11:**135–141.

Miller, W. L. 2000. An overview of aquatic photochemistry as it relates to microbial production. *In* "Microbial Biosystems: New Frontiers" (C. R. Bell, M. Brylinsky, and P. Johnson-Green, Eds.). Atlantic Canada Society for Microbial Ecology, Halifax.

Miller, W. L., and M. A. Moran. 1997. Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. *Limnology and Oceanography* **42:**1317–1324.

Miller, W. L., and R. G. Zepp. 1995. Photochemical production of dissolved inorganic carbon from terrestrial organic matter: Significance to the oceanic organic carbon cycle. *Geophysical Research Letters* **22:**417–420.

Mopper, K., and D. J. Kieber. 2000. Marine photochemistry and its impact on carbon cycling. *In* "The Effects of UV Radiation in the Marine Environment" (S. deMora, S. Demers, and M. Vernet, Eds.), pp. 101–129. Cambridge Univ. Press, Cambridge, UK.

Mopper, K., and W. L. Stahovec. 1986. Sources and sinks of low molecular weight organic carbonyl compounds in seawater. *Marine Chemistry* **19:**305–321.

Mopper, K., X. Zhou, R. J. Kieber, D. J. Kieber, R. J. Sikorski, and R. D. Jones. 1991. Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. *Nature* **353:**60–62.

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography* **42:**1307–1316.

Moran, M. A., and R. G. Zepp. 2000. UV radiation effects on microbes and microbial processes. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman and R. Mitchell, Eds.), pp. 201–228. Wiley, New York.

Moran, M. A., W. M. Sheldon, and J. E. Sheldon. 1999. Biodegradation of riverine dissolved organic carbon in five estuaries of the southeastern United States. *Estuaries* **22:**55–64.

Moran, M. A., W. Sheldon, Jr., and R. G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. *Limnology and Oceanography* **45:**1254–1264.

Naganuma, T., S. Konishi, T. Inoue, T. Nakane, and S. Sukizaki. 1996. Photodegradation or photoalteration? Microbial assay of the effect of UV-B on dissolved organic matter. *Marine Ecology Progress Series* **135:**309–310.

Obernosterer, I., and G. J. Herndl. 2000. Differences in the optical and biological reactivity of the humic and nonhumic dissolved organic carbon component in two contrasting coastal marine environments. *Limnology and Oceanography* **45:**1120–1129.

Obernosterer, I., B. Reitner, and G. J. Herndl. 1999. Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to marine bacterioplankton. *Limnology and Oceanography* **44:**1645–1654.

Opsahl, S., and R. Benner. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. *Limnology and Oceanography* **43:**1297–1304.

Pausz, C., and G. J. Herndl. 1999. Role of ultraviolet radiation on phytoplankton extracellular release and its subsequent utilization by marine bacterioplankton. *Aquatic Microbial Ecology* **18:**85–93.

Pomeroy, L. R. 1974. The ocean's food web: A changing paradigm. *BioScience* **24:**499–504.

Reche, I., M. L. Pace, and J. J. Cole. 1998. Interactions of photobleaching and inorganic nutrients in determining bacterial growth on colored dissolved organic carbon. *Microbial Ecology* **36:**270–280.

Reitner, B., G. J. Herndl, and A. Herzig. 1997. Role of ultraviolet-B radiation on photochemical and microbial oxygen consumption in a humic-rich shallow lake. *Limnology and Oceanography* **42:**950–960.

Salonen, K., and A. Vähätalo. 1994. Photochemical mineralization of dissolved organic matter in lake Skjervatjern. *Environment International* **20:**307–312.

Strome, D. J., and M. C. Miller. 1978. Photolytic changes in dissolved humic substances. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* **20:**1248–1254.

Tranvik, L. J., and S. Bertilsson. 1999. Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. *Ecology Letters* **4:**458–463.

Tranvik, L., and S. Kokalj. 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. *Aquatic Microbial Ecology* **14:**301–307.

Tranvik, L. J., H. Olofsson, and S. Bertilsson. 2000. Photochemical effects on bacterial degradation of dissolved organic matter in lake water. *In* "Microbial Biosystems: New Frontiers" (C. R. Bell, M. Brylinsky, and P. Johnson-Green, Eds.). Atlantic Canada Society for Microbial Ecology, Halifax.

Valentine, R. L., and R. G. Zepp. 1993. Formation of carbon monoxide from the photodegradation of terrestrial dissolved organic carbon in natural waters. *Environmental Science and Technology* **27:**409–412.

Vodacek, A., N. A. Blough, M. D. DeGrandpre, E. T. Peltzer, and R. K. Nelson. 1997. Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. *Limnology and Oceanography* **42:**674–686.

Waiser, M. J., and R. D. Robarts. 2000. Changes in composition and reactivity of allochthonous DOM in a prairie saline lake. *Limnology and Oceanography* **45:**763–774.

Wetzel, R. G., P. G. Hatcher, and T. S. Bianchi. 1995. Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology and Oceanography* **40:**1369–1380.

Zepp, R. G., T. V. Callaghan, and D. J. Erickson. 1998. Effects of enhanced solar ultraviolet radiation on biogeochemical cycles. *Journal of Photochemistry and Photobiology B: Biology* **46:**69–82.

Ziegler, S., and R. Benner. 2000. Effects of solar radiation on dissolved organic matter cycling in a subtropical seagrass meadow. *Limnology and Oceanography* **45:**257–266.

11

The Importance of Organic Nitrogen Production in Aquatic Systems: A Landscape Perspective

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I. INTRODUCTION

Organic matter formation in aquatic systems is generally equated with organic carbon formation due to the preponderance of carbon in the mass of organic matter and its relatively simple conversion to energy units. Organic compounds, however, contain a number of other elements and nitrogen is essential among these (White, 1993). Nitrogen is a key component of proteins and nucleic acids. As these compounds vary in their proportion to fats and

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starches, which have essentially no nitrogen, the amount of nitrogen in organic matter also varies substantially. Expressed in terms of carbon to nitrogen ratios, nitrogen abundance varies from about 4 in bacteria to over 30 in higher plants (Delwiche and Likens, 1977; Kirchman, 1994). Due to the generally low N content of many plants, the ultimate source of much of the reduced carbon produced globally, heterotrophs, including microbes, are frequently nitrogen limited (Meyer and Johnson, 1983; White, 1993). This limitation can, in turn, impact the efficiency of organic matter assimilation and the efficiency of organic matter transfer to higher trophic levels (White, 1993; del Giorgio and Cole, 1998; Vallino *et al*., 1996).

The general focus on carbon in organic matter processing is underscored by the fact that the organisms involved in cycling of organic matter are functionally characterized by their role in organic C transformations. Autotrophs can form organic C from inorganic C using solar energy or chemical energy stored in inorganic compounds (Fenchel and Blackburn, 1979). Heterotrophs rely on organic C ultimately produced by autotrophs. These same terms do not accurately reflect the roles of these organisms in organic N transformations. Microbial heterotrophs, including a diversity of bacteria, can form organic N *de novo* from inorganic N (Fig. 1; Fenchel and Blackburn, 1979) or they can even fix atmospheric N_2 (Fenchel and Blackburn, 1979).

Heterotrophic organic N (HON) production is broadly recognized by soil scientists as a key factor in preventing the N leakage from soils and increasing the N content of organic matter (e.g., Stark and Hart, 1997; Zogg *et al*., 2000). In aquatic systems, the importance of microbes as harvesters of autotrophically produced organic compounds, including organic N, has been well recognized for decades (Paerl, 1978; Azam *et al*., 1983). However, the *de novo* production of organic N by microbes has not been as widely recognized during this same period. Most diagrams of the aquatic N cycle shown in limnology or oceanography texts depict organic N formation from N_2, NH_4, or NO_3 occurring solely by autotrophic organisms and HON is not included (e.g., Goldman and Horne, 1983; Wetzel, 1983; Wada and Hattori, 1991; Millero and Sohn, 1992). Further, many system-level N models ignore HON (Mantoura *et al*., 1988; Boynton *et al*., 1995). Lastly, isotope studies generally ignore the potential impact of HON on ^{15}N signals of allochthonous-derived detritus and its consumers (but see Currin *et al*., 1995; Caraco *et al*., 1998). It is becoming increasingly apparent, however, that heterotrophic microbes are important sources of organic N in aquatic systems and for some systems may represent a larger contributor than do autotrophs (e.g., Kirchman, 1994).

The factors that limit the formation of organic N from inorganic N are likely somewhat different for autotrophic and heterotrophic organisms (Fig. 1). Light can be an important limitation to most aquatic autotrophs (Sverdrup, 1953; MacIsaac and Dugdale, 1972; Cole and Cloern, 1984). On

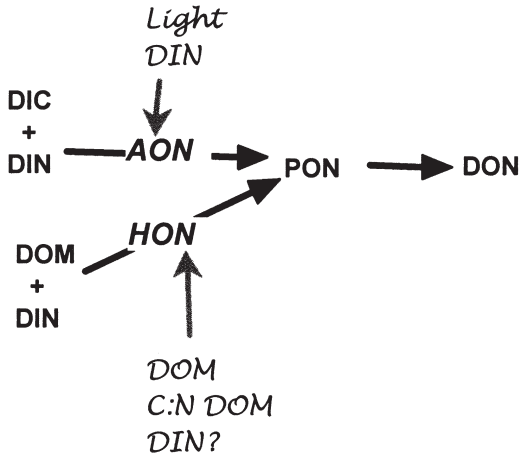

FIGURE 1 Diagram of the formation of particulate and dissolved organic N (PON and DON) by heterotrophic and autotrophic pathways and the likely controls on these two pathways. Autotrophic organic N formation (AON) is accomplished primarily by photosynthetic organisms that form organic carbon from inorganic C, and it is widely recognized that light and inorganic nutrients are key controls on this process. Heterotrophic organic N formation (HON) is accomplished by bacteria and fungi that use organic carbon but combine this premade organic carbon with inorganic nitrogen. The controls on this process include the organic matter supply to the system, the N content of this organic matter, and the inorganic nitrogen supply.

the other hand, organic C supply by allochthonous and autochthonous sources often limits bacterial production (Kirchman 1994). Additionally, heterotrophic organic N formation may depend on the C : N ratio of organic material being used (Zieman *et al*., 1984; Goldman *et al*., 1987; Kirchman, 1994). That is, when the N content of organic matter is low, it is likely that formation of organic N by microbial heterotrophs will be higher. These factors suggest that the relative importance of heterotrophic organic N formation may be greatest in systems with relatively low light and a supply of organic matter with low N content. Such systems tend to have a higher ratio of terrestrial organic matter loads to autochthonous loads (Vannote *et al*., 1980; Webster and Meyer, 1997; Caraco *et al*., 1998; see also Chapter 2).

In this chapter, we develop a simple model that predicts the maximum possible heterotrophic organic N formation as a function of the importance of allochthonous carbon inputs. We then review data from the literature on the relative importance of heterotrophic organic N formation in aquatic systems. This material is presented in a landscape context, following a flow path of organic matter from streams to the open ocean. We consider a variety of evidence to look at heterotrophic organic N formation, including enhancement of decomposition by N additions and ecosystem budgets. We rely, however, primarily on data from ^{15}N addition studies to quantify the

TABLE I Importance of Heterotrophic Organic N (HON) Formation in Aquatic Systems

System	*%HON*	*HON/AON*	*Form*	*Notes*	*Reference*
Kennedy Lake	65	1.9	NH_4	Size	Kirchman (1994)
Flathead Lake	30	0.4	NH_4	Antibiotic	Dodds *et al.* (1992)
Lake Zurich	25	0.3	NH_4	Size	Koster and Juttner (1999)
Average lake	40	0.9	NH_4		
Ems	61	1.6	NH_4	Dark/light	Middleburg and Nieuwenhuize (2000)
Scheldt	45	0.8	NH_4	Dark/light	Middleburg and Nieuwenhuize (2000)
Loire	48	0.9	NH_4	Dark/light	Middleburg and Nieuwenhuize (2000)
Gironde	63	1.7	NH_4	Dark/light	Middleburg and Nieuwenhuize (2000)
Douro	81	4.3	NH_4	Dark/light	Middleburg and Nieuwenhuize (2000)
Rhine	87	6.7	NH_4	Dark/light	Middleburg and Nieuwenhuize (2000)
Ems	39	0.6	NO_3	Dark/light	Middleburg and Nieuwenhuize (2000)
Scheldt	51	1.0	NO_3	Dark/light	Middleburg and Nieuwenhuize (2000)
Loire	22	0.3	NO_3	Dark/Light	Middleburg and Nieuwenhuize (2000)
Gironde	44	0.8	NO_3	Dark/Light	Middleburg and Nieuwenhuize (2000)
Douro	37	0.6	NO_3	Dark/Light	Middleburg and Nieuwenhuize (2000)
Rhine	80	4.0	NO_3	Dark/light	Middleburg and Nieuwenhuize (2000)
Thames	82	4.6	NH_4	Antibiotic	Middleburg and Nieuwenhuize (2000b)
Thames	66	1.9	NO_3	Antibiotic	Middleburg and Nieuwenhuize (2000b)
Hudson	70	2.3	Both	Budget	Caraco *et al.* (1998)
Average river	58	2.1			

Neuse	50	1.0	Both	Phytoplankton demand	Boyer *et al.* (1994)
Chesapeake	10	0.1	NH_4	Size	Kirchman (1994)
Delaware	10	0.1	NH_4	Size	Kirchman (1994)
Long Island Sound	30	0.4	NH_4	Size	Kirchman (1994)
Sapelo Island	78	3.5	NH_4	Size	Hoch and Kirchman (1995)
Chesapeake	5	0.1	NH_4	Size	Hoch and Kirchman (1995)
Average estuary	31	0.9			
Coastal North Atlantic	25	0.3	NH_4	Size	Hoch and Kirchman (1995)
North Atlantic	45	0.8	NH_4	Size	Hoch and Kirchman (1995)
North Atlantic	31	0.4	NH_4	Size	Kirchman (1994)
North Pacific	62	1.6	NH_4	Size	Kirchman (1994)
Subarctic Pacific	45	0.8	NH_4	Size	Kirchman (1994)
Subarctic Pacific	32	0.5	NH_4	Size	Kirchman and Wheeler (1998)
Subarctic Pacific	31	0.4	NO_3	Size	Kirchman and Wheeler (1998)
Average ocean	39	0.7			

Note: HON is expressed both as a percentage of total organic N formation in the system (%HON) and as the ratio of HON to autotrophic organic N formation (AON). All HON measurements except the study of Caraco *et al.* (1988) in the Hudson River and the North Pacific Ocean data are done by ^{15}N uptake of DIN. The form of DIN considered (Form) was NO_3, NH_4, or both together. The bacterial fraction was calculated by size fractionation (size), antibiotic addition, dark/light uptake differences, or, in the case of Boyer *et al.* (1994), total uptake less calculated phytoplankton demand. In all systems where a range of seasonal values is given, we show the numeric average of these values.

importance of heterotrophic organic N formation relative to autotrophic organic N formation (Table I). These studies examine ^{15}N–DIN uptake into organic matter in short-term incubations of water and imply bacterial uptake by size fractionation, antibiotic addition, dark/light differences, or combinations of these methods (Kirchman, 1994). Because most of the ^{15}N uptake studies are performed with ^{15}N–NH_4 as the starting substrate, our focus is on the relative importance of microbes in the conversion of NH_4 to organic N. Recently, however, several studies have examined the importance of ^{15}N–NO_3 uptake and we include these studies in our review. N_2 fixation is not considered as we know of few studies that compare both heterotrophic and autotrophic N_2 fixation estimates for the same system and the consequences for this exclusion are considered in the discussion.

II. MODEL

Where primary production is the sole source of organic matter for heterotrophic N formation, the maximum ratio of heterotrophic organic N (HON) formation to autotrophic organic N (AON) formation can be calculated from autotrophic production (AP), microbial heterotrophic production (MHP), and the C : N ratios of autotrophs ($C : N_{aut}$) and microbial heterotrophs ($C : N_{het}$), such that

$$HON/AON = (MHP/AP) * (C : N_{aut}/C : N_{het}) \quad (1)$$

(after Kirchman 1994). The maximum possible MHP (MHP_{max}) can, in turn, be calculated as a function of AP and bacterial growth efficiency when growth is dependent on autotrophic production (BGE_{aut}), such that

$$MHP_{max} = BGE_{aut} * AP. \quad (2)$$

Thus, substituting (2) into (1),

$$HON/AON = BGE_{aut} * (C : N_{aut}/C : N_{het}). \quad (3)$$

Given a $C : N_{het}$ and $C : N_{aut}$ of 4 and 6 (Kirchman, 1994), respectively, and a BGE_{aut} of 0.4 on low C : N material (Goldman *et al.*, 1987; del Giorgio and Cole, 1998), maximum HON/AON is equal to 0.6.

The maximum value of HON/AON increases in systems with allochthonous carbon inputs. The maximum potential is related directly to the allochthonous organic matter input that is respired in the system (Alloch. Resp.). Additionally, the increased HON will depend on the relative growth efficiency of heterotrophic bacteria on allochthonous material (BGE_{alloch}) and the C : N ratio of allochthonous material ($C : N_{alloch}$), such that

$$HON/AON = BGE_{autoch} * (C : N_{aut}/C : N_{het}) + BGE_{alloch} * (\text{Alloch. Resp.}/AP)(C : N_{alloch}/C : N_{het}). \quad (4)$$

The C : N ratio of material leaving terrestrial systems can vary with nutrient loads to the terrestrial systems and the type of vegetation on those systems (Currie *et al.*, 1996; see also Chapter 2). In general, however, based on the DOC : DON ratio in lysimeter samples, these ratios are between 30 and 50 (Currie *et al.*, 1996); for the purposes of this chapter, we use a value of 40. As the growth efficiency of bacteria is dependent on the C : N ratio of organic matter being used (del Giorgio and Cole, 1998), we used a BGE_{alloch} equal to 0.15 rather than the 0.4 value of BGE_{autoch}. This lower efficiency is in the range of values that were found for growth on natural substrates with a C : N near 40 (del Giorgio and Cole, 1998).

In (4), we have defined all terms as constant except the ratio of allochthonous organic material to AP. Thus, we can combine (4) and the previously cited constants, such that

$$\text{HON/AON} = 0.6 + 1.5 * (\text{Alloch. Resp./AP}). \quad (5)$$

Expressed in terms of percentage of total organic N (%HON) formation,

$$\%\text{HON} = 100 * \text{HON/AON/(1+HON/AON)}. \quad (6)$$

The maximum calculated HON in (2)–(6) is based on primary production being completely respired in the system and all respiration being due to bacteria. Further, all respiration of allochthonous material must be microbial for maximal values to be achieved. Where there is substantial export of primary production or where grazer respiration is large, HON could be far lower than the maximum calculated value. Our calculated maximum HON is dependent on parameters in the model. For example, if our bacterial growth efficiencies are high, then the calculated maximal HON will also be high. Figure 2 shows the magnitude of these possible errors.

III. SYSTEM RESULTS

A. Small Streams

For forested regions, because of the low light and close proximity to the surrounding forest, most streams are dominated by inputs of allochthonous material (Webster and Meyer, 1997). Thus, despite frequently high nutrient loads to these systems (Lovett *et al.*, 2000), primary production is generally quite low and averages about 200 g C m^{-2} yr^{-1} (Lamberti and Steinman, 1997). On the other hand, terrestrial inputs to these same streams are often greater than 1000 g C m^{-2} yr^{-1} and net heterotrophy in these systems is near 200 g C m^{-2} yr^{-1} (Mulholland, 1997; Sinsabaugh, 1997; Webster and Meyer, 1997; Caraco and Cole, 2002). As net heterotrophy is a minimum estimate of Alloch. Resp., this suggests that the ratio of Alloch. Resp./AP in streams is generally greater than 1. Our model suggests that the average maximum

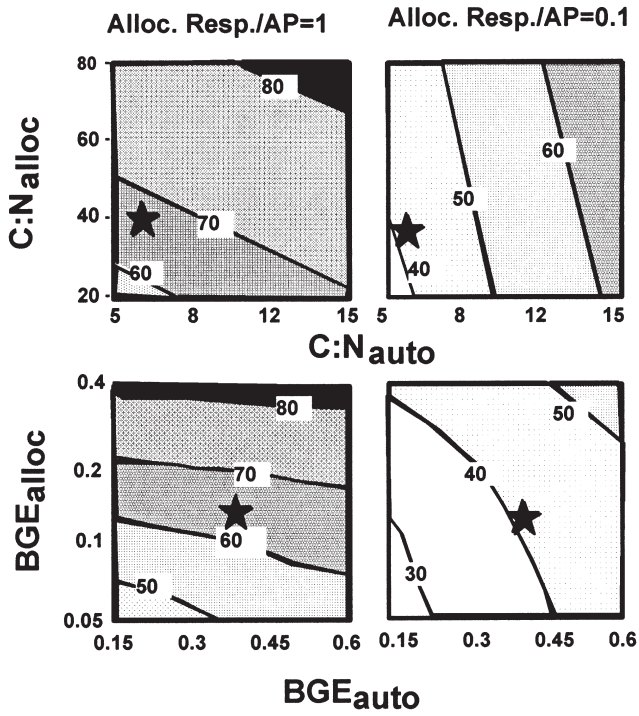

FIGURE 2 Control of the potential relative importance of heterotrophic organic N formation (HON) by the C : N ratio of allochthonous and autochthonous carbon sources (top panels) and by bacterial growth efficiency (BGE) on these two substrates (bottom panels) for two levels of allochthonous carbon respiration (Alloch. Resp.) relative to primary production (AP). In the left panels, Alloch. Resp./AP is set at a value of 1 typical of riverine and stream systems with moderate allochthonous carbon loads. In the right panels, this ratio is set to 0.1, a value that may be representative of these productive estuaries. The values BGE and C : N for allochthonous and autochthonous sources represent the full range generally reported in the literature. In all four panels, the isolines are HON as percentage of total organic N formation in the system (%HON). The stars on each panel represent the values of %HON calculated based on the C : N ratios and BGE values we use in our model (see text). The model predicts maximal potential HON if all AP is respired in the system and all respiration occurs by microbes.

HON/AON should be set at 2.1 and the average maximum %HON should be 67% (Figs. 3 and 4). Variation around these calculated maxima is set by the relative ratio of Alloch. Resp./AP. This ratio varies with watershed vegetation characteristics and discharge of streams (Webster and Meyer, 1997). Small streams surrounded by deciduous forests are likely to have the highest Alloch. Resp./AP ratio and the model would predict the highest %HON.

Our model suggests that microbial organic N formation in streams, in particular in small forested streams, may potentially be quite high. Although

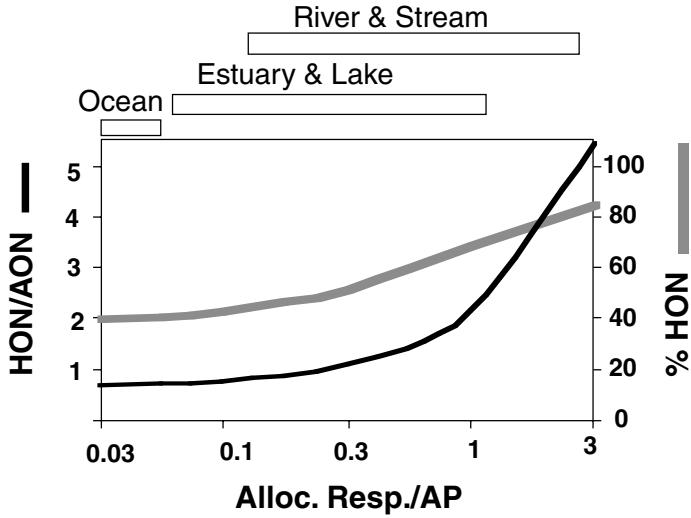

FIGURE 3 Modeled control of potential maximal heterotrophic organic N (HON) formation by the ratio of respiration of allochthonous inputs to autochthonous primary production (Alloch. Resp./AP). HON is expressed both in terms of the ratio of HON to autotrophic organic N formation (left axis and black line) and as the percentage of total organic N formation in the system (right axis and gray line). Also shown is the approximate range of Alloch. Resp./AP across different types of aquatic systems with rivers and streams having the highest ratios and, therefore, the greatest potential relative importance of HON.

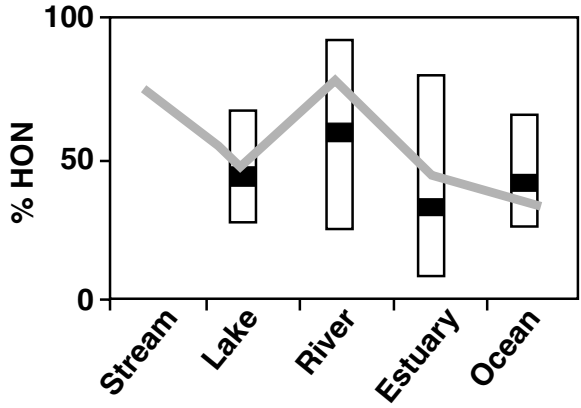

FIGURE 4 Predicted potential maximum and actual measured importance of heterotrophic organic N (HON) formation in various types of aquatic systems. HON is shown as percentage of total organic N formation in the system (%HON). The heavy gray line is the average predicted %HON for the aquatic systems, whereas the shaded region is the range of expected maximum for these systems. The lower range of this shaded area is the same across all systems and represents the maximum predicted HON if respiration of allochthonous material in the system is zero (see Fig. 3). The clear bars represent the range of measured HON in system types and the dark bars represent system averages.

we do not know of data that allow quantification of the %HON, many studies are suggestive. For example, a study in the hyporheic zone of streams showed extremely rapid uptake of $^{15}N–NH_4$ (Hill and Warwick, 1987). The ammonia uptake that occurs in the dark is presumably microbial. Additionally, studies in several streams in forested regions demonstrate that adding nitrogen enhanced decomposition of allochthonous inputs (Howarth and Fisher, 1976; Meyer and Johnson, 1983). The impact of inorganic N on decomposition rates in these experiments suggests that inorganic N is incorporated by microbes and transformed into organic N. Several whole-system $^{15}N–NH_4$ additions to forested streams have provided data suggesting the importance of microbes to organic N formation. First, the exclusion of leaves resulted in lower uptake of NH_4 in a small stream in the Coweeta Forest (Webster *et al*., 2001). A likely explanation of this reduction is decreased microbial uptake in response to the decreased organic input. Second, a $^{15}N–NH_4$ addition study at a second-order stream at Coweeta demonstrated that after 42 days of ^{15}N addition the vast majority of N was in detrital and not primary producer components (Tank *et al*., 2000). After 42 days, however, much of the ^{15}N was unaccounted for and this and the long experimental run make quantification of %HON in this study difficult. Nevertheless, these data and other studies strongly suggest the importance of HON in at least forested steams. For streams in desert or tundra areas, HON may be of less importance (Webster and Meyer, 1997; Wollheim *et al*., 2001).

B. Lakes

Although primary production in some lakes may be light limited due to high DOC or suspended particulates (Cuthbert and del Giorgio, 1992), many lakes appear to be primarily nutrient limited and the supply of N and P in large part determines the productivity of lakes (Smith, 1979; Hecky and Kilham, 1988). Depending on the nutrient input, primary production in lakes can vary from less than 30 to more than 1000 g C m^{-2} yr^{-1}, but for a large number of systems, with moderate nutrient loads, primary production averages about 100 g C m^{-2} yr^{-1} (del Giorgio *et al*., 1999; Caraco and Cole, 2002).

The allochthonous loads to lakes are determined by the organic carbon concentration of streams and groundwater entering lakes and the amount of water entering (water load) from these sources (see also Chapter 6). Both these factors can vary substantially across different regions. On average, however, water load is near 12 m y^{-1} and organic concentration is near 8 mg L^{-1}. Thus, allochthonous organic input should average about 100 g C m^{-2} yr^{-1}, or co-equal to the average autochthonous load (Caraco and Cole, 2002). Not all of this material decomposes in lakes. Rather, considering the average residence times of lakes, about 30% of allochthonous inputs to lakes are decomposed (Dillon and Molot, 1997) or, on average 30 g C m^{-2} yr^{-1}. With

this respiration and a primary production of 100 g C m^{-2} yr^{-1}, the HON/AON ratio should be 1 and the %HON should be 50% [Eqs. (5) and (6) and Figs. 3 and 4]. These values could, however, vary substantially among different lakes and HON would be expected to be greatest in lakes with low nutrient loads or low light but high allochthonous organic matter inputs (Figs. 3 and 4). For example, in some oligotrophic lakes of Quebec, respiration exceeds primary production by as much as 4-times (del Giorgio *et al.*, 1999). Our model suggests that for these oligotrophic lakes HON could be over 80% of total organic N production. On the other hand, eutrophic lakes or lakes with small inputs of allochthonous material would be expected to have a maximum HON of about 37%, the maximum calculated value if allochthonous inputs are near zero.

A limited amount of ^{15}N–DIN uptake data suggests moderate to high HON in lakes relative to AON. In Kennedy Lake, British Columbia, ^{15}N–NH_4 uptake studies indicate that microbial heterotrophic organic N formation is 60% of the total *in situ* production of organic N (Kirchman, 1994). For Flathead Lake, ^{15}N–NH_4 studies during different seasons suggested very different importance of HON with values ranging from 7 to 70% of the total (Dodds *et al.*, 1992), with an average HON value of about 30% of total organic N formation. Lastly, in Lake Zurich, ^{15}N–NH_4 uptake was considered in size categories greater and less than 20 μm and demonstrated that only 25% of ^{15}N uptake was in the smaller size fraction (Koster and Juttner, 1999). As this smaller size fraction includes small autotrophs as well as heterotrophs, it appears that HON in Lake Zurich is less, perhaps substantially less, than 25% of total organic N formation.

Although the data are scarce, the values for lakes are relatively close to the range and average value predicted based on organic C loading. Further, the relatively low HON in Lake Zurich might be expected based on the high primary productivity in this lake and the restricted input of allochthonous material to much of Lake Zurich (Weilenmann *et al.*, 1989).

C. Rivers

Despite generally high nutrient concentrations in rivers as compared to lakes, primary production in these systems appears to be in the same range as that of lakes due to the strong light limitation in these systems (Cole and Cloern, 1984; Roos and Pieterse, 1992). Annual production varies between 10 and 500 g C m^{-2} yr^{-1} and averages about 100 g C m^{-2} yr^{-1} (Capblancq and Dauta, 1978; Bonnetto, 1983; Bonnetto *et al.*, 1981; Saha *et al.*, 1985; Lewis, 1988; Cole *et al.*, 1989, 1992; Webster and Meyer, 1997).

Allochthonous carbon loads to rivers are generally greater than this average primary production. Inputs generally appear to vary between 300 and 3000 g C m^{-2} yr^{-1} and may average close to 1000 g C m^{-2} yr^{-1} (Richey *et al.*, 1990; Findlay *et al.*, 1997; Caraco and Cole, 1999, 2002). Not all of

this organic material is respired within rivers and a majority of the material may, in fact, be exported. Studies suggest, however, that, despite relatively short residence times, about 20% of inputs or 200 g C m^{-2} yr^{-1} may be respired within rivers (Howarth *et al*., 1996; Caraco and Cole, 2002). Given a respiration of allochthonous material that is two-fold greater than primary production, our model suggests, on average, maximum HON/AON should be 3.6 and %HON should be 78% (Figs. 3 and 4). As for other aquatic systems, this value should be higher in rivers with high allochthonous loads and low primary production and lower in systems with low allochthonous loads and high primary production. Rivers with the greatest relative importance of HON formation might, therefore, be expected to be those in regions with extremely high particle loads (Milliman and Meade, 1983) or DOC loads (Ludwig *et al*., 1997) that would both supply organic material and potentially limit primary production through light attenuation. Rivers in continental Europe might be expected to have comparatively low HON due to only moderate organic loads in these areas (Ludwig *et al*., 1997; Caraco and Cole, 1999) and high nutrient loads that could enhance primary production (Caraco and Cole, 1999).

To date, most river studies examining organic N formation have been conducted in continental Europe, but these studies still suggest the high importance of HON. Based on ^{15}N uptake studies in six relatively large European Rivers, the importance of HON varied from 45 to 81% of total organic N formation for NH_4 uptake and from 22 to 80% of total organic N formation for NO_3 uptake (Middleburg and Nieuwenhuize, 2000a, b, Table I). Considering both forms of N together, the average measured value of %HON is 59% of total organic N production (Table I and Fig. 4).

Another line of evidence for high microbial organic N formation in rivers comes from a mass balance study based on the natural abundance ^{15}N and bulk N. This study in the Hudson River, a system with intermediate production and allochthonous loads, suggests that about 70% of the organic N formation is due to heterotrophs and only 30% is associated with autotrophic production (Caraco *et al*., 1998). Thus, both ^{15}N–DIN uptake experiments and bulk ecosystem budgets give %HON values close to the maximum value of 70% that we expect from our model. The range in values could, in part, be due to variation in allochthonous respiration relative to primary production between systems, high grazing in some rivers, or be an artifact caused by incomplete seasonal data for most of the studies to date.

D. Estuaries

As for lakes, primary production in estuaries tends to be nutrient dependent and can vary with nutrient load from 30 to 1000 g C m^{-2} yr^{-1} (Gilmartin, 1964; Cadee and Hegeman, 1974; Cadee, 1978, 1986; Sirois

and Fredrick, 1978; Stockner and Cliff, 1979; Fisher *et al*., 1982; Cole and Cloern, 1984; Harding *et al*., 1986; Nixon *et al*., 1986; Pennock and Sharp, 1986; Randall and Day, 1986; Kuparinen, 1987; Cole *et al*., 1989; Turner *et al*., 1990; Boyer *et al*., 1993). Average primary production is approximately 200 g C m^{-2} yr^{-1}, nearly twice that of lakes and rivers.

Depending on the nature of the estuary, allochthonous organic loads could vary greatly with water inputs from rivers and concentration of organic N in incoming rivers. Given average water loads of about 40 m yr^{-1} (Nixon *et al*., 1996) and total organic C concentrations about 8 mg L^{-1} (Ludwig *et al*., 1997), allochthonous organic C inputs should average about 300 g C m^{-2} yr^{-1}. If respiration of this allochthonous material occurs at a similar rate to lakes (Dillon and Molot, 1997), this suggests that about 10% of allochthonous inputs should be respired within the estuary or 30 g C m^{-2} yr^{-1}. With an Alloch. Resp./AP ratio of 30/200, maximum HON/AON should, on average, be 0.8 and %HON should be near 45% [Eqs. (4) ans (5) and Figs. 3 and 4].

Studies based on ^{15}N uptake of NH_4 into organic matter suggest a large range in the importance of HON in estuaries. %HON values of only 5% were measured in the Delaware Estuary, whereas values as high as 78% were found in the Sapelo Island Estuary (Kirchman, 1994; Hoch and Kirchman, 1995). On average, for the five estuaries for which data are available, 30% of total organic N production is attributable to heterotrophic organic N formation. Although the variation among systems may be driven, in part, by variation in Alloch. Resp./AP ratios, the very low %HON in some estuaries suggests that other factors may be of substantial importance.

In addition to the large variance in %HON in estuaries, what is clear for estuaries, but not rivers, lakes, or ocean systems, is that some estuaries have %HON far lower than the maximum possible. That is, these systems have %HON well below 37%, the maximum value when Alloch. Resp. is equal to zero. One possible reason for the limitation of HON far below the maximum attainable is the high consumption of organic production by grazers, including benthic organisms (Cloern, 1982; Dame, 1993). If these organisms consume much of the autotrophic production and allochthonous carbon loads, a lower percentage is potentially processed by microbial heterotrophs. A second possibility suggested by Kirchman (1994) is that estuaries (as compared to oceanic waters) have dissolved organic matter rich in N-containing amino acids. A third possibility is that the values are near the maximum, but the values of bacterial growth efficiency that we used are higher than the actual values (Fig. 2).

E. Ocean

Oceanic systems vary in production from near 30 to 500 g C m^{-2} yr^{-1} and average near 100 g C m^{-2} yr^{-1} (Valiela, 1984). Although these systems receive some terrestrial carbon, these inputs would only be expected to be

near 3 g C m^{-2} yr^{-1} (Schlesinger and Melack, 1981; Ludwig *et al*., 1997). Thus, given an Alloch. Resp./AP of 0.03, HON in marine systems should be maximally near 40% of total organic N production. The data based on ^{15}N uptake of NH_4 show that microbial uptake in marine systems varies between 30 and 62% of total organic N, with several values exceeding the calculated maximum possible value (Table I).

Although HON values that fall below the maximum possible value are relatively easy to explain (see the discussion on estuaries), it is somewhat more difficult to explain values that exceed this maximum value in the ocean. One possibility is that assumptions underlying our maximum possible calculated HON are incorrect. For example, our value of bacterial growth efficiency could impact greatly our calculated maximum HON formation and a higher efficiency would result in greater calculated HON. Our efficiency value of 0.4 for decomposition of autochthonous material is, however, generally greater than efficiencies measured in the open ocean (del Giorgio and Cole, 1998). If we, in fact, use the BGE of 0.2 found by del Giorgio and Cole (1998) for open-ocean systems, the maximum HON possible would only be near 23% and essentially all of the measured HON to date would be greater than this value.

The high HON measured in the ocean could suggest that there is substantial input and respiration of allochthonous material. Using BGE_{autoch} and BGE_{alloch} of 0.4 and 0.15, respectively, our model suggests that when the %HON is 50%, Alloch. Resp. would need to be 30% of primary production within the system. With lower BGEs, more allochthonous respiration would be needed. Although such high values of allochthonous respiration in the ocean may seem unlikely, the high heterotrophy found in many oligotrophic marine waters is consistent with these high inputs (del Giorgio *et al*., 1997).

IV. DISCUSSION

A. Magnitude and Pattern of Heterotrophic Organic Nitrogen

We hypothesized that the input of terrestrial organic matter relative to the input of autochthonous carbon would be a good predictor of the relative importance of heterotrophic organic N formation in different aquatic systems. Following a flow path from streams to lakes to rivers to estuaries to oceans, this hypothesis would suggest that microbial organic N formation would be highest in wooded streams and rivers, moderate in lakes and estuaries, and lowest in open-water marine systems.

The proposed pattern based on inputs and respiration of allochthonous organic matter cannot be confirmed based on data that presently exist. First, relatively few systems have been studied and many studies do not cover the

entire seasonal cycle. Further, methodological differences among studies make interpretation difficult. Whereas the ^{15}N uptake studies in most of the marine and estuarine studies are based on size fractionation (Kirchman, 1994), river studies have been based on a combination of light–dark differences and antibiotic additions (Middleburg and Nieuwenhuize, 2000a,b). Size fractionation may underestimate HON when fungi or particle-associated bacteria are important (Paerl, 1978; Sinsabaugh and Findlay, 1995). Antibiotics can underestimate HON if they do not completely eliminate bacterial activity but may overestimate HON when autotrophic cyanobacteria are abundant. Another difference among studies is in the substrate considered; whereas the river studies included both NO_3 and NH_4 uptake, most of the marine, estuarine, and the two lake studies are based on NH_4 uptake only. Lastly, our work did not consider the potential importance of N_2 fixation, as we know of few studies that compare both heterotrophic and autotrophic fixation estimates for the same system. We believe, however, this omission may not be significant as rates of N_2 fixation in most aquatic systems are generally less than 0.5 g N m^{-2} yr^{-1} (Howarth *et al.*, 1988), less than 5% of the organic N formation observed in many estuaries, lakes, and the ocean with primary production of 100 g C m^{-2} yr^{-1}.

Despite the fact that data are insufficient to determine with any degree of certainty the cross-system pattern of HON, data are sufficiently abundant to suggest that this process is an important component of organic N formation. Indeed, considering all data together, it appears that close to 50% of the organic N formed in aquatic systems is of heterotrophic rather than autotrophic origin. If this %HON is set at the maximum value determined by Alloch. Resp./AP ratios (e.g., 6), it would suggest that respiration of allochthonous material averages about 30% of primary production in aquatic systems. With average primary production near 100 g C m^{-2} yr^{-1}, this suggests an average net heterotrophy in aquatic systems of approximately 30 g C m^{-2} yr^{-1}.

B. Heterotrophic Organic Phosphorus Formation

Heterotrophic bacteria have the ability to incorporate not only inorganic N but also dissolved inorganic P into organic material. Additionally, as microbes have extremely high P content relative to plants or even phytoplankton (Kirchman, 1994), HOP may be expected to be at least as or even more important than HON. Studies in lakes and marine systems support the importance of HOP in forming new organic P in aquatic systems (Kirchman, 1994).

Based on 10 studies of ^{32}P–PO_4 uptake in lakes, HOP varies from 40 to 98% of total PO_4 uptake and averages near 70% of total inorganic P uptake. For estuaries and coastal waters, HOP varies from 5 to 90% of total

inorganic P uptake and for ocean sites from 39 to 75% of total inorganic P uptake (Kirchman, 1994). The limited available data indicate, therefore, that HOP, like HON, is an important pathway of organic P formation in aquatic systems. More studies on HOP are needed to discern its pattern and controls in diverse ecosystems.

The implications of HOP for quality of organic matter, including quality of DOM, are similar to those of HON (discussion below) as the P content of organic material is often cited as an important determinant of organic matter quality (Sterner, 1995). HON has additional implications for stable isotope research, however, that do not apply to HOP.

C. Implications of Heterotrophic Organic Nitrogen Formation

When HON occurs in systems with inputs of terrestrial organic carbon, there is the potential that this aquatically formed organic N becomes associated with organic C of terrestrial origin. One implication of this aquatic enrichment of terrestrial organic C is that it may alter interpretation of natural-level ^{15}N studies. Standard interpretation of most isotope data implicitly assumes that the ^{15}N signal of organic material, including allochthonous organics, is stable or influenced only by food web shifts (e.g., ca. 3% increases with each trophic transfer; Peterson and Howarth, 1987; Fry, 1991; Fry and Quinones, 1994). Studies in rivers, marshes, and estuaries have shown that the ^{15}N signal in decomposing material with relatively high C : N ratios can be altered substantially by microbial DIN uptake and organic N formation during the decomposition process (Zieman *et al*., 1984; White and Howes, 1994; Currin *et al*., 1995; Caraco *et al*., 1998). In an extreme case, the $\Delta^{15}N$ of oak leaves in the Hudson River changed from –3 to 11% during a 2-month period (Caraco *et al*., 1998). This change affected interpretations of the importance of the origin of organic material in the seston and the importance of allochthonous material as a source of organic matter for consumers in the river. Additionally, the HON may have played a role in creating strong spatial variation in the ^{15}N of the seston, invertebrate grazers, and larval fish in the river (Caraco *et al*., 1998).

A second implication has to do with the N content and potential food quality of dissolved organic material. As the organic N formed by HON can enter the DOM pool by direct leaching from microbial lysis or release during grazing transfers (McCarthy *et al*., 1998), this HON can enrich the N content of DOM in aquatic systems. Terrestrial material exiting soil and entering aquatic systems as DOM often has C : N ratios of near 40 : 1 even in relatively N rich systems (Currie *et al*., 1996). The C : N ratio of DOM in large rivers being exported to coastal waters frequently has far greater N content (Meybeck, 1982; Caraco *et al*., 1998). For example, the C : N of dissolved organic material in the Amazon near 20 and near 8 in the Danube. Much of this N enrichment relative to terrestrial DOM is likely due to HON

in streams, lakes, and rivers themselves. Thus, HON may impact the quality of organic material being exported to coastal seas from the terrestrial environment and this enrichment could influence the rate and extent to which this material is respired in coastal environments (Hedges *et al*., 1997).

REFERENCES

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* **10:**257–263.

Bonnetto, C. A. 1983. Fitoplancton y production primaria del Parana medio. *Ecosur* **10:**79–102.

Bonnetto, C. A., A. A. Bonnetto, and Y. Zalocar. 1981. Contribution al conocimiento limnologiaco del Rio Paraguay en su tramo inverior. *Ecosur* **8:**55–88.

Boyer, J. N., R. R. Christian, and D. W. Stanley. 1993. Patterns of phytoplankton primary productivity in the Neuse River estuary, North Carolina, USA. *Marine Ecology Progress Series* **97:**287–297.

Boynton, W. R., J. H. Garber, R. Summers, and W. M. Kemp. 1995. Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. *Estuaries* **18:**285–314.

Cadee, G. C. 1978. Primary production and chlorophyll in the Zaire River, estuary and plume. *Journal of Sea Research* **12:**368–381.

Cadee, G. C. 1986. Increased phytoplankton primary production in the Marsdiep Area (Western Dutch Wadden Sea). *Journal of Sea Research* **20:**285–290.

Cadee, G. C., and J. Hegeman. 1974. Primary production of phytoplankton in the Dutch Wadden Sea. *Journal of Sea Research* **8:**240–259.

Capblancq, J., and A. Dauta. 1978. Phytoplankton et production primaire de la Riviere Lot. *Annals of Limnology* **14:**85–112.

Caraco, N., and J. Cole. 1999. Regional scale export of C, N, P and sediment: What river data tell us about key controlling variables. *In* "Integrating Hydrology, Ecosystem Dynamics, and Biogeochemistry in Complex Landscapes" (J. D. Tenhunen and P. Kabat, Eds.). Wiley, New York.

Caraco, N. F., and J. J. Cole. 2002. When terrestrial carbon is sent down the river: Importance of allochthonous carbon inputs to the metabolism of lakes and rivers. *In* "Food Webs at the Landscape Level" (G. A. Polis and M. E. Power, Eds.). University of Chicago Press.

Caraco, N. F., G. Lampman, J. J. Cole, K. E. Limburg, M. L. Pace, and D. Fischer. 1998. Microbial assimilation of DIN in a nitrogen rich estuary: Implications for food quality and isotope studies. *Marine Ecology Progress Series* **18:**59–71.

Cloern, J. E. 1982. Does the benthos control phytoplankton biomass in south San Francisco Bay? *Marine Ecology Progress Series* **9:**191–202.

Cole, B. E., and J. E. Cloern. 1984. Significance of biomass and light availability to phytoplankton productivity in San Francisco Bay. *Marine Ecology Progress Series* **17:**15–24.

Cole, J. J., N. F. Caraco, and B. Peierls. 1989. Phytoplankton production in the mid-Hudson. *Hudson River Foundation Report* 003/027/1987A; 1–36.

Cole, J. J., N. F. Caraco, and B. L. Peierls. 1992. Can phytoplankton maintain a positive carbon balance in a turbid, freshwater, tidal estuary? *Limnology and Oceanography* **37:**1608–1617.

Currie, W. S., J. D. Aber, W. H. McDowell, R. D. Boone, and A. H. Magill. 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. *Biogeochemistry* **35:**471–505.

Currin, C. A., S. Y. Newell, and H. W. Paerl. 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: Considerations based on multiple stable isotope analysis. *Marine Ecology Progress Series* **121:**99–116.

Cuthbert, I. D., and P. A. del Giorgio. 1992. Toward a standard method of measuring color in freshwater. *Limnology and Oceanography* **37:**1319–1326.

Dame, R. F. 1993. The role of bivalve filter feeder material fluxes in estuarine ecosystems. *In* "Bivalve Filter Feeders in Estuarine and Coastal Ecosystem Processes" (R. F. Dame, Ed.), pp. 245–269. Springer-Verlag, New York.

del Giorgio P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematic* **29:**503–541.

del Giorgio, P. A., J. J. Cole, and A. Cimbleris. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* **385:**148–151.

del Giorgio, P. A., J. J. Cole, N. F. Caraco, and R. H. Peters. 1999. Dominance of heterotrophic pathways in the plankton of unproductive aquatic systems. *Ecology* **80:**1422–1431.

Delwiche, C. C., and G. E. Likens. 1977. Biological response to fossil fuel combustion products. *In* "Global Chemical Cycles and Their Alterations by Man" (W. Stumm, Ed.), pp. 73–88. Dahlem Konferenzen, Berlin.

Dillon, P. J., and L. A. Molot. 1997. Dissolved organic and inorganic carbon mass balances in central Ontario lakes. *Biogeochemistry* **36:**28–42.

Dodds, W. K., J. C. Priscu, and B. K. Ellis. 1992. Seasonal uptake and regeneration of inorganic nitrogen and phosphorus in a large oligotrophic lake: Size-fractionation and antibiotic treatment. *Journal of Plankton Research* **13:**1339–1358.

Fenchel, T., and T. H. Blackburn. 1979. "Bacteria and Mineral Cycling". Academic Press, New York.

Findlay, S., R. L. Sinsabaugh, D. T. Fischer, and P. Franchini. 1997. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* **1:**227–239.

Fisher, T. R., P. R. Carlson, and R. T. Barber. 1982. Carbon and nitrogen primary productivity in three North Carolina estuaries. *Estuarine, Coastal and Shelf Science* **15:**621–644.

Fry, B. 1991. Stable isotope diagrams of freshwater food webs. *Ecology* **72:**2293–2297.

Fry, B., and R. B. Quinones. 1994. Biomass spectra and stable isotope indicators of trophic level in zooplankton of the northwest Atlantic. *Marine Ecology Progress Series* **112:**201–204.

Gilmartin, M. 1964. The primary production of a British Columbia fjord. *Journal of the Fisheries Research Board of Canada* **21:**505–537.

Goldman, C. R., and A. J. Horne. 1983. "Limnology." McGraw–Hill, New York, 464 pp.

Goldman, J. C., D. A. Caron, and M. R. Dennet. 1987. Regulation of growth efficiency an ammonium regeneration in bacteria by substrate C : N ratio. *Limnology and Oceanology* **32:**1239–1252.

Harding, L. W. J., B. W. Meeson, and T. R. J. Fisher. 1986. Phytoplankton production in two east coast estuaries: Photosynthesis–light functions and patterns of carbon assimilation in Chesapeake and Delaware Bays. *Estuarine, Coastal and Shelf Science* **23:**773–806.

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnology and Oceanology* **33:**796–822.

Hedges, J. I., R. Keil, R. Benner, K. Kvenvolden, and J. Curiale. 1997. What happens to terrestrial organic matter in the ocean. *Organic Geochemistry* **27:**195–212.

Hill, A. R., and J. Warwick. 1987. Ammonium transformations in spring water within the riparian zone of a small woodland stream. *Canadian Journal of Fisheries and Aquatic Sciences* **44:**1948–1956.

Hoch, M. P., and D. L. Kirchman. 1995. Ammonium uptake by heterotrophic bacteria in the Delaware estuary and adjacent coastal waters. *Limnology and Oceanology* **40:**886–897.

Howarth, R. W., and S. G. Fisher. 1976. Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. *Freshwater Biology* **6:**221–228.

Howarth, R. W., R. Marino, J. Lane, and J. J. Cole. 1988. Nitrogen fixation in freshwater, estuarine and marine ecosystems. 1. Rates and importance. *Limnology and Oceanology* **33:**669–687.

Howarth, R. W., R. Schneider, and D. Swaney. 1996. Metabolism and organic carbon fluxes in the tidal freshwater Hudson River. *Estuaries* **19:**848–865.

Kirchman, D. L. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. *Microbial Ecolology* **28:**255–271.

Kirchman, D. L., and P. A. Wheeler. 1998. Uptake of ammonium and nitrate by heterotrophic bacteria and phytoplankton in the sub-arctic Pacific. *Deep Sea Research* **45:**347–365.

Koster, O., and F. Juttner. 1999. NH4 utilization and regeneration rates in freshwater lakes determined by GC–MS of derivatized dihydroindophenol. *Journal of Microbiological Methods* **37:**65–76.

Kuparinen, J. 1987. Production and respiration of overall plankton and ultraplankton communities at the entrance to the Gulf of Finland in the Baltic Sea. *Marine Biolgy* **93:**591–607.

Lamberti, G. I., and A. D. Steinman. 1997. A comparison of primary production in stream ecosystems. *Journal of the North American Benthological Society* **16:**95–103.

Lewis, W. M., Jr. 1988. Primary production in the Orinoco River. *Ecology* **69:**679–692.

Lovett, G. M., K. C. Weathers, and W. V. Sobczak. 2000. Nitrogen saturation and retention in forested watersheds of the Catskill Mountains, NY. *Ecological Applications* **10:**73-84.

Ludwig, W., J.-L. Probst, and S. Kempe. 1997. Predicting the oceanic input of organic carbon by continental erosion. *Global Biogeochemical Cycles* **10:**23–41.

MacIsaac, J. J., and R. C. Dugdale. 1972. Interactions of light and inorganic N in controlling N uptake in the sea. *Deep Sea Research* **19:**209–232.

Mantoura, R. F. C., N. J. P. Owens, and P. H. Burkill. 1988. Nitrogen biogeochemistry and modeling of Carmarthen Bay. *In* "Nitrogen Cycling in Marine and Coastal Marine Environments" (T. H. Blackburn, and J. Sorensen, Eds.), pp. 415–441. Wiley, Chichester.

McCarthy, M. D., J. I. Hedges, and R. Benner. 1998. Major bacterial contribution to marine dissolved organic nitrogen. *Science* **281:**231–234.

Meybeck, M. 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *American Journal of Science* **282:**401–450.

Meyer, J. L., and C. Johnson. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. *Freshwater Biology* **13:**177–183.

Middleburg, J. J., and J. Nieuwenhuize. 2000a. Uptake of dissolved inorganic nitrogen in turbid tidal estuaries. *Marine Ecology Progress Series* **192:**79–88.

Middleburg, J. J., and J. Nieuwenhuize. 2000b. Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate rich Thames Estuary. *Marine Ecology Progress Series* **203:**13–21.

Millero, F. J., and M. L. Sohn. 1992. "Chemical Oceanography." CRC Press, Boca Raton, FL, 531 pp.

Milliman, J. D., and R. H. Meade. 1983. World-wide delivery of river sediment to the oceans. *Journal of Geology* **91:**1–21.

Mulholland, P. J. 1997. Dissolved organic matter concentration and flux in streams. *Jounral of the North American Benthological Society* **16:**131–141.

Nixon, S. W., C. A. Oviatt, J. Frithsen, and B. Sullivan. 1986. Nutrients and the productivity of estuarine and coastal marine ecosystems. *Journal of the Limnological Society of Southern Africa* **12:**43–71.

Nixon, S. W., J. A. Ammerman, L. P. Atkinson, V. M. Berounsky, G. Billen, W. C. Boicourt, W. R. Boynton, T. M. Church, D. M. DiToro, R. Elmgren, J. H. Garber, A. E. Giblin, R. A. Jahnke, N. J. P. Owens, M. E. Q. Pilson, and S. P. Seitzinger. 1996. The fate of nitrogen at the land–sea margin of the North Atlantic Ocean. *In* "Nitrogen Cycling in the North Atlantic Ocean and its Watersheds" (R. W. Howarth, Ed.), pp. 141–180. Kluwer Academic, Dordrecht.

Paerl, H. 1978. Microbial organic carbon recovery in aquatic ecosystems. *Limnology Oceanography* **23:**927–935.

Pennock, J. R., and J. H. Sharp. 1986. Phytoplankton production in the Delaware Estuary: Temporal and spatial variability. *Marine Ecology Progress Series* **34:**143–155.

Peterson, B. J., and R. W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography* **32:**1195–1213.

Randall, J. M., and J. W. Day, Jr. 1986. Effects of river discharge and vertical circulation on aquatic primary production in a turbid Louisiana (USA) estuary. *Journal of Sea Research* **21:**231–242.

Richey, J. E., J. I. Hedges, A. H. Devol, P. D. Quay, L. A. Martinelli, and B. R. Forsberg. 1990. Biogeochemistry of carbon in the Amazon River. *Limnology and Oceanography* **35:**352–371.

Roos, J. C., and A. J. H. Pieterse. 1992. Diurnal variations in the Vaal, a turbid South African river: primary productivity and community metabolism. *Archiv für Hydrobiologie* **124:**459–473.

Saha, L. C., S. K. Choudhary, and N. K. Singh. 1985. Factors affecting phytoplankton productivity and density in the River Ganges at Bhagalpur, India. *Geobios* **12:**63–65.

Schlesinger, W. H., and J. M. Melack. 1981. Transport of organic carbon in the world's rivers. *Tellus* **33:**172–187.

Sinsabaugh, R. L. 1997. Large-scale trends of respiration for stream benthic communities. *Journal of the North American Benthological Society* **16:**119–122.

Sinsabaugh, R. L., and S. Findlay. 1995. Microbial production, enzyme activity and carbon turnover in surface sediments of the Hudson River Estuary. *Microbial Ecology* **30:**127–141.

Sirois, D. L., and S. W. Fredrick. 1978. Phytoplankton and primary production in the Lower Hudson River Estuary. *Estuarine, Coastal and Shelf Science* **7:**413–423.

Smith, V. H. 1979. Nutrient dependence of primary production in lakes. *Limnology and Oceanography* **24:**1051–1064.

Stark, J. M., and S. C. Hart. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* **385:**61–64.

Sterner, R.W. 1995. Elemental stoichiometry of species in ecosystems. *In* "Linking Species and Ecosystems" (C. G. Jones, J. H. Lawton, Eds.), pp. 240–252. Chapman Hall, New York.

Stockner, J. G., and D. D. Cliff. 1979. Phytoplankton ecology of Vancouver Harbor. *Journal of the Fisheries Research Board of Canada* **36:**1–10.

Sverdrup, H. V. 1953. On conditions for vernal blooming of phytoplankton. *Journal du Conseil International pour l'Exploration de lar Mer* **16:**287–295.

Tank, J. L., J. L. Meyer, D. M. Sanzone, P. J. Mulholland, J. R. Webster, B. J. Peterson, W. M. Wolheim, and Leonard, N. E. 2000. Analysis of nitrogen cycling in a forest stream during autumn using a ^{15}N-tracer addition. *Limnology and Oceanography* **45:**1013–1029.

Turner, R. E., N. N. Rabelais, and Z. N. Zhang. 1990. Phytoplankton biomass, production and growth limitations on the Huanghe (Yellow River) continental shelf. *Continental Shelf Research* **10:**545–571.

Valiela, I. 1984. "Marine Ecological Processes." Springer-Verlag, New York.

Vallino, J. J., C. S. Hopkinson, and J. E. Hobbie. 1996. Modeling bacterial utilization of organic matter: Optimization replaces monod growth kinetics. *Limnology and Oceanography* **41:**1591–1609.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37:**130–137.

Wada, E., and A. Hattori. 1991. "Nitrogen in the Sea: Forms, Abundances, and Rate Processes." CRC Press, Boca Raton, FL, 208 pp.

Webster, J. R., and J. L. Meyer. 1997. Organic matter budgets for streams: A synthesis. *Journal of the North American Benthological Society* **16:**141–161.

Webster, J. R., J. L. Tank, J. B. Wallace, J. L. Meyer, S. L. Eggert, T. P. Ehrman, B. R. Ward, B. L. Bennett, P. F. Wagner, and M. E. McTammany 2001. Effects of litter exclusion and wood removal on phosphorus and nitrogen retention in a forest stream. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandle Limnologie* **27:**1337–1340.

Weilenmann, U., C. R. O'Melia, and W. Stumm. 1989. Particle transport in lakes: Models and measurements. *Limnology and Oceanography* **34:**1–18.

Wetzel, R. G. 1983. "Limnology." Saunders, Philadelphia, 767 pp.

White, D. S., and B. L. Howes. 1994. Nitrogen incorporation into decomposing litter of Spartina alterniflora. *Limnology and Oceanography* **39:**133–139.

White, T. C. R. 1993. "The Inadequate Environment: Nitrogen and Abundance of Animals." Springer-Verlag, Berlin, 425 pp.

Wollheim, W. M., B. J. Peterson, L. A. Deegan, J. E. Hobbie, B. Hooker, W. B. Bowden, K. J. Edwardson, D. B. Arscott, A. E. Hershey, and J. Finlay. 2001. Influence of stream size on ammonium and suspended particulate nitrogen processing. *Limnology and Oceanography* **46:**1–13.

Zieman, J. C., S. A. Macko, and A. L. Mills. 1984. Role of seagrasses and mangroves in estuarine food webs: Temporal and spatial changes in stable isotope composition and amino acid content during decomposition. *Bulletin of Marine Science* **35:**380–392.

Zogg, G. P., D. R. Zak, K. S. Pregitzer, and A. J. Burton. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. *Ecology* **81:**1856–1866.

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The Role of Biofilms in the Uptake and Transformation of Dissolved Organic Matter

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I. INTRODUCTION

Biofilms are abundant in a multitude of aquatic environments in which they cover all kinds of inorganic and organic solid surfaces. Biofilms can be defined as microorganisms attached to a surface and embedded in an extracellular gellike matrix of polymeric substances. This matrix is excreted by the microorganisms themselves and may be enriched by molecules adsorbed from the surrounding water.

Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

Biofilms are important in many medical and industrial fields (e.g., Flemming, 1987; Blenkinsopp and Costerton, 1991; Marsh, 1995; Costerton *et al*., 1999; Mittelman, 1999; Flemming and Wingender, 2001a). They are major foci of the self-purification capacity of streams and rivers (Wuhrmann, 1974; Cazelles *et al*., 1991; Grischek *et al*., 1998; Pusch *et al*., 1998) and are made use of in technical waste water treatment (e.g., Bryers and Characklis, 1990). A differentiation between various types of biofilms covering solid surfaces and freely floating organic flocs and colloids is somewhat arbitrary. In a broad sense, particles such as organic aggregates inhabited by bacteria (e.g., marine snow) can be seen as motile biofilms and many of the properties of biofilms on solid surfaces are also valid for these organic particles.

Research from the beginning of the twentieth century showed that the addition of sterile soils or colloidal substances had stimulating effects on various microbial processes in culture media (Söhngen, 1913). Intense research on biofilm bacteria was conducted in the 1930s and 1940s when interest was drawn toward natural systems (e.g., Henrici, 1933), and enhanced growth of bacteria in small vessels compared with natural aquatic environments was observed (ZoBell and Anderson, 1936; Heukelekian and Heller, 1940). It was found then that "surfaces enable bacteria to develop in substrates otherwise too dilute for growth" (Heukelekian and Heller, 1940). ZoBell (1943) speculated that this ability of bacteria depends on the role of surfaces in concentrating nutrients by adsorption, in providing resting places for sessile bacteria, and in retarding the diffusion of exoenzymes and hydrolyzates away from the cell. Despite this early interest, the pure culture of bacterial isolates in suspensions has been the focus of most microbiological research. Geesey *et al*. (1977, 1978) were the first to closely examine bacterial biofilms in mountain streams and to state the numerical dominance of biofilm bacteria in these systems. Lock *et al*. (1984) developed a structural–functional model describing the role of river biofilms in the adsorption and transformation of nutrients and placing these biofilms into the focus of ecological research in running waters. More recently, a shift in paradigms can be observed. The biofilm mode of living (in mixed microbial communities) is now seen as the mode generally preferred for bacterial growth, and it was stressed that these sessile populations predominate in almost all natural ecosystems (Costerton *et al*., 1995; Costerton and Lappin-Scott, 1995). It is therefore necessary to determine how bacteria function as successful members of these interacting communities and as components in the environment (Caldwell, 1995).

The development of biofilms is a succession of processes that can be described in four steps (e.g., Characklis, 1990; Marshall, 1996):

1. *Development of a conditioning film*. This process should depend on surface properties of the substratum and on the charge and polarity of the sorbed matter. It is a relatively fast [half-saturation times of 5–72 s (Armstrong

and Bärlocher, 1989)] physical and chemical process, the kinetics of which can be described by so-called sorption isotherms. By sorbing onto a surface, large macromolecules may significantly alter their quarternary structure [reviewed by Norde (1986)], which may also change their susceptibility to enzymatic attack (Quiquampoix, 2000).

2. *Attachment of bacteria*. At low ionic strength of the medium — as in many freshwaters — bacteria–surface interactions are controlled by the effects of van der Waals attraction and electrostatic repulsion. At high ionic strength — as in seawater — steric interactions between the outer cell surface macromolecules and the substratum gain in importance (van Loosdrecht *et al*., 1989; Rijnaarts *et al*., 1999). Additionally, flagellar and twitching motility of bacteria was found to be essential in the process of attachment by bacteria onto surfaces (Pratt and Kolter, 1998; O'Toole and Kolter, 1998). It seems that extracellular polysaccharides of bacteria are not involved in the adhesion process itself. However, bacterial extracellular polysaccharides are necessary for the development of a biofilm and for the formation of microcolonies (Allison and Sutherland, 1987; Hoyle *et al*., 1993).

3. *Development of a polysaccharide matrix*. Adhered bacteria are able to produce polysaccharides that are not synthesized when they are in the pelagic mode of growth. In the process of adhesion of *Pseudomonas aeruginosa*, the *algC* and *algD* genes that control important enzymes in the alginate synthesis pathway, are up-regulated (Davies *et al*., 1993; Hoyle *et al*., 1993). For the same species it was also found that the formation of type IV pili seems to be necessary to facilitate movement of bacteria along an abiotic surface and to form microcolonies within a developing biofilm (O'Toole and Kolter, 1998). The planktonic-biofilm transformation of several phenotypic expressions of the bacterial genome are controlled by σ factors of the RNA polymerase that initiate transcription (Deretic *et al*., 1994; discussed in Costerton *et al*., 1995).

4. *Growth and detachment of bacteria*. A highly dynamic equilibrium is reached during biofilm maturation. In a flowing system, the hydrodynamic drag will control the biofilm shape (Stoodley *et al*., 1999c) and eventually lead to active detachment of bacteria or passive sloughing of parts of the biofilm. Bacteria in biofilms are often larger and more active than their free-living counterparts. This is true not only for cell-specific activity, but also for biomass specific activity, so that biofilm bacteria in their natural environment also may exhibit shorter doubling times (Kjelleberg *et al*., 1982; Fletcher, 1986; Fischer and Pusch, 1999, 2001).

Much of this research was performed on monospecific biofilms in medical research; it is probable, but not yet substantiated, that the results obtained in these genetic studies can be transferred to natural multispecies biofilms in aquatic environments. In aquatic systems, researchers mostly deal with existing biofilms and with their relationships to the surrounding

water. The focus of this chapter will therefore be on the sorption of dissolved organic matter (DOM) onto and the transformations of DOM in these preexisting biofilms.

II. BIOFILM STRUCTURE

Biofilms exhibit different morphologies depending on the environmental conditions (Lock, 1993; van Loosdrecht *et al*., 1995; Wimpenny and Colasanti, 1997; Stoodley *et al*., 1999a, b, c). In general, laboratory biofilms with one or a few species of bacteria are thicker and less dense in nutrient-rich media than in nutrient-poor media, and they are thinner and denser under high flow velocities than under low flow velocities (e.g., van Loosdrecht *et al*., 1995; Beyenal and Lewandowski, 2000). Mixed species river biofilms growing under high flow velocities of 52–59 cm/sec in an annular biofilm reactor developed a ridged structure (Neu and Lawrence, 1997). The ridges were orientated parallel to the direction of flow. These biofilms were highly heterogeneous; the ridges consisted of individual bacteria and microcolonies embedded in a complex colloidal matrix of polymers. The heterogeneous morphological structure of biofilms is reflected by their diffusive characteristics, as will be discussed in Section IIIB. Recently, it was hypothesized that cell–cell signaling would also play an important role in determining structural complexity of biofilms (Stoodley *et al*., 1999a) and a signaling molecule (acyl homoserine lactone) that is instrumental in the formation of biofilms of *P. aeruginosa* was identified (Davies *et al*., 1998).

A structural and functional model of a biofilm on an inert substratum in an aquatic system (e.g., on sediment particles) is shown in Figure 1. This biofilm exhibits an enlarged surface area with interstitial voids and lacunae (deBeer *et al*., 1994). The formation of biofilm streamers and the flattening of the biofilm under higher flow velocities (Stoodley *et al*., 1999c) are indicated by the arrows at the left margin and on the top of the figure. Regions of varying biofilm thickness are alternating. Regions of the biofilm composed of extracellular polymeric substances (EPS) of different density are symbolized by different shadings. Growth and detachment of bacteria and flocs of EPS are depicted. DOM from the water column is sorbed, and additional DOM is exuded by algae and released by particulate organic matter entrapped in the biofilm. Organic matter is cleaved by extracellular enzymes, some of which are deactivated as a function of time after production or in regions close to the substratum. Bacteria and algae are interacting, as well as colonies of syntrophic or competing bacteria.

In natural streambed biofilms, the biomass of colloidal carbohydrates was found to be 5 times greater than the biomass of bacteria inhabiting the biofilm (Hall and Meyer, 1998). The biofilm volume/bacterial volume ratio is much higher, because the fraction of the biofilm volume actually occupied

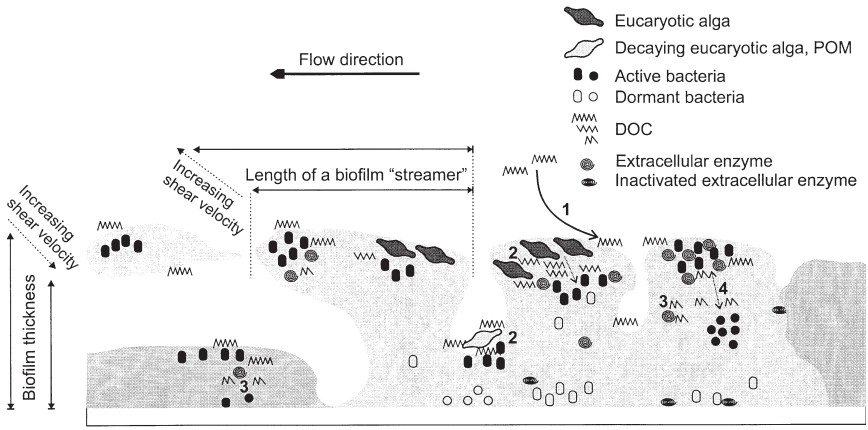

FIGURE 1 Diagrammatic representation of the structure and function of bacterial biofilms. Dissolved organic matter (DOM) is sorbed onto the biofilm (1), additional DOM is released from algae and organic particles (2). The organic matter is cleaved by extracellular enzymes (3). Interactions can occur between clones of syntrophic or competing bacteria (4). See Section II for details.

with solids may be very small (Flemming and Wingender, 2001b). The relatively low biomass therefore shows the gellike structure of the biofilm, whose volume is to a large extent occupied by water.

III. SUPPLY OF DISSOLVED ORGANIC MATTER TO BIOFILM BACTERIA

The retention of DOM in microbial biofilms involves several processes: (A) sorption of a DOM molecule to the biofilm, (B) diffusion into the biofilm, (C) cleavage by extracellular enzymes (in the case of high-molecular-weight organic matter), and (D) uptake and microbial utilization of the DOM molecule.

A. Sorption of Dissolved Organic Matter onto Biofilms

To measure sorption of a dissolved substance (sorbate) to a solid surface (sorbent), the dissolved substance is generally incubated with the solid for a short time (seconds to minutes) at low temperatures. These conditions are chosen to minimize biotic uptake of the dissolved compound (e.g., Henrichs and Sugai, 1993). The partition coefficient

$$K_p = \frac{\%\ \text{adsorbed/gram of solid}}{\%\ \text{dissolved/milliliter of solution}}$$

then gives information about the proportion of the dissolved compound that is adsorbed to the surface. K_p (often referred to as K_d, the distribution

coefficient) not only depends on the properties of the surface (e.g., organic carbon content) and the dissolved compound (charge and polarity) but also on the concentration of the dissolved compound in the solution and the amount of the dissolved compound compared with the amount of solids (Stumm, 1996). From the results of multiple adsorption experiments with different concentrations of the sorbate, typical adsorption isotherms can be calculated. These adsorption isotherms are linear in many environmental situations because in sufficiently diluted systems solute–solute interactions can be ignored in both the sorbent and the sorbate (Karickhoff, 1984). In these cases, K_p is constant and the sorbed phase (S) can be calculated from the concentration in solution (C):

$$S = K_p \times C.$$

If solute–solute interactions occur (e.g., because of the presence of a limited number of sorption sites), then sorption can be modeled by fitting the experimentally derived isotherm to theoretical equations, the Freundlich isotherm

$$S = K \times C^n$$

and the Langmuir isotherm

$$S = \frac{Q^0 \times K \times C}{1 + K \times C},$$

where n is another constant, Q^0 is the maximum sorptive capacity for the sorbent, and K is a specific partition coefficient reflecting the extent of sorption (Podoll and Mabey, 1987; Domenico and Schwartz, 1998). The sorption characteristics can additionally be influenced by cations (e.g., Ca^{2+}) in solution, which may compete with positively charged sorbates for adsorption sites or which can enhance sorption of negatively charged sorbates due to bridging effects with the sorbent (Tipping and Cooke, 1982; Armstrong and Bärlocher, 1989; Henrichs and Sugai, 1993).

Working with the multitude of possible solid surfaces in a natural environment is somewhat simplified by the similar physicochemical properties of the microbial biofilm. In general, these biofilms exhibit a neutral or negative surface charge, the latter being caused by the uronic acids that are common in extracellular polysaccharides (Christensen, 1989). The EPS may further include proteins, nucleic acids, amphiphilic polymeric compounds such as (phospho)lipids, and humic substances (Neu, 1996; Wingender *et al.*, 1999, Flemming and Wingender, 2001b). The chemical heterogeneity of the EPS has recently been made visible by staining the biofilm with fluorescently labeled lectins that bind to specific simple sugars of the biofilm (Wolfaardt *et al.*, 1998; Neu and Lawrence, 1999; Neu, 2000). In general, sorption of DOM to biofilms was found to be higher than that to the clean surface with the biofilm removed (e.g., Armstrong and Bärlocher, 1989). The EPS thus represent a major structural and functional component of biofilms.

For two general reasons it is difficult to distinguish abiotic sorption of DOM onto biofilms from biotic uptake:

1. A comparison of DOM uptake by living and killed biofilms could be accomplished using carbon-free poisons. Sodium azide (N_3Na) and mercury chloride ($HgCl_2$) are the most promising substances for this purpose (Tuominen *et al*., 1994; Ribas *et al*., 1995). These substances can be used in continuous flow systems such as those described in Table II. However, I found that with a $HgCl_2$ concentration of 100 mg/L in perfused sediment cores leucine uptake by bacteria in lower layers of the cores was not completely suppressed, suggesting that $HgCl_2$ was readily adsorbed within the uppermost layers. Additionally, killing the biofilm during experimentation leads to a release of DOM from the biofilm, which masks the actual sorption (Dahm, 1981; Tuominen *et al*., 1994; personal observations). The application of short-term sorption experiments as described earlier in this section therefore seems to be a more useful approach to measure abiotic sorption. However, it should be taken into account that in most of these experiments natural stratification of sediments would be disrupted and existing redox gradients destroyed.

2. In aquatic systems, a physicochemical equilibrium between overlying water and the biofilm is quickly accomplished. By taking up substances, bacteria work against that equilibrium. Thus, the biofilm providing the primary sorption sites for DOM and bacteria using this DOM form an entity. Little is known about the process of formation and decomposition of EPS in living biofilms and whether a "mature" biofilm is a constant, fixed structure or produced and decomposed in a highly dynamic equilibrium. A powerful alternative to measuring sorption onto biofilms is to use labeled model substances. To do so, one can use radioactively labeled substances and determine their fate and distribution in several compartments of the biofilm (biofilm matrix and bacterial biomass), the overlying water (CO_2), or the food web (e.g., Dahm, 1981; Fiebig, 1997; Hall and Meyer, 1998). In this case, sorption to the biofilm and bacterial turnover of the substances can be documented.

In a biofilm, the transport rate of particles and solutes is further influenced by the biofilm matrix itself. It has been calculated that, compared to a clean surface, biofilms may increase convective mass transport near the surface because their compliant nature can increase hydraulic roughness (Bouwer, 1987). Roughness of the biofilm surface in some instances increased eddy diffusion and external mass transfer rate into the biofilm (Siegrist and Gujer, 1985). The viscoelasticity of biofilms has been experimentally demonstrated by changing the hydrodynamic conditions (shear stress and flow velocity) in a flow cell and measuring the structural deformations of biofilms caused by these changes (Stoodley *et al*., 1999c). It was found that biofilms were compliant and readily deformed by changes in shear stress. A decrease

of biofilm thickness by 25% was measured when the shear stress was increased from 0 through 10 N/m^2. This deformation was reversible to some extent — the biofilm returned to its original shape when changes in shear stress were applied below the shear stress at which they were grown. Mass transfer will certainly be influenced by this biofilm deformation. Biofilms also can provide shelter from shear forces and increase surface area for attachment (Bouwer, 1987).

Sorption experiments have been conducted under various experimental conditions, and their results vary depending on whether clean or biofilm-coated sorbents have been used and whether the biofilm was living or had been killed. Armstrong and Bärlocher (1989) demonstrated that sorption on clean natural sediments was higher for positively charged and for uncharged polar amino acids than for negatively charged amino acids. Nonpolar amino acids exhibited intermediate sorption in their study (Table I). Henrichs and Sugai (1993) in their experiments used lower, near natural, amino acid concentrations in seawater sediments covered by a natural biofilm. They also found the highest sorption for the positively charged amino acid lysine; however, they found higher sorption for the negatively charged glutamic acid than for leucine, which is in contrast with the sorption characteristics on clean surfaces (Table I). This finding may be attributed to reactive functional groups in the biofilm, which alter the sorption capacities of the clean surface and provide a greater spatial heterogeneity in the sorption characteristics. Much of the sorption in these experiments was reversible with use of ammonium chloride and/or cesium chloride, demonstrating the functional role of biofilms as cation exchangers as stressed by Freeman *et al.* (1995). However, substantial proportions of the amino acids sorbed onto the biofilm were not extractable by ion exchange. By performing sorption experiments with different amino acid concentrations, Henrichs and Sugai (1993) also found that the sites for this type of irreversible binding were limited. Thus, a chemical equilibrium develops between the sorbate and the

TABLE I Sorption of Amino Acids onto Clean Stream Sediment Surfaces (SiO_2) with the Biofilm Removed

Sorbate (DOM–Fraction)	*Properties of the sorbate*	*Sorption (K_p)*
Arginine, lysine	Positively charged	High
Aspartic acid, glutamic acid	Negatively charged	Low
Glycine, serine, threonine, glutamine, asparagine	Uncharged, but polar	High
Alanine, valine, leucine, isoleucine, phenylalanine	Nonpolar or hydrophobic	Intermediate

From Armstrong, S., and F. Bärlocher, 1989.

sorbent, and further retention of DOM in long-term experiments can be attributed mostly to microbial processes (Section IIIC and Table II).

B. Diffusion of Dissolved Organic Matter into Biofilms

In a flow system with turbulent flow, a laminar boundary layer is formed between the turbulent flow and the biofilm. Mass transfer in the turbulent flow is accomplished by convection and within the biofilm possibly by diffusion. Mass transfer in the boundary layer is accomplished by a mix of diffusion and convective (micro)flow (deBeer *et al*., 1996; Mildenberger, 1999). Mathematical models of biofilm diffusivity assume the existence of a thin diffusive boundary layer above the biofilm. However, some empirical evidence contradicts this assumption. Measurements using nuclear magnetic resonance imaging and particle velocimetry combined with confocal laser scanning microscopy demonstrated that convective flow can be found even within biofilm structures (deBeer *et al*., 1994, Lewandowski *et al*., 1995). Using an oxygen microelectrode Lewandowski (1998) demonstrated that there was only a slight decrease of the mass transfer coefficient above and within a biofilm and a significant reduction only in thick cell clusters. He therefore concluded that biofilms present much less resistance to mass transport than traditionally believed.

The gellike biofilm matrix may be quite unimportant in impeding diffusion in certain circumstances because of the detailed consequences of the diffusion law in special geometries (Koch, 1991). The diffusion coefficient of oxygen in the biofilm of nitrifying trickling filters was calculated to be 40–80% of the value in pure water, with thin biofilms exhibiting higher diffusion resistance than thick biofilms. This was attributed to a higher density of the thin biofilms (Siegrist and Gujer, 1985, 1987). Beyenal and Lewandowski (2000) measured local effective diffusivities of glucose in a three-species artificial biofilm using cathodically polarized microelectrodes, and they spatially integrated the local measurements to give surface averaged effective diffusivities. They found that relative effective diffusivities increased with an increase in glucose concentration and with a decrease in flow velocities. This means that the density of biofilms growing under low nutrient concentrations and high flow velocities — as, for example, in streams and rivers — is probably higher than the density of biofilms in eutrophic lakes or in wastewater treatment plants.

Diffusion into a biofilm may be seen as directed into one dimension. Diffusion time (t) can therefore be calculated as

$$t = \frac{s^2}{2D},$$

where s is the diffusion distance (in centimeters) and D is the diffusion coefficient of a chemical solute (in square centimeters per second) (Berg, 1983).

TABLE II Combined Effects of Sorption on Biofilms and Microbial use on DOC Concentrations, Shown as Retention of DOC (Sorbate) in Perfused Cores (Sorbent)

Sorbate (DOM–Fraction)	*Conditions of incubation*	*Sorbent*	*Retention of the sorbate (% retained)*	*Reference*
<1000 Da	Two columns, residence time 2 h, 21°C	Model drinking-water biofilm on sintered glass beads	High (42.9)	Ribas *et al.* (1995)
1000–10,000 Da			Intermediate-high (27.3)	
>10,000 Da			Low (9.4)	
Humic substances			Low-intermediate (13.9)	
Volatile organic compounds			Wide range, mostly high (5.5–100)	
Total DOC			High (31.9)	
Monosaccharides	1.8 h residence time	Model river biofilm on sintered glass beads	High (59)	Volk *et al.* (1997)
Polysaccharides			High (62)	
Amino acids (free)			High (80)	
Amino acids (combined)			High (48)	
Humic substances			Intermediate (27.1)	
High-molecular-weight DOC (>100 kDa)			High (56.6)	
Total DOC			Intermediate (27.4)	

High-molecular-weight DOC	30 min residence time, *in situ* temperature (6–22°C)	Natural river biofilm (eutrophic lowland river)	High (39)	Fischer et al. (2002)*
Intermediate-molecular-weight DOC			Low (7)	
Low-molecular-weight DOC			High (66)	
Total DOC			Intermediate (14)	
Autochthonous DOC (extracts from epilithic diatoms)	1 h residence time, 21°C	Natural river biofilm (alpine, calcareous, oligosaprobic stream	High (68.7)	Battin et al. (1999)*
Allochthonous DOC (soil extracts)			High (48.5)	
Autochthonous DOC (extracts from epilithic cyanobacteria)		Natural river biofilm (Mediterranean stream)	High (53.2)	
Allochthonous DOC (soil extracts)			High (68.9)	

*Retention measurements coupled with measurements of microbial activity.

If the biofilm is seen simply as a two-dimensional structure on an inert surface, the diffusion time within the biofilm would be

$$t = \frac{s^2}{4D}$$

and in three dimensions, as, for example, in organic particles or thick biofilms, diffusion time would be

$$t = \frac{s^2}{6D}.$$

(Berg, 1983). The diffusion coefficient for a small molecule in water at room temperature is about 10^{-5} cm^2/s. Because diffusion time increases with the square of diffusion distance, the consequence of the above equations is that molecular diffusion is very fast on a small scale and very slow at a large scale (Table III). Given a diffusivity in biofilms of about 50% of that in water, diffusion is probably not the rate-limiting process in thin biofilms, but is certainly a limiting factor in thick biofilms and microbial mats (e.g., Revsbech, 1989). Diffusion limitation can lead to experimental artifacts in laboratory incubations with labeled tracer molecules, as often used for measurements of bacterial production or substrate uptake. In this context, Ploug and Grossart (1999) have measured the thymidine and leucine uptake by bacteria on riverine aggregates incubated individually in a three-dimensional diffusion field versus similar aggregates pooled on the bottom of vials. They found that thymidine and leucine incorporation rates were 5.6- to 5.3-fold lower, respectively, when aggregates were pooled. They attributed this effect to limited (one-dimensional) diffusion of the tracer molecules in vials as opposed to fast (three-dimensional) diffusion into freely floating aggregates.

TABLE III Diffusion Times for One- and Three-Dimensional Diffusion of Leucine and Oxygen Molecules in Water at Room Temperature

	Diffusion time			
	Leucine ($D = 0.73 \times 10^{-5}$)		*Oxygen ($D = 2.24 \times 10^{-5}$)*	
Diffusion distance	*One-dimensional*	*Three-dimensional*	*One-dimensional*	*Three-dimensional*
1 µm	0.68 ms	0.23 ms	0.22 ms	0.074 ms
10 µm	68 ms	23 ms	22 ms	7.4 ms
100 µm	6.8 s	2.3 s	2.2 s	0.74 s
1 mm	11.4 min	3.8 min	3.7 min	1.2 min
1 cm	19.0 h	6.3 h	6.2 h	2.1 h

*Calculated after Berg (1983).

C. Cleavage by Extracellular Enzymes and Microbial Uptake and Utilization

Small molecules diffuse through the biofilm and are taken up by bacteria via membrane permeases or via diffusion through porins (Nikaido and Vaara, 1985). According to Fick's first law, the driving forces for molecular diffusion are the coefficient of diffusion and the concentration gradient. Various strategies can further enhance the availability and uptake of dissolved organic carbon (DOC) by bacteria. Voids and channels can occur in the biofilm and provide ways for convective transport to the deeper biofilm layers (deBeer *et al*., 1994; Lewandowski *et al*., 1995). It may therefore be hypothesized that biofilm bacteria actively communicate to organize biofilm structures that allow continuous nutrient supply via these interstitial voids (cf. Shapiro, 1998). Uptake of DOC can further be enhanced by an increased surface area of the bacterial cell. For example, the anaerobic cellulolytic bacterium *Clostridium thermocellum* bears intricate multienzyme complexes (the cellulosomes) within specialized cell surface organelles. In addition to its enzymatic function, the cellulosome contains functional domains that adhere the bacterium to its particulate cellulosic substrate, so that the enzyme complex can be efficiently used in close proximity to the substrate (Bayer *et al*., 1996). Another way to increase DOC uptake is to quickly remove the organic compound from the internal pool. In this case, the substrate reacts with a phosphorylated enzyme via the phosphoenol phosphotransferase system within the membrane, and a phosphate ester of the substrate is formed and released into the cytoplasm (Gottschalk, 1986). The substrate then appears inside the cell in a chemically modified form, thus not steepening the concentration gradient.

Section IIIA described how amino acids are adsorbed onto the biofilm. However, in natural systems physical sorption and chemical interactions of the sorbed molecules with the biofilm constituents are quickly superseded by microbial activity. Experiments lasting several hours, days, or months have been used to discriminate microbial effects from sorption effects that occur over a time span of a few minutes. In these studies it became clear that, if applied at near natural concentrations, more than 70% of amino acids added to perfused sediment cores were retained in river biofilms (Fiebig, 1992; Fiebig and Marxsen, 1992; Volk *et al*., 1997). However, it may take much longer for this material to be turned over, mineralized, and exported from the cores. Only 14–36% of the sorbed amino acids were mineralized during the first 4 h, the time of the incubation (Fiebig, 1992; Fiebig and Marxsen, 1992). In a long-term experiment using amino acids and hyporheic sediments it took 28 weeks for 88% of the retained carbon to be exported as CO_2 (Fiebig, 1997). The initial rate of immobilization exceeded the rate of export by a factor of 710 in this study. These data demonstrate that biofilms serve as a medium for DOM storage and may accumulate sufficient

nutrients to negate the effects of short-term changes in carbon supply as proposed by Freeman and Lock (1995).

Bacteria living in a biofilm are more or less fixed to a certain position. To sustain their metabolic needs, they therefore depend on a constant flux of organic matter toward their cells. In a recent study, Thompson and Sinsabaugh (2000) have separated biofilms into a polysaccharide matrix fraction and a fraction containing microbial cells and other particulate material. They measured enzyme activity and kinetics of alkaline phosphatase and leucine aminopeptidase in both fractions for both light-exposed and shaded biofilms. They found an average of about 25% of the total activity in the cell free matrix fraction, suggesting that the biofilm matrix was retaining enzymes. They also found that for both enzymes K_m values were significantly higher and V_{max} values were significantly lower in the biofilm matrix than in the cell plus particulate fraction. The authors discuss this phenomenon as an artifact of diagenesis: enzymes released into the environment are conformationally constrained by reactions with the exopolysaccharides, humic substances, or other molecules that reduce their substrate binding capability. However, the matric enzyme activity can comprise a significant fraction of total biofilm activity. Matric enzymes may therefore be regarded as a community resource, uncoupled from the metabolism of individual cells (Thompson and Sinsabaugh, 2000), a function that will be further discussed in the next paragraphs.

By modeling bacterial foraging by means of freely released extracellular enzymes, Vetter *et al*. (1998) defined a "foraging distance" within which 90% of the diffusion current of hydrolysate to the cell is produced. This foraging distance was conservatively modeled to be about 10 μm and would exceed 50 μm in large aggregates and in sediments under conditions of low enzyme inactivation rates (Vetter *et al*., 1998). These calculated distances may have implications for the spatial structures of biofilm communities. If the biofilm is sparsely colonized by single bacteria in distances of more than 10 μm from each other, these bacteria would depend on hydrolysates produced by their own extracellular enzymes. A relatively high proportion of the extracellular enzymes produced may be lost in this case because of inactivation or diffusion into the overlying water. The growth of bacteria in clones forming microcolonies would support a more efficient utilization of extracellular enzymes. Enzymes diffusing farther away from a hypothetical bacterium in the center of the microcolony may then still be used by the clone, because bacteria from the outer margins of the microcolony may benefit from the hydrolysates. On the other hand, bacteria may have developed strategies to avoid excessive production of extracellular enzymes and seek to benefit from enzymes produced by different clones. To do so, they would have to intrude into cell clusters of the other clone and refrain from the production of their own extracellular enzymes. In this scenario, it would not be surprising if the "host" bacteria would have developed defense

mechanisms, such as production of allelochemicals, to hinder the intrusion into their cell clusters. These scenarios would afford extensive interactions between bacteria of the same clone as well as between bacteria of different clones or species. Indeed, cell-to-cell signaling to sense and control cell density ("quorum sensing") is known from bacteria in suspension cultures (Fuqua *et al*., 1994; Dunny and Leonard, 1997) and was recently shown to exist in biofilms as well (Davies *et al*., 1998). Combining the modeling approach (Vetter *et al*., 1998) with the hypotheses of intrabiofilm cell–cell signaling (Davies *et al*., 1998) opens a new perspective for the understanding of the spatial community structure in biofilms.

Gradients of nutrients and oxygen in biofilms additionally promote high diversity, which may ultimately result in functional differences of the bacterial community in biofilms compared with free-floating bacteria. Additionally, increased species diversity may provide spatial and temporal niches not available within monocultures or may create microenvironments within the biofilm (Gieseke *et al*., 2001; Whiteley *et al*., 2001). These thoughts reinforce the need for community-level biofilm studies as opposed to monocultures.

IV. EFFECTS OF THE BIOFILM ON MICROBIAL ACTIVITY

Bacteria afford extracellular enzymes to degrade and effectively use large molecules. Whether biofilm bacteria on inert surfaces develop enzyme patterns different from those of free-living bacteria has rarely been tested (Arnosti, 2000; see Chapter 13). If there was a frequent turnover of the bacterial extracellular polysaccharides in biofilms, the activity of polysaccharases needed for synthesis and degradation of this material can be assumed to be higher in biofilm bacteria than in free-living bacteria. These enzymes can have a marked effect on the structure and the integrity of biofilms (Sutherland, 1999). However, enzymes adsorbed to particle surfaces mostly undergo changes in their confirmation and thus in their kinetics. The optimal catalytic activity shifts to higher pH values when enzymes are adsorbed on negatively charged surfaces (Quiquampoix, 2000). Probably the electrostatic interactions between the positively charged enzymes and negatively charged surfaces lead to an unfolding of the enzymes at pH values below the isoelectric point of the enzymes. Usually, V_{max} decreases and K_m increases in these cases (Quiquampoix, 2000). These restrictions to enzymatic activity on particle surfaces need not necessarily apply to fully hydrated biofilms, but they probably occur in those regions of the biofilms that are located close to a negatively charged substratum. Thus, the often-observed higher activity of biofilm bacteria compared with that of free-living bacteria contrasts with physical and physiological constraints (possible diffusion limitation and deactivation of enzymes) exerted on the bacteria. Whereas in most cases the advantages for growth in a biofilm predominate, they can be offset to some

extent by disadvantages (Fletcher, 1991). Bacteria living close to the substratum should be more strongly affected by those disadvantages than bacteria from outer biofilm layers. Indeed, it has often been found that microorganisms from outer biofilm layers develop higher activities (Paul and Duthie, 1989; Burkholder *et al*., 1990; Fischer *et al*., 1996; Okabe *et al*., 1996).

The type of the biofilm may thereby significantly influence the activity of attached bacteria. In epilithic biofilms, mineral nutrients (Fe, Si, and trace elements) can leach from the substratum and be used by the attached microflora (Wetzel, 1993). However, if the underlying solid substratum can be used as an additional source of carbon and nutrients, bacteria generally exhibit higher activity than those living on a mineral surface. In this context, Sinsabaugh *et al*. (1991) found that ATP content and a suite of extracellular enzyme activities were higher in epixylic biofilms of a boreal river than in epilithic biofilms under similar environmental conditions. Those epixylic biofilms are inhabited by groups of bacteria specialized in the degradation of complex polysaccharides such as cellulose (Reichenbach, 1992; Bayer *et al*., 1996) and polyphenolics such as lignin (Vicuña, 1988; Hendel and Marxsen, 2000). Detrital particles in the hyporheic zone of a prealpine river were important for bacteria as colonizable surface areas and as a source of nutrition and thus had a higher power in predicting total bacterial abundance and production than inorganic particles (Brunke and Fischer, 1999). In pelagic aggregates, intense activity of several ectohydrolases has been revealed (e.g., Smith *et al*., 1992; Grossart and Simon, 1998; but see Müller-Niklas *et al*., 1994; see also Chapter 13). Largely overlooked, these aggregates can contain considerable amounts of interstitial DOC (Alldredge, 2000), which may serve as a carbon source in addition to the particulate fraction. In conclusion, the adverse effects for bacteria living in a biofilm mostly predominate in regions close to the substratum, whereas bacteria in the outer regions of the biofilm or on freely floating organic flocs may benefit from the advantages of continuous substrate supply via sorption to the biofilm.

V. EFFECTS OF DISSOLVED ORGANIC MATTER QUALITY AND QUANTITY ON THE ACTIVITY OF BIOFILM BACTERIA

DOC, whatever its form or origin, either directly or indirectly represents the ultimate source of organic carbon for sustaining the metabolism of heterotrophic bacteria. The metabolic activity of biofilm bacteria can therefore be influenced by the ambient concentration and composition of DOC (e.g. Kaplan and Bott, 1989; Baker *et al*., 1999; Fischer *et al*., 2002; see Chapter 15). Biofilms are able to retain inorganic and organic solutes (e.g., Bencala *et al*., 1984; Mc Dowell, 1985; Fiebig, 1992). As shown in Section IIIC, they can buffer the supply of organic substrates so that short-term

changes in the quality and quantity of DOC need not have an immediate effect on biofilm metabolism (Freeman and Lock, 1995; Fiebig, 1997).

In Table II it is shown that DOM fractions are differentially retained in perfused cores with multispecies biofilms during long-term incubations. This microbially mediated retention is high for low-molecular-weight organic matter, most notably amino acids and mono- and disaccharides, and for the polysaccharide fraction of high-molecular-weight organic matter. It is relatively low for the intermediate-molecular-weight substances predominantly consisting of humic substances. The retention of freshly extracted DOC (Battin *et al*., 1999; Table II) is particularly high, probably because of a high proportion of labile substances in these extracts. Figure 2A depicts an example for DOC retention in a flowthrough incubation of riverine sediments derived from the sixth-order lowland River Spree, Germany (see Table II for details). It can be seen that humic substances are retained to a much lesser extent than the high-molecular-weight compounds (polysaccharides) and the low-molecular-weight compounds (mono- and disaccharides and amino acids). However, the humic substances form the largest group of DOC and can therefore make up a substantial proportion of the total organic matter retained in the cores (Fig. 2A; Volk *et al*., 1997; Fischer *et al*., 2002).

The quality of the DOC available obviously influences bacterial activity in the biofilm. The composition of DOC in natural river water retained in sediment cores has significant effects on bacterial production, whereas changes in the bulk quantity of DOC alters the activity of biofilm bacteria to a lesser extent (Fig. 3A–C; see Chapter 15). Under light conditions, autotrophic algae in the biofilm are a possible source of labile compounds that may be used by biofilm bacteria living in close proximity to the algae. Therefore, bacterial activity often correlates with algal biomass and production in the biofilm (Haack and McFeters, 1982; Chappell and Goulder, 1994; Sobczak, 1996). In a Piedmont stream, biofilm bacteria kept in the light grew twice as fast than those kept in the dark. This was attributed to biofilm-internal DOC cycling mediated by epilithic algae, which had a 5-fold higher biomass in the light than in the dark (Kaplan and Bott, 1989). In a Mediterranean stream, bacterial density and β-glucosidase activity were higher in light-grown biofilms than in dark-grown biofilms. Algal biomass increased with the use of cellobiosic as opposed to xylobiosic polysaccharides, probably because of the presence of high-quality algal exudates (Romani and Sabater, 1999). In the same study, bacteria in dark-grown biofilms responded rapidly to algal activity, although a greater increment in chlorophyll density was necessary to obtain a similar increase in enzymatic activity in light-grown biofilms. This resilience of bacteria in light-grown biofilm is probably related to algal exudates stored in the biofilm. Wetzel (1993) hypothesized that the high productivity of attached microcommunities of algae and bacteria is due to efficient internal recycling of carbon and nutrients. Whereas

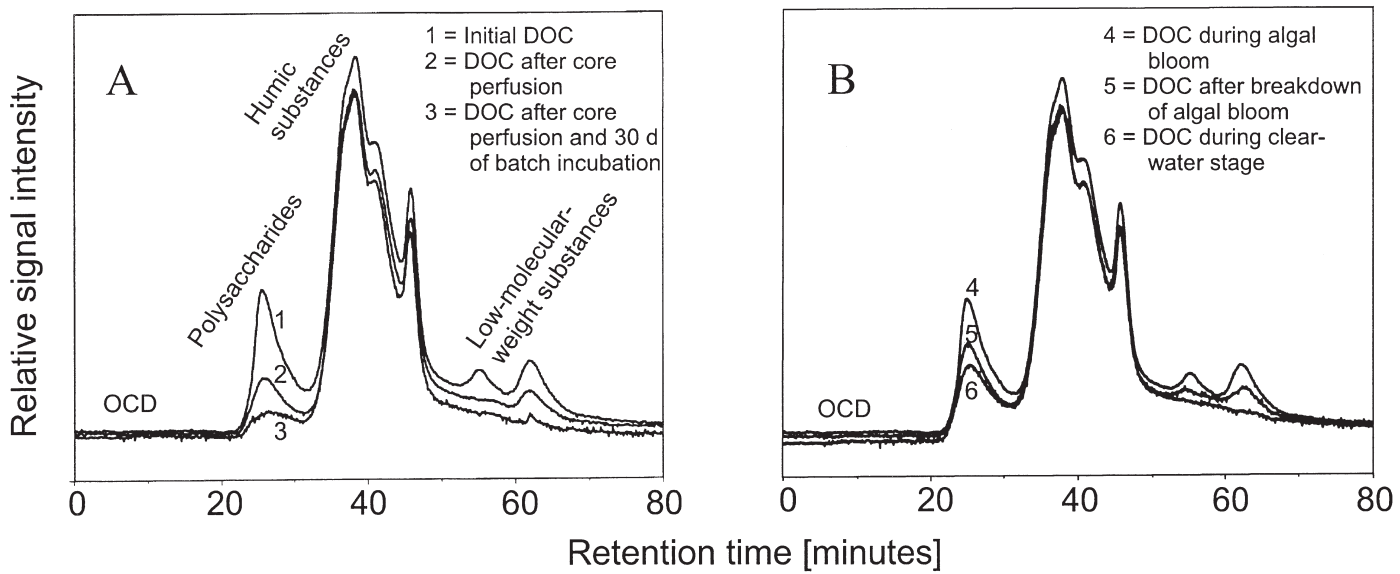

FIGURE 2 (A) Elution diagrams of the DOC composition in Spree water, May 12, 1998, before perfusion through river sediments (upper line, 1), after perfusion (central line, 2), and after core perfusion and subsequent 30 days of batch incubation (lower line, 3). (B) Elution diagrams of the DOC composition in Spree water during an algal bloom May 12, 1998 (upper line, 4), after breakdown of the algal bloom May 26, 1998 (central line, 5), and during clear-water stage June 17, 1998 (lower line, 6).

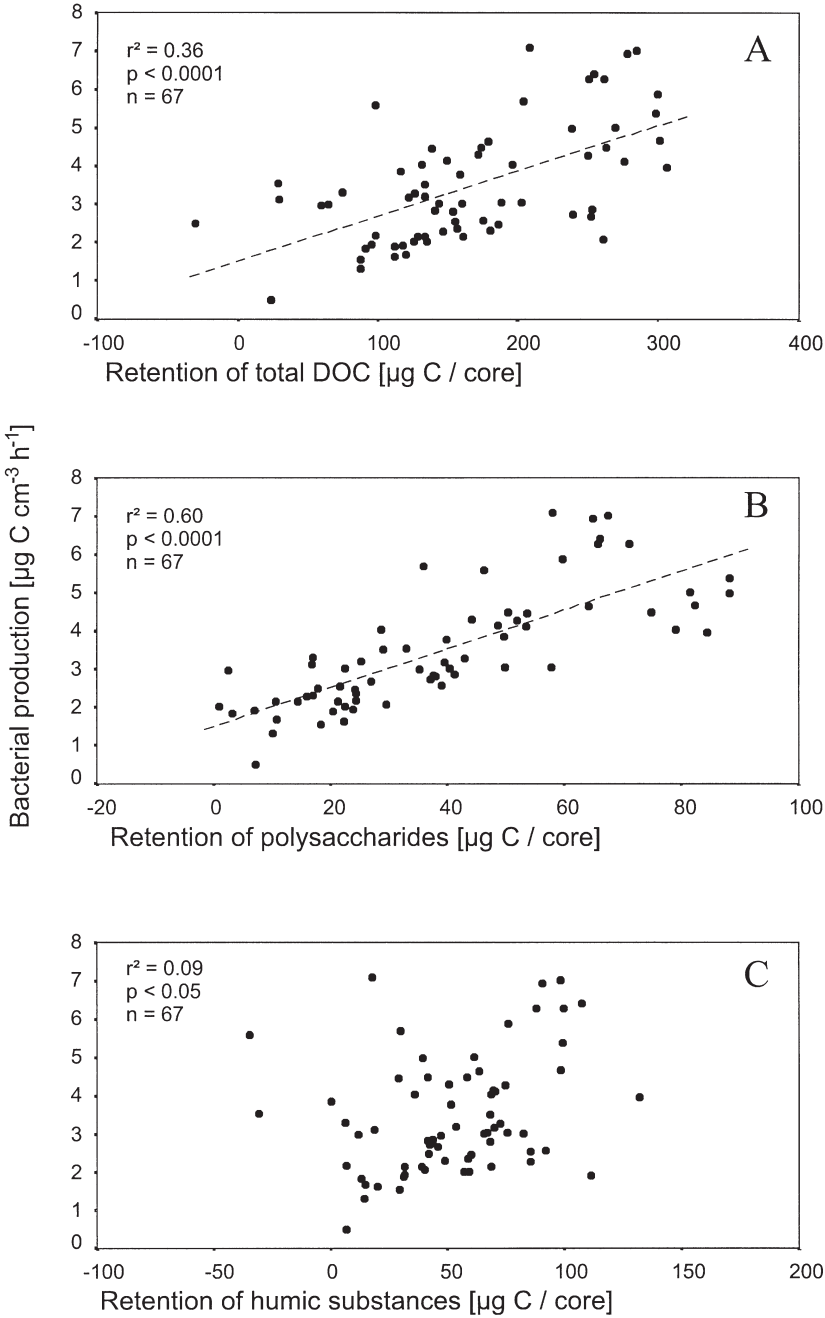

FIGURE 3 Relationships between DOC retention and bacterial production in sediments of the River Spree, Germany, February–November 1998 at temperatures ranging from 7 to 22°C (Fischer *et al*., 2002). (A) Total DOC, (B) polysaccharides, and (C) humic substances.

heterotrophic bacterial biofilms would be largely dependent upon external importation of DOC, the mixed community of auto- and heterotrophs decreases this dependency upon importation from the ambient environment.

VI. ECOSYSTEM CONSEQUENCES

The previous sections of this chapter dealt with biofilm structure and function in a microscopic scale; in Section V the view was extended toward a mesocosm scale. In these mesocosms built as flowthrough reactors, significant retention and/or transformation of DOC was found when inflow and outflow were compared (Ribas *et al*., 1995; Fiebig, 1997; Volk *et al*., 1997; Fischer *et al*., 2002.; Table II). The same condition applied to larger systems built as artificial streambeds (Gantzer *et al*., 1988, 1991). These studies imply that biofilms have the potential to play an important role in the retention and transformation of organic matter from the water column on an ecosystem scale. The relevant processes — adsorption to the biofilm and subsequent bacterial utilization — may occur in natural systems similarly as in laboratory incubations. Processes such as extracellular decomposition of organic macromolecules (e.g., Marxsen and Fiebig, 1993) and selective utilization of specific DOC fractions may thus enable biofilm bacteria to exert a considerable influence on quantity and quality of DOC in natural waters (e.g., Fiebig and Marxsen, 1992; Findlay *et al*., 1993; Findlay and Sobczak, 1996).

Therefore, it seems worthwhile to transfer the ideas gained by mesocosm experiments to larger systems as, for example, to stream sections or to whole rivers. In running waters, sediments are the major sites of bacterial metabolism (Pusch *et al*., 1998; Fischer and Pusch, 2001; see Chapter 4), especially in those rivers with a high proportion of discharge through the hyporheic zone (Findlay, 1995). Their large internal surface area promotes colonization of these sediments by bacterial biofilms (Lock, 1993; Fischer *et al*., 1996; Brunke and Fischer, 1999). These biofilms are supplied with nutrients and oxygen by flowing interstitial water, which originates either from the overlying water column being forced into the sediment interstices by physical processes or from groundwater exfiltration (Hendricks, 1993; Brunke and Gonser, 1997; Pusch *et al*., 1998).

When whole river systems are studied, it is difficult to separate the bacterial influence on longitudinal changes in DOC amount and composition from various other influences such as allochthonous inputs, algal exudation, or photochemical cleavage. However, attempts have been made to investigate the amount and composition of DOC and its availability to bacteria in ecosystem studies performed along the Ogeechee River continuum (Leff and Meyer, 1991; Sabater *et al*., 1993; Sun *et al*., 1997). At low flow situations, the relative availability of DOC to bacteria decreased along the continuum,

which was also reflected by a relative increase in the more refractory DOC fractions and by an increase in total DOC. At high discharges, channel processes were superimposed by inputs of allochthonous DOC from surrounding swamps and the longitudinal patterns of DOC were less clear (Sabater *et al.*, 1993).

Changes in the amount and composition of DOC from the River Spree after an algal bloom are shown in Figure 2B. There is a striking similarity between the sequence of elution diagrams obtained in the laboratory incubations before and after sediment core perfusion (Fig. 2A) and those obtained directly from the river water during and after the algal bloom (Fig. 2B). During the algal bloom, a considerable amount of labile DOC, especially polysaccharides, was released into the river water. In the middle of May, the chlorophyll - a content, as an indicator of algal biomass, decreased dramatically from 45 to 3 µg/L (data not shown). Concomitantly, the amount of labile substances decreased strongly in the river water. It is concluded that this change in riverine DOC composition was caused mainly by biofilm bacteria, analogous to the bacterial decomposition of DOC in the laboratory incubation. Therefore, in a number of ways biofilms exert a strong influence on the carbon biogeochemistry of aquatic ecosystems.

In streams and rivers the surface-bound bacterial activity greatly exceeds the activity of free-living bacteria. The share of the metabolism that can be attributed to biofilm-coated surfaces has usually been calculated for only small (first- and second-order) streams. In these streams, carbon turnover is highest in the hyporheic zone, and less than 1% of the community respiration occurred in the water column (Fuss and Smock, 1996; Naegeli and Uehlinger, 1997). However, biofilm-bound activity can also dominate the heterotrophic metabolism of larger rivers and lakes. It was shown that in the sixth-order section of the blackwater Ogeechee River benthic (biofilm) bacteria accounted for >90% of the system metabolism (Edwards *et al.*, 1990). In the Spree, a sixth-order lowland river with sandy sediments and a mean depth of 1 m, the upper 2 cm of sediment had an areal bacterial production 17- to 35-fold higher than that in the water column (Fischer and Pusch, 2001). Even if the deep sediment layers were not hydrologically connected with the overlying water, biofilm bacteria may exert a major influence on the biogeochemistry of the water column. This was found for shallow Danish lakes, in which the production of bacteria in the biofilm on aquatic plants (epiphyton) was measured. On an areal basis, bacterial production in this epiphytic biofilm was up to 7 times higher than the activity of free-living bacteria (Theil-Nielsen and Søndergaard, 1999). From an ecosystem perspective it seems that in many aquatic systems the water column is the medium that transports carbon and nutrients to the foci of heterotrophic metabolism. These foci are located in the biofilm of the sediments and the epiphyton and serve as important sinks of organic matter in these ecosystems.

VII. CONCLUSIONS

Biofilms enhance bacteria–DOM interactions by several means. Their spatial and chemical heterogeneity provides additional sorption sites for DOM compared with clean surfaces. Their loose architecture with interstitial voids and channels increases diffusivity and to some extent allows convective flow within biofilm structures. Because bacteria metabolize organic matter sorbed to the biofilm, a diffusion flux from the free water to the biofilm is maintained. Large proportions of organic matter sorbed to the biofilm are not instantly turned over but remain in the biofilm as a reservoir, which buffers direct effects of DOM depletion in the water column.

For bacteria, there may be some adverse effects of living in a biofilm, especially in regions close to the substratum; there, bacteria are to some extent cut off from the flux of DOM from the surrounding water. Additionally, bacterial enzymes can be inactivated if they are sorbed to surfaces. On the other hand, fixed positions of bacteria in biofilms support interactions between bacteria either within bacterial clones or between different bacterial species. They take advantage of growing in clones by saving energy spent for the production of extracellular enzymes, and they mutually interact between species in several ways.

Because of the high area of solid surfaces covered with biofilms, these biofilms dominate the heterotrophic metabolism in many aquatic ecosystems. In streams, rivers, and shallow lakes, bacterial activity in epilithic and epiphytic biofilms may be several times higher on an areal basis than the activity of free living bacteria. By the differential use of specific DOM fractions, biofilm bacteria influence the biogeochemical composition of DOM in these ecosystems. Biofilms thus can control biogeochemical fluxes of DOM and are important sinks of organic matter.

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REFERENCES

Alldredge, A. L. 2000. Interstitial dissolved organic carbon (DOC) concentrations within sinking marine aggregates and their potential contribution to carbon flux. *Limnology and Oceanography* **45:**1245–1253.

Allison, D. G., and I. W. Sutherland. 1987. The role of exopolysaccharides in adhesion of freshwater bacteria. *Journal of General Microbiology* **133:**1319–1327.

Armstrong, S., and F. Bärlocher. 1989. Adsorption and release of amino acids from epilithic biofilms in streams. *Freshwater Biology* **22:**153–159.

Arnosti, C. 2000. Substrate specifity in polysaccharide hydrolysis: Contrasts between bottom water and sediments. *Limnology and Oceanography* **45:**1112–1119.

Baker, M. A., C. N. Dahm, and H. M. Valett. 1999. Acetate retention and metabolism in the hyporheic zone of a mountain stream. *Limnology and Oceanography* **44:**1530–1539.

Battin, T. J., A. Butturini, and F. Sabater. 1999. Immobilization and metabolism of dissolved organic carbon by natural sediment biofilms in a Mediterranean and temperate stream. *Aquatic Microbial Ecology* **19:**297–305.

Bayer, E. A., E. Morag, Y. Shoham, J. Tormo, and R. Lamed. 1996. The cellulosome: A cell surface organelle for the adhesion to and degradation of cellulose. *In* "Bacterial Adhesion: Molecular and Ecological Diversity" (M. Fletcher, Ed.), Wiley Series in Ecological and Applied Microbiology; pp. 155–182. Wiley-Liss, New York.

Bencala, K. E., V. C. Kennedy, G. W. Zellweger, A. P. Jackman, and R. J. Avanzino. 1984. Interactions of solutes and streambed sediment. 1. An experimental analysis of cation and anion transport in a mountain stream. *Water Resources Research* **20:**1797–1803.

Berg, H. C. 1983. "Random Walks in Biology." Princeton Univ. Press, Princeton, NJ.

Beyenal, H., and Z. Lewandowski. 2000. Combined effect of substrate concentration and flow velocity on effective diffusivity in biofilms. *Water Research* **34:**528–538.

Blenkinsopp, S. A., and J. W. Costerton. 1991. Understanding bacterial biofilms. *Trends in Biotechnology* **9:**138–143.

Bouwer, E. J. 1987. Theoretical investigation of particle deposition in biofilm systems. *Water Research* **21:**1489–1498.

Brunke, M., and H. Fischer. 1999. Hyporheic bacteria — Relationships to environmental gradients and invertebrates in a prealpine stream. *Archiv für Hydrobiologie* **146:**189–217.

Brunke, M., and T. Gonser. 1997. The ecological significance of exchange processes between rivers and groundwater. *Freshwater Biology* **37:**1–33.

Bryers, J. D., and W. G. Characklis. 1990 Biofilms in water and wastewater treatment. *In* "Biofilms" (W. G. Characklis and K. C. Marshall, Eds.), Wiley Series in Ecological and Applied Microbiology, pp. 671–696. Wiley, New York.

Burkholder, J. M., R. G. Wetzel, and K. L. Klomparens. 1990. Direct comparison of phosphate uptake by adnate and loosely attached microalgae within an intact biofilm matrix. *Applied and Environmental Microbiology* **56:**2882–2890.

Caldwell, D. E. 1995. Cultivation and study of biofilm communities. *In* "Microbial Biofilms" (H. Lappin-Scott and J. W. Costerton, Eds.), Plant and Microbial Biotechnology Research Series 5, pp. 64–79. Cambridge Univ. Press, Cambridge, UK.

Cazelles, B., D. Fontvieille, and N. P. Chau. 1991. Self-purification in a lotic ecosystem: A model of dissolved organic carbon and benthic microorganisms dynamics. *Ecological Modelling* **58:**91–117.

Chappell, K. R., and R. Goulder. 1994. Seasonal variation of epilithic extracellular enzyme activity in three diverse headstreams. *Archiv für Hydrobiologie* **130:**195–214.

Characklis, W. G. 1990. Biofilm processes. *In* "Biofilms" (W. G. Characklis and K. C. Marshall, Eds.), Wiley Series in Ecological and Applied Microbiology, pp. 195–231. Wiley, New York.

Christensen, B. E. 1989. The role of extracellular polysaccharides in biofilms. *Journal of Biotechnology* **10:**181–202.

Costerton, J. W., and H. M. Lappin-Scott. 1995 Introduction to microbial biofilms. *In* "Microbial Biofilms" (H. Lappin-Scott and J. W. Costerton, Eds.), Plant and Microbial Biotechnology Research Series 5, pp. 1–11. Cambridge Univ. Press, Cambridge, UK.

Costerton, J. W., Z. Lewandowski, D. E Caldwell, D. R. Korber, and H. M. Lappin-Scott. 1995. Microbial biofilms. *Annual Review of Microbiology* **49:**711–745.

Costerton, J. W., P. S. Stewart, and E. P. Greenberg. 1999. Bacterial biofilms: A common cause of persistent infections. *Science* **284:**1318–1322.

Dahm, C. N. 1981. Pathways and mechanisms for removal of dissolved organic carbon from leaf leachate in streams. *Canadian Journal of Fisheries and Aquatic Sciences* **38:**68–76.

Davies, D. G., A. M. Chakrabarty, and G. G. Geesey. 1993. Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology* **59:**1181–1186.

Davies, D. G., M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* **280:**295–298.

deBeer, D., P. Stoodley and Z. Lewandowski. 1994. Liquid flow in heterogeneous biofilms. *Biotechnology and Bioengineering* **44:**636–641.

deBeer, D., P. Stoodley, and Z. Lewandowski. 1996. Liquid flow and mass transport in heterogeneous biofilms. *Water Research* **30:**2762–2765.

Deretic, V., M. J. Schurr, J. C. Boucher, and D. W. Martin 1994. Conversion of *Pseudomonas aeruginosa* to mucoidy in cystic fibrosis: Environmental stress and regulation of bacterial virulence by alternative sigma factors. *Journal of Bacteriology* **176:**2773–2780.

Domenico, P. A., and F. W. Schwartz. 1998 "Physical and Chemical Hydrogeology," 2nd ed. Wiley, New York.

Dunny, G. M., and B. A. B. Leonard. 1997. Cell-cell communication in gram-positive bacteria. *Annual Review of Microbiology* **51:**537–564.

Edwards, R. T., J. L. Meyer, and S. E. G. Findlay. 1990. The relative contribution of benthic and suspended bacteria to system biomass, production, and metabolism in a low-gradient blackwater river. *Journal of the North American Benthological Society* **6:**241–250.

Fiebig, D. M. 1992. Fates of dissolved free amino acids in groundwater discharged through streambed sediments. *Hydrobiologia* **235/236:**311–319.

Fiebig, D. M. 1997. Microbiological turnover of amino acids immobilized from groundwater discharged through hyporheic sediments. *Limnology and Oceanography* **42:**763–768.

Fiebig, D. M., and J. Marxsen. 1992. Immobilization and mineralization of dissolved free amino acids by streambed biofilms. *Freshwater Biology* **28:**129–140.

Findlay, S. 1995. Importance of surface-subsurface exchange in stream ecosystems: The hyporheic zone. *Limnology and Oceanography* **40:**159–164.

Findlay, S., and W. V. Sobczak. 1996. Variability in removal of dissolved organic carbon in hyporheic sediments. *Journal of the North American Benthological Society* **15:**35–41.

Findlay, S., D. Strayer, C. Goumbala, and K. Gould. 1993. Metabolism of streamwater dissolved organic carbon in the shallow hyporheic zone. *Limnology and Oceanography* **38:**1493–1499.

Fischer, H., and M. Pusch. 1999. Use of the [^{14}C]leucine incorporation technique to measure bacterial production in river sediments and epiphyton. *Applied and Environmental Microbiology* **65:**4411–4418.

Fischer, H., and M. Pusch. 2001. Comparison of bacterial production in sediments, epiphyton, and the pelagic zone of a lowland river. *Freshwater Biology* **46:**1335–1348.

Fischer, H., M. Pusch, and J. Schwoerbel. 1996. Spatial distribution and respiration of bacteria in streambed sediments. *Archiv für Hydrobiologie* **137:**281–300.

Fischer, H., A. Sachse, C. E. W. Steinberg, and M. Pusch. 2002. Differential retention of dissolved organic carbon by bacteria in river sediments. *Limnology and Oceanography*, in press.

Flemming, H.-C. 1987. Microbial growth on ion exchangers. *Water Research* **21:**745–756.

Flemming, H.-C., and J. Wingender. 2001a. Relevance of microbial extracellular polymeric substances (EPS) — Part II: Technical aspects. *Water Science and Technology* **43:**9–16.

Flemming, H.-C., and J. Wingender. 2001b. Relevance of microbial extracellular polymeric substances (EPS) — Part I: Structural and ecological aspects. *Water Science and Technology* **43:**1–8.

Fletcher, M. 1986. Measurement of glucose utilization by *Pseudomonas fluorescens* that are free-living and that are attached to surfaces. *Applied and Environmental Microbiology* **52:**672–676.

Fletcher, M. 1991. The physiological activity of bacteria attached to solid surfaces. *Advances in Microbial Physiology* **32:**53–85.

Freeman, C., and M. A. Lock. 1995. The biofilm polysaccharide matrix-a buffer against changing organic substrate supply? *Limnology and Oceanography* **40:**273–278.

Freeman, C., P. J. Chapman, K. Gilman, M. A. Lock, B. Reynolds, and H. S. Wheater. 1995. Ion exchange mechanisms and the entrapment of nutrients by river biofilms. *Hydrobiologia* **297:**61–65.

Fuqua, W. C., S. C. Winans, and E. P. Greenberg. 1994. Quorum sensing in bacteria: The LuxR–LuxI family of cell density-responsive transcriptional regulators. *Journal of Bacteriology* **176:**269–275.

Fuss, C. L., and L. A. Smock. 1996. Spatial and temporal variation of microbial respiration rates in a blackwater stream. *Freshwater Biology* **36:**339–349.

Gantzer, C. J., B. E. Rittmann, and E. E. Herricks. 1988. Mass transport to streambed biofilms. *Water Research* **22:**709–722.

Gantzer, C. J., B. E. Rittmann, and E. E. Herricks. 1991. Effect of long-term water velocity changes on streambed biofilm activity. *Water Research* **25:**15–20.

Geesey, G. G., W. T. Richardson, H. G. Yeomans, R. T. Irvin, and J. W. Costerton. 1977. Microscopic examination of natural sessile bacterial populations from an alpine stream. *Canadian Journal of Microbiology* **23:**1733–1736.

Geesey, G. G., R. Mutch, J. W. Costerton, and R. B. Green. 1978. Sessile bacteria: An important component of the microbial population in small mountain streams. *Limnology and Oceanography* **23:**1214–1223.

Gieseke, A., U. Purkhold, M. Wagner, R. Amann, and A. Schramm. 2001. Community structure and activity dynamics of nitrifying bacteria in a phosphate-removing biofilm. *Applied and Environmental Microbiology* **67:**1351–1362.

Gottschalk, G. 1986. "Bacterial Metabolism." Springer Verlag, New York.

Grischek, T., K. M. Hiscock, T. Metschies, P. F. Dennis, and W. Nestler. 1998. Factors affecting denitrification during infiltration of river water into a sand and gravel aquifer in Saxony, Germany. *Water Research* **32:**450–460.

Grossart, H.-P., and M. Simon. 1998. Bacterial colonization and microbial decomposition of limnetic organic aggregates (lake snow). *Aquatic Microbial Ecology* **15:**127–140.

Haack, T. K., and G. A. McFeters. 1982. Microbial dynamics of an epilithic mat community in a high alpine stream. *Applied and Environmental Microbiology* **43:**702–707.

Hall, R. O. Jr., and J. L. Meyer. 1998. The trophic significance of bacteria in a detritus-based stream food web. *Ecology* **79:**1995–2012.

Hendel, B., and J. Marxsen. 2000. Extracellular enzyme activity associated with degradation of beech wood in a central European stream. *International Review of Hydrobiology* **85:**95–105.

Hendricks, S. P. 1993. Microbial ecology of the hyporheic zone: A perspective integrating hydrology and biology. *Journal of the North American Bentholgical Society* **12:**70–78.

Henrichs, S. M., and S. F. Sugai. 1993. Adsorption of amino acid and glucose by sediments of Resurrection Bay, Alaska, USA: Functional group effects. *Geochimica et Cosmochimica Acta* **57:**823–835.

Henrici, A. T. 1933. Studies of freshwater bacteria. 1. A direct microscopic technique. *Journal of Bacteriology* **25:**277–286.

Heukelekian, H., and A. Heller. 1940. Relation between food concentration and surface for bacterial growth. *Journal of Bacteriology* **40:**547–558.

Hoyle, B. D., L. J. Williams, and J. W. Costerton. 1993. Production of mucoid exopolysaccharide during development of *Pseudomonas aeruginosa* biofilms. *Infection and Immunity* **61:**777–780.

Kaplan, L. A., and T. L. Bott. 1989. Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: Effects of temperature, water chemistry, and habitat. *Limnology and Oceanography* **34:**718–733.

Karickhoff, S. W. 1984. Organic pollutant sorption in aquatic systems. *Journal of Hydraulic Engineering* **110:**707–735.

Kjelleberg, S, B. A. Humphrey, and K. C. Marshall. 1982. Effect of interfaces on small, starved marine bacteria. *Applied and Environmental Microbiology* **43:**1166–1172.

Koch, A. L. 1991. Diffusion. The crucial process in many aspects of the biology of bacteria. *Advances in Microbial Ecology* **11:**37–70.

Leff, L. G., and J. L. Meyer. 1991. Biological availability of dissolved organic carbon along the Ogeechee River. *Limnology and Oceanography* **36:**315–323.

Lewandowski, Z. 1998. Structure and function of bacterial biofilms. *Corrosion 98*, **296:**1–15.

Lewandowski, Z., P Stoodley, and S. Altobelli. 1995. Experimental and conceptual studies on mass transport in biofilms. *Water Science and Technology* **31:**153–162.

Lock, M. A. 1993. Attached microbial communities in rivers. *In* "Aquatic Microbiology: An Ecological Approach" (T. E. Ford, Ed.), pp. 113–138. Blackwell, Cambridge, Ma.

Lock, M. A., R. R. Wallace, J. W. Costerton, R. M. Ventullo, and S. E. Charlton. 1984. River epilithon: Toward a structural-functional model. *OIKOS* **42:**10–22.

Marsh, P. D. 1995. Dental plaque. *In* "Microbial Biofilms" (H. Lappin-Scott and J. W. Costerton, Eds.), Plant and Microbial Biotechnology Research Series 5, pp. 282–300. Cambridge Univ. Press, Cambridge, UK.

Marshall, K. C. 1996. Adhesion as a strategy for access to nutrients. *In* "Bacterial Adhesion: Molecular and Ecological Diversity" (M. Fletcher, Ed.), Wiley Series in Ecological and Applied Microbiology, pp. 59–87. Wiley-Liss, New York.

Marxsen, J., and D. M. Fiebig. 1993. Use of perfused cores for evaluating extracellular enzyme activity in streambed sediments. *FEMS Microbiology Ecology* **13:**1–12.

Mc Dowell, W. H. 1985. Kinetics and mechanism of dissolved organic carbon retention in a headwater stream. *Biogeochemistry* **1:**329–352.

Mildenberger, M. 1999. Die Grenzschicht beeinflusst die Abbaurate immobiler Mikroorganismen. *gwf Wasser/Abwasser* **140:**286–291.

Mittelman, M. W. 1999. Recovery and characterization of biofilm bacteria associated with medical devices. *In* "Biofilms" (R. J. Doyle, Ed.), Methods in Enzymology, Vol. 310, pp. 534–551. Academic Press, San Diego.

Müller-Niklas, G., S. Schuster, E. Kaltenböck, and G. J. Herndl. 1994. Organic content and bacterial metabolism in amorphous aggregations of the northern Adriatic Sea. *Limnology and Oceanography* **39:**58–68.

Naegeli, M. W., and U. Uehlinger. 1997. Contribution of the hyporheic zone to ecosystem metabolism in a prealpine gravel-bed river. *Journal of the North American Benthological Society* **16:**794–804.

Neu, T. R. 1996. Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. *Microbiological Reviews* **60:**151–166.

Neu, T. R. 2000. In situ cell and glycoconjugate distribution in river snow studied by confocal laser scanning microscopy. *Aquatic Microbial Ecology* **21:**85–95.

Neu, T. R., and J. R. Lawrence. 1997. Development and structure of microbial biofilms in river water studied by confocal laser scanning microscopy. *FEMS Microbiology Ecology* **24:**11–25.

Neu, T. R., and J. R. Lawrence.1999. Lectin-binding analysis in biofilm systems. *In* "Biofilms" (R. J. Doyle, Ed.), Methods in Enzymology, Vol. 310, pp. 145–152. Academic Press, San Diego.

Nikaido, H., and M. Vaara. 1985. Molecular basis of bacterial outer membrane permeability. *Microbiological Reviews* **49:**1–32.

Norde, W. 1986. Adsorption of proteins from solution at the solid–liquid interface. *Advances in Colloid and Interface Science* **25:**267–340.

Okabe, S., K. Hiratia, Y. Ozawa, and Y. Watanabe. 1996. Spatial microbial distribution of nitrifiers and heterotrophs in mixed-population biofilms. *Biotechnology and Bioengineering* **50:**24–35.

O'Toole, G. A., and R. Kolter. 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Molecular Microbiology* **30:**295–304.

Paul, B. J., and H. C. Duthie. 1989. Nutrient cycling in the epilithon of running waters. *Canadian Journal of Botany* **67:**2302–2309.

Ploug, H., and H.-P. Grossart. 1999. Bacterial production and respiration in pelagic aggregates — A matter of the incubation method. *Aquatic Microbial Ecology* **20:**21–29.

Podoll, R. T., and W. R. Mabey. 1987. Factors to consider in conducting laboratory sorption/desorption tests. *In* "Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems" (K. L. Dickson, A. W. Maki, and W. A. Brungs, Eds.), SETAC Special Publications Series, pp. 99–108. Pergamon Press, New York.

Pratt, L. A., and R. Kolter. 1998. Genetic analysis of *Escherichia coli* biofilm formation: Roles of flagella, motility, chemotaxis and type I pili. *Molecular Microbiology* **30:**285–293.

Pusch, M., D. Fiebig, I. Brettar, H. Eisenmann, B. K. Ellis, L. A. Kaplan, M. A. Lock, M. W. Naegeli, and W. Traunspurger. 1998. The role of microorganisms in the ecological connectivity of running waters. *Freshwater Biology* **40:**453–495.

Quiquampoix, H. 2000. Mechanisms of protein adsorption on surfaces and consequences for extracellular enzyme activity in soil. *In* "Soil Biochemistry," Vol. 10 (J.-M. Bollag and G. Stotzky, Eds.), pp. 282–300. Marcel Dekker, New York.

Reichenbach, H. 1992. The order cytophagales. *In* "The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications," 2nd ed. (A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer, Eds.), pp. 3631–3675, Springer Verlag, New York.

Revsbech, N. P. 1989. Diffusion characteristics of microbial communities determined by use of oxygen microsensors. *Journal of Microbiological Methods* **9:**111–122.

Ribas, F., J. Frias, F. Ventura, L. Mohedano, and F. Lucena. 1995. Assessment of biological activity and fate of organic compounds in a reactor for the measurement of biodegradable organic carbon in water. *Journal of Applied Bacteriology* **79:**558–568.

Rijnaarts, H. H. M., W. Norde, J. Lyklema, and A. J. B. Zehnder. 1999. DLVO and steric contributions to bacterial deposition in media of different ionic strengths. *Colloids and Surfaces B — Biointerfaces* **14:**179–199.

Romani, A. M., and S. Sabater. 1999. Effect of primary producers on the heterotrophic metabolism of a stream biofilm. *Freshwater Biology* **41:**729–736.

Sabater, F., J. L. Meyer, and R. T. Edwards. 1993. Longitudinal patterns of dissolved organic carbon concentration and suspended bacterial density along a blackwater river. *Biogeochemistry* **21:**73–93.

Shapiro, J. A. 1998. Thinking about bacterial populations as multicellular organisms. *Annual Review of Microbiology* **52:**81–104.

Siegrist, H., and W. Gujer. 1985. Mass transfer mechanisms in a heterotrophic biofilm. *Water Research* **19:**1369–1378.

Siegrist, H., and W. Gujer. 1987. Demonstration of mass transfer and pH effects in a nitrifying biofilm. *Water Research* **21:**1481–1487.

Sinsabaugh, R. L., S. W. Golladay, and A. E. Linkins. 1991. Comparison of epilithic and epixylic biofilm development in a boreal river. *Freshwater Biology* **25:**179–187.

Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359:**139–142.

Sobczak, W. V. 1996. Epilithic bacterial responses to variations in algal biomass and labile dissolved organic carbon during biofilm colonization. *Journal of the North American Benthological Society* **15:**143–154.

Söhngen, N. L. 1913. Einfluß von Kolloiden auf mikrobiologische Prozesse. *Centralblatt für Bakteriologie Abteilung II* **38:**621–647.

Stoodley, P., J. D. Boyle, D. deBeer, H. M. Lappin-Scott. 1999a. Evolving perspectives of biofilm structure. *Biofouling* **14:**75–90.

Stoodley, P., I. Dodds, J. D. Boyle, and H. M. Lappin-Scott. 1999b. Influence of hydrodynamics and nutrients on biofilm structure. *Journal of Applied Microbiology Symposium Supplement* **85:**19S–28S.

Stoodley, P., Z. Lewandowski, J. D. Boyle, and H. M. Lappin-Scott. 1999c. Structural deformation of bacterial biofilms caused by short–term fluctuations in fluid shear: An in situ investigation of biofilm rheology. *Biotechnology and Bioengineering* **65:**83–92.

Stumm, W. 1996 Surface chemical theory and predicting the distribution of contaminants in the aquatic environment. Chester C. Kisiel Eleventh Memorial Lecture, University of Arizona, April 5, 1992.

Sun, L., E. M. Perdue, J. L. Meyer, and J. Weis. 1997. Use of the elemental composition to predict bioavailability of organic matter in a Georgia river. *Limnology and Oceanography* **42:**714–727.

Sutherland, I. W. 1999. Polysaccharases in biofilms — sources — action — consequences! *In* "Microbial Extracellular Polymeric Substances: Characterization, Structure, and Function" (J. Wingender, T. R. Neu, and H.-C. Flemming, Eds.), pp. 201–216. Springer-Verlag, Berlin.

Theil-Nielsen, J., and M. Søndergaard. 1999. Production of epiphytic bacteria and bacterioplankton in three shallow lakes. *OIKOS* **86:**283–292.

Thompson, A. J., and R. L. Sinsabaugh. 2000. Matric and particulate phosphatase and aminopeptidase activity in limnetic biofilms. *Aquatic Microbial Ecology* **21:**151–159.

Tipping, E., and D. Cooke. 1982. The effects of adsorbed humic substances on the surface charge of goethite (alpha-FeOOH) in freshwaters. *Geochimica et Cosmochimica Acta* **46:**75–80.

Tuominen, L., T. Kairesalo, and H. Hartikainen. 1994. Comparison of methods for inhibiting bacterial activity in sediment. *Applied and Environmental Microbiology* **60:**3454–3457.

van Loosdrecht, M. C. M., J. Lyklema, W. Norde, and A. J. B. Zehnder. 1989. Bacterial adhesion: A physicochemical approach. *Microbial Ecology* **17:**1–15.

van Loosdrecht, M. C. M., D. Eikelboom, A. Gjaltema, A. Mulder, L. Tijhuis, and J. J. Heijnen. 1995. Biofilm structures. *Water Science and Technology* **32:**35–43.

Vetter, Y. A., J. W. Deming, P. A. Jumars, and B. B. Krieger–Brockett. 1998. A predictive model of bacterial foraging by means of freely released extracellular enzymes. *Microbial Ecology* **36:**75-92.

Vicuña, R. 1988. Bacterial degradation of lignin. *Enzyme and Microbial Technology* **10:**646–655.

Volk, C. J., C. B. Volk, and L. A. Kaplan. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. *Limnology and Oceanography* **42:**39–44.

Wetzel, R. G. 1993. Microcommunities and microgradients: Linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. *Netherland Journal of Aquatic Ecology* **27:**3–9.

Whiteley, M., J. R. Ott, E. A. Weaver, and R. J. C. McLean. 2001. Effects of community composition and growth rate on aquifer biofilm bacteria and their susceptibility to betadine disinfection. *Environmental Microbiology* **3:**43–52.

Wimpenny, J. W. T., and R. Colasanti. 1997. A unifying hypothesis for the structure of microbial biofilms based on cellular automaton models. *FEMS Microbiology Ecology* **22:**1–16.

Wingender, J., T. R. Neu, and H.-C. Flemming. 1999. What are bacterial extracellular polymeric substances? *In* "Microbial Extracellular Polymeric Substances: Characterization, Structure, and Function" (J. Wingender, T. R. Neu, and H.–C. Flemming, Eds.), pp. 1–19. Springer-Verlag, Berlin.

Wolfaardt, G. M., J. R. Lawrence, R. D. Robarts, and D. E. Caldwell. 1998. In situ characterization of biofilm exopolymers involved in the accumulation of chlorinated organics. *Microbial Ecology* **35:**213–223.

Wuhrmann, K. 1974. Some problems and perspectives in applied limnology. *Mitteilungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* **20:**324–402.

ZoBell, C. E. 1943. The effect of solid surfaces upon bacterial activity. *Journal of Bacteriology* **46:**39–56.

ZoBell, C. E., and D. Q. Anderson. 1936. Observations on the multiplication of bacteria in different volumes of stored seawater and the influence of oxygen tension and solid surfaces. *Biological Bulletin* **71:**324–342.

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13

Microbial Extracellular Enzymes and Their Role in Dissolved Organic Matter Cycling

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Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

I. THE ROLE OF EXTRACELLULAR ENZYMES IN CARBON REMINERALIZATION

Heterotrophic bacteria are faced with the fundamental challenge of obtaining sufficient carbon and energy from the organic substrates in their environment. Low-molecular-weight substrates can be transported directly from the surrounding medium into the cell, but these substrates may be present only in low concentrations, and competition for such resources may be high (see Chapter 4 and 9). Higher molecular weight soluble and particulate substrates may be more readily available because most organic matter is initially biosynthesized as macromolecules such as proteins, polysaccharides, and lipid complexes (see Chapters 1, 3, and 5). These substrates, however, must be hydrolyzed outside the cell to sizes small enough to permit transport across the outer membrane. Extracellular enzymes are therefore required to initiate the remineralization of high-molecular-weight organic matter. These enzymes represent the "toolboxes," which enable heterotrophic bacteria to obtain suitable substrates from a diverse array of compounds. Because extracellular enzymes are required in the initial stages of carbon remineralization, any factors affecting their activity or impeding production or use of extracellular enzymes potentially form a bottleneck whose effects will be amplified along the entire remineralization pathway.

Overall, the remineralization process in aquatic systems must be extremely efficient, since only ca. 0.1% of oceanic primary productivity ultimately is buried in marine sediments (Hedges, 1992), and little of the terrestrial DOM brought in by rivers persists in marine waters (see Chapter 5). Radiocarbon measurements have demonstrated, however, that the average age of dissolved organic carbon (DOC) in the water column is on the order of 6000 years, several times greater than the mixing time of the oceans (Williams and Druffel, 1987). Specific factors therefore must impede microbial use of at least a fraction of this DOC. The molecular structure and macro structure and composition of DOC, and the identities and activities of the organisms that initiate carbon turnover, as well as the specific array of enzymatic tools at their disposal, are for the most part unknown; these unknown factors are central limitations in our understanding of carbon cycling.

II. BIOCHEMICAL ASPECTS OF ENZYME PRODUCTION AND ACTIVITY

The outer membrane of Gram-negative bacteria is spanned by porins, trimeric proteins that form inlet channels between the outer membrane and the periplasmic space. The geometry of the porins defines the substrate uptake limit, which is on the order of 600 daltons (Weiss *et al.*, 1991), approximately equivalent to a trisaccharide. Substrates larger than this limit (with few exceptions) must be hydrolyzed outside the outer membrane prior to uptake. Extracellular hydrolysis is carried out by means of extracellular en-

zymes, which are either attached to the outer membrane or released ("free") into the surrounding solution. After transport into the periplasmic space, a substrate may be further hydrolyzed by enzymes located in the periplasmic space and transported across the cell wall. Some methods commonly used to measure extracellular enzymatic activity do not differentiate between the activities of the enzymes in the periplasmic space and those attached to the outer membrane (see Section III), complicating the interpretation of experimental data.

Production and activities of enzymes in general are tightly controlled by a microbial cell, because enzyme production represents an investment of carbon, nitrogen, and energy. Some enzymes are expressed constitutively, but many more are induced only under specific circumstances. Mechanisms by which enzyme expression is controlled have been studied in detail in a number of "model bacteria," including *Escherichia coli*. The intracellular enzymes required for lactose metabolism, for example, are under negative control: transcription is blocked by a specific repressor and can only be induced in the presence of substrate. Metabolism of other carbohydrates in *E. coli* are under positive control, in which an activator plus inducer leads to transcription of specific enzymes (Gottschalk, 1986). Enzyme production can be further regulated by catabolite repression, in which the presence of a substrate (usually an easily metabolized carbon source) may prevent production of enzymes required for metabolism of a more complex substrate. This mechanism of control may affect expression of extracellular enzymes in particular (Priest, 1992). Enzymes involved in anabolic pathways may be controlled by end-product repression, which occurs when a product of a pathway combines with a repressor protein to yield an active repressor, preventing further production of the enzymes that yield the end product. The activity of enzymes, especially those involved in anabolic pathways, may additionally be affected directly by feedback inhibition: end products accumulating in a cell will directly interact with the enzymes involved in their production, impeding further formation of the specific end product (Gottschalk, 1986). The specific mechanisms by which enzyme activities are controlled therefore vary and differ among microorganisms.

In general, hydrolytic enzymes can be classified based on the type of reaction catalyzed, the nature of the enzyme active site, and/or evolutionary relationships among enzymes, as derived from primary sequence data. Among proteases, gross functional distinctions are made between serine proteases, aspartic proteases, cysteine proteases, and metalloproteases. Each of these groups includes a diverse range of enzymes of distinctive size and structure: for example, an aminopeptidase isolated from *B. lichenformis* was found to have a molecular mass of 34,000, whereas an *E. coli* aminopeptidase had a mass of 400,000 (Rao *et al*., 1998).

Glycosyl hydrolases include a similarly wide range of structures. Traditional classification based on substrate specificity can be ambiguous, as some enzymes can hydrolyze a number of distinct substrates, albeit at

rates much lower than those of their "primary" substrates. Classifications of these enzymes are also based on a distinction between cleavage sites, whether from the end of a polysaccharide (exo-acting enzyme) or within the polysaccharide chain (endo-acting enzyme), and on the mode of hydrolysis with reference to the new anomeric carbon, which can occur via an inverting or retaining mechanism (Henrissat and Davies, 1997). Classifications based on amino acid sequence similarities have yielded approximately 77 distinct glycoside hydrolase families (Araki *et al*., 2000).

The structure and function of microbial hydrolytic enzymes, as well as factors controlling enzyme expression at the cellular and subcellular level, have been focal points for numerous studies (e.g., reviews by Warren, 1996; Henrissat and Davies, 1997; White and Rose, 1997; Rao *et al*., 1998). Some studies have focused especially on structure and function of specific enzymes produced by marine bacteria (Feller *et al*., 1992, 1994a, b, 1996, 1998; Svitil *et al*. 1997; Techkarnjanaruk *et al*., 1997; Baty *et al*., 2000a, b; Araki *et al*., 2000). A significant amount of work has been devoted specifically to enzymes of thermophilic and hyperthermophilic marine prokaryotes (e.g., Adams, 1993). Studies of thermophilic and hyperthermophilic enzymes are driven in part by their potential biotechnological applications (Pfu (Stratagene), derived from *Pyrococcus furiosus*, and *Vent* (New England Biolabs), from *Thermococcul litoralis*, both widely used for PCR (polymerase chain reaction), are perhaps the best known examples). In addition, studies of thermophilic or hyperthermophilic enzymes and their mesophilic (and, in a few cases, psychrophilic) counterparts provide insight into factors that control protein stability under extreme conditions (e.g., Feller *et al*., 1994b; Danson and Hough, 1998; Zeikus *et al*., 1998). Regulation and mechanisms of carbohydrate hydrolysis and transport are also a focus of work with hyperthermophiles; as with enzyme hydrolysis, uptake and transport of a substrate via specific systems is highly structure-selective (Xavier *et al*., 1996; Galperin *et al*., 1997). With the exception of thermophilic and hyperthermophilic prokaryotes, however, relatively little is known about the structures and mechanisms of extracellular enzymes produced specifically by marine and freshwater microbes, or about factors controlling the expression and activity of these enzymes at the subcellular level.

This lack of knowledge is especially problematic because a variety of different enzymes are usually required to hydrolyze a complex, high-molecular-weight substrate. The hydrolytic enzymes expressed by prokaryotes are highly sensitive to substrate structural features. Enzymes typically include distinct domains for substrate binding and for catalysis (Warren, 1996); substrates that do not conform to these structural requirements (i.e., tertiary structure, linkage patterns or orientation, order of monomer units, etc.) will not be hydrolyzed by a given enzyme. Several different types of enzymes therefore must act in concert to dismantle complex and/or branched structures, and measuring the activity of just one of these enzymes yields an incomplete picture of organic matter degradation. Limited knowledge of the structure,

function, variety, and expression of aquatic extracellular enzymes is further compounded by a lack of information on the activities, lifetime, and specificity of these enzymes once expressed.

III. MEASURING ENZYME ACTIVITIES IN AQUATIC SYSTEMS

Several general approaches have been used to measure the activities of extracellular enzymes in aquatic systems. These methods typically measure a "potential" activity, inasmuch as a substrate added to a sample to measure enzyme activity is in competition with naturally occurring substrates (whose concentration is usually unknown) for enzyme active sites. The most commonly applied method involves a small substrate proxy, typically consisting of a monosaccharide or an amino acid covalently linked to a small fluorophore; substrates frequently used include methyumbellifery- (MUF-) monosaccharides and 4-methyl-coumainylamide (MCA)- amino acids. Upon hydrolysis of the bond between the monomer and the fluorophore, the fluorophore becomes fluorescent, and hydrolysis is measured as an increase in fluorescence signal with time (Hoppe, 1983; Somville and Billen, 1983).

These commercially available substrates are easy to use, and measurements have been made in a wide range of environments, simplifying intercomparisons among sites. They suffer from a number of limitations, however, because small substrate proxies cannot effectively structurally and spatially represent the three-dimensional structure of high-molecular-weight macromolecules in solution. Bacterial isolates capable of hydrolyzing methylumbelliferyl (MUF) monosaccharides cannot necessarily hydrolyze the corresponding high-molecular-weight polymers that they are supposed to represent (Helmke and Weyland, 1991). In addition, small substrate proxies can be hydrolyzed in the periplasmic space (Martinez and Azam, 1993), whereas macromolecules are too large to enter the periplasmic space prior to hydrolysis. These proxies therefore measure some combination of periplasmic and truly extracellular enzymes.

Kinetic parameters of isolated enzymes determined from model substrates differ significantly from those determined with actual polymers (Feller *et al.*, 1996). Because small substrate analogs likely measure the activities of exoacting extracellular enzymes, which hydrolyze the terminal ends of macromolecules, this lack of correspondence is not particularly surprising. Because prokaryotes typically express a range of enzymes including those with exo- and endo-specificities to hydrolyze a specific macromolecule (Warren, 1996), small substrate analogs can reflect only a portion of enzyme activity. In addition, reliance on a restricted set of substrate analogs masks the diversity of natural structures and substrates used by microbial communities. The importance of organic matter "quality" as a determinant of suitability as a microbial substrate is widely recognized (Westrich and Berner, 1984), but the specific structural and enzymatic factors that determine susceptibility to

degradation remain largely unknown. A further practical problem, especially in high-DOC sediments, relates to the MUF fluorophore, whose fluorescence excitation and emission maxima overlap with those of natural DOC (Green and Blough, 1994). Sorption of fluorophores to surfaces, and fluorescence quenching by natural organic matter, may present additional problems.

In an effort to mitigate some of these problems, new methods have been developed to measure directly the extracellular enzymatic hydrolysis of high-molecular-weight soluble substrates. In enrichment cultures, progressive size changes in macromolecular substrates, as well as the specific bond cleavage sites, have been determined directly with a combination of gel permeation chromatography and nuclear magnetic resonance spectroscopy (Arnosti and Repeta, 1994a; Arnosti *et al*., 1994). Hydrolysis of macromolecules in seawater and sediments has been measured with the use of fluorescently labeled high-molecular-weight soluble substrates. Polysaccharides of differing structure (Arnosti, 1995) and peptides of varying length and composition (Pantoja *et al*., 1997) have been labeled with covalently linked fluorophores. These fluorophores remain attached to the macromolecules, and hydrolysis occurs between polymer bonds. The rate of hydrolysis is measured as the change in molecular weight of the substrate with increasing incubation time. Because these substrates are high-molecular-weight polymers, not proxies, they can be used to investigate possible differences in hydrolysis rates and substrate specificities among peptides differing in composition by a few amino acids (Pantoja and Lee, 1999) and among polysaccharides differing in monomer composition, charge, anomeric linkage, linkage position, and molecular weight (Arnosti, 2000). Furthermore, because the fluorophores used to label the polymers have excitation and emission maxima far removed from those of DOC, background interference from natural DOC is essentially zero.

Another approach is to use ^{14}C -labeled macromolecules such as proteins and to follow the appearance of radioactivity in trichloroacetic acid (TCA)-soluble fraction (as the low-molecular-weight products) along with evolution of $^{14}CO_2$. Conversion of macromolecules to lower molecular weight products, as well as microbial respiration, can be measured using this approach (e.g., Hollibaugh and Azam, 1983). Determining rates of extracellular enzymatic hydrolysis is complicated by the fact that the original protein, as well as label incorporated into high-molecular-weight products, will count as "TCA precipitate," whereas labeled low-molecular-weight cellular products, as well as hydrolyzed source protein, will remain in the TCA-soluble fraction.

High-molecular-weight solid substrates such as hide powder azure, and cellulose, chitin, and agar stained with remazol brilliant blue R have been used in enzyme assays to investigate activities in extracts from sediments (Reichardt, 1986). This technique is one of the few means of examining the particle → dissolved transition in relationship to sedimentary enzyme activity, but the necessity of extracting enzymes from sediments complicates interpretation of the results, because extraction efficiency, as well as the

appropriate conditions to measure enzymatic activity in the extracts, are unknown. Likewise, the relationships between activities measured in a particular buffer and activities occurring in the environment remain unclear.

IV. EXTRACELLULAR ENZYME ACTIVITIES IN THE WATER COLUMN AND SEDIMENT

Despite the limitations of the analytical approaches discussed above, a considerable amount of information has been gained about enzyme activities in aquatic systems (see also Burns and Dick (2002) for further reviews from a range of environments.) Enzyme activities in the water column are often operationally divided into "particle-associated" (activity removed by filtration through a defined pore size); activity associated with "free-living" bacteria (activity remaining after removal of particles by filtration, as above), and "dissolved" activity (in general, activity measurable after passage through 0.2-μm-pore-sized filters). In the water column, particle-associated enzyme activities — presumably the enzyme activities of the particle-attached microbial community — are frequently found to be far higher than enzymatic activities associated with the (operationally defined) free-living microbial community. For example, Smith *et al.* (1992) made parallel measurements of leucine uptake and leu-MCA hydrolysis in marine snow and surrounding seawater and found that leu-MCA hydrolysis rates on particles were frequently two to three orders of magnitude higher than rates in the surrounding seawater. They suggested that particle-associated hydrolytic activity could produce peptides well in excess of bacterial carbon demand for particle-attached bacteria, thereby providing labile substrates for the free-living microbial community. Karner and Herndl (1992) also investigated marine-snow-associated communities and found that the rate of hydrolysis of MUF-β-glucose and MUF-α-glucose, as well as leu-MCA, was considerably higher (typically by a factor of ca. 10) on marine snow than in the ambient water, even though bacterial density and production were similar for free-living and marine-snow-associated bacteria. In the Columbia River estuary, virtually all of the enzyme activity (MUF-β-glucose, -cellobiose, and leu-MCA) was associated with particles removed via 0.3-μm filtration (Crump *et al.*, 1998). The association of high enzyme activities and particles was explored on a theoretical basis by Vetter *et al.* (1998). They developed a model, based on optimal foraging theory and diffusional constraints on aquatic bacteria, which suggests that especially in particles and sediments, release of free enzymes is a viable strategy for fulfilling energy requirements.

Relative enzyme activities associated with particles and with free-living bacterial communities are not constant, however. Particle composition and concentration, as well as the attached microbial community and its associated enzyme activities, can change dramatically as a result of biological and physical processes. For example, Middelboe *et al.* (1995) found that as a

phytoplankton bloom developed in a lake, the relative contributions of free and particle-attached bacteria to MUF-β-glucose and leu-MCA hydrolyzing activity, as well as bacterial biomass and production, changed with time. Initially, free-living bacteria (<3 μm fraction of filtrate) accounted for the majority of bacterial productivity. As the bloom progressed and then collapsed, attached bacteria contributed an increasing fraction of total bacterial numbers and productivity. Riemann *et al*. (2000) observed a similar pattern in a bloom induced in a mesocosm: bacterial numbers, productivity, and enzyme activities shifted from the <1.0 μm fraction to the >1.0 μm size fraction as the bloom progressed.

Although the majority of activities measured in aquatic systems has been found to be associated with the particle- or bacteria-size fraction (e.g., Rosso and Azam, 1987; Vrba *et al*., 1992), dissolved enzymes can at times make substantial contributions to total activity. For example, Vives-Rego *et al*. (1985) found that although most proteolytic activity was associated with bacterial membranes, up to 30% of total hydrolytic activity was contributed by the fraction passing through a 0.2-μm-pore-size filter. There are many other reports of significant contributions of "dissolved" activity in the water column: dissolved aminopeptidase activity ranging from ca. 43–100% of total activity was measured by Jacobsen and Rai (1991) for a lake sampled weekly between September and December. Vrba *et al*. (1992) observed variable contributions of dissolved enzymes to hydrolytic activity in a reservoir over a spring-summer-fall period. The contribution of dissolved to total MUF-α-glucosidase activity was relatively low (average 9.5%) and was not correlated with the much higher contribution of dissolved to total MUF-β-glucosidase activity (average 24% of the total, range 0–65%). Chappell and Goulder (1995) found that the contribution of dissolved enzymes to total activity in two rivers sampled periodically between February and October varied by substrate and river: dissolved MUF-β-glucosidase activity ranged from 18% to 30% in one river, and from 10% to 25% in the second river. The contributions of dissolved enzymes to leu-MCA hydrolysis were even more variable, ranging from 22% to 86% in one river, and from 30% to 98% in the second river. An investigation of laminarinase and xylanase activities along a freshwater–marine transect in the Delaware River and Bay likewise showed that free enzymes could contribute a substantial, but spatially and seasonally varying, portion of total activity (Keith and Arnosti, 2001). Up to 10% of total laminarinase activity was in the free enzyme fraction at all three stations measured in January, but the contribution reached nearly 100% at two of three stations in September. For the same stations and water samples in September, xylanase activity showed different patterns: the free enzyme fraction contributed ca. 70% of total activity upriver, decreasing downriver to ca. 15%. Contributions of dissolved xylanase enzymes to total hydrolysis in January ranged from ca. 0 to 45% at the three stations, and were generally low at the single station measured in June (Keith and Arnosti, 2001). Karner and Rassoulzadegan (1995) also detected significant variations

in the contribution of the <0.1 μm fraction to MUF-α-glucose and MUF-β-glucose hydrolysis. In a coastal marine environment, the contribution of the dissolved enzymes varied between 0 and 100% on 24-h time scales.

The contribution of enzymes in the "dissolved" fraction to total hydrolytic activity in the water column is difficult to generalize, because "dissolved" enzymes may originate from a wide range of sources and processes. Bacteria can release enzymes into solution as a function of substrate concentration or growth phase (Antranikian, 1992). Viral lysis (Karner and Rassoulzadegan, 1995) or zooplankton grazing (Bochdansky *et al*., 1995) may also result in release of free enzymes, and nanoflagellate enzymes may contribute to hydrolytic activity (Nagata and Kirchman, 1992; Karner *et al*., 1994). The "lifetime" of a free enzyme in marine systems is completely unknown; presumably proteins are turned over relatively rapidly (Hollibaugh and Azam, 1983; Pantoja and Lee, 1999), but association with surfaces or particles, as has been observed in soils (Nannipieri *et al*., 1982), may stabilize enzymes and protects them from degradation.

The extent to which dissolved enzymes contribute to total hydrolytic activity in fresh and marine waters may also be affected by differences in freshwater and marine DOC sources and composition. Humic substances characterized by a high phenolic content constitute a large proportion of freshwater DOC (see Chapter 3 and 19). These humics can form complexes with extracellular enzymes, inactivating them, but mild exposure to UV light can lead to enzyme reactivation (Wetzel, 1993). Complexation, transport, and subsequent sunlight-initiated reactivation of enzymes could spatially decouple enzyme production and activity in fresh waters. This scenario is less likely to be significant in marine systems: marine humic substances are chemically distinct and have a much lower phenolic content than freshwater humics. In addition, the higher divalent cation activity of seawater likely strongly reduces humic–enzyme complex formation by interaction of the cations with the humic substances (Wetzel, 1993).

Although distinctions among cell-surface attached, particle/detritus attached, and "free" enzymes in porewaters are important from biochemical, ecological, and geochemical perspectives, they are currently extremely difficult or impossible to separate analytically (see also Sections VII and VIII). Measurements of enzyme activities in sediments are complicated by a range of technical challenges beyond those encountered in the water column. Sediments in general exhibit far higher heterogeneity on much smaller spatial scales, including a wider range of surfaces and microenvironments. The majority of measurements of enzyme activities reported for sediments therefore are actually made using sediments slurried with water. Although making measurements in slurries reduces experimental variability, the relationships between these measurements and rates of processes occurring in the original intact sediments are difficult to establish.

In spite of these caveats, studies of enzyme activities in sediments show some recurring features. Seasonal cycles in primary productivity in temperate

regions are frequently reflected in sedimentary metabolism as well. Sedimentation following the termination of a phytoplankton bloom has been correlated with seasonal maxima in enzyme activities as measured by hide powder azure (Reichardt, 1986), leucine-MCA hydrolysis (Meyer-Reil, 1987), and peptide hydrolysis (Pantoja and Lee, 1999). In general, enzyme activities are maximal in near-surface sediments and decrease with depth (King, 1986; Meyer-Reil, 1987; Mayer, 1989), but subsurface maxima have frequently also been reported, likely in conjunction with infaunal organisms or "hot spots" in microbial activity due to rapid mixing or heterogeneous distribution of particulate organic carbon in sediments (e.g., Mayer, 1989; Arnosti, 1995; Pantoja and Lee, 1999). In deep ocean sediments, enzyme activities measured using a range of substrate proxies likewise are usually maximal in surface or near-surface sediments and decrease with sediment depth (Poremba and Hoppe, 1995; Boetius *et al*., 1996). Enzyme activities also tend to decrease with increasing water column depth (Poremba and Hoppe, 1995; Boetius and Damm, 1998), an observation that has been linked to substrate limitations for deep-sea microbial communities (Deming and Yager, 1992; Meyer-Reil and Koster, 1992).

V. PATTERNS OF ENZYME ACTIVITIES

A few general patterns of enzyme activity emerge from both water column and sediment studies: in the majority of cases where both MUF-glucosidase and peptidase (either leu-MCA or L-leucyl β-naphthylamine, LLBN) activities have been measured in the same samples, peptidase activity has been found to be far higher than MUF-glucose activity; MUF-β-glucosidase activity is also usually higher than MUF-α-glucosidase activity. This pattern applied to both free-living and attached bacteria in a lake, where MUF-β-glucose) and leu-MCA activities for the free-living bacteria ranged from ca. 5 to 32 nmol L^{-1} h^{-1} and from ca. 100 to 305 nmol L^{-1} h^{-1}, and for attached bacteria from ca. 0 to 16 and from 81 to 353 nmol L^{-1} h^{-1}, respectively (Middelboe *et al*., 1995). A similar pattern was observed for marine snow by Smith *et al*. (1992) and Karner and Herndl (1992), who found that leu-MCA activities were usually higher than MUF-β-glucose activities by a factor of 10–100 or more, whereas MUF-β-glucose activities were usually a factor of 2–8 greater than those of MUF-α-glucose. Smith *et al*. (1992) reported particle-attached leu-MCA activities frequently in excess of 1000 μmol mL^{-1} h^{-1}, with MUF-β-glucosidase activities ranging from ca. 0.01 to 130 μmol L^{-1} h^{-1}. Karner and Herndl (1992) measured considerably lower rates (leu-MCA hydrolysis on the order of 0.5 to 9 μmol mL^{-1} h^{-1}, still approximately 100 times faster than both MUF-α- and MUF-β-glucose). In the Columbia River estuary, leu-MCA activity was generally higher than MUF-β-glucose activity by a factor of approximately 10, with leu-MCA hydrolysis rates ranging up to a maximum of ca.

30 μmol $L^{-1} h^{-1}$ (Crump *et al.*, 1998). The same pattern was observed during a phytoplankton bloom induced in a mesocosm, where maximum leu-MCA hydrolysis rates reached ca. 1065 nmol $L^{-1} h^{-1}$, MUF-β-glucosidase reached ca. 50 nmol $L^{-1} h^{-1}$, and MUF-α-glucosidase activity reached 21 nmol $L^{-1} h^{-1}$ (Riemann *et al.*, 2000).

In sediments leu-MCA activities are frequently significantly higher than MUF-glucose activities. MUF-monosaccharide potential hydrolysis rates in coastal and temperate sediments vary over several orders of magnitude, spanning a range from ca. 2 to 1300 nmol $cm^{-3} h^{-1}$ (King, 1986; Meyer-Reil, 1986). Rates reported in deep sea sediments are much lower (ca. 0.005–0.50 nmol $cm^{-3} h^{-1}$ (Boetius and Lochte, 1994; Boetius, 1995). Leu-MCA hydrolysis rates in deep-sea sediments are typically much higher than MUF-glucose rates (e.g., 10–60 nmol $cm^{-3} h^{-1}$ across a series of stations from 135 m to 1680 m water depth in the northeast Atlantic (Poremba and Hoppe, 1995).) In contrast to the usual pattern, leu-MCA activities have also been observed to increase with increasing water depth in some polar sediments (Boetius and Lochte, 1996; Boetius and Damm, 1998).

In a few instances, different relationships between MUF-glucose and leu-MCA (or LLBN) hydrolysis have been reported. For example, Münster (1991) detected similar levels of leu-MCA and MUF-glucosidase activity in a polyhumic lake. Christian and Karl (1995) found that the relative relationship of MUF-β-glucose to LLBN hydrolytic activity varied significantly between the southern ocean (Antarctic peninsula), the Pacific subtropical gyre (station ALOHA), and the equatorial Pacific. While LLBN activity was on average ca. 7–11 nmol $L^{-1} h^{-1}$ at all three sites, MUF-β-glucose hydrolysis was very low (0.02–0.03 nmol $L^{-1} h^{-1}$) in the southern ocean and Pacific subtropical gyre, and quite high (average of 63 nmol $L^{-1} h^{-1}$) in the equatorial Pacific.

Ratios of LLBN (or leu-MCA) to MUF-glucose activities have been hypothesized to relate to the relative importance and/or bioavailability of proteins and polysaccharides to microbial communities (e.g., Christian and Karl, 1995; Fukuda *et al.*, 2000). While the observation that amino acids tend to decrease more rapidly than carbohydrates in sinking particles (e.g., Wakeham *et al.*, 1997) supports the idea that protein-containing components of marine organic matter may be more rapidly remineralized than carbohydrate-containing components, the connection between particle composition and potential hydrolysis rates is poorly defined. Efforts to define the link between potential hydrolysis rates — measured with small soluble substrate proxies — and particle composition are greatly hampered by the fact that the extent to which potential hydrolysis rates actually reflect hydrolysis rates of macromolecules is unknown. Martinez *et al.* (1996) suggested that the generally "low" rates measured with MUF α- and β-glucose may in fact underestimate actual enzyme activities. Potential hydrolysis rates measured using fluorescently labeled polysaccharides in seawater and sediments investigated to date (e.g., Hoppe *et al.*, 2002; Keith and Arnosti, 2001) have generally been higher than hydrolysis rates measured using MUF-glucose

substrates. In addition, Pantoja and Lee (1999) found that hydrolysis rates measured using leu-MCA were considerably lower than hydrolysis rates of larger peptides, suggesting that potential hydrolysis rate measurements made with small substrate proxies do not effectively represent oligo- and polymer hydrolysis.

Basing general estimates of polymer hydrolysis rates primarily on measurements made with a specific substrate proxy such as MUF-glucose is problematic for a number of other reasons as well. As discussed in Section III, small substrate proxies such as MUF-glucose likely measure the activities only of exo-acting extracellular enzymes, so the activities of endo-acting extracellular enzymes, which cleave polymers midchain, are not included in these measurements. Furthermore, although glucose is commonly detected in aquatic systems, it is only one of a wide range of carbohydrates present (Cowie and Hedges, 1984; see Chapter 4), and — as studies using a wider range of MUF-monosaccharides have shown (King, 1986) — MUF-glucose activities are not even representative of other MUF-monosaccharide activities. Hydrolysis of MUF-glucose substrates therefore reflects the activity of only a small fraction of the glycolytic extracellular enzymes present in a given system.

Similar caveats apply to the commonly observed pattern of more rapid hydrolysis of β-linked than α-linked MUF-monosaccharide substrates, reported in fresh and marine waters, as well as in sediments. These reports show, for example, that in surface waters of a lake sampled repeatedly during July and August, MUF-β-galactosidase activity was found to exceed MUF-α-galactosidase activity on 8 of 10 sample dates, while MUF-β-mannosidase activity exceeded MUF-α-mannosidase activity on 5 of 9 sample dates. MUF-β-glucosidase activity was more rapid than MUF-α-glucosidase activity on all 13 dates sampled (Münster, 1991). A comparison of potential enzyme activities in three rivers showed that V_{max} for MUF-β-glucosidase exceeded the V_{max} of MUF-α-glucosidase in most, but not all, cases (Sinsabaugh *et al*., 1997). In samples collected from the surface to depths of 4000 m in the Pacific Ocean, MUF-β-glucosidase was generally, but not always, higher than MUF-α-glucosidase activity (Koike and Nagata, 1997). Investigations of sediments have frequently also reported MUF-β-glucosidase activities considerably higher than MUF-α-glucosidase activities (e.g., Poremba, 1995; Boetius and Damm, 1998).

The relative ratio of MUF-α- and MUF-β-glucose hydrolysis has been used as an indication of the proportion of "more refractory" or structural carbohydrates present in a given sample (e.g., Karner and Rassoulzadegan, 1995). The underlying idea is that cellulose, a polymer of β-linked glucose, is a structural polysaccharide and tends to be more refractory than starch, an α-linked glucose polymer that some phytoplankton accumulate as an energy reserve. However, energy storage polysaccharides can also consist of β-linked glucose, as in the case of laminarin, an energy storage polymer of diatoms (Painter, 1983). The cellulose/starch comparison additionally fails

to account for the fact that cellulose is insoluble and contains highly ordered crystalline regions, whereas starch is a soluble polysaccharide.

A more fundamental problem with all of the field methods used to date is that they leave open several critical questions about the extracellular enzymes themselves. A measurement of a high potential hydrolysis rate could be due to a high enzyme turnover number, a large quantity of enzymes, and/or a broad substrate affinity such that a given enzyme can hydrolyze a wide range of related substrates. Furthermore, some types of enzymes may have longer effective "lifetimes" in the water column or in sediments, such that a hydrolysis rate measurement may reflect a significant contribution of "relict" enzymes. Any or all of these factors could affect a potential hydrolysis rate measurement. Extrapolating from activities measured with two or three substrate proxies to the activities of broad ranges of enzymes, and further extrapolating these data to determine the importance of whole classes of organic matter to entire microbial communities is therefore highly uncertain.

An indication of our limited knowledge of aquatic enzyme activities is provided by a recent study that suggests that the spectrum of enzyme activities expressed in the water column and in sediments may differ substantially. The hydrolysis of six polysaccharides (pullulan, laminarin, xylan, fucoidan, chondroitin sulfate, and arabinogalactan) differing in monomer composition, charge, molecular weight, and linkage pattern were compared in sediments and seawater (Arnosti, 2000). Not only relative rates but also patterns of enzyme activity differed between seawater and surface sediments. At two distinct sites, Skagerrak (57° 50.0′ N, 09° 38.7′ E) and Svalbard (79° 42.8′ N, 11° 05.2′ E), pullulan, a soluble linear glucose polysaccharide, was essentially unhydrolyzed in bottom water (Fig. 1A), although it was rapidly hydrolyzed in sediments (Fig. 1B). Arabinogalactan (a mixed polymer of galactose and arabinose) and fucoidan (a sulfated fucose polysaccharide) were likewise not measurably hydrolyzed in bottom water, although they were measurably hydrolyzed in sediments. Molecular biological studies have suggested that microbial communities colonizing particles and those which are free-living in the water column are fundamentally different (DeLong *et al.*, 1993); sedimentary and biofilm communities also appear to be distinct from water-column communities (Llobet-Brossa *et al.*, 1998; see Chapter 12); these differences may extend to patterns of enzyme expression.

VI. RELATIONSHIPS BETWEEN HYDROLYSIS AND UPTAKE

The precise relationship between potential hydrolysis rates measured with externally added substrates and the rates at which complex microbial communities in marine systems hydrolyze and ultimately remineralize a spectrum of organic macromolecules actually available as substrates is unknown. Extracellular enzymatic hydrolysis is frequently regarded as the rate-limiting step in remineralization of organic carbon (e.g., King, 1986;

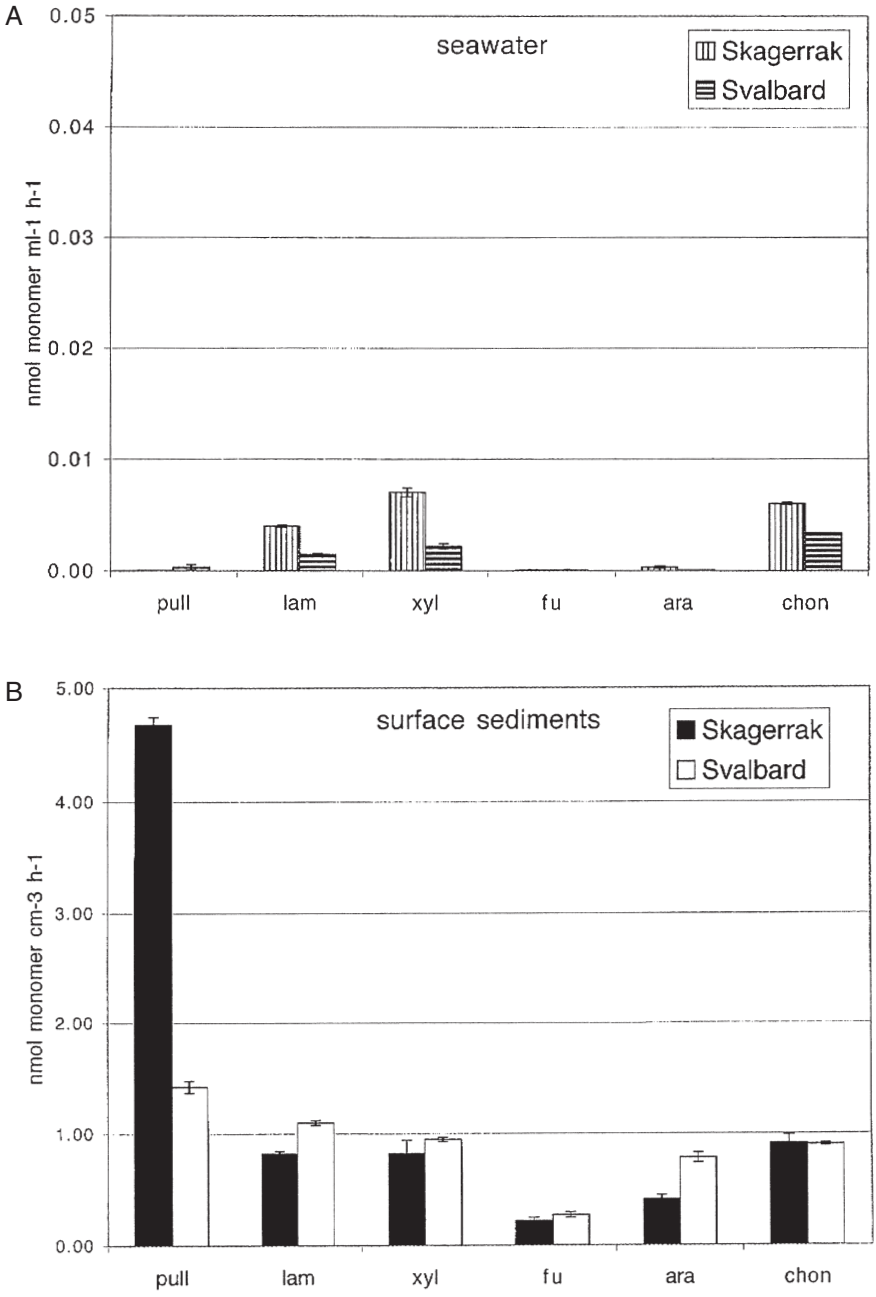

FIGURE 1 Potential hydrolysis rates of six structurally distinct polysaccharides in seawater (A) and surface sediments (B) from Skagerrak and Svalbard. Pull = pullulan, lam = laminarin, xyl = xylan, fu = fucoidan, ara = arabinogalactan, chon = chondroitin sulfate. Note the differences in scale on the *y* axes for sediments and for seawater. [Skagerrak data from Arnosti (2000).]

Meyer-Reil, 1987; Hoppe, 1991), though direct experimental evidence is lacking. In particular, several laboratory and field investigations have shown that at least in certain defined cases, extracellular enzymatic hydrolysis rates can outpace uptake of hydrolysis products. In a laboratory study, anaerobic bacterial cultures were enriched on pullulan, a high-molecular-weight linear glucose polysaccharide. The polysaccharide was rapidly removed from the medium, and sequential changes in substrate molecular weight, as well as bond cleavage positions, were followed with gel permeation chromatography and nuclear magnetic resonance spectroscopy. Production of oligosaccharides from the polysaccharides via extracellular enzymatic hydrolysis clearly outpaced uptake of the newly formed oligosaccharides, which increased in concentration until the substrate was exhausted and the microbial population "caught up" with the substrate (Fig. 2; Arnosti and Repeta, 1994a; Arnosti *et al*., 1994). Investigations of defined cultures of cellulolytic rumen bacteria

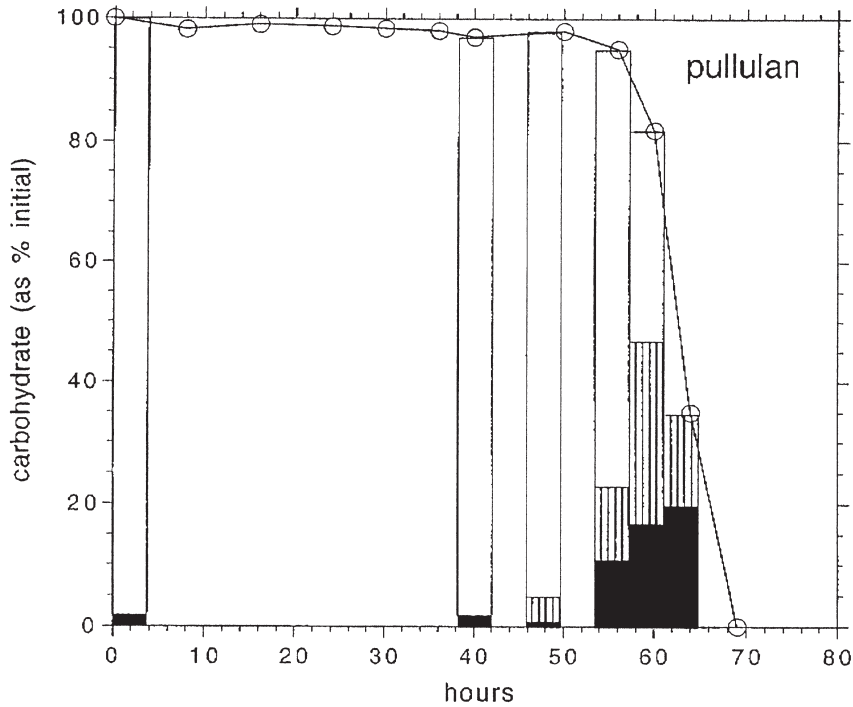

FIGURE 2 Degradation of pullulan (MW: 200,000) in replicate anaerobic enrichment cultures of marine bacteria from anoxic sediments. Open circles show total pullulan concentrations remaining in the medium at each time point. The molecular weight distribution of the pullulan is shown by the stacked bars: white: >10,000 Da, stripes: 5000 Da, black: <1200 Da. Note that the lower molecular weight fraction progressively accumulated between 50 and 64 h. [Data from Arnosti *et al*. (1994).]

have likewise shown that the rate of extracellular hydrolysis of cellodextrins did not limit bacterial growth rates (Shi and Weimer, 1996).

Field studies point in a similar direction: field comparisons of peptide hydrolysis rates and amino acid turnover in coastal sediments showed that amino acid production could exceed uptake by a factor of approximately 8 (Pantoja and Lee, 1999). A comparison of potential enzyme activities and sedimentary amino acid and carbohydrate inventories in sediments from the Ross Sea also showed that potential hydrolysis rates on time scales of hours should in theory rapidly deplete sedimentary amino acid and carbohydrate inventories (Fabiano and Danovaro, 1998). In deep-sea sediments, Poremba (1995) also found that potential enzyme activities in theory could exceed total sedimentary carbon input by a factor of 200. Finally, Smith *et al.*'s (1992) investigation of potential hydrolysis rates and amino acid uptake in marine snow demonstrated that the particle-associated bacteria were potentially producing amino acids far in excess of their own carbon demand.

VII. SOME KEY QUESTIONS

A. What Factors Impede Enzymatic Hydrolysis and/or Substrate Transport?

Accumulation of extracellular enzymatic hydrolysis products from pullulan in enrichment cultures and evidence that a range of polysaccharides, peptides, and substrate proxies can be rapidly hydrolyzed in sediments and seawater seem inconsistent with the persistence of molecularly identifiable carbohydrates and amino acids as components of organic matter in particles and sediments (Cowie and Hedges, 1984; Hedges *et al.*, 1988), and a significant carbohydrate contribution to DOC (Benner *et al.*, 1992). Even freshly produced DOC can resist remineralization. Incubation of ^{13}C-labeled DOC produced during a phytoplankton bloom in a mesocosm demonstrated that approximately 25–35% of the newly produced DOC resisted remineralization even after 2.5 years' additional incubation, and addition of nutrients and labile carbon (Fry *et al.*, 1996). Particularly in light of studies suggesting that planktonic as well as sedimentary bacteria are frequently carbon-limited (e.g., Deming and Yager, 1992; Kirchman and Rich, 1997), carbohydrate and amino-acid containing components of organic matter should be rapidly hydrolyzed and remineralized. What factors then may impede enzymatic hydrolysis or substrate uptake, and why is some fraction of dissolved organic matter geochemically "old"? Perhaps much of the naturally available organic carbon is a "poor fit," possibly because of biological or chemical modifications (Fig. 3a). Because many enzymes are produced only in the presence of an inducer, perhaps the specific structures required to induce a particular enzyme are simply not present in sufficient concentrations (Fig. 3b). In this

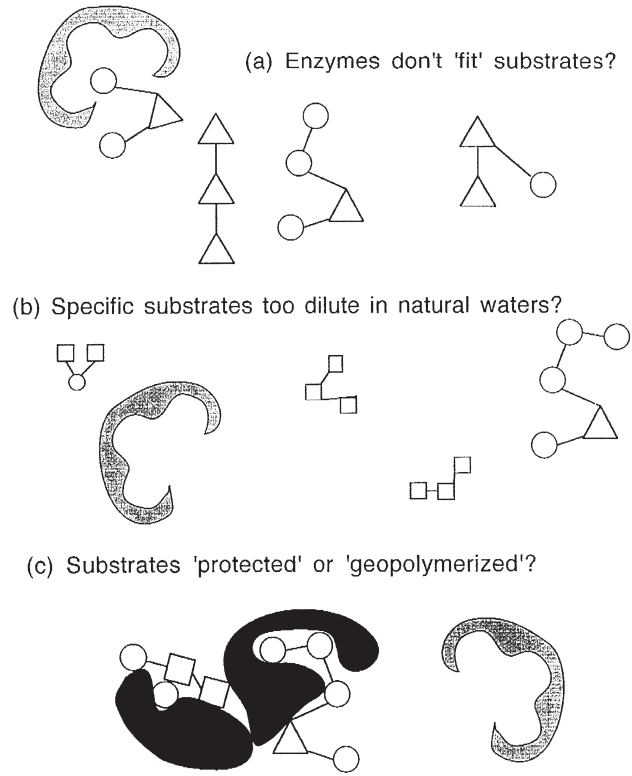

FIGURE 3 Several hypothetical scenarios which might explain resistance of organic macromolecules to extracellular enzymatic hydrolysis: (a) natural substrates are not a good "fit" for enzyme active sites, perhaps because of biological or chemical modifications; (b) specific substrates are too dilute to induce enzymes under most circumstances; (c) substrates are physically "protected" from hydrolysis (e.g., Mayer, 1994; Keil *et al.*, 1994). Enzymes may also be complexed, hindering their activities (Wetzel, 1993).

case, significant production of a specific enzyme may occur only rarely — or upon addition of external substrates and/or substrate proxies. Another possibility would be that activity of an enzyme might be inhibited by complexation of the substrate (Mayer 1994; Keil *et al.*, 1994) (Fig. 3c) or of the enzyme itself (Wetzel, 1993). Geochemical transformations (Keil and Kirchman, 1994) or complexation (Nagata and Kirchman, 1996; Borch and Kirchman, 1999), which slows the degradation of organic matter, may occur on very short time scales; such structural alterations or complexation may also impede enzyme induction or cause a "misfit" in an enzyme's catalytic or binding domain. Keil and Kirchman (1993) demonstrated that glycosylation of the protein ribulose-1,5-diphosphate carboxylase (Rubisco) that example, slowed turnover by factors of ca. 30–200 relative to Rubisco, that was not glycosylated.

Evidence that a significant portion of DOC has a nominal molecular weight below 1000 (Benner *et al*., 1992) also raises the possibility that the "problem" for microbial communities may lay not only in enzymatic hydrolysis, but also in substrate uptake. A molecular weight of 1000 is relatively close to the nominal uptake limit of a microbial porin (Weiss *et al*., 1991). Some porins, such as maltoporin, can in fact transport maltooligosaccharides up to maltoheptaose (molecular weight 1152) (Benz, 1988). Because many transport systems are highly structure-specific (Schirmer *et al*., 1995), however, structurally "unfamiliar" substrates may not be transported as readily across microbial membranes. This point is illustrated by a comparative study of the degradation of a range of disaccharides and maltooligosaccharides by enrichment cultures of anaerobic marine bacteria, in which a galactose-arabinose disaccharide (MW 312) persisted in the medium and was ultimately degraded at less than half the rate of any of the other di- or oligosaccharides, despite the fact that this disaccharide does not require extracellular hydrolysis prior to uptake (Fig. 4; Arnosti and Repeta, 1994b). Uptake of the same disaccharide, added to *in situ* lake water mesocosms, was also found to be slow (Meon and Juettner, 1999).

Physical or chemical modification of a substrate may additionally selectively affect transformation or uptake: Keil and Kirchman (1992) compared the degradation of Rubisco uniformly labeled with ^{3}H amino acids produced via *in vitro* translation to Rubisco that was reductively methylated with ^{3}H-methane. Although both Rubisco preparations were hydrolyzed to lower molecular weights at approximately the same rate, little of the methylated protein was assimilated or respired. The presence of one substrate may also inhibit uptake of another, as has been demonstrated for anaerobic rumen bacteria. Transport and metabolism of the monosaccharides xylose and arabinose were strongly reduced in *Ruminococcus albus* in the presence of cellobiose (a disaccharide of glucose), likely because of repression of pentose utilization in the presence of the disaccharide. Glucose, in contrast, competitively inhibited xylose transport and showed noncompetitive inhibition of arabinose transport, likely because of inactivation of arabinose permease (Thurston *et al*., 1994).

B. What Fraction of Extracellular Enzyme Activity Is Not Measured Using Current Methods?

As discussed in Section V, the spectrum of substrates and substrate proxies currently used to measure enzyme activities in marine systems is limited and most likely does not adequately represent the macromolecules actually available to aquatic microbial communities. Despite progress in recent years, our knowledge of the molecular structure and composition of dissolved organic matter is still only rudimentary. Although carbohydrates constitute an estimated 25–50% of DOC in seawater (Benner *et al*., 1992),

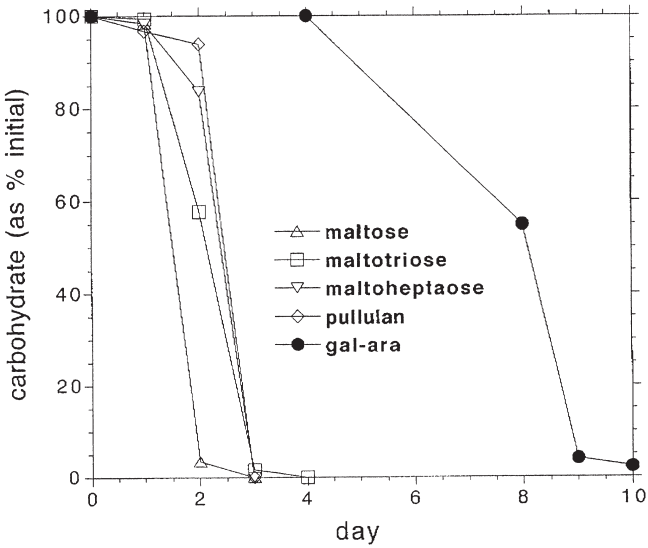

FIGURE 4 Degradation of carbohydrates in replicate anaerobic enrichment cultures of marine bacteria. Note that equivalent molar carbon concentrations of each substrate were added to the cultures. Maltose = glucose disaccharide (MW 342), maltotriose = glucose trisaccharide (MW 504), maltoheptaose = glucose heptasaccharide (MW 1152), pullulan = glucose polymer (MW 200,000), gal-ara = arabinogalactose disaccharide (MW 312). Note that the lowest molecular weight substrate persisted in the medium for the longest period of time. [Data from Arnosti and Repeta (1994b) and Arnosti *et al.* (1994).]

neutral aldoses constitute only 10–20% of this fraction (Skoog and Benner, 1997). The substrate proxies most commonly used to measure carbohydrate hydrolysis rates in aquatic systems, however, are MUF-α- and β-glucose, and relatively few studies have included a broader spectrum of even neutral monosaccharides. Much of the diversity in enzyme specificity and activity likely cannot be measured with the limited set of substrate proxies currently at hand.

C. Which Organisms Are Responsible for Hydrolysis and Uptake?

Understanding the factors controlling organic matter turnover is complicated by possible differences in population distributions of organisms responsible for enzyme production and product uptake. Although relationships between enzyme V_{max} and bacterial productivity have been reported (Sinsabaugh *et al.*, 1997), correlations between total bacterial numbers or bacterial production and enzyme activities are frequently found to be variable or weak (e.g., Mayer, 1989; Boetius *et al.*, 1996, 2000). Vrba *et al.* (1992) observed that MUF-α-glucosidase activity correlated with bacterial biomass measured in a freshwater reservoir over the course of three seasons,

whereas MUF-β-glucosidase activity showed no such correlation. Over several annual cycles, Chappell and Goulder (1995) compared enzyme activities and microbial parameters in two rivers, finding significant relationships between a range of microbial parameters and several enzyme activities for only one of two rivers. Chrøst *et al.* (1986) measured aminopeptidase as well as endopeptidase activity in a lake over an annual cycle and found no correlation between enzyme activities and the occurrence of proteolytic bacteria. A series of six lakes studied by Jacobsen and Rai (1991) also showed no correlation between aminopeptidase activity and bacterial numbers.

Attempts to correlate net microbial parameters with measurements of the activities of specific enzymes are complicated by the fact that only a fraction of a microbial community is likely responsible for production of the enzymes in question. Studies of anaerobic microbial communities from rumen have demonstrated that specific members of a consortium may produce extracellular enzymes, while a broader range of the community can take up the hydrolysis products of the enzymes ("cross-feeding") (Russell, 1985; Cotta, 1992). Measurements of hydrolysis and uptake in marine systems may therefore include a broad range of organisms taking up the radiolabeled simple substrates used to measure incorporation, and a much smaller number of organisms which produce extracellular enzymes. Studies of rumen organisms have additionally shown that some members of the rumen community preferentially take up disaccharides over monosaccharides (Bernier and Stutzenberger, 1987; Thurston *et al.*, 1993; Kajikawa and Masaki, 1999), most likely because of increased resulting energy yield. Metabolic measurements based on monosaccharide turnover therefore might not accurately reflect the activities of key bacteria. In addition, contributions of dissolved enzymes to total activity may also obscure correlations with specific measurements of microbial activity, because (as discussed above) dissolved enzymes can originate from a variety of sources and processes not directly related to local microbial activities.

The differing abilities of bacteria to use specific simple substrates in fact forms the basis of a variety of "profiling" systems such as Biolog. Recent studies have also highlighted the point that the bacteria which are capable of producing extracellular enzymes likely differ in their ability to hydrolyze and use specific high-molecular-weight substrates. Martinez *et al.* (1996) examined enzyme activities in 44 bacterial strains that they isolated from two seawater samples. Using six substrate analogs, they found that the isolates expressed a distinct "enzyme profile." Enzyme activities were also compared in four seawater samples collected from the same location over a period of 7 days. Although total bacterial abundance was relatively constant over the 7-day period, cell-specific hydrolysis rates of the six enzyme proxies varied relative to one another (on an absolute scale), by a factor of ca. 4. Shifts in microbial populations have been found to coincide with changes in enzyme activities expressed during mesocosm enrichment experiments

(Pinhassi *et al*., 1999; Riemann *et al*., 2000). Increases in enzyme activity and bacterial growth were associated with colonization of particles, particularly by members of the *Cytophaga* and α-proteobacteria (Riemann *et al*., 2000). Cottrell and Kirchman (2000) directly demonstrated that the organisms metabolizing a high-molecular-weight substrate (protein) were not necessarily the same as the organisms using lower molecular weight hydrolysis products (amino acids). Using microautoradiography and fluorescent *in situ* hybridization, they showed that members of *Cytophaga-Flavobacteria* were predominant among water-column bacteria taking up protein, chitin, and *N*-acetylglucoseamine but accounted for little amino acid uptake.

Even among individual cells of a single population, up-regulation of genes encoding extracellular enzymes, as well as synthesis of the enzymes themselves, have been shown to vary significantly. Baty *et al*. (2000a) used a GFP reporter gene to visualize *chiA* gene expression, and a precipitating fluorescent substrate analog to simultaneously investigate chitinase activity among individual cells in a culture of *Pseudoalteromonas* sp. Strain S91. Of the cells that attached to the solid chitin substrate, 41.5% were up-expressed for the *chiA* chitinase gene. Just 11.6% of the cells adhering to the chitin substrate, however, expressed chitinolytic activity. Baty *et al*. (2000a) hypothesized that the surface-associated cells that did not express chitinase were utilizing the hydrolysis products produced by the cells that did express the enzyme (see also Fischer, this volume). The intraspecific "cross-feeding," they hypothesized, may allow for more efficient use of resources by the population as a whole. Further molecular and microbiological, as well as bio- and geochemical investigations will be required to link enzyme production and activity more directly to substrate metabolism by specific organisms in aquatic environments.

D. How Rapidly Are Particles Hydrolyzed to Soluble Substrates, and What Role Is Played by Free Enzymes in Sediments?

A major experimental issue to be addressed is the rate and means by which particles are hydrolyzed and solubilized to provide substrates for heterotrophic bacteria, and the role of free enzymes in this process. Burns (1982) reviewed the possible locations and origin of enzyme activities in soils, and particularly underscored the potential importance of enzyme-humic complexes in microbial catalysis of substrates. As Burns (1982) discussed, enzymes associated with soil particles or humic substances are not subject to the same biochemical and physical restraints as are enzymes newly produced by microbial cells. Soil-held (or sediment-held) enzymes may therefore play a catalytic "trigger" role in substrate degradation, providing critical signals about substrate availability to the local microbial community. The conceptual model presented by Vetter *et al*. (1998) suggested that release of free enzymes into the environment may in fact represent

"active chemosensing" by aquatic bacteria in particles and sediments and is a viable means of obtaining substrates. Vetter and Deming (1999) isolated bacteria from marine sediments and demonstrated that these bacteria could obtain sufficient carbon and energy to grow when physically separated from solid substrates (i.e., when growth depended exclusively on hydrolysis products produced by free enzymes). "Relict" extracellular enzymes may also remain active as sediments are progressively buried, representing a contribution to sedimentary enzyme activity that is spatially and temporally separated from microbial activity at a specific depth horizon.

VIII. RESEARCH NEEDS

A few key points will enable us to begin addressing the factors controlling production, function, and activities of extracellular enzymes in aquatic systems:

- Isolation and physiological characterization of mesophilic and psychrotolerant/psychrophilic heterotrophic bacteria
- Biochemical and genetic investigations the structure, function, and regulation of specific extracellular enzymes.

While the production, structure, regulation, and function of extracellular enzymes of thermophiles and hyperthermophiles have been and continue to be the focus of numerous investigation (see Adams, 1993), comparatively little is known about the extracellular enzymes of the organisms ultimately responsible for initiating organic matter remineralization in most aquatic environments. Although culture-based investigations can only provide a partial view of microbial diversity (see also Chapter 14), detailed investigations of at least a few marine and/or freshwater organisms are essential to determine the extent to which mechanisms of enzyme activity, expression, and control established in "classical" organisms (*E. coli, B. subtilius*) also apply in the aquatic world.

- New and more diverse methods to measure extracellular enzyme activity
- Further detailed structural characterizations of dissolved organic carbon.

More realistic and diverse methods are needed to measure the activities of extracellular enzymes on a wide range of substrates, such as glycoproteins, lipid complexes, and heterogeneous particulate and dissolved organic matter. Although much has been learned with the techniques at hand, little may be gained if we simply continue applying the same methods to slightly different sample sets around the world. Because of the inherent limitations of pure-culture isolation techniques, we can only expect to isolate a small

fraction of the organisms active in the water column and sediments. It is therefore essential to have specific means of measuring extracellular enzyme activities in "real world" samples with complex substrates. To develop these substrates, we require more specific information about the molecular structure and composition of DOC, all the way to the bond-linkage and bond-orientation level.

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REFERENCES

Adams, M. W. W. 1993. Enzymes and proteins from organisms that grow near and above 100°C. *Annual Review of Microbiology* **47:**627-658.

Antranikian, G. 1992. Microbial degradation of starch. *In* "Microbial Degradation of Natural Products" (G. Winkelman, Ed.) pp. 27–56. VCH, New York.

Araki, T., S. Hashikawa, and T. Morishita. 2000. Cloning, sequencing, and expression in *Escherichia coli* of the new gene encoding b-1,3-xylanase from a marine bacterium, *Vibrio* sp. Strain XY-214. *Applied and Environment Microbiology* **66:**1741–1743.

Arnosti, C. 1995. Measurement of depth- and site-related differences in polysaccharide hydrolysis rates in marine sediments. *Geochimica et Cosmochimica Acta* **59:**4247–4257.

Arnosti, C. 2000. Substrate specificity in polysaccharide hydrolysis: Contrasts between bottom water and sediments. *Limnology and Oceanography* **45:**1112–1119.

Arnosti, C., and D. J. Repeta. 1994a. Extracellular enzyme activity in anaerobic bacterial cultures: Evidence of pullulanase activity among mesophilic marine bacteria. *Applied and Environmental Microbiology* **60:**840–846.

Arnosti, C., and D. J. Repeta. 1994b. Oligosaccharide degradation by anaerobic marine bacteria: Characterization of an experimental system to study polymer degradation in sediments. *Limnology and Oceanography* **39**:1865–1877.

Arnosti, C., D. J. Repeta, and N. V. Blough. 1994. Rapid bacterial degradation of polysaccharides in anoxic marine systems. *Geochimica et Cosmochimica Acta* **58:**2639–2652.

Baty, A. M., III, C. C. Eastburn, Z. Diwu, S. Techkarnjanaruk, A. E. Goodman, and G. G. Geesey. 2000a. Differentiation of chitinase-active and non-chitinase-active subpopulations of a marine bacterium during chitin degradation. *Applied and Environmental Microbiology* **66:**3566–3573.

Baty, A. M., III, C. C. Eastburn, S. Techkarnjanaruk, A. E. Goodman, and G. G. Geesey. 2000b. Spatial and temporal variations in chitinolytic gene expression and bacterial

biomass production during chitin degradation. *Applied and Environmental Microbiology* **66:**3574–3585.
Benner, R., J. D. Pakulski, M. McCarthy, J. I. Hedges, and P. G. Hatcher. 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* **255:**1561–1564.
Benz, R. 1988. Structure and function of porins from gram-negative bacteria. *Annual Review of Microbiology* **42:**359-393.
Bernier, R., and F. Stutzenberger. 1987. Preferential utilization of cellobiose by *Thermomonospora curvata*. *Applied and Environmental Microbiology* **53:**1743–1747.
Bochdansky, A. B., S. Puskaric, and G. J. Herndl. 1995. Influence of zooplankton grazing on free dissolved enzymes in the sea. *Marine Ecology Progress Series* **121:**53–63.
Boetius, A. 1995. Microbial hydrolytic enzyme activities in deep-sea sediments. *Helgolander Meeresuntersuchungen* **49:**177–187.
Boetius, A., and K. Lochte. 1994. Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. *Marine Ecology Progress Series* **104:**299–307.
Boetius, A., and K. Lochte. 1996. High proteolytic activities of deep-sea bacteria from oligotrophic polar sediments. *Archiv für Hydrobiologie* **48:**269–276.
Boetius, A., and E. Damm. 1998. Benthic oxygen uptake, hydrolytic potentials and microbial biomass at the Arctic continental slope. *Deep-Sea Research I* **45:**239–275.
Boetius, A., S. Scheibe, A. Tselepides, and H. Thiel. 1996. Microbial biomass and activities in deep-sea sediments of the eastern Mediterranean: Trenches are benthic hotspots. *Deep-Sea Research I* **43:**1439–1460.
Boetius, A., T. Ferdelman, and K. Lochte. 2000. Bacterial activity in sediments of the deep Arabian Sea in relation to vertical flux. *Deep-Sea Research II* **47:**2835–2875.
Borch, N. H., and D. L. Kirchman. 1999. Protection of protein from bacterial degradation by submicron particles. *Aquatic Microbiol Ecology* **16:**265–272.
Burns, R. G. 1982. Enzyme activity in soil: Location and a possible role in microbial ecology. *Soil Biology and Biochemistry* **14:**423–427.
Burns, R. G., and R. P. Dick, Eds. 2002 "Enzymes in the Environment." Dekker, New York.
Christian, J. R., and D. M. Karl. 1995. Bacterial ectoenzymes in marine waters: Activity ratios and temperature responses in three oceanographic provinces. *Limnology and Oceanography* **40:**1042–1049.
Chappell, K. R., and R. Goulder. 1995. A between-river comparison of extracellular-enzyme activity. *Microbial Ecology* **29:**1–17.
Chróst, R. J., R. Wcislo, and G. Z. Halemejko. 1986. Enzymatic decomposition of organic matter by bacteria in an eutrophic lake. *Archiv für Hydrobiologie* **107:**145–165.
Cotta, M. A. 1992. Interaction of ruminal bacteria in the production and utilization of maltooligosaccharides from starch. *Applied and Environmental Microbiology* **58:**48–54.
Cottrell, M. T., and D. L. Kirchman. 2000. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Applied and Environmental Microbiology* **66:**1692–1697.
Cowie, G. L., and J. I. Hedges. 1984. Carbohydrates sources in a coastal marine environment. *Geochimica et Cosmochimica Acta* **48:**2075–2087.
Crump, B. C., J. A. Baross, and C. A. Simenstad. 1998. Dominance of particle-attached bacteria in the Columbia River estuary, USA. *Aquatic Microbial Ecology* **14:**7–18.
Danson, M. J., and D. W. Hough. 1998. Structure, function, and stability of exoenzymes from the Archaea. *Trends in Microbiology* **6:**307–314.
DeLong, E. F., D. G. Franks, and A. L. Alldredge. 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnology and Oceanography* **38:**924–934.
Deming, J. W., and P. L. Yager. 1992. Natural bacterial assemblages in deep-sea sediments: Towards a global view. *In* Deep-Sea Food Chains and the Global Carbon Cycle. Kluwer, Dordrecht, the Netherlands.

Fabiano, M., and R. Danovaro. 1998. Enzymatic activity, bacterial distribution, and organic matter composition in sediments of the Ross Sea (Antarctica). *Applied and Environmental Microbiology* **64:**3838–3845.

Feller, G., T. Lonhienne, C. Deroanne, C. Libioulle, J. Van Beeummen, and C. Gerday. 1992. Purification, characterization, and nucleotide sequence of the thermolabile α-amylase from the Antarctic psychrotroph *Alteromonas haloplanctis* A23. *Journal of Biology Chemisty* **267:**5217–5221.

Feller, G., E. Narinx, J. L. Arpigny, Z. Zekhnini, J. Swings, and C. Gerday. 1994a. Temperature dependence of growth, enzyme secretion and activity of psychrophilic Antarctic bacteria. *Applied Microbiology and Biotechnology* **41:**477–479.

Feller, G., F. Payan, F. Theys, M. Qian, R. Haser, and C. Gerday. 1994b. Stability and structural analysis of α-amylase from the Antarctic psychrophile *Alteromonas haloplanctis* A23. *European Journal of Biochemistry* **222:**441–447.

Feller, G., E. Narinx, J. L. Arpigny, M. Aittaleb, E. Baise, S. Genicot, and C. Gerday. 1996. Enzymes from psychrophilic organisms. *FEMS Microbiological Reviews* **18:**189–202.

Feller, G., O. Le Bussy, and C. Gerday. 1998. Expression of psychrophilic genes in mesophilic hosts: Assessment of the folding state of a recombinant α-amylase. *Applied and Environmental Microbiology* **64:**1163–1165.

Fry, B., C. S. Hopkinson, Jr., A. Nolin, B. Norrman, and U. L. Zweifel. 1996. Long-term decomposition of DOC from experimental diatom blooms. *Limnology and Oceanography* **41:**1344–1347.

Fukuda, R., Y. Sohrin, N. Saotome, H. Fukuda, T. Nagata, and I. Koike. 2000. East-west gradient in ectoenzyme activities in the subarctic Pacific: Possible regulation by zinc. *Limnology and Oceanography* **45:**930–939.

Galperin, M. Y., K. M. Noll, and A. H. Romano. 1997. Coregulation of β-galactoside uptake and hydrolysis by the hyperthermophilic bacterium *Thermotoga neapolitana*. *Applied and Environmental Microbiology* **63:**969–972.

Gottschalk, G. 1986. "Bacterial Metabolism." Springer-Verlag, Berlin.

Green, S. A., and N. V. Blough. 1994. Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. *Limnology and Oceanography* **39:**1903–1916.

Hedges, J. I. 1992. Global biogeochemical cycles: Progress and problems. *Marine Chemistry* **39:**67–93.

Hedges, J. I., W. A. Clark, and G. L. Cowie. 1988. Organic matter sources to the water column and surficial sediments of a marine bay. *Limnology and Oceanography* **33:**1116–1136.

Helmke, E., and H. Weyland. 1991. Effect of temperature on extracellular enzymes occurring in permanently cold marine environments. *Kieler Meeresforschungen Sonderheft* **8:**198–204.

Henrissat, B., and G. Davies. 1997. Structural and sequence-based classification of glycoside hydrolases. *Current Opinions in Structural Biology* **7:**637–644.

Hollibaugh, J. T., and F. Azam. 1983. Microbial degradation of dissolved proteins in seawater. *Limnology and Oceanography* **28:**1104–1116.

Hoppe, H.-G. 1983. Significance of exoenzymatic activities in the ecology of brackish water: Measurements by means of methylumbelliferyl-substrates. *Marine Ecology Progress Series* **11:**299–308.

Hoppe, H.-G. 1991. Microbial extracellular enzyme activity: A new key parameter in aquatic ecology. *In* "Microbial Enzymes in Aquatic Environments." Springer-Verlag, Berlin.

Hoppe, H.-G., C. Arnosti, and G. J. Herndl. 2002. Ecological significance of bacterial enzymes in the marine environment. *In* "Enzymes in the Environment" R. G. (Burns, and R. P. Dick, Eds.). Dekker, New York.

Jacobsen, T. R., and H. Rai. 1991. Aminopeptidase activity in lakes of differing eutrophication. *In* "Microbial Enzymes in Aquatic Ecosystems" (R. J. Chrøst Ed.), pp. 144–164, Springer-Verlag, New York.

Kajikawa, H., and S. Masaki. 1999. Cellobiose transport by mixed ruminal bacteria from a cow. *Applied Environmental Microbiology* **65:**2565–2569.

Karner, M., and G. J. Herndl. 1992. Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria. *Marine Biology* **113:**341–347.

Karner, M., and F. Rassoulzadegan. 1995. Extracellular enzyme activity: Indications for high short-term variability in a coastal marine ecosystem. *Microbial Ecology* **30:**143–156.

Karner, M., C. Ferrier-Pages, and F. Rassoulzadegan. 1994. Phagotrophic nanoflagellates contribute to occurrence of a-glucosidase and aminopeptidase in marine environments. *Marine Ecology Progress Series* **114:**237–244.

Keil, R. G., and D. L. Kirchman. 1992. Bacterial hydrolysis of protein and methylated protein and its implications for studies of protein degradation in aquatic systems. *Applied Environmental Microbiology* **58:**1374–1375.

Keil, R. G., and D. L. Kirchman. 1993. Dissolved combined amino acids: Chemical form and utilization by marine bacteria. *Limnology and Oceanography* **38:**1256–1270.

Keil, R. G., and D. L. Kirchman. 1994. Abiotic transformation of labile protein to refractory protein in seawater. *Marine Chemistry* **45:**187–196.

Keil, R. G., D. B. Montlucon, F. G. Prahl, and J. I. Hedges. 1994. Sorptive preservation of labile organic matter in marine sediments. *Nature* **370:**549–552.

Keith, S. C., and C. Arnosti. 2001. Extracellular enzyme activity in a river-bay-shelf transect: Variations in polysaccharide hydrolysis rates with substrate and size class. *Aquatic Microbial Ecology* **24:**243–253

King, G. M. 1986. Characterization of b-glucosidase activity in intertidal marine sediments. *Applied Environmental Microbiology* **51:**373–380.

Kirchman, D. L., and J. H. Rich. 1997. Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean. *Microbial Ecology* **33:**11–20.

Koike, I., and T. Nagata. 1997. High potential activity of extracellular alkaline phosphatase in deep waters of the central Pacific. *Deep-Sea Research II* **44:**2283–2294.

Llobet-Brossa, E., R. Rossello-Mora, and R. Amann. 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. *Applied Environmental Microbiology* **64:**2691–2696.

Martinez, J., and F. Azam. 1993. Periplasmic aminopeptidase and alkaline phosphatase activities in a marine bacterium: Implications for substrate processing in the sea. *Marine Ecology Progress Series* **92:**89–97.

Martinez, J., D. C. Smith, G. F. Steward, and F. Azam. 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquatic Microbial Ecology* **10:**223–230.

Mayer, L. M. 1989. Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. *Limnology and Oceanography* **34:**973–981.

Mayer, L. M. 1994. Surface area control of organic carbon accumulation in continental shelf sediments. *Geochimica et Cosmochimica Acta* **58:**1271–1284.

Meon, B., and F. Juettner. 1999. Concentrations and dynamics of free mono- and oligosaccharides in a shallow eutrophic lake measured by thermospray mass spectrometry. *Aquatic Microbial Ecology* **16:**281–293.

Meyer-Reil, L.-A. 1986. Measurement of hydrolytic activity and incorporation of dissolved organic substrates by microorganisms in marine sediments. *Marine Ecology Progress Series* **31:**143–149.

Meyer-Reil, L.-A. 1987. Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments. *Applied Environmental Microbiology* **53:**1748–1755.

Meyer-Reil, L.-A., and M. Koster. 1992. Microbial life in pelagic sediments: The impact of environmental parameters on enzymatic degradation of organic material. *Marine Ecology Progress Series* **81:**65–72.

Middelboe, M., M. Sondergaard, Y. Letarte, and N. H. Borch. 1995. Attached and free-living bacteria: Production and polymer hydrolysis during a diatom bloom. *Microbial Ecology* **29:**231–248.

Munster, U. 1991. Extracellular enzyme activity in eutrophic and polyhumic lakes. *In* "Microbial Enzymes in Aquatic Ecosystems" (R. J. Chrøst Ed.), pp. 96–122 Springer-Verlag, New York.

Nagata, T., and D. L. Kirchman. 1992. Release of macromolecular organic complexes by heterotrophic marine flagellates. *Marine Ecology Progress Series* **83:**233–240.

Nagata, T., and D. L. Kirchman. 1996. Bacterial degradation of protein adsorbed to model submicron particles in seawater. *Marine Ecology Progress Series* **132:**241–248.

Nannipieri, P., B. Ceccanti, C. Conti, and D. Bianchi. 1982. Hydrolases extracted from soil: Their properties and activities. *Soil Biol. Biochem.* **14:**357–263.

Painter, T. J. 1983. Algal polysaccharides. *In* "The Polysaccharides" (G. O. Aspinall, Ed.), Vol. 2. pp. 195–285. Academic Press, New York.

Pantoja, S., and C. Lee. 1999. Peptide decomposition by extracellular hydrolysis in coastal seawater and salt marsh sediment. *Marine Chemistry* **63:**273–291.

Pantoja, S., C. Lee, and J. F. Marecek. 1997. Hydrolysis of peptides in seawater and sediments. *Marine Chemistry* **57:**25–40.

Pinhassi, J., F. Azam, J. Hemphälä, R. A. Long, J. Martinez, U. L. Zweifel, and A. Hagstrom. 1999. Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquatic Microbial Ecology* **17:**13–26.

Poremba, K. 1995. Hydrolytic enzymatic activity in deep-sea sediments. *FEMS Microbial Ecology* **16:**213–222.

Poremba, K., and H.-G. Hoppe. 1995. Spatial variation of benthic microbial production and hydrolytic enzymatic activity down the continental slope of the Celtic Sea. *Marine Ecology Progress Series* **118:**237–245.

Priest, F. G. 1992. Synthesis and secretion of extracellular enzymes in bacteria. *In* "Microbial Degradation of Natural Products." VCH, New York.

Rao, M. B., A. M. Tanksale, M. S. Ghatge, and V. V. Deshpande. 1998. Molecular and biotechnological aspects of microbial proteases. *Microbiology and Molecular Biology Reviews* **62:**597–635.

Reichardt, W. 1986. Enzymatic potential for decomposition of detrital biopolymers in sediments from Kiel Bay. *Ophelia* **26:**369–384.

Riemann, L., G. F. Steward, and F. Azam. 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Applied Environmental Microbiology* **66:**578–587.

Rosso, A. L., and F. Azam. 1987. Proteolytic activity in coastal oceanic waters: Depth distribution and relationship to bacterial populations. *Marine Ecology Progress Series* **41:**231–240.

Russell, J. B. 1985. Fermentation of cellodextrins by cellulolytic and noncellulolytic rumen bacteria. *Applied and Environmental Microbiology* **49:**572–576.

Schirmer, T., T. A. Keller, Y.-F. Wang, and J. P. Rosenbusch. 1995. Structural basis for sugar translocation through maltoporin channels at 3.1 Å resolution. *Science* **267:**512–514.

Shi, Y., and P. J. Weimer. 1996. Utilization of individual cellodextrins by three predominant ruminal cellulolytic bacteria. *Applied Environmental Microbiology* **62:**1084–1088.

Sinsabaugh, R. L., S. Findlay, P. Franchini, and D. Fisher. 1997. Enzymatic analysis of riverine bacterioplankton production. *Limnology and Oceanography* **42:**29–38.

Skoog, A., and R. Benner. 1997. Aldoses in various size fractions of marine organic matter: Implications for carbon cycling. *Limnology and Oceanography* **42:**1803–1813.

Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359:**139–142.

Somville, M., and G. Billen. 1983. A method for determining exoproteolytic activity in natural waters. *Limnology and Oceanography* **28:**109–193.

Svitil, A. L., S. M. N. Chadhain, J. A. Moore, and D. L. Kirchman. 1997. Chitin degradation proteins produced by the marine bacterium Vibrio harveyi growing on different forms of chitin. *Applied Environmental Microbiology* **63:**408–413.

Techkarnjanaruk, S., S. Pongpattanakitshote, and A. E. Goodman. 1997. Use of a promoterless *lacZ* gene insertion to investigate chitinase gene expression in the marine *bacterium Pseudoalteromonas* sp. Strain S9. *Applied Environmental Microbiology* **63:**2989–2996.

Thurston, B., K. A. Dawson, and H. J. Strobel. 1993. Cellobiose versus glucose utilization by the ruminal bacterium *Ruminococcus albus*. *Applied Environmental Microbiology* **59:**2631–2637.

Thurston, B., K. A. Dawson, and H. J. Strobel. 1994. Pentose utilization by the ruminal acterium *Ruminococcus albus*. *Applied Environmental Microbiology* **60:**1087–1092.

Vetter, Y. A., and J. W. Deming. 1999. Growth rates of marine bacterial isolates on particulate organic substrates solubilized by freely released extracellular enzymes. *Microbial Ecology* **37:**86–94.

Vetter, Y. A., J. W. Deming, P. A. Jumars, and B. B. Krieger-Brockett. 1998. A predictive model of bacterial foraging by means of freely released extracellular enzymes. *Microbial Ecology* **36:**75-92.

Vives-Rego, J., G. Billen, A. Fontigny, and M. Somville. 1985. Free and attached proteolytic activity in water environments. *Marine Ecology Progress Series* **21**:245–249.

Vrba, J., J. Nedoma, K. Simek, and J. Seda. 1992. Microbial decomposition of polymeric organic matter related to plankton development in a reservoir: Activity of α, β-glucosidase, and β-*N*-acetlyglucosaminidase and uptake of *N*-acetylglucoseamine. *Archiv für Hydrobiologie* **126:**193–211.

Wakeham, S. G., C. Lee, J. I. Hedges, P. J. Hernes, and M. L. Peterson. 1997. Molecular indicators of diagenetic status in marine organic matter. *Geochimica et Cosmochimica Acta* **61:**5363–5369.

Warren, R. A. J. 1996. Microbial hydrolysis of polysaccharides. *Annual Review of Microbiology* **50:**183–212.

Weiss, M. S., U. Abele, J. Weckesser, W. Welte, E. Schiltz, and G. E. Schulz. 1991. Molecular architecture and electrostatic properties of a bacterial porin. *Science* **254:**1627–1630.

Westrich, J. T., and R. A. Berner. 1984. The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested. *Limnology and Oceanography* **29:**236–249.

White, A., and D. R. Rose. 1997. Mechanism of catalysis by retaining β-glycosyl hydrolases. *Current Opinions in Structural Biology* **7:**645–651.

Wetzel, R. G. 1993. Humic compounds from wetlands: Complexation, inactivation, and reactivation of surface-bound and extracellular enzymes. *Internationale Vereinigung für Theoretische und Angewandte Limnologie Verhandlungen* **25:**122–128.

Williams, P. M., and E. R. M. Druffel. 1987. Radiocarbon in dissolved organic matter in the central north Pacific Ocean. *Nature* **330:**246–248.

Xavier, K. B., L. O. Martins, R. Peist, M. Kossmann, W. Boos, and H. Santos. 1996. High-affinity maltose/trehalose transport system in the hyperthermophilic archaeon *Thermococcus litoralis*. *Journal of Bacteriology* **178:**4773–4777.

Zeikus, J. G., C. Vielle, and A. Savchenko. 1998. Thermozymes: Biotechnology and structure-function relationships. *Extremophiles* **2:**179–183.

14

Linkages between Dissolved Organic Matter Composition and Bacterial Community Structure

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I. INTRODUCTION

There has long been interest in the generation, transport, and fate of dissolved organic matter (DOM) within aquatic ecosystems (Thurman, 1986). The fate of DOM is integrally linked to the microorganisms that are

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responsible for carrying out a wide array of processes. Energy transfer and materials cycling through different trophic levels are fundamental to ecosystem success. The notion that bacteria provide a link in the flow of energy and materials is a relatively recent addition to ecosystems theory: the microbial loop (Fig. 1) (Pomeroy, 1974; Azam *et al*., 1983). Despite the significant roles of DOM and bacteria in the ecosystem-level metabolism of aquatic systems (Hopkinson *et al*., 1989; Smith and Hollibaugh, 1993), little is known about the extent to which DOM influences the distribution and abundance of microorganisms (Thomas, 1997).

Our knowledge of bulk microbial processes has advanced throughout the years, concurrent with advances in methodologies. There are many studies examining community respiration, production, cell turnover, substrate incorporation, and ectoenzyme activity in relation to DOM characteristics, but these generally treat the microbial community as a "black box." In general, we know what goes into and comes out of the box, but we have no idea what organisms are involved in actively facilitating the transfers. This aggregation of bacteria into one group was due in large part to the poor culturability of naturally occurring bacteria on laboratory media. In fact, 90–99% of the bacteria are estimated to be unculturable using traditional microbiological methods (Amann *et al*., 1995). Due in part to the difficulties involved in culturing bacteria, few studies linking bacterial community structure to environmental processes, such as DOM turnover, have been

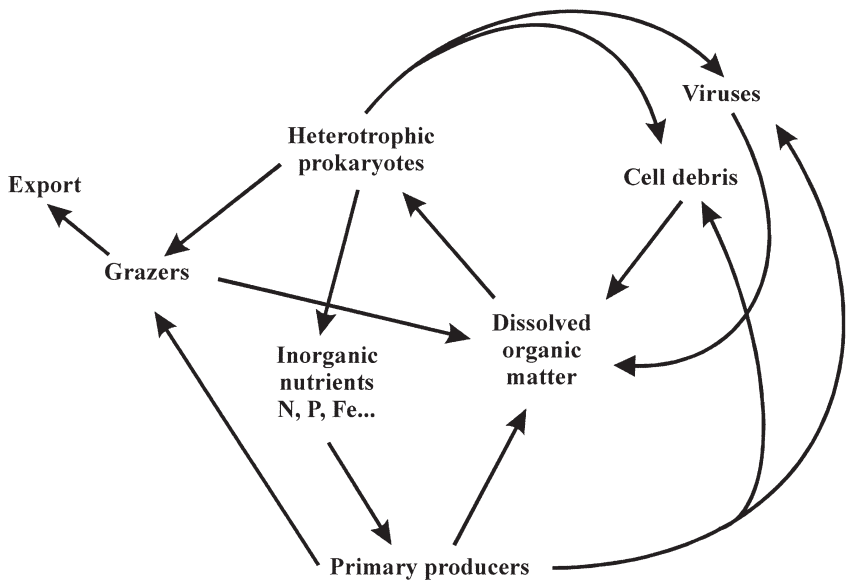

FIGURE 1 Schematic representation of the microbial loop. (Adapted from Fuhrman, 1999).

conducted. Over the last 10 years there has been an explosion of new techniques that have allowed microbial ecologists to begin answering questions relating to community composition. It is time to begin linking both structural and functional studies to get a better understanding of ecosystem dynamics. In many situations biogeochemical fluxes may be spearheaded by a few key taxa (Verity and Smetacek, 1996) whose taxonomy, life history, and physiology may be important but are poorly understood.

If we make the assumption that free-living bacteria are growing at the expense of DOM while particle-attached bacteria are growing on particulate organic matter (POM) (an assumption that is not always likely to be the case), there are a number of studies that provide hints to the taxonomic affiliations of bacteria that process organic matter. DeLong *et al.* (1993) observed that distinct bacterial populations were associated with DOM (free-living bacteria) versus POM in a coastal marine ecosystem. The free-living fraction was dominated by α-Proteobacteria and the POM-associated community consisted mainly of Cytophaga, Planctomyces, or γ-Proteobacteria (DeLong *et al.*, 1993). Similarly, this distinction between bacterial communities associated with DOM and POM has been noted by researchers in a number of other aquatic ecosystems (Albright *et al.*, 1996; Acinas *et al.*, 1999; Crump *et al.* 1999; Crump and Baross, 2000). However, studies focusing on identifying the bacteria that use different constituents of DOM are only now being conducted. Thus, the focus of this chapter is to examine studies in both marine and freshwater ecosystems that attempt to link specific members of the bacterial community with specific aspects of DOM processing.

II. METHODOLOGICAL SHIFT

There have been recent, significant shifts in the types of techniques used to understand the composition of bacterial communities. In the past, taxonomic studies of bacteria have focused on physiological and morphological characteristics associated with organisms grown in pure culture. These types of studies, however, were found to be lacking as it became clear that the majority of bacterial groups and species are not amenable to culture with standard culture methods (Ward *et al.*, 1992; Amann *et al.*, 1995). In fact, the vast majority (90–99%)of microbial species are not culturable (Amann *et al.*, 1995). Consequently, characterizations of the bacterial communities based on these techniques alone were biased by specific cultivation conditions and the resistance of many species to cultivation; hence true estimates of community composition were unattainable. A reliable means of identifying species is crucial to understanding the relationship between bacterial diversity and ecological processes. In response to the inability to completely understand environmental microbial diversity via culture-dependent methods, alternative techniques for identification were sought.

A. Advent of Molecular Methods

To address the seemingly inherent unculturability of bacteria from natural environments, researchers turned to molecular biological techniques to identify bacteria. Carl Woese and others focused on the 16S rRNA gene in the reformulation of the prokaryotic phylogenetic classification system and establishment of the Bacteria, Archaea, and Eukarya as fundamental lineages of descent (Fig. 2) (Woese, 1987). Of the many possible targets within the bacterial cell for genetic analysis, the 16S gene is uniquely suited because it is found in all Bacteria and Archaea and because it is composed of both conserved and variable regions, which allows for comparisons of both distantly and closely related organisms. Taken together, these characteristics result in the 16S molecule being a stable and robust chronometer of phylogenetic relationships (Woese, 1987; Pace, 1997). From the earliest applications of this approach, researchers began finding remarkable numbers of uncultivated microorganisms in a wide variety of ecosystems from hot-spring habitats (Ward *et al*., 1990) to oligotrophic ocean waters (Giovannoni *et al*., 1990; Schmidt *et al*., 1991). For the majority of sequences recovered in these studies, the similarity to sequences from previously described organisms was less than 90%, reinforcing the notion that prior studies into the bacterial diversity of natural environments had only scratched the surface.

B. Application of 16S rRNA-Based Methods

There are several different methods based on 16S rRNA sequences that can be used to study bacterial community structure. The first approach involves extracting bulk nucleic acids from samples and then using this material in subsequent molecular analyses. A large number of these methods involve the polymerase chain reaction (PCR). Using PCR, target gene sequences

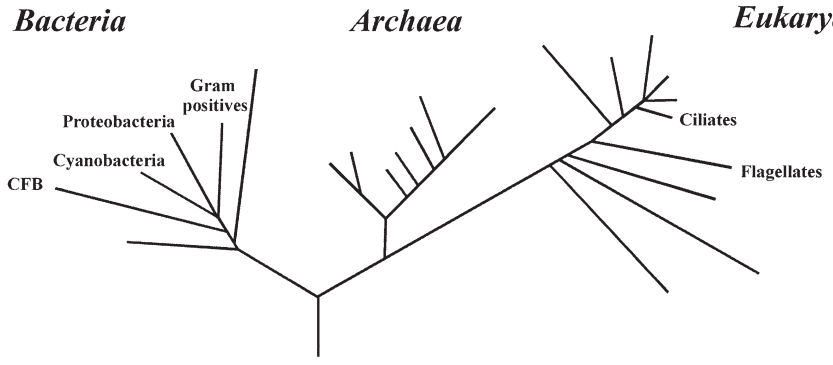

FIGURE 2 Rooted universal phylogenetic tree highlighting major components of the microbial loop. CFB indicates the *Cytophaga-Flavobacter-Bacteriodes* lineage. (Adapted from Woese, 1994).

(in this case, 16S rRNA genes) can be amplified to provide sufficient quantities for analysis. This asset can also be a drawback, however, as differential amplification of particular gene sequences within a sample can result in under- or overrepresentation in the final product (Suzuki and Giovanonni, 1996). Although these effects can be minimized through careful control of reaction parameters (Polz and Cavanaugh, 1998), it is generally held that PCR-based methods are not quantitative.

The cloning and sequencing of bacterial 16S genes has been the most prevalent method for identifying unculturable bacteria from environmental samples. Although a very effective means for gathering information about which gene sequences are present in a community is to create and analyze a clone library, it is a laborious process, so methods to more rapidly characterize bacterial diversity have been developed. More rapid techniques have been developed; among these denaturant gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE), terminal restriction fragment length polymorphism (T-RFLP), randomly amplified polymorphic DNA (RAPD), and ribosomal intergenic spaces analysis (RISA) are the most commonly applied methods. With both DGGE/TGGE and T-RFLP, the first step involves a PCR reaction in which a specially modified primer is used. In the case of DGGE/TGGE, a GC clamp is added to the primer that slows the migration of the PCR product when it is moved electrophoretically through a gel containing a gradient of either denaturants (DGGE) or temperature (TGGE). Mobility through this gradient is characteristic of the chemical make-up of the sample, and samples exhibit differential mobility based on their sequence, implying taxonomic differences (Muyzer, 1999). In T-RFLP analysis, a fluorescently labeled marker is attached to the PCR primer to produce amplicons with a fluorescent tag. The fluorescently labeled PCR products are then digested with a restriction enzyme that cuts the products at specific sites, depending on the 16S rRNA sequence (Liu *et al*., 1997; Marsh, 1999). Other community fingerprinting methods include RISA and RAPD. RISA targets variability in the size of the region between two rRNA genes within the bacterial genome. Because the sequence in this region is more variable than that within the rRNA gene, it can be used to characterize the relationship between closely related organisms (Acinas *et al*., 1999). RAPD markers are produced by PCR using short oligonucleotide primers of random sequence. Different patterns arise when differences in sequences of genomic regions result in differences between complementary primer annealing sites among bacterial species (Parker *et al*. 1998).

Within the category of bulk nucleic acid techniques, there are a group of methods that do not involve PCR. Instead of using PCR to amplify the 16S rRNA gene this type of analysis involves the design and hybridization of probes targeting specific sequences that provide information about the identity of the organism. The probe is labeled with a radioactive or fluorescent tag and then hybridized to the bulk DNA sample. Through comparisons to

a standard of known concentration, the relative abundance of the specific gene sequence within the community can be calculated. Using techniques such as dot blot hybridization, many different levels of the phylogenetic hierarchy, from domain to strain, can be targeted (Embley and Stackebrandt, 1996).

The second category of molecular methods is used to target the 16S gene within intact bacterial cells. Compared with those just described, the whole cell methods do not involve the extraction of DNA or RNA from the cells. Rather, with fluorescent *in situ* hybridization (FISH), nucleic acid probes are applied to intact cells and then the probe hybridizes to its target sequence inside the cell (Pernthaler *et al*. 1998; Glöckner *et al*. 1999; Moter and Gobel, 2000; see also Chapter 9). An interesting variation on the FISH technique combines it with microautoradiography (Lee *et al*., 1999; Ouverney and Fuhrman, 1999; Cottrell and Kirchman, 2000). Using this combined approach, cells metabolizing the radioactively labeled substrates can be identified and quantified.

Since the initial application of molecular techniques targeting the 16S rRNA molecule, there have been many advances in the field. First, more rapid techniques to sequence 16S genes are now widely available, allowing researchers to sequence hundreds of genes in a much shorter period of time. Second, large databases (i.e., GenBank and the Ribosomal Database Project) now exist for the storage of sequences collected from across the globe, allowing for easy comparison of newly recovered sequences to an ever-increasing library of sequences. Third, as a result of advances in sequencing methods and gene data libraries, it is easier to develop probes that target particular microbial groups at various phylogenetic levels, as well as at the level of physiological function.

Cloning and sequencing have provided a great deal of information on the bacteria that are not culturable by current means. Until very recently, however, researchers using the 16S rRNA approach were not able to link the presence of a particular bacterium or bacterial sequence with activity or biogeochemical function. Without this ability to link the two, knowledge regarding bacterial diversity has not been included in discussions of the cycling of DOM in aquatic ecosystems. This is beginning to change in light of the recent combined application of molecular biological and biogeochemical techniques to link bacterial community composition and activity.

III. RELATIONSHIP BETWEEN BACTERIAL COMMUNITY STRUCTURE AND DISSOLVED ORGANIC MATTER COMPOSITION

A. Marine Studies

Much of the early work into the structure of aquatic bacterial communities was conducted in marine settings with molecular techniques. Giovannoni

et al. (1990) discovered a previously undescribed lineage of marine bacteria in the Sargasso Sea (the "SAR11" lineage), providing the first example of a widespread and abundant bacterial group with no cultured representatives. Other significant findings include the discovery of Archaea in water column samples by DeLong (1992) and the identification of dominant bacterial groups in the ocean [Fuhrman *et al*., 1993; Massana *et al*., 1997; Fuhrman and Davis, 1997; Field *et al*., 1997; Glöckner *et al*., 1999; see Giovannoni and Rappé, (2000) for a wider review of this literature]. Although these studies, along with many others, indicated a large amount of heretofore unrecognized microbial diversity, they did not link the presence of the particular 16S rRNA genes with the biogeochemical function of the bacterial species. Recently, however, this situation has changed as a number of researchers have begun to simultaneously apply techniques from molecular biology and biogeochemistry to discover structure and function relationships between bacteria and the cycling of DOM in aquatic systems.

Studies attempting to link community structure and biogeochemical function can be divided into two general types based on the type of organic matter used. In the first approach, natural bacterial communities are exposed to defined components of DOM, such as chitin, protein, amino acids, and carbohydrates (Table I). In the second approach, the response of bacterial communities to enrichment with various forms of less defined, natural DOM (i.e., high- and low-molecular weight DOM, lignocellulose, and humic acids) is examined (Table II). One of the first studies to use the defined DOM component approach was that of Pinhassi *et al*. (1999). In this study, the effect of addition of protein and starch to bacterial community structure in seawater mesocosms was tracked using whole genome probes constructed from 31 phylogenetically different cultured isolates. They observed no significant changes in community composition in the mesocosm amended with starch. However, in the protein-amended mesocosm, there was a significant increase in the abundance of 10 of the 31 strains, increased bacterial productivity, and enhanced exoenzyme activity, led by a 16-fold increase in protease activity. The 10 most abundant strains included 4 from the α-Proteobacteria, 2 from the γ-Proteobacteria, and 4 from the Cytophag–Flavobacter–Bacteriodes (CFB) lineage. When taken together, these 10 strains had a growth rate up to 5 times greater than that of the overall community, suggesting that at least some members of the bacterial community can rapidly adapt to changes in the supply of organic matter.

Ouverney and Fuhrman (1999) made another application of the defined DOM component approach in which the uptake of radiolabeled amino acids by a coastal bacterioplankton community was monitored with a combination of microautoradiography and fluorescent *in situ* hybridization (STARFISH), tracking the abundance and activity of bacterial cells from α-Proteobacteria and CFB lineages. The results indicate that 80% of the cells from each subgroup use the labeled amino acid mixture. When taken together these two

TABLE I Responses of Marine and Freshwater Bacterial Communities to Experimental Enrichment with Various Defined DOM Components

Reference	*System*	*Type of DOM*	*Process/rate measurement*	*Molecular methods*	*Bacterial cultures required*	*Duration*	*Response of community*	*Effect on community composition*
Pinhassi *et al.* (1999)	Marine	Starch and protein (bovine serum albumin)	Bacterial production and ectoenzyme activity	Whole-genome DNA probes	Yes	7 days	3-fold increase in bacterial production and 6- to 14-fold increase in ectoenzyme activity due to protein addition	α- and γ-Proteobacteria and *Cytophaga–Flavobacter* isolates accounted for majority of nucleoid containing cells (<89%)
Ouverney and Fuhrman (1999)	Marine	Radiolabeled amino acids	Autoradiography	FISH	No	3 h	≤90% of total cells uptake amino acids	α-Proteobacteria and *Cytophaga–Flavobacter* account for at least 40% of total amino acid uptake

Ouverney and Fuhrman (2000)	Marine	Radiolabeled amino acids	Autoradiography	FISH	No	13–19 h	Amino acids taken up by 1–30% of Bacteria and Archaea	Archaea comprise 3–43% of total cells α- and γ-
Cottrell and Kirchman (2000)	Marine		Autoradiography	FISH	No	1–26 h	>80% of cells actively taking up substrate could be identified	Proteobacteria and *Cytophaga–Flavobacter* dominate the uptake of the four DOM components
Foreman *et al.* (1999)	Freshwater	Leucine, glucose, and humic acid	Bacterial production, ectoenzyme activity, BIOLOG GN and ECO	Oligonucleotide probes, RAPD, whole community DNA–DNA hybridizations	No	48 hours	Increased productivity, higher V_{max} and lower K_m values and overall lower diversity of substrate usage in the LDOC cultures	Dissimilar communities developed in association with the different carbon sources

TABLE II Responses of Marine and Freshwater Bacterial Communities to Experimental Enrichment with Various Defined DOM Components.

Reference	*System*	*Type of DOM*	*Process/rate measurement*	*Molecular methods*	*Bacterial cultures required*	*Duration*	*Response of community*	*Effect on community composition*
González *et al.* (1996, 1999)	Marine	Lignin	Mineralization of radiolabeled, synthetic lignin	Oligonucleotide probes	Yes	25 days	Approximately 1/3 of lignin mineralized by 3 bacterial isolates	Bacterial isolates from α- and γ-Proteobacteria accounted for up to 55% of total community DNA
Covert and Moran (2002)	Marine	Whole, HMW, and LMW DOM	Bacterial respiration	16S rDNA clone libraries and T-RFLP	No	6 weeks	LMW DOM 4–10x more bioavailable	HMW DOM community dominated by α- and β-Proteobacteria; LMW DOM community dominated by γ- and ε-Proteobacteria
Weinbauer and Höfle (1998)	Freshwater	LMW DOM, virus-reduced total DOM, virus-rich total DOM	Bacterial production, BIOLOG, API ZYM	Monoclonal antibodies	No	40 h	HMW DOM more bioreactive than LMW DOM	(PX54) β-Proteobacteria K-strategist; (PU7718) γ-Probeobacteria r-strategist
Methe and Zehr (1999)	Freshwater	Natural DOC	Water chemistry and acidification	16S rRNA cloning and sequencing	No	N/A	N/A	β-Proteobacteria and γ-Proteobacteria correlated with DOC

groups accounted for around 40% of the total amino acid used by Bacteria. Using STARFISH, Ouverney and Fuhrman (2000) showed that Archaea are active components of the microbial loop in oceanic waters with archaeal cells making up 3–43% of the community whereas 1–24% of the Archaea are actively uptaking amino acids.

Cottrell and Kirchman (2000) used MICRO-FISH, a combined micro-autoradiography and fluorescent *in situ* hybridization method similar to STARFISH, to assay the identity of coastal marine bacteria that degraded several defined DOM components. The hypothesis that all heterotrophic bacteria can use low-molecular-weight (LMW) DOM, while only a subset of bacteria capable of producing extracellular enzymes can degrade high-molecular-weight (HMW) compounds was tested with radiolabeled substrates representing LMW (amino acids and *N*-acetylglucosamine) and HMW (chitin and protein) components of DOM. Oligonucleotide probes for α-, β-, and γ-Proteobacteria and CFB were used to assess changes in community structure in response to the different substrates. In general, no single group of bacteria dominated the use of all the DOM components tested. Rather, there were distinct patterns of use that fell along phylogenetic lines. Bacteria from the CFB cluster were over-represented (substrate use relative to overall abundance) in assemblages degrading chitin, *N*–acetylglucosamine, and protein. The α-Proteobacteria were over-represented in the use of amino acids, a finding that is consistent with Ouverney and Fuhrman (1999). For both β- and γ-Proteobacteria, patterns of abundance and substrate use were similar, suggesting an overlap of the ecological functions of these groups. β-Proteobacteria are a subgroup within the γ-Proteobacteria (Woese, 1987), suggesting that functional attributes can be correlated with changes in phylogenetic composition.

Cottrell and Kirchman (2000) concluded that current biogeochemical models, which condense heterotrophic bacteria into one compartment, need revision. From their findings that physiological roles may group with taxonomic status, the authors concluded that the carbon cycle in the Delaware Bay might be best described by the consideration of activity and abundance of three groups of heterotrophic bacteria (i.e., α-Proteobacteria, CFB, and β- and γ-Proteobacteria). However, they stated that this is not a general rule for all systems and further investigation of structure–function relationships at different levels of the phylogenetic hierarchy should be conducted.

In contrast to the use of individual DOM constituents, another approach involves the use of structurally complex mixtures of organic matter as substrates for enrichment. González *et al*., (1996) assessed the composition of the bacterial community in seawater microcosms enriched with a HMW lignin mixture. Isolates recovered from these microcosms were used for design of whole genome and oligonucleotide probes, which were then used to characterize the composition of the microcosm community. Three isolates, two from γ-Proteobacteria and one from the *Roseobacter* clade of α-

Proteobacteria, were found to account for up to 50% of the community DNA and up to a third of the total mineralization of lignin in these enrichments (González *et al*., 1999). The community composition varied considerably between the microcosms, which might be attributed to differences in inoculum composition, differences in ecological interactions, or a combination of the two (González *et al*., 1999).

Covert and Moran (2002) characterized the bacterial communities that used the HMW and LMW fractions of natural DOM in an estuarine system. DOM was fractionated into the HMW and LMW fractions using ultrafiltration with a 1000 molecular weight cutoff filter, and the use of DOM fractions was assessed by measuring bacterial respiration in enrichments of each fraction. The LMW fractions supported 4–10 times greater respiration than the HMW fractions. Characterization of the community was made through the analysis of 16S rDNA clones from each enrichment, and the results showed clear differences between the enrichment communities. The predominant phylotypes recovered from the LMW clone library were γ-Proteobacteria from the genus *Pseudomonas* and ε-Proteobacteria related to *Arcobacter nitrofigilis*, both of which have been recovered from other marine systems. Clones from these groups accounted for greater than 75% of the LMW clone library. In the HMW clone library, the clones were distributed among a larger number of phylotypes than in the LMW clone library. Two groups, one related to the *Rhizobium–Agrobacterium* group from the α-Proteobacteria lineage and β-Proteobacteria identical to *Janthinobacterium lividum*, comprised ~50% of the HMW clone library.

B. Freshwater Studies

The advent of the molecular techniques described above has led to a growing body of literature identifying the numerically dominant marine bacteria. Researchers in freshwater systems have lagged behind their marine counterparts in applying molecular techniques (Hiorns *et al*., 1997; Nold and Zwart, 1998). Recently, however, several phylogenetic studies have been carried out for lakes (Bahr *et al*., 1996; Hiorns *et al*., 1997; Øvreås *et al*., 1997; Tuomi *et al*., 1997; Lindstrom, 1998; Pernthaler *et al*., 1998; Zwart *et al*., 1998a, b; Höfle *et al*., 1999; Konopka *et al*., 1999), lake snow aggregates (Weiss *et al*., 1996), river snow aggregates (Bockelmann *et al*., 2000), and rivers and streams (Kenzaka *et al*., 1998; Foreman, 1999; Leff, 2000). What has emerged from these studies is the existence of globally distributed freshwater bacterial clades. Urbach *et al*. (2001) compiled data from molecular studies of pelagic bacteria in oxygenated lakes and found that the majority of the taxa were dominated by members of the α-Proteobacteria subclass. β-Proteobacteria have been found wide ranging areas — the Antarctic, Adirondack lakes, an Alpine lake in Austria, rivers in Japan, and sludge flocculates (Pernthaler *et al*., 1998, Voytek *et al*., 1998, Kenzaka *et al*.,

1998). Other commonly represented divisions in freshwater systems include members of the α-Proteobacteria, Actinomycetales, Verrucomicrobiales, *Rhizobium–Agrobacterium*, and Cytophaga–Flavobacteria (Bahr *et al*., 1996; Hiorns *et al*., 1997; Methe *et al*., 1998; Pernthaler *et al*., 1998; Zwart *et al*., 1998a, b).

Studying phosphatase activity in activated sludge particles from a wastewater treatment plant, Van Omman Kloeke and Geesey (1999) found that the Cytophaga–Flavobacteria group represented 17–20% of the total diamidinophenylindole (DAPI) staining population on these particles. *Cytophaga*-related species have also been found by others to dominate in sludge flocs (Manz *et al*., 1996) and in freshwater mesocosms (van Hannen *et al*., 1999). According to Bergey's manual (Reichenbach, 1989), Cytophaga species are capable of degrading complex macromolecules, mainly various proteins and polysaccharides, including cellulose, agar, chitin, pectin, and starch. However, it is reported that these organisms are also capable of degrading highly recalcitrant substances.

Inland water systems are more diverse and dynamic than marine systems (see Chapter 18); therefore, it becomes apparent that more emphasis should be placed on understanding microbial community dynamics in these highly variable systems. Lakes and rivers are intimately associated with their watersheds and hence receive a large influx of material from the surrounding terrestrial environments. This linkage affects the biogeochemical cycles and processes within the system as oftentimes members of the terrestrial community end up in the aquatic environment.

Scarce as the above studies in freshwater microbial diversity may be, investigations that attempt to link phylogenetic distribution to actual processes or environmental variables are even more rare. Methe and Zehr (1999) examined bacterioplankton diversity in six Adirondack lakes in relation to lake water chemistry. Their emphasis was on acidification but they also examined other water quality parameters, including dissolved organic carbon (DOC). DOC concentrations were found to be positively correlated with the abundance of γ- and β-Proteobacteria, and also with the ACK1 clade of the β-Proteobacteria. β-Proteobacteria have also been found in other systems with high DOC [i.e., activated sludge wastewater treatment systems (Wagner *et al*., 1994)].

Weinbauer and Höfle (1998) postulated that the quality and quantity of the DOM fractions in Lake Pluβsee influenced the growth of specific bacterial strains and hence the overall community composition. Using monoclonal antibodies to two bacterial strains, *Comamonas acidovorans* PX54 (β-Proteobacteria) and *Aeromonas hydrophila* PU7718 (γ-Proteobacteria), they found that the abundance and growth rate of PX54 was greatest on the LMW fraction of the DOM pool whereas PU7718 did better on the total DOM fraction. They went on to suggest that based on life strategies, PX54 could be described as a K-strategist or equilibrium

population that grew better on more refractory DOM and that PU7718 was an r-strategist or opportunistic population.

In a series of continuous culture experiments, Foreman (1999) manipulated DOC characteristics and hydraulic turnover time of water samples collected from a eutrophic river. Divergence in community structure was assessed using RAPD fingerprinting, whole community DNA–DNA hybridization, taxon-specific probing, and scanning electron microscopy. RAPD results indicated a high degree of dissimilarity between the cultures receiving labile dissolved organic carbon (LDOC) versus those receiving recalcitrant dissolved organic carbon (RDOC) inputs. Whole community hybridization showed that divergence between LDOC and RDOC amended cultures increased with reactor turnover time. The γ-Proteobacteria probe had the greatest binding affinity for bacterial cells in the cultures, followed by the δ-, β- and α-Proteobacteria probes. Binding patterns for all probes were similar across treatments, showing that short-term shifts in community composition did not involve significant changes at the division level. Based on scanning electron microscopic observations, bacteria from LDOC and RDOC amended cultures were morphologically distinct. The findings suggest that short-term responses in community function associated with a carbon source were affected through structural changes in the phylotypic composition of the community.

IV. CONCLUSIONS

Lindstrom (1998) has suggested that freshwater systems should be more stable structurally than their marine counterparts because of the origin and composition of their respective organic matter pools, but this finding has not been substantiated. In freshwater systems a large pool of recalcitrant carbon is probably always present, which is capable of sustaining a background population, whereas in marine systems labile materials excreted by phytoplankton and other biotic breakdown products occur sporadically. Hence it may be more difficult to maintain a stable community structure in marine environments (see also Chapter 19). In a study of stability in methanogenic reactors they concluded that functional stability is possible even under conditions of changing community dynamics (Fernandez *et al*., 1999).

Functional processes are essentially the same in both freshwater and marine systems, the differences become apparent when the emphasis is placed on the source of the organic matter (OM). The source of this OM affects the rate at which it is used not the overall process of use (Wetzel, 2000). These rates differ because of the greater influx of humic or recalcitrant materials in inland systems versus the dominating inputs of autochthonous OM in marine systems. These studies show that bacterial populations do respond to differences in the DOM pool. The challenge now lies in increasing the scope of these studies to find patterns in the spatiotemporal distribution

of aquatic bacteria that can be linked to their ecological niche. We need to move from studies that focus on describing phylotypic diversity toward more integrated studies to explain these patterns of diversity. In addition to the consumption of DOM by bacteria in aquatic ecosystems, recent reports indicate that bacteria may play a crucial role in the production of refractory DOC (Ogawa *et al*., 2001).

The availability of organic and inorganic resources and removal of cells by grazing or viral loss are major mechanisms involved in bacterial production and mortality, and thus it is conceivable that these may also regulate microbial population dynamics and diversity (Weinbauer and Höfle, 1998). Hollibaugh (1994) suggested that there are probably phylogenetic differences between bacterial communities using different types of OM because of the different metabolic capabilities required to assimilate these various forms of OM. In addition to variations in resources driving changes in abundance of microbial populations, there are other factors that may also be important in structuring microbial communities. Physical forces such as dispersal, disturbance, salinity, pH, oxygen concentrations, and pressure all contribute to defining habitat conditions. Biotic forces such as competition, predation, and parasitism can also be potential regulators of community composition.

In the past, microbial diversity has been disregarded out of methodological necessity, but now we have the capabilities to open the "black box" and explore the linkages that exist between specific fractions of the microbial community and the niche they occupy. As we attempt to synthesize what is now known it becomes quite clear that we know very little definitively, and what we do know seems, at times, to be contradictory.

Nold and Zwart (1998) and Glöckner *et al*. (1999) have compiled comparisons between the diversity of bacterioplankton in lakes and oceans to compare patterns related to the genetic composition of the various phylotypes. Several cosmopolitan groups, such as α- and γ- Proteobacteria, can be found throughout both freshwater and marine environments, and in benthic as well as pelagic zones. Other groups such as β-Proteobacteria appear to be more common in freshwater environments whereas members of the CFB group are more prevalent in marine systems. For our purposes, in comparing the bacteria associated with particular fractions of the DOM pool the problem is not tractable anymore. If we were to break down the DOM into simple LMW fractions and complex HMW fractions and look for patterns in microbial assemblages based upon these carbon sources, we find that several species again appear to be widely distributed (Fig. 3), while others are more restricted. This begs the question, are the more abundant groups necessarily more important for DOM processing? Or, is it only certain groups that have an advantage in metabolizing DOM? Are consortial activities necessary or significant? What becomes apparent when trying to make generalizations of this sort is the lack of information that we have at present.

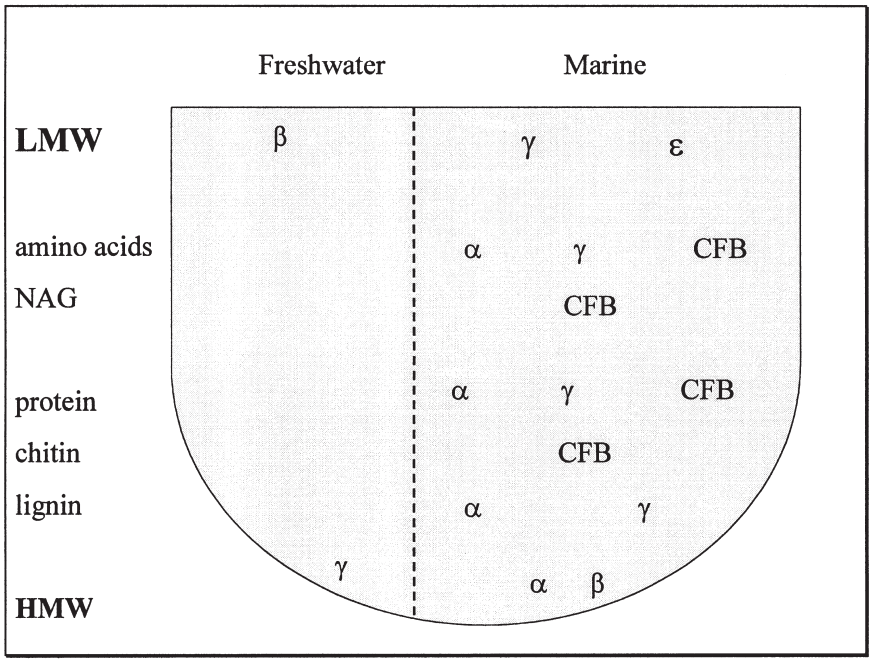

FIGURE 3 Distribution of bacterial groups implicated in the use of a variety of DOM components and size classes. α, β, γ, and ε refer to subclasses of the Proteobacteria; CFB indicates the *Cytophaga-Flavobacter-Bacteriodes* lineage NAG, *N*-acetylglucosamine.

Our future challenge is to conduct studies to test the many unanswered questions of microbial ecology, including the following: are particular species necessary and/or sufficient to perform specific functions within a community? What is the level, if any, of functional redundancy in a bacterial community? How rapidly do shifts in bacterial community structure occur in response to changing environmental conditions? What level of the phylogenetic hierarchy should we be looking at when trying to address these questions (i.e., communities, populations, individual groups, families, or the ill-defined species level)? And, what effect do lateral gene transfer mechanisms have on adaptations to variations in quantity and quality of DOM?

Martinus Beijerinck, one of the early pioneers of microbial ecology, stated, "everything is everywhere, the environment selects" (cited in Atlas and Barta, 1993). Our task is to determine what aspects of the particular environments are crucial to this selection process.

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REFERENCES

Acinas, S. G., J. Anton, and F. Rodríguez-Valera. 1999. Diversity of free-living and attached bacteria in offshore western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. *Applied and Environmental Microbiology* **65:**514–522.

Albright, L. J., J. B. Pernthaler, R. Amann, B. Sattler, F. O. Glöckner, A. Wille, and R. Psenner. 1996. Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. *Applied and Environmental Microbiology* **62:**2138–2144.

Amann, R. I., W. Ludwig, and K.- H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* **59:**143–169.

Atlas, R. M., and R. Barta. 1993. "Microbial Ecology Fundamentals and Applications." Benjamin/Cummins, New York, 561 pp.

Atlas, R. M., and R. Barta. 1998. "Microbial Ecology: Fundamentals and Applications," 4th ed. Benjamin/Cummings, Menlo Park, CA.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* **10:**257–263.

Bahr, M. J. E. Hobbie, and M. Sogin. 1996. Bacterial diversity in an arctic lake: A freshwater SAR11 cluster. *Aquatic Microbial Ecology* **11:**271–277.

Bockelmann, U., W. Manz, T. R. Neu, and U. Szewzyk. 2000. Characterization of the microbial community of lotic organic aggregates ('river snow') in the Elbe River of Germany by cultivation and molecular methods. *FEMS Microbiology Ecology* **33:**157–170.

Cottrell, M. T., and S. L. Kirchman. 2000. Natural assemblages of marine Proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Applied and Environmental Microbiology* **66:**1692–1697.

Covert, J. S. and M. A. Moran. 2001. Molecular characterization of estuarine bacterial communities using high- and low-molecular weight fractions of dissolved organic carbon. *Aquatic Microbial Ecology* **25:**127–139.

Crump B. C., and J. A. Baross. 2000. Characterization of the bacterially-active fraction in the Columbia River estuary. *Marine Ecology Progress Series* **206:**13–22.

Crump B. C., E. V. Armbrust, and J. A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. *Applied and Environmental Microbiology* **65:**3192–3204.

DeLong, E. F. 1992. Archaea in coastal marine environments. *Proceedings of the National Academy of Sciences* **89:**5685–5689.

DeLong, E. F., D. G. Franks, and A. L. Alldredge. 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnology and Oceanography* **38:**924–934.

Embley, T. M., and E. Stackebrandt. 1996. The use of 16S ribosomal RNA sequences in microbial ecology. *In* "Molecular Approaches to Environmental Microbiology". (R. W. Pickup, J. R. Saunders, Eds.), pp. 39–61. E. Horwood, New York.

Fernandez. A., S. Huang, S. Seston, J. Xian, R. Hickey, and J. Tiedje. 1999. How stable is stable? Function versus community composition. *Applied and Environmental Microbiology* **65:**3697–3704.

Field, K. G., D. Gordon, T. Wright, M. Rappé, E. Urbach, K. Vergin, and S. J. Giovannoni. 1997. Diversity and depth-specific distribution of SAR11 cluster rRNA genes from marine planktonic bacteria. *Applied and Environmental Microbiology* **63:**63–70.

Foreman, C. M. 1999. Dissolved organic carbon: Importance as a regulator of microbial communities. Doctoral dissertation, University of Toledo, Department of Biology, 292 pp.

Fuhrman, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* **399:**541–548.

Fuhrman, J. A., and A. A. Davis. 1997. Widespread Archaea and novel Bacteria from the deep sea as shown by 16S rRNA sequences. *Marine Ecology Progress Series* **150:**275–285.

Fuhrman, J. A., K. McCallum, and A. A. Davis. 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans. *Applied and Environmental Microbiology* **59:**1294–1302.

Giovannoni, S. J., and M. Rappé. 2000. Evolution, diversity, and molecular ecology of marine prokaryotes. *In* "Microbial Ecology of the Oceans" (D. Kirchman, Ed.)., Wiley Sons, New York.

Giovannoni, S. J., T. B. Brtischgi, C. L. Moyer, and K. G. Field. 1990. Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345:**60–63.

Glöckner, F. O., B. M. Bernhard, and R. Amann. 1999. Bacterioplankton compositions of lakes and oceans: A first comparison based on fluorescence in situ hybridization. *Applied and Environmental Microbiology* **65:**3721–3726.

González, J. M., W. B. Whitman, R. E. Hodson, and M. A. Moran. 1996. Identifying numerically abundant culturable bacteria from complex communities: An example from a lignin enrichment culture. *Applied and Environmental Microbiology* **62:**4433–4440.

González, J. M., R. E. Hodson, and M. A. Moran. 1999. Bacterial populations in replicate marine enrichment cultures: Assessing variability in abundance using 16S rRNA-based probes. *Hydrobiologia* **401:**69–75.

Hiorns, W. D., B. A. Methe, S. A. Nierzwicki-Bauer, and J. P. Zehr. 1997. Bacterial diversity in Adirondack Mountain lakes as revealed by 16S rRNA gene sequences. *Applied and Environmental Microbiology* **63:**2957–2960.

Höfle, M. G., H. Haas, and K. Dominik. 1999. Seasonal dynamics of bacterioplankton community structure in a eutrophic lake as determined by 5S rRNA analysis. *Applied and Environmental Microbiology* **65:**3164–3174.

Hollibaugh, J. T. 1994. Relationship between thymidine metabolism bacterioplankton community metabolic capabilities and sources of organic matter used for growth. *Microbial Ecology* **28:**117–131.

Hopkinson, C. S., B. Sherr, and W. J. Wiebe. 1989. Size fractionated metabolism of coastal microbial plankton. *Marine Ecology Progress Series* **51:**155–166.

Kenzaka, T., N. Yamaguchi, K. Tani, and M. Nasu. 1998. rRNA-targeted fluorescent in situ hybridization analysis of bacterial community structure in river water. *Microbiology* **144:**2085–2093.

Konopka, A., T. Bercot, and C. Nakatsu. 1999. Bacterioplankton community diversity in a series of thermally stratified lakes. *Microbial Ecology* **38:**126–135.

Lee, N., P. H. Nielsen, K. H. Andreasen, S. Juretschko, J. L. Nielsen, K.–H. Schleifer, and M. Wagner. 1999. Combination of fluorescenet *in situ* hybridization and microautoradiography – a new tool for structure-function analyses in microbial ecology. *Applied and Environmental Microbiology* **65:**1289–1297.

Leff, L. G. 2000. Longitudinal changes in microbial assemblages of the Ogeechee River. *Freshwater Biology* **43:**605–615.

Lindstrom, E. S. 1998. Bacterioplankton community composition in a boreal forest lake. *FEMS Microbiology Ecology* **27:**163–174.

Liu, W.-T., T. L. Marsh, H. Cheng, and L. J. Forney. 1997. Characterization of microbial diversity by determining terminal fragment length polymorphisms of genes encoding 16S rRNA. *Applied and Environmental Microbiology* **63:**4516–4522.

Manz, W., R. Amann, W. Ludwig, M. Vancanney, and K.-H. Schleifer. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum *Cytophaga-Flavobacter-Bacteroides* in the natural environment. *Microbiology* **142:**1097–1106.

Marsh, T. L. 1999. Terminal restriction fragment length polymorphism (T-RFLP): An emerging method for characterizing diversity among homologous populations of amplification products. *Current Opinion in Microbiology* **2:**323–327.

Massana, R., A. E. Murray, C. M. Preston, and E. F. DeLong. 1997. Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Applied and Environmental Microbiology* **63:**50–56.

Methe, B. A., W. D. Hiorns, and J. P. Zehr. 1998. Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes. *Limnology and Oceanography* **43:**368–374.

Methe, B.A., and J. P. Zehr. 1999. Diversity of bacterial communities in Adirondack lakes: Do species assemblages reflect lake water chemistry? *Hydrobiologia* **401:**77–96.

Moter, A., and U. B. Gobel. 2000. Fluorescent in situ hybridization (FISH) for direct visualization of microorganisms. *Journal of Microbiological Methods* **41:**85–112.

Muyzer, G. 1999. DGGE/TGGE a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology* **2:**317–322.

Nold, S.C., and G. Zwart. 1998. Patterns and governing forces in aquatic microbial communities. *Aquatic Ecology* **32:**17–35.

Ogawa, H., Y. Amagai, I. Koike, K. Kaiser, and R. Benner. 2001. Production of refractory dissolved organic matter by bacteria. *Science* **292:**917–920.

Ouverney, C. C., and J. A. Fuhrman. 1999. Combined microautoradiography–16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types in situ. *Applied and Environmental Microbiology* **65:**1746–1752.

Ouverney, C. C., and J. A. Fuhrman. 2000. Marine planktonic Archaea take up amino acids. *Applied and Environmental Microbiology* **66:**4829–4833.

Øvreås, L., L. Forney, F. L. Daae, and V. Torsvik. 1997. Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Applied and Environmental Microbiology* **63:**3367–3373.

Pace, N. R. 1997. A molecular view of the microbial diversity and the biosphere. *Science* **276:**734–740.

Parker, P. G., A. A. Snow, M. D. Schug, G. C. Booton, and P. A. Fuerst 1998. What molecules can tell us about populations: Choosing and using a molecular marker. *Ecology* **79:**361–382.

Pernthaler, J., F. O. Glockner, S. Unterholzner, A. Alfreider, R. Psenner, and R. Amann. 1998. Seasonal community and population dynamics of pelagic bacteria and Archaea in a high mountain lake. *Applied and Environmental Microbiology* **64:**4299–4306

Pinhassi, J., F. Azam, J. Hemphälä, R. A. Long, J. Martinez, U. L. Zweifel, and Å. Hagström. 1999. Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquatic Microbial Ecology* **17:**13–26.

Polz, M. F., and C. M. Cavanaugh. 1998. Bias in template-to-product ratios in multitemplate PCR. *Applied and Environmental Microbiology* **64:**3724–3730.

Pomeroy, L. R. 1974. The ocean's food web: A changing paradigm. *Bioscience* **24:**499–504.

Reichenbach, H. 1989. *In* "Bergey's Manual of Systematic Bacteriology, Vol. 3" (Staley, J. T., M. P. Bryant, N. Pfennig, and J. G. Holt, Eds.), pp. 2015–2021. Lippincott Williams & Wilkins, Baltimore.

Schmidt, T. M., E. F. DeLong, and N. R. Pace. 1991. Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. *Journal of Bacteriology* **173:**4371–4378.

Smith, S. V., and J. T. Hollibaugh. 1993. Coastal metabolism and the oceanic organic carbon balance. *Reviews of Geophysics* **31:**75–89.

Suzuki, M. T. and S. J. Giovanonni. 1996. Bias caused by template annealing in the application of mixtures of 16SrRNA genes by PCR. *Applied and Environmental Microbiology* **62:**625–630.

Thomas, J. D. 1997. The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshwater Ecology* **38:**1–36.

Thurman, E. M. 1986. "Organic Geochemistry of Natural Waters." Junk, Dordrecht.

Tuomi, P., T. Torsvik, M. Heldal, and G. Bratbak. 1997. Bacterial population dynamics in a meromictic lake. *Applied and Environmental Microbiology* **63:**2181–2188.

Urbach, E., K. L. Vergin, L. Young, A. Morse, G. L. Larson, and S. J. Giovannoni. 2001. Unusual bacterioplankton community structure in ultra-oligotrophic Crater Lake. *Limnology and Oceanography*. **46:**557–572.

van Hannen, E. J., G. Zwart, M. P. van Agterveld, H. J. Gons, J. Ebert, and H. J. Laanbroek. 1999. Changes in bacterial and eukaryotic community structure after mass lysis of filamentous *Cyanobacteria* associated with viruses. *Applied and Environmental Microbiology* **65:**795–801.

Van Omman Kloeke, F., and G. G. Geesey. 1999. Localization and identification of populations of phosphatase-active bacterial cells associated with activated sludge flocs. *Microbial Ecology* **38:**201–214.

Verity, P. G., and V. Smetacek. 1996. Organism life cycles, predation, and the structure of the marine pelagic ecosystems. *Marine Ecology Progress Series* **130:**277–293.

Voytek, M. A., B. B. Ward, and J. C. Priscu. 1998. The abundance of ammonium-oxidizing bacteria in Lake Bonney, Antarctica determined by immunofluorescence, PCR and in situ hybridization. *In* "Ecosystem Dynamics in a Polar Desert. The McMurdo Dry Valleys, Antarctica" (J. C. Priscu, Ed.), pp. 217–228. Am. Geophys. Union, Washington, DC.

Wagner, M., R. Erhart, W. Manz, R. Amann, H. Lemmer, D. Wedi, and K.-H Schleifer. 1994. Development of an rRNA-targeted oligonucleotide probe specific for the genus Acinetobacter and its application for in situ monitoring in activated sludge. *Applied and Environmental Microbiology* **60:**792–800.

Ward, D. M., R. Weller, and M. M. Bateson. 1990. 16S rRNA sequences reveal uncultured microorganisms in a natural community. *Nature* **345:**63–65.

Ward, D. M., M. M. Bateson, R. Weller, and A. L. Ruff-Roberts. 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. *Advances in Microbial Ecology* **12:**219–286.

Weinbauer, M. G., and M. G. Höfle. 1998. Distribution and life strategies of two bacterial populations in a eutrophic lake. *Applied and Environmental Microbiology* **64:**3776–3783.

Weiss, P., B. Schweitzer, R. Amann, and M. Simon. 1996. Identification in situ and dynamics of bacteria on limnetic organic aggregates. *Applied and Environmental Microbiology* **62:**1998–2005.

Wetzel, R. G. 2001. "Limnology-Lake and River Ecosystems." Academic Press, San Diego, 850 p.

Woese, C. R. 1987. Bacterial evolution. *Microbiological Reviews* **51:**221–271.

Woese, C. R. 1994. There must be a prokaryote somewhere: Microbiology's search for itself. *Microbiological Reviews* **58:**1–9.

Zwart, G., D. Hiorns, B. A. Methe, M. P. Van Agterveld, R. Huismans, S. C. Nold, J. P. Zehr, and H. L. Laanbroek. 1998a. Nearly identical 16S rRNA sequences recovered from lakes in North American and Europe indicate the existence of clades of globally distributed freshwater bacteria. *Systematic and Applied Microbiology* **21:**546–556.

Zwart, G., R. Huismans, M. P. van Agterveld, Y. Van de Peer, P. DeRijk, H. Eenhourn, G. Muyzer, E. J. van Hannen, H. J. Gons, and H. J. Laanbroek. 1998b. Divergent members of the bacterial division Verrucomicrobiales in a temperate freshwater lake. *FEMS Microbiology Ecology* **25:**159–169.

15

Bacterial Response to Variation in Dissolved Organic Matter

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I. INTRODUCTION

Dissolved organic matter (DOM) is often the largest input of organic carbon to aquatic ecosystems, and benthic or pelagic bacteria presumably mediate many of the transformations of DOM into biomass or inorganic components. Changes in the quantity or composition of DOM may affect several aspects of bacterial metabolism, including growth rates, respiration, enzymatic degradation and demand for inorganic nutrients. Both the rate of supply and the composition of the DOM pool are highly variable and controlled by a variety of extrinsic and intrinsic factors. Most of our information on supply and variability deals with the carbon component, that is, the dissolved organic carbon (DOC), whereas other elements (nitrogen in particular) may have different sources (see Chapter 11). A few general patterns relating to DOC supply have emerged, for instance, that soil type and the actual flow path of water through the soil profile control the

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concentration of DOC delivered to streams (Boyer *et al*., 1997; Brooks *et al*., 1999). Also, the relative abundance of phytoplankton and their grazers, or the seasonal pattern of littoral macrophyte growth and senescence, will influence autochthonous DOC (see Chapter 1). There can be rapid changes in DOM following precipitation (Easthouse *et al*., 1992), seasonal changes, even in large aquatic systems such as the Hudson River (Findlay *et al*., 1996), and longer term regional trends in DOM (Forsberg, 1992).

II. BACTERIAL RESPONSE VARIABLES

Recognizing the dynamic nature of DOM inputs and composition, it becomes important to know which of the microbial response variables are most sensitive to variation in DOM quantity or composition. Differences in the absolute magnitude of the response variables will suggest how DOM variation might affect net ecosystem retention of DOM versus loss to transport or respiration. Specifically, if the response of production is small relative to the response of respiration, then new DOM inputs are more likely to be respired rather than retained as microbial biomass with potential transfer to higher trophic levels. Also, the processes linked to microbial transformation of DOM such as demand for inorganic nutrients may be more or less strongly affected by variations in DOM supply. Bacterial immobilization of inorganic nutrients during decomposition of organic material depends strongly on the stoichiometry of the organic substrate while at the system level; heterotrophic demand for inorganic nutrients is related to the total quantity of decomposable organic carbon (see Chapter 11).

Aside from differences in the absolute magnitude of variation in bacterial production, respiration, degradative ability, and so on, the relative range in these response variables delimits the relative importance of factors other than organic carbon supply in regulation of the various processes. For instance, if production only varies half as widely as respiration in response to some manipulation or gradient of DOM, it suggests that production may be more tightly controlled by exogenous factors such as temperature (e.g., Pomeroy *et al*., 1991), whereas respiration has the scope to vary over a wider range. Such a difference in responsiveness among variables might also suggest that the physiological processes underlying production are more tightly constrained than the processes underlying respiration. The converse observation, that is, production varies more than respiration, would have different implications, but the point is that examination of both the absolute and the relative response of several microbial processes can contribute to our understanding of the consequences of fluctuations in DOM. Such fluctuations in DOM supply are now widely recognized, but historical views of DOM as a relatively refractory material have hindered consideration of biological

consequences. Moreover, future environmental change, driven by climate, land use, or other processes, will broaden the existing range of DOM inputs to aquatic ecosystems.

In this chapter, I consider two independent, but potentially covarying variables that may affect microbial responses: the bulk concentration of DOM and the known or inferred composition of that DOM. For clarity of presentation, concentration and composition are treated as being independent, but one of the pressing questions is whether there is any relationship between these important variables. There are at least two potential patterns relating bulk DOM concentration and the fraction capable of supporting bacterial metabolism (Fig. 1). If one assumes differences in DOM concentration are largely driven by additions of relatively hard-to-metabolize compounds such as humic materials, then increases in concentration will be accompanied by relatively small increases in the bioavailable fraction. The other extreme assumes increases in concentration are caused by inputs of either more of the compounds already present or novel, assimilable compounds. Then it seems reasonable to expect a linear or power function to relate the bioavailable quantity to bulk concentration. This scenario, increasing compositional diversity as concentration increases, is analogous to the initial slope of a species-area curve, because as bulk DOM concentration increases a greater diversity of compounds becomes abundant enough to support

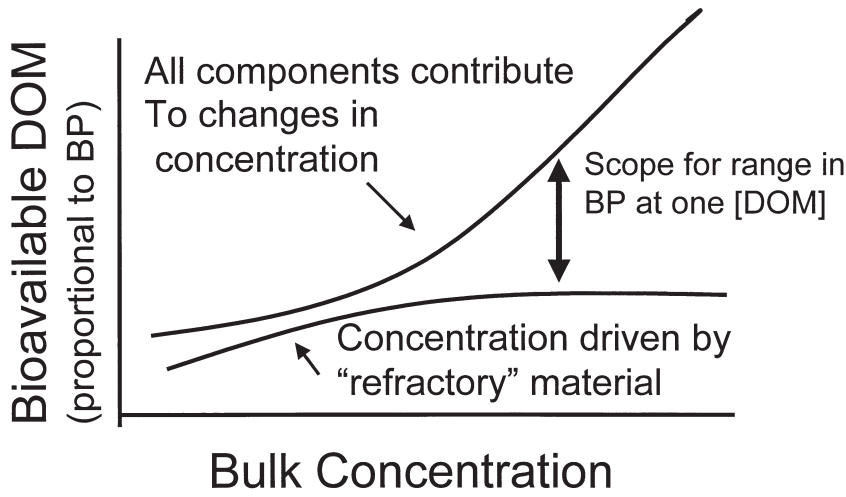

FIGURE 1 Potential relationships between bioavailable DOM and bulk DOM. The lower curve assumes increases in bulk DOM are largely driven by the addition of relatively refractory material; the upper curve assumes increases in bulk concentration are due to the addition of either novel compounds or compounds similar to those already present. The space between the two curves allows microbial metabolism to vary widely within a narrow range of bulk DOM concentration.

microbial metabolism. In contrast, if the increase in concentration is driven by increases in less available compounds, production will increase slightly, if at all. Obviously, production must eventually plateau due to inorganic nutrient limitation or other factors. For comparison the cross-system dataset analyzed by Søndergaard and Middleboe (1995) would appear as a straight line with a slope of roughly 0.2. The differences in predicted bioavailable DOM across concentration gradients under these scenarios may, in part, explain the disparate observations of bulk DOM effects on microbial metabolism. At low concentrations, there may be a significant increase in bioavailable carbon (and bacterial production) as concentration increases, but the divergence between curves at higher bulk concentrations allows for a multitude of patterns between bulk DOM and bacterial growth.

The effects of variation in DOM concentration and/or composition can be examined via comparative studies of systems known (or suspected) to vary in the type of DOM being supplied or by direct manipulation (usually addition) of specific dissolved compounds.

The dependent variables to be considered include production of new bacterial cells, respiration, demand for inorganic nutrients, and shifts in degradative activity (Fig. 2). Production and respiration are often looked at together by calculating a total assimilation or bacterial growth efficiency but are treated separately here because they do not necessarily covary linearly and may be constrained by different factors. All the response variables are quantitatively important processes in most aquatic ecosystems. For instance, bacterial secondary production can be as large or greater than primary production and seems to be at least 30% of primary production in planktonic systems (Cole *et al.*, 1988). Similarly, the heterotrophic immobilization of

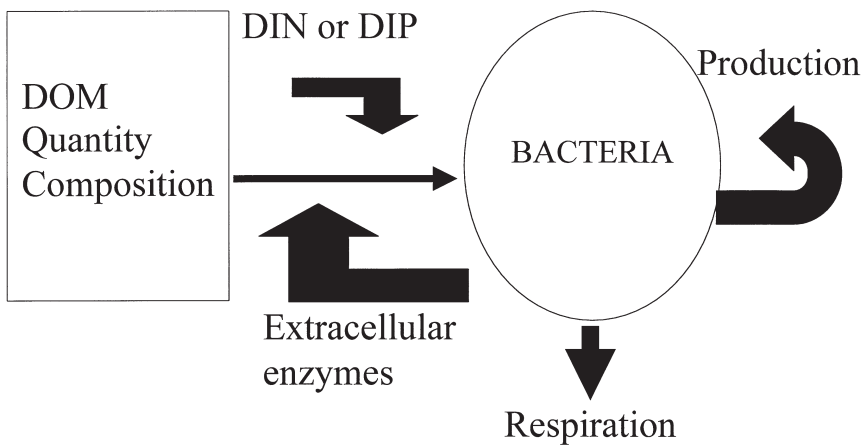

FIGURE 2 Schematic of independent (DOM quantity and composition) and dependent variables (nutrient demand, bacterial production and respiration, and degradative activity).

inorganic nutrients can partially control nutrient fluxes in aquatic systems (Caraco *et al*., 1998) and affects the availability of those dissolved compounds for other trophic levels (Bratbak and Thingstad, 1985).

A. Growth Rate

In part because DOM is recognized as a major component of material flow in aquatic ecosystems, there have been many attempts to link its abundance (concentration) with the dynamics of microbial communities. In the tidal freshwater Hudson River, we have nearly 10 years of data on both DOM and bacterial production collected at temporally intensive and spatially distributed stations (Findlay *et al*., 1996). Despite a roughly four-fold range in DOM concentration, there is no correlation between bulk DOM and bacterial production (Fig. 3). Comparing among systems, Coffin *et al*. (1993) present values for bacterial production and bulk DOM for a range of nearshore marine ecosystems and also report no correlation. Similarly, Kelly *et al*. (1999) found no correlation between total DOM and bacterial growth when examining variation among water masses. del Giorgio and Davis (Chapter 17) found no correlation between the proportion of available carbon and the absolute concentration. Søndergaard and Middleboe (1995) found that the proportion of labile carbon did not vary with total DOC concentrations across a 15-fold range in bulk DOC.

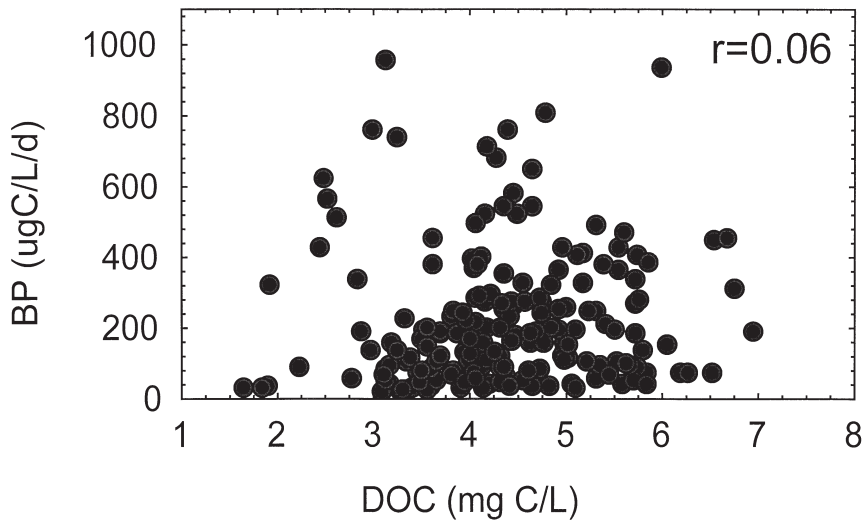

FIGURE 3 Relationship between Hudson River planktonic bacterial production and ambient bulk DOC. Data are derived from biweekly sampling of a single station near Kingston, New York over a 10-year span.

Various dilution experiments have been used to evaluate relationships between concentration and production in the absence of any potential change in composition. Amon and Benner (1996) isolated high-molecular-weight DOM from a nearshore Gulf of Mexico sample and made three different dilutions to directly examine concentration effects in the absence of any difference in DOM composition. They found a roughly linear increase in bacterial production and respiration with increasing concentration although the slopes for both processes were less than 1 : 1. These results suggest bacteria growing under these conditions were carbon limited and could take advantage of higher DOM concentrations although the fact that the slope was less than 1 suggests the bioavailable fraction was substantially smaller than the total pool size. Sobczak (1999) diluted stream water from a site known to consistently exhibit DOM removal and metabolism along hyporheic flow paths. He found that the proportional removal of DOM by sediment biofilm communities was unaffected by dilution, with 13% DOM removal at ambient concentration and 16% removal when stream water was diluted to half the ambient concentration. This result together with the observation that *in situ* loss rates were correlated with ambient concentrations (Findlay and Sobczak, 1996) suggests that concentration governs the initial removal step, perhaps via concentration-dependent adsorption to biofilm surfaces.

Where there have been positive correlations between bulk DOM and microbial activity, these are often associated with new inputs that both increase the ambient concentration and are likely to supply novel compounds. For instance, Kaplan and Bott (1989) show diel periodicity in bacterial growth as stream water DOM increases in mid-afternoon. The rise in DOM apparently originates as organic exudates from benthic algae and many of these compounds are thought to be highly available to heterotrophic bacteria (see Chapter 1). In another example, McKnight *et al.* (1993) showed an increase in planktonic bacteria during a DOM flush occurring at the time of snowmelt in the Rocky Mountains. This event, although increasing background DOM concentration, also represents an input of fresh organic matter and thus is a compositional shift as well as a quantitative change.

Overall, although there are examples of increasing bacterial production in sites of higher DOM concentration (Biddandah *et al.*, 1994), many of these differences are associated with differences in additional factors such as compositional changes, temperature, or nutrient availability and most cannot be solely related to increased concentration. Modeling approaches suggest the rate of supply rather than the ambient concentration is the predominant factor controlling bacterial production (see Chapter 18), but, in general, we do not have sufficient data on loading rates and subsequent metabolism to properly test this hypothesis. The inability to consistently document a correlation between bulk DOM and bacterial production has led researchers to conclude either that bulk DOM is unavailable for microbial assimilation and/or that variation in specific, possibly minor, components and constituents

regulates the metabolism of bulk DOM by microbes (see Chapters 4 and 9; Weiss and Simon, 1999; Marmonier *et al.*, 1995).

Two general approaches have been applied to reveal potential connections between specific components of bulk DOM and bacterial metabolism. The first approach uses natural variability in DOM over time or space to look for corresponding variation in bacterial growth. The second approach uses manipulations (almost always additions, but see Foreman *et al.*, 1998) of specific compounds to generate variability in DOM composition. Examining bacterial response to natural variability in DOM usually presumes that the locations (or times) sampled vary in the relative contribution of DOM from different sources. For example, Sun *et al.* (1996) measured bacterial growth and DOM composition in longitudinal transects down the Ogeechee River, Georgia, to ascertain which components of terrestrial DOM were being degraded during transport to the sea. They found a correlation between the relative abundance of aliphatic carbon and bacterial growth rates, suggesting that this class of dissolved organic matter was particularly important in supporting the planktonic bacterial community. Using a combination of experiments and natural variation, Sobczak (1999) collected water from different stream ecosystems and used replicated mesocosms to simulate hyporheic flow paths. There was differential removal of DOM from stream water along these flow paths and corresponding differences in bacterial production (Fig. 4; see also Chapter 12). Bacterial production per unit carbon supplied was actually highest for the stream with intermediate DOM concentration;

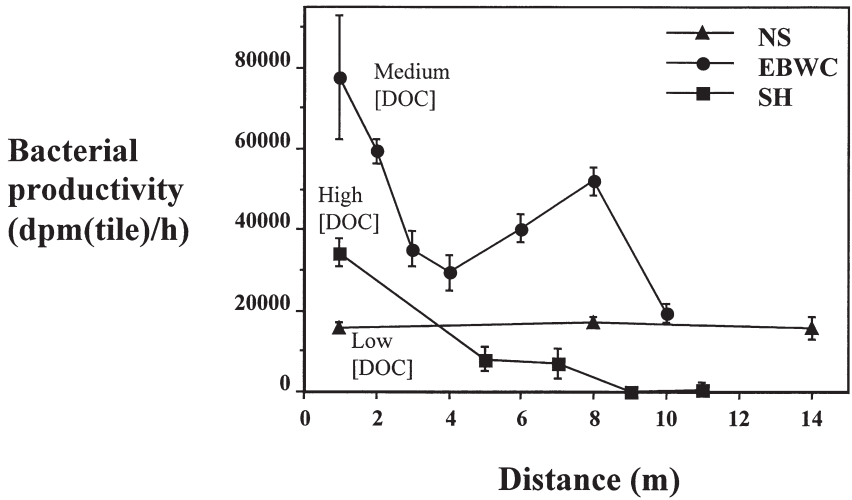

FIGURE 4 Patterns of bacterial growth along hyporheic flow paths varying in concentration of DOM. The integrated production along a flow path is not directly related to initial stream water DOM, highlighting the importance of differences in composition among streams.

supporting the idea that individual components are more important than bulk concentration, in regulating bacterial growth.

Experimental manipulations of specific compounds have proven powerful for identifying carbon limitation in aquatic microbial communities. In most cases, addition of presumably labile DOM (simple sugars, amino acids) has been found to stimulate bacterial growth. For example, Carlson and Ducklow (1996) added simple amino acids and glucose to Sargasso Sea water and found several-fold increases in net bacterial production. These increases in bacteria occurred with glucose additions despite vanishingly low concentrations of inorganic nutrients.

B. Respiration

Aside from adding defined compounds, experimental additions of natural DOM mixtures suspected to vary in lability have helped test ideas about the contribution of various DOM sources to aquatic ecosystems. In a nice example using manipulation of natural DOM sources, Battin *et al.* (1999) used flowthrough microcosms to measure the relative uptake rates of allochthonous and autochthonous DOM by stream sediments. They documented greater than fivefold differences or more in uptake and respiration, depending on whether the DOM was extracted from soil or periphyton. Moreover, they were able to show, via transplant experiments, several cases where prior exposure to a particular source of DOM increased the ability of that community to metabolize the DOM supplied. There appears to be some preadaptation of microbial catabolic capacity when these stream biofilms were re-exposed to a "familiar" type of DOM. Similarly, the response of heterotrophic bacteria to carbon or nutrient addition was greatest when the source community was particularly active (Foreman *et al.*, 1998). Kaplan *et al.* (1996) showed that fixed film bioreactors, colonized on one water source, were unable to rapidly metabolize DOC in water from another source.

The majority of studies examining natural variability or experimental manipulation suggest the response to specific DOM components (known or presumed) is much stronger than the response to changes in bulk concentrations. These findings underscore the need to identify which components of DOM are generating the metabolic heterogeneity we observe in the real world.

C. Enzymatic Degradation

The degradative capability of microbial communities should also be sensitive to variations in DOM supply and composition. The majority of bulk DOM is comprised of molecules too large for direct transport across cell

membranes so extracellular enzymes play a crucial role in carbon acquisition (see Chapter 13). Relative activities of a range of extracellular enzymes have been used to "fingerprint" DOM from different river systems (Hopkinson *et al*., 1998). Similarly, Christian and Karl (1995) used the range and absolute activity of β-glucosidase and leucine aminopeptidase across a latitudinal gradient of oceanic DOM to argue that high-latitude bacterial communities are more dependent on peptides and amino acids than equatorial communities. Use of extracellular enzyme activity (EEA) as fingerprints relies on the assumption that as the composition of the bulk DOM pool fluctuates, bacteria have the capacity to express a different set of enzymes to gain carbon from the new mixture. Foreman *et al*. (1998) added a wide array of model compounds to waters from the Maumee River and generally observed quick and dramatic increases in the enzymes responsible for degrading the polymeric substrates. For example, there was a three-fold increase in β-glucosidase activity following amendment of river water with cellobiose. These changes in EEA occur quickly, often as fast as cell turnover times. In microcosms of Hudson River water, activities of certain enzymes had increased manyfold in the first 16 hours since the addition of particular source waters (Findlay *et al*., 1998). This suggests the extant community had the capacity to induce these enzymes and their synthesis did not require (but may be enhanced by) growth of previously dormant cells or replacement of species. This view presumes small quantities of extracellular enzymes ("scout" enzymes) are always present, allowing monomer products to induce further enzyme synthesis. More specialized enzymes have a more restricted distribution among taxa (Martinez *et al*., 1996) and so sufficient time for accumulation of cells capable of synthesizing these enzymes would be necessary before significant shifts in degradation of these substrates could occur. It seems reasonable that if the compounds responsible for fluctuations in DOM composition are degraded by enzymes widely distributed among bacterial taxa the ability to respond to such fluctuations will be high and compounds are likely to be metabolized. Alternatively, if fluctuations in DOM are due to novel compounds or those requiring enzymes restricted to a few taxa or consortia, not abundant in the extant community, these compounds may well escape metabolism and downstream transport of these compounds create links among ecosystems (see Chapter 4).

The time scale of responsiveness clearly affects the ability of a microbial community to respond to dynamic fluxes of DOM and a major unanswered question is how common is the necessity for a taxonomic shift before detectable changes in degradative ability are evident? Also, the time scale of observation will dictate which of these responses are documented, with short-term bioassays probably reflecting the extant community, whereas seasonal changes may include taxonomic replacement. Any particular question will have a logical time scale and this should govern the time span under consideration.

D. Demand for Inorganic Nutrients

Dissolved organic matter is often low in nitrogen or phosphorus relative to microbial cell N and P quotas and so complete metabolism of the organic carbon component would require an external nutrient source. In actuality, only a fraction of the total organic carbon can be metabolized on time scales relevant to terrestrial–freshwater–estuarine transport processes (see Chapter 17). Because the available carbon is less than the bulk carbon, the imbalance between C : N (or C : P) of the bulk DOM pool and microbial cells is not as wide as the gross C : N : P analyses of DOM might suggest. In fact, the C : N ratio of DOM has proven to be a powerful predictor of the growth efficiency of planktonic bacteria (Kroer, 1993), suggesting that much of the organic nitrogen in DOM is at least as available as the organic carbon compounds. The apparent dependence of DOM degradation on external nutrients varies widely among experiments, revealing the entire spectrum of results from strong stimulation to no net effect following the addition of N, P, or a combination of the two. For example, Brett *et al.* (1999) showed persuasively that the addition of dissolved inorganic phosphorus (DIP) greatly stimulated net bacterial production (measured as final bacterial biomass) in microcosms of Castle Lake water. This stimulation occurred in the dark pointing to a direct stimulation of bacterial growth rather than an indirect effect of enhanced phytoplankton production. In contrast, Carlson and Ducklow (1996) found no stimulation of bacterial growth in Sargasso Sea water following the addition of dissolved inorganic nitrogen (DIN) or DIP but found a large response to the addition of labile DOM. Surprisingly, the addition of inorganic nutrients to relatively nutrient-rich Hudson River water led to significant increases in bacterial production but no change in respiration (Roland and Cole, 1999). Moreover, alkaline phosphatase activity in the Hudson River is relatively high despite 0.5–1 μM SRP. The contrasting results in these extreme cases cannot be simply resolved by differences in background DIN or DIP.

Elser *et al.* (1995) conducted nutrient amendment experiments in a range of aquatic systems and frequently found stimulation of net bacterial production. In actuality, the degree of stimulation was much greater for lakes than for coastal waters, suggesting that nutrient supply to lakes will exert greater control over bacterial carbon metabolism than nutrient supply to coastal waters. The mechanism underlying this differential susceptibility to nutrient amendment is unclear (and the observation requires a more rigorous test), but it has significant consequences for carbon degradation during transport from terrestrial to freshwater to marine ecosystems.

Our present inability to predict where or when inorganic nutrients should (or should not) lead to large differences in bacterial growth suggests that our current understanding is inadequate and we need new models of the interaction among inorganic nutrients, organic matter composition, and microbial

consumers. Thingstad (see Chapter 16) shows that the dividing line between inorganic nutrient and carbon limitation in a model system is related to the intensity of grazing on the bacteria. Sinsabaugh and Foreman (Chapter 18) argue that the relative allocation of enzymes to carbon, nitrogen, or phosphorus acquisition is under energetic constraint and so not simply predictable from variables such as exogenous nutrient availability. The ability of heterotrophic microbes to obtain N and P (and other elements) from organic as well as inorganic sources allows for multiple nutrient sources and fluctuating controls on microbial growth (Schweitzer and Simon, 1995). Moreover, the "recycling effects" of grazers make it less likely that simple large-scale relationships between bacterial growth and inorganic nutrients will emerge. del Giorgio and Davis (see Chapter 17) could not find a relationship between the quantity of degradable carbon and ambient inorganic nutrients. Therefore, simple relationships between nutrients and bacterial growth are less likely than the well-known phosphorus–phytoplankton relationships.

III. TIME SCALE AND SCOPE OF BACTERIAL RESPONSE VARIABLES

Microbial communities have the capacity to respond in a quantitatively significant manner to a wide range of variations in DOM supply, and episodic variations in DOM are a common feature of ecosystems (e.g., Hinton *et al*., 1997). For both these reasons, it is necessary to consider the time scale and scope of the response variables. For purposes of discussion, I define the capacity of a particular process to respond to variation in DOM as the product of speed and scope. Speed is simply the response time for a given variable and would be measured as the percentage change per unit time. Bacterial cell division, requiring genome replication, might be slower than enzyme induction so growth would respond with less speed than degradative ability. Scope refers to the range of response, and different variables must be standardized (dividing the range by the mean) to remove differences in absolute magnitude. Variables that fluctuate rapidly over a broad range obvious have a greater capacity to affect DOM fluxes than processes responding slowly or over a narrow range. Clearly, if one variable is constrained (for whatever reason) to be less responsive than others, then the potential diversion of organic carbon among the pathways will be more restricted. For instance, if induction of extracellular enzymes is constrained by cell physiology or bacterial community composition, then infrequent inputs of macromolecular organic compounds will be less susceptible to degradation/assimilation in a particular habitat than if enzyme activity can respond rapidly and across a wide range. Similarly, if bacterial production has greater capacity for response than respiration, it is more likely that a pulse input of DOM can be diverted to new cell production rather than respiration.

Relevant time scales of response range from extremely rapid shifts in transport of monomers (seconds to minutes), through induction of extracellular enzymes, activation of dormant cells, or accumulation of bacterial cells (hours to days), and finally, probably slowest, shifts in bacterial community structure (10's of hours to many days). The speed at which extracellular enzyme activities can shift with variation in DOM is crucial for understanding/predicting the likelihood that macromolecules will be degraded in particular habitats. In a set of DOM manipulations, Foreman *et al.* (1998) and Findlay *et al.* (1998) found significant shifts in EEA within 24 h of the addition of new types of DOM. This relatively rapid response suggests that planktonic bacteria in a water mass have the capacity to degrade macromolecules during transport in large rivers or in most lentic systems. In small streams, the frequent contact between surface waters and bed sediments or hyporheic sediments offers the opportunity for abiotic adsorption to "hold" DOM in place, susceptible to later enzymatic attack (Freeman and Lock, 1995; Findlay and Sobczak, 1996; see also Chapter 12). The slowest response time for bacterial communities will occur when shifts in community structure are necessary for degradation of a DOM source. If explosive growth of an existing subpopulation or novel colonists is required to attack particular compounds or suites of compounds, then shifts in metabolic capacity are constrained to be slower than rates of population increase. Under natural conditions, such changes in relative or absolute cell abundance are likely to take 10's of hours to days or longer. Growth rate responses of individual populations (days to weeks) may be faster than the community-level integration (weeks to months) important for complete degradation of some DOM (see Chapter 12). The time scales for the various response variables considered here are fairly well understood, ranging from enzyme induction to shifts in community structure. Only for cases where a substantial shift in community structure is a prerequisite will the speed of response limit the capacity of microbes to respond to variability in DOM.

The second aspect of capacity for response is the scope or range. As argued above, if certain process variables are subject to multiple constraints, then it seems less likely that those pathways will be major diversions of DOM under conditions of variable DOM concentration or composition. For example, both the production and the respiration of planktonic bacteria generally correlate with phytoplankton production, but the slopes of the production and respiration relationships differ among systems. Using a wide array of data from the literature, del Giorgio and Cole (1998) show that growth responds over a narrower range than respiration and this leads to relatively lower bacterial growth efficiency in less productive ecosystems. Low bacterial growth efficiency at low net primary production (NPP) implies many unproductive systems will be net heterotrophic despite an apparent lack of major loads of allochthonous carbon.

For riparian–stream–river ecosystems, we do not have a large empirical database on bacterial respiration, growth, and degradative enzyme capacity

similar to the data available for planktonic systems. To look for patterns in the relative range of these variables, I will use a set of experimental results. Two of the four examples are from published works on manipulation of DOM to planktonic bacteria (Findlay *et al*., 1998; Foreman *et al*., 1998), whereas the other two deal with manipulations of DOM supply to sediment or biofilm communities. In one manipulation, a series of hyporheic sediment cores were perfused with either low-DOM spring water or moderate-DOM stream water to provide two different "background" DOM concentrations. Replicate cores were further amended with a presumably labile DOM mixture [glucose+bovine serum albumin (BSA)] or a more refractory DOM (tannic acid) providing six treatments (low and moderate background DOM, each with no further addition, labile addition, or "refractory" addition). As expected, the addition of organic carbon compounds yielded significant shifts in bacterial production, oxygen consumption, and extracellular enzyme activity, and the magnitude of response to organic matter amendments was greater for the spring water treatments than for the moderate-[DOM] stream water treatments. In a similar experiment, Fischer (see Chapter 12) showed that river biofilms preferentially use specific fractions of DOM, possibly from phytoplankton exudates. In the other manipulation, Findlay *et al*. (2001) took advantage of land use–driven differences in DOM to examine microbial response. Artificial substrates incubated in riparian groundwater collected from different flow path locations across three land uses showed significant differences in bacterial production and shifts in enzyme activities. Given the small dataset, it is wise to consider any conclusions as tentative, but the exercise is intended to illustrate what can be learned from considering the relative response ranges for these variables.

Comparing across DOM treatments for the hyporheic core experiments, the range in respiration was greater than the range in carbon production, whereas the ranges were roughly equal in Foreman *et al*.'s (1998) river DOM amendment study (Fig. 5). The range in production was greater than the range in respiration across types of riparian water. In these studies, five common enzyme activities were also measured (leucine aminopeptidase, phosphatase, esterase, β-glucosidase, and α-glucosidase), allowing a comparison of their relative responsiveness. Two patterns are evident, although the dataset is too small to justify any statistical analysis. All enzyme activities are as responsive and usually substantially more responsive than either production or respiration (Fig. 5). The enzymes potentially playing a role in nutrient acquisition (leucine aminopeptidase and phosphatase) show a large range, although most of these studies are conducted against a fairly high-nutrient background. These patterns again suggest an important interplay between carbon degradation and inorganic nutrient availability and the strength of this interaction does not seem consistently predictable from ambient DIN or DIP concentrations. The observation that enzymes are generally as variable, if not more variable, than cell growth or respiration suggests many communities have the capacity to alter their array of extra-

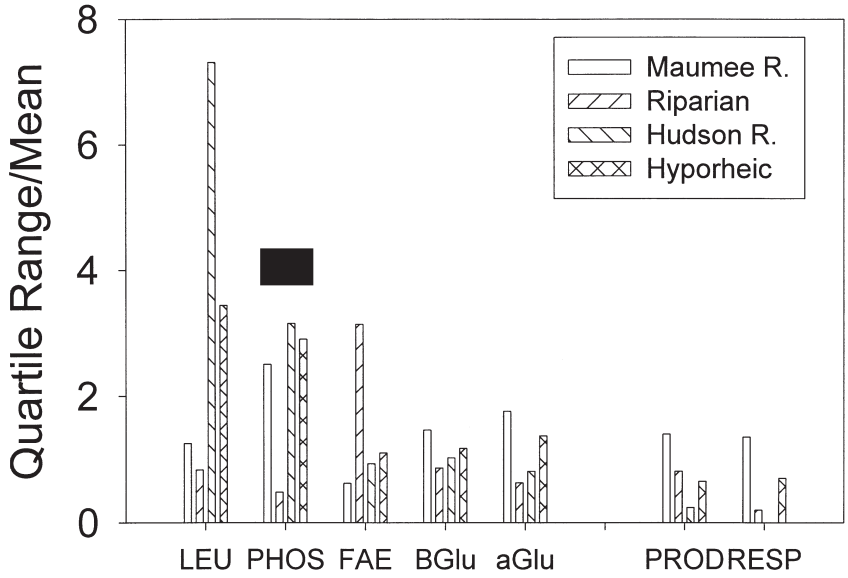

FIGURE 5 Relative scopes (measured as quartile range/median) for an array of extracellular enzymes and for bacterial production and respiration. Dependent variables shown along the X-axis are (left to right) leucine aminopeptidase, phosphatase, fatty acid esterase, β-glucosidase, α-glucosidase, bacterial production, and bacterial respiration. Data are derived from four DOM experimental amendments.

cellular enzymes without necessarily replacing the community. Therefore, extracellular enzyme activity (EEA) will be one of the more dynamic responses to fluctuations (in time or space) in DOM composition.

IV. CONCLUSIONS

1. The range in bulk DOM among aquatic ecosystems is only about one order of magnitude and appears (at best) weakly related to variability in bacterial growth and assimilation. In contrast, the apparent range in bacterial production at similar DOM can range over three orders of magnitude. Apparently, whatever process(es) constrain DOM concentrations have less effect on the relative abundance of specific components of DOM.

2. The availability of inorganic nutrients has significant effects on bacterial growth, even in ecosystems where ambient nutrient concentrations suggest they should not be limiting. Therefore, the role of DIN (DIP) in affecting bacterial growth will not be simply predictable and may be a function of DOM carbon composition, nutrient content, ambient inorganic nutrient concentration or prevalence of grazers.

3. Extracellular enzymes appear more responsive (greater scope) than bacterial production or respiration, implying that there is sufficient dynamic range for these enzymes to catabolize shifting sources of DOM. Experiments show these enzymes can change almost as quickly as cell growth, implying that there does not necessarily have to be a shift in bacterial community structure in order for enzymes to attack a shifting pool of DOM.

4. Of the commonly assayed enzymes, the two with the highest activity and greatest scope have a role in N or P acquisition. This pattern again suggests that the availability of inorganic or organic nutrients has a particularly strong regulatory role for degradative activity.

REFERENCES

Amon, R. W,. and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography* **41:**41–51.

Battin, T. J., A. Butturini, and F. Sabater. 1999. Immobilization and metabolism of dissolved organic carbon by natural sediment biofilms in a Mediterranean and temperate stream. *Aquatic Microbial Ecology* **19:**297–305.

Biddandah, B., S. Opsahl, and R. Benner. 1994. Plankton respiration and carbon flux through bacterioplankton on the Louisiana shelf. *Limnology and Oceanography* **39:**1259–1275.

Boyer, E. W., G. M. Hornberger, K. E. Bencala, and D. McKnight. 1997. Response characteristics of DOC flushing in an alpine catchment. *Hydrological Processes* **11:**1635–1647.

Bratbak, G., and T. F. Thingstad. 1985. Phytoplankton–bacteria interactions: An apparent paradox? Analysis of a model system with both competition and commensalism. *Marine Ecology Progress Series* **25:**23–30.

Brett, M. T., F. S. Lubnow, M. Villar-Argaiz, A. Müller-Solger, and C. R. Goldman. 1999. Nutrient control of bacterioplankton and phytoplankton dynamics. *Aquatic Ecology* **33:**135–145.

Brooks, P. D., D. M. McKnight, and K. E. Bencala. 1999. The relationship between soil heterotrophic activity, soil dissolved organic carbon (DOC) leachate, and catchment-scale DOC export in headwater catchments. *Water Resources Research* **35:**1895–1902.

Caraco, N. F., G. Lampman, J. J. Cole, K. E. Limburg, M. L. Pace, and D. Fischer. 1998. Microbial assimilation of DIN in a nitrogen rich estuary: Implications for food quality and isotope studies. *Marine Ecology Progress Series* **167:**59–71.

Carlson, C. A., and H. W. Ducklow. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. *Aquatic Microbial Ecology* **10:**69–85.

Christian, J. R., and D. M. Karl. 1995. Bacterial ectoenzymes in marine waters: Activity ratios and temperature responses in three oceanographic provinces. *Limnology and Oceanography* **40:**1042–1049.

Coffin, R. B., J. P. Connolly, and P. S. Harris. 1993. Availability of dissolved organic carbon to bacterioplankton examined by oxygen utilization. *Marine Ecology Progress Series* **101:**9–22.

Cole, J. J., S. Findlay, and M. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: A cross-system overview. *Marine Ecology Progress Series* **43:**1–10.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic ecosystems, a review. *Annual Review of Ecology and Systematics* **29:**503–541.

Easthouse, K. B., J. Mulder, N. Christopherses, and H. M. Seip. 1992. Dissolved organic carbon fractions in soil and stream water during variable hydrological conditions at Birkenes, Southern Norway. *Water Resources Research* **28:**1585–1596.

Elser, J. J., L. B. Stabler, and R. P. Hassett. 1995. Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: A comparative study. *Aquatic Microbial Ecology* **9:**105–110.

Findlay, S., and W. V. Sobczak. 1996. Variability in removal of dissolved organic carbon in hyporheic sediments. *Journal of the North American Benthological Society* **15:**35–41.

Findlay, S., M. L. Pace, and D. Fischer. 1996. Spatial and temporal variability in the lower food web of the tidal freshwater Hudson River. *Estuaries* **19:**866–873.

Findlay, S., R. L. Sinsabaugh, D. T. Fischer, and P. Franchini, P. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* **1:**227–239.

Findlay, S., J. M. Quinn, C. W. Hickey, G. Burrell, and M. Downes. 2001. Effects of land-use and riparian flowpath on delivery of dissolved organic carbon to streams. *Limnology and Oceanography* **46:**345–355.

Foreman, C. M., P. Franchini, and R. L. Sinsabaugh. 1998. The trophic dynamics of riverine bacterioplankton: Relationships among substrate availability, ectoenzyme kinetics and growth. *Limnology and Oceanography* **43:**1344–1352.

Forsberg, C. 1992. Will an increased greenhouse impact in Fennoscandia give rise to more humic and colored lakes? *Hydrobiologia* **229:**51–58.

Freeman, C., and M. A. Lock. 1995. The biofilm polysaccharide matrix: A buffer against changing organic substrate supply? *Limnology and Oceanography* **40:**273–278.

Hinton, M. J., S. L. Schiff, and M. C. English. 1997. The significance of storms for the concentration and export of dissolved organic carbon from two precambrian shield catchments. *Biogeochemistry* **36:**67–88.

Hopkinson, C., I. Buffam, J. Hobbie, J. Vallino, R. Hodson, M. A. Moran, J. Covert, E. Smith, J. Baross, B. Crump, B. Eversmeyer, F. Prahl, M. Perdue, S. Findlay, and K. Foreman. 1998. Terrestrial inputs of organic matter to coastal ecosystems: an intercomparison of chemical characteristics and bioavailability. *Biogeochemistry* **43:**211–234.

Kaplan, L. A., and T. L. Bott. 1989. Diel fluctuations in bacterial activity on streambed substrata during vernal algal blooms: Effects of temperature, water chemistry, and habitat. *Limnology and Oceanography* **34:**718–733.

Kaplan, L. A., F. Ribas, J. C. Joret, C. Volk, J. Frias, and F. Lucena. 1996. "Measurement of Biodegradable Organic Matter with Biofilm Reactors." AWWA Research Foundation, Denver.

Kelly, C. A., J. D. Pakulski, S. I. H. Sandvik, R. B. Coffin, R. C. Downer, Jr., P. Aas, M. M. Lyons, and W. H. Jeffrey. 1999. Phytoplanktonic and bacterial carbon pools and productivities in the Gerlache Strait, Antarctica, during early Austral Spring. *Microbial Ecology* **38:**296–305.

Kroer, N. 1993. Bacterial growth efficiency on natural dissolved organic matter. *Limnology and Oceanography* **38:**1282–1290.

Marmonier, P., D. Fontvieille, J. Gibert, and V. Vanek. 1995. Distribution of dissolved organic carbon and bacteria at the interface between the Rhone River and its alluvial aquifer. *Journal of the North American Benthological Society* **14:**383–393.

Martinez, J., D. C. Smith, G. F. Steward, and F. Azam. 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquatic Microbial Ecology* **10:**223–230.

McKnight, D. M., R. L. Smith, R. A. Harnish, C. L. Miller, and K. E. Bencala. 1993. Seasonal relationships between planktonic microorganisms and dissolved organic material in an alpine stream. *Biogeochemistry* **21:**39–59.

Pomeroy, L. R., W. J. Wiebe, D. Deibel, R. J. Thompson, G. T. Rowe, and J. D. Pakulski. 1991. Bacterial responses to temperature and substrate concentration during the Newfoundland spring bloom. *Marine Ecology Progress Series* **75:**143–159.

Roland, F. and J. J. Cole. 1999. Regulation of bacterial growth efficiency in a large turbid estuary. *Aquatic Microbial Ecology* **20:**31–38.

Schweitzer, B., and M. Simon. 1995. Growth limitation of planktonic bacteria in a large mesotrophic lake. *Microbial Ecology* **30:**89–104.

Sobczak, W.V. 1999. Microbial metabolism of dissolved organic carbon in stream hyporheic zones. Ph.D. Dissertation, Cornell University, Ithaca, New York.

Søndergaard, M., and M. Middleboe. 1995. A cross-system analysis of labile dissolved organic carbon. *Marine Ecology Progress Series* **118:**283–294.

Sun, L., E. M. Perdue, J. L. Meyer, and J. Weis. 1996. Using elemental composition to predict bioavailability of dissolved organic matter in a Georgia River. *Limnology and Oceanography* **42:**714–721.

Weiss, M., and M. Simon. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: The relative significance of amino acids and carbohydrates. *Aquatic Microbial Ecology* **17:**1–12.

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SECTION

THREE

APPROACHES TO SYNTHESIS

16 Physiological Models in the Context of Microbial Food Webs

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I. INTRODUCTION

The question of turnover of dissolved organic matter (DOM) has so many aspects that most models focus only on some limited subset of all the relevant issues. With emphasis on the consumption aspect, there are models concentrating on the trophic mechanisms controlling bacterial biomass and growth rate (Thingstad *et al*., 1997), on bacterial production of ectoenzymes hydrolyzing polymers to monomers (Billen and Fontigny, 1987), or on how growth yield changes under different growth conditions (Vallino *et al*., 1996). On the DOM production side, there is the potential for at least one model for each of the many mechanisms of production suggested. This includes passive leakage (Bjørnsen, 1988) or active excretion (Myklestad *et al*., 1989;

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Obernosterer and Herndl, 1995) from phytoplankton; release by viral lysis (Bratbak *et al*., 1992; Middelboe *et al*., 1996; Gobler *et al*., 1997; Bratbak *et al*., 1998); and egestion, excretion, or sloppy feeding from zooplankton (Fenchel, 1982; Jumars *et al*., 1989). To understand DOM dynamics, both the consumption and the production side must be understood. To get closer to such an integrated view on system functioning, we must consider the interface between food web models and models focusing on bacterial physiology.

II. TROPHIC MECHANISMS CONTROLLING BACTERIAL CONSUMPTION

Fundamental to any model for bacterial DOM consumption is the equation

$$\mathrm{BCD} = \mu B / Y_{\mathrm{BC}}, \tag{1}$$

defining bacterial carbon demand BCD as the ratio between bacterial production μB and bacterial yield Y_{BC} on the carbon substrates used. Production is the product of a specific growth rate μ and biomass B, and yield Y_{BC} is the number of biomass units formed per unit of carbon substrate consumed. A full understanding of how BCD varies thus requires an understanding of the environmental conditions influencing each of μ, B, and Y_{BC}.

Although many discussions on this theme have a tendency to, explicitly or implicitly, assume growth rate to be carbon limited in natural aquatic environments, there is an increasing body of evidence supporting the idea that this is not necessarily the case in the photic zone. There are many reports of P-limited bacteria in P-deficient areas (Thingstad *et al*., 1993; Pomeroy *et al*., 1995; Cotner *et al*., 1997; Rivkin and Anderson, 1997; Thingstad *et al*., 1998; Zohary and Robarts, 1998). There is recent evidence of N-limited bacteria in the deep chlorophyll maximum of an N-deficient area (Skoog *et al*., 1999), and a suggestion of bacteria being Fe limited in Fe-deficient areas (Tortell *et al*., 1996). A hypothesis for the mechanisms leading to shifts between carbon and mineral-nutrient-limited growth can be analyzed using an idealized food web structure as in Figure 1 and assuming simple Lotka–Volterra equations for the trophic interactions between the functional groups of organisms (Thingstad *et al*., 1997). Assuming mineral nutrient limitation of bacterial growth rate, food consumption to be proportional to food concentration, and steady state in the part of the system contained within the shaded area of Figure 1, bacterial production can be shown to be proportional to the square of ciliate biomass C (Thingstad *et al*., 1997). The squared relationship can intuitively be understood when realizing that, in the food web of Figure 1, increasing ciliate biomass will have two multiplicative effects on bacterial production: one effect on bacterial growth rate μ via predation on the phytoplankton competitor to the bacteria, and one

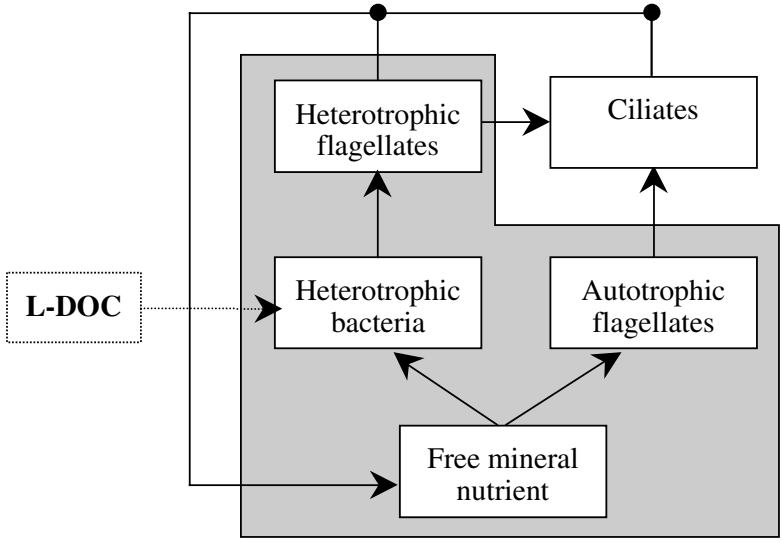

FIGURE 1 Idealized microbial food web illustrating the flow of a growth-limiting element from the free mineral form into heterotrophic bacteria and phytoplankton, here represented by autotrophic flagellates. The limiting element is further transported into heterotrophic flagellates and ciliates, representing the phagotrophic predators on the two groups of osmotrophs. In the analysis in the text, the part inside the shaded area is assumed to be in internal steady state. Growth rates of heterotrophic bacteria are assumed to be potentially limited by either labile dissolved organic matter (L-DOC) or by the free mineral nutrient.

on bacterial biomass B via predation on the bacterivorous heterotrophic flagellates. The formulas derived by Thingstad *et al.* (1997) are

$$\mathrm{BCD}_{\mathrm{N}}^{*} = (Y_{\mathrm{BC}} Y_{\mathrm{H}})^{-1} \frac{\alpha_{\mathrm{B}}}{\alpha_{\mathrm{A}} \alpha_{\mathrm{H}}} \delta_{\mathrm{A}} \delta_{\mathrm{H}} = (Y_{\mathrm{BC}} Y_{\mathrm{H}})^{-1} \frac{\alpha_{\mathrm{B}}}{\alpha_{\mathrm{A}} \alpha_{\mathrm{H}}} \alpha_{\mathrm{C}}^{2} C^{2}, \tag{2}$$

where Y_{H} is the yield of heterotrophic flagellates on bacterial prey and α_{B}, α_{A}, α_{H}, and α_{C} are the affinity constants or clearance rates of bacteria, auto- and heterotrophic flagellates, and ciliates, respectively. The second equality sign uses the assumption that ciliates prey unselectively on auto- and heterotrophic flagellates so that their specific loss rates δ_{A} and δ_{H} are both equal to $\alpha_{\mathrm{C}} C$. The superscript * and subscript N on BCD are used to indicate the assumptions of steady state and mineral-nutrient-limited bacterial growth rate, respectively.

The formula for $\mathrm{BCD}_{\mathrm{N}}^{*}$ in Eq. (2) was derived under the assumption that the bacterial growth rate was mineral nutrient limited. If the supply rate of labile organic carbon from allochthonous and autochthonous sources is insufficient to meet this demand, the pool of labile dissolved organic carbon (DOC) will eventually be depleted and the bacteria will become carbon

limited. This implies that in an autotroph–heterotroph succession where biomass is shifted from phytoplankton to their ciliate predators, the squared dependence on ciliate biomass should lead to an accelerating increase in BCD_N [Eq. (2)]. A shift to carbon-limited bacterial growth would then be expected if the system's autochthonous production of organic substrates for bacterial growth does not increase as rapidly with ciliate biomass as does the potential bacterial carbon demand. After a transition period, one would thus expect a new situation where the carbon demand of carbon-limited bacteria BCD_C^* equals the supply rate ψ_C of labile DOC : $BCD_C^* = \psi_C$. Any more exact evaluation of the timing of the shift in bacterial growth rate limitation would obviously require an understanding of how ψ_C varies with shifts in food web structure.

It is illustrative to summarize the above analysis in the dimensionless number $\eta = \dfrac{BCD_N^*}{BCD_C^*}$, which for the simple model in Figure 1 becomes

$$\eta = (Y_{BC}Y_H)^{-1}\frac{\alpha_B}{\alpha_A\alpha_H}\frac{\alpha_A\alpha_H}{\psi}. \tag{3}$$

Values of $\eta > 1$ thus correspond to steady states with carbon-limited bacterial growth rate, whereas values of $\eta < 1$ correspond to mineral nutrient limitation. Equation (3) illustrates how "everything depends on everything" in a steady-state situation. The value of η is a function not only of the ratio between production rate of organic bacterial substrates and the product of loss rates of the bacterial predators and competitors to higher predators, but also of all the parameters representing physiological properties of bacteria, bacterial competitors, and bacterial predators.

This way of thinking uses a technique referred to as pseudo or hybrid steady-state modeling. It combines the assumption that part of the system (free mineral nutrient concentration, bacteria, and auto- and heterotrophic flagellates) is in internal steady state, with the assumption that the conditions for this steady state change over time. In this case, there are two variable conditions: the total amount of limiting element available for distribution within the steady-state part of the system and the rate at which the limiting element is drawn out of the steady-state part by an increasing ciliate popuation. The steady state may thus shift over time. In the case of Eq. (2), this means that ciliate biomass is a function of time $C = C(t)$. The technique combines the strength of steady-state models allowing analytical expressions like Eq. (2) with a description of system changes over time. Although the use of such a hybrid technique is seldom stated explicitly, it is used in almost any dynamic model. In ecological models, the use of Michaelis–Menten kinetics for nutrient uptake is a typical example where the familiar relationship is derived under the assumption that the enzyme–substrate complex is in pseudo steady state with respect to the external nutrient concentration. Intuitively, there are also longer time constants involved in such systems

linked to, for example, seasonal shifts in temperature, nutrient loss by sedimentation balancing nutrient import to the photic zone, and changes in the N : P ratio based on nitrogen fixation and denitrification. The border between the dynamic part and the steady-state part would change, depending on the focus of interest and temporal perspective in each case. In the present case, the condition for the subdivision in Figure 1 must be that the system of nutrients, bacteria, and flagellates must have a fast internal dynamics compared to the changes in ciliate biomass. Despite the highly idealized nature of these models, meso- (Thingstad *et al*., 1999a) and microcosm (Thingstad *et al*., 1999b) experiments indicate that such an approach may provide a reasonable first-order approximation to microbial dynamics. This seems to suggest that they contain at least some of the basic principles of trophic control of bacterial production.

With the highly idealized food web structure of Figure 1, it is easy to find situations where more details are needed to account for observed food web behavior. An important example is the occurrence of phytoplankton not consumed by ciliates. A case of particular interest would be diatoms, which, when silicate is available and in the absence of an efficient predator population, can potentially invade the system in Figure 1. This would allow mineral nutrient competition to remain independent of a buildup of ciliate biomass. In such a situation, silicate availability would thus be expected to have a negative effect on bacterial carbon demand. Such a slightly expanded perspective can be represented by adding diatoms and copepods to the food web of Figure 1 as suggested in Figure 2. In the case of the bacterial and diatom growth rate being limited by the same mineral nutrient, and the part of the food web inside the shaded area is in approximate internal equilibrium, bacterial production becomes proportional to the square of mesozooplankton biomass (Thingstad, 2000a). The theoretical consequence is that

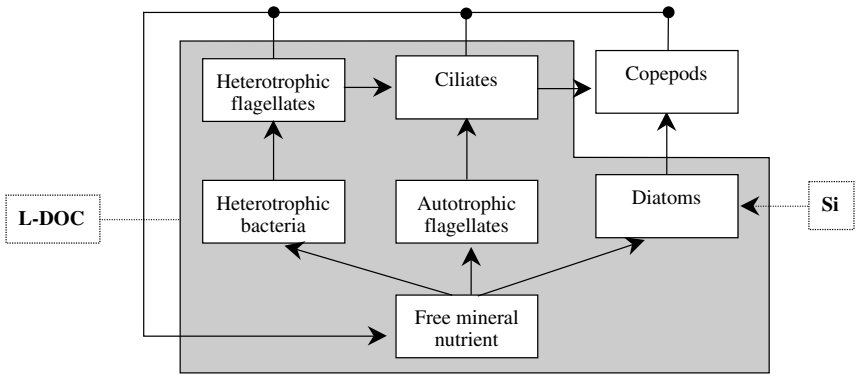

FIGURE 2 Expansion of the model in Figure 1 by adding a silicate-requiring diatom competitor for the mineral nutrient and copepods as a predator on diatoms and ciliates.

the dynamics for changes in BCD_N is driven by the long characteristic time scale for mesozooplankton development, rather than by the fast dynamics of ciliate growth. Interestingly, this also gives a situation where labile organic carbon and silicate have symmetric roles in the food web, restricting the flow of mineral nutrients through the "microbial" and "classical" sides of the food web, respectively (Thingstad and Rassoulzadegan, 1999).

III. ORGANIC FORMS OF LIMITING ELEMENT (LABILE DISSOLVED ORGANIC NITROGEN AND DISSOLVED ORGANIC PHOSPHORUS)

In the idealized models of Figures 1 and 2, we assumed mineral nutrients and organic carbon to come from chemically independent substrate sources. Particularly in the case of nitrogen, being carried by substances like amino acids, this is unrealistic and we need to look into the effect of incorporating organic forms of the mineral nutrients into the structure. For bacteria with a biomass carbon : nitrogen ratio of $(C:N)_B$ consuming an organic substrate with carbon : nitrogen ratio $(C:N)_S$, respiring a fraction ρ of the carbon and incorporating the rest $(1-\rho)$, mass balance gives the stoichiometric relationship:

$$\Delta N_I = \frac{\Delta C}{(C:N)_S} - (1-\rho)\frac{\Delta C}{(C:N)_B}, \tag{4}$$

where ΔN_I is the amount of inorganic nitrogen forms excreted (if positive) or consumed (if negative) when an amount ΔC of organic carbon is consumed (Parnas, 1975; Billen, 1984; Goldman *et al.*, 1987). If we measure bacterial biomass in terms of its N content, we have $Y_{BC} = (1-\rho)(C:N)_B^{-1}$, and we can rewrite Eq. (4) into a form linking it more closely to the previous discussion:

$$BND_I = (Y_{BC} - (C:N)_S^{-1})BCD, \tag{5}$$

where BND_I is bacterial demand for (or production of) inorganic nitrogen. These purely mass balance considerations do not, however, take into explicit consideration the effects of a possible phytoplankton–bacteria competition for the mineral nutrient. To take a closer look at this, we use the food web in Figure 3 where there is a supply rate ψ_N of DON, utilizable by bacteria only. Carbon demand of N-limited bacteria consuming all available N then becomes

$$BCD_N^* = Y_{BC}^{-1}(\psi_N + Y_H^{-1}\frac{\alpha_B}{\alpha_A\alpha_H}\alpha_C^2\, C^2). \tag{6}$$

The bacteria will remain nitrogen limited as long as BCD_N^* is smaller than the carbon supply rate $\psi_C + (C:N)_S\psi_N$. The surplus production of organic carbon would accumulate at a rate R:

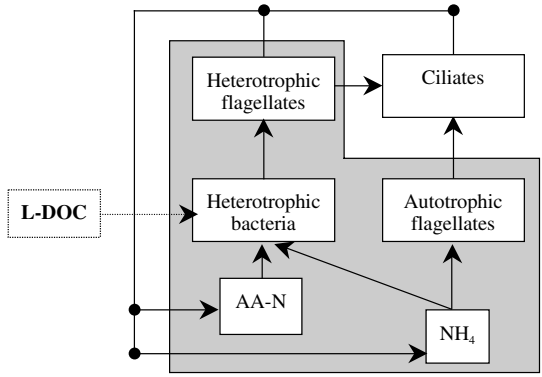

FIGURE 3 Expanding the model in Figure 1 by adding an organic form of the limiting mineral nutrient, assumed here to be amino acids only consumed by the heterotrophic bacteria.

$$R = \psi_C + (C:N)_S\psi_N - Y_{BC}^{-1}(\psi_C + Y_H^{-1}\frac{\alpha_B}{\alpha_A\alpha_H}\alpha_C^2\, C^2) \quad \text{for } R \geq 0. \qquad (7)$$

Equation (7) thus gives a theory for the ability of N-limited bacteria to consume the potentially degradable organic carbon produced as a function of the production rates for labile DOC and DON and of algal-bacterial competition for mineral forms of nitrogen.

The effect of the DON supply is given by the two middle terms in Eq. (7), which combined give $((C:N)_S - Y_{BC}^{-1})\psi_N$. Because this contribution may be positive or negative, the organic nitrogen supply ψ_N may shift the transition point between nitrogen- and carbon-limited bacterial growth to either higher or lower values of ciliate biomass, as compared to the situation with ψ_C as the only supply of organic C. Again, one would need a model for ψ_C and ψ_C to give a more detailed theory for the transition point.

IV. BACTERIAL PHYSIOLOGY

In the idealized framework outlined above, bacterial physiology is represented by two parameters only. The yield coefficient Y_{BC} gives the stoichiometric link between consumption of organic carbon and the limiting mineral nutrient, whereas the specific affinity constant α_B defines the volume of water cleared for limiting mineral nutrient per unit bacterial biomass, per unit time.

A. What Determines α_B?

In a model where biomass is defined as the cell content of the limiting element, uptake = growth (disregarding possible complications due to leak-

age from the cells). The specific affinity constant α_B is the proportionality constant between substrate concentration and substrate uptake (growth). In a Michaelis–Menten representation of growth versus substrate concentration, the affinity constant would correspond to the slope μ_{max}/K at zero substrate concentrations, μ_{max} being the maximum growth rate and K the half saturation constant. One can use the argument that by decreasing substrate concentration sufficiently, one will eventually reach a level where the cell's uptake system is efficient enough to capture all molecules that reach the cell surface. The rate-limiting step will then be the physical process of diffusion (Jumars *et al.*, 1993). A spherical cell of radius r will then have a specific affinity constant:

$$\alpha_B = \frac{3D}{\sigma r^2}, \tag{8}$$

where σ is the volume-specific content of the limiting nutrient (Thingstad and Rassoulzadegan, 1999). If the cells are diffusion limited, there is thus nothing to gain for the cell by constructing more refined uptake systems; the only way to improve the uptake is by reducing σ, reducing r, or modifying the shape to increase the surface : volume ratio. Based on experiments with uptake of carbon sources, Koch and Wang (1982) found bacteria not to be subject to diffusion limitation. Looking at orthophosphate uptake, Thingstad and Rassoulzadegan (1999) suggested, however, that bacteria in P-limited marine environments might be closer to diffusion limitation than indicated from Koch's work with carbon substrates. The recent claim that concentration of bioavailable orthophosphate in P-limited freshwater systems is less than 1 nmol P L^{-1} (Hudson *et al.*, 2000) is consistent with such a view. The specific growth rate of a P-limited osmotroph using orthophosphate at concentration N as the only P source is $\mu = \alpha N$. A growth rate of 1 day^{-1} and an orthophosphate concentration of 1 nmol P^{-1} L^{-1} imply an osmotroph affinity constant $\alpha \approx 0.04$ L nmol P^{-1} h^{-1}. This is close to what one would estimate from Eq. (8) for a small spherical phytoplankton cell of 1 μm radius and a biomass with Redfield C : P composition (Thingstad and Rassoulzadegan, 1999). Bacteria would be expected to have a smaller radius, but a higher specific P content. Using Eq. (8), a radius of one half that of the phytoplankton above combined with a doubling of specific P content would correspond to a theoretical affinity constant $\alpha_B \approx 0.04\ 2^2/2 = 0.08$ L nmol P^{-1} h^{-1}.

An interesting aspect of Eq. (8) was discussed by Jumars *et al.* (1993). The only factor directly influenced by temperature is the diffusion constant D, which is a function of water viscosity. The temperature dependence of this physical process is less than the Q_{10} of 2.7–3 normally found for biological processes. In our expression for BCD_N^* [Eq. (2)], however, such an argument would apply to both α_B in the numerator and α_A in the denominator; the effect on affinity constants would therefore be expected to cancel out. From the preceding arguments, clearance rates and affinity constants

could have different temperature dependence. Because there is one clearance rate (α_H) in the denominator and two (α_C^2) in the numerator, Eq. (2) suggests that the effect of temperature on BCD_N^* might follow that of clearance rates, rather than that of affinity constants. This example illustrates the importance of being able to distinguish between direct effects on organisms, as opposed to indirect effects mediated through food web interactions.

B. Variations in the Yield Coefficient Y_{BC}

The expression $Y_{BC} = (1 - \rho)(C:N)_B^{-1}$ for bacterial yield (in units of biomass N formed per units of substrate C consumed) summarizes how yield is a function of both respiration and biomass composition. That both the respiration coefficient ρ and the biomass composition $(C:N)_B$ change with growth rate is well known from classical chemostat studies. During carbon-limited growth, the yield measured in biomass carbon units decreases at low growth rates because an increasing fraction of the substrate is used for maintenance purposes. Under mineral nutrient limitation, the yield in cell units increases because the cell quota of the limiting element normally decreases with decreasing growth rate (Veldkamp, 1976), and at least in the case of N limitation, there can be an increase in the C : N ratio due to a storage of C-rich intracellular reserve materials (Dawes and Senior, 1973).

With a variable Y_{BC}, the last term in Eq. (7) becomes variable, and the distinct transition point between mineral-nutrient-limited and carbon-limited growth disappears. In such a case, one would get a transition zone in which an increase in, for example, the supply rate ψ_C of labile DOC would lead to a decrease in Y_{BC}, rather than in a positive value for the accumulation rate R. The existence of such a transition zone can be demonstrated in chemostat cultures with bacteria. An example is the work by Pengerud *et al.* (1987) where *Pseudomonas putida* was grown in chemostats arranged along a gradient with increasing glucose concentration in the medium reservoir, the potentially limiting mineral nutrient being phosphate. With C limitation, the molar ratio of glucose-C : phosphate-P consumption was 140, meaning that all P was consumed for a reservoir C : P ratio greater than 140. There was, however, no free glucose observed in the culture until medium C : P increased beyond 300, corresponding to a factor of more than 2 between the smallest and largest value of Y_{BC}. This study also included chemostats with a heterotrophic nanoflagellate as bacterial predator, with the diatom *Skeletonema costatum* as a bacterial competitor, and with all three in combinations. Except for the ciliate predator, this was thus an experimental model of the idealized food web in Figure 1. The results confirmed not only the existence of a "flexibility zone" for Y_{BC}, but also the theoretical prediction that bacterial consumption of labile DOC (glucose) should be restricted in the three-species community where bacteria are "sandwiched" between predatory control of their biomass and competitive control of their growth rate.

Considering nitrogen, phosphorus, and organic carbon together, one can also show that there is a range for substrate $(C : N : P)_s$ ratios for which all three substrates will be depleted (Martinussen and Thingstad, 1987; Thingstad, 1987). The extent and placement of this range differ for different species (Martinussen and Thingstad, 1987). A mixed natural community would be expected to have an even larger flexibility due either to the combined effect of coexisting species or to the replacement of dominating species in the transition regions between types of limitation. If there are homeostatic mechanisms such as nitrogen fixation/denitrification that drive the systems toward stoichiometric balance (Tyrrell, 1999), one would expect that the occurrence of such multiple nutrient depletion is more the rule than the exception. Special biogeochemical conditions or strongly biasing anthropogenic influences are perhaps necessary to drive the systems away from this state.

Flexibility in Y_{BC} can be modeled in different ways. The classical model of Pirt (1982) or its modification by Cajal-Medrano and Maske (1999) gives descriptions of the yield, allowing for both carbon- (energy) and mineral-nutrient-limited growth. Touratier *et al.* (1999) derived a model of bacterial growth, taking into account the effects on yield of both a variable C : N ratio in the substrate and a variable growth rate. Another approach, which gives a good fit to many observations, is to adapt a Droop-type formulation (Droop, 1974), linking growth rate to cell quota of the limiting nutrient (Nyholm, 1976; Vadstein *et al.*, 1988). Because organic carbon is respired for both growth and maintenance purposes, adaptation of the Droop formulation requires some special consideration (Thingstad, 1987). Although they are able to mimic the observed flexibility, such formulations are not really providing much insight into the underlying cellular mechanisms. The most complete model of bacterial growth focusing on these aspects is probably the one by Vallino *et al.* (1996), which considers bacterial growth as an optimization problem with constraints posed by substrate concentration, free energy, electron balance, and C : N ratio.

V. DISSOLVED ORGANIC MATTER PRODUCTION SIDE

In the preceding discussions, we assumed labile DOC and DON to be produced at rates ψ_C and ψ_N, respectively, without discussing their sources and how the production rate and the composition of the produced material would be expected to vary with food web structure. The important differences among different models can be illustrated by some examples. One potential model is that DOC production is an overflow mechanism occurring in mineral-nutrient-limited phytoplankton not able to use the photosynthetically produced organic carbon for biomass production due to lack

of N and/or P (Williams, 1990; see also Chapter 1). This would lead to the situation where phytoplankton produce C-rich bacterial substrates under conditions where they have to compete with the bacteria for mineral nutrients (Bratbak and Thingstad, 1985). Whether this is the mechanism, or there is a passive leakage from phytoplankton (Bjørnsen, 1988), any production dominated by phytoplankton would imply that production is reduced in the phase of an autotroph–heterotroph succession where biomass distribution shifts away from phytoplankton. In our food web from Figure 1, reduction in DOC production due to lowered phytoplankton biomass would coincide in time with the increase in potential for its consumption due to increasing ciliate biomass [the C^2 relationship of Eq. (2)]. A shift to carbon limitation of the bacteria in this phase would thus be a fairly robust feature of such models. If, on the other hand, production increases with predator biomass or with predation rate, production and consumption potential increase together, making the timing of a shift to C limitation more difficult to predict. Also, predatory processes and viral lysis presumably release organic material resembling biomass in chemical composition, whereas excretion from phytoplankton may, at least in principle, be dominated by C-rich compounds. If viral lysis is a quantitatively important part of the production process, the situation is even less clear because there is no generally accepted theory for the mechanisms controlling the partitioning of loss between predation and viral lysis. Using a simple model that combines unselective protozoan predation with host-specific lytic bacteriophages, one can derive a theory where loss by viral lysis compensates for the difference in growth rate between coexisting bacteria with different nutrient affinities (Thingstad, 2000b). The partitioning of bacterial loss between the two processes then becomes a function of the difference in bacterial nutrient affinities. Such a theory thus links DOC release to diversity in a somewhat unanticipated manner: The amount of bacterial production being recirculated via a "viral loop" back to bacterial substrates (Bratbak *et al.*, 1992) becomes a function of differences in physiological properties (nutrient affinities) inside the bacterial community. It is then the existence of lytic viruses that allows the coexistence of different bacteria limited by the same substrate, but it is the difference in nutrient affinities between the coexisting bacteria that determines viral abundance and the amount of bacterial production being shunted away from the predatory food chain.

In the present situation, it is a rather unattractive idea to try to construct a detailed mathematical description of all the suggested mechanisms for DOM production. The expectation would be that this could rapidly lead into a jungle of poorly supported hypotheses, poorly quantified parameters, and a dependence on species composition, which is another poorly defined aspect of the present models. A possible alternative would be to explore the use of more aggregated descriptions. Two simple alternative hypotheses of this kind would be that production is either (1) proportional to plankton

biomass or (2) proportional to the rate of transfer of matter between the components of the food web. Because rates in Lotka–Volterra formulations scale as the square of biomass (e.g., as concentration of prey × concentration of predator), hypothesis (2) would lead to an expected increase in production more or less proportional to the square of plankton biomass. Because C-limited bacteria would be expected to have a BCD_C equal to the system's production rate of labile DOC, a squared relationship between biomass and DOC production implies that we can construct a theory for how bacterial production varies as a function of (a) how much plankton biomass there is in the food web and (b) how this is distributed between functional groups. The result, based on the idealized food web of Figure 1, is qualitatively sketched in Figure 4, where bacterial production by carbon-limited bacteria increases as the square of total plankton biomass, whereas production of mineral-nutrient-limited bacteria increases as the square of ciliate biomass. The realized bacterial production for a given combination of total biomass and ciliate biomass is the minimum of the two.

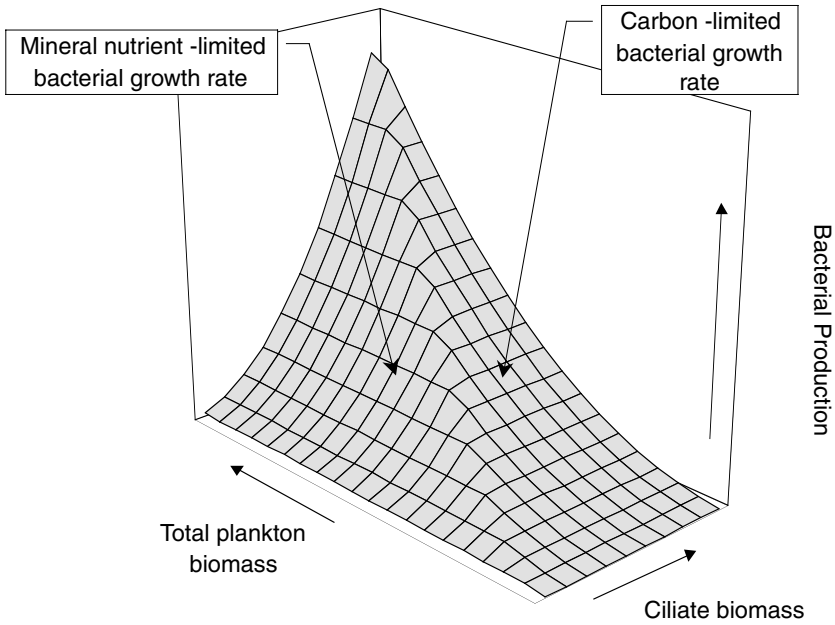

FIGURE 4 Integrated model for bacterial production illustrating qualitatively the suggested proportionality with the square of total plankton biomass for C-limited bacteria, and proportionality with the square of ciliate biomass for mineral-nutrient-limited bacteria. In this model, C-limited growth occurs for food web structures with a high ciliate : total plankton biomass.

VI. CONCLUSIONS

This chapter illustrates how idealized models can be used to analyze the way in which food web mechanisms control whether bacteria become carbon or mineral nutrient limited. This could be summarized in a dimensionless "limitation index" η [Eq. (3)], where values of $\eta < 1$ correspond to situations of mineral nutrient limitation and values of $\eta > 1$ to carbon (energy) limitation. Accumulation of labile DOC occurring for $\eta < 1$ would imply that an unused resource is available in the system. When bacterial yield on the carbon substrates is assumed constant, a well-defined transition between the two types of limitation occurs for $\eta = 1$. A variable bacterial yield can, however, be caused at the cell level by changes in the fraction of substrate respired or by changes in biomass composition, as well as at the community level by changes in species composition. Flexibility in yield may thus be perceived as a kind of homeostatic mechanism allowing the system to remain in a state with $\eta = 1$, exploiting the otherwise unused resource of accumulating organic substrates. Flexible yield thus allows for an extended range of states that combine bacteria close to mineral nutrient limitation with consumption of all labile DOC. Beyond the borders of this range of homeostasis, degradable organic substrates may accumulate or bacteria may become carbon limited. A better description of these transitions will require the combination of more sophisticated physiological models with food web models that contain realistic descriptions of the rate and quality at which labile organic substrates are produced. Because systems being inside or close to these transition zones may turn out to be the rule rather than the exception, the ecological importance of such models may be great.

ACKNOWLEDGMENT

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REFERENCES

Billen, G. 1984. Heterotrophic utilization and regeneration of nitrogen. *In* "Heterotrophic Activity in the Sea" (J. E. Hobbie, and P. J. LeB., Williams, Eds.), pp. 313–356. Plenum, New York.

Billen, G., and A. Fontigny. 1987. Dynamics of a *Phaeocystis* dominated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. *Marine Ecology Progress Series* **37:**249–257.

Bjørnsen, P. K. 1988. Phytoplankton exudation of organic matter: Why do healthy cells do it? *Limnology and Oceanography* **33:**151–154.

Bratbak, G., and T. F. Thingstad. 1985. Phytoplankton–bacteria interactions: An apparent paradox? Analysis of a model system with both competition and commensalism. *Marine Ecology Progress Series* **25:**23–30.

Bratbak, G., M. Heldal, T. F. Thingstad, B. Riemann, and O. H. Haslund. 1992. Incorporation of viruses into the budget of microbial C-transfer. A first approach. *Marine Ecology Progress Series* **83:**273–280.

Bratbak, G., A. Jacobsen, and M. Heldal. 1998. Viral lysis of *Phaeocystis pouchetii* and bacterial secondary production. *Aquatic Microbial Ecology* **16:**11–16.

Cajal-Medrano, R., and H. Maske. 1999. Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton — revisiting the Pirt model. *Aquatic Microbial Ecology* **19:**119–128.

Cotner, J. B., J. W. Ammerman, E. R. Peele, and E. Bentzen. 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. *Aquatic Microbial Ecology* **13:**141–149.

Dawes, E., and P. Senior. 1973. The role and regulation of energy reserve polymers in microorganisms. *Advances in Microbial Physiology* **10:**135–266.

Droop, M. R. 1974. The nutrient status of algal cells in continuous culture. *Journal of the Marine Biological Association* **55:**825–855.

Fenchel, T. 1982. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. *Marine Ecology Progress Series*. **8:**225–231.

Gobler, C. J., D. A. Hutchins, N. S. Fisher, E. M. Cosper, and S. A. Sañudo-Wilhelmy. 1997. Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. *Limnology and Oceanography* **42:**1492–1504.

Goldman, J. C., D. A. Caron, and M. R. Dennett. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C : N ratio. *Limnology and Oceanography* **32:**1239–1252.

Hudson, J. J., W. D. Taylor, and D. W. Schindler. 2000. Phosphate concentration in lakes. *Nature* **406:**54–56.

Jumars, P. A., D. L. Penry, J. A. Baross, M. J. Perry, and B. W. Frost. 1989. Closing the microbial loop: Dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. *Deep Sea Research* **36:**483–495.

Jumars, P., J. Deming, P. Hill, L. Karp-Boss, P. L. Yager, and W. Dade. 1993. Physical constraints on marine osmotrophy in an optimal foraging context. *Marine Microbial Food Webs* **7:**121–161.

Koch, K., and C. Wang. 1982. How close to the theoretical diffusion limit do bacterial uptake systems function? *Archives of Microbiology* **131:**36–42.

Martinussen, I., and T. F. Thingstad. 1987. Utilization of N, P, and organic C by heterotrophic bacteria. II. Comparison of experiments and a mathematical model. *Marine Ecology Progress Series* **37:**285–293.

Middelboe, M., N. O. G. Jorgensen, and N. Kroer. 1996. Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. *Applied and Environmental Microbiology* **62:**1991–1997.

Myklestad, S., O. Holm-Hansen, K. M. Vørum, and B. E. Volcani. 1989. Rate of release of extracellular amino acids and carbohydrates from the marine diatom *Chaetocheros affinis*. *Journal of Plankton Research* **11:**763–773.

Nyholm, N. 1976. A mathematical model for microbial growth under limitation by conservative substrates. *Biotechnology and Bioengineering* **18:**1043–1056.

Obernosterer, I., and G. Herndl. 1995. Phytoplankton extracellular release and bacterial-growth — dependence on the inorganic N–P ratio. *Marine Ecology Progress Series* **116:**247–257.

Parnas, H. 1975. Model for decomposition of organic material by microorganisms. *Soil Biology and Biochemistry* **7:**161–169.

Pengerud, B., E. F. Skjoldal, and T. F. Thingstad. 1987. The reciprocal interaction between degradation of glucose and ecosystem structure. Studies in mixed chemostat cultures of marine bacteria, algae, and bacterivorous nanoflagellates. *Marine Ecology Progress Series* **35:**111–117.

Pirt, S. J. 1982. Maintenance energy: A general model for energy-limited and energy-sufficient growth. *Archives of Microbiology* **133:**300–302.

Pomeroy, L. R., J. E. Sheldon, W. M. J. Sheldon, and F. Peters. 1995. Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. *Marine Ecology Progress Series* **117:**259–268.

Rivkin, R., and M. Anderson. 1997. Inorganic nutrient limitation of oceanic bacterioplankton. *Limnology and Oceanography* **42:**730–740.

Skoog, A., B. Biddanda, and R. Benner. 1999. Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. *Limnology and Oceanography* **44:**1625–1633.

Thingstad, T. F. 1987. Utilization of N, P, and organic C by heterotrophic bacteria. I. Outline of a chemostat theory with a consistent concept of maintenance metabolism. *Marine Ecology Progress Series* **35:**99–109.

Thingstad, T. F. 2000a. Control of bacterial growth in idealised food webs. *In* "Microbial Ecology of the Ocean" (D. L. Kirchman, Ed.), pp. 229–259. Wiley, New York.

Thingstad, T. F. 2000b. Elements of a theory for the mechanisms controlling abundance, diversity and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnology and Oceanography* **45:**1320–1328.

Thingstad, T. F., and F. Rassoulzadegan. 1999. Conceptual models for the biogeochemical role of the photic zone food web, with particular reference to the Mediterranean Sea. *Progress in Oceanography* **44:**271–286.

Thingstad, T. F., E. F. Skjoldal, and R. A. Bohne. 1993. Phosphorus cycling and algal–bacterial competition in Sandsfjord, western Norway. *Marine Ecology Progress Series* **99:**239–259.

Thingstad, T. F., Å. Hagstrøm, and F. Rassoulzadegan. 1997. Export of degradable DOC from oligotrophic surface waters: Caused by a malfunctioning microbial loop? *Limnology and Oceanography* **42:**398–404.

Thingstad, T. F., U. L. Zweifel, and F. Rassoulzadegan. 1998. P-limitation of both phytoplankton and heterotrophic bacteria in the northwest Mediterranean. *Limnology and Oceanography* **43:**88–94.

Thingstad, T. F., H. Havskum, H. Kaas, D. Lefevre, T. G. Nielsen, B. Riemann, and P. J. LeB. Williams. 1999a. Bacteria–protist interactions and organic matter degradation under P-limited conditions. Comparison between an enclosure experiment and a simple model. *Limnology and Oceanography* **44:**62–79.

Thingstad, T. F., M. Pérez, S. Pelegri, J. Dolan, and F. Rassoulzadegan. 1999b. Trophic control of bacterial growth in microcosms containing a natural community from northwest Mediterranean surface waters. *Aquatic Microbial Ecology* **18:**145–156.

Tortell, P. D., M. T. Maldonado, and N. M. Price. 1996. The role of heterotrophic bacteria in iron-limited ocean ecosystems. *Nature* **383:**330–332.

Touratier, F., L. Legendre, and A. Vézina. 1999. Model of bacterial growth influenced by substrate C : N ratio and concentration. *Aquatic Microbial Ecology* **19:**105–118.

Tyrrell, T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature* **400:**525–531.

Vadstein, O., A. Jensen, Y. Olsen, and H. Reinertsen. 1988. Growth and phosphorus status of limnetic phytoplankton and bacteria. *Limnology and Oceanography* **33:**489–503.

Vallino, J. J., C. S. Hopkinson, and J. E. Hobbie. 1996. Modelling bacterial utilization of dissolved organic matter: Optimization replaces Monod growth kinetics. *Limnology and Oceanography* **41:**1591–1609.

Veldkamp, H. 1976. "Continuous Culture in Microbial Physiology and Ecology." Patterns of Progress. Meadowfield Press, Durham, UK.

Williams, P. J. L. 1990. The importance of losses during microbial growth: Commentary on the physiology, measurement, and ecology of the release of dissolved organic material. *Marine Microbial Food Webs* **4:**175–206.

Zohary, T., and R. D. Robarts. 1998. Experimental study of microbial P limitation in the eastern Mediterranean. *Limnology and Oceanography* **43:**387–395.

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Patterns in Dissolved Organic Matter Lability and Consumption across Aquatic Ecosystems

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Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

I. INTRODUCTION

The key components in the cycling of organic matter in the biosphere are its production, transformation, and degradation. In aquatic ecosystems, heterotrophic bacteria participate in all three components and are primarily responsible for the bulk of degradation of organic matter. Exoenzymatic breakdown, uptake of small molecules, and the eventual intracellular oxidation of organic molecules to CO_2 and inorganic constituents are the principal pathways of organic matter degradation in aquatic systems. Microbial action not only results in the remineralization of the dissolved organic matter (DOM), but also in its transformation in such a way that the DOM pool owes much of its chemical structure to microbial activity (Moran *et al.* 2000). The biological availability of DOM to microbes is a key property that determines the turnover and eventual fate of this organic matter pool.

It is now well accepted that the DOM is composed of various pools, each characterized by its distinct chemical composition and by differences in its availability to microbes (Middleburg *et al.*, 1993). The turnover of these pools varies widely, ranging from minutes to hundreds of years or even longer. The relative abundance of these different pools in the bulk DOM determines the overall bioavailability of the organic matter within a given time frame. From a geological standpoint, it is important to understand the long-term degradation dynamics of DOM, which, in turn, determines the long-term storage of organic matter in soils, sediments, and deep oceanic waters. From an ecological point of view, however, there is interest in determining the amount of DOM that can be utilized by microorganisms at much shorter time frames, typically ranging from hours to weeks or perhaps months. Organic matter is also an important conduit for nutrients in aquatic ecosystems, and the short-term degradation of DOM is implicated in the cycling and bioavailability of P, N (see also Chapters 8, 11, and 16), and other important bioreactive elements. In addition, important physical and chemical processes, such as sorptive and transport processes, "compete" with metabolism on these time scales. This chapter focuses on large-scale patterns in DOM biological availability and consumption at times scales shorter than one year.

The short-term, *in situ* bioavailability of organic matter is a complex function of intrinsic factors, such as the chemical characteristics of the DOM itself, which include of molecular weight distribution, the nutrient contents, and the relative contribution of broad classes of compounds and are determined by the source and the diagenetic state of the matter (Amon *et al.*, 2001). The utilization of DOM and its apparent lability are also affected by extrinsic factors regulating the metabolism of bacteria and, therefore, the utilization of the organic matter by the bacterial community. These factors include temperature, the availability of inorganic and trace nutrients, trophic interactions within microbial food webs, and even the

phylogenetic composition of bacterial assemblages (Thingstad *et al*., 1997; see also Chapter 16).

DOM "lability" is an entirely operational term generally used in the context of intrinsic properties of the DOM, but these are often intimately linked to extrinsic regulatory factors, and it is sometimes difficult to separate their independent effects on DOM consumption, even in *in vitro* bioassays. Most of these bioassays involve following the changes in DOM concentration [usually measured as dissolved organic carbon (DOC)] with time in the absence of light and any new source of DOM (i.e., algal photosynthesis). Some studies also follow oxygen consumption, the production of carbon dioxide, or bacterial biomass production to indirectly determine organic matter consumption. These bioassays do not yield an estimate of lability that is only dependent on intrinsic DOM properties, because factors other than just the quality of the DOM vary, for example, incubation temperature, the initial concentration of inorganic nutrients, and even the nature of the microbial inoculum.

The overall patterns of DOM distribution among aquatic systems are fairly well characterized (Findlay and Sinsabaugh, 1999; see Chapter 6), but we know much less about the variability in labile carbon in time and across systems. Søndergaard and Middelboe (1995) collected published measurements of DOM lability from a variety of aquatic systems and concluded that, on average, 15% of the DOM in a variety of aquatic ecosystems was labile and that the percentage of labile DOM increased with increasing concentrations. However, because DOM lability is operationally defined as organic matter consumed by bacteria within a given period of time, any discussion of DOM bioavailability must be placed within the context of time. In the synthesis by Søndergaard and Middelboe (1995), for example, data from incubations ranging from several days to several weeks were pooled. Dramatic changes in consumption rates of DOM are known to occur in batch incubations during the first two weeks, corresponding to rapid depletion of small and highly labile pools and increasing use of less labile pools over time (Markosova, 1991; Osinga *et al*., 1997). The greatest differences in the patterns of DOM consumption among samples are observed over this initial period, which is perhaps the most ecologically relevant due to potentially rapid fluctuations in supply.

The purpose of this chapter is to explore cross-system patterns in DOM bioavailability with data obtained from *in vitro* bioassays. We extend previous attempts to assess large-scale patterns in DOM lability (Søndergaard and Middelboe, 1995), by quantitatively synthesizing and analyzing the published data on DOM lability in natural aquatic systems. Our main objective was to search for large-scale patterns in DOM lability across aquatic systems and to address a set of specific questions: (1) Does DOM lability vary predictably acrossamong aquatic ecosystems? (2) Do the kinetics of DOM consumption vary systematically across among systems? (3) How does DOM consumption

in laboratory bioassays compare to *in situ* DOM consumption by heterotrophic bacteriamicrobes? To address any of these and other broad questions effectively, we must consider explicitly the dynamics of DOM consumption over time. We have made an effort to express the kinetics of DOM consumption and the lability values from different studies in a comparable form by taking into consideration both incubation time and temperature.

II. DATA COLLECTION

We have surveyed the post-1970 literature for published estimates of DOM lability in natural waters, by systematically reviewing 15 major journals that regularly publish research on aquatic microbiology and biogeochemistry (Table I). Additionally, we have extracted data from papers cited therein. We only collected data obtained from laboratory bioassays that have followed the disappearance of natural DOC with time, generally in dark incubations of reinoculated, filtered water in batch incubations (i.e., Tranvik, 1988; Findlay *et al*., 1992). Many investigators have explored DOM lability indirectly by measuring the resulting bacterial productionor

TABLE I Summary of Data Collected from the Literature, Including Type of System, Source of the DOC Used in the Bioassay, Range of Ambient DOC, Number of Individual Time Courses Extracted from That report, and the Reference

System	*DOM source*	*DOM mg/L*	*Number of experiments*	*Reference*
Lake				
Lake Superior, Minnesota	2, 3	1.6–2.0	4	Aas and Hicks (1993)
Star Lake, Vermont	4	0.2–1.9	6	Allen (1976)
Various lakes, Canada	1		3	Engstrom (1987)
Lake Mindelsee, Austria	1	10.0–12.0	2	Geller (1986)
Lake Ontario, Canada	1	11.1–20.9	3	Markosova (1991)
Shibakusa-daira, Japan	1	12.0	1	Satoh and Abe (1987)
Various lakes, Denmark	1	3.4–6.5	4	Søndergaard (1984)
Frederiksborg Slottso, Denmark	1	13.5	1	Søndergaard and Borch (1992)
Frederiksborg Slottso, Denmark	1	12.1–14.3	15	Søndergaard *et al.* (1995)
Various lakes, Sweden	1	4.9–30.8	10	Tranvik (1988)
Various lakes, Sweden	1	3.0–10.0	2	Tranvik and Jorgensen (1995)
Humic lakes, Sweden	4	108.0	2	Tranvik and Kokalj (1998)
Lakes Uberlingen and Constance, Germany	1	1.6–2.4	2	Weiss and Simon (1999)
Lake Ortrasket, Sweden	1	6.0	2	Wikner *et al.* (1999)

(*continued*)

TABLE I (*continued*)

System	*DOM source*	*DOM mg/L*	*Number of experiments*	*Reference*
River				
Rio Negro and Rio Solimoes, Brazil	1	4.5–9.6	2	Amon and Benner (1996a)
Rio Negro, Brazil	1	9.9	2	Amon and Benner (1996b)
Various rivers, South Carolina	3		3	Benner and Vaun McArthur 1988)
Hudson River, New York	1	2.0–4.0	1	Findlay *et al.* (1992)
Aquifer, Denmark	1	20.0	1	Gron *et al.* (1992)
White Clay Creek, Pennsylvania	3	6.9–11.1	2	Kaplan and Bott (1983)
Perdido River, Texas	1	5.0–10.0	2	Kroer (1993)
Speed River, United States	2	30.8–37.5	3	Lock and Hynes (1976)
Appalachian stream, North Carolina	1		4	Qualls and Haines (1992)
York River, Virginia	1	4.3–10.0	5	Raymond and Bauer, (2000)
Three Rivers, Belgium	1	3.5–13.3	12	Servais *et al.* (1989)
Various Streams, Pennsylvania	1	1.7–9.3	6	Volk *et al.* (1997)
Experimental stream	2	35.6	2	Wetzel and Manny (1972)
Marsh				
Okefenokee Swamp	2	46.2–57.7	1	Bano *et al.* (1997)
Range Point Marsh, Florida	1	3.0–12.1	3	Coffin *et al.* (1993)
Various tidal marshes, United States	1	3.2–10.7	31	del Giorgio and Newell, (in prep.)
Various Marshes, North Carolina	2	0.6	1	Linley and Newell (1984)
Sapelo Island, Georgia	2	6.0–9.0	3	Moran and Hodson (1989)
Sapelo Island, Georgia	2	5.9	2	Moran and Hodson (1994)
Estuary				
Gulf of Mexico, Texas	1	1.8–5.4	12	Amon and Benner (1996a)
Danube River, Romania	1	3.1	1	Becquevort *et al.* (2002)
Chesapeake Bay, Maryland	2		1	Blum and Mills (1991)
Gulmar Fjord, Sweden	3	7.0	1	Carlsson *et al.* (1993)
Santa Rosa Sound, Florida	1	3.0–12.1	3	Coffin *et al.* (1993)
Chesapeake and Delaware Bays	1	3.2–10.7	12	del Giorgio and Newell, (in prep.)
Parker River Estuary, Massachusetts	3	1.6–3.0	5	Fry *et al.* (1996)

(*continued*)

TABLE I (*continued*)

System	*DOM source*	*DOM mg/L*	*Number of experiments*	*Reference*
Estuary				
Gulf of Mexico, Texas	1	5.0–10.0	5	Kroer (1993)
Roskilde Fjord, Denmark	1	6.8–8.2	4	Middelboe *et al.* (1992)
Various estuaries, Georgia	1	3.0–4.5	5	Moran *et al.* (1999)
Cape Peninsula, South Africa	3		1	Newell and Lucas (1981)
Sagami and Tokyo Bays, Japan	1	2.0–6.0	5	Ogura (1975)
Gulf of Trieste, Italy	2		1	Peduzzi and Herndl (1991)
York River Estuary, Virginia	1	3.4–10.0	39	Raymond and Bauer (2000)
Ore Estuary, Sweden	1	7.2	2	Wikner *et al.* (1999)
Gulf of Riga, Europe	1	5.4–8.1	8	Zweifel (1999)
Experimental system	1	2.2	1	Zweifel *et al.* (1996)
Marine				
Mid-continental Shelf, Georgia	3	4.0	3	Biddanda (1988)
Sargasso Sea	1	0.7–0.8	3	Carlson and Ducklow (1996)
Ross Sea, Antarctica	1	0.9	2	Carlson *et al* (2002)
Eastern North Pacific Ocean	3	0.8–2.1	6	Cherrier *et al.* (1996)
Sargasso Sea	1	0.5–0.8	3	Hansell *et al.* (1995)
Coastal Ocean, The Netherlands	4		1	Janse *et al.* (1999)
Salt River Canyon, U.S. Virgin Islands	2		1	Kenworthy *et al.* (1989)
North Atlantic Ocean	1	1.6–2.4	3	Kirchman *et al.* (1991)
North Equatorial Pacific Ocean	1	1.0	1	Ogura (1972)
Wadden Sea, The Netherlands	3		2	Osinga *et al.* (1997)
Bedford Basin, Canada	3		1	Pett (1989)
North Atlantic Ocean	4	4.0–6.0	2	Turley and Lochte (1990)
Bothnian Sea, Europe	1	3.5	1	Zweifel *et al.* (1993)
Bothnian Sea, Europe	1	3.7–4.7	12	Zweifel *et al.* (1995)

consumption of CO_2. We did not include these data because they involve assumptions about bacterial growth efficiency, biomass, and carbon contents, which add uncertainty to the comparisons. DOM lability has also been explored using continuous bioreactors (i.e., Volk *et al.*, 1997), but the

resulting data are not strictly comparable to those from standard regrowth bioassays because the retention time of DOC in a bioreactor is not equivalent to the incubation time in a standard regrowth batch incubation. Many studies report only the initial and final DOM concentrations, whereas others report the time course of change. We have included both types of data in our analyses. In addition, we have collected data from bioassays in terms of actual DOC concentration with time, as well as in terms of the percentage of DOC consumed within a given interval of time. The bulk of the data that we have collected correspond to experiments using batch bioassays to assess the consumption of natural DOC. The resulting dataset includes data from 62 papers, with 57 individual bioassay time courses for lakes, 45 for rivers, 41 for marshes, 106 for estuaries, and 41 for marine samples (Table I). In addition, and for comparative purposes, we have collected lability data for specific types of DOM, including molecular size fractions, and DOM derived directly from algae or macrophytes.

III. DATA ANALYSIS

In batch incubations, the DOM becomes increasingly enriched with less reactive fractions as the time course progresses, resulting in a continuous decrease in the consumption rate with increasing incubation time. Hence, first-order models (i.e., Berner, 1964) are seldom appropriate to describe the kinetics of natural DOM consumption. A model that considers a discrete number of reactive groups (multi-G model) has been proposed as an alternative to the first-order model (Billen, 1982, Connolly *et al*., 1992) and requires that the number of groups and their reactivities be determined empirically as curve-fitting parameters (Middleburg *et al*., 1993). Alternatively, DOM consumption can be assumed to change continuously, rather than discretely, as the different reactivity pools are depleted. A power function usually fits well the decline in the rates of DOM consumption with incubation time (Middleburg *et al*., 1993).

A typical bioassay includes a nonlinear decline in DOC concentration with time (Fig. 1) that can be described as a series of declining first-order rates of DOC consumption. The latter can be approximated using a negative hyperbola. In turn, the proportion of DOC consumed with time can be approximated with a positive hyperbola. We focus on the parameters of the fitted hyperbola in this chapter. To compare the average lability of DOM from different aquatic systems, it is necessary to account for differences in incubation time. This is particularly important when short-term (<15 days) lability is analyzed, because the differences in consumption are most evident during this period. One of the main problems with DOM lability data is that, although there are many published bioassays, there are relatively few of these that provide detailed time courses, and most studies report DOC

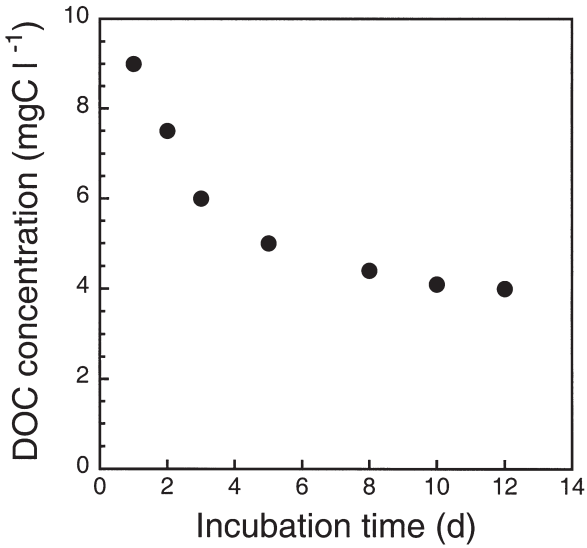


FIGURE 1 Example of a typical DOCM consumption bioassay, where the decline in DOCM concentration is followed with time in regrowth experiments. This bioassay was performed on water from Lake Ontario, extracted from Markosova (1991).

consumption or percentability after a fixed incubation time. This becomes a major problem because (1) the kinetics of DOC consumption are as important as the actual value of lability to understand differences among systems (see also Chapter 15 and (2) the absence of detailed time courses does not allow comparison of lability estimates among studies that use different fixed incubation times.

Another major problem is that bioassays have been conducted over a wide range of incubation temperatures. Temperature is an overriding factor determining bacterial metabolic rates, so that varying incubation temperatures might mask patterns in lability due to differences in the nature of the DOM. Therefore, to assess cross-system differences in DOM lability, we have standardized all the rates to 20°C assuming a Q_{10} of 2.1, empirically determined by Raymond and Bauer (2000) for the York River (Virginia) for a range of temperatures from 5 to 26°C. This Q_{10} is in good agreement with our own observations in bioassays of samples from coastal salt marshes, the Delaware Bay, and the Atlantic coast (del Giorgio and Newell, in prep.). This standardization enabled us to investigate how the average percentage of DOM consumed and the rate of consumption vary among different aquatic systems, once the effect of temperature has been removed.

To partly overcome the problem of nonexistent or incomplete time courses, we have chosen to pool all the available time courses for each of five major ecosystem types investigated, thus generating a system-average relationship

of percentage of DOC consumed or DOC consumption rate versus incubation time. We have grouped all the bioassays into lake, river, estuarine, marsh, and marine samples, and the individual data of all bioassays for each of these systems were aggregated into nine discrete incubation time categories: 0–1 day, >1–4 day, >4–7 days, >7–10 days, >10–15 days, >15–25 days, >25–40 days, >40–100 days, >100 days. We have then averaged all the percentage of DOC consumed valuse within each category and obtained an "average" time course of percentage of DOC consumed for each major system investigated.

Likewise, for each bioassay for which we had a time course, we calculated the temperature-corrected linear rate of consumption between pairs of observations and grouped the resulting consumption rates into one of the incubation time categories described above. Thus, for each of the five ecosystem categories, we have obtained an "average" rate of consumption at discrete incubation intervals. We then fitted power equations to each of these "average" time courses. Even though each average time course was constructed with data from numerous different bioassays, in some of the time intervals there may have been few or no data at all, and there were gaps in most of the time courses. We should also note that the dataset used to assess the proportion of DOM consumed over time is not identical to that used to explore DOM consumption rates, because not all data collected from the literature were in the form of DOC concentration versus time. A substantial portion of the papers only report percentage of DOC consumed or the consumption rate, but not the actual DOC concentration.

IV. PATTERNS OF DISSOLVED ORGANIC MATTER LABILITY AMONG SYSTEMS

The curve of percentage of DOC consumed versus time for each type of system can be approximated well with a power equation (Fig. 2; and see Table II for the parameters of the four power equations), and the shapes of these four curves is were very different: lake and marine samples were characterized by significantly ($P < 0.05$) higher intercepts than river and estuarine samples, which in turn, had higher slopes, significantly different from all others in the case of estuaries (Table II). The high intercepts that describe lake and marine DOM lability reflect a relatively high proportion of DOM consumed within the first 2 days of incubation, which was, on average, three- to fivefold higher than in rivers and estuarine samples. These initial differences propagate into major differences in short-term DOC lability among the systems; an average of 22 and 26% of the DOM in lake and marine samples, respectively, appeared to be consumed in bioassays after 1 to 3 days, whereas an average of 9–10% was consumed in rivers, marsh, and estuarine samples within in the same interval (Table III). These

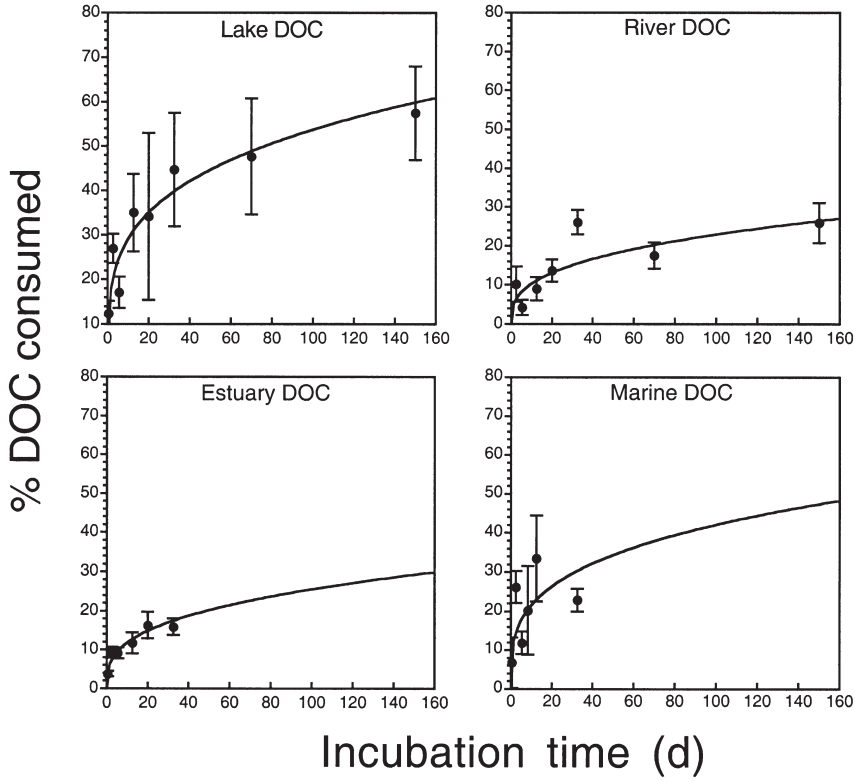


FIGURE 2 Average percentage of DOC consumed as a function of incubation time in regrowth experiments for lake, river, and estuarine, and marine samples. The various time courses of DOC consumption that were extracted from published reports were standardized to 20°C and then pooled to obtain an average percentage of DOC consumed unsumed at each incubation time category. Each point in the graph thus represents the average (± SDSE) of all the available reports of percentage of DOC consumed for that particular incubation time in each system. The parameters of the fitted power regression line are given in Table II.

differences persisted in longer time courses and were still evident after 2 weeks of incubation (Table III). These patterns suggest that there may be major and systematic differences in the average DOM lability among lakes, rivers, estuaries, and oceans. Not only does the lake DOM appear to be initially more labile than that of rivers and estuaries, but extrapolation using the fitted power equations (Table II) suggests that these differences in DOC lability should persist over even longer time frames.

The data on percentage of DOM consumed for marsh sites were sparse and there were gaps in the time coverage, especially in the long time intervals, and it was difficult to fit a represents power equation for marsh data. The available bioassays of marsh marine DOM showed low initial (1- to 3-day incubation) lability (approximately 9%), but extremely high lability

TABLE II Change in Percentage of DOC Consumed (%DOC) with Incubation Time (days) for All Data Grouped into Five Main System Categories

System	*Intercept (a)*	*Slope (b)*
Lake	12.20	0.32
River	2.27	0.55
Estuary	5.37	0.33
Marine	10.91	0.29

Note: The percentage of DOC consumption rate (y) versus the incubation time (x) relationship fits a power model $y = ax^b$. There were insufficient marsh time points to fit a power equation.

TABLE III The Average DOC Concentration (±Standard Error) for the Five Major System Categories in Our Dataset and the Proportion of This DOC Consumed in Bioassays

System	*DOC*	*%DOC consumed 1- to 3-day incubation*	*%DOC consumed 10- to 15-day incubation*
Lake	9.14 ± 0.57	21.69 ± 2.54	27.84 ± 9.01
River	9.07 ± 0.74	10.20 ± 4.54	9.01 ± 3.01
Marsh	6.62 ± 0.22	9.19 ± 1.41	36.55 ± 2.08
Estuary	4.60 ± 0.10	9.33 ± 1.38	11.70 ± 2.72
Marine	2.06 ± 0.18	26.20 ± 4.04	33.48 ± 11.10

Note: The percentage of labile DOC in samples from each type of system were averaged (±SE) over two incubation periods, 1–3 days, and 10–15 days. All %DOC consumed values have been standardized to 20°C (see text).

thereafter, reaching over 34% in 10–15 days (Table III). Taken over the course of 2 weeks, the lability of marsh DOM did not seem to be lower than that reported for lake and marine samples, and about threefold higher that the average for rivers and estuaries.

V. LABILITY OF SPECIFIC TYPES OF DISSOLVED ORGANIC MATTER

In addition to collecting natural DOM bioassay results, we have gathered data on bioassays using DOM derived from particular sources. The most extensive data of this type that we could gather were results from bioassays of algal-derived DOM, which showed a pattern of short-term lability remarkably similar to that described for lake and marine samples (Fig. 3), with an average of 21% DOC consumed within the first 1–3 days of incubation, reaching over 36% after 10–15 days of incubation. Over longer incubations, algal-derived DOM appeared to be more labile than both marine and fresh-

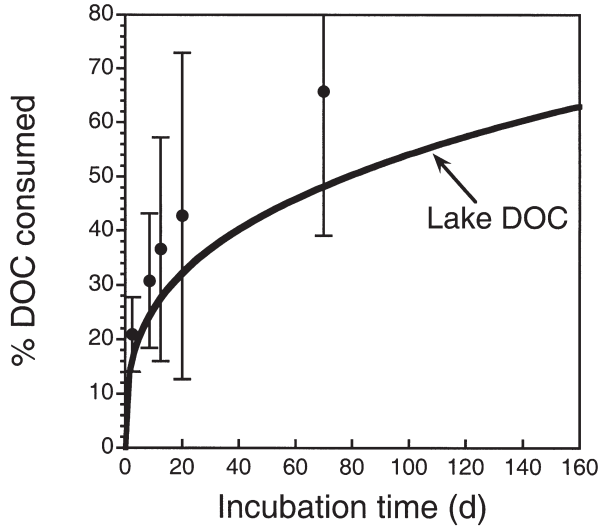


FIGURE 3 Average percentage DOC consumed with incubation time in bioassays of algal-derived DOM. Each point in the graph represents the average (± SE) of all the available reports of percentage of algal DOC consumed for that particular incubation time. The power regression for lakes from Figure 2 is superimposed for comparison.

water bulk DOM (Fig. 3). It is generally assumed that algal-derived DOC is the main source of highly labile DOC in the water column (Søndergaard and Middelboe, 1995; see also Chapter 1), particularly in lakes, where terrestrially derived DOM is assumed to be more recalcitrant. Our results support this assumption, but also suggest that over short time scales, the algal-derived DOM is not necessarily more labile than the *in situ* DOM pool, which is itself a complex heterogeneous mixture of algal- and nonalgal-derived carbon. A limited dataset on the consumption of DOM derived from aquatic macrophytes (e.g., sea grasses and marsh plants) also showed relatively high lability (data not shown), certainly higher that for most ambient DOM samples, and even higher than that reported for algal-derived DOM. This would also suggest that vascular DOM is not necessarily refractory, as it is often assumed, and that ambient DOM that is mostly derived from vascular vegetation should not be *a priori* presumed to be less labile.

VI. CROSS-SYSTEM PATTERNS IN DISSOLVED ORGANIC MATTER CONSUMPTION

There also appeared to be systematic differences in the patterns of consumption rates in bioassays from different systems. The average rates of consumption declined as a power function of incubation time for all systems

(Fig. 4), but the shape of the curves differed among systems, as shown by the parameters of the nonlinear regressions (Table IV). In particular, marine samples were characterized by a high intercept, reflecting high initial rates of consumption. However, the decline in the rates with incubation time was much faster in marine samples than in samples from any other system, as shown by a much steeper slope of the relationship (Table IV). Consequently, the inflection point in the curve of consumption rate versus time occurs much sooner in marine samples (Fig. 4), and after 20 days of incubation, consumption rates were extremely low. In contrast, the decline in consumption rate was more gradual for rivers and estuaries (Fig. 3).

The average kinetics of consumptionse thus suggest major differences in the relative proportions of highly labile, semilabile, and recalcitrant DOM among aquatic systems. Marine samples, for example, appear to have a

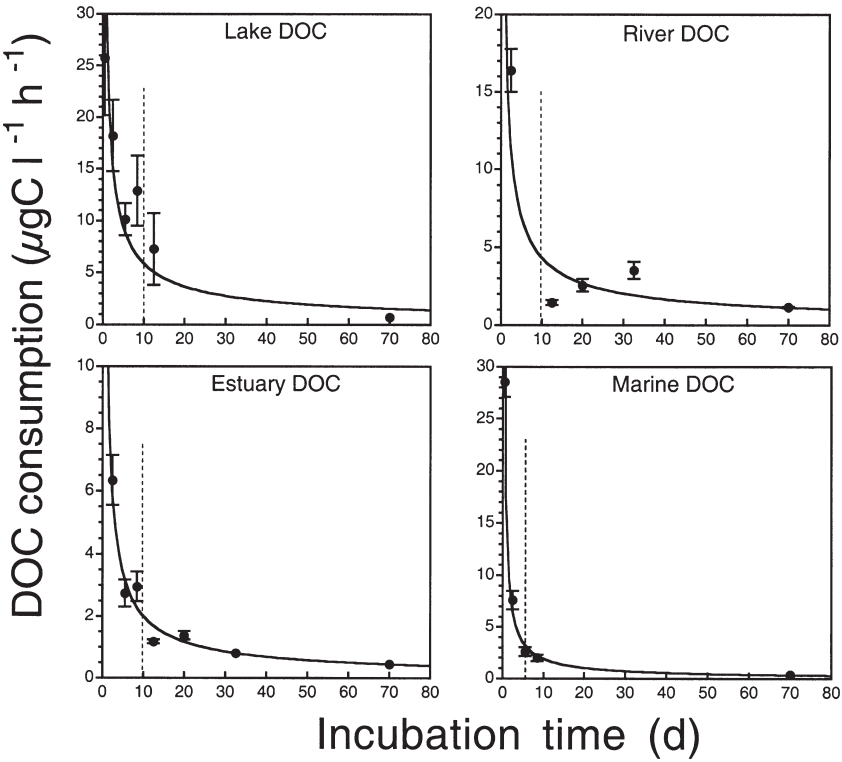


FIGURE 4 Relationship between the average linear rates of DOC consumption and incubation time in DOC bioassays. Each point represents the average (± SED) linear rate of consumption of all the available data within a given incubation interval for each system. All data have been standardized to 20°C (see text). The solid line is the least squares fit of a power model to the data, and the parameters of the equations are given in Table IV. The dotted line approximately marks the point of inflection in the curve.

TABLE IV Change in DOC Consumption Rate (μg C L^{-1} h^{-1}) with Incubation Time (days) for All Data Grouped into Five Main System Categories

System	*Intercept (a)*	*Slope (b)*
Lake	12.20	0.32
River	2.27	0.55
Estuary	5.37	0.33
Marine	10.91	0.29

Note: The consumption rate (y) versus the incubation time (x) relationship fits a power model $y = ax^b$. There were insufficient marsh time points to fit a power equation.

relatively high proportion of highly labile DOM, which leads to high initial rates of bacterial consumption. As a result, although the total DOM pool is relatively small in most marine samples relative to other aquatic systems, it can still support relatively high rates of microbial metabolism. The dramatic drop in consumption rates in marine samples after a few days suggests that the pool of semilabile DOM is generally small in these samples and that most of the marine DOM is divided between highly labile or refractory pools. In contrast, the pool of highly reactive DOM appears to be relatively smaller for estuaries and rivers, but the more gradual decline in consumption rates suggests the presence of a large semilabile DOM pool in these systems. Overall, these patterns are most likely linked to differences in the origin and pathways of DOM among the five major systems, but these underlying mechanisms are unclear and need further investigation, as we discuss below.

VII. PATTERNS OF LABILITY ALONG DISSOLVED ORGANIC MATTER GRADIENTS

Søndergaard and Middelboe (1995) had previously concluded that the proportion of labile DOM tended to increase with system productivity and with the total amount of DOM. We did not have enough data to assess whether there were patterns in DOM lability along primary productivity or chlorophyll gradients, but we could assess whether the proportion of labile DOM varied systematically with concentration. For this analysis, we grouped all the temperature-corrected bioassay results into three incubation time categories (<3 days, 3–8 days, and 8–16 days), so that we could at least partially remove the effect of length of incubation on the apparent lability. There was no relationship between the proportion of labile DOM and the total DOM concentration, regardless of how we aggregated the data (Fig. 5). There was essentially a three order of magnitude range in the proportion of labile DOM, at comparable incubation temperature and length, which was independent of concentration. The differences in lability among ecosystem types

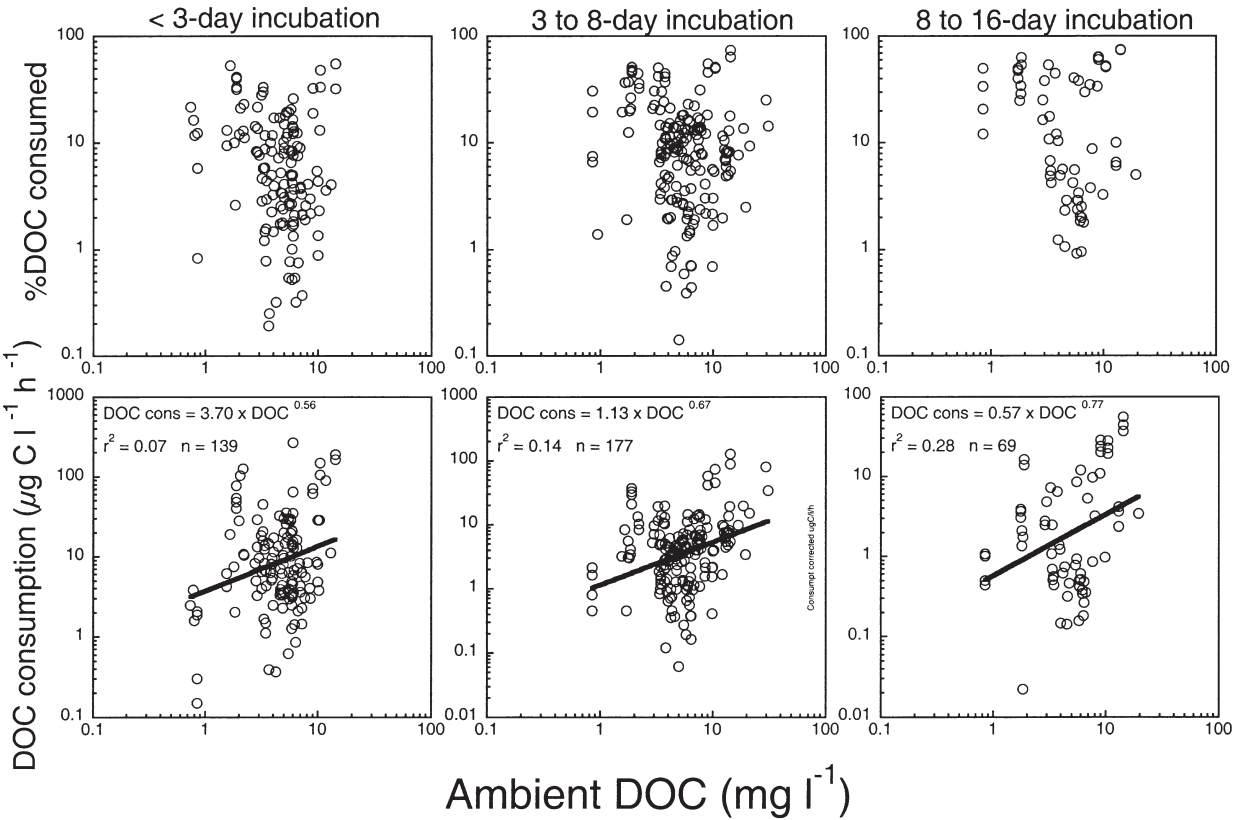


FIGURE 5 Percentage of DOC consumed (top panels) and rate of DOC consumption (bottom panels) as functions of the initial DOC concentration, separated into three incubation periods. Each point is an individual observation from a bioassay, and the data from all systems are shown together.

were, thus, not linked to the absolute amount of DOM, but rather to differences in intrinsic properties, which, in turn, would appear to be independent of the actual concentration. Alternatively, the differences in lability may be related to differences in inorganic nutrient availability or other factors that are not directly linked to the chemical composition and quality of the DOM. We discuss some of these factors in the following sections.

A common assumption in aquatic ecology is that larger total DOM pools represent a larger short-term resource for heterotrophic bacteria, but this assumption is not supported by our data. The rate of DOM consumption tends to increase with total concentration (Fig. 5, bottom panels), but these relationships are only marginally significant. If the rate of consumption of labile DOM is any indication of standing stock of this organic matter, we must conclude that the absolute amount of labile DOM is only marginally correlated with the total concentration.

VIII. TEMPORAL PATTERNS IN DISSOLVED ORGANIC MATTER LABILITY

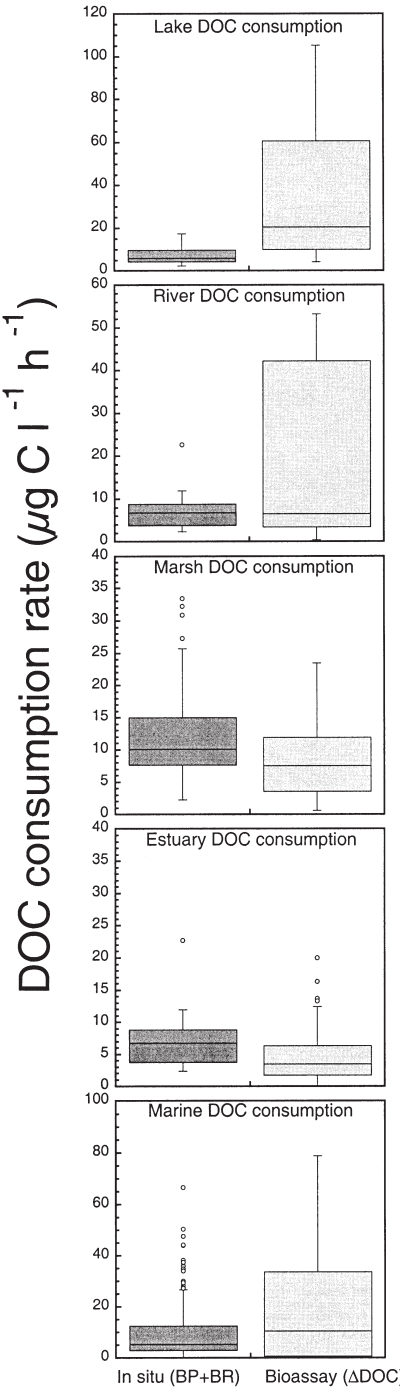
We did not have enough data at hand to draw any generalizations about temporal variation in DOM lability within systems, but the few studies that have explicitly addressed this issue suggest that there may be as much variability within systems as there is across systems (Søndergaard *et al*., 1995). Factors such as increased heterotrophic activity in the summer (Marmonier *et al*., 1995) and algal blooms (Weiss and Simon, 1999) affect the apparent DOM lability within a given system. In addition, seasonal flooding can transport labile organic matter that has accumulated in the upper soil horizons to lakes and streams. For example, Baker *et al*. (2000) found that up to 45% of the DOC transported to streams in groundwater can be biologically labile during snowmelt (Stepanauskas *et al*., 2000) and that lability then decreases substantially. Spring floods have been found to increase the overall lability of the organic matter in rivers (Michaelson *et al*., 1998), lakes (Kling, 1995), and estuaries (Wikner *et al*., 1999). The cross-system comparisons that we have attempted above represent only average trends and cannot take into consideration the variability of DOM lability within a given system.

IX. BIOASSAY VERSUS METABOLIC ESTIMATES OF DISSOLVED ORGANIC MATTER CONSUMPTION

The extent to which patterns of consumption in batch experiments reflect the possible rates of *in situ* consumption by heterotrophic bacteria is unclear, because the latter are extremely difficult to measure directly. There are innumerable reports of bacterial production in aquatic systems, but

because bacterial growth efficiency is highly variable (del Giorgio and Cole, 1998), these data alone do not provide a reliable estimate of total bacterial carbon consumption and, therefore, of the DOC that is bioavailable in the shortterm. The total organic carbon consumption by bacteria can be approximated by combining measurements of bacterial production (BP) and bacterial respiration (BR) in natural water samples. Both types of measurements usually require some form of incubation of the samples and, in this sense, do not truly represent "*in situ*" rates, but because they target the metabolism of the native assemblage they do reflect ambient processes better than bioassays. del Giorgio and Cole (1998) synthesized published reports of simultaneous measurements of BP and BR that can be used to roughly estimate the range of "*in situ*" carbon consumption by bacteria in different aquatic systems. This range can then be compared to the average rates of consumption in lability bioassays. Although *in situ* consumption is seldom in steady state with supply, in natural waters the DOM is being constantly replenished with both labile and recalcitrant materials. In contrast, the bioassay experiments are static so that the most labile fractions are quickly depleted and thus, as we discussed above, consumption rates quickly decline. Therefore, the only portion of the bioassay that can be compared to metabolic rates is the initial stage when the pool of labile DOM may still reflect *in situ* conditions.

We aggregated the rates of consumption for all bioassays for which incubation times less than 1.5 days were reported to derive a median and range of initial rates of consumption in bioassays for each ecosystem type. We then compared these rates with the range of total bacterial DOC consumption, calculated as the sum of simultaneous measurements of BP and BR in the global dataset assembled by del Giorgio and Cole (1998) and to which we have added over 100 unpublished measurements taken in marshes and estuaries (del Giorgio and Newell, in prep.), for a total of 478 pairs of observations. For all systems, except lakes, there was a relatively good agreement (within a factor of 2) between the median initial rates of consumption in bioassays and the median rates of consumption "*in situ*" calculated from BR and BP measurements. This suggests that, in most cases, short-term bioassay experiments may capture the essence of the bacteria–DOM interaction that occurs in natural waters. In lakes, however, the median for consumption in bioassay experiments was 4 times higher than the median BP+BR measurements (Fig. 6), suggesting a sampling bias. BP and BR measurements cover a wide variety of lake types, including many oligotrophic, clear-water lakes, whereas bioassays have been preferentially carried out in high-DOM lakes, which also tend to have higher bacterial metabolism. An interesting feature is that the ranges of both the metabolic and bioassay-based rates of bacterial DOM consumption in marine samples are similar to those for estuarine and marsh samples, despite the fact that marine areas have smaller DOM pools and are generally much less productive.


DOC consumption rate (μg C l -1 h -1)
Lake DOC consumption
River DOC consumption
Marsh DOC consumption
Estuary DOC consumption
Marine DOC consumption
In situ (BP+BR)
Bioassay (ΔDOC)

X. FACTORS THAT INFLUENCE DISSOLVED ORGANIC MATTER LABILITY IN BIOASSAYS

The conditions imposed in bioassays clearly depart from those *in situ*, and yet our data also show relatively good agreement between median rates of consumption from metabolic measurements of "*in situ*" assemblages and the median consumption measured in bioassays. DOM lability is operationally defined, and the apparent lability depends greatly on issues such as the length of the incubation and the initial bioassay conditions, which include temperature, the size and type of the bacterial inoculum, and the addition of inorganic nutrients. Assessment of lability, and particularly the comparison of samples from different types of ecosystems, should, in theory, consider all these factors. In practice, this is difficult, because there is no standard protocol to determine lability and the approaches vary greatly. In this chapter, we have attempted to standardize at least for incubation time and temperature, but even this is complicated by the fact that we have had to assume a fixed Q_{10} for all samples and have had to lump together very heterogeneous data in order to explore the kinetics of DOM consumption in bioassays.

In addition to temperature, the availability of inorganic nutrients may play a central role in the dynamics of DOM degradation and consumption (see Chapter 16). We have insufficient data to assess whether the differences among systems in the apparent lability may result from major in nutrient availability; the linkages between nutrient and organic matter geochemistry are discussed in detail in other chapters (see Caraco and Cole, Findlay and Sinsabaugh). Most of the long-term bioassays, with some exceptions (i.e., Moran *et al*., 2000), are conducted without any inorganic nutrient addition, and most do not report the ambient nutrient concentrations. Some studies have shown increased DOM degradability after the N and P addition of (i.e., Zweifel e*t al*., 1995, Wikner *et al*., 1999), but not all nutrient additions result in increased consumption (Carlsson *et al*., 1993). The lability of DOM from marine samples, even from extremely oligotrophic sites, appears to be, on average, higher than that of estuaries, which typically have much higher ambient nutrient concentrations, suggesting that intrinsic DOM properties

FIGURE 6 Summary of measurements of *in situ* rates of bacterial DOC consumption, calculated as the sum of bacterial production and respiration, and *in vitro* rates of DOC consumption, calculated from declines in DOC during batch incubations. Box-and-whisker plots show median, and upper/lower quartiles (box), and the range of values (bars). Extreme outliers are denoted by open circles. The *in situ* consumption rates are from the global dataset of simultaneous measurements of bacterial production and respiration collected by del Giorgio and Cole (1998), with the addition of unpublished data (see text). Because the *in situ* rates have been measured under a range of temperature, for this comparison the bioassay rates have not been corrected for temperature.

may play a greater role. Nutrient availability may also be a problem in the actual determination of lability. In ambient water, there is a constant replenishment of nutrients, which may accelerate consumption relative to *in vitro* incubations, leading to a systematic underestimation of lability.

Another key factor that determines the lability of ambient DOM is exposure to light and UV (see Chapter 10). The majority of the bioassays reported here were conducted in the dark, and, in this sense, they do not mimic the ambient conditions, because the surface water DOM pool is exposed to at least some light through mixing in the water column as it is being degraded by microbes. Even in cases where the DOM was pretreated with UV (Moran *et al*., 2000), the actual microbial consumption proceeds in the dark with no further exposure.

In addition to chemical and physical factors, the composition of the assemblage may influence the apparent lability. There is strong evidence that regrowth experiments favor opportunistic bacterial species and generally lead to low microbial diversity (Giovannoni and Rappé, 2000). The link between bacterial diversity and the capacity of the bacterial assemblage to collectively degrade and consume DOM has so far been difficult to establish. Earlier studies (Tranvik, 1988; Tranvik and Hofle, 1987) showed no effect of the inoculum on the rates of consumption in batch experiments with lake water. There is now increasing evidence, however, that different bacterial phylogenetic groups selectively degrade or consume broad chemical categories of ambient DOM (Cottrell and Kirchman, 2000; see also Chapter 9), which would suggest that composition might indeed play a role in the apparent lability. In this regard, it is interesting to note that a much larger fraction of riverine DOM can be consumed by bacteria during a short transit time within bioreactors (Volk *et al*., 1997) than in long-term batch bioassays. Bioreactors differ from regrowth bioassays in having much higher microbial biomass relative to DOM inputs. However, it is also likely that bioreactors develop a more diverse and stable bacterial assemblage (see also Chapter 12), and one hypothesis is that this more diverse assemblage is collectively more effective in utilizing ambient DOM than the less diverse assemblage that typically develops in batch bioassays. This is a hypothesis that remains to be tested in the future and has great ecological implications.

There have been some attempts to find chemical or physical correlates of short-term consumption that can be used as indices of biological lability (Amon *et al*., 2001). For example, there is indication that bioavailability (i.e., in terms of milligrams bacterial biomass per milligrams of DOC consumed) is correlated to the elemental composition in terms of C, N, H, and O, to the aliphatic contents, and to the overall degree of reduction of the DOM (Sun *et al*., 1997; Hopkinson *et al*., 1998; Hunt *et al*., 1999). However, there is still no general index that is applicable to all different aquatic systems, and it is unlikely that a single index will be able to account for variation in the multiple factors that simultaneously determine DOM

bioavailability. This is one of the reasons that the lability of ambient DOM must still be empirically determined with *in vitro* bioassays.

XI. CONCLUSIONS

In spite of the shortcomings that we have discussed previously, there are several robust trends that have emerged from our analysis. There appear to be systematic differences in DOM lability among ecosystem types, once the results from bioassays are standardized for time and temperature. Extrapolation from the regression models that we have developed (Table II), which combine all the available data, suggest that the proportion of DOC that can be consumed within 5 days at 20°C is, on average, 6% in river samples, 9% in estuarine samples, 17% in marine samples, and 20% in lake samples. These differences persist over longer incubations, and after 20 days our models predict that, on average, 12% of DOC is consumed in river samples, 15% in estuarine samples, 26% in marine samples, and 32% in lake samples.

The patterns in consumption during bioassays appear to be very different in marine and lake samples compared to estuaries and freshwaters. In particular, although the total DOM pool is considerably smaller in marine areas, this material is clearly capable of supporting short-term rates of bacterial metabolism that are comparable to those measured in richer systems with higher concentrations. The composition of marine DOM in terms of the proportion of highly reactive versus less reactive and recalcitrant pools appears to be distinct from other aquatic systems, a pattern that is most likely related to the origin of the DOM. In marine areas most of the DOM either is produced locally, directly or indirectly from algal production or forms part of the large background pool of old, recalcitrant DOM that cycles in the ocean. There is radiocarbon evidence that bacteria utilize a significant amount of this old DOC, particularly in open oceans (Cherrier *et al*., 1999). Photooxidation of old, recalcitrant DOM may be an important source of highly reactive DOM in surface marine waters (Bano *et al*., 1998). In lability bioassays, which are typically conducted in the dark, the products of photooxidation will rapidly be consumed and will not be replenished, and this may, in part, explain the sharp decline in consumption rate that characterizes marine samples. In estuaries, marshes, and many lakes, there are a wider variety of DOM sources, including terrestrial and aquatic vascular vegetation and sediments (see Chapters 2 and 6). Perhaps the different kinetics of consumption are a reflection not only of the absolute input of organic matter, but also of the diversity of sources supplying the pool.

It is interesting to note that the highest lability was observed at both ends of the concentration spectrum (i.e., low-DOM marine and high-DOM lake samples), and there are important ecological consequences to this

observation. Although DOM is unquestionably the main source of substrate to support bacterial metabolism in aquatic systems, there is often a weak or even no relationship at all between bulk DOM concentration and bacterial metabolism (Sherr *et al*., 2001; see also Chapter 15). As a whole, DOC concentrations vary by about 60-fold across most aquatic ecosystems (including our dataset), from less than 1 mg C L^{-1} in open ocean areas to exceptionally over 50 mg C L^{-1} in highly dystrophic lakes and marshes. Our dataset shows that the concentration of labile carbon that can be consumed within a period of approximately 1 week may vary by almost 4 orders of magnitude across the same gradient, from 0.07 to over 20 mg L^{-1}. Because the proportion of labile DOC is so variable, any given concentration of DOC can yield a large range in the apparent amount of short-term bioavailable organic matter. It is not surprising, then, that bulk DOM is often a poor predictor of bacterial metabolism. All the lines of evidence converge to suggest the importance of intrinsic DOM characteristics in regulating bacterial metabolism in aquatic ecosystems.

Another interesting result is that DOM from tidal marshes does not appear to be less labile, on average, than either lake or marine DOM. This is somewhat surprising because it has been generally assumed that the organic carbon budget and flow in coastal and freshwater marshes is dominated by both particulate and dissolved detrital organic matter derived from the breakdown of vascular vegetation, which is generally considered highly recalcitrant. The source of the labile DOM in tidal marshes is unclear, but leaching from both vascular plants and epiphytic communities may be a major source of reactive DOM in these systems. Our own data further suggest that DOM derived from vascular vegetation is, on average, as labile as that originating from phytoplankton or other sources.

One of the main problems when interpreting lability data and in detecting meaningful patterns among systems and along environmental gradients is that most often the available data are difficult to compare because of the variety of approaches that are used. Lability measurements are, in essence, relatively simple, but are nevertheless fraught with technical problems. The greatest challenge still is to accurately determine small changes within the relatively large background pool of DOC, and progress will have to be made in this area if we are to improve our understanding of consumption. To fill the current gaps in our understanding, it will be important in the future to conduct large-scale lability comparisons using uniform approaches, with an emphasis on short-term consumption.

REFERENCES

Allen, H. L. 1976. Dissolved organic matter in lake water: Characteristics of molecular weight size-fractions and ecological implications. *Oikos* **27:**64–70.

Amon, R. M. W., and R. Benner. 1996a. Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography* **41:**41–51.

Amon, R. M. W., and R. Benner. 1996b. Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River System. *Geochimica et Cosmochimica Acta* **6:**1783–1792.

Amon, R. M. W., H.-P. Fitznar, and R. Benner. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnology and Oceanography* **46:**287–297.

Baker, M. A., H. M. Valett, and C. N. Dahm. 2000. Organic carbon supply and metabolism in shallow groundwater ecosystem. *Ecology* **81:**3133–3148.

Bano, N., M. A. Moran, and R. E. Hodson. 1997. Bacterial utilization of dissolved humic substances from a freshwater swamp. *Aquatic Microbial Ecology* **12:**233–238.

Bano, N., M. A. Moran, and R. E. Hodson. 1998. Photochemical formation of labile organic matter from two components of dissolved organic carbon in a freshwater wetland. *Aquatic Microbial Ecology* **16:**95–102.

Becquevort, S., T. Bouvier, C. Lancelot, G. Cauwet, G. Deliat, V. N. Egorov, and V. Popovichev. 2002. The seasonal modulation of organic matter utilization by bacteria in the Danube–Black Sea mixing zone. *Estuarine, Coastal and Shelf Science* **54:**337–354.

Benner, R., and J. Vaun McArthur. 1988. Effects of temperature on microbial utilization of lignocellulose detritus in a chemically impacted stream. *Microbiol Ecology* **16:**323–330.

Biddanda, B. A. 1988. Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. II. Microbial metabolism. *Marine Ecology Progress Series* **42:**89–95.

Billen, G. 1982. Modeling the processes of organic matter degradation and nutrients recycling in sedimentary systems. *In* "Sediment Microbiology" (D. B. Nedwell and C. M. Brown, Eds.), pp. 15–52. Academic Press, London.

Blum, L. K., and A. L. Mills. 1991. Microbial growth and activity during the initial stages of seagrass decomposition. *Marine Ecology Progress Series* **70:**73–82.

Carlson, C. A., and H. W. Ducklow. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. *Aquatic Microbial Ecology* **10:**69–85.

Carlson, C. A., N. R. Bates, D. A. Hansell, and H. W. Ducklow. 1999. Estimation of bacterial respiration and growth efficiency in the Ross Sea, Antarctica. *Aquatic Microbial Ecology* **19:**229–244.

Carlsson, P., A. Z. Segatto, and E. Granéli. 1993. Nitrogen bound to humic matter of terrestrial origin — a nitrogen pool for coastal phytoplankton. *Marine Ecology Progress Series* **97:**105–116.

Cherrier, J., J. E. Bauer, and E. R. M. Druffel. 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. *Marine Ecology Progress Series* **139:**267–279.

Cherrier, J., J. E. Bauer, E. R. M. Druffel, R. B. Coffin, and J. P. Chanton. 1999. Radiocarbon in marine bacteria: Evidence for the ages of assimilated carbon. *Limnology and Oceanography* **44:**730–736.

Coffin, R. B., J. P. Connolly, and P. S. Harris. 1993. Availability of dissolved organic carbon to bacterioplankton examined by oxygen utilization. *Marine Ecology Progress Series* **101:**9–22.

Connolly, J. P., R. B. Coffin, and R. E. Landeck. 1992. Modeling carbon utilization by bacteria in natural water systems. *In* "Modeling the Metabolic and Physiologic Activities of Microorganisms" (C. J. Hurst, Ed.), pp. 249–276.

Cottrell, M. T., and D. L. Kirchman. 2000. Natural assemblage of marine proteobacteria and members of *Cytopha–Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Applied and Environment Microbiology* **66:**1692–1697.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in aquatic systems. *Annual Review of Ecology and Systematics* **29:**503–541.

Engstrom, D. R. 1987. Influence of vegetation and hydrology on the humus budgets of Labrador lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **44:**1306–1314.

Findlay, S., and R. L. Sinsabaugh. 1999. Unraveling the sources and bioavailability of dissolved organic matter in lotic aquatic ecosystems. *Marine and Freshwater Research* **50:**781–790.

Findlay, S., M. L. Pace, D. Lints, and K. Howe. 1992. Bacterial metabolism of organic carbon in the tidal freshwater Hudson Estuary. *Marine Ecology Progress Series* **89:**147–153.

Fry, B., C. S. Hopkinson, A. Nolin, B. Norrman, and U. L. Zweifel. 1996. Long-term decomposition of DOC from experimental diatom blooms. *Limnology and Oceanography* **41:**1344–1347.

Geller, A. 1986. Comparison of mechanisms enhancing biodegradability of refractory lake water constituents. *Limnology Oceanography* **31:**755–764.

Giovannoni, S., and M. Rappé. 2000. Evolution, diversity, and molecular ecology of marine prokaryotes. *In* "Microbial Ecology of the Oceans" Wiley–Liss, New York.

Grøm, C., J. Tørslør, H. J. Albrechtsen, and H. M. Jensen. 1992. Biodegradability of dissolved organic carbon in groundwater from an unconfined aquifer. *Science of the Total Environment* **117/118:**241–251.

Hansell, D. A., N. R. Bates, and K. Gundersen. 1995. Mineralization of dissolved organic carbon in the Sargasso Sea. *Marine Chemistry* **51:**201–212.

Hopkinson, C. S., I. Buffam, J. Hobbie, J. Vallino, M. Perdue, B. Eversmeyer, F. Prahl, J. Covert, R. Hodson, M. A. Moran, E. Smith, J. Baross, B. Crump, S. Findlay, and K. Foreman. 1998. Terrestrial inputs of organic matter to coastal ecosystems: An intercomparison of chemical characteristics and bioavailability. *Biogeochemistry* **43:**211–234.

Hunt, A. P., J. D. Parry, and J. Hamilton-Taylor. 1999. Further evidence of elemental composition as an indicator of the bioavailability of humic substances to bacteria. *Limnology and Oceanography* **45:**237–241.

Janse, I., M. van Rijssel, A. Ottema, and J. C. Gottschalk. 1999. Microbial breakdown of *Phaeocystis* mucopolysaccharides. *Limnology and Oceanography* **44:**1447–1457.

Kaplan, L. A., and T. L. Bott. 1983. Microbial heterotrophic utilization of dissolved organic matter in a piedmont stream. *Freshwater Biology* **13:**363–377.

Kenworthy, W. J., C. A. Currin, M. S. Fonseca, and G. Smith. 1989. Production, decomposition, and heterotrophic utilization of the seagrass *Halophila decipiens* in a submarine canyon. *Marine Ecology Progress Series* **51:**277–290.

Kirchman, D. L., Y. Suzuki, C. Garside, and H. W. Ducklow. 1991. High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. *Nature* **352:**612–615.

Kling, G. W. 1995. Land–water linkages: The influence of terrestrial diversity on aquatic systems. *In* "Role of Biodiversity in Arctic and Alpine Tundra Ecosystems" (F. S. Chapin and C, Körner, Eds.), pp. 297–310. Springer-Verlag, New York.

Kroer, N. 1993. Bacterial growth efficiency on natural dissolved organic matter. *Limnology and Oceanography* **38:**1282–1290.

Linley, E. A. S., and R. C. Newell. 1984. Estimates of bacterial growth yield based on plant detritus. *Bulletin of Marine Science* **35:**409–425.

Lock, M. A., and H. B. N. Hynes. 1976. The fate of "dissolved" organic carbon derived from autumn-shed maple leaves (*Acer saccharum*) in a temperate hard-water stream. *Limnology and Oceanography* **21:**436–443.

Markosova, R. 1991. Growth of bacterioplankton on dissolved organic carbon in Hamilton Harbor and Western Lake Ontario. *Water Pollution Research Journal of Canada* **26:**173–185.

Marmonier, P., D. Fontvieille, J. Gilbert, and V. Vanek. 1995. Distribution of dissolved organic carbon and bacteria at the interface between the Rhone River and its alluvial aquifer. *Journal of the North American Benthological Society* **14:**382–392.

Michaelson, G. J., C. L. Ping, G. W. Kling, and J. E. Hobbie. 1998. The character and bio-

activity of dissolved organic matter at thaw and in the spring runoff waters of the arctic tundra north slope, Alaska. *Journal of Geophysical Research* **103:**939–946.

Middelboe, M., B. Nielsen, and M. Søndergaard. 1992. Bacterial utilization of dissolved organic carbon (DOC) in coastal waters — determination of growth yield. *Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie* **37:**51–61.

Middleburg, K. J., T. Vlug, and F. J. W. A. van der Nat. 1993. Organic matter mineralization in marine systems. *Global and Planetary Change* **8:**47–58.

Moran, M. A., and R. E. Hodson. 1989. Formation and bacterial utilization of dissolved organic carbon derived from detrital lignocellulose. *Limnology and Oceanography* **34:**1034–1047.

Moran, M. A., and R. E. Hodson. 1994. Dissolved humic substances of vascular plant origin in a coastal marine environment. *Limnology and Oceanography* **39:**762–771.

Moran, M. A., W. M. Sheldon, Jr.,r, and J. E. Sheldon. 1999. Biodegradation of riverine dissolved organic carbon in five estuaries of the southeastern United States. *Estuaries* **22:**55–64.

Moran, M. A., W. A. S. Sheldon, Jr., and R. G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. *Limnology and Oceanography* **45:**1254–1264.

Newell, R. C., and M. I. Lucas. 1981. The quantitative significance of dissolved and particulate organic matter released during fragmentation of kelp in coastal waters. *Kieler Meeresforschungen Sonderheft* **5:**356–359.

Ogura, N. 1972. Rate and extent of decomposition of dissolved organic matter in surface seawater. *Marine Biology* **13:**89–93.

Ogura, N. 1975. Further studies on decomposition of dissolved organic matter in coastal seawater. *Marine Biology* **31:**101–111.

Osinga, R., K. A. de Vries, W. E. Lewis, W. von Raaphorst, L. Dijkhuizen, and F. C. von Duyl. 1997. Aerobic degradation of phytoplankton debris dominated by *Phaeocystis* sp. in different physiological stages of growth. *Aquatic Microbial Ecology* **12:**11–19.

Peduzzi, P., and G. J. Herndl. 1991. Decomposition and significance of seagrass leaf litter (*Cymodocea modosa*) for the microbial food web in coastal waters (Gulf of Trieste, Northern Adriatic Sea). *Marine Ecology Progress Series* **71:**163–174.

Pett, R. J. 1989. Kinetics of microbial mineralization of organic carbon from detrital *Skeletonema costatum cells*. *Marine Ecology Progress Series* **52:**123–128.

Qualls, R. G., and B. L. Haines. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. *Soil Science Society of America Journa*l **56:**578–586.

Raymond, P. A., and J. E. Bauer. 2000. Bacterial utilization and transport of DOC in a temperate estuary: Implications for export to the coastal ocean. *Aquatic Microbial Ecology* **22:**1–12.

Satoh, Y., and H. Abe. 1987. Dissolved organic matter in colored water from mountain bog pools in Japan II. Biological decomposability. *Archiv für Hydrobiologie* **111:**25–35.

Servais, P. A., A. Anzil, and C. Ventresque. 1989. Simple method for determination of biodegradable dissolved organic carbon in water. *Applied and Environment Microbiology* **55:**2732–2734.

Sherr, E. B., B. F. Sherr, and T. J. Cowles. 2001. Mesoscale variability in bacterial activity in the Northeast Pacific Ocean off Oregon, USA. *Aquatic Microbial Ecology* **25:**21–30.

Søndergaard, M. 1984. Dissolved organic carbon in Danish lakes: Concentration, composition, and lability. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* **22:**780–784.

Søndergaard, M., and M. Middelboe. 1995. A cross-system analysis of labile dissolved organic carbon. *Marine Ecology Progress Series* **118:**283–294.

Søndergaard, M., B. Hansen, and S. Markager. 1995. Dynamics of dissolved organic carbon lability in a eutrophic lake. *Limnology and Oceanography* **40:**46–54.

Stepanauskas, R., H. Laudoin, and N. O. G. Jorgensen. 2000. High DON bioavailability in boreal streams during a spring flood. *Limnology and Oceanography* **45:**1298–1307.

Sun, L., E. M. Perdue, J. L. Meyer, and J. Weis. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. *Limnology and Oceanography* **42:**714–721.

Thingstad, T. F., A. Hagström, and F. Rassoulzadegan. 1997. Accumulation of degradable DOC in surface waters: Is it caused by a malfunctioning microbial loop? *Limnology and Oceanography* **42:**398–404.

Tranvik, L. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. *Microbiol Ecology* **16:**311–322.

Tranvik, L. J., and M. G. Hofle. 1987. Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. *Applied and Environment Microbiology* **53:**482–488.

Tranvik, L., and N. O. G. Jørgensen. 1995. Colloidal and dissolved organic matter in lake water: Carbohydrate and amino acid composition, and ability to support bacterial growth. *Biochemistry* **30:**77–97.

Tranvik, L., and S. Kokalj. 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. *Aquatic Microbial Ecology* **14:**301–307.

Turley, C. M., and K. Lochte. 1990. Microbial response to the input of fresh detritus to the deep sea. *Paleogeography, Paleoclimatology, Paleoecology* **89:**2–23.

Volk, C. J., C. B. Volk, and L. A. Kaplan. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. *Limnology and Oceanography* **42:**39–44.

Weiss, M., and M. Simon. 1999. Consumption of labile dissolved organic matter by limnetic bacterioplankton: The relative significance of amino acids and carbohydrates. *Aquatic Microbial Ecology* **17:**1–12.

Wetzel, R. G., and B. A. Manny. 1972. Decomposition of dissolved organic carbon and nitrogen compounds from leaves in an experimental hard-water stream. *Limnology and Oceanography* **19:**927–931.

Wikner, J., R. Cuadros, and M. Jansson. 1999. Differences in consumption of allochthonous DOC under limnic and estuarine conditions in a watershed. *Aquatic Microbial Ecology* **17:**289–299.

Zweifel, U. L. 1999. Factors controlling accumulation of labile dissolved organic carbon in the Gulf of Riga. *Eastuarine, Coastal and Shelf Science* **48:**357–370.

Zweifel, U. L., B. Norrman, and A. Hagström. 1993. Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. *Marine Ecology Progress Series* **101:**23–32.

Zweifel, U., J. Wikner, A. Hagström, E. Lundberg, and B. Norrman. 1995. Dynamics of dissolved organic carbon in a coastal ecosystem. *Limnology and Oceanography* **40:**299–305.

Zweifel, U. L., N. Blackburn, and A. Hagström. 1996. Cycling of marine dissolved organic matter. I. An experimental system. *Aquatic Microbial Ecology* **11:**65–77.

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Integrating Dissolved Organic Matter Metabolism and Microbial Diversity: An Overview of Conceptual Models

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Aquatic Ecosystems: Interactivity of Dissolved Organic Matter

I. INTRODUCTION

Research advances by both conception and technological innovation. The former shapes the questions that are asked and orders the interpretation of data; the latter expands the range of questions that are addressable and the volume of data that can be collected. The dynamics of heterotrophic microbial communities is a topic long hampered by technological limitations. As those limitations have eased, the complexity and diversity associated with the microbial processing of dissolved organic nutrients has become a large template for research. The increasing flow of data from this research begs the need for conceptual models that order the information (Azam, 1998). In this chapter, we present an overview of the principal conceptual models associated with various perspectives on microbial community organization and dissolved organic matter (DOM) processing. Both "pattern" models and "process" models are included. We have classified these models into three research domains: the community structure domain, the biogeochemical domain, and the trophic domain (Fig. 1). As we cover each domain, we try to identify empirical gaps and opportunities for bridging across domains. We also highlight differences between freshwater and marine systems when they are relevant to the range of application of various models.

II. COMMUNITY STRUCTURE DOMAIN

A. Biofilms

The predilection of bacteria to associate with surfaces is the primary organizing principle of community structure. ZoBell (1943) presented a conceptual model of this organization: surfaces concentrate nutrients; bacterial attachment to surfaces maximizes concentration gradients and reduces diffusion distances; packing cells on surfaces improves retention of extracellular enzymes. Over the next 40 years, the study of attached microbial communities became an integral part of several fields (see Chapter 12). The importance of periphyton, or aufwuchs, in stream metabolism was established (Hynes, 1970; Geesey *et al*., 1978; Stevenson *et al*., 1996). Comparative studies of community organization and activity in relation to substratum produced a taxonomy for attached communities: epilithon, epiphyton,

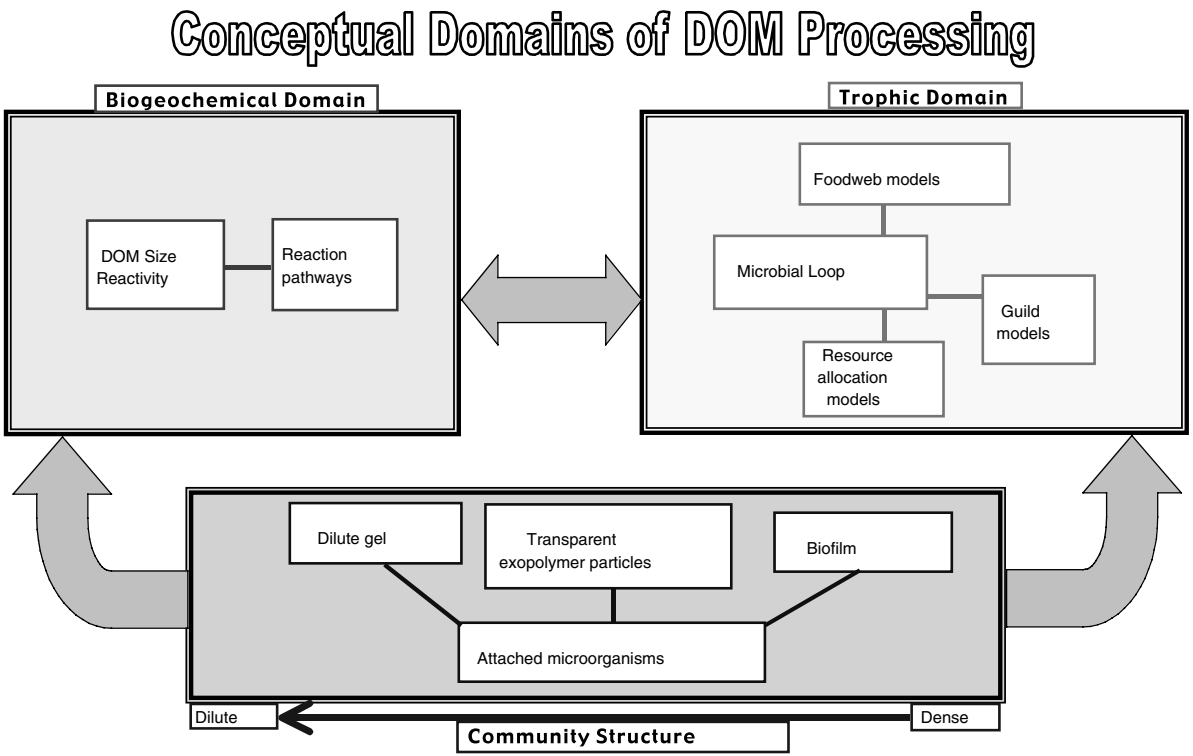

FIGURE 1 Principal research domains for investigations of DOM dynamics in relation to bacteria community organization. Within each domain a fundamental concept has become the basis for models of increasing sophistication. For the community structure domain, the founding concept is the affinity of bacteria and DOM for surfaces; for the biogeochemical domain, the founding concept is the relationship between molecular size and reactivity; for the trophic domain, the founding concept is the microbial loop.

epipsammon, and epixylon (Lock, 1993). In the 1980s, this research, along with a lot of microbiological work from soils and sediments, was unified by a new paradigm, the biofilm (Characklis and Marshall, 1990).

The biofilm concept developed in environmental engineering and quickly spread to microbial ecology. By emphasizing the organizational commonalities of attached microbial communities, it bridged disciplinary perspectives and generated new conceptions of the processing of dissolved and particulate organic matter (e.g., Lock *et al*., 1984; Costerton *et al*., 1995).

Biofilms are complex communities of microorganisms attached to a surface and enveloped in a microbially produced, exopolymer matrix (Christensen and Characklis, 1990; Lock, 1994). This matrix, although composed largely of exopolysaccharides (EPS), also contains protein, nucleic acid, and humic compounds. The EPS forms a hydrated gel (Jahn and Neilsen, 1993; Costerton *et al*., 1995); gradients within the matrix lead to the formation of consortia that integrate the community and enhance metabolism (Costerton *et al*., 1995; Paerl and Pinckney, 1996; Wimpenny and Colasanti, 1997). Functions of the EPS include an adhesive for the attachment of cells (Headley *et al*., 1995), a lattice that allows heterotrophic and autotrophic organisms to create microenvironments (Gieseke *et al*., 2001; Whitely *et al*., 2001), an ion exchange medium (Freeman *et al*., 1995), a trap for soluble and particulate nutrients (Fiebig and Marxsen, 1992), and a framework that enhances retention of extracellular enzymes (Sinsabaugh and Linkins, 1988; Thompson and Sinsabaugh, 2000). Reservoirs of nutrients retained within the matrix buffer responses to fluctuations in water column delivery (Lock *et al*., 1984; Freeman and Lock, 1995; Battin *et al*., 1999). Studies have highlighted the role of pores and microchannels in the transport of solutes through the biofilm (de Beer and Stoodley, 1995; de Beer *et al*., 1996; Neu and Lawrence, 1997), the significance of cell to cell signaling in community operation (Davies *et al*., 1998), and the phenotypic transformations that accompany cellular attachment (Davies *et al*., 1993; Goodman and Geesey, 2000).

In freshwater ecosystems, particularly streams and wetlands, biofilms account for a large portion of heterotrophic metabolism, as well as primary production (Edwards *et al*., 1990; see Chapter 12), acting as both sources and sinks for DOM. As the depth of the overlying water in the system increases, attached communities account for a declining share of system metabolism.

B. Suspended Exopolymer Particles

A biofilm is commonly visualized as a two-dimensional matrix layered on a solid surface. However, aggregates of exopolymer, detritus, and cells also form in the water column through a variety of physical, chemical, and biotic processes (Ward *et al*., 1994; Grossart *et al*., 1997; Chapter 12). These aggregates are variously described as flocs or "snow". A type of aggregate

called transparent exopolymer particles (TEP) has been described. TEP are found in marine (McIntyre *et al*., 1995) and limnetic waters (Grossart *et al*., 1997). Operationally, TEP are defined as discrete particles >0.4 μm in diameter, formed from acidic polysaccharide precursors released by phytoplankton (Zhou *et al*., 1998). After staining with alcian blue, TEP appear as strings, disks, or gellike films that contain enmeshed bacteria (Passow and Alldredge, 1995). The existence of gelatinous particles in the water column has important ramifications for microbial growth and carbon transformations in planktonic systems (Decho, 1990; Smith *et al*., 1992). These particles have many of the same properties and characteristics as attached biofilms, including the ability to support diverse types of bacterial metabolism through creation of microgradients (Alldredge, 2000). In addition to providing nuclei for nutrient sorption and fostering heterotrophic metabolism, such particles represent a vector for export of DOM from surface waters to the benthic zone (Passow and Alldredge, 1994) and for delivery of DOM directly to zooplankters (Decho, 1990; Wotton, 1994; Lemke and Bowen, 1998).

C. Planktonic Communities

The architecture of biofilms and exopolymer particles, in more attenuated form, ramifies throughout the water column (Azam, 1998). Phytoplankton and zooplankton release polymeric material (Nagata, 2000; see Chapter 1); cells lyse; humic macromolecules form. These events provide fuel for heterotrophic metabolism, nuclei for sorption and aggregate formation, and a scaffold to which bacteria are linked. Consequently, the distribution of DOM and cells on a microscale is heterogeneous yet structured. Within this dilute gel, there is a continuum of organic particle size — monomers, polymers, viruses, colloids, aggregates, cells — spanning 7 orders of magnitude in mass (Azam, 1998).

D. Synthesis

The distribution of DOM and bacteria in aquatic environments is ordered by the affinity of both for surfaces and by the gel-creating properties of exopolymers and other macromolecules (Fig. 2). Although biofilm communities attached to a solid phase substratum are often considered distinct from planktonic communities, it is also useful to consider the aquatic habitat as an aqueous gel whose density varies spatiotemporally. As gel density (DOM per unit volume) increases, microgradients become more pronounced and diffusion distances shrink. With increased structure and nutrient availability, the potential for consortial development rises, leading to enhancement of community metabolism and integration. Thus, microbial diversity may increase as well as numbers. Extrapolating to larger scales,

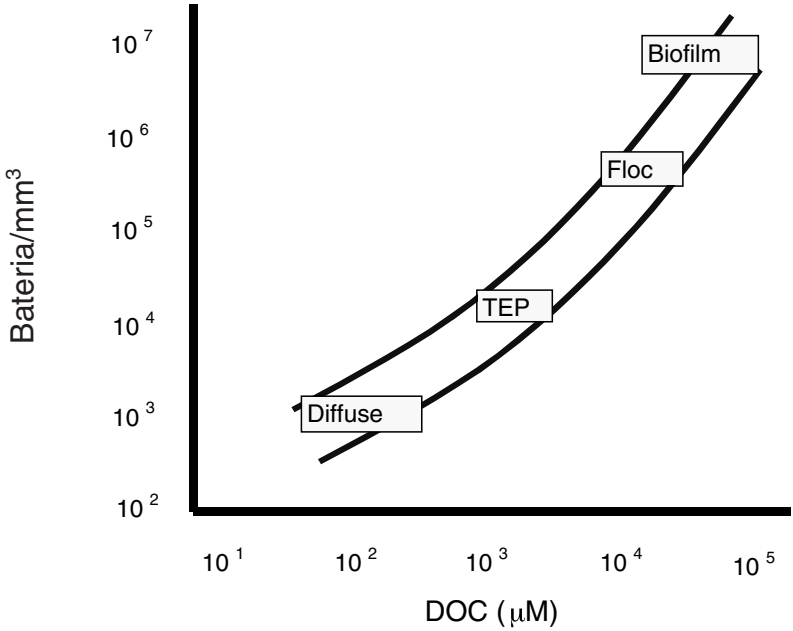

FIGURE 2 Aquatic systems organized as an aqueous gel. The propensity of diverse macromolecules to coalesce imparts a gellike structure on aqueous environments. Bacteria and nutrients associate with this lattice to create microenvironments that enhance community metabolism and diversity. As a result, bacterial abundance may decline more rapidly with dilution than DOM.

the prediction is that systems with a high surface area to water volume ratio and environmental conditions conducive to organisms that produce abundant exopolymer will have the highest turnover rates for DOM.

III. BIOGEOCHEMICAL DOMAIN

A. Size–Reactivity

The heterogeneity of DOM is the product of both the diversity of sources and the range of biogeochemical reactions to which it is subject. Generalizations about composition and fate are difficult. One of the most useful was proposed by Amon and Benner (1996), who synthesized information from spectrometric analyses and microbiological assays of DOM from marine and riverine systems in the form of a diagenetic relationship called the size–reactivity model. In this model, the most recalcitrant DOM is comprised of low molecular weight aliphatic molecules. DOM fractions of larger molecular size are increasingly more bioavailable and photoreactive (Amon and Benner, 1996; see Chapter 5).

The size–reactivity model has generated a lot of discussion, because it seems counterintuitive to those accustomed to thinking about DOM processing from a trophic perspective where large molecules must be degraded into small monomeric ones for uptake. Thus, perceptions of size–reactivity relationships depend on whether you focus on what is consumed (trophic perspective) or what is left behind (diagenetic perspective; Fig. 3). In part, the paradox is a manifestation of the heterogeneity of DOM in that every size class includes both reactive and unreactive molecules, but it is also a manifestation of scale: small scale trophic observations versus larger scale diagenetic patterns. For any particular system, what you see probably depends on whether DOM inputs are dominated by new carbon or old carbon. Because freshwater systems tend to be more dynamic and more eutrophic than marine ones, the diagenetic pattern may be more difficult to see in those systems.

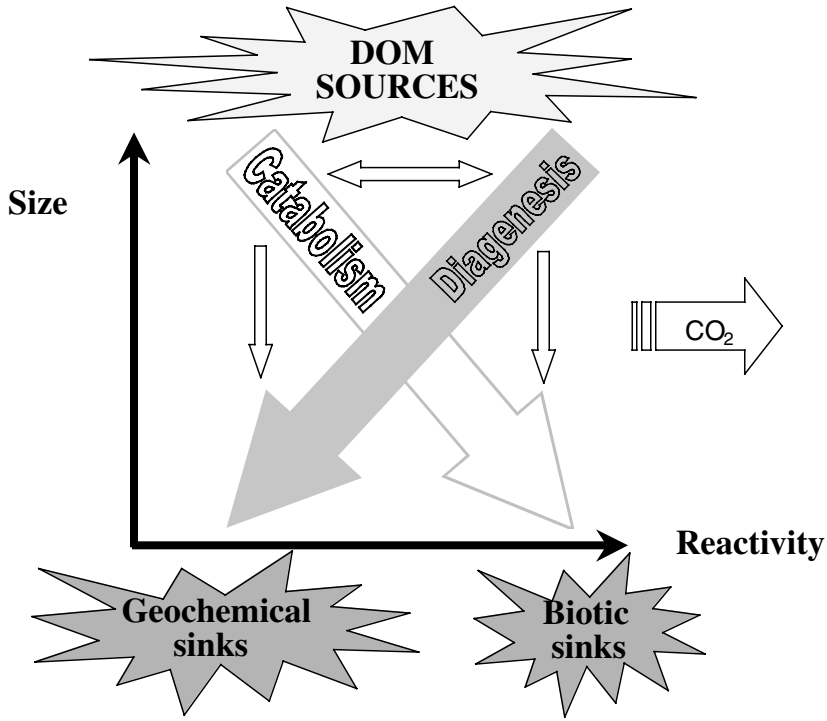

FIGURE 3 Diagenetic versus trophic perspectives on the relationship between molecular size and reactivity. Perceptions of size–reactivity relationships depend on whether the focus is on what is consumed or what remains. From the trophic perspective, small molecules turn over more rapidly than polymers, which turn over more rapidly than humic complexes. From the diagenetic perspective, large molecules are progressively stripped of reactive moieties, leaving behind small refractory residuals.

B. Biogeochemical Process Pathways

The size–reactivity model is a "big-picture" description of DOM processing. To understand the flow of organic nutrients into bacteria, these relationships must be examined at higher resolution. The principal questions are (1) what chemical characteristics of DOM make it potentially bioavailable and (2) what biogeochemical and hydrodynamic processes proffer DOM to bacteria? This approach complements the diagenetic one in that the focus is on what is consumed rather than what remains. Operationally, the goal is to identify the pathways through which constituents of the DOM pool are assimilated. These pathways can be studied on a range of scales. At the ecosystem level, the interest is describing the principal biogeochemical process paths through which DOM flows to bacteria, and presumably contrasting these among systems. At the community scale, the interest is identifying the metabolic pathways through which various classes of substrates are consumed in a manner analogous with descriptions of cellular metabolism. At both scales, there is potential to connect the heterogeneity of DOM with bacterial diversity.

At the ecosystem scale, Findlay and Sinsabaugh (1999) proposed that carbon flow from DOM to bacteria moves along four major pathways, each defined by their first, and presumably rate-determining, steps: (1) direct uptake, (2) ectoenzyme-mediated uptake, (3) photolysis-mediated uptake, and (4) sorption-mediated uptake (Fig. 4).

Direct uptake is the assimilation of an organic molecule without mediation by an external process. Small monomeric or oligomeric substrates (e.g., amino acids, fatty acids, saccharides, nucleosides) up to a few hundred

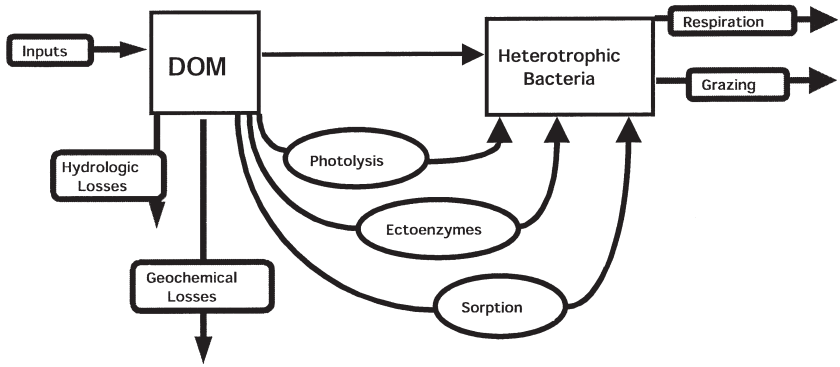

FIGURE 4 Pathways for DOM consumption. There are four types of pathways or processes, differentiated by their first and presumably rate-limiting steps, that connect DOM to bacterial assimilation: (1) direct uptake, (2) ectoenzyme-mediated uptake, (3) photolysis-mediated uptake, and (4) sorption-mediated uptake. The significance of each pathway for a particular system is assessed by measuring the kinetics of its first reaction.

daltons in size can be consumed. Because such substrates are useful to a wide variety of heterotrophs their residence time in the DOM pool is short (Bunte and Simon, 1999; see Chapter 9). Long distance transport is unlikely, so the source of these compounds is generally secretion and lysis of nearby cells (see Chapter 1). For individual compounds, uptake rates are determined by substrate concentration and microbial "demand." The process can be quantified using isotopically labeled substrates, and for comparative purposes, the results can be expressed in terms of Monod kinetics. (Methods are detailed in several chapters in Kemp *et al*., 1993.)

For ectoenzyme-mediated uptake, the rate-defining step is the degradation of macromolecules into assimilable products by the action of extracellular enzymes (Chróst, 1990; Münster and De Haan, 1998; Burns, 2001; see Chapter 13). These enzymes may be hydrolytic or oxidative, with varying degrees of specificity, and reaction products may include sources of nitrogen, phosphorus, and other nutrients as well as carbon. Enzyme activities are commonly measured using colorigenic or fluorigenic substrates (Hoppe, 1993; Sinsabaugh *et al*., 1999), although isotopically labeled compounds are sometimes used for *in situ* measurements, and results are often presented in terms of Michaelis–Menten models (see Chapter 13).

In photolysis-mediated uptake, the rate-defining step is the generation of assimilable products from the photooxidation of DOM. Most macromolecular DOM contains photoreactive moieties that differ in identity and abundance among DOM sources and along diagenetic gradients (see Chapter 10). The absorption of solar radiation causes chemical oxidation reactions that can generate products for microbial assimilation, as well as inorganic carbon (Moran and Zepp, 1997; Wetzel *et al*., 1995). However, random oxidations can also lead to condensation reactions that reduce bioavailability (Tranvik and Kokalj, 1998; Opsahl and Benner, 1998). The net effects of photoreaction on DOM bioavailability in various systems have yet to be generalized. The flow of nutrients through the photolytic pathway is generally presented in the form of dose–response curves with microbial production or respiration as the metric (Moran and Zepp, 2000).

Sorption-mediated uptake is probably the least quantified uptake pathway. The sorption of dissolved molecules to exopolymers, cell fragments, humic colloids, biofilms, and particle surfaces concentrates carbon and other nutrients (Johnson *et al*., 1994; Lock, 1994; see Chapters 7 and 12). As described earlier, the association of bacteria with these structures creates a heterogeneous distribution of microbial cells and microbial activities, and the turnover of organic matter associated with these structural elements constitutes a pathway for directing DOM into bacteria. DOM sorption alters bioreactivity and residence time (e.g., Fiebig, 1997; Freeman and Lock, 1995), but also condenses DOM into units that may be directly consumed by protists and animals (Wotton, 1994; Lemke and Bowen, 1998), thereby diverting organic matter from the microbial loop to higher trophic levels.

C. Metabolic Pathways

At the ecosystem level, the principal goals of the biogeochemical process approach are to trace the mass flow of carbon and nutrients along particular process paths and to estimate mass balances. If we reduce the scale of interest from the ecosystem to the community, the focus shifts to descriptions of the extracellular biochemical pathways that process specific DOM components (Fig. 5). For this overview, we restrict our discussion to general observations and conjectures about various classes of substrates rather than attempt to review each of the pathways shown in Fig. 5.

Monomers, polysaccharides, and polypeptides are generally considered the major fuels for community metabolism (see Chapter 9). There are data on utilization rates in various systems, but not enough to make generalizations about differences among various types of aquatic ecosystems (see Chapter 17). Even less information is available on the underlying enzymology. Within systems, the activities of commonly measured ectoenzymes like β-glucosidase (βG) and leucine aminopeptidase (LAP) can vary over 3 orders of magnitude (Foreman *et al*., 1998; Sinsabaugh *et al*., 1997; Fukuda *et al*., 2000). Among systems, the ratio of the two varies by 5 orders of magnitude. The mix of biotic and environmental factors that underlie this variation is not well known (Foreman *et al*., 1998), and these enzymes, along with phosphatase (Ammerman, 1991), are the most studied.

Although βG and LAP are sometimes used as indicators of the relative magnitude of polysaccharide and polypeptide consumption (e.g., Christian and Karl, 1995), the obvious point that these catalyze only two of the many extracellular reactions needed for polysaccharide and polypeptide degradation bears emphasis (Sinsabaugh and Foreman, 2001; see Chapter 13). Given that enzyme construction is energetically expensive and that the materials they contain are lost to the organism when the enzyme enters the environment, it is reasonable to assume that expression of inducible enzymes is indicative of a net positive nutrient and energy flow to the cell. If true, the economics of polysaccharide degradation may differ from that of polypeptide degradation. Compared to polysaccharides, polypeptides are "simple" linear polymers composed of about two dozen different alpha amino acids joined by identical linkages. Polysaccharides are more structurally diverse: several different linkages are common, permitting branching. The variety of monomers is also higher because saccharides have more anomeric carbons as well as multiple reactive sites that can be oxidized, reduced, or substituted. This difference in structural complexity means that polysaccharide degradation potentially requires a larger number of enzymes than polypeptide degradation. The implications include: (1) metabolic costs of polysaccharide degradation may be higher than those for polypeptides, both in terms of the number of enzymes that need to be expressed and the quantity of assimilable substrate released; (2) expression of extracellular glycosidases

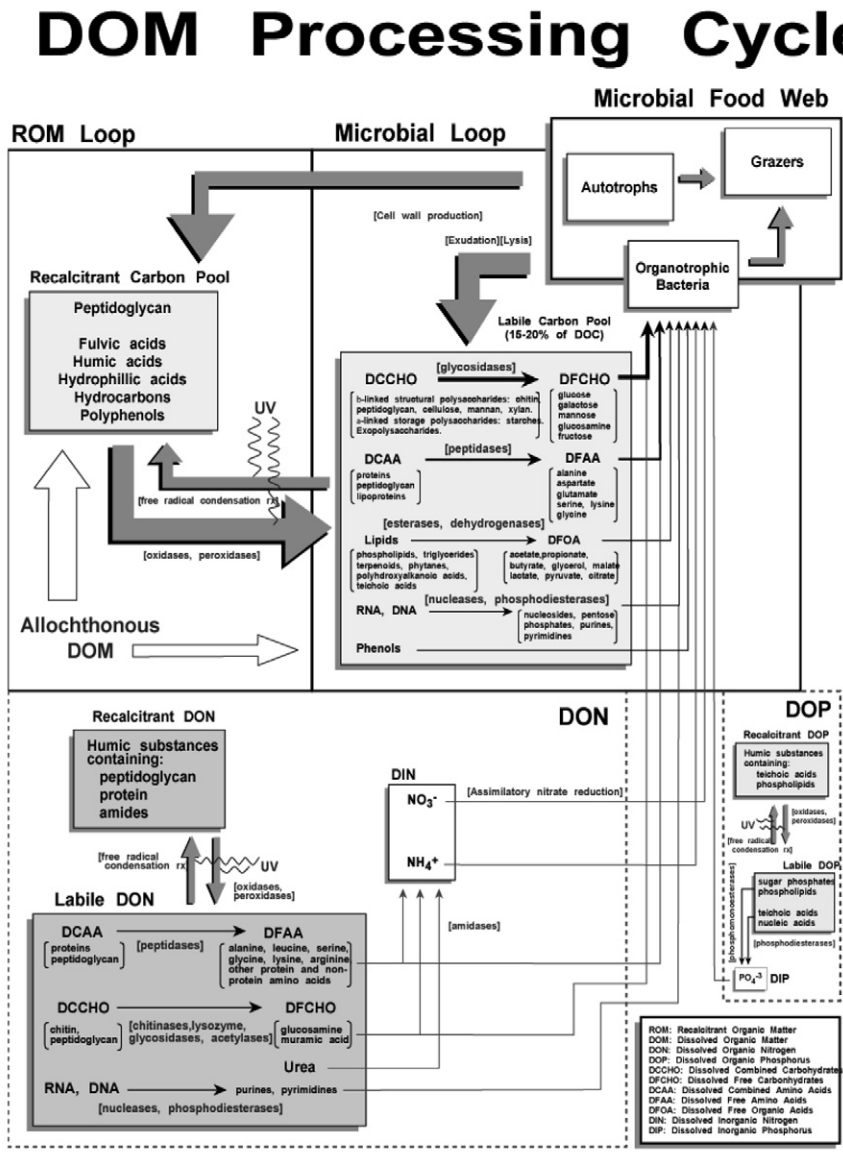

FIGURE 5 Extracellular metabolism of DOM. The diagram represents the principal pathways for the extracellular degradation and assimilation of DOM

may be more tightly regulated than that of peptidases; and (3) the polysaccharide degradation capability of individual taxa may be more specialized or restricted than polypeptide capabilities. These conjectures are consistent with present knowledge such as the apparent preference of heterotrophic bacteria for amino acids (see Chapter 9), but to our knowledge, they have not been investigated.

D. Recalcitrant DOM

Identifying the pathways for labile substrate utilization helps connect DOM composition with community metabolism and perhaps diversity as well, but it is also interesting to consider what makes some DOM components recalcitrant. There appear to be two classes of recalcitrant substrates. Polyphenols, largely humic and fulvic acids, are the residues from microbial decomposition of plant litter (Thurman, 1986; Stevenson, 1994; McKnight and Aiken, 1998). Stable diagenetic products (SDP) are small, reduced aliphatic molecules that appear to be the end product of microbial metabolism (Ogawa *et al*., 2001). From a geochemical perspective, these two recalcitrant fractions are entangled, but from the perspective of community metabolism they are quite distinct.

Phenolic moieties are degraded by a variety of monoxygenases, dioxygenases, and peroxidases (Hammel, 1997). A key feature of these enzymes is their capacity to attack a broad range of substrates. These enzymes have received a lot of study in terrestrial systems for their role in lignin degradation, litter breakdown, and humification. Their role in regulating soil carbon storage in relation to nitrogen deposition and global climate change has been highlighted (Freeman *et al*., 2001, Sinsabaugh *et al*., 2002). In aquatic systems, phenol oxidase and peroxidase activities are seldom measured, although some data exist for benthic particulate organic matter (POM) and biofilms, and to a much lesser extent, the water column (see review by Münster and De Haan, 1998). [An exception to this lack of information is the oxygenases of proteobacteria, which have been extensively studied in the context of degradation of xenobiotic compounds like polychlorinated biphenyls, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, and polycyclic aromatic hydrocarbons (Wackett and Hershberger, 2001).]

The knowledge gap between terrestrial and aquatic systems is significant because of accumulating evidence that humic DOM is less recalcitrant than once thought (Bano *et al*., 1997; Carlson *et al*., 1993; Tranvik, 1998; Moran *et al*., 1999). In some freshwater systems, this material is a major trophic resource. In others, its presence inhibits bacterial activity by inhibiting extracellular enzymes, reducing growth, and increasing respiration (Wetzel, 1991, 1992; Foreman *et al*., 1998; Foreman, 1999). The net effect on community metabolism appears to depend on the magnitude of autochthonous DOM inputs relative to inputs of plant-derived DOM. The input ratio determines the type of bacteria selected and thus the type of DOM that is

degraded (see Chapter 14). In oligotrophic systems, the availability of micronutrients may also limit degradative potential because oxidative enzymes contain iron- or copper-containing prosthetic groups.

Although the products of humification may be described as conditionally recalcitrant (apparent recalcitrance varying with community composition, oxygen availability, solar radiation, and residence time), SDP is the refractory residue of microbial metabolism. It represents the difference between what bacteria consume and what the environment proffers. This asymmetry is a consequence of cellular organization and the economics of nutrient acquisition.

Microbial infallibility with regard to biodegradation applies only on the largest scales. A bacterium contains a limited amount of genetic information, most of which is translated into enzymes that are organized into catabolic and synthetic pathways that generate energy and construct biomolecules (White, 1995). It is estimated that 70% of these enzymes catalyze redox reactions (Wackett and Hershberger, 2001), which generally have lower substrate specificity than other types of enzymatic reactions. To acquire materials for these cellular pathways, bacteria also deploy enzymes outside the cell. For any particular cell, the number and type of enzymes varies, but for energetic reasons the range of enzymes deployed extracellularly is far smaller than the range deployed intracellularly.

The principal determinant of lability for DOM components is the cost, measured by the number of enzyme-catalyzed reaction steps and the yield of substrate per enzyme expressed, required to transform a particular class of molecules into an intermediate metabolite. Thus, extracellular enzyme activity is skewed toward hydrolases that release monosaccharides, amino acids, and organic acids from polymers. The deployment of less specific, oxidative enzymes to generate organic nutrients appears more taxonomically and spatiotemporally restricted, apparently because the energetic return in terms of intermediate metabolites is lower. Organisms using this less efficient strategy are more likely to be prominent in environments that have low rates of labile DOM input. Within this strategy, the probability of generating intermediary metabolites through nonspecific oxidation reactions declines with molecular size: cracking a large humic colloid is more likely to yield useful substrate than fracturing a small aliphatic molecule. At the ecosystem level, the net result is a pool of SDP, which represents the differential between extracellular biogeochemical reactions that randomize the molecular weight distribution and composition of DOM, and cellular reactions that structure it.

E. Synthesis

At the largest scale, the DOM pool can be resolved into four functional pools: labile, semilabile, recalcitrant, and refractory (Fig. 6). This classification reflects both chemical composition, measured in units of intermediatory

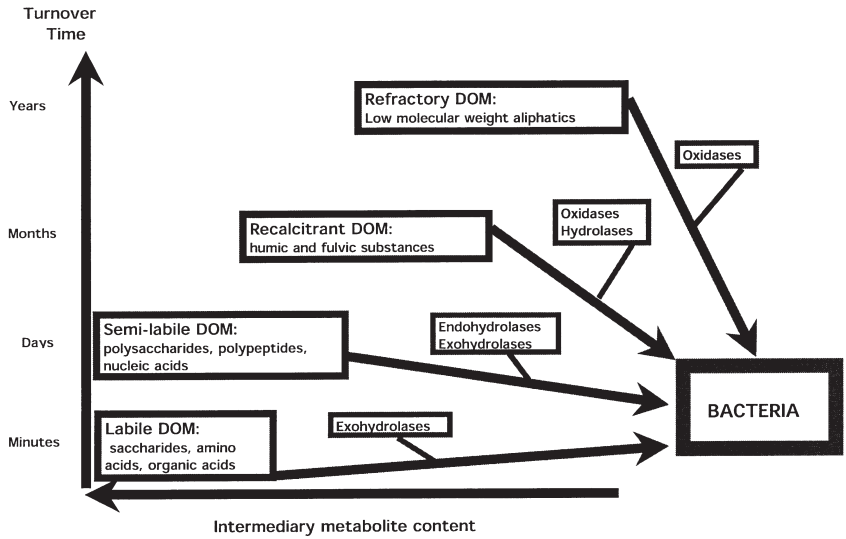

FIGURE 6 A functional classification for DOM. The bioavailability of DOM is a function of its intermediary metabolite content, and the number and type of enzymes needed to generate intermediary metabolites. Turnover time increases as the content of intermediary metabolites declines and the heterogeneity of linkages increases.

metabolites, and the number and type of reactions needed to release intermediary metabolites for bacterial consumption. In this scheme, labile DOM consists of intermediary metabolites, and molecules that can be transformed into intermediary metabolites in a single reaction step. Examples are saccharides, amino acids, and their oligomers. Semilabile DOM is defined as polymers of intermediary metabolites that are made available for assimilation using multicomponent hydrolase systems. Examples include polypeptides, polysaccharides, nucleic acids, and phospholipids. Recalcitrant DOM comprises humic substances. These substances can include a high proportion of intermediary metabolites, but because of their irregular structure, nonspecific photooxidation or biochemical oxidation steps are needed to access intermediary metabolites. Refractory DOM is composed of SDP that contain a low abundance of intermediary metabolites. The photoreactivity of this material is low and biochemical oxidation using extracellular enzymes is not energetically cost effective.

This four-pool pattern emerges from the asymmetries of intracellular versus extracellular reactions. However, within a particular system, DOM dynamics are affected by the availability of solar radiation, the rate of production of labile and semilabile DOM, and other factors that exert strong selective pressure on bacterial community composition, thereby influencing the relative turnover rates of the various DOM classes. Because these factors vary somewhat predictably on a geographic scale from upland streams,

to rivers, estuaries, continental margins, and mid-ocean, large scale patterns in the processing of recalcitrant DOM may emerge.

IV. TROPHIC DOMAIN

A. Microbial Loop Concept

Before 1974, bacteria and grazing protists were not generally regarded as major players in marine foodweb dynamics. Pomeroy (1974) challenged this view by proposing that these organisms were integral players in the transfer of energy in pelagic systems. Azam *et al.* (1983) developed this proposal into a conceptual framework for aquatic microbial foodwebs: the microbial loop hypothesis. The microbial loop describes the role bacterioplankton play in transferring phytoplankton-derived carbon, in the form of DOM, to the metazoan food chain. In the microbial loop pathway, carbon flows from DOM to bacteria to protists to metazoa. Many studies have confirmed that the microbial loop is a major pathway for the transfer of energy from primary producers to higher trophic levels (Pomeroy and Wiebe, 1988; Ducklow, 2000; Hart *et al.*, 2000). At the same time, the microbial loop also serves as a sink for organic carbon and a regenerator of nutrients (Kirchman, 2000).

The microbial loop concept has been the prevailing paradigm for marine microbial food webs for two decades and has stimulated work on DOM sources and composition, rates of biomass production, transfer efficiencies, and respiratory losses (Benner, 1998; del Giorgio and Cole, 2000; Ducklow, 2000; Williams, 2000). The only major modification has arisen from new information on the abundance and ecology of viruses (Wilhelm and Suttle, 1999; Fuhrman, 1999, 2000).

In many aquatic environments, virioplankton abundance exceeds that of bacterioplankton by an order of magnitude (Bergh *et al.*, 1989). For marine systems, it has been estimated that 20% of bacterioplankton and 3–5% of phytoplankton mortality is attributable to viral infection (Suttle, 1994), and that infection may influence the composition of the microbial community (Bratbak *et al.*, 1994; Steward *et al.*, 1996; Fuhrman, 1999). Modeling studies of microbial foodwebs predict that viral lysis increases both carbon mineralization and bacterial production, and decreases carbon flow to nanoplankton grazers (Fuhrman and Suttle, 1993). Viruses may have a greater effect on the transfer of carbon to higher trophic levels in oligotrophic environments where OM recycling dominates, than in mesotrophic environments where more primary production is available to support metazoan populations (Murray and Eldridge, 1994). In a recent review, Wommack and Colwell (2000) present a revised microbial loop model that explicitly incorporates the role of virioplankton.

B. Marine versus Inland Systems

Although the microbial loop was developed for marine pelagic systems, it has often been applied to inland waters. However, differences between the two environments may affect its relative importance for the movement of dissolved carbon to higher trophic levels. In streams and most lakes, shallow water increases the relative importance of benthic food webs and increases coupling between the planktonic and benthic food webs (Meyer, 1994). Because a large portion of both primary production and microbial heterotrophic production is associated with surfaces and particles (see Chapters 1 and 12), detritivores, grazers, and collectors are common, and their feeding creates a direct path for transfer of microbial carbon to metazoans. Another difference is that major DOM sources and sinks often lie outside the boundaries of the system, so the "loop" is more open. Not least, terrestrially derived DOM differs in composition from that generated *in situ* by microbial primary producers (Hopkinson *et al*., 1998; see Chapters 1, 3, and 5).

The implications of DOM source heterogeneity on the organization and trophic efficiency of microbial communities are only beginning to be appreciated. From a chemical standpoint, it may be hard to show that DOM derived from one source is more heterogeneous or complex than DOM from another, but bacteria can clearly tell the difference. Expose an extant microbial community to a new DOM source and it begins to reorganize almost immediately (see Chapter 14), and it may take several weeks to establish a new metabolic equilibrium. Because many freshwater systems experience diel (see Chapter 4), seasonal and hydrologic modulations in DOM flux from multiple sources (see Chapter 6), community metabolism and biodiversity may be more closely linked to the heterogeneity and dynamics of DOM sources than to bulk composition or concentration. One way to represent the effects of source heterogeneity on DOM processing and community metabolism is to partition heterotrophic bacteria into guilds.

C. Guild Models

In many freshwater systems, humic substances account for most of the DOM pool (Thurman, 1986). This material provides a reservoir of slowly metabolized nutrients that coexist and interact with a labile pool of rapidly consumed monomers and polymers (Tranvik, 1992, 1998; Søndergaard and Middleboe, 1995; Münster and De Haan, 1998; see Chapter 19). The literature clearly illustrates the amphitrophic nature of humic DOM; it acts variously as a significant energy source for bacteria (Tranvik and Hofle, 1987; Tulonen *et al*., 1992; Moran and Hodson, 1994; Volk *et al*., 1997; Jannson, 1998) and as an inhibiter of growth and metabolic activity (Moran and Hodson, 1990; Tranvik, 1992; Foreman *et al*., 1998). This range of

effects suggests that bacterial populations vary in their tolerance for humics as well as in their ability to metabolize them.

Microcosm studies have shown that humic DOM selects heterotrophic bacterial populations that contrast in enzyme kinetics, productivity, cell size, and phylotype with those selected by labile substrates (see Chapter 14). Because many systems receive multiple DOM inputs that vary in composition and abundance, it is likely that divergent phenotypes coexist in bacterioplankton (Pomeroy and Wiebe, 1988; Tranvik, 1992; see Chapter 9). This diversity can be difficult to describe. Although latent phylotypic diversity may be high, there is extensive functional redundancy both within and across classes (Konopka *et al*., 1999; Wackett and Hershberger, 2001), although some evidence suggests that there may be more correspondence between phenotype and phylotype than previously thought (see Chapter 9). Moreover, at any particular moment, community activity is dominated by a relatively small number of populations that may turn over quickly in response to environmental change. These observations suggest that it may be useful to represent bacterioplankton as a set of guilds, each defined by their capacity to compete effectively for particular DOM sources.

The simplest guild model recognizes humic and labile DOM as different resources. This approach is common in trophic models, reflecting well-documented differences in pool size and turnover rate for labile and recalcitrant constituents of DOM (e.g., Münster and De Haan, 1998). To create a guild model, we make a further assumption that to maintain a place within the community, a bacterial population must compete effectively for one or the other of these resources. This premise leads to hypotheses about what constitutes a competitive strategy for exploitation of humic versus labile DOC pools, which in turn form the basis for partitioning heterotrophic bacteria into guilds whose dynamics can be explored through simulation.

D. DOC–Enzyme–Microbe Interaction Model

The DOC/enzyme/microbe interaction (DEMI) model divides bacterioplankton into two functional guilds, opportunists and decomposers, and DOC into two pools, labile and recalcitrant. In the context of the model, labile DOC is defined as directly assimilable monomers (saccharides, amino acids, and organic acids) and readily hydrolyzed polymers (polysaccharides, proteins, and nucleic acids). Because these substrates turn over rapidly, thus are unlikely to be transported far, most of the carbon in this pool will be autochthonous lysates and exudates, or allochthonous leachates from storms or seasonal litter fall. Recalcitrant DOC is defined as humic substances created by oxidative reactions among proteins, polysaccharides, hydrocarbons, and phenolic molecules. For inland waters, recalcitrant DOC is largely of allochthonous origin.

This model assumes a more simplified classification of the DOM pool than the one described in Section III. The labile and semilabile DOM fractions shown in Fig. 6 have been combined to form the labile DOC pool; the recalcitrant DOC (RDOC) pool remains the same as that defined in Fig. 6; and the refractory DOC pool is not part of the model.

Opportunists are defined as consumers of labile DOC (LDOC). The designation refers to their hypothesized capacity to assimilate substrates at low concentration and to the observation that LDOC sources tends to be ephemeral relative to the "background" of RDOC that buffers the metabolism of many freshwater ecosystems (Tranvik, 1998; see Chapter 19). Other proposed characteristics of opportunists include small cell size, low half-saturation constants for substrate uptake (K_s) and ectoenzyme activity (K_m), and high growth efficiencies, because they do not express extracellular enzymes in large quantities and there is less DNA to replicate.

Decomposers are capable of degrading RDOC. Decomposer growth is presumed to require the production of a variety of hydrolytic and oxidative enzymes; therefore, cell sizes are larger and growth is slower. Because their carbon and nutrient sources are concentrated in complexes that reach colloidal size, K_m and K_s values are assumed to be higher than those of opportunists. Decomposers also consume LDOC, but in the context of the model, their lower affinity enzyme systems put them at a competitive disadvantage relative to opportunists when the abundance of LDOC is low. Decomposers are protected from competitive exclusion by opportunists by the inhibitory effect that the phenolic components of RDOC exert on opportunist growth. In the simulation, this effect is expressed as a half-saturation constant, K_i.

To run the DEMI simulation (Fig. 7), initial values are assigned to each stock and to the rates of LDOC and RDOC input. Values for the four stocks (two guilds, two DOC pools) are in units of micrograms of carbon per liter; units for inputs are micrograms of carbon per liter per hour. The simulations run in 1 h time steps. In general, the model predicts that (1) for any fixed set of kinetic parameters (opportunist K_s, decomposer K_s, opportunist K_i), the equilibrium abundance of opportunists and decomposers depends only on the input rates of LDOC and RDOC (Fig. 8a) and (2) for any fixed set of input rates, the equilibrium concentrations of LDOC and RDOC depend only on the K_s and K_i parameters associated with decomposers and opportunists (Fig. 8b).

The model, as simple as can be made, illustrates how bacterial productivity and enzyme activity can be modulated by rates of DOC input from varied sources through competitive selection. As the relative abundance of the two guilds changes in response to DOM inputs, biotic parameters (productivity, growth efficiency, and ectoenzyme kinetics) vary widely, while RDOC and LDOC concentrations remain comparatively static. This outcome is consistent with field observations from freshwater systems,

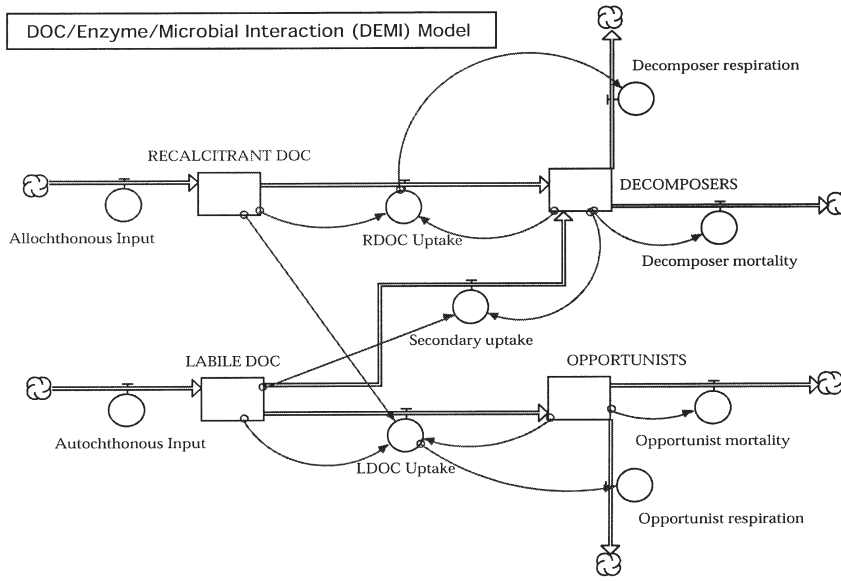

FIGURE 7 Simulation model for the functional organization of heterotrophic bacterioplankton. Inputs of labile autochthonous DOC and recalcitrant allochthonous DOC support the growth of two bacterial guilds. Decomposers use both RDOC and LDOC for growth. Relative to opportunists they are slow growing, resistant to humic inhibition, and produce a wide array of extracellular enzymes, including oxidative ones. Opportunists use LDOC for growth. Relative to decomposers, they turn over rapidly and have high growth efficiencies. They are able to out compete decomposers for LDOC, but are susceptible to humic inhibition.

which show wide variations (2–3 orders of magnitude) in productivity and enzyme activity both within and among systems while DOC concentration varies comparatively little (Cole *et al.*, 1988; Sinsabaugh *et al.*, 1997; Foreman *et al.*, 1998; Sinsabaugh and Foreman, 2001). This effect is difficult to simulate when bacteria are lumped into a single box.

The alternative explanation for the phenomenon is that bacterial communities respond physiologically to changes in DOM inputs rather than by replacing populations. Bacteria obviously have some capacity to physiologically adapt, but their reservoir of genetic information is limited, and fundamental parameters such as uptake rates and substrate affinities are determined by cell size and enzyme structure, which may be difficult to modulate. Microcosm studies suggest that changes in productivity and enzyme kinetics measured 24–48 h after DOM amendment are associated with displacements in community composition as measured by DNA comparison techniques (see Chapter 14).

The DEMI model also accounts for the amphitrophic effects of humic substances observed across systems by assuming that populations vary in

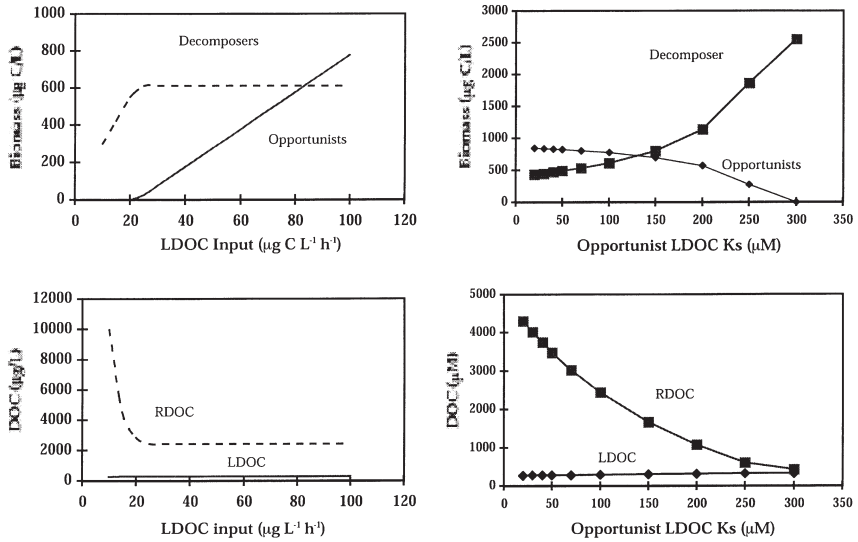

FIGURE 8 Sensitivity analysis of DEMI model. Left panel: Allochthonous input is fixed at 10 μg C/(L1 h) as autochthonous input is varied. For opportunists K_s = 100 and K_i = 5000 μg C/L. For decomposers, K_s for RDOC = 5000 and K_s for LDOC = 500 μg C/L. Equilibrium abundances of decomposers and opportunists are shown in the upper figure and equilibrium concentrations of RDOC and LDOC are shown in the lower figure. The simulations show that autochthonous and allochthonous input rates control bacterial biomass and that bacterial abundance can vary widely at identical DOC concentrations. Right panel: Kinetic parameters for decomposers are fixed while the opportunist K_s varies. Allochthonous input = 10 and autochthonous input = 100 μg C/(L1 h). Decomposer K_s values for LDOC and RDOC are 500 and 5000 μg C/L, respectively. Opportunist K_i = 5000 μg C/L. The upper figure shows that the competitive advantage of opportunists relative to decomposers is dependent on their differences in K_s. The lower figure shows that the equilibrium abundance of RDOC is strongly dependent on the relative abundance of the opportunist and decomposer guilds.

their physiological tolerance for phenols. In the model, phenolic inhibition is associated with the opportunist guild. This premise is consistent with field observations that freshwater systems in which RDOC inputs appear to dominate have bacterial communities that tolerate and consume phenols, whereas those that receive substantial LDOC inputs appear to have communities whose activities are inhibited by phenolic compounds (Tranvik, 1998; Foreman, 1999).

This simple model can even simulate dystrophy and cometabolism. Dystrophy occurs when LDOC inputs are very low relative to RDOC. Under those conditions, opportunists disappear and decomposer growth slows, allowing RDOC to accumulate to high concentrations. Cometabolism occurs when increases in LDOC inputs support higher decomposer abundance, drawing down the RDOC pool (Fig. 8).

E. Resource Allocation Models

Connecting microbial diversity to DOM heterogeneity by representing the community as guilds selected by input sources is one approach for modeling bacterioplankton organization. However, a more comprehensive model of heterotrophic bacterial dynamics should include the processing of both organic and inorganic nutrients.

There is abundant, though fragmented, information on the mechanisms that integrate organic and inorganic nutrient acquisition (Kirchman, 2000). Relationships between phosphatase activity and inorganic P, peptidase activity and inorganic N, chitinase activity and inorganic N, and phenol oxidase activity and inorganic N have been described in varied systems (e.g., Chróst and Rai, 1993; Foreman *et al*., 1998; Carreiro *et al*., 2000; Sinsabaugh *et al*., 2002). Such observations led Sinsabaugh and Moorhead (1994) to propose a model for the functional organization of microdecomposer communities based on optimal resource allocation among C, N, and P acquisition pathways. The model, called microbial allocation of resources among community indicator enzymes (MARCIE), is based on the premise that the production of extracellular enzymes is often controlled by induction–repression mechanisms tied to substrate availability. At the community level, this type of regulation resembles an optimal resource allocation strategy for maximizing community production. The model links mass loss from decomposing litter with the activities of C-acquiring enzymes (e.g., cellulases and hemicellulases), the production of which is constrained by resources expended in the acquisition of N and P. In this model, ratios of N-acquisition activity (e.g., chitinase, urease, and peptidase) to C-acquisition activity and P-acquisition activity (e.g., phosphatase) to C-acquisition activity become indicators of relative nutrient availability. The model has been used in various contexts to understand the processes that relate nutrient availability to decomposition rates (Jackson *et al*., 1995; Tank *et al*., 1998; Carreiro *et al*., 2000). As information has accumulated, the original model has been steadily modified (Moorhead and Sinsabaugh, 2000; Sinsabaugh *et al*., 2001). For example, it is now clear that N availability differentially affects the activities of enzymes that degrade polyphenols and polysaccharides (Carreiro *et al*., 2000; Sinsabaugh *et al*., 2002); consequently, these C flow pathways need to be separated to accurately represent the effects of N on carbon acquisition and litter decay.

Although, the MARCIE model has been applied to bacterioplankton, success has been limited (Sinsabaugh *et al*., 1997; Foreman *et al*., 2001). For microdecomposers of plant detritus, the available trophic resources are known and the enzymology of their degradation has been extensively studied. For bacterioplankton, resources are more structurally diffuse and more temporally dynamic, and basic information about the regulation and trophic significance of potentially important enzyme pathways is lacking.

The principal sources of carbon for bacterioplankton are saccharides and amino acids. To acquire these substrates, bacteria produce extracellular glycosidases and peptidases; the most readily measured are α- and β-glucosidase and leucine aminopeptidase (Sinsabaugh and Foreman, 2001). Glucosidase activities respond positively to saccharide availability and leucine aminopeptidase activities respond positively to protein availabilities (Foreman *et al*., 1998; Stepanauskas *et al*., 1999), although glucosidase activities are more dynamic (Kroer *et al*., 1994; Christian and Karl, 1995; Sinsabaugh *et al*., 1997; Foreman *et al*., 1998). The activities of both enzyme classes have been correlated with bacterial production (Hoppe *et al*., 1988; Münster, 1991; Chróst and Rai, 1993; Müller-Niklas *et al*., 1994; Sinsabaugh *et al*., 1997; Foreman *et al*., 1998) and the ratio of β- glucosidase to leucine aminopeptidases has been used as a metric for comparing the trophic basis of bacterial production across systems (Christian and Karl, 1995; Foreman *et al*., 1998; Fukuda *et al*., 2000).

A complicating factor is that amino acids are sources of both carbon and nitrogen: peptidase activities can be repressed by inorganic N as well as induced by protein. [A similar situation exists for the aminosaccharides that comprise chitin and peptidoglycan; however, β-*N*-acetylglucosaminidase activity appears less prominent in DOM systems than in POM (Sinsabaugh and Foreman, 2001).] The degradation of dissolved combined amino acids by bacteria also shares some features with the degradation of the lignin and humus components of POM. For decomposer fungi, humified lignin is a source of N and a barrier to C acquisition. The production of oxidative enzymes that degrade the aromatic components of plant litter is induced by low N availability and repressed by high N availability. In effect, inorganic N reduces the efficiency of C acquisition (Carreiro *et al*., 2000). A similar phenomenon may occur for bacterioplankton when inorganic N reduces peptidase expression.

Humic substances are abundant in DOM as well as POM, but the role of phenol oxidases in DOM metabolism has been little studied (Münster and De Haan, 1998). Foreman (1999) found that phenol oxidase activity in water from five contrasting Michigan streams was correlated with phenol concentration and that bacterial production in systems with significant humic DOC input was stimulated by the addition of phenols, suggesting that phenols may be an important growth substrate for some bacterial guilds.

It is clear that a resource allocation model that describes bacterioplankton production by integrating nutrient flows will look quite different than one for the microdecomposers of plant litter. Because there are few data on the distribution and regulation of amidohydrolase and phenol oxidase activities by bacterioplankton, the regulatory nexus linking organic and inorganic nutrient acquisition is incomplete. From available evidence, we propose that a model that links bacterial production to organic and inorganic nutrient availabilities through pathways mediated by phosphatase,

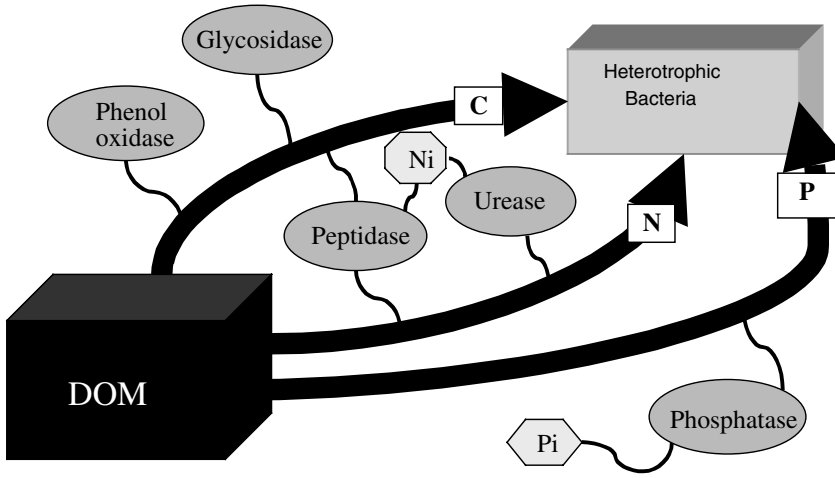

FIGURE 9 A resource allocation model for DOM acquisition by bacterioplankton. The model is based on the premise that bacteria optimize nutrient acquisition by altering the expression of extracellular enzymes in response to environmental availabilities. In this view, glycosidases, peptidases, and phenol oxidases mediate carbon acquisition pathways. Carbon acquisition is constrained by the need for N and P. Because peptidase and amidohydrolase (urease) activities generate assimilable N, their activities are influenced by inorganic N availability. Phosphatase activity is regulated by inorganic P availability in relation to demand. The model provides a scheme for comparing the relative nutrient limitations of bacterial communities in relation to production.

amidohydrolase, phenol oxidase, glucosidase, and aminopeptidase activities may provide a framework for analyzing the functional organization of bacterioplankton (Fig. 9).

F. Food Web Models

Guild and resource allocation models represent bottom-up approaches that attempt to relate bacterial community organization to DOM characteristics. However, other interactions such as predation and viral lysis also affect community organization and thus DOM processing (Carpenter *et al.*, 1998; Hessen, 1998). Thingstad (2000) developed a series of models that describe the regulation of bacterial growth within a food web context. In these models, bacterivorous predators affect production by controlling biomass through direct consumption and by influencing growth rate through competition for inorganic nutrients. These interactions create lags in response and can constrain the bacterial response to changes in carbon availability (see Chapter 16).

G. Synthesis

Trophic models improve our understanding of the microbial loop and its connection with the metazoan food web. By resolving the black box that represents bacteria into functional compartments, we can reach a deeper understanding of DOM processing in varied systems. Guild, resource allocation, and food web models all illustrate that competition for macronutrients among a highly diverse group of organisms, each with a limited range of response, is sufficient to account for the spatiotemporal heterogeneity observed in natural systems. Selective pressures on community composition, arising from both the bottom and the top, affect rates of bacterial growth and respiration, and the rate of carbon transfer to other trophic levels and to "downstream" systems. The challenge is to develop tractable but "realistic" representations of heterotrophic diversity that include more than one box.

V. SUMMARY

From an empirical perspective, there is much to learn about the fate of DOM and its relationship to community organization, diversity, and productivity, on both fine and coarse scales. However, from a conceptual standpoint, we are rapidly creating a synthetic framework for this information. Over the past three decades, each of the three approach domains described in this chapter — community structure, biogeochemical, and trophic — have generated models that represent major insights into microbial ecology and DOM processing. Starting from observations about the affinity of bacteria for surfaces, the community structure approach has led to the establishment of the biofilm concept and to the realization that DOM structures the whole aqueous environment on a micrometer scale. The biogeochemical approach began with analyses of DOM characteristics, which led to development of the size–reactivity model and to a steadily improving picture of the mechanics of bacterial nutrient transformation. The trophic approach can be traced to realization of the importance of bacteria in marine food webs — a finding that led to the microbial loop concept, then to a variety of higher resolution models that elucidate how DOM source dynamics, competition for nutrients, and grazing control bacterial diversity and metabolism.

Present models, taken collectively, provide a general picture of the structural and functional organization of bacteria in relation to DOM processing. Within each of the three research domains, there is abundant opportunity to extend resolution, particularly as knowledge of the ecology of individual taxa grows. Even in their present state, existing concepts appear sufficient to account for most phenomena associated with the diagenesis and metabolism of DOM.

REFERENCES

Alldredge, A. L. 2000. Interstitial dissolved organic carbon (DOC) concentrations within sinking marine aggregates and their potential contribution to carbon flux. *Limnology and Oceanography* **45:**1245–1253.

Ammerman, J. W. 1991. Role of ecto-phosphohydrolases in phosphorus regeneration in estuarine and coastal ecosystems. *In* "Microbial Enzymes in Aquatic Environments" (R. J. Chróst, Ed.), pp. 60–76. Springer, New York.

Amon, R. M. W., and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography* **41:**41–51.

Azam, F. 1998. Microbial control of oceanic carbon flux: The plot thickens. *Science* **280:**694–696.

Azam, F., T. Fenchel, J. Field, J. Gray, L. Meyer-Reid, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* **10:**257–263.

Bano, N., M. A. Moran, and R. E. Hodson. 1997. Bacterial utilization of dissolved humic substances from a freshwater swamp. *Aquatic Microbial Ecology* **12:**233–238.

Battin, T. J., A. Butturini, and F. Sabater. 1999. Immobilization and metabolism of dissolved organic carbon by natural sediment biofilms in a Mediterranean and temperate stream. *Aquatic Microbial Ecology* **19:**297–305.

Benner, R. H. 1998. Cycling of dissolved organic matter in the ocean. *In* "Aquatic Humic Substances", (D. O. Hessen and L. J. Tranvik, Eds.), pp. 317–331. Springer-Verlag, Berlin.

Bergh, O., K. Y. Borsheim, G. Bratbak, and M. Heldal. 1989. High abundance of viruses found in aquatic environments. *Nature* **340:**467–468.

Bratbak, G., F. Thingstad, and M. Heldal. 1994. Viruses and the microbial loop. *Microbial Ecology* **28:**209–221.

Bunte, C., and M. Simon. 1999. Bacterioplankton turnover of dissolved free monosaccharides in a mesotrophic lake. *Limnology and Oceanography* **44:**1862–1870.

Burns, R. G., Ed. 2001. "Enzymes in the Environment." Dekker, London.

Carlson, P., A. Z. Segatto, and E. Granéli. 1993. Nitrogen bound to humic matter of terrestrial origin — A nitrogen pool for coastal phytoplankton? *Marine Ecology Progress Series* **97:**105–116.

Carpenter, S. R., J. J. Cole, J. F. Kitchell, and M. L. Pace. 1998. Impact of dissolved organic carbon, phosphorus and grazing on phytoplankton biomass and production in experimental lakes. *Limnology and Oceanography* **43:**73–80.

Carreiro, M. M., R. L. Sinsabaugh, D. A. Repert, and D. F. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* **81:**2359–2365.

Characklis, W. G., and K. C. Marshall, Eds. 1990. "Biofilms." Wiley, New York.

Christensen, B. E., and W. G. Characklis. 1990. Physical and chemical properties of biofilms. *In* "Biofilms" (W. G. Characklis, and K. C. Marshall, Eds.), pp. 93–130. Wiley, New York.

Christian, J. R., and D. M. Karl. 1995. Bacterial ectoenzymes in marine waters: Activity ratios and temperature responses in three oceanographic provinces. *Limnology and Oceanography* **40:**1042–1049.

Chróst, R. J. 1990. Microbial ectoenzymes in aquatic environments. *In* "Aquatic Microbial Ecology" (J. Overbeck and R. J. Chróst, Eds.), pp. 47–78. Springer, New York.

Chróst, R. J., and H. Rai. 1993. Ectoenzyme activity and bacterial secondary production in nutrient-impoverished and nutrient-enriched freshwater mesocosms. *Microbial Ecology* **25:**131–150.

Cole, J. J., S. Findlay, and M. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: A cross-system overview. *Marine Ecology Progress Series* **43:**1–10.

Costerton, J. W., Z. Lewandowski, D. E. Caldwell, D. R. Korber, and H. M. Lappin-Scott. 1995. Microbial biofilms. *Annual Review of Microbiology* **49:**711–745.

Davies, D. G., A. M. Chakrabarty, and G. G. Geesey. 1993. Exopolysaccharide production in biofilms: Substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology* **59:**1181–1186.

Davies, D. G., M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* **280:**295–298.

de Beer, D., and P. Stoodley. 1995. Relation between the structure of an aerobic biofilm and mass transport phenomena. *Water Science and Technology* **32:**11–18.

de Beer, D., P. Stoodley, and Z. Lewandowski. 1996. Liquid flow and mass transport in heterogeneous biofilms. *Water Research* **30:**2762–2765.

Decho, A. W. 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology Annual Review* **28:**73–153.

del Giorgio, P. A., and J. J. Cole. 2000. Bacterial energetics and growth efficiency. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman Ed.), pp. 289–326. Wiley–Liss, New York.

Ducklow, H. W. 2000. Bacterial production and biomass in the oceans. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 85–120. Wiley–Liss, New York.

Edwards, R. T., J. L. Meyer, and S. E. G. Findlay. 1990. The relative contribution of benthic and suspended bacteria to system biomass, production, and metabolism in a low-gradient blackwater river. *Journal of the North American Benthological Society* **6:**241–250.

Fiebig, D. M. 1997. Microbiological turnover of amino acids immobilized from groundwater discharged through hyporheic sediments. *Limnology and Oceanography* **42:**763–768.

Fiebig, D. M., and J. Marxsen. 1992. Immobilization and mineralization of dissolved free amino acids by streambed biofilms. *Freshwater Biology* **28:**129–140.

Findlay, S., and R. L. Sinsabaugh. 1999. Unraveling the sources and bioavailability of dissolved organic matter in aquatic ecosystems. *Marine and Freshwater Research* **50:**781–790.

Foreman, C. M. 1999. Dissolved organic carbon: Importance as a regulator of bacterioplankton communities. Ph.D. Dissertation, University of Toledo.

Foreman, C. M., P. Franchini, and R. L. Sinsabaugh. 1998. The trophic dynamics of riverine bacterioplankton: Relationships among substrate availability, ectoenzyme kinetics and growth. *Limnology and Oceanography* **43:**1344–1352.

Freeman, C., and M. A. Lock. 1995. The biofilm polysaccharide matrix: A buffer against changing substrate supply? *Limnology and Oceanography* **40:**273–278.

Freeman, C., P. J. Chapman, K. Gilman, M. A. Lock, B. Reynolds, and H. S. Wheater. 1995. Ion exchange mechanisms and the entrapment of nutrients by river biofilms. Hydrobiologia **297:**61–65.

Freeman, C., N. Ostle, and H. Kang. 2001. Peatland phenol oxidase: An enzymic latch on a global carbon store. *Nature* **409:**149.

Fuhrman J. A. 1999. Marine viruses and their biogeochemical and ecological effects. *Nature* **399:**541–548.

Fuhrman, J. A. 2000. Impact of viruses on bacterial processes. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 327–350. Wiley–Liss, New York.

Fuhrman, J. A. and C. A. Suttle. 1993. Viruses in marine planktonic systems. *Oceanography* **6:**50–62.

Fukuda, R., Y. Sohrin, N. Saotome, H. Fukuda, T. Nagata, and I. Koike. 2000. East–west gradient in ectoenzyme activities in the subarctic Pacific: Possible regulation by zinc. *Limnology and Oceanography* **45:**930–939.

Geesey, G. G., R. Mutch, J. W. Costerton, and R. B. Green. 1978. Sessile bacteria: An important component of the microbial population in small mountain streams. *Limnology and Oceanography* **23:**1214–1223.

Gieseke, A., U. Purkhold, M. Wagner, R. Amann, and A. Schramm. 2001. Community structure and activity dynamics of nitrifying bacteria in a phosphate-removing biofilm. *Applied and Environmental Microbiology* **67:**1351–1362.

Goodman, A. E., and G. G. Geesey. 2000. Gene expression of cells attached to surfaces. *In* "Biofilms: Recent Advances in Their Study and Control" (L. V. Evans, Ed.), pp. 71–80. Harwood Academic, Reading, UK.

Grossart, H.-P., M. Simon, and B. E. Logan. 1997. Formation of macroscopic organic aggregates (lake snow) in a large lake: The significance of transparent exopolymer particles, phytoplankton, and zooplankton. *Limnology and Oceanography* **42:**1651–1659.

Hammel, K. E. 1997. Fungal degradation of lignin. *In* "Driven by Nature: Plant Litter Quality and Decomposition" (G. Cadisch, and K. E. Giller, Eds.), pp. 33–46. CAB International, Wallingford.

Hart, D. R., L. Stone, and T. Berman. 2000. Seasonal dynamics of the Lake Kinneret food web: The importance of the microbial loop. *Limnology and Oceanography* **45:**350–361.

Headley, J. V., K. M. Peru, J. R. Lawrence, and G. M. Wolfaardt. 1995. MS/MS identification of transformation products in degradative biofilms. *Analytical Chemistry* **67:**1831–1837.

Hessen, D. O. 1998. Food webs and carbon cycling in humic lakes. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 259–278. Springer-Verlag, Berlin.

Hopkinson, C., I. Buffam, J. Hobbie, J. Vallino, R. Hodson, M. A. Moran, J. Covert, E. Smith, J. Baross, B. Crump, B. Eversmeyer, F. Prahl, M. Perdue, S. Findlay, and K. Foreman. 1998. Terrestrial inputs of organic matter to coastal ecosystems: An intercomparison of chemical characteristics and bioavailability. *Biogeochemistry* **43:**211–234.

Hoppe, H.-G. 1993. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. *In* "Handbook of Methods in Aquatic Microbial Ecology" (P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole, Eds.), pp. 423–432. Lewis Publishers, Ann Arbor, MI.

Hoppe H., S. Kim, and K. Gocke. 1988. Microbial decomposition in aquatic environments: Combined process of extracellular enzyme activity and substrate uptake. *Applied and Environmental Microbiology* **54:**784–790.

Hynes, H. B. N. 1970. "The Ecology of Running Waters." University of Toronto Press, Toronto.

Jackson, C., C. Foreman, and R. L. Sinsabaugh. 1995. Microbial enzyme activities as indicators of organic matter processing rates in a Lake Erie coastal wetland. *Freshwater Biology* **34:**329–342.

Jahn, A., and P. H. Neilsen. 1993. Extraction of extracellular polymeric substances (EPS) from biofilms using a cation exchange resin. *Water Science and Technology* **32:**157–164.

Jannson, M. 1998. Nutrient limitation and bacteria–phytoplankton interactions in humic lakes. *In* "Aquatic Humic Substances" D. O. Hessen and L. J. Tranvik, Eds.), pp. 177–195. Springer-Verlag, Berlin.

Johnson, B. D., K. Kranck, and D. K. Muschenheim. 1994. Physicochemical factors in particle aggregation. *In* "The Biology of Particles in Aquatic Systems" R. S. Wotton, Ed.), pp. 75–96. Lewis Publishers, Ann Arbor, MI.

Kemp, P. F., B. F. Sherr, E. B. Sherr, and J. J. Cole. 1993. Handbook of Methods in Aquatic Microbial Ecology." Lewis Publishers, Ann Arbor, MI.

Kirchman, D. L. 2000. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacterial. *In* "Microbial Ecology of the Oceans" D. L. Kirchman, Ed.), pp. 261–288. Wiley, New York.

Konopka, A., T. Bercot, and C. Nakatsu. 1999. Bacterioplankton community diversity in a series of thermally stratified lakes. *Microbial Ecology* **38:**126–135.

Kroer, N., N. O. G. Jørgensen, and R. B. Coffin. 1994. Utilization of dissolved nitrogen by heterotrophic bacterioplankton: A comparison of three ecosystems. *Applied and Environmental Microbiology* **60:**4116–4123.

Lemke, M. J., and S. H. Bowen. 1998. The nutritional value of organic detrital aggregate in the diet of fathead minnows. *Freshwater Biology* **39:**447–453.

Lock, M. A. 1993. Attached microbial communities in rivers. *In* "Aquatic Microbiology: An Ecological Approach" (T. E. Ford, Ed.). Blackwell Scientific, Oxford.

Lock, M. A. 1994. Dynamics of particulate and dissolved organic matter over the substratum

of water bodies. *In* "The Biology of Particles in Aquatic Systems" (R. S. Wotton, Ed.). pp. 137–160. Lewis Publishers, Ann Arbor, MI.

Lock, M. A., R. R. Wallace, J. W. Costerton, R. M. Ventullo, and S. E. Charlton, 1984. River epilithon: Toward a structural-functional model. *Oikos* **42:**10–22.

McIntyre, S., A. L. Alldredge, and C. C. Gottschalk. 1995. Accumulation of marine snow at density discontinuities in the water column. *Limnology and Oceanography* **40:**449–468.

McKnight, D. M., and G. R. Aiken. 1998. *In* Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 9–37. Springer-Verlag, Berlin.

Meyer, J. L. 1994. The microbial loop in flowing waters. *Microbial Ecology* **28:**195–199.

Moorhead, D. L., and R. L. Sinsabaugh. 2000. Simulated patterns of litter decay predict patterns of extracellular enzyme activities. *Applied Soil Ecology* **14:**71–79.

Moran, M. A., and R. E. Hodson. 1990. Bacterial production on humic and nonhumic components of dissolved organic carbon. *Limnology and Oceanography* **35:**1744–1756.

Moran, M. A., and R. E. Hodson. 1994. Support of bacterioplankton production by dissolved humic substances from three marine environments. *Marine Ecology Progress Series* **110:**241–247.

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography* **42:**1307–1316.

Moran, M. A. and R. G. Zepp. 2000. UV radiation effects on microbes and microbial processes. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 201–228. Wiley–Liss, New York.

Moran, M. A., W. M. Sheldon, and J. E. Sheldon. 1999. Biodegradation of riverine dissolved organic carbon in five estuaries of the southeastern United States. *Estuaries* **22:**55–64.

Müller-Niklas, G., S. Schuster, E. Kaltenböck, and G. J. Herndl. 1994. Organic content and bacterial metabolism in amorphous aggregations of the northern Adriatic Sea. *Limnology and Oceanography* **39:**58–68.

Münster, U. 1991. Extracellular enzyme activity in eutrophic and polyhumic lakes. *In* "Microbial Enzymes in Aquatic Environments" (R. J. Chróst, Ed.), pp. 25–59. Springer-Verlag, New York.

Münster, U., and H. De Haan. 1998. The role of microbial extracellular enzymes in the transformation of dissolved organic matter in humic waters. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 199–257. Springer-Verlag, Berlin.

Murray, A. G., and P. M. Eldridge. 1994. Marine viral ecology: Incorporation of bacteriophage into the microbial planktonic food web paradigm. *Journal of Plankton Research* **16:**627–641.

Nagata, T. 2000. Production mechanisms of dissolved organic matter. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 121–152. Wiley–Liss, New York.

Neu, T. R., and J. R. Lawrence. 1997. Development and structure of microbial biofilms in river water studied by confocal laser scanning microscopy. *FEMS Microbiology Ecology* **24:**11–25.

Ogawa, H., Y. Amagai, I. Koike, K. Kaiser, and R. Benner. 2001. Production of refractory dissolved organic matter by bacteria. *Science* **292:**917–920.

Opsahl, S., and R. Benner. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. *Limnology and Oceanography* **43:**1297–1304.

Paerl, H. W., and J. L. Pinckney. 1996. A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. *Microbial Ecology* **31:**225–247.

Passow, U., and A. L. Alldredge. 1994. Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. *Marine Ecology Progress Series* **113:**185–198.

Passow, U., and A. L. Alldredge. 1995. A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). *Limnology and Oceanography* **40:**1326–1335.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. *Bioscience* **24:**499–504.
Pomeroy, L. R., and W. J. Wiebe. 1988. Energetics of microbial food webs. *Hydrobiologia* **159:**7–18.
Sinsabaugh, R. L., and C. M. Foreman. 2001. Activity profiles of bacterioplankton in a eutrophic river. *Freshwater Biology* **46:**1–12.
Sinsabaugh, R. L., and A. E. Linkins. 1988. Exoenzyme activity associated with lotic epilithon. *Freshwater Biology* **20:**249–261.
Sinsabaugh, R. L., and D. L. Moorhead. 1994. Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. *Soil Biology and Biochemistry* **26:**1305–1311.
Sinsabaugh, R. L., S. Findlay, P. Franchini, and D. Fischer. 1997. Enzymatic analysis of riverine bacterioplankton production. *Limnology and Oceanography* **42:**29–38.
Sinsabaugh, R. L., M. J. Klug, H. P. Collins, P. E. Yeager, and S.,O. Peterson. 1999. Characterizing soil microbial communities. *In* Standard Soil Methods for Long-term Ecological Research" (G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins, Eds.), pp. 318–348. Oxford University Press, New York.
Sinsabaugh, R. L., M. M. Carreiro, and S. Alvarez. 2001. Enzyme and microbial dynamics during litter decomposition. *In* Enzymes in the Environment" (R. Burns, Ed.). Dekker, New York.
Sinsabaugh, R. L., M. M. Carreiro, and D. A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry*, in press.
Smith, D. C., M. Simon, A. L. Alldredge, and F. Azam. 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359:**139–142.
Søndergaard, M., and M. Middleboe. 1995. A cross-system analysis of labile dissolved organic carbon. *Marine Ecology Progress Series* **118:**283–294.
Stepanauskas, R., H. Edling, and L. J. Tranvik. 1999. Differential dissolved organic nitrogen availability and bacterial aminopeptidase activity in limnic and marine waters. *Microbial Ecology* **38:**264–272.
Stevenson, F. J. 1994. "Humus Chemistry. Genesis, Composition, Reactions." Wiley, New York, 496 pp.
Stevenson, R. J., M. L. Bothwell, and R. L. Lowe, Eds. 1996. Algal Ecology: Freshwater Benthic Ecosystems." Academic Press, New York.
Steward, G. F., D. C. Smith, and F. Azam. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi seas. *Marine Ecology Progress Series* **131:**287–300.
Suttle, C. A. 1994. The significance of viruses to mortality in aquatic microbial communities. *Microbial Ecology*. **28:**237–
Tank, J. L., J. R. Webster, E. F. Benfield, and R. L. Sinsabaugh. 1998. Effect of leaf litter exclusion on microbial enzyme activity associated with wood biofilms in streams. *Journal of the North American Benthological Society* **17:**95–103.
Thingstad, T. F. 2000. Control of bacterial growth in idealized food webs. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 229–260. Wiley, New York.
Thompson, A., and R. L. Sinsabaugh. 2000. Matric and particulate phosphatase and leucine aminopeptidase activity in limnetic biofilms. *Aquatic Microbial Ecology* **2:**151–159.
Thurman, E. M. 1986. "Organic Geochemistry of Natural Waters." Nijhoff Boston, 497 pp.
Tranvik L. J. 1992. Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. *Hydrobiologia* **229:**107–114.
Tranvik, L. J. 1998. Degradation of dissolved organic matter in humic waters by bacteria. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 259–278. Springer-Verlag, Berlin.
Tranvik, L. J., and M. G. Hofle. 1987. Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. *Applied and Environmental Microbiology* **53:**482–488.

Tranvik, L. J., and S. Kokalj. 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. *Aquatic Microbial Ecology* **14:**301–307.

Tulonen, T., K. Salonen, and L. Arvola. 1992. Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. *Hydrobiologia* **229:**239–252.

Volk, C. J., C. B. Volk, and L. A. Kaplan. 1997. Chemical composition of biodegradable dissolved organic matter in streamwater. *Limology and Oceanography* **42:**39–44.

Wackett, L. P., and C. D. Hershberger. 2001. "Biocatalysis and Biodegradation: Microbial Transformation of Organic Compounds." ASM Press, Washington, DC.

Ward, G. M., A. K. Ward, C. N. Dahm, and N. G. Aumen. 1994. Origin and formation of organic and inorganic particles in aquatic systems. *In* "The Biology of Particles in Aquatic Systems" (R. S. Wotton, Ed.), pp. 45–74. Lewis Publishers, Ann Arbor, MI.

Wetzel, R. G. 1991. Extracellular enzymatic interactions in aquatic ecosystems: Storage, redistribution and interspecific communication. *In* Microbial Enzymes in Aquatic Environments" (R. J. Chróst, Ed.), pp. 25–59. Springer-Verlag, New York.

Wetzel, R. G. 1992. Gradient-dominated ecosystems: Sources and regulatory functions of dissolved organic matter in freshwater ecosystems. *Hydrobiologia* **229:**181–198.

Wetzel, R. G., P. G. Hatcher, and T. S. Bianchi. 1995. Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology and Oceanography* **40:**1369–1380.

White, D. 1995. "The Physiology and Biochemistry of Prokaryotes." Oxford University Press, New York.

Whiteley, M., J. R. Ott, E. A. Weaver, and R. J. C. McLean. 2001. Effects of community composition and growth rate on aquifer biofilm bacteria and their susceptibility to betadine disinfection. *Environmental Microbiology* **3:**43–52.

Wilhelm, S. W., and C. A. Suttle. 1999. Viruses and nutrient cycles in the sea. *BioScience* **49:**781–787.

Williams, P. J. B. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 153–200. Wiley–Liss, New York.

Wimpenny, J. W. T., and R. Colasanti. 1997. A unifying hypothesis for the structure of microbial biofilms based on cellular automaton models. *FEMS Microbiology Ecology* **22:**1–16.

Wommack, K. E., and R. R. Colwell. 2000. Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* **64:**69–104.

Wotton, R. S. 1994. Particulate and dissolved organic matter as food. *In* "The Biology of Particles in Aquatic Systems" (R. S. Wotton, Ed.), pp. 235–288. Lewis Publishers, Ann Arbor, MI.

Zhou, J., K. Mopper, and U. Passow. 1998. The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnology and Oceanography* **43:**1860–1871.

ZoBell, C. E. 1943. The effect of solid surfaces upon bacterial activity. *Journal of Bacteriology* **46:**39–56.

19

Dissolved Organic Carbon: Detrital Energetics, Metabolic Regulators, and Drivers of Ecosystem Stability of Aquatic Ecosystems[1]

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I. INTRODUCTION

Trophic structure has dominated evaluations of the rates of energy fixation by primary producers in terrestrial and aquatic pelagic communities, and the rates of transfer efficiencies of this energy to higher trophic levels. Original promulgations of the effects of energy flow on trophic structure emphasized restrictions on trophic complexity by limitations of energy transfers among trophic levels (Lindeman, 1942; Hutchinson, 1959; Wetzel,

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1995). These relationships of pelagic trophic structure and energy fluxes, however, consider solely particulate organic carbon — ingestion of particulate organic matter (POM). Flows of energy within the trophic structures and their variants specifically addressed predation, that is, ingestion of particles of organic matter. Decades of ensuing study of feeding relationships have greatly increased our understanding. In particular, studies have addressed variations in the size of ingested POM, morphological aspects of ingestion (e.g., filtration and gape), and avoidance of ingestion (e.g., transparency/visibility and interference by cellular or body projections). Many additional excellent studies have addressed behavioral capabilities of organisms for movements within the pelagic zone in relation to refuges or escape from predators, nutritional differences in particulate food, and other factors. Despite recognition that many organisms ingest variable amounts of particulate detritus (i.e., dead particulate organic matter) and that particulate detritus usually dominates living POM of the plankton (e.g., Saunders, 1972), quantitative measures of consumption, assimilation, and residual egestion of detrital POM and its associated microbes remain practically unknown.

Clearly, from the inception of evolving ecological constructs, early trophic dynamics, particularly among lakes, emphasized integration and interdependence among biotic components internally, but these dynamics were entirely predation based as formulated by the innovative theoretical developments of Lindeman and Hutchinson (see detailed reviews by Wetzel, 1995, 2001). Trophic structure and energy fluxes were evaluated predominantly in pelagic regions on the basis of ingestion of particulate organic matter by living organisms and the effects of consumption on the population dynamics of trophic levels. Insightful community analyses, particularly in relatively simplified ecosystems such as springs, indicated the importance of detrital feeding as an important pathway (e.g., Odum, 1957; Teal, 1957). As the metabolism of community components was analyzed with increasing accuracy, however, flux pathways and rates of transfer of organic carbon demonstrated a number of complexities and inconsistencies that could not be explained within the conventional food-web paradigms even when the demonstrated importance of particulate detritus transfers was included for particulate feeding animals. Early carbon budgets demonstrated the importance of allochthonous dissolved organic carbon (DOC) from terrestrial and littoral production (Table I). For example, the total annual budget of carbon fluxes in Lawrence Lake, Michigan, demonstrated that much of the DOC was relatively recalcitrant DOC derived from structural tissues of terrestrial and higher aquatic plants, even though higher aquatic plants colonized only some 10% of the benthic area (Wetzel *et al*., 1972; Otsuki and Wetzel, 1974a, b; Wetzel and Otsuki, 1974). Importantly, most of the heterotrophic respiration of organic matter occurred in the sediments: a massive net evasion of CO_2 to the atmosphere that was many times in excess of the primary productivity of phytoplankton was recorded. In Mirror Lake, New Hampshire,

TABLE I Total Annual Budget of Carbon Fluxes in Lawrence Lake, Michigan

Components	*Carbon ($g\ m^{-2}\ yr^{-1}$)*	*Percentage*
Inputs		
Autochthonous		
Phytoplankton	43.4	19.1
Submersed macrophytes	87.9	38.8
Epiphytic algae	37.9	16.8
Epipelic algae	2.0	0.9
Algal secretion and autolysis	14.7	6.5
Littoral plant secretion	5.5	2.5
Heterotrophy	2.8	1.2
Dark CO_2 fixation	7.1	3.1
Allochthonous		
Stream & groundwater DOC	21.0	9.3
Stream particulate organic carbon (POC)	4.1	1.8
Shoreline litter	0.01	0.0
	226.4	100.0
Outputs		
Respiration		
Benthic respiration	117.5	54.6
Bacterial respiration of DOC	20.6	9.6
Bacterial respiration of POC	8.6	4.0
Algal respiration	13.0	6.1
Permanent sedimentation	14.8	6.9
Coprecipitation of DOC with $CaCO_3$	2.0	1.0
Outflow		
Dissolved	35.8	16.5
Particulate	2.8	1.3
	215.1	100.0

Source: Derived from data in Wetzel *et al.* (1972), Otsuki and Wetzel (1974), and Burkholder and Wetzel (1989).

much less productivity from littoral plants and associated attached algae was reported: allochthonous organic carbon was also an important input and benthic respiration was a major export (Table II; care is needed because this budget is balanced by the difference for rates of sedimentation).

Metabolism of particulate detritus (nonliving) and especially dissolved organic matter from many pelagic and nonpelagic autochthonous and from allochthonous sources dominates both material and energy fluxes (Fig. 1). The main points of emphasis are that the pelagic region is but a portion of the whole lake ecosystem; in relation to loading and fluxes of dissolved organic matter (DOM), allochthonous and littoral sources are critical because of their chemical differences from that produced by algal photosynthesis. The

TABLE II Annual Organic Carbon Fluxes in Mirror Lake, New Hamsphire

Components	*Carbon* ($g\ m^{-2}\ yr^{-1}$)	*Percentage of subtotal*
Inputs		
Autochthonous		
Phytoplankton	56.5	69.5
Epilithic algae	2.2	2.7
Epipelic algae	0.6	0.7
Epiphytic algae	0.06	0.07
Macrophytes	2.5	3.1
Dark CO_2 fixation	2.1	2.6
Allochthonous		
With precipitation	1.4	1.7
Shoreline litter	4.3	5.3
Stream DOC	10.5	12.9
Stream POC	1.15	1.4
	81.31	100.0
Outputs		
Respiration		
Phytoplankton	19.1	23.5
Zooplankton	12.0	14.8
Macrophytes	1.0	1.2
Attached algae	1.16	1.4
Benthic invertebrates	2.8	3.4
Fish	0.2	0.2
Sediment bacteria	17.3	21.3
Planktonic bacteria	4.9	6.0
Permanent sedimentation	10.7[a]	13.2
Outflow		
Dissolved	10.87	13.4
Particulate	0.78	1.0
Insect emergence	0.5	0.6
	81.31	100.0

Source: From unpublished data from M. Jordan, G. E. Likens, and B. Petersen and from Likens (1985).

[a]Estimated by difference.

modes of senescence and death of biota are also of considerable importance to the rates and pathways of degradation and energetic utilization. For example, the continual slow senescence and release of dissolved organic matter from a higher aquatic plant is very different from the relatively instantaneous biochemical death and release of DOC from a bacterium or alga. A reluctant realization is that although predation by ingestion of living particulate organic matter can be significant at times, it is not the predominant cause of mortality of organisms (review by Wetzel, 2001). Non-

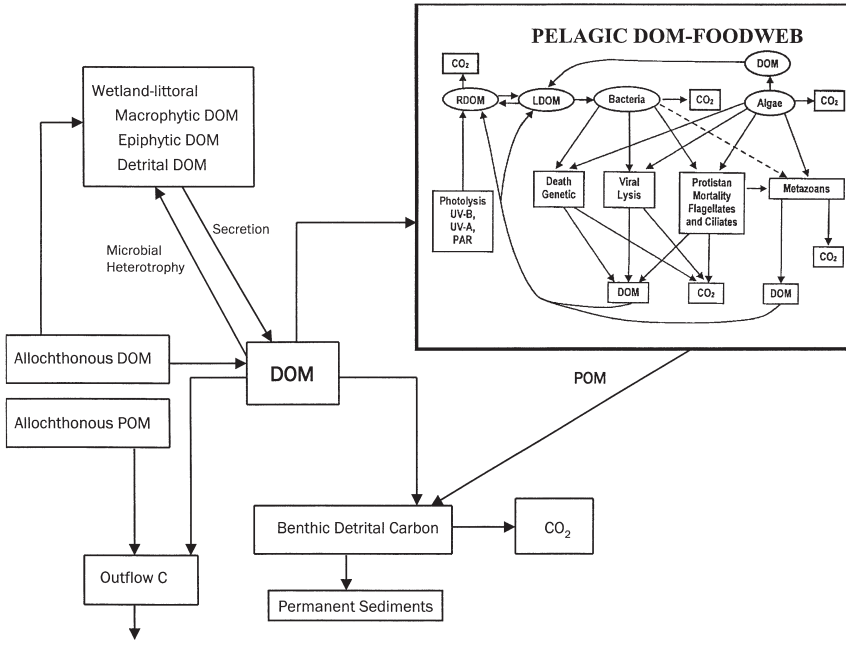

FIGURE 1 Detrital structure and primary fluxes of dissolved organic carbon in lake ecosystems with the food-web structure of predominantly pelagic components (box) that reflects the importance of the microbiota, the microbial loop, and physical processes by which most other organic matter inputs are metabolized heterotrophically or degraded by physical processes. DOM denotes dissolved organic matter, with labile (LDOM) and recalcitrant (RDOM) components; POM denotes particulate organic matter.

predatory death and metabolism of nonliving detrital particulate organic matter and of dissolved organic matter by prokaryotic and protistan heterotrophs dominate in all aquatic ecosystems. In addition, and also of ecosystem significance to the fluxes of DOC, assimilation efficiencies of ingested food are modest at best under natural conditions (usually much less than 50%; see review by Wetzel, 2001), and much of the ingested organic matter is released or egested as both dissolved and particulate detrital organic matter. In addition, there exist massive amounts of organic matter, commonly exceeding 90% of the total organic matter metabolized, that are produced within or imported to the ecosystems and are metabolized but are never predated by particulate-ingesting metazoans. The evolution of this insight and recognition of its reality have been agonizingly slow, and are largely ignored in the evaluations and management of aquatic ecosystems. The evaluation of organic carbon budgets of rivers, particularly among low- and medium-order streams, demonstrated that running waters are heterotrophic and allochthonously mediated ecosystems (e.g., Table III), and assisted in erosion of the autochthonous dogma. Nonetheless, despite organic carbon

TABLE III Annual Mean Concentrations of Organic Matter in Transport in the Fifth- to Sixth-Order Ogeechee River, Georgia

	Mean AFDW (mg L^{-1})		*Percentage of total organic matter*	
Dissolved organic matter		25.4		96.3
Particulate organic matter				
Amorphous material, bacteria	0.30		1.14	
Amorphous material, protozoans	0.04		0.15	
Amorphous material, other	0.52		1.98	
Vascular plant detritus	0.03		0.11	
Algae (mostly diatoms)	0.06		0.23	
Fungi	0.014		0.05	
Animals	0.002		0.01	
Total particulate organic matter		0.97		3.7
Total organic matter in transport		26.37		100.0

Source: Data from Benke and Meyer (1988).

budgets that indicate up to 99% of total organic matter fluxes within aquatic ecosystems are detritus based, the predation-based paradigms continue to prevail as the primary constructs of ecosystem operations (e.g., Hairston and Hairston, 1993), whereas in reality they are the energetic and thermodynamic minority. It also must be noted in any discussion of relative rates and quantities of organic matter, that the annual time period is the only meaningful interval in comparative quantitative analyses of material and energy fluxes at population, community, and ecosystem levels (Wetzel, 1995). Essentially all inland water ecosystems are microbially based heterotrophic ecosystems in which heterotrophy utilization, largely of DOC, within lake and stream *ecosystems* greatly exceeds autochthonous autotrophic production.

In providing an overview and syntheses of the evaluations of organic carbon, a general concensus has emerged along seven directions of thought concerning the functions of dissolved organic matter in aquatic ecosystems. The discussions represent a culmination of work that has evolved from studies of the past 30 years. Two important changes in thought emerge. Clearly, the inland water systems are being treated as integrated ecosystems with the increasing and full appreciation that the metabolism of lakes and rivers is dependent largely or to a major extent on metabolism in the adjacent drainage basin. This paradigm had, of course, been long realized in the case of phosphorus and nitrogen loading, but was not the case for organic matter until relatively recently. Second, commonality of function is found amid the plethora of individual habitat diversity (Wetzel, 1995, 2000a). This important merger is especially evident in the recognition that processes are functionally similar in freshwater and marine ecosystems. Differences

are apparent at the sources of organic matter, which affect the rates of utilization, not the operation of utilization. The rates of utilization differ because of the dominance of more recalcitrant organic substrates in inland water ecosystems, as discussed herein.

II. SEVEN DIRECTIONS OF DOM THOUGHT AND UNDERSTANDING

Direction 1. The observed heterotrophic biotic productivity of most lakes and rivers, as estimated in detailed organic carbon budgets discussed herein and in subsequent works, could not possibly be supported by the autochthonous organic carbon generated by pelagic primary productivity within these waters. Among the earliest detailed quantitative budgets that considered dissolved as well as particulate organic carbon from autochthonous, allochthonous, and littoral sources and compartments of utilization and losses were those of Lawrence Lake (Wetzel *et al*., 1972) and of Mirror Lake [reviewed in Likens (1985) and Wetzel (1983, 2001)]. The aquatic heterotrophic productivity must be supplemented by external allochthonous organic matter imported from terrestrial and land–water interface regions. Therefore, from the standpoint of a lake or a river *per se*, bounded by the shoreline water demarcation, the lake or river is a net heterotrophic ecosystem that decomposes much more organic matter than is produced within those boundaries.

The annual time period is the only interval of relevance in comparative analyses of productivity and utilization of organic matter (Wetzel, 1995). A major portion of the total autochthonous annual photosynthetic production, even in the pelagic regions, occurs during cold, low-light periods when consumption by zooplankton and higher animals is reduced or virtually absent. Although that noningested production may be of little immediate consequence to the higher animal heterotrophs, it is of major importance, and usually dominates, energy flows within the pelagic region. Furthermore, nonpredatory heterotrophic metabolism completely dominates energy flows of the whole lake or river ecosystem. Large amounts of dissolved and non-ingested particulate organic matter enter the detrital pool for the dominant microbial heterotrophy.

Utilization of organic matter by metazoan predation, however, represents only a small portion of the whole pool and a very small source of DOM. Material and energy fluxes of egested particulate and soluble organic matter within the pelagic zone and the rest of aquatic ecosystems by nonmetazoan heterotrophs are largely ignored or discussed in terms of conversion back to particulate organic matter of sufficient size for ingestion by predation of metazoans (Wetzel, 1995). The common thesis that living net production in organisms, particularly algae and microbes as well as metazoans, die largely by ingestive predation is not only unsubstantiated, but also unreasonable.

Many if not most organisms, particularly bacteria, fungi, and algae, as well as higher organisms, simply mature physiologically, senesce, and die, and then enter the combined particulate and dissolved organic detrital pool for utilization by microbial heterotrophs. Biochemical death of many cells and organisms is genetically programmed and fixed within relatively narrow physiological sequences. Turnover of dissolved organic matter occurs many times prior to predation: large portions of the organic matter are converted to CO_2 heterotrophically before predation, which has much slower generation times, can respond to changes in particulate organic matter.

Senescence and mortality of bacteria, algae, and other microbes from viral infections is large (40 to >50%). Clearly viral mortality leads to major evasions of dissolved organic matter as cellular integrity is compromised (e.g., Suttle, 1994; Fuhrman, 2000). Average virus-to-bacteria ratios of 50:1 have been observed with a high direct correlation between abundance of viruses and bacteria (cf. review by Wetzel, 2001). Most estimates indicate viral phages lyse about one fourth of the bacterial production per hour (Heidal and Bratbak, 1991). Phage-induced mortality of bacterioplankton in Lake Konstanz, Germany, for example, was estimated to vary between 1 and 24% of total mortality over an annual period (Simon *et al*., 1998). Other viral mortality estimates of bacterioplankton and phytoplankton are in a similar or higher range (11–66%) for lakes, coastal waters, and rivers (e.g., Suttle, 1994; Fuhrman and Noble, 1995; Mathias *et al*., 1995; Wommack and Colwell, 2000).

Loss of cellular integrity upon senescence or viral parasitism results in a rapid large loss of soluble cellular organic matter, often exceeding 40% of the total organic carbon within the first 24 h (e.g., Krause, 1962; Otsuki and Wetzel, 1974b). DOM lost at this stage often consists of simple, non-structural organic compounds of relatively high energy and availability to microbes.

Similarly, ingestion of bacteria, and pico- and ultraplankton by protists, which purportedly, although erroneously, "short circuit" metabolism in the "microbial loop," is a major diversion of organic carbon away from animal higher trophic levels. Among some communities, such as higher aquatic plants and their attached periphytic microbial complex, most (>90%) of the total organic matter production (foliage, sloughed tissues, excreted DOM, rooting tissues, and others) never enters a metazoan digestive tract, analogous to the situation in terrestrial ecosystems (Wetzel, 1990, 1995). Functionally, organic carbon cycling in terrestrial and inland aquatic ecosystems is similar.

Direction 2. A large portion, usually >90%, of the organic matter imported from allochthonous and littoral/wetland sources to these aquatic ecosystems is predominantly in dissolved or colloidal form. Although a portion of the dissolved organic compounds may aggregate and shift to a particulate and hence gravitoidal form that may sediment out of the water, most of the imported dissolved organic matter is dispersed within the water

and is moved about with the hydrodynamic movements within the water body. This dispersion of DOM is important owing to retention in zones of utilization or modification by physical processes (e.g., photolysis) within the aquatic ecosystems that may not be the case with particulate organic matter that is subject to gravitoidal sedimentation. Great seasonal and daily variations can occur in those hydrodynamics, particularly in streams and rivers. In lakes, daily and seasonal variations are generally appreciably slower and smaller than in rivers. Retention times, which can be important for effective utilization by enzymatic hydrolysis, tend to be longer in lakes than in streams.

Direction 3. Much of the dissolved organic matter originates from lignin, cellulose, and related structural precursor compounds of higher plants. These substances are abundantly produced in true lake and river ecosystems — that is, where the ecosystem includes the drainage basin and organic matter produced photosynthetically within it. The productivity of terrestrial vegetation and aquatic plants associated with the land–water interface region is manyfold (usually several orders of magnitude) greater per unit area than that of algae. Organic substances from higher plant tissues are abundant, chemically complex, and relatively recalcitrant to rapid biological degradation (e.g., Thurman, 1985; Haslam, 1998). During oxidative and anaerobic degradation, these compounds are modified by microbial activities in detrital masses, including standing dead tissues that can remain in an oxidative aerial environment for months or years. Much of the dissolved organic compounds released from partial decomposition of the plant tissues and from associated microbial degradation products is leached and partially degraded en route toward recipient lakes and streams. This microbial modification continues during partial decomposition in terrestrial soils and hydrosoils of wetlands. Once within land–water interface regions, water containing dissolved organic compounds moves, often diffusely, through dense aggregations of living emergent and submersed aquatic plants and massive amounts of particulate, largely plant derived, particulate detritus. The enormous surface areas of these habitats support large aggregations of rapidly growing microbial communities. During transport through these microbial metabolic sieves associated with wetlands and littoral areas, appreciable further selective degradation of more labile dissolved organic constituents occurs before final movement into the receiving lake body or river channel *per se*.

Direction 4. Massive microbial degradation of DOM occurs within lakes and streams despite the general recalcitrance of the organic matter. Because of limited accessibility of large portions of these predominantly humic and fulvic acid molecules to enzymatic hydrolysis, degradation rates are slow. Thus, many commonly acidic macromolecules have long turnover times and relatively long residence times once they enter the lakes or streams. Dissolved macromolecules are often of considerable age (years), but are mixed with variable and rapidly changing inputs of younger humic and

nonhumic substances of more recent synthesis. Studies indicate that humic substances, particularly relatively recalcitrant fulvic acids, can be generated by algae and contribute to the multitude of diverse compounds that constitute the collective dissolved organic matter (McKnight *et al*., 1991, 1994). This source is particularly important in littoral areas, where attached and sessile algal productivity is often several orders of magnitude greater than that of phytoplankton (e.g., Wetzel, 1990, 1996). Because of the recalcitrance of these dominating dissolved organic compounds, the dissolved organic matter can reside *within* lakes and rivers for long periods of time (months and years).

Strong budgetary evidence in early lake studies indicated that, despite continual loading, the dissolved organic matter did not accumulate and losses by aggregation and sedimentation are small. However, large quantities of CO_2 evade to the atmosphere (e.g., Otsuki and Wetzel 1974; Kling, *et al*., 1992; recently verified globally; cf. Cole *et al*., 1994). This CO_2 evasion from the pelagic zones of lakes is always in excess, often manifold, of autochthonous photosynthetic organic carbon production by phytoplankton on an annual basis. When the organic matter inputs from allochthonous, wetland, and littoral sources and their loading to the sediments are included, as they must be in any lake ecosystem analysis, then the CO_2 evasion from the lake is always greatly in excess of autochthonous production.

For decades in aquatic ecology, the apparent chemical recalcitrance of humic substances that dominated the instantaneous bulk DOC of standing and running waters led to the belief that these compounds were poorly used by microbiota. To be sure, loss rates are slow, but consistently in the range of 0.5–2% per day under many different environmental conditions (Fig. 2). Often rates of degradation of total dissolved organic matter are greater than 2% per day.

Direction 5. Physical processes, such as partial or complete photochemical modification of organic macromolecules, can result in major alterations in biological availability of portions of complex, heterogeneous dissolved organic compounds. These photochemical processes can result in the following changes:

(a) Alterations of enzymatic accessibility by the macromolecules. In particular, cross-linking of polypeptide chains with polyphenolic humic substances can lead to enzymatic inhibition or reduction of activity (e.g., Wetzel, 1991; Boavida and Wetzel, 1998; Haslam, 1998).

(b) Partial photolysis of humic macromolecules, particularly with the generation of volatile fatty acids and related simple compounds that serve as excellent substrates for bacterial degradation (e.g., Stewart and Wetzel, 1981; Wetzel *et al*., 1995; see Chapter 10). It is important to recognize that of the total photolytic irradiance, about half of the

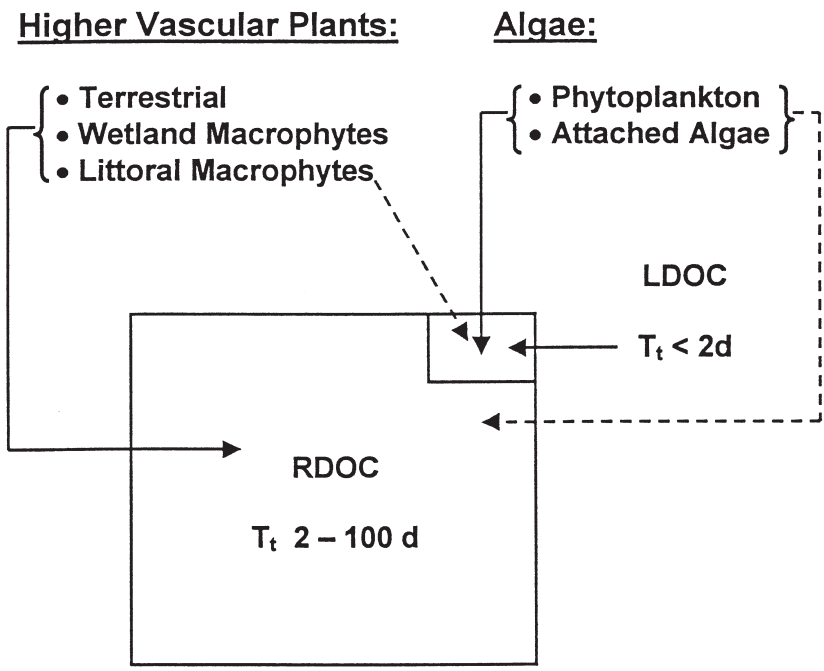

FIGURE 2 Common sources of organic carbon and rates of mineralization of labile (LDOC) and recalcitrant (RDOC) dissolved organic carbon in lakes and streams.

partial photolysis of organic substrates results from UV-B (285–300 nm) and UV-A (320–400 nm) irradiance. Transmittance and photolytic activity from UV-B and UV-A is restricted largely to the surface waters. In contrast, photosynthetically active radiation (PAR, 400–720 nm), although much weaker energetically than UV, penetrates to much greater depths. Although photolysis of organic compounds is appreciably less than that induced by UV at surface waters, the photolytic generation of simple substrates is appreciable by PAR as well as by UV (Fig. 3). Results of such studies indicate that over half of some substrates cleaved from the humic macromolecules are generated by PAR irradiance.

(c) Degradation of dissolved organic nitrogen and phosphorus compounds to release inorganic nutrient compounds such as nitrate, ammonia, and phosphate (e.g., Manny *et al.*, 1971; Moran and Zepp, 1997; Vähätalo *et al.*, 2000).

(d) Complete photolysis of humic substances to CO and CO_2, accompanied by some dissociation to dissolved inorganic bicarbonate as well as evasion of some CO_2 to the atmosphere. Previous studies on the photolytic degradation of dissolved organic matter suggested that

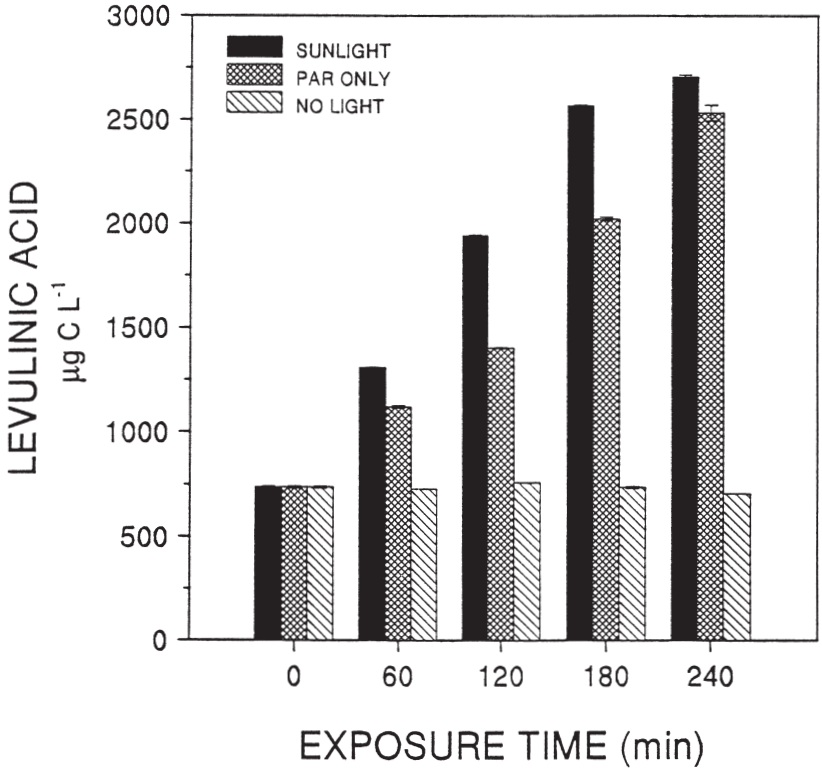

FIGURE 3 Net generation of levulinic acid from the partial photolysis of sterile whole leachate (0.2 µm pore size filtrate) of the emergent macrophyte *Juncus effusus* (10 mg DOC L^{-1}) after 4 weeks of microbial decomposition at 20°C in the dark. Exposed to full natural sunlight (total insolation over the 4 h period = 13.05 mol m^{-2} of UV-B, UV-A, and PAR), to PAR only, and incubated simultaneously in the dark. Error bars = SD; n = 3 each. Modified from Wetzel, 2001.

the dominant components of solar irradiance were UV-B and UV-A, and that PAR above 400 nm was of little consequence. Many of these studies, however, were not performed under sterile conditions and, as a result, the findings were confounded by nearly instantaneous microbial utilization of organic compounds generated with rapid degradation and generation of CO_2. Moreover, many of the DOM sources of these studies had been exposed to natural sunlight for long (e.g., weeks) and noncomparable periods of light. Contemporary research indicates that although UV-B is significant, photolysis by UV-A can contribute to more than half of photochemical mineralization (Vähätalo *et al.*, 2000; Wetzel, 2000b, 2002). For example, from nearly 200 separate photolytic experiments on DOM from different waters and plants under different conditions, the UV-B

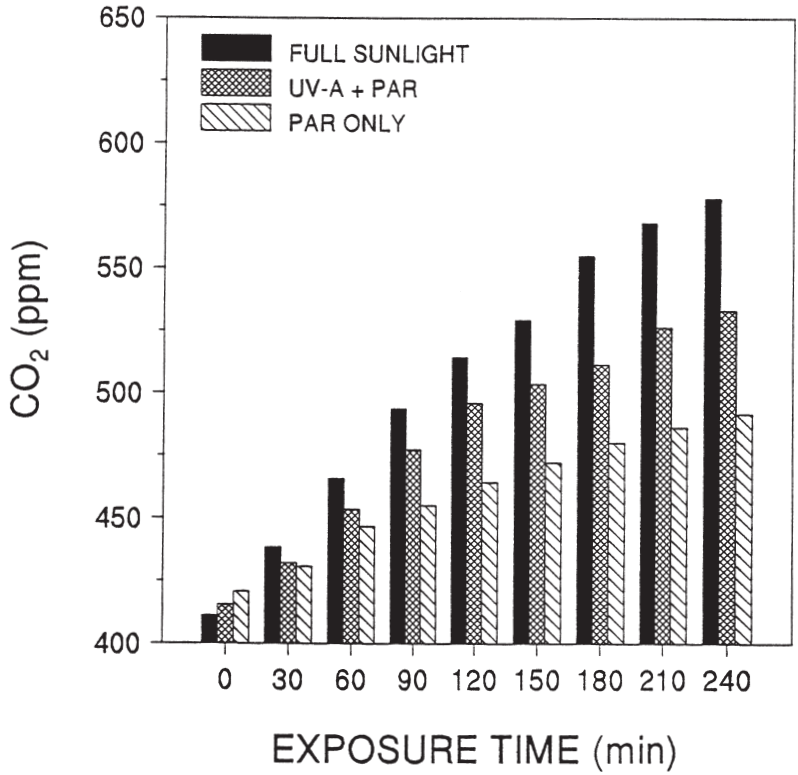

FIGURE 4 Photolytic degradation of sterile dissolved organic matter (leachate from *Juncus effusus*, 0.2 μm pore size filtrate) to CO_2 under replicated, aseptic conditions exposed to full sunlight (UV-B + UV-A + PAR; 15.53 mol m^{-2} over the 4 h period), to UV-A + PAR, and to PAR only.

portion of the spectrum was always most effective in complete photodegradation to CO_2, but UV-A was also highly effective with small differences from the photolytic capacities of UV-B (Fig. 4). PAR is also highly effective in photolytic degradation of DOM to CO_2: about one quarter to one half of the collective photolysis can be attributed to the largely blue portion of the PAR spectrum (Fig. 5).

Both partial photolysis by PAR of DOC accompanied by the generation of volatile fatty acids and complete photolysis accompanied by the generation of large quantities of CO_2 are important findings because of the much lower extinction rates of PAR in water in comparison to those of ultraviolet irradiance. Photolytic processes, so important to nutrient cycling, are therefore not restricted to the uppermost strata of a few centimeters of aquatic ecosystems, but rather affect much of the variable volume of the photic zone.

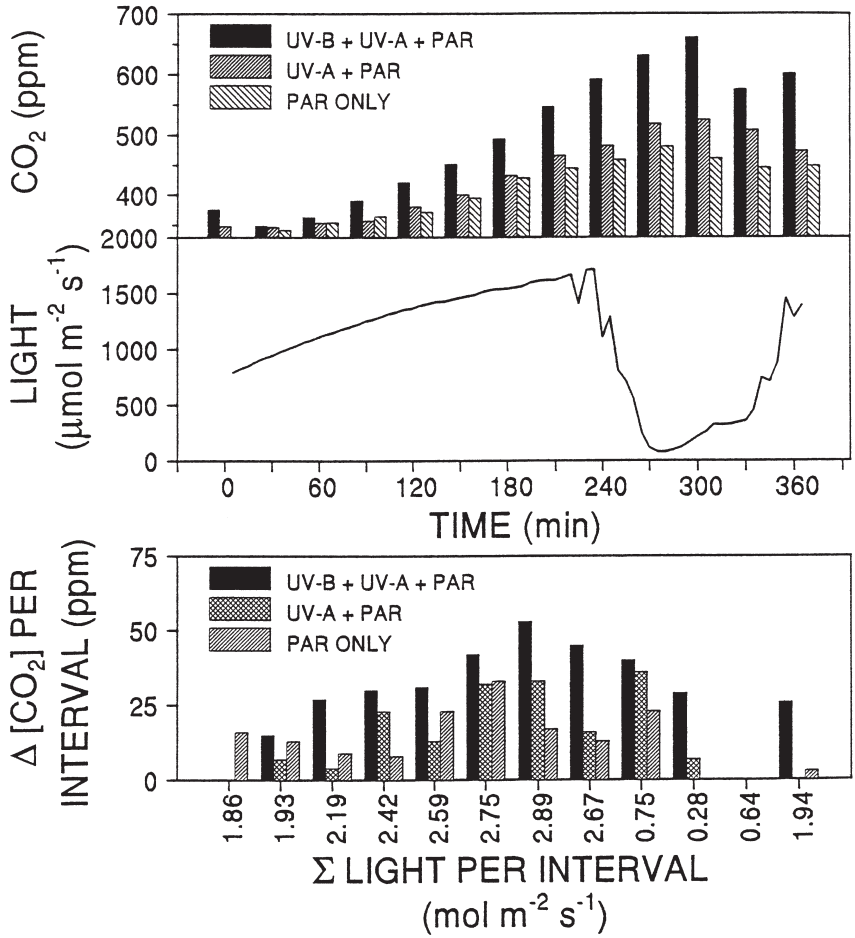

FIGURE 5 Photolytic degradation of sterile aquatic plant leachate (*Juncus effusus*, 0.2 µm pore size filtrate after 20 weeks of microbial decomposition) to CO_2 under replicated, aseptic conditions exposed to full sunlight (UV-B + UV-A + PAR), to UV-A + PAR only, and to PAR only (upper). A severe rainstorm occurred during the incubations, which reduced light severely for an hour (middle). The net change in CO_2 production per amount of light received per interval under these conditions (lower).

Furthermore, the photolytic responses are very rapid. For example, when natural sunlight was attenuated very rapidly, as by a severe thunderstorm, the rate of photolytic degradation of DOC to CO_2 declined precipitously (Fig. 5, upper), but the photolytic capacities of PAR declined more rapidly than did the effects of UV-B (Fig. 5, lower). The precise chemical degradation processes involved in photolysis of dissolved macromolecules, particularly the helical humic substances, represent a major void in our understanding. Interdisciplinary collaboration among chemists and biologists is essential to

progress effectively in our understanding of biological implications of organic molecular structure.

Direction 6. Less direct but important biogeochemical interactions of dissolved organic matter in aquatic systems are also important but poorly studied at the ecosystem level. Continued intensive study of natural dissolved organic substances in aquatic ecosystems is also resulting in improved understanding of the many ways in which these diverse compounds, particularly humic compounds, can interact with other important metabolic components. For example, dissolved organic compounds can function in the following ways:

(a) Interact with inorganic compounds, particularly in complexation reactions such as chelation (reviewed in Wetzel, 2001). Depending on the concentration ratios of complexing DOM to inorganic elements, the mode of organic complexation, biological availability, and, in some cases, elemental toxicity can be increased or decreased.

(b) Interact with other organic compounds, such as peptidization, and alter biological susceptibility to enzymatic hydrolysis. For example, membrane properties, such as lipid hydrophobicity, can be altered by humic substances and in turn can affect enzyme hydrolysis rates and nutrient transport mechanisms (e.g., Lemke *et al*., 1995, 1998). In a most interesting interaction, humic substances can complex with proteins, particularly both freely soluble and membrane-bound enzymes, with noncompetitive inhibition (Wetzel, 1992, 1993). Enzymes can be stored for long periods (days and weeks) in this complexed, inactive state, be redistributed in the ecosystem with water parcel movements, and be reactivated by partial photolytic cleavage by ultraviolet irradiance (Fig. 6; Wetzel, 1991, 1995, 2001; Boavida and Wetzel, 1998).

(c) Alter chemical properties such as redox and pH. A predominance of humic acids can result in an organic acidity that can influence, and at times exceed, inorganically derived acidity from natural or anthropogenic sources (reviewed in Wetzel, 2001).

(d) Microbially reduced humic substances can, upon entering less reduced zones of sediments, serve as electron donors for the microbial reduction of several environmentally significant electron donors (Lovley *et al*., 1999). Once microbially reduced, humic substances can transfer electrons to various Fe(III) or Mn(IV) oxide forms abiotically and recycle the humic compounds to the oxidized form, which can then accept more electrons from the humic compound-reducing microorganisms.

(e) Change physical properties such as selective modifications of light penetration. The well-known selective attenuation of light by chromophoric dissolved organic matter (cf. Wetzel, 2001) can further modify biogeochemical cycling in numerous ways. Such modification of the

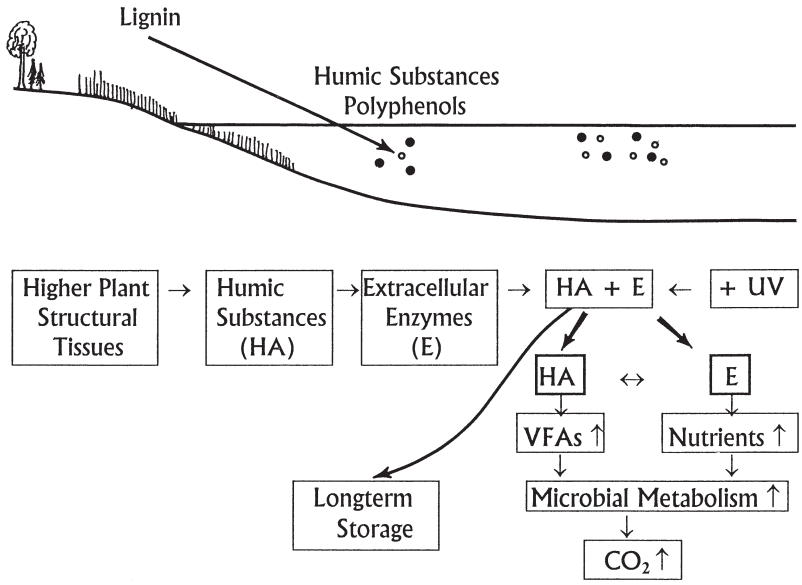

FIGURE 6 Potential interactive pathways and processes of humic substances emanating from decomposition products of higher plant tissues with extracellular and surface-bound enzymes and photolytic reactions, particularly with UV irradiance. Humic acid–enzyme complexes can be stable for long periods (weeks and months) and subsequently reactivated upon exposure to weak UV light. Further photolysis can cleave simple compounds from the macromolecules for subsequent utilization by microbes.

light regimes can alter rates of photosynthesis, hormonal activities, migratory distribution, and reproductive behaviors. Absorption of ultraviolet irradiance by humic substances can protect organisms from genetic damage as well as modify macromolecules and enhance bioavailability of organic substrates.

Direction 7. The seventh direction of DOM understanding is one that I consider to be of major importance. Much of the present emphasis on DOM of aquatic ecosystems correctly addresses how this abundant reservoir of recalcitrant dissolved organic carbon can be utilized in microbial metabolism and respiration, and can influence microbial nutrient regeneration. The commonly observed incomplete photolysis of DOC is critical to accelerated utilization of these macromolecules, but is clearly not mandatory. Portions of the complex DOM pools, including fractions of humic and fulvic acid compounds, are degraded, but total degradation rates are clearly slow. I have argued for years that such chemical organic recalcitrance of DOM is instrumental in providing a thermodynamic stability to metabolism within lake, reservoir, wetland–littoral land–water interface, and river ecosystems (Wetzel, 1983, 1984, 1992, 1995, 2001). Stability in this sense refers to a

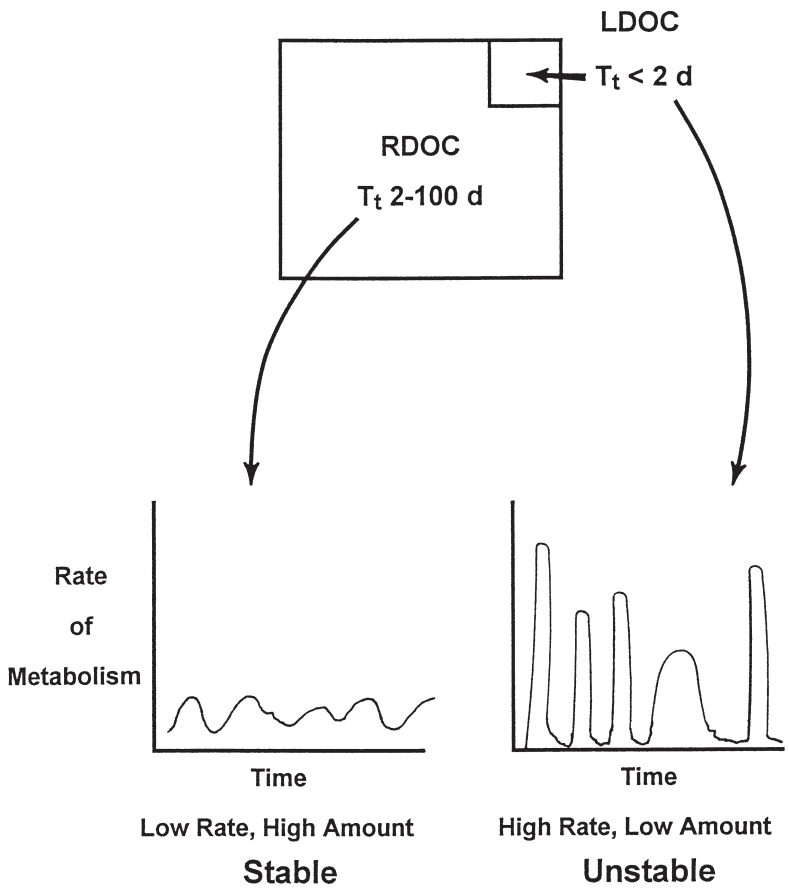

FIGURE 7 Relative rates of metabolism of the large, dominating pool of RDOC versus the small instantaneous pool of LDOC compounds that are largely under the supply control of highly ephemeral algal communities.

mean low variance of collective ecosystem metabolism. The chemical recalcitrance is truly a "brake' on ecosystem metabolism, and that brake is critical for maintenance of stability.

Most of the detrital organic pool, both particulate and dissolved phases, of inland aquatic ecosystems consists of residual organic compounds of plant structural tissues. The more labile organic constituents of complex dissolved and particulate organic matter are commonly hydrolyzed and metabolized more rapidly than more recalcitrant organic compounds that are less accessible enzymatically (Fig. 7). The result is a general increase in concentration of the more recalcitrant compounds, commonly exceeding 80% of the total, with slower rates of metabolism and turnover. These recalcitrant compounds, however, are metabolized at rates slowed and regulated in large part by their molecular complexity and bonding structure.

In every detailed *annual* organic carbon budget of lake and river ecosystems, the heterotrophic metabolism of the ecosystem cannot be supported by organic matter generated by phytoplankton. At least severalfold support of the total metabolism is by organic subsidies from the land–water interface communities and allochthonous production. From the standpoint of metabolic stability, it is particularly important that most of the organic carbon is dissolved and relatively recalcitrant, which ameliorates the violent oscillations so characteristic of the pelagic particulate components of the ecosystem. In addition, much of the particulate organic matter formed in the dominating land–water interface regions of lake and river ecosystems is displaced to reducing, anoxic environments of the littoral and profundal sediments. The dissolved organic carbon, largely of higher plant origins, provides the stability and is the currency for the quantitatively more important detrital pathways in aquatic ecosystems (Fig. 8). The same underpinnings of that stability prevail in terrestrial ecosystems and likely much if not most of the marine ecosystem.

Detritus includes nonliving particulate, colloidal, and dissolved organic matter, and metabolically size affects only the rates of hydrolytic attack (Wetzel, 1995). Inland aquatic ecosystems collect organic matter, particularly

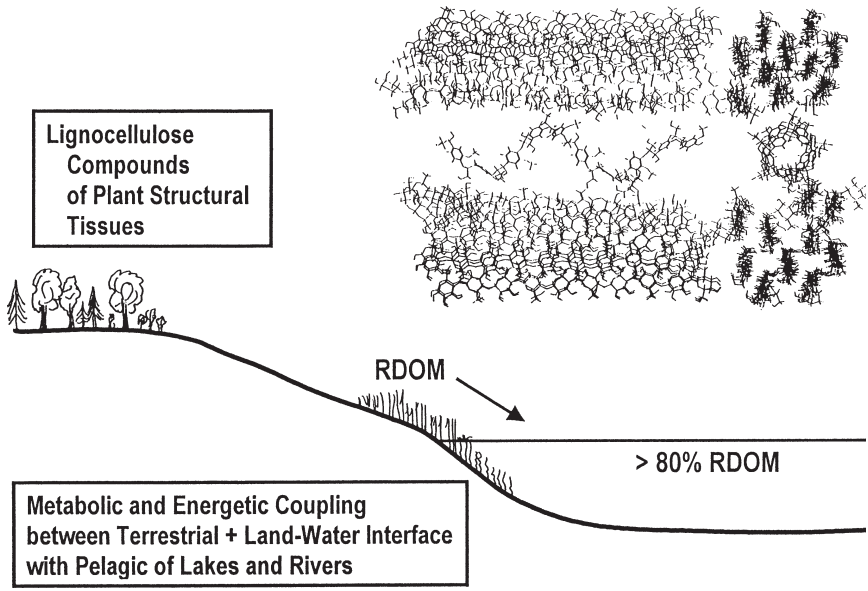

FIGURE 8 Lignocellulose compounds of higher plant structural tissues from terrestrial and land–water interface regions form a major source of RDOM within aquatic ecosystems, and a major metabolic coupling between the drainage basin and aquatic ecosystems. Chemical structure indicates a humic macromolecule (P. Hatcher, personal communication).

in dissolved forms, from terrestrial, wetland, and littoral sources in quantities that supplement if not exceed those produced autochthonously. Rates of utilization of that organic matter are slowed by a combination of chemical recalcitrance as well as displacement to anoxic environments. From a eutrophication viewpoint, lakes have been referred to as "algal bowls" (Vallentyne, 1974). Functionally, however, inland aquatic ecosystems are heterotrophic and functionally are detrital bowls, not algal bowls.

In long-term evolutionary scales, humans now have the abilities to intervene rapidly in this interdependent relationship and alter the stability of the rates of metabolism of organic matter. For example, reduction of ozone in the stratosphere and associated increased UV irradiance could lead to accelerated photolytic degradation of macromolecules of DOM by both abiotic and biotic pathways to CO_2. In addition, the photolytic enhancement of substrates for bacterial metabolism by UV photolysis can result in accelerated rates of biogeochemical cycling of nutrients and stimulated

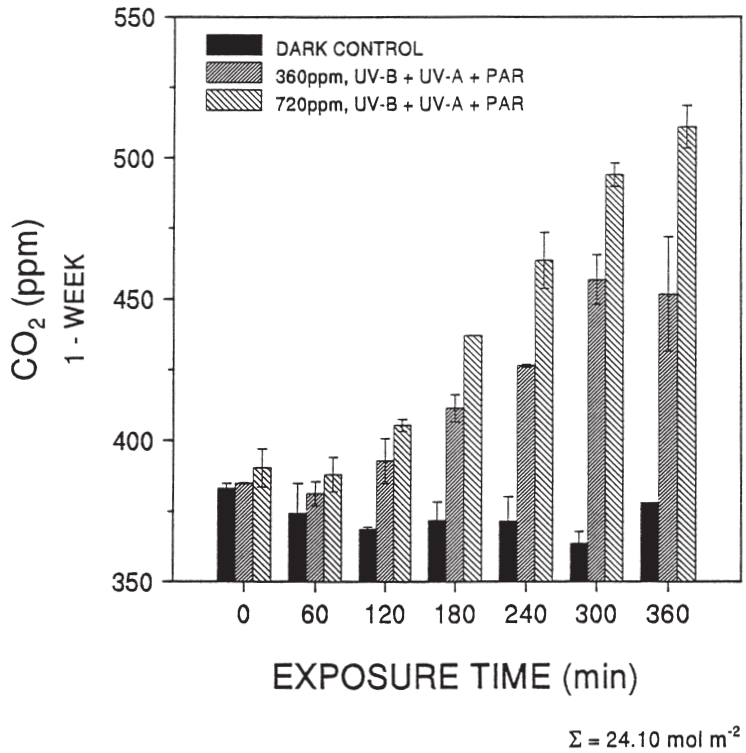

FIGURE 9 Release of CO_2 by complete photolytic degradation of sterile leachate from leaves of the riverine popular tree (*Populus tremuloides*), grown under ambient atmospheric CO_2 and doubled CO_2 concentrations, after one week of microbial decomposition.

productivity of the ecosystems. In addition to decreasing the metabolic stability of the lakes and streams, the enhanced microbial respiration will certainly lead to enhance generation of CO_2 and evasion to the atmosphere.

Moreover, as the concentrations of CO_2 in the atmosphere increase, largely from anthropogenic combustion of fossil fuels, plant growth commonly accelerates. For example, CO_2 concentrations will double to ca. 720 ppm in the 21st century. Experimental enhancement of CO_2 to 720 ppm leads to increased rates of photosynthesis and growth by about 35% of some emergent wetland plants in just a few months, and commonly leads to nitrogen limitations. As a result C : N ratios increase, often doubling, and common structural tissues, particularly lignin content, increase. As these plants grown in enriched CO_2 decay, DOM leached into the surface waters contain larger amounts of dissolved humic substances per unit mass. Photolysis of this DOM from plants grown under enriched atmospheric CO_2 resulted in a conspicuous increase in CO_2 release among both terrestrial

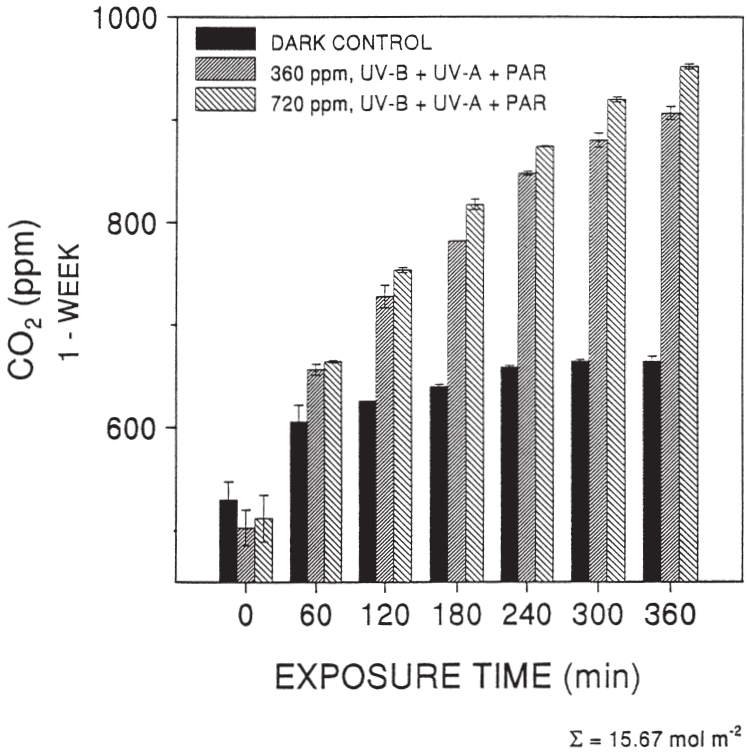

FIGURE 10 Release of CO_2 by compete photolytic degradation of sterile leachate from leaves of the cattail (*Typha latifolia*), grown under ambient atmospheric CO_2 and doubled CO_2 concentrations, after 1 week of microbial decomposition.

plants (Fig. 9) and emergent aquatic plants (Fig. 10). Clearly, photodegradation of dissolved organic substrates is a major process that can alter rates of biogeochemical cycling in aquatic ecosystems.

REFERENCES

Benke, A. C., and J. L. Meyer. 1988. Structure and function of a backwater river in the southeastern USA. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **23:**1209–1218.

Boavida, M.-J., and R. G. Wetzel. 1998. Inhibition of phosphatase activity by dissolved humic substances and hydrolytic reactivation by natural UV. *Freshwater Biology* **40:**285–293.

Burkholder, J. M., and R. G. Wetzel. 1989. Epiphytic microalgae on a natural substratum in a hardwater lake: Seasonal dynamics of community structure, biomass and ATP content. *Archiv fur Hydrobiologie/Suppl.* **83:**1-56.

Cole, J. J., N. F. Caraco, G. W. Kling, and T. K. Kratz. 1994. Carbon dioxide supersaturation in the surface waters of lakes. *Science* **265:**1568–1570.

Coveney, M. F., and R. G. Wetzel. 1995. Biomass, production, and specific growth rate of bacterioplankton and coupling to phytoplankton in an oligotrophic lake. *Limnology and Oceanography* **40:**1187–1200.

Fuhrman, J. A. 2000. Impact of viruses on bacterial processes. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 327–350. Wiley–Liss, New York.

Fuhrman, J. A., and R. T. Noble. 1995. Viruses and protists cause similar bacterial mortality in coastal seawater. *Limnology and Oceanography* **40:**1236–1242.

Hairston, N. G., Jr., and N. G. Hairston. 1993. Cause–effect relationships in energy flow, trophic structure, and interspecific interactions. *American Naturalist* **142:**379–411.

Haslam, E. 1998. "Practical polyphenolics: From Structure to Molecular Recognition and Physiological Action." Cambridge University Press, Cambridge, UK, 422 pp.

Heidal, M., and G. Bratbak. 1991. Production and decay of viruses in aquatic environments. *Marine Ecology — Progress Series* **72:**205–212.

Hutchinson, G. E. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? *American Naturalist* **93:**145–159.

Kling, G. W., G. W. Kipphut, and M. C. Miller. 1992. The flux of CO_2 and CH_4 from lakes and rivers in arctic Alaska. *Hydrobiologia* **240:**23–36.

Krause, H. R. 1962. Investigation of the decomposition of organic matter in natural waters. FAO Fisheries *Biology Report* 34(FB/R34), pp. 1–19

Lemke, M. J., P. F. Churchill, and R. G. Wetzel. 1995. Effect of substrate and cell surface hydrophobicity on phosphate utilization in bacteria. *Applied Environmental Microbiology* **61:**913–919.

Lemke, M. J., P. F. Churchill, and R. G. Wetzel. 1998. Humic acid interaction with extracellular layers of wetland bacteria. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limonologie* **26:**1621–1624.

Likens, G. E., Editor. 1985. "An Ecosystem Approach to Aquatic Ecology: Mirror Lake and Its Environment." Springer-Verlag, New York, 516 pp.

Lindeman, R. L. 1942. The trophic–dynamic aspect of ecology. *Ecology* **23:**399–417.

Lovley, D. R., J. L. Fraga, J. D. Coates, and E. L. Blunt-Harris. 1999. Humics as an electron donor for anaerobic respiration. *Environmental Microbiology* **1:**89–98.

Manny, B. A., M. C. Miller, and R. G. Wetzel. 1971. Ultraviolet combustion of dissolved organic nitrogen compounds in lake waters. *Limnology and Oceanography* **16:**71–85.

Mathias, C. B., A. K. T. Kirschner, and B. Velimirov. 1995. Seasonal variations of virus abundance and viral control of the bacterial production in a backwater system of the Danube River. *Applied Environmental Microbiology* **61:**3734–3740.

McKnight, D. M., G. R. Aiken, and R. L. Smith. 1991. Aquatic fulvic acids in microbially based ecosystems: Results from two Antarctic desert lakes. *Limnology and Oceanography* **36:**998–1006.

McKnight, D. M., E. D. Andrews, R. L. Smith, and R. Dufford. 1994. Aquatic fulvic acids in algal-rich Antarctic ponds. *Limnology and Oceanography* **39:**1972–1979.

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography* **42:**1307–1316

Odum, H. T. 1957. Trophic structure and productivity of Silver Springs, Florida. *Ecological Monographs* **27:***55*–112.

Otsuki, A., and R. G. Wetzel. 1974a. Calcium and total alkalinity budgets and calcium carbonate precipitation of a small hard-water lake. *Archiv für Hydrobiologie* **73:**14–30.

Otsuki, A., and R. G. Wetzel. 1974b. Release of dissolved organic matter by autolysis of a submersed macrophyte, *Scirpus subterminalis*. *Limnology and Oceanography* **19:**842–834.

Rich, P. H., and R. G. Wetzel. 1978. Detritus in lake ecosystems. *American Naturalist* **112:**57–71.

Saunders, G. W. 1972. The transformation of artificial detritus in lake water. *Memorie di Istituto Italiano Idrobiologie* **29**(Suppl.):261–288.

Simon, M., M. M. Tilzer, and H. Müller. 1998. Bacterioplankton dynamics in a large mesotrophic lake. I. Abundance, production and growth control. *Archiv für Hydrobiologie* **143:**385–407.

Stewart, A. J., and R. G. Wetzel. 1981. Dissolved humic materials: Photodegradation, sediment effects, and reactivity with phosphate and calcium carbonate precipitation. *Archiv für Hydrobiologie* **92:**265–286.

Suttle, C. A. 1994. The significance of viruses to mortality in aquatic microbial communities. *Microbial Ecology* **28:**237–243.

Teal, J. M. 1957. Community metabolism in a temperate cold spring. *Ecological Monographs* **27:**283–302.

Thurman, E. M. 1985. "Organic Geochemistry of Natural Waters." Nijhoff, Dordrecht, 497 pp.

Vähätalo, A. V., M. Salkinoja-Salonen, P. Taalas, and K. Salonen. 2000. Spectrum of the quantum yield for photochemical mineralization of dissolved organic carbon in a humic lake. *Limnology and Oceanography* **45:**664–676.

Vallentyne, J. R. 1974. The algal bowl — Lakes and man. Miscellaneous Special Publication 22, Department of the Environment, Ottawa, 185 pp.

Wetzel, R. G. 1983. "Limnology," 2nd ed. Saunders, Philadelphia, 860 pp.

Wetzel, R. G. 1984. Detrital dissolved and particulate organic carbon functions in aquatic ecosystems. *Bulletin of Marine Science* **35:**503–509.

Wetzel, R. G. 1990. Land–water interfaces: Metabolic and limnological regulators. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **24:**6–24.

Wetzel, R. G. 1991. Extracellular enzymatic interactions in aquatic ecosystems: Storage, redistribution, and interspecific communication. *In* "Microbial Enzymes in Aquatic Environments" (R. J. Chróst, Ed.), pp. 6–28. Springer-Verlag, New York.

Wetzel, R. G. 1992. Gradient-dominated ecosystems: Sources and regulatory functions of dissolved organic matter in freshwater ecosystems. *Hydrobiologia* **229:**181–198.

Wetzel, R. G. 1993. Humic compounds from wetlands: Complexation, inactivation, and reactivation of surface-bound and extracellular enzymes. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **25:**122–128.

Wetzel, R. G. 1995. Death, detritus, and energy flow in aquatic ecosystems. *Freshwater Biology* **33:**83–89.

Wetzel, R. G. 1996. Benthic algae and nutrient cycling in standing freshwater ecosystems. *In* "Algal Ecology: Benthic Algae in Freshwater Ecosystems" (R. J. Stevenson, M. Bothwell, and R. Lowe, Eds.), pp. 641–667. Academic Press, New York.

Wetzel, R. G. 2000a. Freshwater ecology: Changes, requirements, future demands. *Limnology* **1:**3–11.

Wetzel, R. G. 2000b. Natural photodegradation by UV–B of dissolved organic matter of different decomposing plant sources to readily degradable fatty acids. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **27:**2036–2043.

Wetzel, R. G. 2001. "Limnology: Lake and River Ecosystems." Academic Press, San Diego, 1006 pp.

Wetzel, R. G. 2002. Origins, fates, and ramifications of natural organic compounds of wetlands. *In* "Proceedings of a conference on sustainability of wetlands and water resources: How well can riverine wetlands continue to support society into the 21st century?" (M. M. Holland, M. L. Warren, and J. A. Stanturf, Eds.), pp. 183–189. Gen. Tech. Rep. SRS-50, U.S. Dept. of Agriculture, Forest Service, Asheville, NC.

Wetzel, R. G., P. H. Rich, M. C. Miller, and H. L. Allen. 1972. Metabolism of dissolved and particulate detrital carbon in a temperate hard-water lake. *Memorie di Istituto Italiano Idrobiologie* **29**(Suppl.):185–245.

Wetzel, R. G., and A. Otsuki. 1974. Allochthonous organic carbon of a marl lake. *Archiv für Hydrobiologie* **73:**31–56.

Wetzel, R. G., P. G. Hatcher, and T. S. Bianchi. 1995. Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology and Oceanography* **40:**1369–1380.

Wommack, K. E., and R. R. Colwell. 2000. Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Review* 6**4:**69–114.

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Dissolved Organic Matter: Out of the Black Box into the Mainstream

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I. INTRODUCTION

The last 20 years have brought dramatic changes in perceptions of the role of dissolved organic matter (DOM) in food webs and ecosystem function. These changes have been catalyzed by major progress on two fronts: first, our expanding ability to deconstruct the "black boxes" often used to represent

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both DOM and the bacterial community; and second, an emerging global picture of DOM reactivity within ecosystems and DOM interactions with multiple elements of connected ecosystems. With technological advances in analytical chemistry and molecular biology, operational categories of DOM and microbes are being resolved into ever finer divisions. Now, we face the challenge of deciding how much detail is sufficient or necessary to accurately model system dynamics at various scales. On the second front, it has become clear that a significant proportion of DOM is reactive, both in terms of microbial metabolism and in myriad environmental interactions with the abiotic elements of terrestrial and aquatic ecosystems. The result is that DOM experiences highly variable dynamics as it moves through ecosystem subunits (e.g., wetlands, soil horizons) and across major system boundaries (terrestrial to aquatic and freshwater to marine). Collectively these developments have largely supplanted the conception that DOM is "refractory" or "conservative," a view based on earlier assessments that DOM turnover was slow in relation to microbial dynamics and hydrologic retention times.

Contributions to this emerging appreciation of the multitude of interactions that affect DOM generation, reactivity, consumption and transport have come from several fields. In this chapter, we attempt to synthesize these contributions into a unified framework using interactivity as the core theme (Fig. 1). Some interactions are primarily external, such as those forced by large-scale edaphic, physical and hydrologic elements, including soil, land form, climate, physiography, and solar radiation. Within the system, the abiotic interactions of DOM affect the transparency of water, the microscale structure of the aqueous medium, and the availability and mobility of inorganic nutrients and other compounds. At the same time, interactions between DOM and the microbial community underlie system metabolism and influence the composition of DOM exported to linked ecosystems.

The interaction of DOM with physical processes is often viewed in one direction: the physical environment imposing constraints on DOM supply, retention, and reactivity. The most obvious example is how the spatial arrangement of landscape units (wetlands and reactive soil horizons) and their individual physiography (lake littoral area and system depth) affect net DOM production–consumption and DOM transport to hydrologically linked ecosystems. Perhaps less appreciated is the ability of DOM to affect the internal physical characteristics of the aquatic environment by altering the gel state and transparency of the aqueous medium, and mediating the reactivity of inorganic nutrients, metals, toxic organic compounds, and enzymes. These small scale physical effects feed back on DOM–bacteria interaction in several ways. For example, DOM can restrict primary production by absorbing light, while DOM photoreactions alter the bioavailability of complex substrates. Another example is the role of high molecular weight DOM in organizing community structure and promoting diversity.

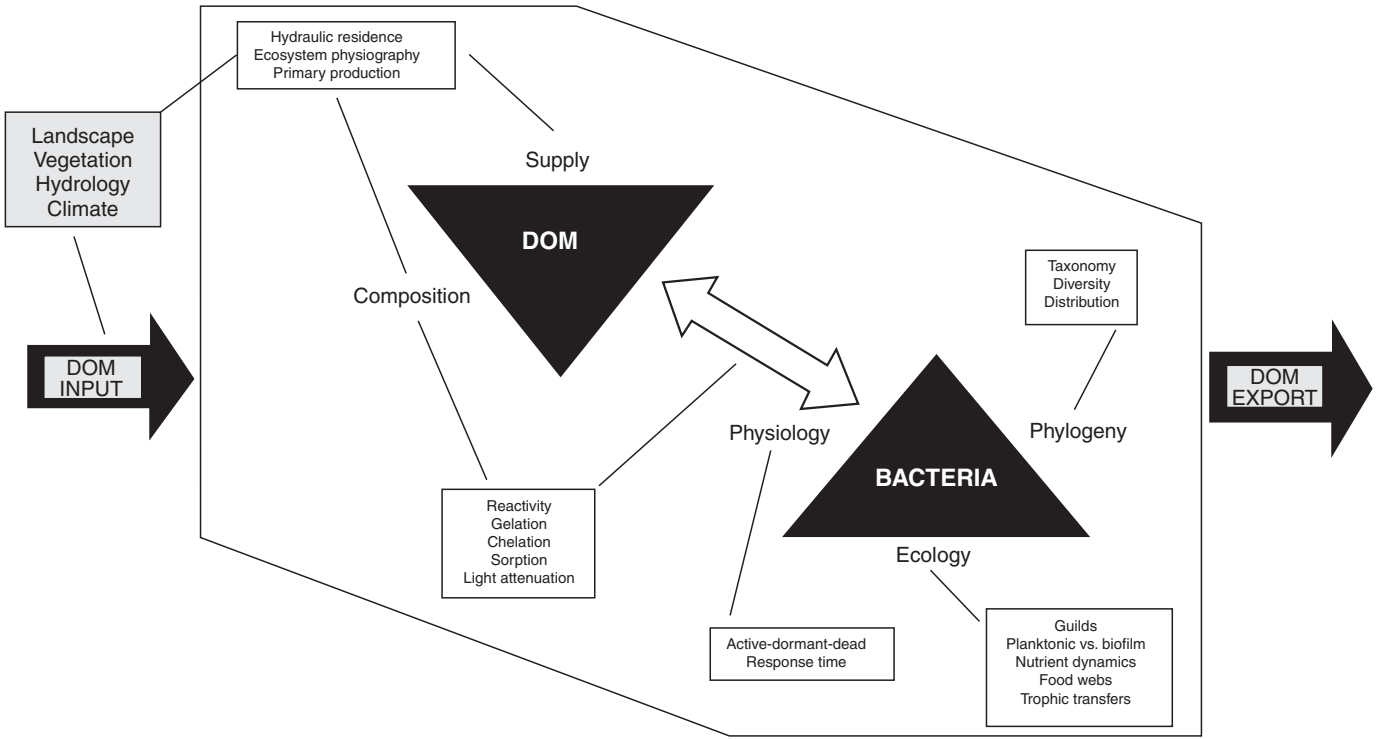

FIGURE 1 Conceptual diagram highlighting the principlal interactions that affect the supply, composition, metabolism, and transport of DOM.

DOM–microbe interactions are intense in both directions. DOM characteristics influence microbial community structure and function, while microbial community metabolism and composition influence DOM production, characteristics, and fate. A critical control on the polarity of these interactions is the dynamics of DOM inputs in relation to microbial response times. Considerable progress has been made in resolving the interactions between the DOM and bacterial black boxes by connecting specific DOM characteristics with specific aspects of microbial community structure or metabolism. These efforts proceed along multiple tracks: partitioning DOM into ecologically meaningful fractions linked to corresponding guilds of bacteria, describing the catabolic capabilities of individual taxa, linking extracellular enzyme kinetics with DOM composition, and investigating the interaction between extracellular polymers and cells that create highly organized biofilms. Underlying these research lines are observations that most of the activity in a bacterial community is concentrated among a small fraction of the cells (and perhaps the taxa) that are present. How this is possible in the face of grazing, lysis, and transport is an open question.

We have organized our synthesis into two broad categories: DOM–microbial and DOM–physicochemical interactions (Fig. 1). Although these categories are fairly distinct at large scales, they become increasingly difficult to parse at fine scales, and this is reflected in our synthesis.

II. DOM–MICROBIAL INTERACTIONS (Fig. 2)

A. Osmotrophy

Osmotrophs are organotrophic organisms that acquire nutrients from their environment one molecule at a time. Osmotrophs include most members of the bacteria and archaea domains, as well as fungi and several protistan groups. Except in association with coarse organic particles where eukaryotic fungi and oomycetes are prominent, prokaryotes dominate heterotrophic metabolism, so are the focus of the text that follows. We use the term bacteria loosely, recognizing that heterotrophic archaea may well be major consitituents of the microbial loop of many systems.

The ecology of osmotrophs is at the center of DOM dynamics. That ecology is largely a manifestation of size. To assimilate an adequate supply of nutrients on a molecular scale requires a high surface area to volume ratio. As this ratio increases, so does the energetic cost of locomotion (Vogel, 1996), which must be balanced against the limitations of remaining still and acquiring nutrients through passive diffusion. This is one reason why osmotrophs are most abundant and active at sites where nutrients are concentrated: mineral surfaces like sediment or boulders, organic surfaces like detritus or macrophytes, and macromolecular aggregates like humic colloids and transparent extracellular polymer (TEP).

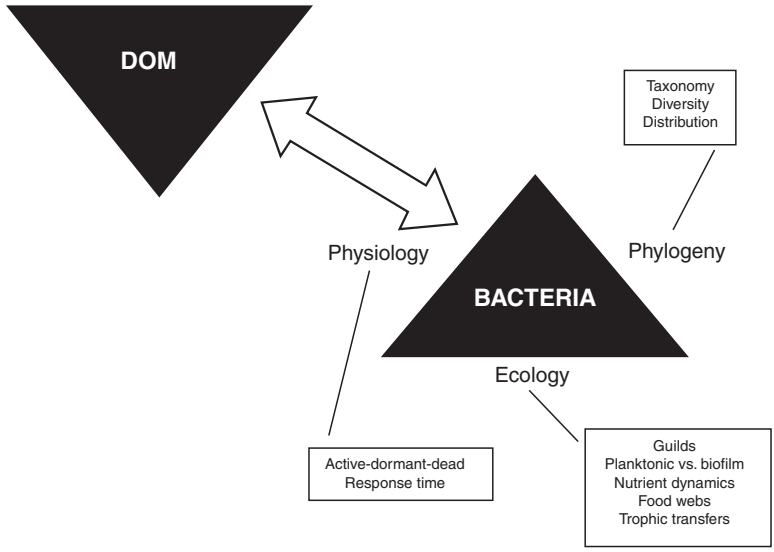

FIGURE 2 DOM–microbial interactions.

The association of cells with concentrations of organic matter is largely a function of physicochemial affinities, but selection has also favored organisms that alter their phenotypes in response to surfaces (see Chapter 12). The association of diverse organisms at surfaces has generated co-evolutionary pressures that across geologic time have produced a capacity for symbiotic integration so remarkable that biofilm communities are often likened to the tissues of metazoans or described as superorganisms. The capacity for consortial organization in conjunction with the structural re-engineering of the local environment through exopolysaccharide production appears to stabilize diversity and promote community metabolism. Consequently, the interaction of bacteria with surfaces and other physical elements of the environment exerts a major influence on DOM flux through aquatic ecosystems.

B. Nutrient Acquisition

Bacteria acquire nutrients by passive diffusion and active transport. The first requires no energy expenditure by the cell, but intake is limited by diffusion barriers and concentration gradients. Active transport requires energy expenditure to carry materials into the cell against concentration gradients. The efficiency of the process is shown by the low concentration of monomers (micromolar), particularly monosaccharides, within the DOM pool and by their rapid turnover (minutes to hours; see Chapters 4 and 9). Energy costs are higher still if the cell expresses extracellular enzymes or

chelators to generate or concentrate substrate for assimilation (Münster and De Haan 1998; see Chapter 13).

The physiological problem is how to distribute cellular resources to obtain the energy and materials needed for maintenance and growth (del Giorgio and Cole, 2000; Kirchman, 2000; see Chapter 18). The more steps needed to transform a nutrient into an intermediate metabolite, the more costly it is. For individual cells, the range of response to the resource allocation problem is limited by its store of genetic information, the maintenance of which also imposes costs at the expense of growth. The window for physiological adaptation is further limited by viral lysis, grazing, competition, and hydraulic residence time, all of which establish the threshold level of productivity required to hold an active place within the community.

For active cells, assimilation of substrate is tied to concentration and to the characteristics of the biochemical machinery that bind, transform, or transport it. The process is often described using the Monod or Michaelis–Menton model. The key ecological point is that any particular acquisition system functions optimally (minimum cost per unit assimilated) only within a constrained range of substrate abundance. Organisms with higher substrate affinities outcompete those with lower affinities as concentration drops (see Chapter 18). When substrate is abundant, organisms with low affinity systems bring in substrate at lower cost. At the community level, the pattern that emerges from the competition for substrate is that half-saturation constants tend to mirror substrate availability.

C. Substrate Preferences

Availability is not the only constraint on substrate consumption. The value of a substrate is also related to the resources needed to convert the molecule into an intermediary metabolite. Thus in aerobic environments, monosaccharides and amino acids are readily consumed; under anaerobic conditions, heterotrophic metabolism is largely fueled by small organic acids. Some taxa consume other types of substrates as long as they are reasonably abundant. Important examples include β-proteobacteria that consume phenols, a significant component of DOM inputs originating from plant material, and methylotrophs that consume single carbon compounds produced by anaerobic metabolism, photochemical reactions, and oxidation of methyl and methoxy substituents (Giovannoni and Rappé, 2000).

Current evidence indicates that glucose (free and combined) is the most abundant and ubiquitous neutral monosaccharide in DOM, with lesser amounts of galactose, fructose, and others (see Chapters 4, 5, and 9). The ubiquity of glucose is not surprising given its predominance in both structural and storage polysaccharides. Among charged sugars, it seems likely that glucosamine, found in chitin and peptidoglycan, and ribose phosphate, derived from RNA, would be most common, although these sugars have been detected only sometimes. Among amino acids, those found in peptidoglycan

and those abundant in algal protein (alanine, aspartate, glutamate, glycine, leucine, lysine, and serine) appear most abundant in DOM. Ectoenzyme assays generally corroborate the chemical analyses: α- and β-glucosidase are the most readily detected glycosidases; aminopeptidases with preferences for hydrophobic, basic, and hydroxylated side chains appear to be the most readily measured (Sinsabaugh and Foreman, 2001; see Chapters 13, 15, and 18). Substrate utilization profiles of culturable bacteria generally show the strongest responses to saccharides and their derivatives, with lesser responses to amino and volatile organic acids (Sinsabaugh and Foreman, 2001).

The preference of many aerobic heterotrophs for monosaccharides and amino acids reflects the ease with which these molecules can enter intermediary metabolism. Field and microcosm studies of planktonic systems indicate that growth on amino acids is generally more efficient than growth on monosaccharides (del Giorgio and Cole, 2000; Kirchman, 2000; see Chapter 9). Amino acids have a C : N ratio similar to biomass and a somewhat higher energy content. Monosaccharides provide carbon, but they may increase the need for heterotrophic organic nitrogen fixation, which may impose additional metabolic cost (see Chapters 11 and 15). Moving back a step from monomers to polymers, it is also possible that extracellular hydrolysis of polypeptides is more energetically efficient than polysaccharide hydrolysis, particularly for free-living planktonic cells, because fewer types of enzymes are required. These considerations highlight only a few of the myriad options for carbon, nitrogen, and phosphorous acquisition from DOM, but they are sufficient to make it clear how diverse microbial taxa may coexist on a complex resource (see Chapters 9 and 13).

D. Continua versus Categories

On a microbial scale, the aqueous environment is a continuum in which the density and composition of organic matter varies from point to point. Distributed throughout the medium, organic matter, spanning several orders of magnitude in molecular size, confounds distinctions between dissolved and particulate, and even nonliving and living: monomers, intermediary metabolites, oligomers, fulvic acids, polymers, enzymes, humic acids, viruses, organometal colloids, cell fragments, lysed cells, dead cells, and inactive cells (Azam, 1998; see Chapter 2).

DOM is the product of biotic activity, released to the environment by secretion, excretion, diffusion, and lysis, produced during normal metabolism and during turnovers (Nagata, 2000; see Chapter 1). Ultimately, lysis, by whatever agent, introduces all cellular components into the environment. Consequently, DOM should reflect biomass composition, but its identity is modified by myriad biogeochemical reactions of varying rates and selectivity (Williams, 2000). The larger the spatiotemporal displacement from the point of production, the more randomized the composition and the lower the content of intermediary metabolites recognizable by the nutrient acquisition

systems of cells. The asymmetry between the mostly random biogeochemical reactions of the environment and the mostly ordered biochemical reactions of cells imprints a large-scale pattern on DOM, represented biogeochemically by the size–reactivity model (Amon and Benner, 1996) or trophically by compartment models that variously parse DOM into labile, semilabile, recalcitrant, and refractory categories (see Chapter 18).

Although DOM components are distributed over continua of molecular size, composition, and reactivity, it is possible to recognize meaningful categories, and an understanding of the dynamics of these categories is essential for modeling. For fine-scale questions about bacterial responses, the relevant categories are structurally defined biomolecules, particularly monosaccharides and their polymers and amino acids and their polymers (see Chapters 4 and 18). Other fine-scale questions may involve functionally defined categories, for example, chelators, contaminants, signalling molecules, and photoreactive molecules (see Chapters 7 and 10). With increasing scale, the categories become operationally defined on the basis of their reactivity (labile, semilabile, recalcitrant, and refractory) or chemical properties [humic acids, fulvic acids, dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), etc.; see Chapters 2 and 11]. Additionally there can be multiple interactions between bacterial DOM consumption, inorganic nutrient demand, and grazing intensity (see Chapter 16). As interest in the myriad roles of DOM in natural systems expands, so does the number of useful categories for both DOM and the bacterial community. The issue confronting modelers is how much resolution is needed to address particular questions about food web organization or biogeochemical process?

E. Population Ecology and the Red Queen Paradox

Once assimilated, DOM contributes to respiration, growth, and reproduction. Respiration completes the carbon cycle, returning organic carbon to the dissolved inorganic carbon pool. Growth and reproduction, usually lumped as production, determine how much carbon is potentially available to grazers, but production also determines whether a population persists within the system. Estimates of protistan grazing and viral lysis from a number of planktonic systems indicate that bacterial communities turn over every day or two (Fuhrman, 2000; Ducklow, 2000). The magnitude of these processes and, in some systems, the hydraulic residence time establish a productivity threshold for persistence.

Persistence is further complicated by the dynamics of DOM inputs. Ephemeral sources create a boom and bust economy for bacterial populations. Like spatial heterogeneity, temporal dynamics are thought to promote diversity within the bacterial community by mitigating competitive interactions.

However, what happens when the productivity of a population falls below the minimum needed for persistence? [Red Queen to Alice: "Now here you see, it takes all the running you can do to keep in the same place." *Alice Through the Looking Glass*, Lewis Carroll.] The first answer — extinction — is belied by an apparently ubiquitous phenomenon of aquatic systems: only a small fraction, typically less than a quarter of the bacteria you can enumerate, are active, on the basis of their ability to react to dyes that bind to nucleic acids, that act as substrates for esterases, or that act as electron acceptors (del Giorgio and Cole, 1998, 2000; Ducklow, 2000). It is also becoming clear that, for bacteria, dead is a relative condition. Biochemical activity and the capacity to reproduce do not necessarily covary. Cells may be active and growing, alive but metabolically dormant, or dead but partly functional.

The red queen paradox: How do populations persist if they are not actively reproducing (Ducklow, 2000)? Clearly there must be mechanisms for withdrawing from the game. Inactive cells may not be subject to viral attack; they may also evade grazers by being small and by not emitting chemical cues that attract grazers. Small size also extends settling times. For lotic systems, input of cells from upstream may offset downstream losses. The full range of adaptations and processes at work remain fuzzy, but they must be effective given the generally high latent diversity of microbial systems.

F. Community Organization

The organization, diversity, and activity of microbial communities is intimately linked with the heterogeneity and dynamics of DOM. These fine-scale interactions, extrapolated to the ecosystem scale, strongly influence DOM retention, DOM export, and net production. The organizational templates for microbial communities are gradients in electron donors, electron acceptors, and nutrients. The initial template may be a physical one, for example, a hydrologic flow path, a point source input, an exposed surface, or a light extinction gradient, but successional processes within the community superimpose new gradients and establish patterns of biotic activity. One solution to Hutchinson's paradox of the plankton is the realization that ecologically significant gradients exist on micrometer scales, even within a medium that appears to be homogeneous from an anthropocentric perspective. The realization that DOM is continuously distributed over a wide range of molecular size and that many types of macromolecules have amphipathic properties has provided a conceptual link that connects planktonic communities to biofilms along a continuum of extracellular structure (Decho, 1990; Azam, 1998; see Chapter 18).

Presumably, microenvironments that are extensively structured have more potential to establish and sustain electrochemical and nutrient gradients, and thus are able to support higher biotic diversity. Furthermore, high

concentrations of DOM and microorganisms promote the development of consortia which yoke biodiversity and synergize community metabolism (Pearl and Pinckney, 1996). Biofilms are the epitome of this type of organization (see Chapter 12). Because they are attached to a solid surface and subject to boundary layer effects, it is possible to maintain steep electrochemical gradients and buffer metabolism through the internal recycling of captured nutrients (Christensen and Characklis, 1990). Free-floating particles like flocs or lower density TEP are more open structures with less potential to create the gradients that are the foundation of a consortial organization. At the other end of the spectrum are planktonic communities that inhabit a comparatively dilute though heterogeneous medium. The principal population interactions in freshwater are competition and predation (Strom, 2000; Thingstadt, 2000; see Chapter 16), which may impart a guild-type organization on the community. In this environment, a dozen or two populations may account for a large portion of the metabolism.

Evidence is accumulating that DOM composition selects specific taxa (see Chapters 9 and 14). Models show that the relative input rates of labile and humic DOM can determine which guilds of bacteria dominate community metabolism (see Chapter 18). Because there may be multiple input sources, varying in magnitude and composition, each community represents a solution to a cacophony of selective pressures. Source heterogeneity in terms of DOM composition or timing probably contributes to greater active diversity, unless response is limited by other environmental factors (e.g., low temperature or low pH). From this perspective, ambient DOM concentration and composition are products of the interaction between input sources and community activity. This conception makes clear the limitations of attempts to model microbial activity in terms of static analyses of DOM abundance or composition.

At the ecosystem scale, the significance of microbial community organization is that it determines the magnitude and efficiency of carbon transfer to other portions of the food web. High density communities like biofilms and flocs can be directly grazed by metazoans, which divert microbial and detrital carbon out of the microbial loop (Wotton, 1994). In dilute planktonic systems, much of the carbon reaches metazoans through a microbial foodweb; after two to three trophic transfers, only a small fraction of microbial production remains.

G. Response Times

The extent to which DOM abundance and composition can be considered as end products of microbial activity rather than as drivers depends on the relationship between source dynamics and community response time. At one extreme, steady inputs, stable environmental conditions, and long residence time promote a dynamic equilibrium state in which DOM characteristics can be viewed as the product of source–activity interaction. At the other

extreme, rapidly fluctuating inputs that vary in quality and quantity on time scales comparable to those required for physiological adaptation and community succession create a state in which DOM can be considered to be driving community activity. The ability of bacteria to respond to these fluctuations depends on whether response requires a shift in a constitutive process, expression of different genetic products, or a change in taxonomic composition (see Chapter 15).

The relevant time scales for microbial response extend from minutes to weeks. In general, the up and down regulation of gene expression is faster than the cell cycle, the cell cycle is faster than population turnover time, and population turnovers are faster than community integration. Thus, individual bacteria can respond almost immediately to changes in DOM charcteristics, but it may take several weeks for a closely integrated community, like a biofilm, to attain something approaching a new equilibrium state.

III. DOM–PHYSICOCHEMICAL INTERACTIONS (Fig. 3)

The interactions of DOM with the physical environment mediate biotic interactions across scales ranging from the molecular to the landscape. On small scales, the focus is on how DOM alters the physical environment by binding to surfaces, absorbing solar radiation, providing microstructure, and mediating the availability or activity of biologically significant molecules. On large scales, the perspective inverts and questions center on how the physical

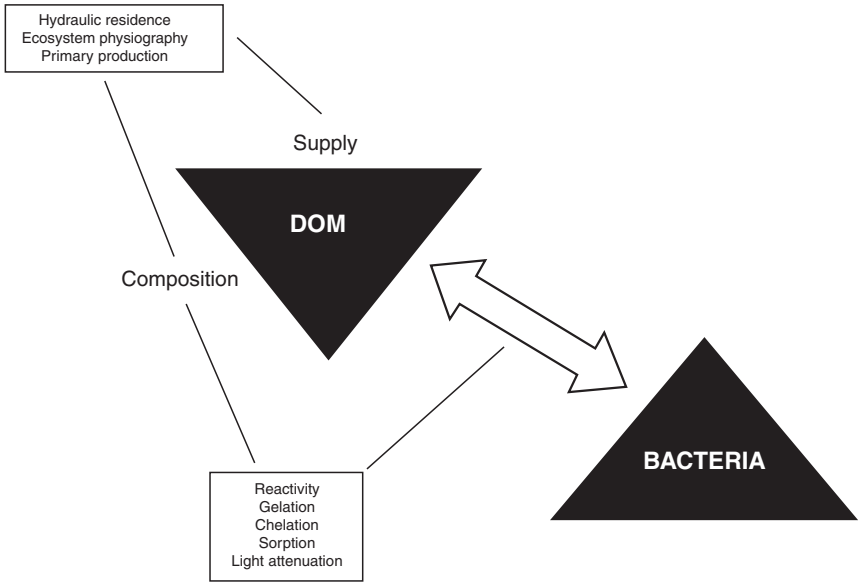

FIGURE 3 DOM–physicochemical interactions.

environment of the ecosystem or surrounding landscape affects production, fate, and transport of DOM.

A. DOM as Elements of System Structure

Small-scale interactions of DOM with the physicochemical environment affect the distribution and activity of microbial communities within systems and influence material fluxes by absorbing solar radiation (see Chapters 2 and 10), mediating the mobility of inorganic nutrients, enzymes, and other molecules (see Chapters 3, 5, 8, 11, and 19), and imposing a macromolecular architecture on the aqueous medium (see Chapters 12 and 18).

Organic molecules that contain unsaturated carbon bonds absorb solar radiation in the UV-B, UV-A, and visible ranges. In general, the more double bonds there are, the more chromatic the molecule is. Chromatic DOM can protect aquatic organisms from UV radiation, but highly colored humic molecules limit primary production by reducing the depth of the photic zone (Lean, 1998). In lentic systems, the absorption of solar radiation within the upper zone of the water column by DOM alters heat distribution, promoting thermal stratification. Reduced photic zones and enhanced stratification impact almost every aspect of the ecosystem, including primary production, nutrient availability, oxygen distribution, and trophic structure (Jones, 1998).

On a finer scale, components of DOM affect the availability and mobility of biologically significant molecules. Chelation of metals like iron, copper, and zinc helps maintain micronutrient availability within the water column (Jones, 1998; see Chapter 8); similar reactions may mitigate the toxicity of copper, aluminum, and mercury (Jones, 1998; Lean, 1998; Lyderson, 1998). Hydrophobic organic contaminants associate with naturally produced DOM. This partitioning may sequester contaminants from degradation and reduce rates of bioaccumulation (see Chapter 7). Extracellular enzymes are structurally stabilized but kinetically compromised by noncompetitive interactions with DOM (Chróst, 1990). Some of these interactions are reversible; in such cases, DOM acts as a vector that moves enzymes across systems (Wetzel, 1991). DOM also contains nitrogen and phosphorus, in both organic form and as complexes with inorganic ions (Jones, 1998; see Chapters 8 and 11). Phosphorus associated with DOM may be more mobile than inorganic P, and ratios of C : P and C : N approaching that of bacteria cells enhance bacterial growth (see Chapter 9).

Macromolecules such as exopolysaccharides and humic condensates affect the distribution of microorganisms and nutrients within the water column by adding microstructure. Many planktonic bacteria and algae secrete polysaccharides. In some circumstances, exopolysaccharide production is a substantial portion of gross production. The adaptive significance of this activity is unclear. Possibilities include the dumping of excess photosynthate,

creating a protective capsule against grazers and viruses, trapping nutrients and enzymes, and slowing sedimentation rate. Some of this exopolysaccharide is resistant to degradation because of its heterogeneous composition and may persist for years (see Chapter 1), providing nucleation sites for TEP and flocs.

The contribution of high molecular weight humic condensates to the microstructure of the water column is perhaps less known than that of exopolysaccharides. The capacity of humic material to bind ions, nutrients, and enzymes is well known (see Chapters 7 and 19), but interactions between high molecular weight complexes and virions or bacteria have received little study. Some bacteria appear to have binding affinities for these complexes, using them as nutrient sources or electron acceptors. Other bacteria are inhibited by interactions with humic substances. Given their abundance, it appears likely that humic substances play a greater role in modulating community structure and activity in inland waters than is generally appreciated. The view of humic material as "conservative" or "recalcitrant" comes largely from the narrow perspective of bacterial assimilation. In a broader biogeochemical context, it is highly reactive.

B. Reactivity

DOM participates in several reactions that occur in the extracellular environment. In contrast to intracellular reactions, environmental reactions are more or less nonspecific (excepting some enzymatic reactions), unregulated, and opportunistic. For heuristic purposes, these reactions can grouped into three categories: hydrolytic, redox, and associative.

Hydrolytic reactions are catalyzed by extracellular hydrolases and mineral surfaces (Chróst, 1990; Hoffman, 1990). For enzymatic reactions, a defined substrate or moiety must match the catalytic site of a specific enzyme. The most studied examples in aquatic systems are glycosidases, peptidases, and phosphatases (Münster and De Hann, 1998; see Chapter 13). In general, hydrolytic reactions break the relatively labile C−N and C−O bonds that link monomers, generating lower molecular weight products more suitable for microbial consumption.

Oxidation reactions are catalyzed by enzymes and mineral surfaces (particularly iron and manganese oxides) or result directly from the absorption of solar radiation (Hoffman, 1990; Miller, 1998). Much of the enzymatic activity, though nonspecific, appears directed toward the breakdown of phenolic moieties (Münster and De Haan, 1998). For such residues, a catalyst plus molecular oxygen, or a highly reactive oxygen radical is sufficient. Photolytic reactions require structures that absorb radiation in the UV range such as aromatic rings and other unsaturated carbon skeletons. The most difficult bonds to break are aliphatic C−C bonds. Generally, it takes both a catalyst and a highly reactive oxidant to break the bond. Be-

cause oxidative reactions are nonspecific, they are integral to both the creation and the degradation of humic substances, thus they can either enhance or reduce the bioavailability of DOM (see Chapter 10).

DOM can also act as an electron acceptor for biotically mediated oxidation reactions. Many active microorganisms, particularly phototrophs, produce reductants in excess of metabolic needs that must be regenerated by transfering electrons to acceptors in the environment via membrane-spanning reductases (Price and Morel, 1990). It has been discovered that some iron-reducing bacteria use humic and fulvic acids as terminal electron acceptors for their respiratory transport systems (Coates *et al*., 1998).

Associative reactions involve the non-covalent binding of DOM molecules with one another or with other components of the system. As discussed in the previous section, these reactions, affect every aspect of DOM metabolism, microbial community organization, and cross system transport.

Although rates and mechanics vary widely, abiotic hydrolytic, redox, and associative reactions occur in all systems. The diagenetic processing of DOM can be described as an interaction (or alternation) of stochastic environmental reactions with selective biotic reactions, the former continually rearranging DOM components and the latter consuming the most valuable components proffered. The result is that DOM is progressively stripped of intermediary metabolites, nitrogen, and phosphorus, leaving a low molecular weight residue of aliphatic carbon skeletons (see Chapter 5).

C. DOM Sources

At the global scale, there are clear patterns of DOM concentration and flux related to regional climate, either directly through hydrologic effects or indirectly through vegetation (see Chapters 2 and 6). At the catchment scale, the interactions of DOM with landscape elements control DOM input to aquatic ecosystems (George *et al*., 1999). Inputs of organic matter to aquatic systems are categorized as autochthonous or allochthonous. Allochthonous DOM inputs are generally easier to quantify because they are associated with hydrologic inputs. This connection ties DOM supply and its residence time within the system to watershed hydrology and climate (Curtis, 1998). Within these large scale constraints, rates of DOM delivery depend on the density of detrital carbon within the watershed and the nature of the delivery path (see Chapters 2 and 6). As a generalization, the higher the density of stored carbon and the closer the delivery path to the soil surface, where the organic horizons lie, the higher the DOM content of influent water. Systems that meet these critieria include those with extensive wetlands and those with a high water table, created either by abundant precipitation or by an impermeable soil horizon such as permafrost or clay. As water tables drop, hydrologic flow paths may intersect mineral soil horizons, which, depending on their composition, tend to act as sinks rather than sources of DOM,

resulting in selective removal of the more hydrophobic constituents of DOM (see Chapters 2 and 3).

Allochthonous DOM inputs can be highly dynamic; a large fraction of the annual flux may enter during a relatively brief time period (e.g., Books *et al.*, 1999). Much of this dynamism is controlled by large-scale physical features of ecosystems and pulsed hydrologic events such as snowmelt and storms. The ability of the aquatic microbial community to respond depends strongly on the physiological and genetic controls on metabolic capacity (see Chapter 15). This interplay of physical loading and biotic processing influences the quantity and composition of DOM export.

Virtually all watersheds of any size have been altered by human activity. The removal of vegetation, propagation of impermeable surfaces, and installation of drainage networks alters hydrologic flows and their contact with organic matter reservoirs. Development also has the effect of replacing diffuse sources of DOM with point sources. Wastewater effluents may contain substantial amounts of DOM and are often enriched in DON and DOP relative to natural influents (see Chapter 2).

Allochthonous sources account for most of the DOM moving through many ecosystems and may support most of the heterotrophic metabolism as well. However, by definition this material has experienced biogeochemical reactions, so on average is less labile than DOM newly produced within the system. The principal sources of autochthonous DOM production vary across systems: periphyton dominate in streams, macrophytes in lentic systems, and phytoplankton in the ocean. The relative importance of these autochthonous sources depends in large part on ecosystem physiography. Using literature values, Bertilsson and Jones (Chapter 1) estimated that 95% of freshwater lakes and wetlands receive >97% of their autochthonous DOM from macrophytes, because most of the surface area of these systems falls in the littoral zone. In small streams, primary production is dominated by biofilm communities where production is constrained by riparian shading and physical substrate stability. Thus, landscape elements and their arrangement, together with the physical characteristics of aquatic systems, exert strong control on the absolute magnitude of DOM supply and the relative importance of allochthonous versus autochthonous sources.

D. Source Effects on Communities

The nature and timing of DOM inputs influence both community organization and metabolism (see Chapter 15), which in turn affect DOM concentration and export at the ecosystem scale. The relationship of source heterogeneity to system function is one of the least studied aspects of DOM dynamics. For planktonic communities, source effects are direct and immediate. Attached communities also respond and adapt to local DOM inputs, but the capacity of biofilms to self-organize within a secreted polymer matrix

mitigates short-term responses to external fluctuations in DOM characteristics (see Chapter 12).

Source effects operate on many scales. Aitkenhead and McDowell (2000) reported that the magnitude of DOC export from watersheds is strongly linked to the C : N ratio of soil organic matter within the watershed. The reasons for this are not known, but a clear implication is that heterotrophic nitrogen fixation (see Chapter 11) will be greater in microbial communities downstream of high C : N watersheds. Smaller scale examples of source effects include algal blooms, seasonal litterfall, macrophyte growth and senescence, and spates (Burney, 1994).

E. The Big Picture (Fig. 4)

The interplay between the external, large-scale processes that regulate DOM supply and composition, and the internal, small-scale processes that regulate microbial community structure and metabolism is a major conceptual, measurement, and modeling challenge. DOM concentrations vary over a relatively narrow range despite the diversity of DOM sources, the multiple controls on DOM delivery to aquatic ecosystems, and the highly flexible response capacity of microbes. This convergence is surprising if the focus is on the heterogeneity that exists within individual processes, but less surprising if the focus is on the commonalities of supply and metabolism or, more holistically, on the web of interactivity that affects DOM. Both perspectives are essential: the former provides the understanding of how the world works, and the latter offers the potential for generalization and prediction.

In this regard, it is interesting to note that the systems most likely to experience highly variable source dynamics (i.e., small streams and shallow standing water systems) are those in which biofilms account for most of the microbial activity, whereas in large rivers and lakes, as well as the ocean, planktonic communities dominate metabolism. For small systems with high throughputs, biofilm organization provides a buffer for system metabolism analogous to that accorded to the planktonic communities of large systems by large mass and low ratios of surface area to water volume.

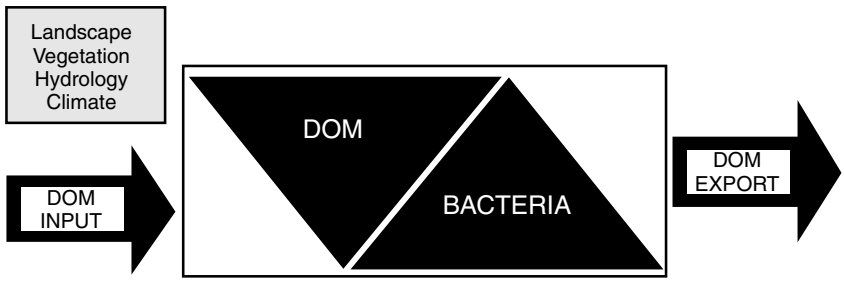

FIGURE 4 The big picture.

Microbial community organization is not the only biotic relationship that grades with system size. The metabolism of small upstream systems is dominated by allochthonous DOM that originates from terrestrial systems. With high throughputs and short hydraulic residence times, DOM dynamics often exceed microbial community response times and all but the most reactive material passes downstream unaltered. In such systems, lags in microbial response and the buffer of biofilm organization suggests that microbial activity may not be easily related to bulk DOM composition when viewed in snapshots (cf. Chapter 15). In large systems, DOM fluxes become less variable and residence time increases. The response time of heterotrophic planktonic communities may match or exceed DOM dynamics. DOM composition becomes a product of microbial activity. The result, again, is that bulk DOM composition cannot be easily related to microbial activity. As DOM moves downstream, its composition is affected by both source dynamics and microbial dynamics. Within any particular system, bulk composition will more closely reflect whichever is faster. The multitude of potential relationships between DOM quantity and composition generate a broad range of microbial metabolism and community composition over time and space (see Chapters 9, 15, and 18).

The DOM output of one system becomes an input to the next. Because the character of major inputs influences the composition and diversity of microbial communities, community activities may respond in a potentially predictable fashion along such a gradient. The complication is that downstream systems can differ in hydrologic, physical, and chemical properties, and may receive DOM inputs from varied sources. The new selective regime may obscure patterns of connectivity.

For inland waters, the role of DOM in system connectivity has become an important concept. Most of the DOM moving downstream is humic material that has a turnover time that appears to exceed the hydraulic residence time of most of the systems through which it passes. As it moves, it contributes to longitudinal integration by acting as a dynamic reservoir for carbon and other nutrients. However, the extent of this effect may be limited. On geographic scales, there is little evidence that DOM concentration increases downstream. Estimates of DOM production rates for terrestrial watersheds suggest that only a small fraction (ca. 1%) of the terrestrial DOM that reaches aquatic systems is transported to the ocean (Benner, 1998). These large-scale observations suggest that humic DOM is more dynamic than generally perceived. The pattern is consistent with a growing body of literature that details the participation of humic material in biotic, photolytic, and associative reactions. The geographic pattern of stable concentration and composition emerges from highly dynamic biogeochemical interactions that consume, transform, immobilize, and reconstitute this material as it moves oceanward.

The dynamism of these reactions may be most apparent at major system boundaries. The transport of DOM from terrestrial to freshwater systems

and from freshwater to marine systems appears to stimulate turnover. Material that was resistant to degradation in its previous environment appears more labile in the new system, apparently because the processes and players have changed. Terrestrial DOM entering aquatic ecosystems may be subject to rapid metabolism (Findlay *et al*., 1998) even if the DOM has persisted in the terrestrial ecosystem for long periods of time (Cole and Caraco, 2001). When DOM moves from subsurface to surface water systems, it becomes subject to photoreactions. Terrestrial DOM is especially reactive because of its high aromaticity (see Chapters 3, 5, and 10). The composition of heterotrophic bacterial communities changes across the boundary. These taxonomic substitutions, which extend to at least the class level, have functional correlates that are only beginning to be explored. When DOM transits from large rivers into the nearshore marine environment, it moves from a system that is predominantly heterotrophic into one that is predominantly autotrophic. In addition to high category changes in the composition of heterotrophic bacteria assemblages and increased exposure to solar radiation, autochthonous production may prime the turnover of recalcitrant molecules via cometabolism or organize consortia through production of exopolymer.

Although much remains to be learned, it is clear that our perception of the role of dissolved organic matter in aquatic ecosystems has completely inverted in 30 years. In the old paradigm, DOM was a residue of biological and geochemical activity. Similarities in concentration and composition across systems were taken as evidence of inertness. As the black boxes labeled "DOM" and "Bacteria" became increasingly resolved, the range of DOM reactivity and the role of heterotrophic bacteria in ecosystem process expanded. DOM components less susceptible to biotic degradation have the greatest diversity of interactions with abiotic processes such as adsorption and photolysis. In the new paradigm, DOM is perhaps the most interactive component of aquatic ecosystems: similarities across systems emerge from the collective interaction of myriad biogeochemical processes.

REFERENCES

Aitkenhead, J. A., and W. H. McDowell. 2000. Soil C : N ratio as a predictor of annual riverine DOC flux at local and global scales. *Global Biogeochemical Cycles* **14:**127–138.

Amon, R. M. W., and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography* **41:**41–51.

Azam, F. 1998. Microbial control of oceanic carbon flux: The plot thickens. *Science* **280:**694–696.

Benner, R. H. 1998. Cycling of dissolved organic matter in the ocean. *In* "Aquatic Humic Substances" (D. O. Hessen and L. J. Tranvik, Eds.), pp. 317–331. Springer-Verlag, Berlin.

Brooks, P. D., D. M. McKnight, and K. E. Bencala. 1999. The relationship between soil heterotrophic activity, soil dissolved organic carbon (DOC) leachate, and catchment-scale DOC export in headwater catchments. *Water Resources Research* **35:**1895–1902.

Burney, C. M. 1994. Seasonal and diel changes in particulate and and dissolved organic matter. *In* "The Biology of Particles in Aquatic Systems" (R. S. Wotton, Ed.), pp. 97–135. Lewis Publishers, Ann Arbor, MI.

Christensen, B. E., and W. G. Characklis. 1990. Physical and chemical properties of biofilms. *In* "Biofilms" (W. G. Characklis, and K. C. Marshall, Eds.), pp. 93–130. Wiley, New York.

Chróst, R. J. 1990. Microbial ectoenzymes in aquatic environments. *In* "Aquatic Microbial Ecology" (J. Overbeck, and R. J. Chróst, Eds.), pp. 47–78. Springer-Verlag, New York.

Coates, J. D., D. J. Ellis, E. L. Blunt-Harris, C. V. Gaw, E. E. Roden, and D. R. Lovely. 1998. Recovery of humic-reducing bacteria from a diversity of environments. *Applied and Environmental Microbiology* **64:**1504–1509.

Cole, J. J., and N. F. Caraco. 2001. Carbon in catchments: Connecting terrestrial carbon losses with aquatic metabolism. *Marine and Freshwater Research* **52:**101–110.

Curtis, P. J. 1998. Climatic and hydrologic control of DOM concentration and quality in lakes. *In* "Aquatic Humic Substances" (D. O. Hessen, and L. J. Tranvik, Eds.), pp. 93–106. Springer-Verlag, Berlin.

del Giorgio, P. A., and J. J. Cole. 2000. Bacterial energetics and growth efficiency. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 289–326. Wiley–Liss, New York.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. *Annual Review of Ecology and Systematics* **29:**503–541.

Decho, A. W. 1990. Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes. *Oceanography and Marine Biology Annual Review* **28:**73–153.

Ducklow, H. W. 2000. Bacterial production and biomass in the oceans. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 85–120. Wiley–Liss, New York.

Findlay, S., R. L. Sinsabaugh, D. T. Fischer, and P. Franchini. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* **1:**227–239.

Fuhrman, J. A. 2000. Impact of viruses on bacterial processes. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 327–350. Wiley–Liss, New York.

Gergel, S. E., M. G. Turner, and T. K. Kratz. 1999. Dissolved organic carbon as an indictor of the scale of watershed influence on lakes and rivers. *Ecological Applications* **9:**1377–1390.

Giovannoni, S., and M. Rappé. 2000. Evolution, diversity, and molecular ecology of marine prokarytoes. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 47–84. Wiley–Liss, New York.

Hoffman, M. R. 1990. Catalysis in aquatic environments. *In* "Aquatic Chemical Kinetics: Reaction Rates of Processes in Natural Waters" (W. Stumm, Ed.), pp. 71–112. Wiley, New York.

Jones, R. I. 1998. Phytoplankton, primary production and nutrient cycling. *In* "Aquatic Humic Substances" (D. O Hessen and L. J. Tranvik, Eds.), pp. 145–176. Springer-Verlag, Berlin.

Kirchman, D. L. 2000. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacterial. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 261–288. Wiley, New York.

Lean, D. 1998. Attenuation of solar radiation in humic waters. *In* "Aquatic Humic Substances" (D. O. Hessen, and L. J. Tranvik, Eds.), pp. 109–124. Springer-Verlag, Berlin.

Lyderson, E. 1998. Humus and acidification. *In* "Aquatic Humic Substances" (D. O. Hessen, and L. J. Tranvik, Eds.), pp. 63–92. Springer-Verlag, Berlin.

Miller, W. L. 1998. Effects of UV radiation on aquatic humus: Photochemical principles and experimental considerations. *In* "Aquatic Humic Substances" (D. O. Hessen, and L. J. Tranvik, Eds.), pp. 125–144. Springer-Verlag, Berlin.

Münster, U., and H. De Haan. 1998. The role of microbial extracellular enzymes in the transformation of dissolved organic matter in humic waters. *In* "Aquatic Humic Substances" (D. O. Hessen, and L. J. Tranvik, Eds.), pp. 199–257. Springer-Verlag, Berlin.

Nagata, T. 2000. Production mechanisms of dissolved organic matter. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 121–152. Wiley–Liss, New York.

Paerl, H. W., and J. L. Pinckney. 1996. A mini-review of microbial consortia: Their roles in aquatic production and biogeochemical cycling. *Microbial Ecology* **31:**225–247.

Price, N. M., and F. M. M. Morel. 1990. Role of extracellular enzymatic reactions in natural waters. *In* "Aquatic Chemical Kinetics: Reaction Rates of Processes in Natural Waters" (W. Stumm, Ed.), pp. 235–258. Wiley, New York.

Sinsabaugh, R. L., and C. M. Foreman. 2001. Activity profiles of bacterioplankton in a eutrophic river. *Freshwater Biology* **46:**1–12.

Strom, S. L. 2000. Bactivory: Interactions between bacteria and their grazers. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 351–386. Wiley–Liss, New York.

Thingstadt, T. F. 2000. Control of bacterial growth in idealized food webs. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 229–260. Wiley–Liss, New York.

Vogel, S. 1996. "Life in Moving Fluids," 2nd ed. Princeton University Press.

Wetzel, R. G. 1991. Extracellular enzymatic interactions in aquatic ecosystems: Storage, redistribution and interspecific communication. *In* "Microbial Enzymes in Aquatic Environments" (R. J. Chróst, Ed.), pp. 25–59. Springer-Verlag, New York.

Williams, P. J. B. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material. *In* "Microbial Ecology of the Oceans" (D. L. Kirchman, Ed.), pp. 153–200. Wiley–Liss, New York.

Wotton, R. S. 1994. Particulate and dissolved organic matter as food. *In* "The Biology of Particles in Aquatic Systems" (R. S. Wotton, Ed.), pp. 235–288. Lewis Publishers, Ann Arbor, MI.

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