THE ENERGETIC IMPORTANCE OF TERRESTRIAL CARBON IN LAKE ECOSYSTEMS

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ABSTRACT

Over the past decade, ecologists have begun to appreciate how subsidies – matter and energy transported from outside the boundaries of an ecosystem – influence population dynamics, community structure, and ecosystem processes. Terrestrial ecosystems export large quantities of dissolved organic matter (DOM) to nearby aquatic ecosystems. This DOM surpasses the amount of living biomass and thus constitutes a source of potential energy for inland waterbodies.

The energetic importance of terrestrial DOM in lakes is ultimately determined by aquatic microbial metabolism. I exposed bacteria to leachates from different soils to test the hypothesis that the source and supply of terrestrial DOM are strong determinants of aquatic microbial metabolism. Overall, metabolism increased with DOM concentrations, but productivity was differentially affected by DOM source. Phosphorus content, which may be linked to the identity of terrestrial vegetation, was indicative of DOM quality for freshwater bacteria.

Inputs of DOM to lakes are variable through time and linked to hydrological events. I addressed how variable DOM supply influences bacterial productivity (BP) using field experiments and simulation models. Time-integrated BP was 2-5X higher in pulsed versus pressed treatments even though both received the same amount of DOM. Simulation models suggest that these differences may be linked a combination of factors, including mortality and rapid uptake labile DOM.

Inputs of terrestrial DOM may modify lake ecosystem processes. CO_2 flux and BP increased linearly over an experimental DOM gradient, but were not influenced by DOM-correlated attributes (i.e., shading and nutrients). These results suggest that the carbon content of terrestrial DOM may be an important subsidy, which can influence whether recipient aquatic ecosystems function as sources or sinks of atmospheric CO_2.

Using naturally-occurring stable isotope ratios, I evaluated how terrestrial DOM influences carbon cycling in a comparative study of northeastern U.S. lakes. Epilimnetic CO_2 concentrations increased with dissolved organic carbon (DOC), indicating that terrestrial-derived DOM was assimilated by lake biota. However, isotope evidence suggests that the assimilated terrestrial-carbon was used rather inefficiently. Nevertheless, phytoplankton did not comprise the entire diet of crustacean zooplankton, suggesting that DOM may serve as an energetic subsidy for higher trophic levels in lake food webs.

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GENERAL INTRODUCTION

Ecologists have long recognized that ecosystems are spatially connected by the flux of materials and energy. Recently though, there has been growing appreciation for how nutrients, detritus, and organisms from donor habitats influence ecological attributes of recipient habitats (e.g., Polis et al. 1997; Loreau et al. 2003). Such allochthonous subsidies alter population abundances (Sabo and Power 2002; Stapp and Polis 2003), structure communities (Murakami and Nakano 2002; Takada et al. 2002), increase food chain length (Pimm and Kitching 1987), induce trophic cascades (Nakano et al. 1999; Henschel et al. 2001), and influence food web stability (Huxel and McCann 1998; Takimoto et al. 2002). Subsidies also affect key ecosystem processes such as primary productivity (Sánchez-Piñero and Polis 2000) nutrient cycling (Helfield and Naiman 2001) and whole system metabolism (Smith and Hollibaugh 1997).

One well-recognized class of subsidies is the transport of organic material from terrestrial to aquatic ecosystems. For example, many headwater streams are fueled almost entirely by inputs of terrestrial-derived *particulate* organic matter (POM). Experimental manipulations of leaf litter supply have demonstrated that these allochthonous inputs are strong drivers of species diversity, community structure, and the overall productivity of stream ecosystems (e.g., Wallace et al. 1999; Hall et al. 2000). In addition, most aquatic ecosystems, including lakes, estuaries, and coastal marine environments, receive large inputs of terrestrial-derived *dissolved* organic matter (DOM) (Goni et al. 1997; Raymond and Bauer 2001; Aitkenhead-Peterson et al. 2003). In temperate latitudes, up to 20% of terrestrial primary productivity is exported to nearby waterbodies as DOM (Neff and Asner 2001). This DOM constitutes a large carbon pool that typically exceeds the amount of living biomass in aquatic ecosystems by an order of magnitude (Wetzel 1984; Thomas 1997; Fig. 1). As such, terrestrial-derived DOM is a potentially important resource subsidy that may influence the structure and function of aquatic ecosystems.

Despite being a large allochthonous input, the energetic importance of terrestrial DOM in aquatic ecosystems remains unclear. Historically, DOM has been considered a low quality resource because consists of lignin-rich, high molecular weight humic substances, with high carbon : nutrient ratios (e.g., McKnight et al. 1997). Furthermore, it is difficult to identify the effects of terrestrial DOM as a resource subsidy because it simultaneously modifies numerous chemical and physical aspects of aquatic ecosystems, including light penetration, UV stress, pH, and metal toxicity (e.g., Williamson et al. 1999, see Fig. 2). Finally, unlike POM, most organisms in aquatic ecosystems do not readily consume DOM (but see Roditi et al. 2000). Instead, it is commonly viewed that bacteria are the major link between DOM and higher trophic levels (Pomeroy 1974; Azam et al. 1983). Therefore, a major goal of my thesis has been to gain a better understanding of factors that influence how aquatic bacteria utilize terrestrial DOM and how this assimilated material flows in lake food webs.

The energetic importance of terrestrial DOM in lakes is likely to be controlled by aquatic microbial metabolism. Two potentially important factors that may influence aquatic bacterial metabolism are the bulk concentration of terrestrial DOM and the source of terrestrial DOM. Bulk concentrations of DOM, which vary across the landscape due to a variety of watershed attributes (see Chapter 1), should affect the metabolism of carbon-limited aquatic bacteria. In addition, aquatic microbial metabolism may be affected by different sources of DOM that vary in their chemical attributes. In Chapter 1, I used laboratory experiments to examine how the source and supply of DOM influences the productivity, respiration, and growth efficiency of heterotrophic bacteria from lake ecosystems. Overall, I found that bacterial productivity (BP) and bacterial respiration (BR) increased linearly with bulk DOM concentration. However, BP was also affected by the phosphorus content of DOM, which may be determined by the type of terrestrial vegetation. These results confirm that freshwater bacteria exploit DOM as an alternate carbon resource, but also highlight that DOM may serve as an important nutrient subsidy for microbial metabolism.

The metabolic activity of aquatic bacteria may also be affected by the temporal variability of DOM supply rate. DOM accumulates in soils on land during prolonged dry periods, and is then flushed to nearby aquatic ecosystems following episodic hydrologic events (Boyer et al. 1997; Judd and Kling 2002). Although rare, these events are responsible for a majority of the DOC export from terrestrial to aquatic ecosystems (Hinton et al. 1997; Buffam et al. 2001). In Chapter 2, I used field experiments and simulations models to explore how variability in terrestrial DOM supply influences aquatic BP. Pulse additions of terrestrial DOM to field mesocosms caused large, but short-lived, peaks in BP. In addition, cumulative BP was 2–5 X higher in mesocosms receiving Pulse versus Press resource supply even though both treatments received the same total amount of DOM. These empirical results demonstrate that variability in DOM supply had a larger influence on BP than differences in DOM quality. I developed a three-compartment simulation model to identify potential mechanisms that would help explain why variability in resource supply had such a large effect on BP. Simulations captured the general behavior of BP under Pulse and Press conditions, however, I was unable to identify parameters that explained the observed differences in cumulative BP. In future studies, I plan to investigate whether the incorporation of a more realistic mortality term affects the outcome of cumulative BP.

Microbial assimilation of terrestrial DOM may be responsible for modifying the metabolism of recipient lake ecosystems. This view stems from the observation that many lakes are supersaturated with CO_2 (e.g., Cole et al. 1994), a condition that arises when community respiration exceeds local primary productivity. In Chapter 3, I tested this hypothesis using a series of mesocosm experiments in New England lakes. Prior to conducting my experiments, there were a number of studies that documented positive correlations between dissolved organic carbon (DOC) and CO_2 (Hope et al. 1996; Prairie et al. 2002; Sobek et al. 2003). However, to my knowledge, there were no experimental tests of this causal relationship. I found that peak CO_2 flux from experimental mesocosms in two lakes increased linearly over a broad DOM gradient. I conducted two additional studies to test whether DOM-correlated attributes were responsible for the observed change in metabolism along the subsidy gradient. First, although terrestrial DOM reduced light transmittance, experimental shading revealed that this was not responsible for the observed patterns of CO_2 flux. Second, organically bound nitrogen (N) and phosphorus (P) accompanied DOM inputs, but experimental nutrient additions made mesocosms net sinks, rather than sources of atmospheric CO_2. In combination with comparative lake surveys, my experimental results provide support that terrestrial-derived DOM is a resource subsidy that can determine whether lake ecosystems functions as sources or sinks of atmospheric CO_2.

Chapters 1 – 3 demonstrate that terrestrial-derived DOM can serve as a supplemental resource for heterotrophic bacteria and influence whether lakes function as sources or sinks of atmospheric CO_2. However, the degree to which terrestrial DOM subsidizes plankton food webs remains unclear. I conducted a comparative survey of 70 lakes throughout the northeastern U.S. along a gradient of terrestrial-derived DOM. I used naturally occurring stable isotopes of CO_2, particulate organic matter (POM), and crustacean zooplankton to make inferences about the efficiency of terrestrial carbon flow in lake food webs. Stable isotope ratios of CO_2 became progressively depleted in ^{13}C relative to the atmosphere, suggesting that phytoplankton rely more on heterotrophically-respired CO_2 with increasing concentrations of terrestrial DOM. The strength of this signal implies that the importance of phytoplankton production was not diminished along the DOM gradient and suggests that DOM may not be an important resource subsidy for higher trophic levels in lake food webs. However, zooplankton diets did not consist entirely of phytoplankton carbon. Instead, results suggest that POM and zooplankton were probably subsidized by terrestrial carbon in lakes with both low and high concentrations of DOC.

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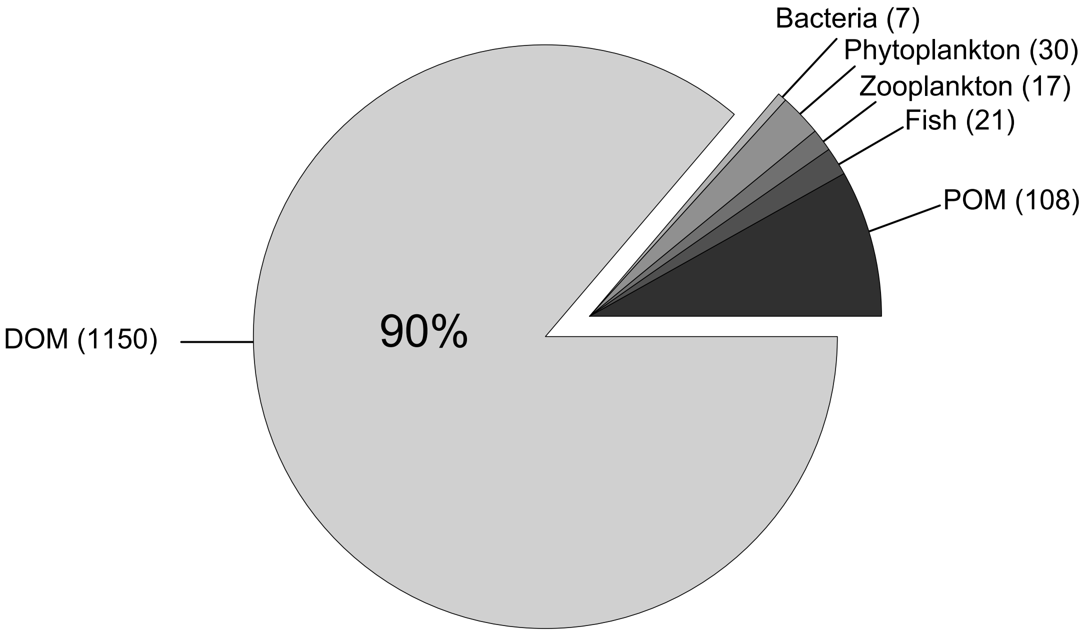
FIGURE CAPTIONS

Figure 1. Carbon standing stock in the water column of Mirror Lake, NH (Jordan and Likens 1985). Mirror Lake is an oligotrophic waterbody with relatively low DOC (225 μmol/L). Values in parentheses represent areal carbon concentrations (mM C/m^2).

Figure 2. A gradient of terrestrial-derived DOM in lake water samples. The jar on the far left represents lake water with ambient DOM concentrations (600 μmol DOC/L). The jars to the right came from experimental mesocosms that were amended with terrestrial-derived DOM (see Chapter 3). The jar on the far right has a DOC concentration of 2400 μmol/L.


Bacteria (7)
Phytoplankton (30)
Zooplankton (17)
Fish (21)
POM (108)
DOM (1150)
90%

CHAPTER 1: THE SOURCE AND SUPPLY OF TERRESTRIAL ORGANIC MATTER AFFECTS AQUATIC MICROBIAL METABOLISM

ABSTRACT

Aquatic ecosystems are connected to their surrounding watersheds through inputs of terrestrial-derived dissolved organic matter (DOM). The assimilation of this allochthonous resource by recipient bacterioplankton has consequences for food webs and the biogeochemistry of aquatic ecosystems. We used laboratory batch experiments to examine how variation in the source and supply of DOM affects the productivity, respiration, and growth efficiency of heterotrophic lake bacterioplankton. We created six different DOM leachates from soils beneath near-monotypic tree stands in a temperate deciduous-coniferous forest. We then exposed freshwater microcosms containing a natural microbial community to a supply gradient of each DOM source. Depending on the DOM source, we documented a 2–5X linear increase in bacterial productivity (BP) and a 3–10X linear increase in bacterial respiration (BR) over a 14 mg L^{-1} range in dissolved organic carbon (DOC). Bacterial metabolism was also influenced by the chemical composition of the DOM source as indicated by an exponential decline in the carbon-specific productivity with the carbon : phosphorus ratio of terrestrial leachates. Together, results from our short-term laboratory experiment confirm that terrestrial-derived DOM can be an important carbon resource for aquatic bacterioplankton. Moreover, our results suggest that dissolved organic phosphorus (DOP) content, which may be linked to the identity of terrestrial vegetation, is indicative of DOM quality and influences the productivity of freshwater bacterioplankton.

INTRODUCTION

The metabolic activities of heterotrophic bacteria have important implications for the functioning of aquatic ecosystems. From a biogeochemical perspective, bacterial respiration (BR) constitutes a major carbon flux in freshwater and marine ecosystems (Biddanda et al. 2001; Gonzalez et al. 2003). From a food web perspective, bacterial productivity (BP) converts reduced carbon into biomass that can be ingested by protists and macrozooplankton (Langenheder and Jürgens 2001; Sherr and Sherr 2002). BP and BR are metabolic activities that are fueled primarily through the consumption of dissolved organic matter (DOM). In aquatic ecosystems, DOM is supplied to bacteria both locally by primary producers (Bertilsson and Jones 2003) and externally by detrital inputs from terrestrial ecosystems (Aitkenhead-Peterson et al. 2003). Comparative studies reveal that BP is positively correlated with net primary productivity (NPP) across fresh and saltwater ecosystems (Cole et al. 1988), but is usually 70-90% less than NPP (Ducklow et al. 2002; Gaedke et al. 2002). This observation suggests that bacterial metabolism may be tightly coupled to the local production of photosynthetically-derived DOM. Bacterial carbon demand, however, often exceeds NPP. For example, BR alone is greater than NPP in most oligotrophic waterbodies (del Giorgio et al. 1997) and BP:NPP ratios can sometimes exceed unity in freshwater ecosystems (Jansson et al. 2000; Karlsson et al. 2002; Waiser and Robarts 2004). These results suggest that bacterial metabolism can be decoupled from local primary productivity. One potential explanation for this decoupling is that bacterial carbon demand may be subsidized by terrestrial-derived DOM.

Terrestrial ecosystems export large quantities of DOM to inland, estuarine, and coastal marine waterbodies (Findlay et al. 1998; Goni et al. 1997; Neff and Asner 2001). However, terrestrial-derived DOM is typically considered a poor-quality resource for aquatic bacteria because it is relatively old (Raymond and Bauer 2001) and comprises humic compounds with low nutritional content (McKnight and Aiken 1998). Nevertheless, growing evidence suggests that bacterial metabolism may be supported in part by terrestrial DOM. First, numerous laboratory studies have directly demonstrated that aquatic bacteria grow on terrestrial fractions of DOM (Bano et al. 1997; Moran and Hodson 1994; Tranvik 1988). Second, community respiration tends to exceed local primary productivity in lakes as with increasing concentrations of terrestrial DOM (Hanson et al. 2003), suggesting that allochthonous carbon may be consumed by heterotrophic bacteria. Third, experimental stable isotope enrichment of Wisconsin lakes revealed that 35-70% of the bacterial biomass was derived from terrestrial DOM (Kritzberg et al. 2004). Despite these studies, however, the relationship between aquatic bacterial metabolism and terrestrial DOM inputs remains unclear (Findlay 2003).

Two potentially important factors that may influence aquatic bacterial metabolism are the bulk concentration of terrestrial DOM and the source of terrestrial DOM. In freshwater ecosystems, bulk DOM concentrations range over two orders of magnitude (Mulholland 2003) and can be explained in part by watershed attributes including wetland cover (Engstrom 1987; Gergel et al. 1999), soil properties (McClain et al. 1997; Nelson et al. 1993), drainage area (Rasmussen et al. 1989), forest cover (Goodale et al. 2000, Canham et al. *in press*), and hydrology (Hinton et al. 1997; Boyer et al. 2000) . Given the high demand for organic carbon by heterotrophic bacteria (Kirchman and Rich

1997), BP and BR may be expected to increase along DOM concentration gradients. However, bacterial metabolism is also affected by the chemical attributes of different DOM sources (Vallino et al. 1996, Sun et al. 1997). Variation in the chemical attributes of aquatic DOM may be influenced by parent vegetation within the watershed (Grieve and Marsden 2001). The chemistry of plant material differs among taxonomic groups (Likens and Bormann 1970) and is reflected in the surrounding litter and soil organic matter (Preston and Trofymow 2000; Finzi et al. 2001; Quideau et al. 2001). Furthermore, the chemical composition of soil DOM is often similar to the total soil organic matter (Strobel et al. 2001) because the solid and soluble soil organic matter phases tend to be in equilibrium with one another (Zsolnay 1996). Soil microbes may modify chemical attributes of DOM (Marschner and Kalbitz 2003; Smolander and Kitunen 2002), but it is still this material that is exported to nearby aquatic ecosystems (Neff and Asner 2001).

This study examined how aquatic bacteria respond to variation in the source and supply of terrestrial DOM. First, we created different DOM sources from soils under different types of terrestrial vegetation. Then, using batch cultures, we measured BP, BR, and bacterial growth efficiency (BGE) of a lake microbial community on varying concentrations (i.e., supply) of the different DOM sources. We predicted that BP, BR, and BGE would increase with DOM concentration. However, we predicted that the relationship between bacterial metabolism and DOM concentration would be modified by the chemical attributes of the different DOM sources. We used a combination of univariate and multivariate statistical approaches to help identify DOM chemical attributes that had a strong influence on aquatic microbial metabolism.

METHODS

Soil Collection and DOM preparation— We created six different DOM leachates from the organic (Oa/A) soil horizons underneath near-monoculture stands of some of the most common trees in New England forests: pine (*Pinus strobus*), hemlock (*Tsuga canadensis*), beech (*Fagus grandifolia*), maple (*Acer saccharum*), birch (*Betula alleghaniensis* and *B. populifolia*), and oak (*Quercus rubra* and *Q. velutina*). We collected soils from a total of six different sites all located within 100 km of Hanover, NH, USA. In addition, all of the collection sites were ≤ 200 m from a stream or lake. The soils represented a mixture of Inceptisols and Entisols, and based on texture were classified as fine sandy loams and sandy loams.

To create each DOM leachate, we dried the soils at 60°C, pooled equal amounts of soil (100 g total) from multiple sites, and then extracted the organic matter for 48 h in 0.1N NaOH (Schnitzer 1982). We removed particulate material (> 0.7 μm) via serial filtration and then dialyzed (500 D cellulose ester, Spectrum Labs) the leachates in distilled water buffers for 24 h to reduce concentrations of salts and inorganic nutrients (Vinebrooke and Leavitt 1998, Lennon 2004). We bottled the dialyzed leachates and had them gamma-irradiated (25 kGy dose, SteriGenics International, Westerville, OH, USA) in order to kill soil-associated microbes while still maintaining the chemical integrity of the organic matter (McNamara et al. 2003).

Leachate Characterization— We analyzed each DOM leachate for a suite of inorganic and organic attributes. We measured DOC with a Tekmar-Dohrmann TIC/TOC analyzer after H_2SO_4 digestion. We measured total nitrogen (TN) and total phosphorus (TP) spectrophotometrically after persulfate digestion (APHA 1998). In addition, we measured 15 major elements with inductively coupled plasma (ICP) atomic emission spectroscopy (AES) on a Spectro Analytical model FTM-08 ICPOES (see Table 1). We measured polyphenolic compounds using the Prussian Blue assay (Price and Bulter 1977) against a tannic acid standard (Herrera-Silveira and Ramirez-Ramirez 1996; Thoss et al. 2002). We measured humic acids as the amount of precipitated DOC after acidifying the leachate to pH 2 with H_3PO_4. We measured protein content using the BCA method Walker 1996 with a Compat-Able Protein Assay Preparation kit (Pierce Biotechnology, Rockford, IL). We measured high molecular weight DOM (HMWDOM) as the amount of DOC retained in 12000–14000 D dialysis tubing after 24 hours of dialysis.

We tested for differences in chemical attributes among DOM sources using both univariate and multivariate statistics. For individual chemical attributes, we used univariate one-way ANOVA with Tukey's HSD tests. We adjusted the α value from 0.05 to 0.0028 with a Bonferroni correction to account for multiple comparisons. We also used principal components analysis (PCA) to describe the chemical characteristics of the DOM sources using 16 of the 18 chemical attributes (magnesium and manganese were excluded because of incomplete data). We used a correlation matrix of untransformed mean values in the PCA analysis because it standardizes data and thus does not overemphasize large values (Manly 1994).

Experimental design— We used batch cultures to quantify BP, BR, and BGE on different sources and supplies of DOM. Each experimental unit consisted of a 1 L polycarbonate bottle filled with 450 mL of 0.22 μm-filtered lake water. We then inoculated each experimental unit with a 50 mL aliquot of 2.7 μm-filtered (Whatman GF/D) lake water containing a natural microbial assemblage. We obtained lake water and the microbial community from Norford Lake (Orange Co., VT, USA), which is an oligo-mesotrophic water body (chlorophyll *a* = 2.8-5.1 μg L^{-1}) with relatively low DOC (2.4-3.3 mg L^{-1}) (Lennon, unpublished data). We manipulated DOM supply by exposing 11 experimental units to a concentration gradient ranging from 3-17 mg L^{-1} DOC. Using an unreplicated regression design, we then crossed the DOM supply manipulation with six different DOM sources (pine, hemlock, beech, maple, birch and oak). We incubated all 66 experimental units in environmental chambers at 20°C without light. We estimated BP 36 hrs after the initiation of the experiment by measuring the uptake and incorporation of ^{3}H-leucine into bacterial protein (Kirchman 1993). We estimated BR as the change in dissolved oxygen concentrations between 24 and 48 h using spectrophotometric methods with corrections for color interference (Roland and Cole 1999). We used a respiratory quotient of one to convert oxygen consumption to carbon evolution. We calculated BGE as (BP) / (BP + BR).

We used multiple linear regression with indicator variables to evaluate the response of BP, BR, and BGE to different concentrations and sources of DOM. We used DOM concentration (measured as DOC) as a continuous predictor and DOM source as a qualitative predictor of bacterial metabolism (see indicator variables in Neter et al. [1996], pp 455-490). We examined residuals for assumptions of normality and homogeneity of variance, and used diagnostic tests (Studentized residuals, leverage, Cook's D, and Dffits) to identify potential outliers. We then calculated confidence limits

(*CL*) around the differences between the slopes and intercepts for each possible pairing of DOM sources to determine whether DOM source affected bacterial metabolism.

$$CL = (b_i - b_j) \pm \left(t_{\alpha[n-1]} \cdot \sqrt{\sigma^2{}_{b_i} + \sigma^2{}_{b_j} - 2\ (\sigma_{b_i\ b_j})} \right) \qquad \text{(eq. 1)}$$

where b_i and b_j are parameter estimates for two of the six DOM sources, t is the critical value from the Student's t-distribution for a given α and sample size (n) used to create the parameters, $\sigma^2{}_{b_i}$ and $\sigma^2{}_{b_i}$ are the variances for each parameter estimate, and $\sigma_{b_i\ b_j}$ is the covariance between the two parameter estimates (Neter et al. 1996). We adjusted α with a Bonferroni correction to account for the 15 pairwise comparisons determine whether different DOM sources affected the parameters of each metabolic response variable. We concluded that bacterial metabolism was not affected by DOM sources if adjusted confidence limits (99%) around the difference of parameter estimates ($b_i - b_j$) contained zero.

We defined the slopes of BP and BR vs. DOC as rates of carbon-specific metabolism, then attempted to explain variance in carbon-specific metabolism as a function of DOM chemistry. First, we used the output from the PCA of the DOM chemistry to explain carbon-specific metabolism. We used eigenvectors from the first three PC axes as individual predictors of the BP- and BR-DOC slopes. Second, we used individual chemical properties as predictors of carbon-specific metabolism. We narrowed down the number of individual predictors by constructing a correlation matrix comparing all of the chemical attributes to the BP- and BR-DOC slopes. We considered all individual chemical variables that had high correlation coefficients (> |0.7|, see Fowler

1998) with BP- and BR-DOC slopes as potential predictors of carbon-specific metabolism. We used curve fitting (SigmaPlot v. 8.0, 2002) to describe the relationships between carbon-specific metabolism and the selected group of chemical predictor variables. We used SAS (SAS 1999) for all other statistical procedures.

RESULTS

***Chemical composition of DOM*—**The DOM sources were chemically different from one another. All chemical attributes were different at $\alpha = 0.05$ (one-way ANOVA). However, cadmium, HMWDOM, and TN were not significantly different between DOM sources after applying a Bonferroni correction for multiple comparisons (Table 1). The PCA also indicated that the six DOM sources had different chemical compositions. The first three PC axes explained 86% of the chemical variation in the different DOM sources (Table 2, Fig. 1). Hemlock DOM was positively correlated with PCA axis 1 (iron and lead), while maple DOM was negatively correlated with PCA axis 1 (potassium and TN). Oak DOM was positively correlated with PCA axis 2 (HMWDOM and sodium), while birch and maple DOM were negatively correlated with PCA axis 2 (cadmium and zinc). Finally, beech DOM was negatively correlated with PCA axis 3 (TP).

***Bacterial productivity*—**Bacterial productivity was affected by both DOC concentration and the different DOM sources. The indicator variables multiple regression model explained 88% of the variation in BP ($F_{11, 71} = 37.8$, $P < 0.0001$). Carbon-specific productivity was approximately 3-fold higher on beech and oak DOM sources than on maple and pine DOM sources (Table 3). Carbon-specific productivity was intermediate on hemlock and birch DOM sources and could not be statistically distinguished from carbon-specific productivity on the other DOM sources (Table 3).

We removed one BP data point from the multiple regression model. Bacterial productivity in the beech treatment measured 17.8 $\mu g \bullet L^{-1} \bullet h^{-1}$ at 15 mg L^{-1} DOC, creating a humped-shape relationship (see Fig. 2). Diagnostic checks from the multiple regression (Studentized residuals, leverage, Cook's D, and Dffits) indicated that this data point weighed heavily in the analysis. When this point was included, however, we could fit the data well for this leachate by including a polynomial term in a regression analysis:

$$BP_{(beech)} = -3.7 + 3.9\,(DOC) - 0.16\,(DOC)^2,\ R^2 = 0.93,\ P < 0.0001 \qquad \text{(eq. 2)}$$

Eigenvectors from the PCA were poor predictors of carbon specific productivity ($P > 0.05$). Instead, our correlation analyses identified three chemical attributes as potential predictors of carbon-specific productivity: TP ($r = 0.90$), HMWDOM ($r = 0.73$), and potassium ($r = 0.72$). We did not identify models where HMWDOM or potassium was a significant predictor of carbon-specific productivity ($P > 0.05$), but carbon-specific productivity was explained by phosphorus content. We represent this relationship as an exponential function of the carbon : phosphorus ratio (C:P) (see Fig. 3):

$$\text{Carbon-specific productivity} = 0.6 + 3599\, e^{(-0.15\, *\, C:P)},\ R^2 = 0.99,\ P < 0.0003 \qquad \text{(eq. 3)}$$

Phosphorus content was not significantly correlated with any of the other chemical attributes measured on the six DOM sources ($P > 0.05$).

Bacterial respiration**—**Bacterial respiration increased seven-fold over the DOC concentration gradient, but was not affected by DOM source (Fig. 2, Table 3). Multiple regression using DOC concentration and DOM source as predictors explained 67% percent of the variation in BR ($F_{11, 70}$ =10.9, $P < 0.0001$). We did not attempt to use DOM chemical attributes to explain variance in carbon-specific respiration because there were no differences among the different DOM sources (Table 3).

Bacterial growth efficiency**—**BP and BR were significantly correlated with one another in all treatments except maple DOM (Table 4). Therefore, BGE was not affected by DOM concentration or DOM source ($R^2 = 0.23$, $F_{11, 63}$ =1.4, $P = 0.20$). BGE averaged 0.54 ± 0.022 (SE) for all samples.

DISCUSSION

Terrestrial-derived dissolved organic matter (DOM) can serve as carbon and nutrient subsidies for bacterioplankton in both freshwater and marine ecosystems. The consumption of this allochthonous resource should influence bacterial metabolism and may in turn have consequences for food webs and the biogeochemistry of aquatic ecosystems. This study examined how variation in the source and supply of DOM affects the metabolism of lake bacterioplankton. Both bacterial productivity (BP) and bacterial respiration (BR) increased with the supply of terrestrial DOM, but only BP was affected by the DOM source. Our results suggest that phosphorus content, which may be linked to the identity of terrestrial vegetation, is a chemical attribute that is indicative of DOM quality influencing the productivity of freshwater bacterioplankton.

Bacterial metabolism on different DOM supply— We predicted that BP and BR would generally increase with additions of DOM because heterotrophic bacterioplankton are often carbon limited (Kirchman and Rich 1997). However, we did not assume that metabolic rates would be constant along the DOM gradient. For example, radiotracer studies (i.e., ^{14}C-labeled glucose, leucine, acetate) commonly indicate that that carbon uptake rates of microbial communities saturate at high substrate concentrations according to Michaelis-Menten dynamics (Wright and Hobbie 1965, Garnier and Benest 1991, Münster 1993). Such kinetic constraints could affect the functional response of bacteria to DOM concentrations. In addition, the functional relationship between metabolism and DOM could be shaped by the relative concentrations of co-limiting nutrients (e.g., nitrogen, phosphorus, etc.). However, we found little evidence that bacterial metabolism saturated with increasing DOM concentration. Instead, depending on the DOM source, there was a 2–5X linear increase in BP and a 3–10X linear increase in BR along the experimental DOM concentration gradient (Table 3, Fig. 2).

Only in the beech treatment did it appear that metabolic rates might have changed along the DOM concentration gradient (Fig. 2). At the highest DOM concentration, BP in the beech treatment was 63% lower than predicted based on the linear equation in Table 3. The inclusion of the outlier data point resulted in a hump-shaped relationship so that BP actually decreased at the highest DOM concentration instead of saturating at a maximum level of productivity. One potential explanation for this hump-shaped pattern is that bacteria depleted a majority of the labile substrate over the incubation period, resulting in low rates of BP by the end of the experiment. This hypothesis seems plausible given that carbon-specific productivity was highest for bacteria in the beech DOM treatment (see Table 3 and ***Bacterial metabolism on different DOM sources*** below).

The positive relationship between bacterial metabolism and DOM concentration in our study is consistent with some trends found in freshwater and marine ecosystems. For example, both BP and BR of coastal microbial communities increased nearly linearly when exposed to three levels of high molecular weight carbon (>1 kDa) ranging from 1.3 – 3.6 mg DOC L^{-1} (Amon and Benner 1996). Similarly, BR in the Bay of Biscay increased over a relatively narrow DOC gradient (0.9 – 1.3 mg L^{-1}) that was associated with a coastal upwelling event (Gonzalez et al. 2003). In lake communities, bacterial biomass production increased with experimental additions of aged humic material (Eiler et al. 2003). Finally, cross-system analyses of batch culture experiments have shown that the proportion of labile DOC (Søndergaard and Middelboe 1995) and rate of DOC consumption (del Giorgio and Davis 2003) increase with bulk DOC content.

However, a considerable number of studies have failed to detect any pattern between bacterioplankton metabolism and bulk DOM concentration. For example, there was no relationship between either bacterial growth or BP along a DOC gradient (2.5–9.1 mg L^{-1}) in 20 Quebec lakes (Smith and Prairie 2004). BP was likewise unaffected by DOC concentrations in tropical coastal lagoons (Farjalla et al. 2002). Finally, 10 years of data have revealed that there is no relationship between BP and DOC (1.5–7 mg L^{-1}) on tidal freshwater stretches of the Hudson River (Findlay 2003). The null relationships found in this latter group of studies suggest that factors besides bulk DOM content, such as DOM chemistry, may be important in driving the metabolism of heterotrophic bacterioplankton.

Contrary to our predictions, BGE did not increase over our DOM concentration gradient. Under low DOM supplies, we anticipated that most assimilated carbon would be devoted to maintenance costs resulting in relatively low BGE (Russell and Cook 1995). Occasionally, BGE does not increase with substrate concentration because resources are not always energy-rich enough for bacteria to reduce all of the available organic matter into cellular carbon (del Giorgio and Cole 1998). However, this explanation cannot account for the lack of pattern between BGE and DOM since BP always responded positively to increased DOM concentrations (Fig. 2, Table 4). Instead, the lack of BGE response in this study most likely reflects our experimental approach. Bacterial growth can often be unconstrained on a given resource in batch cultures of relatively short duration. Under such conditions, BP and BR will often be correlated (e.g., Table 4) leading to a relatively constant BGE. In contrast, bacterial growth is often constrained by resource availability in nature and increased maintenance costs can generate variance in BGE (del Giorgio and Cole 1998). Nevertheless, our results are consistent with the empirical trend that BGE plateaus at approximately 0.5 when BP is > 5 $\mu g\ C \cdot L^{-1} \cdot d^{-1}$ (del Giorgio and Cole 1998).

Chemical attributes of different DOM sources— Most of the DOM in freshwater ecosystems is derived from DOM in the soils of terrestrial ecosystems (Thurman 1985). The chemical attributes of soil DOM are affected by a number of factors including soil properties, sorption, biodegradation, parent material, and terrestrial vegetation (Qualls and Haines 1992; Chantigny 2003). Terrestrial vegetation can have a large influence on the chemistry of soil DOM via litterfall, root exudates, and local microbial interactions (Kalbitz et al. 2000). Based on this underlying assumption, we made different DOM sources by leaching organic material from soils beneath relatively monotypic stands of the most common tree taxa in New England forests.

Our leaching approach created DOM sources with different chemical characteristics (Table 1, 2, Fig. 1). It is important to note however, that it was not our objective to test hypotheses about how the identity of terrestrial vegetation influences DOM chemistry. Nevertheless, attributes of our DOM sources are consistent with some vegetation-soil chemistry patterns reported in the literature. For example, our hemlock leachate had high concentrations of aluminum, iron, and lead (Table 1; Figure 1), consistent with soils in coniferous forests (David and Driscoll 1984; LaZerte and Scott 1996) and likely reflecting the fact that metals are mobilized by the production of organic acids in hemlock stands (Dijkstra et al. 2001). Conifers also have low foliar nitrogen relative to most deciduous species and thus produce litter with high C:N ratios (Templer et al. 2003). This pattern was reflected in our hemlock leachate (lowest nitrogen concentration), but not in the pine leachate (intermediate nitrogen concentration). Finally, our maple DOM source had relatively high concentrations of nitrogen, calcium, and magnesium, but low concentrations of aluminum, consistent with the chemical attributes commonly associated with soils in sugar maple stands (Minocha et al. 2000; Dijkstra and Smits 2002; Lovett and Mitchell 2004). Despite similarities between our DOM chemistry and the soil chemistry patterns in the literature, we must be cautious about using our data to link specific forest compositions to aquatic microbial metabolism. In addition to the short duration of our small-scale laboratory study, our approach does not address whether the chemical signal of different DOM sources would be preserved along land-water flowpaths of DOM (Sommer et al. 1997).

Bacterial metabolism on different DOM sources**—** Variation in the chemical composition of DOM can modify aquatic microbial metabolism. For example, rates of bacterial metabolism are affected by various DOM properties including oxidation state (Vallino et al. 1996), molecular weight (Weiss and Simon 1999), humic content (Moran and Hodson 1990), percentage of aliphatic compounds (Sun et al. 1997), and nutrient content (Hunt et al. 2000, Castillo et al. 2003). Our results suggest that the phosphorus content of DOM is an important predictor of carbon-specific productivity. We observed a 3X exponential decline in carbon-specific productivity with phosphorus content expressed as C:P (Fig. 3). Since we did not manipulate phosphorus directly, it is possible that this trend represents a correlation with other unmeasured attributes of our DOM sources. However, multiple lines of evidence suggest that phosphorus content should be an important attribute influencing DOM quality for aquatic bacteria.

Phosphorus often limits or co-limits bacterial metabolism in freshwater (Currie and Kalff 1984; Drakare 2002; Pace and Cole 1996) and marine ecosystems (Rivkin and Anderson 1997; Sala et al. 2002). The high phosphorus demand of aquatic bacteria is reflected by their low biomass C:P ratios (14-70 by mass; Sala et al. 2002; Vrede et al. 2002). In contrast, water column ratios of DOC : dissolved organic phosphorus (DOP) are typically much higher (16–300 by mass; Hopkinson et al. 1997; McKnight et al.

1997). The C:P ratios of the DOM used in this study (53–106 by mass) overlapped with literature-reported C:P ratios for bacterial biomass. As a result, carbon-specific productivity was relatively high on DOM sources with low C:P ratios. However, carbon-specific productivity dropped when substrate C:P ratios moved outside the reported stoichiometric range required for optimum bacterial biomass production (14–70 by mass; Fig. 3). Together, this information suggests that phosphorus limitation of bacterial biomass production can be alleviated by variation in DOM sources. Higher phosphorus concentrations may allow bacteria to consume relatively recalcitrant carbon molecules (Benner et al. 1988), possibly through increased extracellular enzyme production (Sinsabaugh et al. 1997).

In contrast with BP, we found no effect of different DOM sources on carbon-specific respiration. There are two potential explanations for this lack of pattern. First, we may not have been able to detect an underlying effect of different DOM sources due to inherent variability associated with BR measurements. For example, the coefficient of variation (CV) for replicate BP measurements on an experimental unit is only 3% whereas the CV for replicate BR measurements on an experimental unit is 35% (data not shown). This variability should not influence our parameter estimates (Miller 1986), but would increase the confidence interval around the parameter estimates and thus reduce our ability to detect true effects of different DOM sources on BR. Second, overall BR might not be affected by different DOM sources due to slight decoupling of catabolic and anabolic respiratory reactions (del Giorgio and Cole 1998). BP and BR were not perfectly correlated (Table 4) and thus anabolic respiratory costs could be masked by the independent effect of different DOM sources on catabolic respiratory costs.

In contrast with some studies, we found that BGE was not affected by DOM with varying chemical attributes. For example, BGE can be influenced by resource characteristics such as mineral nutrient content (Benner et al. 1988), molecular weight (Tranvik 1990), amino acid content (Carlson and Ducklow 1996), and carbon : nutrient ratios (Goldman and Dennett 2000). However, these patterns are not always consistent between studies (del Giorgio and Cole 1998). Often, BGE is correlated with factors that drive BP. For example, BP was more sensitive to environmental conditions than BR in the Hudson River, and thus was largely responsible for observed changes in BGE (Roland and Cole 1999). However, the magnitudes of response for BP and BR to DOM manipulations in this study were roughly equal (Table 4). As such, BGE remained relatively constant despite substantial variation in key chemical attributes of DOM that sometimes affect bacterial metabolism.

Conclusions— We used laboratory regrowth experiments to explore how aquatic bacterial metabolism was affected by different concentrations and sources of terrestrial-derived DOM. Terrestrial DOM is often the largest pool of carbon in lake ecosystems and therefore is a large source of potential energy for bacteria. However, terrestrial DOM is often perceived as a low-quality substrate because it is often old (Raymond and Bauer 2001) and comprised of high molecular weight aromatic compounds (McKnight and Aiken 1998). Our results challenge the view that terrestrial DOM is a low quality substrate. BP and BR increased linearly over broad DOM concentration gradients. Furthermore, bacterial metabolism was affected by variation in the chemical attributes of terrestrial DOM. Specifically, our results suggest that inputs of certain DOM may alleviate phosphorus-limited biomass production. DOM sources made from beech, oak, and hemlock soils had elevated concentrations of phosphorus that resulted in high rates of carbon-specific production. However, data linking vegetation type and phosphorus content of soil DOM are limited (Chantigny 2003). More detailed studies are needed before explicit links can be made between terrestrial vegetation, DOM chemistry, and aquatic microbial metabolism. Field surveys of aquatic bacterial metabolism in watersheds with contrasting forest composition would be one approach to test the results from this laboratory study.

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Table 1. Chemical attributes of six different DOM sources used in the bacterioplankton regrowth experiments. Values represent the mean (± SE) of replicate samples from each DOM stock solution. All concentrations are in μg/mg carbon. We used univariate one-way ANOVA with Tukey's HSD to test for chemical differences between DOM sources. We adjusted the α value from 0.05 to 0.0028 with a Bonferrnoni correction to account for the 18 multiple comparisons. Shared letters within a row indicate DOM sources which similar chemical attributes. † = *P*-values that would be significant at an α of 0.05, but were not considered significant after Bonferroni correction. * = concentrations outside the range of our standard curve.

Element or Compound	Beech	Oak	Hemlock	Birch	Maple	Pine	ANOVA Results		
							df	F	*P*
Aluminum	8.6 (0.19) c	15.8 (0.91) b	40.9 (2.27) a	15.5 (0.70) b	2.6 (0.09) d	12.0 (0.31) bc	5, 17	160.4	<0.0001
Cadmium	0.004 (0.0008) ab	0.003 (0.0010) b	0.004 (0.0007) ab	0.005 (0.0013) ab	0.008 (0.0007) a	0.004 (0.0007) ab	5, 17	3.39	0.0386 †
Calcium	31.4 (1.57) c	47.1 (3.01) b	32.3 (2.75) c	24.9 (2.26) c	121 (4.7) a	21.6 (2.07) c	5, 26	152.2	<0.0001
Chromium	0.03 (0.004) b	0.03 (0.005) b	0.076 (0.0036) a	0.06 (0.008) a	0.004 (0.001) c	0.03 (0.001) b	5, 17	46.0	<0.0001
Copper	0.14 (0.004) ab	0.20 (0.007) a	0.16 (0.002) ab	0.08 (0.003) bc	0.19 (0.007) a	0.22 (0.043) a	5, 17	7.11	0.0026
HMWDOM	849 (15.3) a	873 (45.8) a	864 (62.2) a	680 (8.2) a	756 (33.6) a	779 (24.9) a	5, 16	3.23	0.049 †
Humic Acid	292 (7.4) c	366 (6.1) ab	397 (2.8) a	382 (7.3) ab	334 (13.0) bc	368 (19.0) ab	5, 17	12.6	0.0002
Iron	21.5 (0.33) c	17.7 (0.28) d	41.4 (0.023) a	37.7 (0.10) b	6.34 (0.141) f	15.6 (0.20) e	5, 19	2045	<0.0001
Lead	0.25 (0.012) b	0.06 (0.002) d	0.35 (0.005) a	0.33 (0.017) a	0.07 (0.012) d	0.19 (0.011) c	5, 17	127.4	<0.0001

Element or Compound	Beech	Oak	Hemlock	Birch	Maple	Pine	ANOVA Results		
							df	F	*P*
Magnesium	0.38 (0.013) d	2.6 (0.14) b	*	*	6.8 (0.16) a	2.1 (0.06) c	3, 11	594.5	<0.0001
Manganese	3.1 (0.10) b	4.1 (0.12) a	2.4 (0.04) c	3.9 (0.10) a	*	2.0 (0.09) c	4, 14	95.2	<0.0001
Nitrogen, Total	92.0 (1.27) a	107.4 (5.7) a	81.7 (3.35) b	90.4 (3.06) a	96.5 (5.26) a	92.6 (1.59) a	5, 23	6.88	0.0112 †
Phosphorus, Total	18.8 (0.29) a	18.3 (0.30) ab	17.2 (0.33) b	14.7 (0.44) c	9.4 (0.27) e	10.8 (0.22) d	5, 17	276.9	<0.0001
Polyphenol	361 (4.7) ab	309 (3.2) d	364 (10.1) ab	378 (2.5) a	348 (4.7) bc	347 (13.3) bc	5, 17	29.3	<0.0001
Potassium	10.4 (0.30) b	12.9 (0.02) a	8.2 (0.08) c	6.4 (0.21) d	8.7 (0.14) c	8.4 (0.16) c	5, 17	162.2	<0.0001
Protein	103 (3.8) d	149 (4.5) a	109 (2.7) cd	114 (1.6) bcd	121 (2.1) bc	127 (4.2) b	5, 17	24.4	<0.0001
Sodium	322 (3.7) c	350 (4.0) b	444 (6.0) a	187 (2.5) f	204 (2.5) e	243 (2.8) d	5, 25	827.1	<0.0001
Zinc	0.38 (0.004) b	0.23 (0.009) d	0.24 (0.006) d	0.34 (0.005) c	0.33 (0.008) c	0.42 (0.006) a	5, 17	135.3	<0.0001

Table 2. Weightings for the first three principal components generated from a matrix of 16 chemical attributes for six different DOM sources. Chemistry data represent untransformed concentrations which were scaled between leachates to µg (mg DOC)$^{-1}$. HMWDOM = high molecular weight dissolved organic matter.

Chemical attributes	**PCA 1**	**PCA 2**	**PCA 3**
Potassium	-0.381	0.351	-0.177
Total nitrogen	-0.303	0.095	0.210
Copper	-0.265	0.163	0.054
Protein	-0.259	0.200	0.338
Calcium	-0.247	-0.137	0.068
Cadmium	-0.102	-0.356	0.133
Zinc	-0.044	-0.311	-0.271
HMWDOM	-0.024	0.420	-0.223
Sodium	0.143	0.400	-0.088
Humic acids	0.161	0.085	0.572
Total phosphorus	0.179	0.221	-0.448
Aluminum	0.287	0.244	0.209
Polyphenolics	0.290	-0.297	-0.109
Chromium	0.352	0.095	0.229
Iron	0.369	0.069	0.128
Lead	0.381	-0.070	-0.042
Percent variance explained	42	29	15

Table 3. Parameter estimates for bacterial productivity and bacterial respiration (mean ± SE) along a DOC (mg L^{-1}) concentration gradient for six different DOM sources. Shared letters in the asterisk (*) column identify parameters that are not statistically different from one another as determined by pairwise comparisons with 99% confidence limits. In the text, we refer to the slopes as the rates of carbon-

DOM source	**Bacterial Productivity**						**Bacterial Respiration**					
	Intercept			Slope			Intercept			Slope		
	mean	SE	*	mean	SE	*	mean	SE	*	mean	SE	*
Beech	2.0	(1.63)	ab	1.8	(0.24)	a	-3.5	(4.30)	a	2.2	(0.55)	a
Oak	-0.1	(1.46)	a	1.6	(0.20)	ab	-2.8	(4.39)	a	1.8	(0.60)	a
Hemlock	5.9	(1.45)	b	1.1	(0.19)	abc	1.9	(4.37)	a	1.9	(0.59)	a
Birch	3.6	(0.98)	ab	0.8	(0.12)	bc	-2.4	(2.97)	a	1.5	(0.36)	a
Maple	3.3	(1.53)	ab	0.6	(0.22)	c	4.0	(4.61)	a	1.0	(0.67)	a
Pine	5.0	(0.98)	b	0.6	(0.17)	c	-1.5	(4.25)	a	1.8	(0.53)	a

Table 4. Correlation between bacterial respiration (BR) and bacterial productivity (BP) for six different sources of terrestrial-derived DOM.

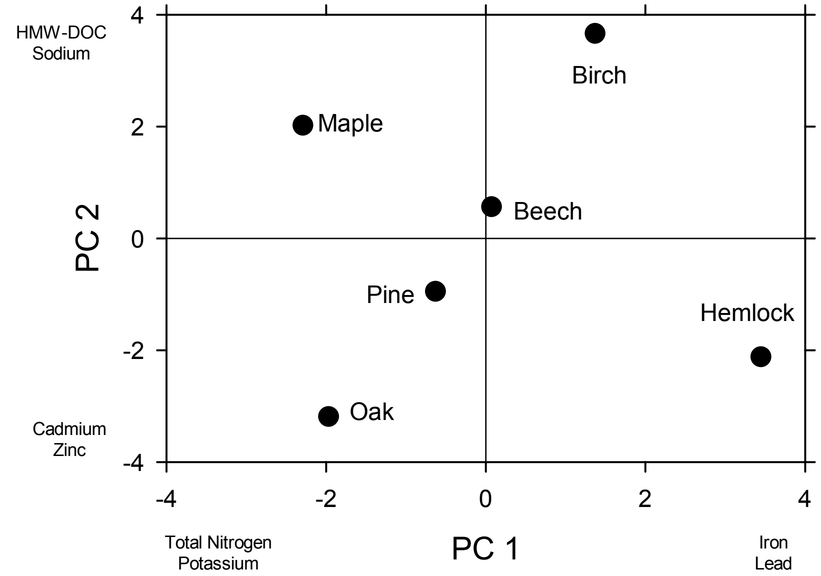
DOM Source	**Correlation Coefficient (*r*)**	***P*-value**
Pine	0.62	0.0404
Birch	0.80	0.0032
Hemlock	0.68	0.0203
Beech	0.90	0.0004
Oak	0.78	0.0045
Maple	0.07	0.8473

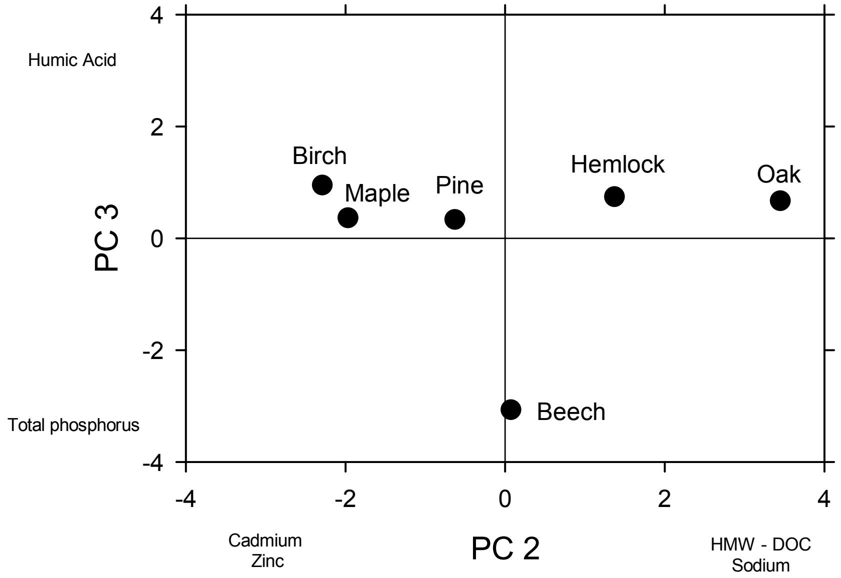
FIGURE CAPTIONS

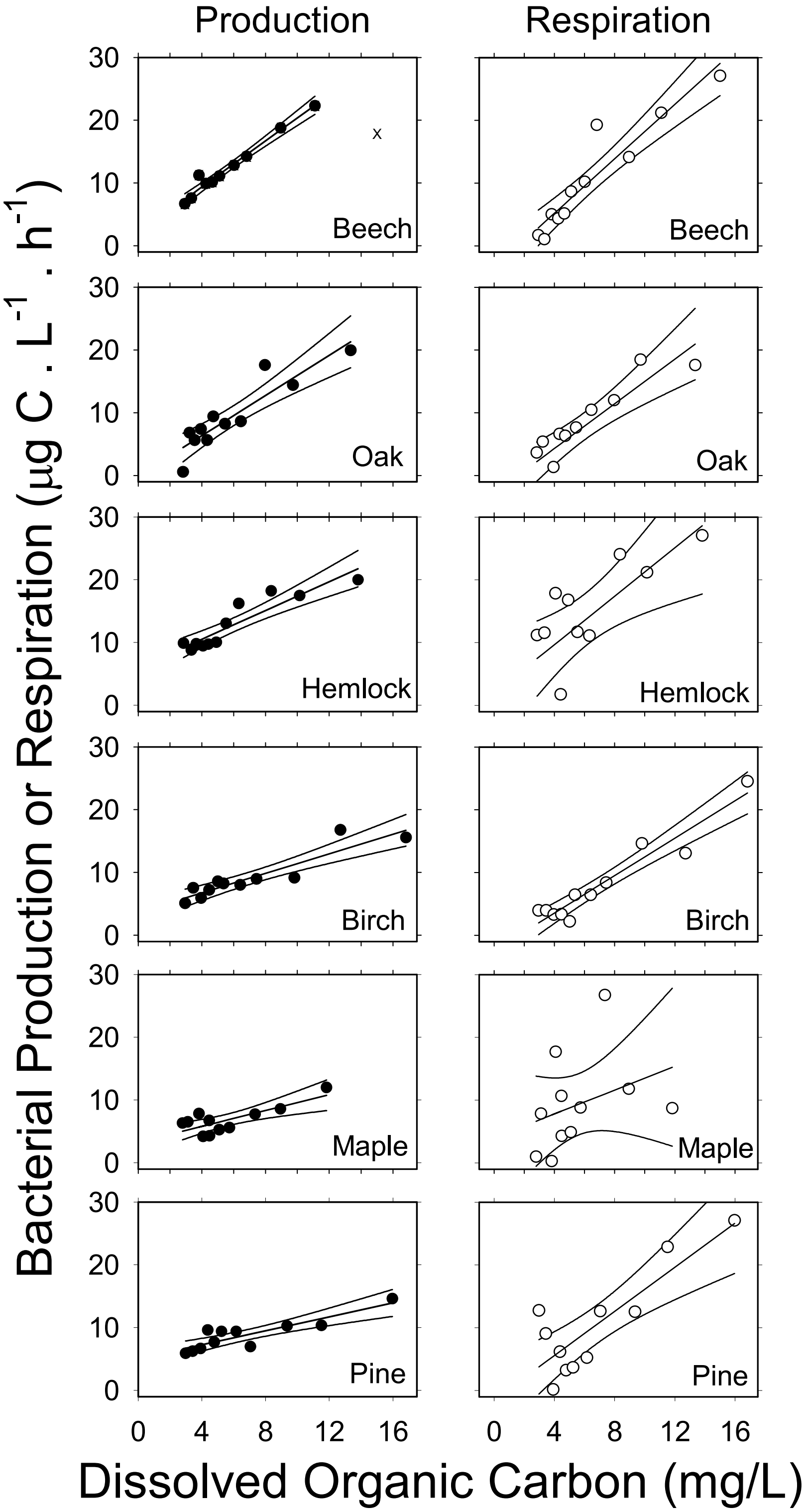
Fig. 1. Principal components analysis (PCA) on the correlation matrix for the chemical attributes of the six terrestrial DOM sources used in the bacterioplankton regrowth experiment. Points are shown for the first three principal components axes (top panel: PC2 vs. PC1 and bottom panel: PC3 vs. PC2).

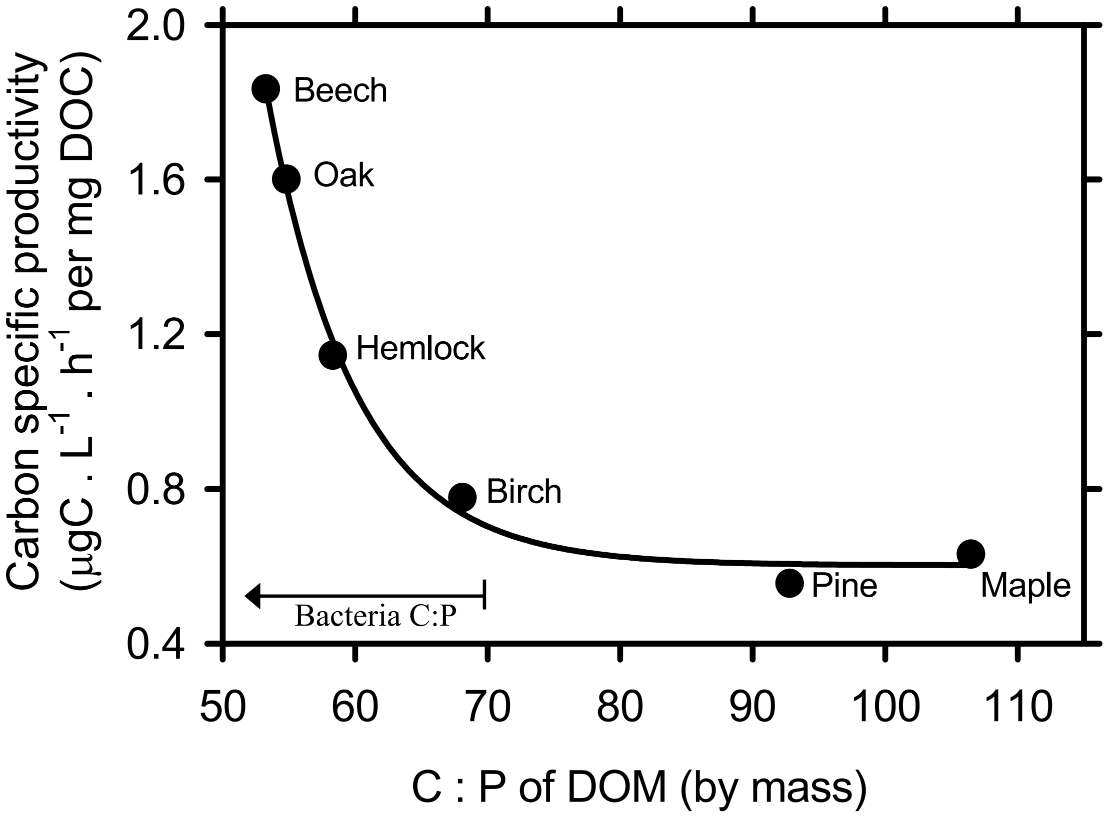
Fig 2. Bacterial productivity (●) and bacterial respiration (○) in response to different concentrations and sources of terrestrial-derived DOM. A productivity outlier (X) was detected and removed from the multiple regression analysis, but was included in a separate polynomial regression (see text). Carbon-specific bacterial productivity (i.e., the slopes) is ranked from highest to lowest along the vertical panels. Lines represent the predicted values and 95% confidence intervals from the multiple regression models.

Fig. 3. Carbon-specific bacterial productivity, the slope of the BP versus DOC relationship, decreased exponentially with the C:P ratio (mass) of the different DOM sources. Shaded area represents literature-reported range for the C:P ratio of aquatic bacterial biomass (Chrzanowski et al. 1996; Vrede et al. 2002).


HMW-DOC
Sodium
Cadmium
Zinc
PC 2
4
2
0
-2
-4
-4
-2
0
2
4
Total Nitrogen
Potassium
PC 1
Iron
Lead
Birch
Maple
Beech
Pine
Hemlock
Oak


Humic Acid
Total phosphorus
PC 3
4
2
0
-2
-4
-4
-2
0
2
4
Cadmium
Zinc
PC 2
HMW - DOC
Sodium
Birch
Maple
Pine
Hemlock
Oak
Beech


Production
Respiration
Bacterial Production or Respiration (μg C . L^{-1} . h^{-1})
Dissolved Organic Carbon (mg/L)
Beech
Oak
Hemlock
Birch
Maple
Pine
x
0
10
20
30
0
4
8
12
16


2.0
1.6
1.2
0.8
0.4
50
60
70
80
90
100
110
Carbon specific productivity
(μgC . L-1 . h-1 per mg DOC)
C : P of DOM (by mass)
Beech
Oak
Hemlock
Birch
Pine
Maple
Bacteria C:P

CHAPTER 2: CONSEQUENCES OF TEMPORAL VARIABILITY IN RESOURCE SUPPLY FOR AQUATIC MICROBIAL PRODUCTIVITY: INSIGHT FROM FIELD EXPERIMENTS AND SIMULATION MODELING

ABSTRACT

Temporal variability in resource supply affects numerous biological processes, including several important ecosystem functions. One example of temporal variability in resource supply is the export of dissolved organic carbon (DOC) from terrestrial to aquatic ecosystems. Terrestrial-derived DOC is an important allochthonous resource for aquatic bacteria, but few studies have addressed how its characteristically variable supply rate influences patterns of microbial metabolism. In this study, we used field experiments and simulation models to explore freshwater bacterioplankton responses to Pulse and Press additions of terrestrial DOC. Pulse additions of terrestrial DOC to field mesocosms caused large, but short-lived, peaks in BP. In addition, cumulative BP was 2 – 5 X higher in mesocosms receiving Pulse versus Press resource supply although both treatments received the same total amount of DOC. We then used a three compartment simulation model to identify potential mechanisms that would explain why variability in resource supply had such a large effect on BP. The model effectively captured the temporal dynamics of DOC and BP observed in the field experiments. These dynamics were produced for a wide range of parameter values, suggesting that bacterial responses to variable resource supply may be found under a variety of environmental conditions. In addition, there are many parameter combinations that cause cumulative BP in Pulse treatments to exceed cumulative BP in Press treatments. Together, our field and model

results suggest that the timing and magnitude of terrestrial DOC export may have important consequences for bacterial metabolism and carbon flow in aquatic ecosystems.

INTRODUCTION

Environmental variability can have major consequences for biological processes by influencing the rate, timing, and quality of resource supply to consumers. For example, temporal variability in resource supply affects population dynamics (Orland and Lawler 2004), competitive interactions (Gebauer et al. 2002), species coexistence (Grover 1997), and food web dynamics (Ostfeld and Keesing 2000). Temporal variability in resource supply may also have important implications for ecosystem processes. A recent synthesis described how fluctuating environmental conditions create pulses, or “hot moments”, of ecosystem activity (McClain et al. 2003), which constitute major biogeochemical fluxes of energy and nutrients (Lodge et al. 1994; Xu and Baldocchi 2004).

Pulses in ecosystem activity are often the result of episodic hydrological events that activate or mobilize growth-limiting resources (Lodge et al. 1994; Fierer and Schimel 2003; McClain et al. 2003). For example, drying-wetting cycles within terrestrial ecosystems stimulate nitrogen and phosphorus mineralization (Pulleman and Tietema 1999; Chepkwony et al. 2001), methane oxidation (West and Schmidt 1998), N_2O emissions (Brumme et al. 1999), and carbon dioxide flux (Franzluebbers et al. 2000). Hydrological variability also plays a critical role in redistributing resources among ecosystems (Odum et al. 1995; Benke et al. 2000; Baron et al. 2002; Grimm et al. 2003). For example, dissolved organic carbon (DOC) accumulates in soils during prolonged dry periods, but is flushed to nearby aquatic ecosystems following episodic hydrologic events (Boyer et al. 1997; Judd and Kling 2002). These rare events are often responsible for a majority of the DOC export from terrestrial to aquatic ecosystems (Boyer 1997; Hinton et al. 1997; Buffam et al. 2001). Once in aquatic habitats, terrestrial-derived DOC may serve as an important, albeit variable, resource that can influence ecosystem processes (e.g., Jansson et al. 2000; Chapters 1, 2, 3).

Heterotrophic bacteria perform important functions in aquatic ecosystems through their assimilation of DOC. In particular, the production of bacterial biomass represents a potentially important pathway for linking detrital energy to higher trophic levels (Cole and Pace 1995; Chapter 3). Heterotrophic bacteria in aquatic ecosystems rely on two distinct sources of DOC for producing new biomass. First, aquatic bacteria utilize DOC that is generated locally by phytoplankton and macrophytes (e.g., Bertilsson and Jones 2003). Locally-produced DOC is considered a high quality resource, but concentrations are generally low and the supply rate is dictated by the metabolic activity of primary producers (Baines and Pace 1991). Second, aquatic bacteria utilize DOC derived from surrounding terrestrial ecosystems (Moran et al. 1994; Eiler et al. 2003; Chapter 2). Terrestrial-derived DOC is considered a lower quality resource because it consists of high molecular weight humic substances (McKnight et al. 1997). However, episodic hydrologic events export relatively fresh DOC that is more labile than the bulk DOC found in aquatic ecosystems during the intervening periods (Bergstrom and Jansson 2000; Crump et al. 2003). Therefore, the importance of terrestrial-derived DOC for aquatic bacteria may be determined by the ability of bacteria to take advantage of short-term resource availability.

Growing evidence suggests that terrestrial-derived DOC is an important resource subsidy that can determine whether aquatic ecosystems function as sources or sinks of atmospheric CO_2 (del Giorgio et al. 1999; Hanson et al. 2003; Lennon 2004). However, few studies have addressed how bacteria, the primary consumers of terrestrial DOC, are affected by variability in the supply of this allochthonous resource. Therefore, we explored aquatic bacterial responses to variability in resource supply and resource quality using both field experiments and simulation modeling. The field study assessed how variability in resource supply and resource quality affect bacterial productivity (BP). We then used simulation models to identify attributes of bacteria and DOC that might explain how microorganisms respond to resource variability.

FIELD MANIPULATIONS OF RESOURCE VARIABILITY

***Methods* —** We used replicate *in situ* mesocosms to examine how resource quality and variability in resource supply affect aquatic bacterial productivity (BP). We deployed 20 mesocosms in Norford Lake, Vermont (Orange County) from 23 July to 3 August 2002. Norford Lake is a small (8 ha), oligo-mesotrophic water body (chlorophyll *a* = 2.8–5.1 µg/L) with a relatively low concentration of dissolved organic carbon (DOC; 2.4–3.3 mg/L). Mesocosms consisted of 25 L polyethylene bags suspended from styrofoam rafts. We filled each mesocosm with unfiltered lake water obtained from depth-integrated samples taken from the surface to 1.5 m. We let the mesocosms equilibrate for 3 d prior to initiating experiments.

We conducted a 12 d field experiment using a 2 x 2 design that manipulated resource quality and variability in resource supply. We replicated each experimental treatment, plus a Control treatment (no resource addition), four times for a total of 20 mesocosms. We obtained the two different qualities of terrestrial-derived DOC by leaching organic matter from soils underneath near-monoculture stands of white pine (*Pinus strobes*) and American beech (*Fagus grandifolia*) (see Chapter 2 for details). A laboratory regrowth study demonstrated that carbon-specific productivity of aquatic bacteria from Norford Lake was 3X higher on beech-derived DOC than on pine-derived DOC, primarily due to differences in leachate phosphorus content (Chapter 2). Therefore, we refer to the beech leachate as high-quality DOC and the pine leachate as low-quality DOC.

We manipulated variability in resource supply using a contrast between Press and Pulse additions. In the Press treatment, we added 10 mg of carbon from the resource stocks to each mesocosm once every 24 hrs for 10 d ($0.4\ \mathrm{mg\ C} \cdot \mathrm{L}^{-1} \cdot \mathrm{d}^{-1}$). In the Pulse treatment, we added 100 mg of carbon resource to mesocosms only at beginning of the experiment ($4\ \mathrm{mg\ C} \cdot \mathrm{L}^{-1}$). In both the Pulse and Press treatments, we had added a total of 100 mg of carbon to all experimental mesocosms.

We measured bacterial productivity (BP) and DOC daily throughout the experiment. We estimated BP by measuring the uptake and incorporation of ^{3}H-leucine into bacterial protein (Kirchman 1993) and DOC with a Tekmar-Dohrmann TIC/TOC analyzer after H_2SO_4 digestion. DOC measurements confirmed carbon manipulations and monitored losses of carbon by bacterial use and photooxidation (e.g., Granelli et al. 1998)

We analyzed the resulting time series data in two ways. First, we determined the effects of resource quality, variability in resource supply, and time on BP and DOC using repeated measures ANOVA (SAS PROC MIXED with an AR(1) covariance matrix). Second, to determine whether treatments differentially affected total bacterial production during the experiment, we calculated cumulative BP for each mesocosm using trapezoidal integration (trapz command in Matlab version 6.3, MathWorks, Natick, MA), standardized these estimates by subtracting the mean cumulative production in the control mesocosms, and then used two-way ANOVA to test for treatment effects (SAS PROC GLM).

***Results and discussion* —** DOC concentrations were significantly affected by our experimental manipulations (Fig. 1). Although DOC concentrations in the low and high quality resource treatments were similar over the duration of the experiment (RM-ANOVA, time x quality, $P = 0.10$), our manipulation of variability in resource supply had a significant effect on DOC dynamics (time x variability, $P < 0.0001$). DOC may have also been affected by photooxidation or by bacterial uptake, but these processes seem to have been masked by the relatively large fraction of recalcitrant carbon in the bulk DOC pool (Fig. 1).

Bacterial productivity responded significantly to both resource quality and variability in resource supply over the course of the experiment (Fig. 2; RM-ANOVA, time x quality x variability, $P < 0.0001$). We observed large, short-lived peaks in BP following Pulse resource additions, although the peak in the low quality Pulse treatment was approximately half as large as the peak in the high quality Pulse treatment (Fig. 2). In contrast, BP remained low in the Press treatments for both the low and high quality resource additions (Fig. 2).

Resource quality and variability in resource supply also interacted to determine cumulative BP (Fig. 3; two-way ANOVA, quality x variability, $P = 0.039$). Cumulative BP was higher in the Pulse treatment than in the Press treatment, but was approximately twice as high in systems that received a high quality Pulse than a low quality Pulse. In contrast, cumulative BP was low in the Press systems regardless of resource quality (Fig. 3).

Thus, our field experiment revealed that BP is affected by both resource quality and variability in resource supply. In particular, variability in resource supply had a strong effect on both the temporal dynamics and cumulative amount of BP. Bacterial productivity increased 20 - 37X immediately following pulse additions of DOC depending on resource quality. Although this pulsed response was short-lived (48 hrs), it translated into large differences in cumulative BP across treatments. For example, cumulative BP was 2-5X greater in Pulse versus Press DOC treatments depending on resource quality. These results suggest that episodic inputs of terrestrial DOC can be readily exploited by aquatic bacteria and can have longer-term consequences for microbial metabolism. Interestingly, bacteria were much less efficient at using DOC resources supplied at relatively constant rate, suggesting that the importance of resource quality is conditional upon the variability in resource supply.

We found these results somewhat surprising and wanted to identify a set of potential mechanisms to explain why variability in resource supply had such a large effect on BP. We constructed a mathematical model to simulate the responses of heterotrophic bacteria to manipulations in resource variability. In particular, we were interested in identifying conditions that created temporal dynamics in BP and DOC comparable to those observed in the field experiment. We were also interested in identifying specific attributes of bacteria and/or DOC (i.e., quality) that might explain the observed difference between cumulative BP in Pulse versus Press resource treatments.

SIMULATIONS OF VARIABLE RESOURCE SUPPLY

Description **—** We constructed a three-compartment simulation model to explore how aquatic bacteria respond to resource quality and variability in resource supply (Fig. 4). The structure of the model is analogous to some soil models in that it distinguishes between different carbon pools based on their bioactivity (e.g., Parton 1988). Specifically, the labile carbon pool (L) is immediately available for bacterial uptake, whereas the recalcitrant carbon pool (R) has a longer residence time and is not available for bacterial uptake. Refractory carbon becomes labile carbon based on a rate of mobilization (m).

Carbon becomes available to bacteria in two ways. First, a constant supply of labile carbon is added to the system (I_i) to simulate the release of phytoplankton-derived DOM (Baines and Pace 1991). A second source of external carbon (I_e) simulates additions of terrestrial-derived DOM. I_e enters the system at either a constant rate (Press) or as a one-time addition (Pulse). A fraction of I_e is refractory (r) and unavailable for bacterial uptake; the remainder goes to the labile carbon pool and is available for bacterial uptake.

Labile carbon is taken up by bacteria according to Michaelis-Menten dynamics, where V_{max} is the maximum rate of resource uptake and K_m is the half saturation constant. Based on bacterial growth efficiency (BGE), a fraction of assimilated carbon is used to build bacterial biomass (*B*), while the rest is lost to respiration. Bacterial carbon is lost to death (*d)* due to viral lysis or grazing (*lg*) (Noble and Fuhrman 1995). Carbon is lost from the system when death is due to grazing, but is recycled to the labile carbon pool when due to viral lysis.

The system of equations is written as:

$$\frac{dR}{dt} = I_e \; r - m \; R \qquad \text{(eq. 1)}$$

$$\frac{dL}{dt} = I_i \; + \; (1-r)\, I_e \; + \; m\, R \; + \; lg\, (d\, B) \; - \; V_{\max} \left(\frac{L}{L + K_m} \right) B \qquad \text{(eq. 2)}$$

$$\frac{dB}{dt} = BGE \;\; V_{\max} \left(\frac{L}{L + K_m} \right) \, B \; - \; d\, B \qquad \text{(eq. 3)}$$

We calculated per capita bacterial productivity (BP) as:

$$BP = BGE \;\; V_{\max} \left(\frac{L}{L + K_m} \right) \qquad \text{(eq. 4)}$$

We implemented these equations in Matlab version 6.3 (MathWorks, Natick, MA) using an adaptive step size Runge-Kutta algorithm (Jackson et al. 2000).

Simulations — We ran all simulations for 12 d to match the field experiments. As in the field, total I_e was equal for Pulse and Press simulations. However, in Pulse simulations, all of I_e was added on day 2 of the simulation, whereas I_e was added continuously throughout Press simulations beginning on day 2.

We evaluated model sensitivity by first manipulating the model parameters independently and then conducting Monte Carlo simulations with multiple factors using parameter distributions estimated from the literature (Table 1). We also ensured that the simulation outcomes were robust to a broad range of initial conditions for the three state variables (not reported here).

Numerical experiments: time courses— Our first objective was to explore the temporal trajectories of BP and DOC (the sum of the refractory and labile carbon pools, $L + R$) in response to Press and Pulse additions of I_e. We then evaluated qualitatively how variation in each of the model parameters might have contributed to the patterns observed in the field experiments. We were particularly interested in parameters that controlled the peak height of BP in the Pulse treatments and parameters that kept BP at consistently low levels in the Press treatments. We also assessed how individual parameters affected the dynamics of DOC in the Pulse and Press treatments, and whether these simulation outcomes matched the trends observed in our field experiment.

Our simulations captured the general behavior of DOC observed in the field (compare Fig. 1 and Fig. 5). In the Pulse treatment, total DOC increased rapidly following the addition of external carbon then slowly declined through the rest of each simulation. In the Press treatment, DOC increased steadily with continuous additions of external carbon. Overall, DOC was relatively insensitive to variation in BGE, I_i, d, and lg (Fig. 5). In contrast, DOC concentrations decreased in both Pulse and Press simulations when the fraction of refractory carbon (*r*) from the external carbon supply decreased, presumably due to the greater lability of the total pool. DOC concentrations also declined with increasing *m*, the mobilization of refractory carbon to labile carbon. Finally, DOC responded to parameters affecting bacterial C uptake. For example, we found that DOC concentrations did not decline over time when simulations were run with the lowest V_{max} (1.0 μg C · L^{-1} · d^{-1}). Similarly, DOC concentrations were slightly higher when simulations were run with the highest K_m (1000 μg C/L) (see Fig. 5). These results suggest that aquatic bacteria can affect bulk DOC concentrations, even when a majority of the DOC pool is considered refractory.

Our simulations also captured the observed behavior of BP in the field experiments, particularly for the Pulse treatment (compare Fig. 2 and Fig. 6). BP peaked soon after the addition of external carbon in the Pulse treatment. The amplitude of the peaks was affected by attributes of both the DOC and bacteria. With regard to DOC attributes, peak height of BP was negatively affected by the fraction of carbon input that was refractory (*r*), but was less responsive to the rate at which refractory carbon became mobile (*m*). With regard to bacteria attributes, peak height of BP increased with BGE. In addition, we found that were threshold responses of BP to manipulations of V_{max} and K_m. Specifically, there were no peaks in BP at the lowest V_{max} and highest $K_{m.}$ Unlike the field experiments, there was a small increase in BP at the beginning of most Press simulation runs (Fig. 6). However, this level of BP was not maintained throughout the simulation despite the constant supply of external carbon. In both Pulse and Press simulations, BP recovered to background levels within 2-3 d of the initial external carbon addition.

Together, the time courses of our simulation models matched up quite well with our observation from the field. Based on our simulations, we infer that the DOC used in our field experiments was largely refractory and had a relatively slow rate of mobilization. In addition, BP in our simulations behaved similarly to field observations for a wide range of parameter values. This suggests that the BP responses in our field experiments are likely to be observed under a variety of environmental conditions.

***Numerical experiments: cumulative BP*—** We also used extensive multifactor experiments with the simulation model to identify potential mechanisms for why cumulative BP was significantly higher in Pulse versus Press treatments in the field experiment. As with the field experiment, we calculated cumulative BP for time series of Pulse and Press simulations using trapezoidal integration. Also, to test the hypothesis that differences in cumulative BP could be attributed to the functional response of bacterial predators, we ran simulations with a squared death term (dB^2) to approximate a saturating predator functional response. In all, we ran 240,000 simulations. For each simulation, we used the ratio of cumulative BP in the Press scenario to cumulative BP in the Pulse scenario to express the difference between treatments. We then used variance components analysis (SAS PROC VARCOMP) to evaluate which parameters and interactions are responsible for differences in cumulative productivity between treatments.

Many parameter combinations led to cumulative BP in Pulse treatments exceeding cumulative BP in Press treatments. When we used a linear death rate function, the ratio of cumulative BP between Pulse and Press treatments ranged from 0.1 to 9.0 (Fig. 7). Half of the ratios were within the range of 0.996 to 1.001, indicating that, in many situations, cumulative BP was unaffected by variability in resource supply. When cumulative BP was affected by resource supply, the effect was due to higher order interactions: only four one-, two-, and three-way effects explained more than 1% of the variability in the data set, and 86% of the variation was unexplained by these effects (Table 2).

Results were slightly different when we analyzed the simulations generated with quadratic death rates. Although the median value of cumulative BP between pulse and press treatments was still 1.0, there was much less variance among simulations (Fig. 7). As a result, one-, two-, and three-factor variance components explained ~40% of the variation in the Pulse : Press ratio (Table 2). Components including V_{max}, I_i, and r were most important (Table 2).

Together, these results suggest that although we captured the qualitative behavior of our experiments, the simulation model was not effective at identifying the potential mechanisms creating the observed differences in BP or cumulative BP. Although cumulative BP was frequently higher in Press versus Pulse treatments, our variance components analysis indicated that this variance was explained largely by higher-order interactions. Finally, the simulations also indicate that discrepancies between cumulative BP in Pulse versus Press treatments may be influenced by the functional response of bacterial predators, that determine death rates.

SYNTHESIS

This study used field experiments and computer simulations m to explore how aquatic bacteria respond to resource variability. In particular, we examined how bacterial productivity was affected by DOC quality and variability in DOC supply rate. In the field, BP responded strongly to variability in resource supply. Pulse additions of terrestrial DOC caused large, but short-lived, peaks in BP. Cumulative BP was 2 – 5 X higher in mesocosms receiving Pulse versus Press treatments despite the fact that both treatments received the same total amount of DOC. Moreover, it was only in the Pulse treatments that BP was affected by resource quality. Together, these results support the argument that organisms experience and respond to variability in environmental conditions (Ruel and Ayres 1999; Benedetti-Cecchi 2003).

Our simulation model was effective at reproducing the behaviors of DOC and BP observed in the field. The model suggests that the DOC source used in our experiment was largely refractory and was mineralized by bacteria at a relatively slow rate. Nevertheless, BP peaked in response to Pulse additions of DOC and was consistently low throughout the simulations in the Press treatment for a wide range of parameter values, suggesting that the pattern observed in the field may be found under a diversity of conditions.

Our simulation model predicted that cumulative BP could be up to 9X higher in Pulse than in Press treatments (Fig. 7). However, we were unable to identify single parameters, or even combinations of parameters, that determined when cumulative BP in Pulse treatments exceeded BP in Press treatments. In addition, the median value for the ratio of cumulative BP in Pulse and Press treatments was 1.0. This implies that 1) our field results regarding cumulative BP were anomalous, or 2) there are important aspects missing from our simulation model.

One feature of our model that might account for discrepancies between the field and simulation results is bacterial death rates. The functional form of the death rates (*dB* versus dB^2) had a large effect on the range of cumulative BP outcomes (Fig. 7). Moreover, we know that bacterial death rates can take on more complicated functional forms (Jürgens et al. 1997) and that there may be time lags in the numerical response of different grazers depending on their generation times. Therefore, one future avenue for this research is to incorporate a more complicated, but realistic grazing function into the simulation model.

One important aspect of nature that cannot be easily incorporated into our model is the functional diversity of heterotrophic bacteria (Balser et al. 2002; Wellington et al. 2003). In this study, we treated the bacterial community as homogenous. The composition and activity of bacteria, however, changes through time and is affected by environmental conditions including the availability of carbon resources (Lindstrom 2001; Smith and del Giorgio 2003; Eiler et al. 2003; Yannarell et al. 2003). In addition, many microbes are metabolically inactive as a means of coping with low resource availability or stressful abiotic conditions (White 1995). Episodic DOC inputs may be an important external cue that awakens heterotrophic bacteria from dormancy. In contrast, constant inputs of DOC at low concentrations may not be sufficient to stimulate the metabolic activity of some bacteria. Recent advances that allow community characterization provide a means to test this hypothesis (e.g., Boschker and Middelburg 2002; Torsvik and Øvreås 2002).

Regardless of the exact mechanism, variability in resource supply had strong effects on the temporal dynamics and cumulative biomass production of aquatic bacteria.

These results are important given recent evidence showing that lake food webs are supported in part by terrestrial carbon that is mobilized by aquatic bacteria (Pace et al. 2004). However, the importance of terrestrial carbon in lake food webs is variable through time (Grey et al. 2001) and could potentially be linked to the timing of terrestrial DOC inputs. If so, climate-induced alterations in the connectivity of terrestrial and aquatic ecosystems (e.g., Schindler et al. 1997) could affect the energetic importance of allochthonous carbon sources for aquatic food webs.

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Table 1. Notation, description, values, units, and references for the parameters that we used in our three-compartment simulation model. For parameter values, we have listed the nominal values and the range of values (in parentheses) used in our simulations. *1*: approximates carbon loading from the field experiment; *2*: Baines and Pace (1991), Bertilsson and Jones (2003); *3*: Söndergaard, and Middelboe (1995); del Giorgio and Davis (2003); *4*: Raymond and Bauer (2000); Wetzel (2003); *5* Bianchi et al. (1998); *6*: Overbeck 1994; Bianchi et al. (1998); Kirchman and Rich (1997); Kisand and Tammert (2000); *7*: del Giorgio and Cole (1998); *8*: Fuhrman and Noble (1995); *9*: Fuhrman and Noble (1995). Thingstad and Lignell (1997)

Parameter	**Description**	**Parameter Values**	**Units**	**References**
I_e	External C supply	500 (10 – 2000)	μg C / simulation	*1*
I_i	Internal C supply	1 (0.1 – 100)	$\mu g\ C \cdot L^{-1} \cdot d^{-1}$	*2*
r	Recalcitrant fraction of I_e	0.9 (0.5 – 0.99)	Proportion	*3*
m	Mobilization rate ($R \rightarrow L$)	0.05 (0.01 – 0.25)	Proportion per day	*4*
V_{max}	Maximum uptake rate	100 (0.5 – 1000)	$\mu g\ C \cdot L^{-1} \cdot d^{-1}$	*5*
K_m	Half-saturation constant	1 (0.5 – 10000)	μg C / L	*6*
BGE	Bacterial growth efficiency	0.25 (0.05 – 0.75)	Proportion of C uptake	*7*
d	Bacterial death rate	0.15 (0.01 – 0.5)	Proportion of B per day	*8*
lg	Lysis : grazing ratio	0.5 (0.0 – 1.0)	Proportion of d	*9*

Table 2. Variance components analysis for the ratio of cumulative bacterial productivity in Pulse scenarios to Press scenarios. The model examined the effects of parameters in one-way, two-way, and three-way combinations only. Only factors that explained ≥1% of the total variation are listed below. **A** = results from linear death rates, **B** = results from polynomial (squared) death rates.

A)	**Variance term**	**Variance Component**	**Fraction of Explained Variance**
	Error	93.5×10^{-4}	0.858
	$V_{max} * I_i$	2.8×10^{-4}	0.026
	$V_{max} * I_i * r$	2.8×10^{-4}	0.026
	$I_i * r * d$	2.6×10^{-4}	0.024
	$V_{max} * I_i * I_e$	1.2×10^{-4}	0.011
	Total	109.0×10^{-4}	1.000

B)	**Variance term**	**Variance Component**	**Fraction of Explained Variance**
	Error	207.0×10^{-4}	0.610
	$V_{max} * I_i$	21.6×10^{-4}	0.063
	V_{max}	17.6×10^{-4}	0.052
	$r * V_{max} * I_i$	12.9×10^{-4}	0.038
	$r * I_i$	10.5×10^{-4}	0.031
	$I_e * V_{max} * I_i$	9.7×10^{-4}	0.028
	$r * V_{max}$	7.6×10^{-4}	0.022
	I_i	5.8×10^{-4}	0.017
	S	5.7×10^{-4}	0.017
	r	5.4×10^{-4}	0.016
	$S * r * V_{max}$	4.8×10^{-4}	0.014
	$S * V_{max}$	4.5×10^{-4}	0.013
	$V_{max} * K_m * I_i$	3.4×10^{-4}	0.010
	Total	339.3×10^{-4}	1.000

FIGURE CAPTIONS

Fig. 1. Concentrations of dissolved organic carbon (DOC) in replicate mesocosms during the field experiment. Data are means ± SEM.

Fig. 2. Response of bacterial productivity to different DOM treatments during the field experiment. Vertical dashed line represents the time DOM treatments were initiated. Data are means ± SEM.

Fig. 3. Cumulative bacterial production in the experimental DOM treatments corrected for cumulative bacterial production in the control mesocosms. Data are means ± SEM.

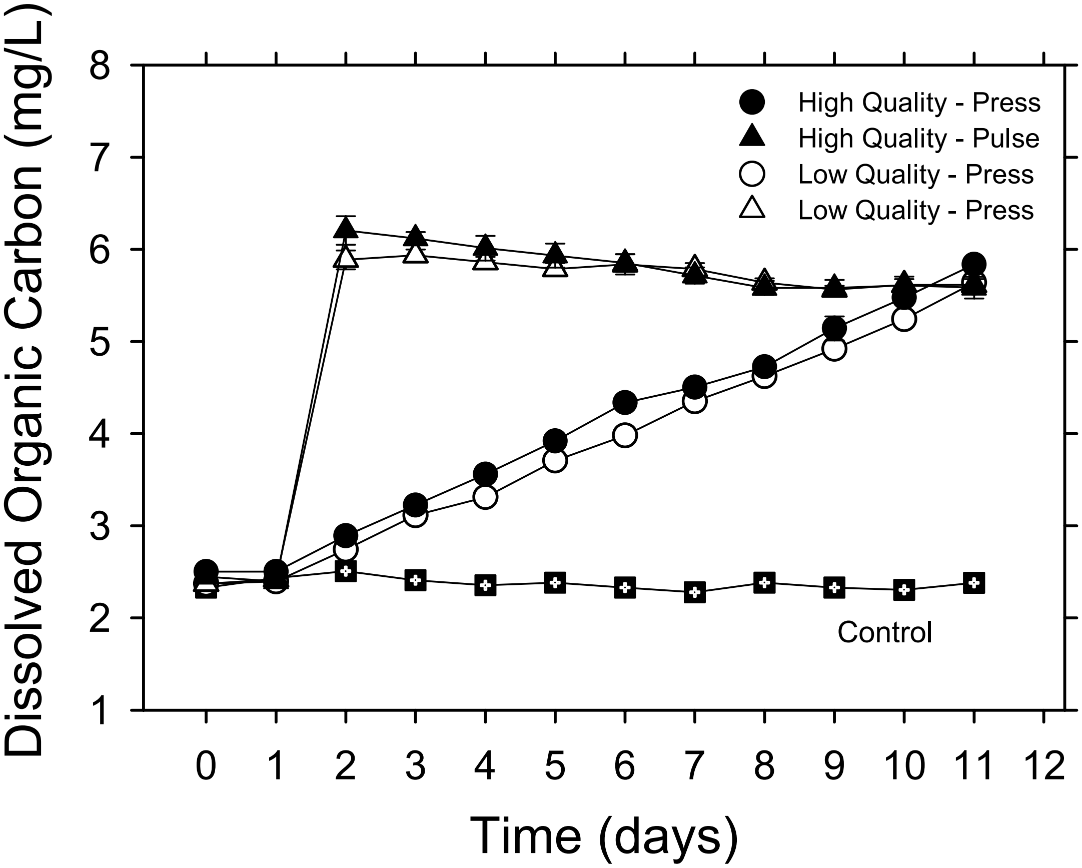
Fig. 4. The three-compartment simulation model used to explore bacterial responses to variability in DOM quality and supply rate.

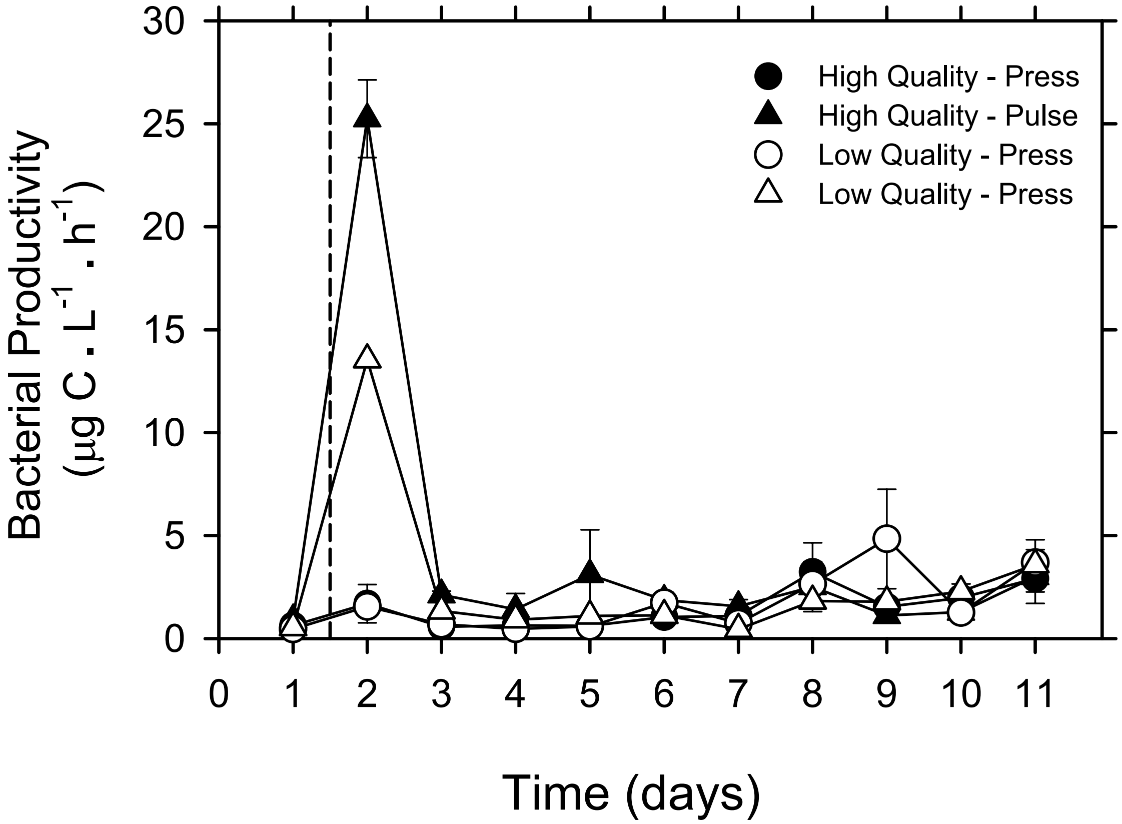
Fig. 5. Temporal dynamics of DOC (sum of the labile + refractory pools) in our simulations. Columns represent Pulsed (left) and Pressed (right) supplies of carbon resources. Rows represent responses to the manipulation of each of the model's eight parameters.

Fig. 6. As Fig. 5, but for bacterial productivity (BP).

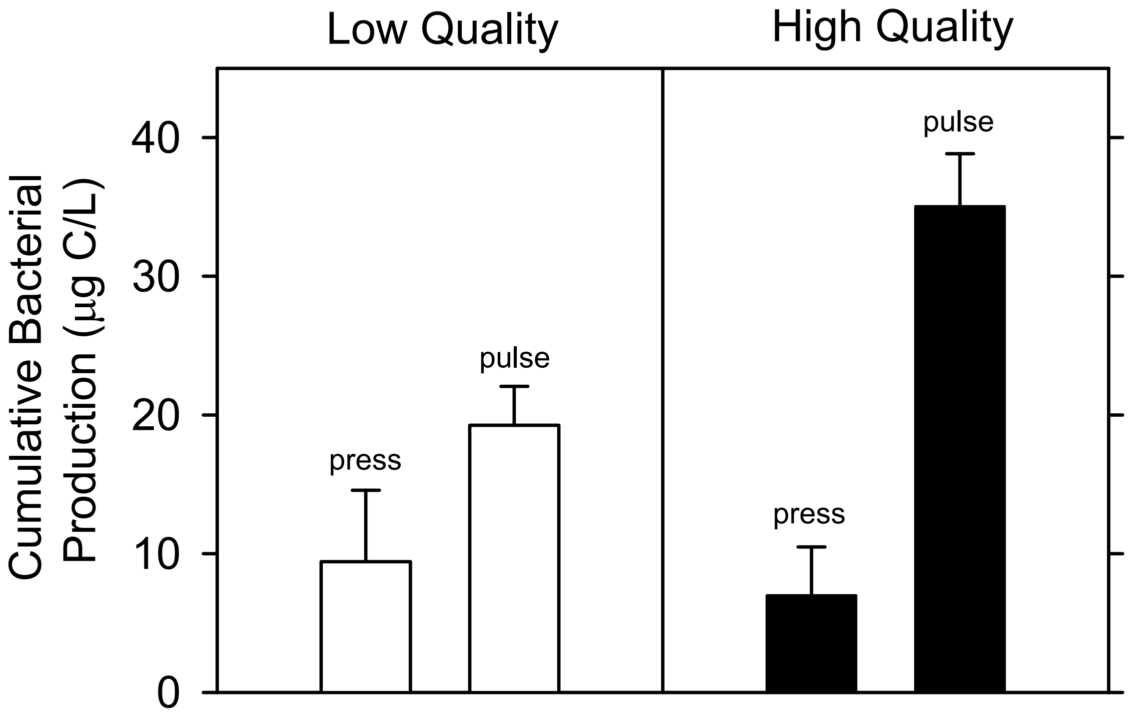
Fig. 7. Distributions of the ratio of cumulative bacterial production in Pulse : Press resource supply scenarios for 240,000 factorial manipulations of all eight model

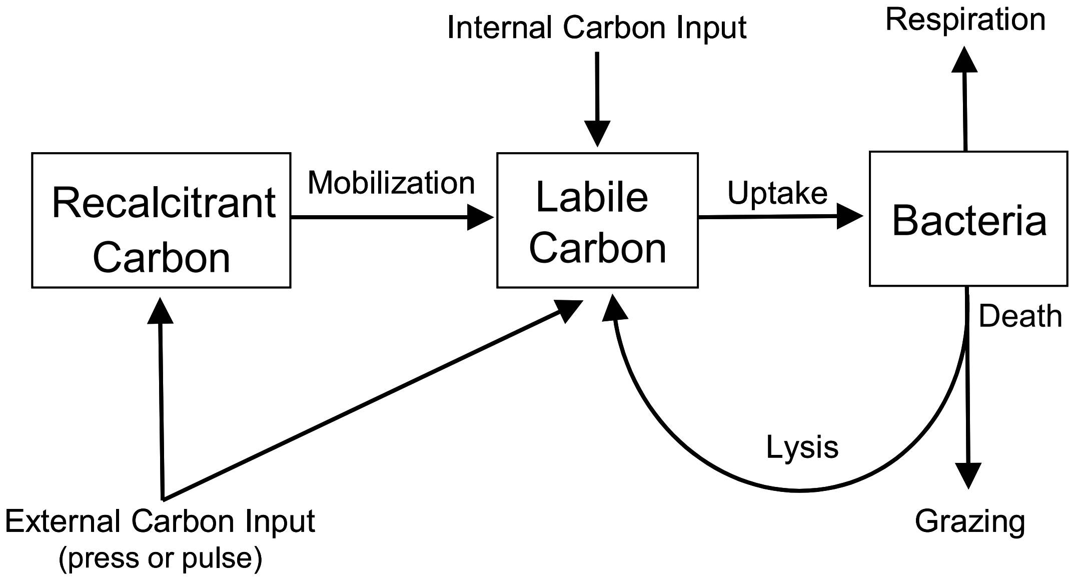
parameters. The lower panel shows the Log_2-transformed data from the top panel. Black bars (*dB*) represent simulations with death rates that are a linear function of bacterial biomass and the grey bars (*d B*^2) represent simulations with death rates that are a squared function of bacterial biomass.

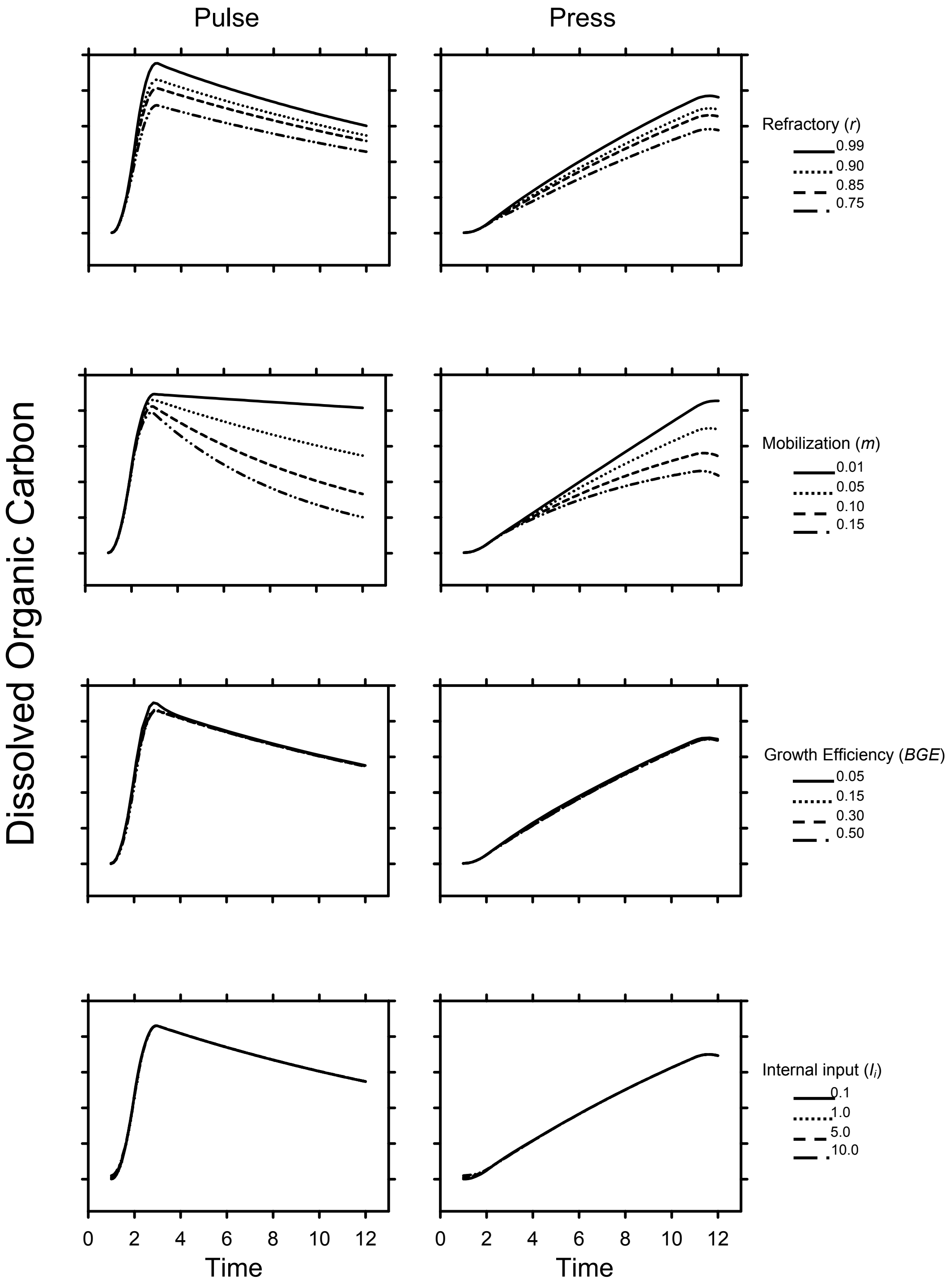

Dissolved Organic Carbon (mg/L)
1
2
3
4
5
6
7
8
Time (days)
0
1
2
3
4
5
6
7
8
9
10
11
12
● High Quality - Press
▲ High Quality - Pulse
○ Low Quality - Press
△ Low Quality - Press
Control



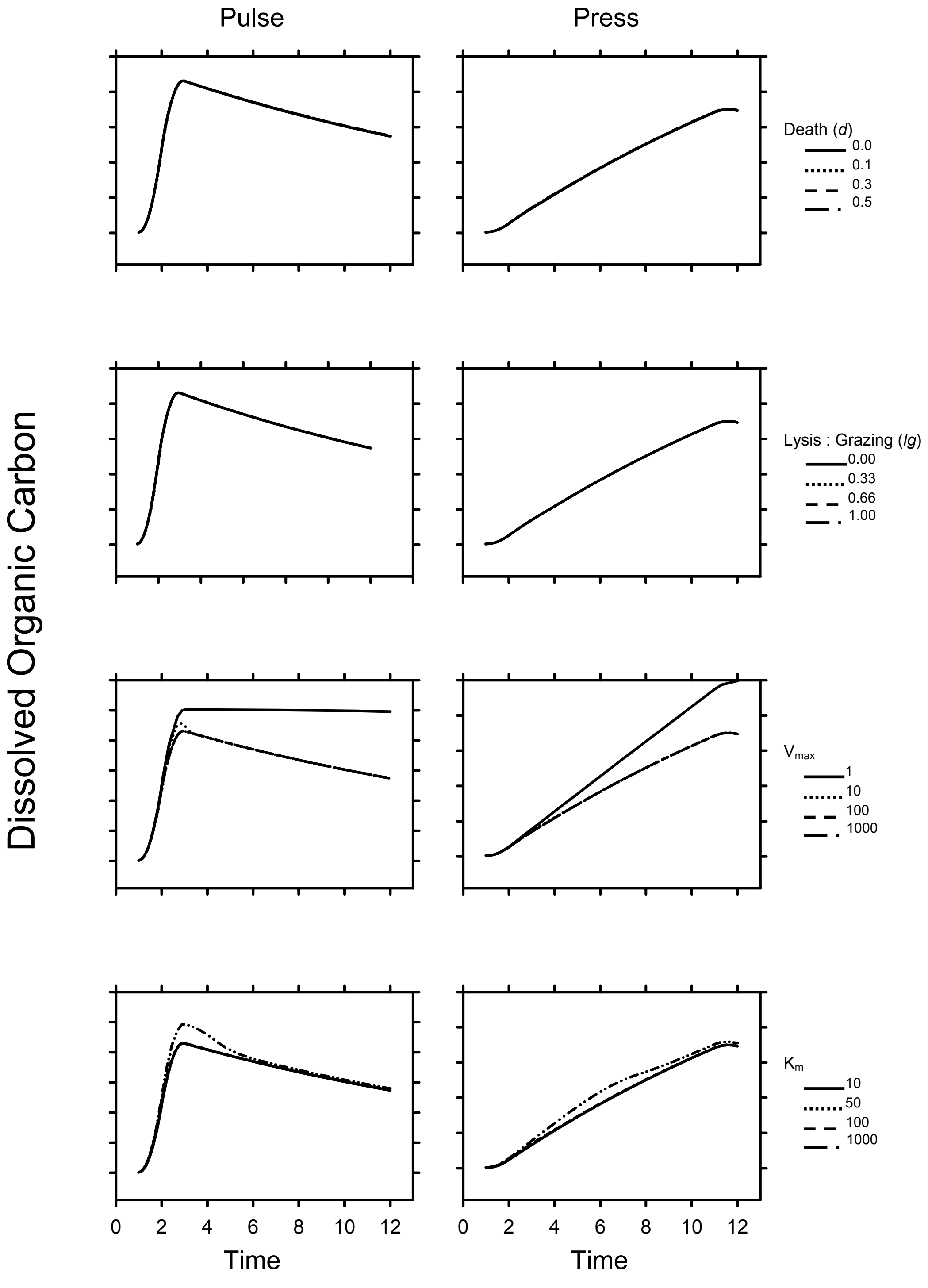
30
25
20
15
10
5
0
Bacterial Productivity
(µg C . L^{-1} . h^{-1})
0
1
2
3
4
5
6
7
8
9
10
11
Time (days)
● High Quality - Press
▲ High Quality - Pulse
○ Low Quality - Press
△ Low Quality - Press


Low Quality
High Quality
Cumulative Bacterial Production (μg C/L)
0
10
20
30
40
press
pulse
press
pulse

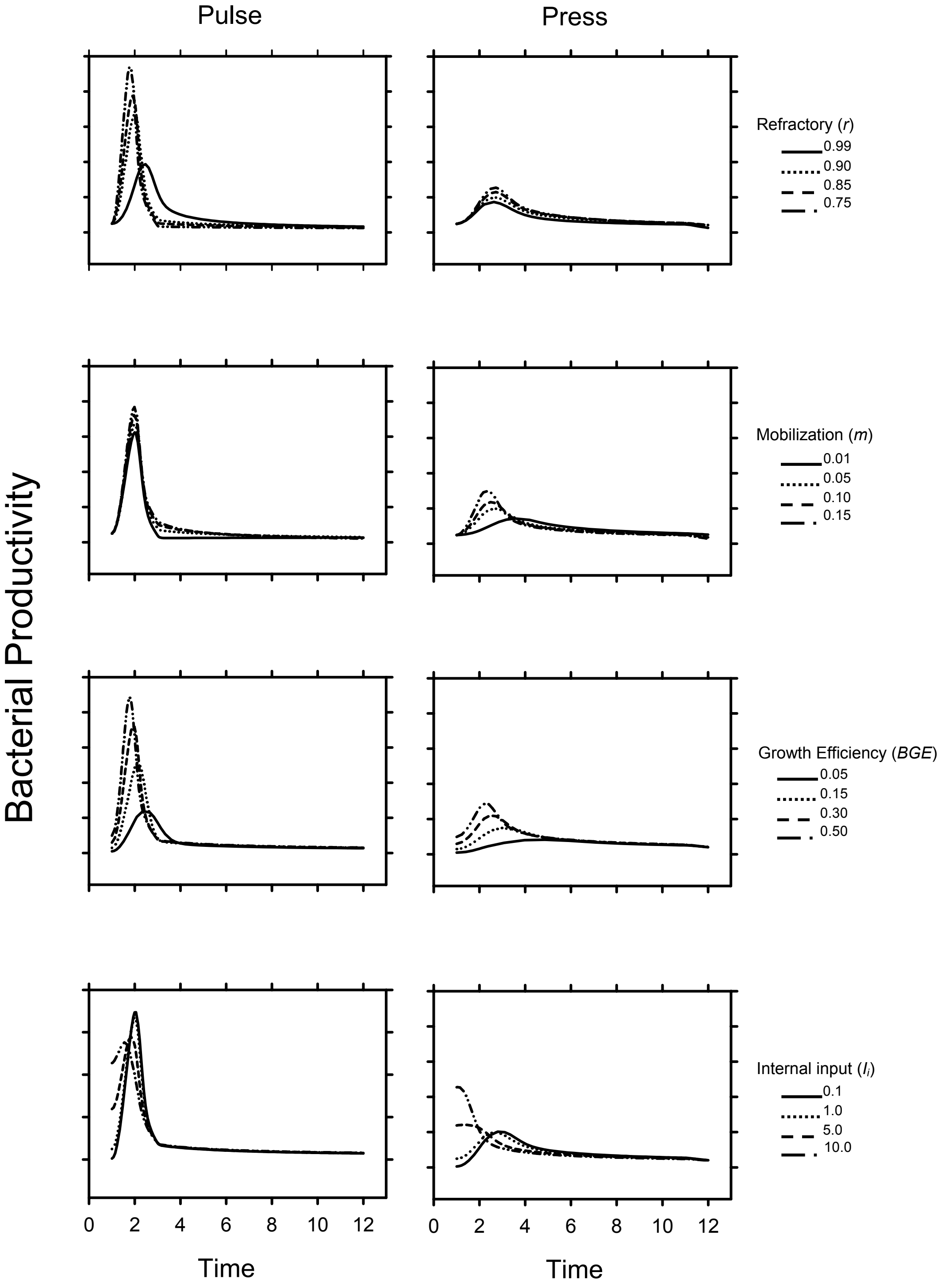

Internal Carbon Input
Respiration
Recalcitrant Carbon
Mobilization
Labile Carbon
Uptake
Bacteria
Death
Lysis
External Carbon Input (press or pulse)
Grazing



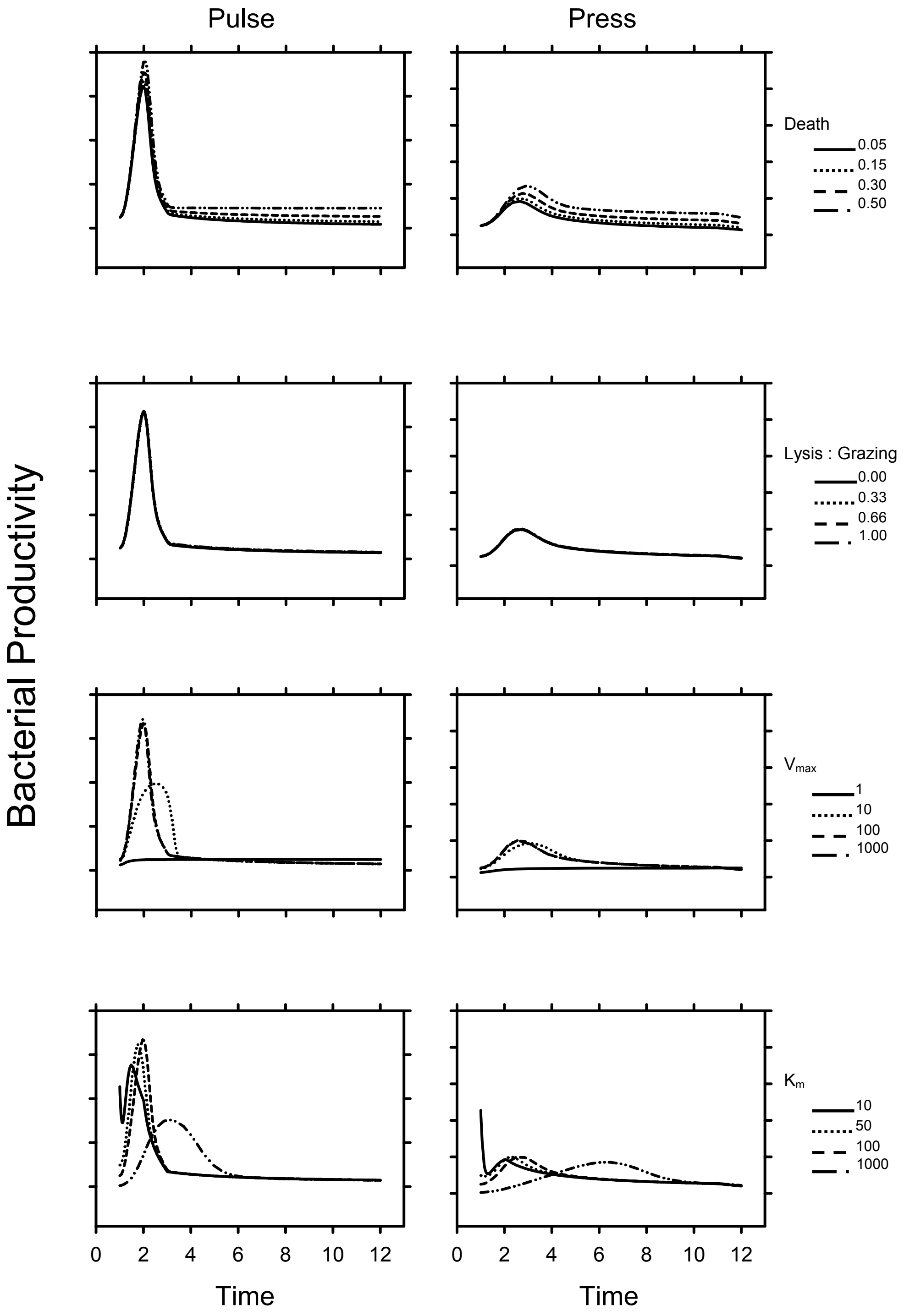
Pulse
Press
Dissolved Organic Carbon
Refractory (*r*)
0.99
0.90
0.85
0.75
Mobilization (*m*)
0.01
0.05
0.10
0.15
Growth Efficiency (*BGE*)
0.05
0.15
0.30
0.50
Internal input (I_i)
0.1
1.0
5.0
10.0
0
2
4
6
8
10
12
Time
0
2
4
6
8
10
12
Time

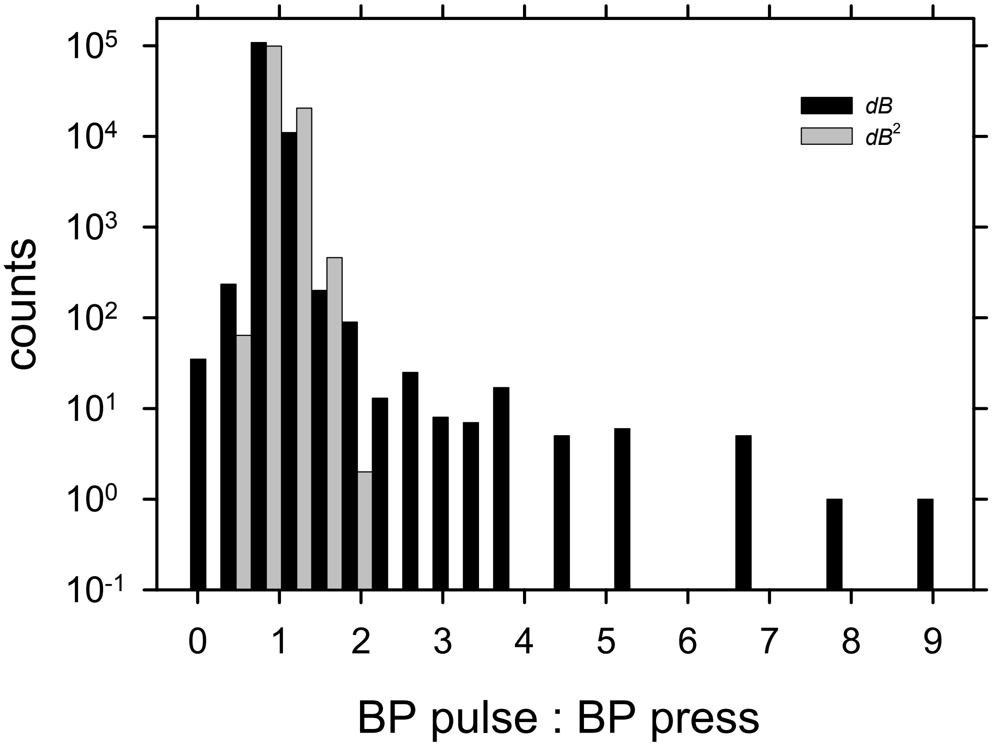


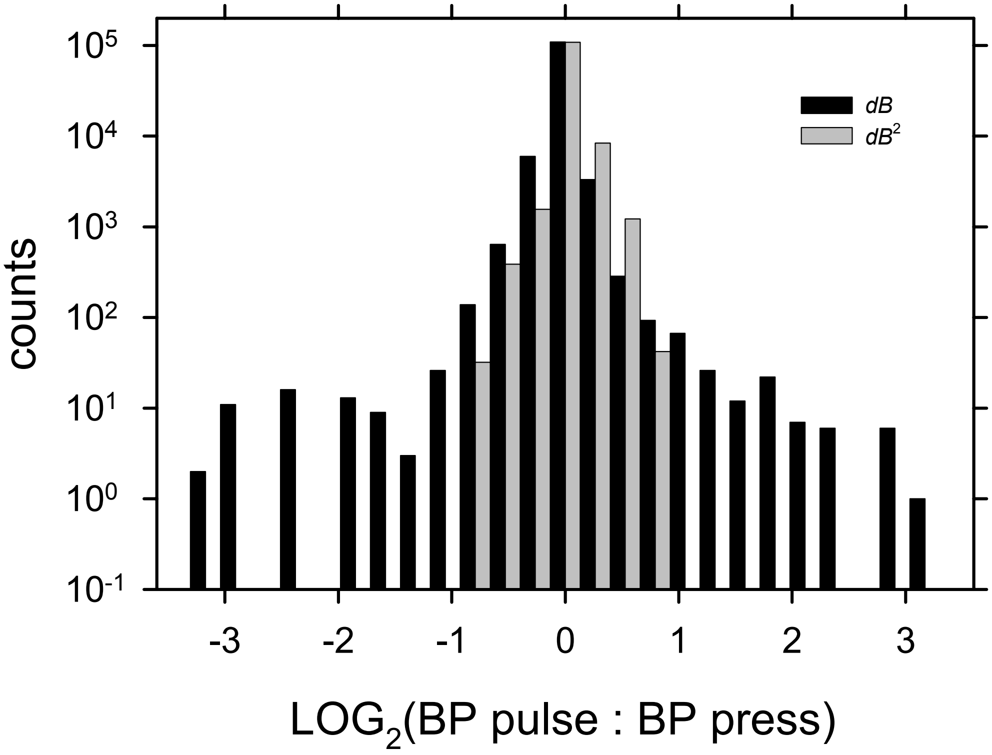
Pulse
Press
Dissolved Organic Carbon
Death (d)
0.0
0.1
0.3
0.5
Lysis : Grazing (lg)
0.00
0.33
0.66
1.00
V_{max}
1
10
100
1000
K_m
10
50
100
1000
0
2
4
6
8
10
12
Time
0
2
4
6
8
10
12
Time



Pulse
Press
Bacterial Productivity
Refractory (*r*)
0.99
0.90
0.85
0.75
Mobilization (*m*)
0.01
0.05
0.10
0.15
Growth Efficiency (*BGE*)
0.05
0.15
0.30
0.50
Internal input (I_i)
0.1
1.0
5.0
10.0
0 2 4 6 8 10 12
0 2 4 6 8 10 12
Time
Time


Pulse
Press
Death
0.05
0.15
0.30
0.50
Lysis : Grazing
0.00
0.33
0.66
1.00
V_{max}
1
10
100
1000
K_m
10
50
100
1000
Bacterial Productivity
0
2
4
6
8
10
12
Time
Time


counts
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
dB
dB^2
0
1
2
3
4
5
6
7
8
9
BP pulse : BP press


counts
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
dB
dB^2
-3
-2
-1
0
1
2
3
LOG2(BP pulse : BP press)

CHAPTER 3: EXPERIMENTAL EVIDENCE THAT TERRESTRIAL CARBON SUBSIDIES INCREASE CO_2 FLUX FROM LAKE ECOSYSTEMS

ABSTRACT

Subsidies are donor-controlled inputs of nutrients and energy that can affect ecosystem level processes in a recipient environment. Lake ecosystems receive large inputs of terrestrial carbon (C) in the form of dissolved organic matter (DOM). DOM inputs may energetically subsidize heterotrophic bacteria and determine whether lakes function as sources or sinks of atmospheric CO_2. I experimentally tested this hypothesis using a series of mesocosm experiments in New England lakes. In the first experiment, I observed that CO_2 flux increased by 160% four days following a 1000 μM C addition in the form of DOM. However, this response was relatively short lived, as there was no effect of DOM enrichment on CO_2 flux beyond eight days. In a second experiment, I demonstrated that peak CO_2 flux from mesocosms in two lakes increased linearly over a broad DOM gradient (slope for both lakes = 0.02 ± 0.001 mM $CO_2 \bullet m^{-2} \bullet d^{-1}$ per μM DOC, mean ± SE). Concomitant changes in bacterial productivity and dissolved oxygen strengthen the inference that increasing CO_2 flux resulted from the metabolism of DOM. I conducted two additional studies to test whether DOM-correlated attributes were responsible for the observed change in plankton metabolism along the subsidy gradient. First, terrestrial DOM reduced light transmittance, but experimental shading revealed that this was not responsible for the observed patterns of CO_2 flux. Second, organically bound nitrogen (N) and phosphorus (P) accompanied DOM inputs, but experimental nutrient additions (without organic C) made mesocosms net sinks, rather than sources of atmospheric CO_2. Together, these results suggest that C content of terrestrial DOM may

be an important subsidy for freshwater bacteria that can influence whether recipient aquatic ecosystems are sources or sinks of atmospheric CO_2.

INTRODUCTION

Ecologists have long recognized that ecosystems are spatially connected by the flux of materials and energy. Recently though, there has been growing appreciation for how nutrients, detritus, and organisms from donor habitats influence ecological attributes in recipient habitats (Polis et al. 1997; Jefferies 2000; Schindler and Scheuerell 2002; Kawaguchi et al. 2003). Such allochthonous subsidies alter population abundances (Sabo and Power 2002; Stapp and Polis 2003), structure communities (Murakami and Nakano 2002; Takada et al. 2002), increase food chain length (Pimm and Kitching 1987), induce trophic cascades (Nakano et al. 1999; Henschel et al. 2001), and influence food web stability (Huxel and McCann 1998; Takimoto et al. 2002). Subsidies also affect key ecosystem processes such as primary productivity (Sánchez-Piñero and Polis 2000) nutrient cycling (Helfield and Naiman 2001) and whole system metabolism (Smith and Hollibaugh 1997).

One well-recognized class of subsidies is the transport of organic matter from terrestrial to aquatic ecosystems (see Polis et al. 1997). In temperate latitudes, up to 20% of terrestrial primary productivity may be exported to nearby waterbodies as dissolved organic matter (DOM) (Hope et al. 1994; Neff and Asner 2001). There, terrestrial-derived DOM constitutes a large carbon (C) pool that often surpasses the amount of living biomass (Thomas 1997) and particulate organic matter (POM) by an order of magnitude (Wetzel 1984). Despite being a large allochthonous input, terrestrial DOM is generally considered a low quality resource for aquatic food webs. The chemical composition of terrestrial DOM is heterogeneous, but is typically comprised of lignin-rich, high molecular weight humic substances, with high C : nutrient ratios (McKnight et al. 1997; Hopkinson et al. 1998). Moreover, terrestrial C recovered in aquatic ecosystems can be old (1000-4000 yrs; see Aiken et al. 1996; Raymond and Bauer 2001), suggesting that some DOM fractions are quite recalcitrant. Nevertheless, many studies have demonstrated that aquatic bacteria consume terrestrial DOM (e.g., Tranvik 1990; Bano et al. 1997; Sun et al. 1997) with the aid of extracellular enzymes (Arnosti 2003). However, bacterial productivity and growth efficiencies are generally higher on more labile, autochthonous sources of organic matter (Moran and Hodson 1990; Moran and Hodson 1994; del Giorgio and Cole 1998; Wehr et al. 1999).

Microbial assimilation of terrestrial DOM may be responsible for modifying the metabolism of recipient lake ecosystems. This view stems from the observation that many lakes are supersaturated with CO_2 (e.g., Cole et al. 1994), a condition that arises when community respiration exceeds local primary productivity. However, additional factors besides assimilation of terrestrial DOM may contribute to lakes being net sources of atmospheric CO_2. First, other sources of allochthonous C subsidize lakes and may contribute to CO_2 supersaturation. For example, lakes receive inputs of organic C from precipitation (Richey and Wissmar 1979; Likens et al. 1985), sediments (Bachmann et al. 2000; Biddanda and Cotner 2002), and particulate litterfall (Wissmar et al. 1977; France and Peters 1995). Second, lakes may be supersaturated with CO_2 because they receive groundwater or surface water inputs that are supersaturated with CO_2 (Dillon and Molot 1997; Jones et al. 2001). Third, terrestrial DOM is abiotically oxidized by solar radiation

(Osburn et al. 2001) and can account for 10-25% of the total C mineralization in lake ecosystems (Jonsson et al. 2001; Pers et al. 2001). Fourth, terrestrial DOM attenuates photosynthetically active radiation (Bukaveckas and Robbins-Forbes 2000) and contains organically bound nutrients (Williamson et al. 1999), which may modify system metabolism even if bacterial are not directly subsidized by the C content of terrestrial DOM inputs.

The primary aim of this study was to test whether terrestrial-derived DOM is a subsidy that could be responsible for patterns of observed CO_2 efflux from lake ecosystems. Specifically, experiments were designed to 1) examine the temporal dynamics of CO_2 flux under contrasting DOM regimes, 2) describe the functional relationship between CO_2 flux and terrestrial DOM loading in different lakes, and 3) test how non-energetic attributes of terrestrial DOM (i.e., light and nutrients) affect lake CO_2 flux.

MATERIALS AND METHODS

Study sites and experimental mesocosms— I used mesocosm experiments in New England lakes to test multiple hypotheses regarding DOM enrichment on C metabolism in plankton communities. Mesocosm studies are sometimes criticized because they lack ecological complexity and appropriate scale, but were useful here because they create a potential bridge between small-scale laboratory studies (see del Giorgio and Davis 2003) and comparative whole-ecosystem studies (e.g., Caraco and Cole 2000; Prairie et al. 2002; Sobeck et al. 2003). In the summer of 2001, I deployed mesocosms in Childs Pond, VT (Orange Co.) and Storrs Pond, NH (Grafton Co.). Both lakes are small (14 and

8 ha, respectively) and slightly eutrophic (chlorophyll *a* = 10-15 μg L^{-1}). The pH of Childs Pond (7.0) is lower than Storrs Pond (7.5) and is reflected by differences in alkalinity (136 and 1395 μeq L^{-1}, respectively). Replicate mesocosms consisted of 25 L polyethylene bags suspended from styrofoam rafts. Each cylindrical bag was 0.5 m long and open at the surface (0.5 m^2) for gas exchange. I filled mesocosms with whole-lake water obtained from 1.5 m depth-integrated water samples. After filling, I let the mesocosms equilibrate for two days prior to initiating experiments.

Preparation and characterization of terrestrial leachate— I obtained terrestrial organic matter from the organic (Oa/A) horizon of hydric soils on the shoreline of Childs Pond. Organic matter was leached from the soil in 0.1 N NaOH (Schnitzer 1982) for 48 h, centrifuged (2500 rpm), and sterilized (120°C) to eliminate soil-associated bacteria. I removed particulate material (> 0.7 μm) from the leachate via serial filtration. I dialyzed the leachate (500 D cellulose ester, Spectrum Labs) in a distilled water buffer for 24 h to reduce concentrations of salts and inorganic nutrients (Vinebrooke and Leavitt 1998) and to neutralize the pH.

I characterized the final leachate for a suite of elemental and organic properties. I measured DOC with a Tekmar-Dohrmann TIC/TOC analyzer after H_2SO_4 digestion. Total nitrogen (TN) and total phosphorus (TP) were measured spectrophotometrically after persulfate digestion; soluble reactive phosphorus was measured similarly, but without persulfate digestion (APHA 1998). Total inorganic N was measured as the sum of nitrate-N (NO_3+NO_2) plus ammonium-N using a Lachat QuickChem autoanalyzer. Organic N and P were estimated as the difference between total and inorganic fractions of each respective element. I measured polyphenolic compounds using the Prussian Blue method against a tannic acid standard (Herrera-Silveira and Ramírez-Ramírez 1996). I quantified humic acids as precipitated DOC after acidifying the leachate to pH 2 with H_3PO_4. I measured protein content using the BCA method (Walker 1996) with a Compat-Able Protein Assay Preparation kit (Pierce Biotechnology, Rockford, IL). I measured high molecular weight DOM as DOC retained in 12000–14000 D dialysis tubes.

***Experimental designs*—** Two main experiments were conducted in this study. First, I monitored the temporal dynamics of CO_2 flux under ambient and enriched DOM levels in Childs Pond. The ambient DOM treatment consisted of three replicate mesocosms with background concentrations of DOM (650 μM DOC). The ambient DOM treatment consisted of three replicate mesocosms with enough leachate added to increase ambient DOC concentrations by 60% (1650 μM DOC). I then measured CO_2 flux daily for the following ten days. I used univariate one-way repeated measures ANOVA (RM-ANOVA) to test for the main effect of DOM enrichment on CO_2 flux over the duration of the experiment. I used trapezoidal integration to determine how much terrestrial C was lost to the atmosphere via CO_2 flux between the two DOM treatments.

In the second experiment, I described the relationship between peak CO_2 flux and DOC concentration. Based on results from the temporal dynamics experiment, I defined peak flux as the point of maximum CO_2 flux following DOM enrichment. I used 18 mesocosms in each lake to construct a DOM gradient ranging from ambient concentrations to approximately 2500 μM DOC. I used multiple regression to determine whether DOC (continuous variable) and lake identity (dummy variable) had a significant effect on CO_2 flux, dissolved oxygen, and bacterial productivity (BP). I also used multiple regression to assess how DOC and lake identity influenced dissolved inorganic carbon (DIC), pH, a_{440}, and chlorophyll *a*. I used partial correlation coefficients to assess the relative contribution of DOC and lake identity to the regression models. I used SAS for all statistical analyses (SAS 1999).

I conducted two smaller experiments to independently assess how shading and nutrient mineralization may have influenced changes in CO_2 flux along the DOM gradient. First, I ran an experiment to evaluate how DOM-mediated light attenuation affected CO_2 flux. I entirely covered two mesocosms, having ambient DOM concentrations, with black polyethylene bags. I then compared CO_2 flux, dissolved oxygen, and BP in these two shaded mesocosms to three unshaded mesocosms with ambient DOM concentrations. I quantified shading with a Li Cor quantum sensor. The shading experiment was conducted in Storrs Pond during the peak flux experiment. Second, I conducted a nutrient enrichment experiment to assess whether mineralization of organically bound N and P could have accounted for patterns of CO_2 flux along the DOM gradient. I tested this by measuring CO_2 flux from 18 mesocosms that were enriched with inorganic nutrients at a ratio and concentrations found in the terrestrial leachate (Table 1). Nine of the 18 mesocosms received inorganic P alone (KH_2PO_4) and the remaining nine mesocosms received both inorganic P and N (KH_2PO_4 and NH_4NO_3). The inorganic nutrient experiment was conducted in Childs Pond during the peak flux experiment.

Measurements— I measured CO_2 flux using the headspace equilibrium method (Kling et al. 1992). In this procedure, 100 mL of air was left in a 2000 mL bottle filled with 1900 mL of lake water. I obtained $p\mathrm{CO}_{2\,\mathrm{(water)}}$ by sampling the headspace with a gas-tight syringe assuming equilibrium had been reached after shaking the bottle vigorously for 2 min. I obtained $p\mathrm{CO}_{2\,\mathrm{(atmosphere)}}$ from the air approximately 2 m above the lake. $p\mathrm{CO}_2$ was measured with a calibrated CID (CI-301) infrared gas analyzer (IRGA). An index of CO_2 flux was then calculated as:

$$\mathrm{CO}_2 \text{ flux} = k\,[\mathrm{CO}_{2\mathrm{(water)}} - \mathrm{Kh} \text{ x } \mathrm{CO}_{2\mathrm{(eq)}}] \qquad \text{(eq. 1)}$$

where k is the gas piston velocity, $\mathrm{CO}_{2\mathrm{(water)}}$ is CO_2 in the lake water, Kh is Henry's constant at ambient temperature, and $\mathrm{CO}_{2\mathrm{(eq)}}$ is CO_2 in the lake water if it were in atmospheric equilibrium. Our best estimates of k at low wind speeds come from studies that measure gas evasion using experimental additions of inert gases. In this study, I used a gas piston velocity of 0.63 m d^{-1}, which is an empirically derived estimate based on whole lake additions of SF_6 gas to Mirror Lake, NH (Cole and Caraco 1998). This parameter value is consistent with estimates of k found in lakes in different geographic locations using a similar SF_6 approach (Crusius and Wanninkhof 2003).

$p\mathrm{CO}_2$ measurements were taken from mesocosms in random order once per day between 1000 and 1200 h. Reasonable estimates of CO_2 flux can be obtained from this type of sampling because $p\mathrm{CO}_2$ in the atmosphere and water only exhibit a small amount of diel variation. For example, $p\mathrm{CO}_{2\,\mathrm{(atmosphere)}}$ near Childs Pond during the time of the mesocosm experiments ranged between 350–380 μatm over a 24 h period (n =1093, data not shown). Likewise, Cole and Caraco (1998) found no temporal trend in $p\mathrm{CO}_{2\,(\mathrm{water})}$ over a 24 h period, but there was a slight (2 μM) difference in $\mathrm{CO}_{2(\mathrm{water})}$ when dawn and dusk samples were pooled. The magnitude of these differences is small though when compared to the range of $p\mathrm{CO}_{2\,(\mathrm{water})}$ reported in this mesocosm study (30–2200 μatm) and among natural lakes (e.g., 300–4000 μatm; Sobek et al. 2003).

I measured dissolved oxygen and temperature with a YSI model 85 water quality monitor and pH with an Orion model 230-A pH meter. I measured light attenuation on 0.7μm-filtered water samples at 440-nm (a_{440}) using a 10-cm quartz cuvette. I estimated BP by measuring the uptake and incorporation of radiolabeled leucine into bacterial protein (Kirchman 1993). I measured phytoplankton biomass as chlorophyll *a* after cold methanol extraction (APHA 1998). I measured DIC with the IRGA as the amount of CO_2 in the headspace following the addition of 2 mL of concentrated $\mathrm{H_2SO_4}$ to 100 mL of lake water.

RESULTS

***Temporal dynamics of CO_2 flux*—**CO_2 flux was significantly different between ambient and enriched DOM treatments in the temporal dynamics experiment (RM-ANOVA, $P <$ 0.0001, $F_{9,\,90} = 24.4$, Fig. 1). CO_2 flux increased 100% 24 h after the DOM addition (day 2) and peaked 48 h later (day 4) at 27.5 ± 1.24 mM • m^{-2} • d^{-1}. CO_2 flux then decreased sharply and by day 8 there were no longer any significant differences between the two DOM treatments (t-tests, $P > 0.05$). Using trapezoidal integration, I estimated that an additional 60.0 mM m^{-2} of C was lost to the atmosphere over 10 days due to DOM enrichment (40.7 mM m^{-2} of C was lost under ambient conditions). Based on this calculation, one would expect there to be a ~33 μM (3.4%) reduction in DOC. There was no detectable decline in DOC (regression, $F_{1, 14} = 4.34$, $P=0.06$, positive relationship) or color (regression $F_{1, 25} = 0.07$, $P = 0.79$) over time, but there was a 145 μM (9%) drop in mean DOC concentration between days 2 and 3 (Fig. 1).

CO_2 flux along DOM gradients**—** Peak CO_2 flux (day 4, determined from the temporal dynamics experiment, Fig. 1) increased linearly over a broad range of DOC in both Childs Pond and Storrs Pond (Fig. 2). The slopes of this relationship were identical, but the intercept of Storrs Pond was significantly greater than that of Childs Pond (Table 2). Dissolved oxygen declined with DOC in both systems, though at a greater rate in Childs Pond compared to Storrs Pond (DOM x Lake interaction, Table 2). Bacterial productivity increased with DOM enrichment, but at a lower rate in Childs Pond (significant DOM x Lake interaction, Table 2). DIC remained constant (340-350 μM) in Storrs Pond along the DOM gradient, but increased with DOM in Childs Pond (33 to 72 μM, Table 2).

Effects of shading on CO_2 flux**—** DOM additions attenuated light (a_{440}; Table 2), but light was affected more by experimental shading (90% reduction). Experimental shading increased CO_2 flux (t-test, $t = 6.14$, $df = 3$, $P < 0.009$) and decreased dissolved oxygen (t-test, $t = 6.33$, $df = 3$, $P < 0.008$), but had no significant effect on BP (t-test, $t = 1.28$, $df = 3$, $P < 0.29$) when compared to unshaded mesocosms with ambient DOM levels. Nevertheless, the data points from the shaded mesocosm fell within the 95% confidence intervals generated by the DOM gradient analysis in Storrs Pond (see Fig. 2).

Effects of inorganic nutrient enrichment on CO_2 flux—Terrestrial DOM contains other elements besides C that may have influenced CO_2 flux (Table 1). Inorganic P additions alone did not cause CO_2 in the mesocosms to deviate from atmospheric equilibrium (-0.3 ± 0.58 mM $CO_2 \bullet m^{-2} \bullet d^{-1}$, [mean ± SE], one sample t-test, t = 0.52, df = 8, *P* = 0.62). However, additions of both inorganic N and P caused mesocosms to be undersaturated with CO_2 (-5.0 ± 1.28 mM $CO_2 \bullet m^{-2} \bullet d^{-1}$, [mean ± SE], one sample t-test, t = 3.89, df = 8, *P* = 0.005, Fig. 2).

DISCUSSION

Donor-controlled inputs of terrestrial subsidies should relax plankton resource limitation and potentially alter lake ecosystem processes. This study tested whether bacterial utilization of terrestrial-derived DOM can explain why many lakes are supersaturated with CO_2. DOM enrichment had strong effects on the dynamics and processes of plankton communities. Bacterial productivity increased with DOM supply suggesting that microbial metabolism of the terrestrial subsidy was responsible for patterns of CO_2 efflux. Although light attenuation and inorganic nutrients were positively correlated with subsidy supply, these factors were not responsible for the observed shifts in DOM-enriched systems. In the following section, I discuss my experimental findings and place them into context of recent comparative studies.

Temporal dynamics of CO_2 flux—DOM enrichment had a strong, but short-lived effect on CO_2 flux. I estimated that 3.4% of the added terrestrial C was lost to the atmosphere over the 10 day experiment. This loss most likely reflects the rapid use and subsequent exhaustion of the labile DOM pool. Accordingly, DOC levels dropped during the period of peak CO_2 flux (Fig. 1). After day 3 however, DOC remained constant suggesting that most of the added leachate constituted recalcitrant C. These results agree with DOC utilization studies in riverine ecosystems where a small fraction of the DOC pool (~2.5%) was consumed within a five day period while the remaining labile DOC pool (5-7.5%) was consumed over a longer period of time (~1 month; Raymond and Bauer 2000).

Whole-lake observations also demonstrate that CO_2 flux is highly variable through time. In temperate lakes, most variability occurs during spring and autumn mixing events where there are pronounced peaks in CO_2 flux (e.g., Striegl et al. 2001). CO_2 flux is more stable during periods of summer stratification (Kelly 2001), but this is not true for all lakes. For example, $pCO_{2(\mathrm{water})}$ fluctuated more through time in high vs. low DOC lakes (Hope et al. 1996), suggesting that terrestrial DOM loading rates influence temporal trends in CO_2 flux.

DOM loading rates are variable through time and are strongly influenced by precipitation. For example, Hinton et al. (1997) found that 30-70% of the annual DOC export to streams in central Ontario was associated with episodic storm events. Here, leachate additions simulated a pulse of terrestrial DOM that might accompany a precipitation event. Terrestrial DOM additions affected CO_2 flux for approximately eight days (Fig. 1). This duration is longer than the frequency of growing-season rain events in Hanover, NH (3.8 ± 0.13 d, http://lwf.ncdc.noaa.gov), suggesting that the temporal variability of CO_2 flux may be dampened by frequent inputs of terrestrial DOM associated with precipitation. Future studies should examine how the timing, frequency, and magnitude of DOM inputs influence CO_2 flux. Such information would provide insight into the importance of terrestrial subsidies for lake metabolism.

CO_2 flux along DOM gradients — Many studies have documented that lakes are supersaturated with CO_2. Fewer studies have attempted to link patterns of CO_2 flux and DOC concentrations. I found that peak CO_2 flux increased linearly over a range of DOC that captures most of the natural variation found for lakes worldwide (Fig. 2). These results qualitatively agree with a number of recent comparative lake surveys. First, in North America, Hope et al. 1996) found that pCO$_2$ increased linearly with DOC (150–1600 μM) in Wisconsin lakes. Likewise, Prairie et al. (2002) found that CO_2 flux increased linearly over a smaller range of DOC (167-933 μM) in Quebec lakes. A positive DOC-CO_2 flux relationship has also been reported for Swedish lakes (Jonsson et al. 2003), even for systems up to 2700 μM DOC (Sobek et al. 2003). Collectively, these results support the hypothesis that terrestrial DOM subsidies alter aquatic ecosystem function and cause lakes to be supersaturated with CO_2. Moreover, there is currently no evidence that CO_2 flux plateaus with increasing concentrations of DOM.

Despite these findings, it is currently difficult to predict when lakes switch from being sinks to sources of atmospheric CO_2. Based on ratios of bacterial productivity : primary productivity, it was originally proposed that Scandinavian lakes become net heterotrophic at ~850 μM DOC (Jansson et al. 2000). These estimates agree with observations made for Wisconsin lakes (~850 μM; Hanson et al. 2003). However, recent surveys indicate Swedish lakes become supersaturated with CO_2 at much lower DOC concentrations (~100 μM; Jonsson et al. 2003; Sobek et al. 2003). In this study, lake identity influenced when mesocosms became supersaturated with CO_2. In Childs Pond, $pCO_{2\,(\mathrm{water})}$ was at equilibrium with $pCO_{2\,(\mathrm{atmosphere})}$ in mesocosms with ambient DOM (640 μM DOC; Fig. 2). This transition point falls in the middle of literature reported values above. In contrast, Storrs Pond may always be supersaturated with CO_2 as indicated by the positive intercept of the DOC–CO_2 flux relationship (Table 2, Fig. 2). This result indicates that benthic respiration and CO_2-supersaturated water inputs may be important sources of DIC in Storrs Pond (see Kling et al. 1992; Jones et al. 2001).

Shading and CO_2 flux— Experimental additions of DOM significantly reduced light penetration (Table 2). This may have created light limitation for phytoplankton (e.g., Klug 2002), which in turn may have favored CO_2 supersaturation. At first glance, the results suggest that the light attenuating properties of DOM be an important driver of CO_2 flux. For example, shading increased CO_2 flux and decreased dissolved oxygen concentrations. However, CO_2 flux data from the shaded mesocosms lie well within the 95% confidence intervals generated by the regression analysis (see Fig. 2) indicating that the energetic content of the terrestrial DOM had a larger effect on CO_2 flux than its optical properties. This interpretation agrees with a recent whole-lake C enrichment experiment, which found that the structure and function of a sucrose-enriched lake behaved similarly to a humic reference lake (Blomqvist et al. 2001).

Inorganic nutrients and CO_2 flux — Terrestrial DOM contained other elements besides C that are known to influence lake ecosystem metabolism (Table 1). In particular, the mineralization or dissociation of organically bound N and P could affect rates of photosynthesis and respiration. In fact, indicators of lake fertility (i.e., TP, total TN, and chlorophyll *a*) are often positively correlated with DOC (Nürnberg and Shaw 1998). However, inputs of N and P alone did not account for the observed pattern of increasing CO_2 flux along the DOM gradient (Fig. 2). Inputs of inorganic P alone had no effect on CO_2 flux, whereas inputs of both inorganic N and P caused mesocosms to be undersaturated with CO_2 (Fig. 2). These results highlight how lakes respond differently to inorganic vs. organic subsidies. In general, inorganic nutrient enrichment tends to stimulate primary productivity in lake ecosystems and thus favor net autotrophy. However, it appears that lake CO_2 flux may be less responsive to inorganic nutrients than organic material. For example, maximum CO_2 efflux was 38 mM $CO_2 \bullet m^{-2} \bullet d^{-1}$ in this study and can be as high as 50 mM $CO_2 \bullet m^{-2} \bullet d^{-1}$ in natural lakes (Kling et al. 1992; Cole et al. 2000). However, even under high levels of inorganic nutrient loading, CO_2 influx was -5 mM $CO_2 \bullet m^{-2} \bullet d^{-1}$ in this study and is rarely less than -10 mM $CO_2 \bullet m^{-2} \bullet d^{-1}$ in natural lakes (Kling et al. 1992; Cole et al. 2000). In other words, lakes may have a tendency to be larger sources than sinks of atmospheric CO_2.

DOM quality and lake metabolism — The origin, age, and composition of terrestrial-derived DOM affects bacterial DOM consumption and could possibly influence patterns of lake CO_2 flux. Two chemical attributes that affect bacterial DOM consumption are size and stoichiometry. It has been traditionally viewed that bacteria consume low molecular weight DOM (LMW DOM) more readily than high molecular weight DOM (HMW DOM) because it is moved more readily across cell membranes. An alternate interpretation is that HMW DOM is more bioreactive because it represents a diagenetically “fresher” bacterial substrate (i.e., the size-reactivity continuum model; Amon and Benner 1996). Regarding stoichiometry, it is generally thought that DOM quality is negatively correlated with C:N and C:P ratios because N and P are nutrients that can be used for synthesizing nucleic acids and ATP. In fact it has been shown that bacterial growth rates (Hunt et al. 2000) and growth efficiencies (Goldman et al. 1987) are predictably higher on DOM sources with low C:N ratios.

Based on these two chemical criteria, the DOM source used in this study can be regarded as a high quality subsidy for planktonic bacteria. The leachate had a high percentage of HMW DOM (92%), which in the context of size-reactivity continuum model, is consistent with the leachate being created from fresh organic litter. In addition, the DOM had relatively low C:N and C:P ratios (9 and 160, respectively) compared to ratios found in the DOM of lake water (C:N = 7-170, C:P = 14-4000; Sun et al. 1997; Anesio et al. 2000). It is possible that different patterns of CO_2 flux would emerge if this experiment was conducted with different DOM sources. For example, bacterial productivity (but not respiration) increases on DOM sources with more P (Lennon and Pfaff, in preparation). Future comparative studies might explain more variation in DOC-CO_2 flux relationships by including N and P data as covariates in their analyses.

Conclusions and implications — Terrestrial DOM modifies chemical and physical features of lakes including light regimes, pH, thermocline depth, UV stress, and nutrient status (see Williamson et al. 1999). Terrestrial DOM also represents a potentially large energy source for plankton communities. Results here suggest that the C content of terrestrial DOM serves as a subsidy for planktonic bacteria, which can ultimately affect whether lakes function as sources or sinks of atmospheric CO_2. From a biogeochemical perspective these findings are important because they provide mechanistic insight into the net heterotrophy phenomenon, which has been observed in a diversity of aquatic habitats including large expanses of the open ocean (Hoppe et al. 2002). However, this study does not demonstrate that terrestrial C flows through plankton food webs once it is assimilated by aquatic bacteria. To date, empirical evidence provides conflicting results about the relative importance of DOM as an energetic subsidy for higher trophic levels in lake ecosystems (Grey et al. 2001; Cole et al. 2002). This issue deserves further attention especially since current trends in climate change and land-use alter DOM export from terrestrial to aquatic ecosystems (France et al. 2000; Freeman et al. 2001).

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Table 1. Chemical characteristics of terrestrial leachate used for DOM enrichment experiments in Childs and Storrs Ponds. All values are from concentrated leachate. Mean values (mM) and standard errors (SE) are based on 3 – 8 replicate subsamples of the leachate. Ratios of carbon, nitrogen, and phosphorus were calculated from the mean value of each element.

Leachate Characteristic	mM	SE
Carbon	198	36.8
Nitrogen, Total	22.7	2.43
Nitrogen, Inorganic	16.4	0.00
Nitrogen, Organic	9.8	0.32
Phosphorus, Total	1.2	0.03
Phosphorus, Soluble Reactive	0.32	0.004
Phosphorus, Organic	0.91	0.025
Polyphenolic compounds	22.1	1.53
Humic Acids	121	1.0
Protein	10.1	0.47
High molecular weight DOC	183	48.5
Carbon : Total Nitrogen	8.7	
Carbon : Total Phosphorus	161	
Total Nitrogen : Total Phosphorus	18.5	

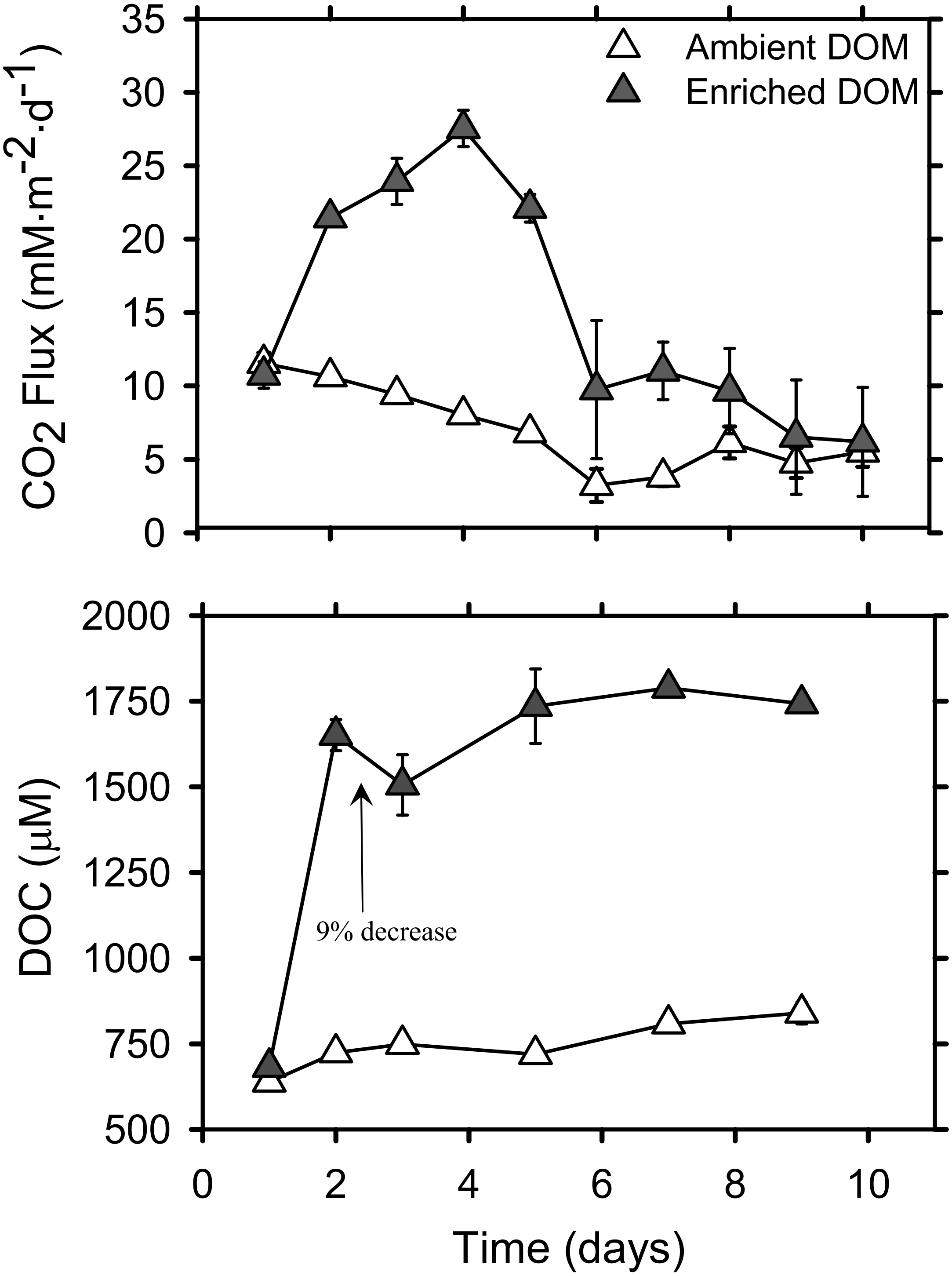
Table 2. Parameter estimates ($B_0 – B_3$) and summary statistics (R^2 and partial *r*) for terrestrial DOM enrichment experiments in two New England lakes. Predictor variables in the multiple regression were DOC (continuous variable) and lake identity (dummy variable). Significant parameter estimates ($P < 0.05$) and marginally significant parameter estimates (*, $0.05 < P < 0.1$) are indicated by numerical values (means ± SE) in each cell; ns means a parameter estimate was not significant. A significant B_0 means that the intercept was different from zero. A significant B_1 means that the slope (DOC) was different from zero. A significant B_2 means that there was a change in the intercept between lakes. A significant B_3 means that there was a change in slopes between ponds (i.e., a DOC x Lake interaction). R^2 represents the coefficient of determination from each multiple regression model. Partial correlation coefficients (partial *r*) estimate the relative contribution of DOC and lake identity to the overall multiple regression.

Response Variable	**Intercept (B_0)**	**DOC (B_1)**	**Lake (B_2)**	**DOC x Lake (B_3)**	**R^2**	**Partial *r* (DOC)**	**Partial *r* (lake)**
CO_2 flux (mM • m^{-2} • d^{-1})	10.7 ± 1.77	0.015 ± 0.0013	-23.5 ± 2.77	ns	0.94	0.98	-0.95
Dissolved O_2 (μM)	222 ± 2.2	-0.01 ± 0.002	30.8 ± 3.47	-0.009 ± 0.0024	0.90	-0.90	0.87
Bacterial productivity (μmol C d^{-1})	ns	0.02 ± 0.003	ns	-0.01 ± 0.004	0.62	0.83	-0.45
Dissolved inorganic carbon (μM)	348 ± 2.6	ns	-331 ± 4.0	0.02 ± 0.003	0.99	0.59	-0.99
pH	7.5 ± 0.02	$-9E^{-5} \pm 1.8E^{-5}$	-0.4 ± 0.038	ns	0.96	-0.75	-0.97
LOG_{10} chlorophyll *a* (μg L^{-1})	1.8 ± 0.19	$2\ E^{-4} \pm 1.2\ E^{-4}$ *	-0.56 ± 0.240	ns	0.50	0.53	-0.47
Color (a_{440})	-1.3 ± 0.27	$9\ E^{-3} \pm 2\ E^{-4}$	-2.9 ± 0.42	ns	0.99	0.99	-0.95

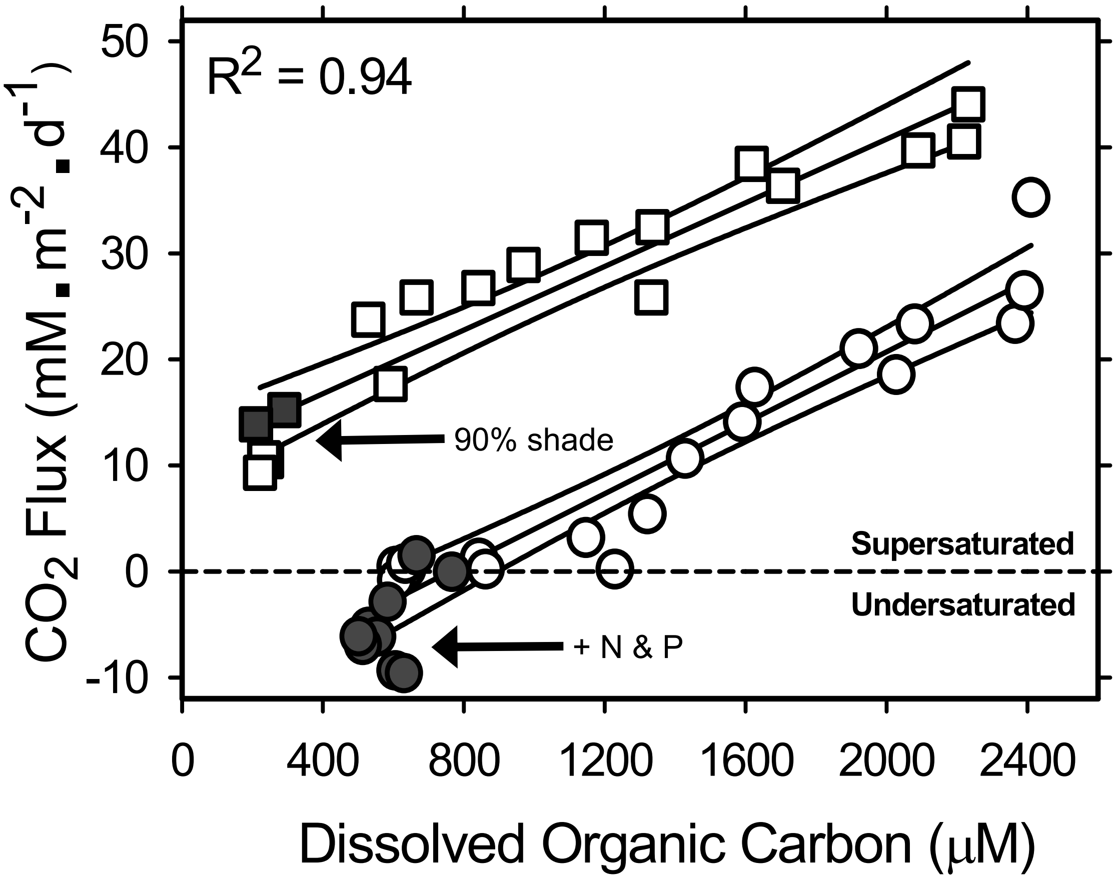
FIGURE CAPTIONS

Figure 1. Temporal dynamics of CO_2 flux (upper panel) and DOC (lower panel) from in situ aquatic mesocosms in Childs Pond, Vermont with ambient (△, 650 μM DOC) and enriched (▲, 1650) levels of terrestrial-derived DOM. DOM enrichment had a significant effect on CO_2 flux over the ten-day experiment (RM-ANOVA, $P < 0.0001$, $F_{9, 90} = 24.4$). Data points represent mean ± SE.

Figure 2. Peak CO_2 flux from aquatic mesocosms four days after creating a terrestrial DOM gradient. Multiple regression revealed that mesocosms in both Childs Pond (○) and Storrs Pond (□) responded identically (i.e., same slopes) to DOM enrichment (Table 2). DOM colored the water (Table 2), but independent experiments showed that the increased CO_2 flux was not due to manipulated light reduction (■). I tested whether mineralization of organically bound nutrients may have increased CO_2 flux by adding inorganic N and P to mesocosms (no organic carbon) at a ratio and concentrations found in the terrestrial leachate (Table 1). Additions of P alone did not move systems from equilibrium (one sample t-test, $t = 0.52$ $df = 8$, $P = 0.62$, data not shown), but additions of both N and P caused mesocosms in Childs Pond to be undersaturated with CO_2 (●, one sample t-test, $t = 3.89$, $df = 8$, $P = 0.005$). Lines are from least squares regression and calculations of 95% confidence intervals.

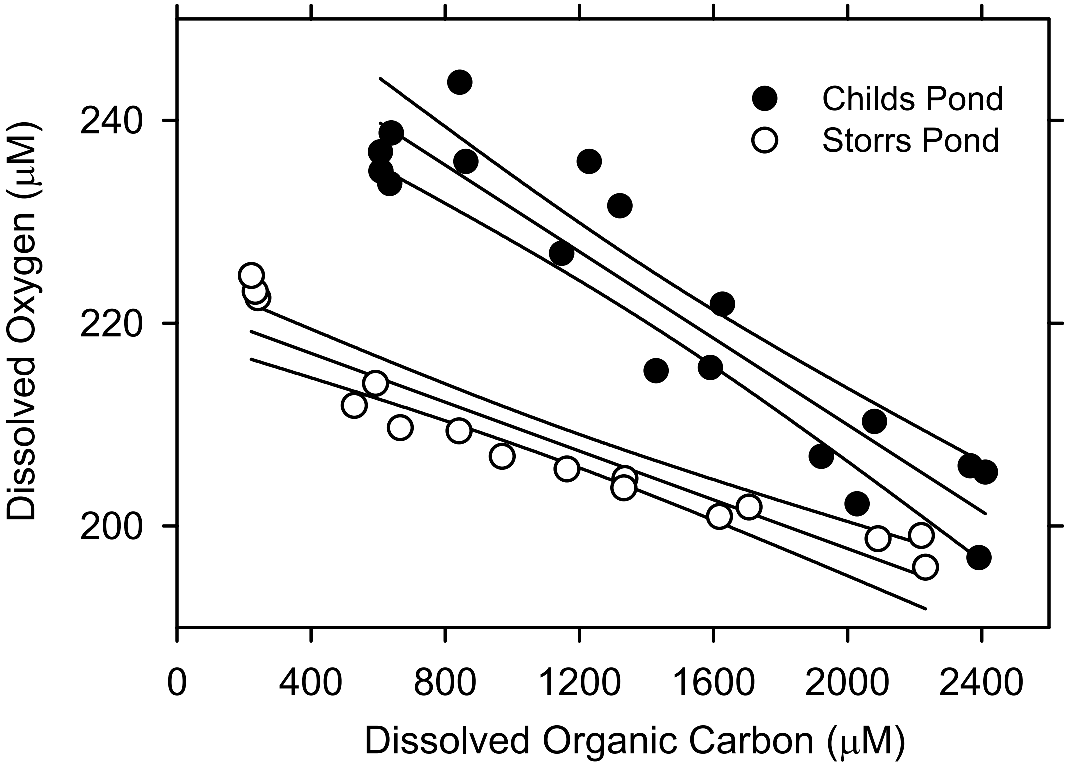


35
30
25
20
15
10
5
0
CO2 Flux (mM·m-2·d-1)
△ Ambient DOM
▲ Enriched DOM
2000
1750
1500
1250
1000
750
500
DOC (μM)
9% decrease
0
2
4
6
8
10
Time (days)

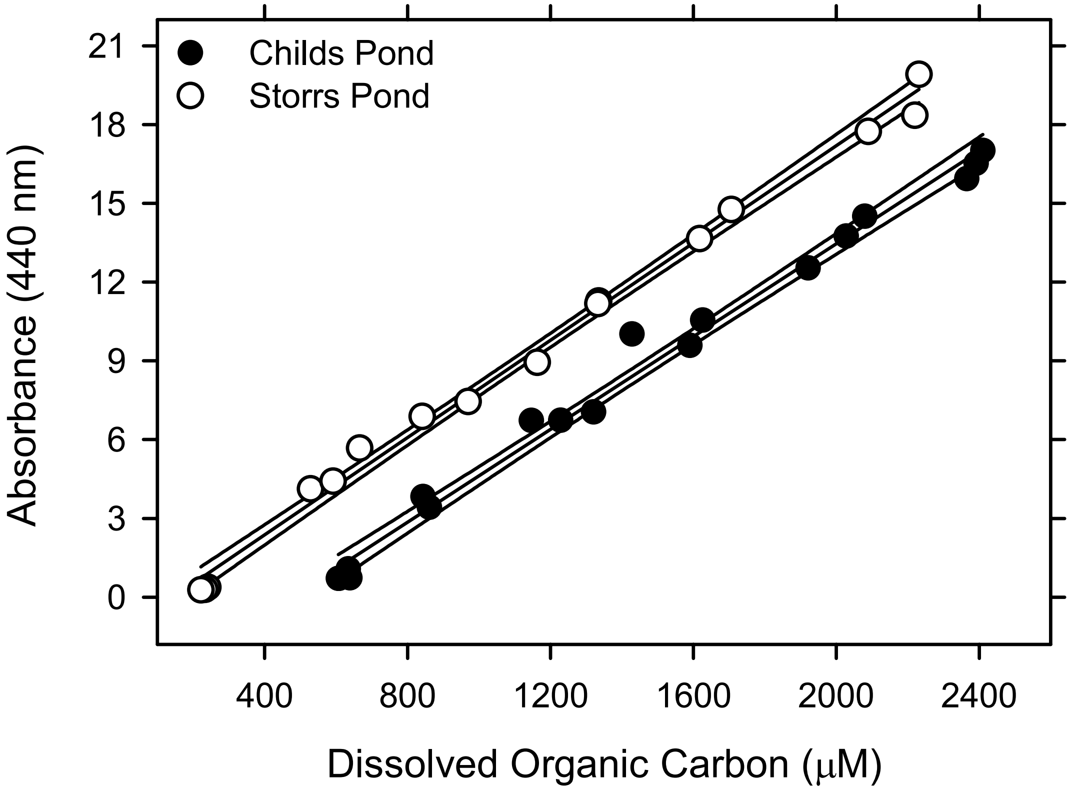

R^2 = 0.94
CO$_2$ Flux (mM ▪ m^{-2} ▪ d^{-1})
50
40
30
20
10
0
-10
90% shade
Supersaturated
Undersaturated
+ N & P
0
400
800
1200
1600
2000
2400
Dissolved Organic Carbon (µM)

APPENDIX

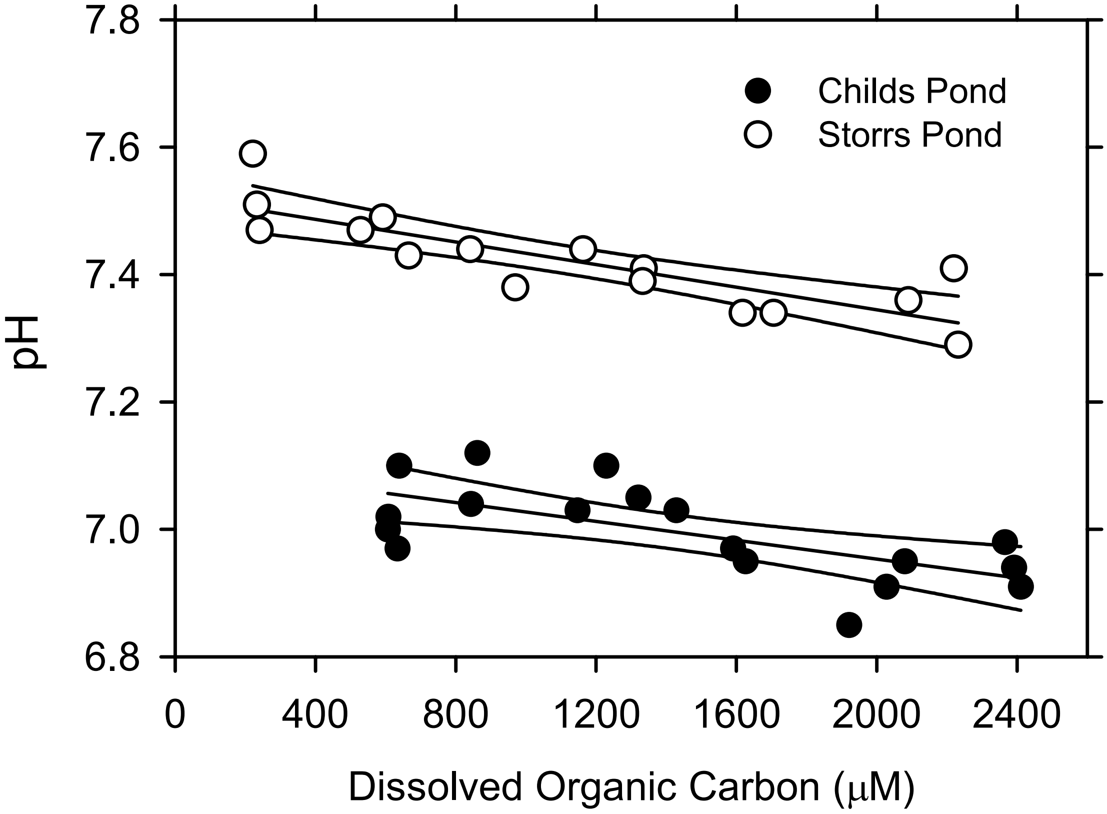
Leachate Characteristic	mM		SE	Sample size
Aluminum	1.03		0.009	2
Arsenic	<1.33	*	n/a	2
Cadmium	<0.04	*	n/a	2
Calcium	0.92		0.000	2
Carbon	197.6		36.85	6
Chromium	<0.19	*	n/a	2
Copper	<0.31	*	n/a	2
Iron	0.93		0.008	2
Lead	<0.48	*	n/a	2
Magnesium	0.19		0.019	2
Manganese	0.03		0.004	2
Nickel	<0.34	*	n/a	2
Nitrogen, Total	22.71		2.439	6
Nitrogen, Ammonia	11.49		0.000	2
Nitrogen, $NO_3 + NO_2$	4.93		0.000	2
Nitrogen, Organic	9.82		0.323	6
Phosphorus, Total	1.23		0.026	7
Phosphorus, Soluble Reactive	0.32		0.004	8
Phosphorus, Organic	0.91		0.025	7
Potassium	0.29		0.059	2
Silicon	0.59		0.000	2
Sodium	9.90		0.090	2
Zinc	0.45		0.019	2
Polyphenolic compounds	22.1		1.53	3
Humic Acids	120.5		1.01	3
Protein	10.1		0.47	3
High molecular weight DOC	182.6		48.50	3
Carbon : Total Nitrogen	8.7			
Carbon : Total Phosphorus	160.7			
Total Nitrogen : Total Phosphorus	18.5			
Carbon : Organic Nitrogen	20.1			
Carbon : Organic Phosphorus	217.1			
Organic Nitrogen : Organic Phosphorus	10.8			

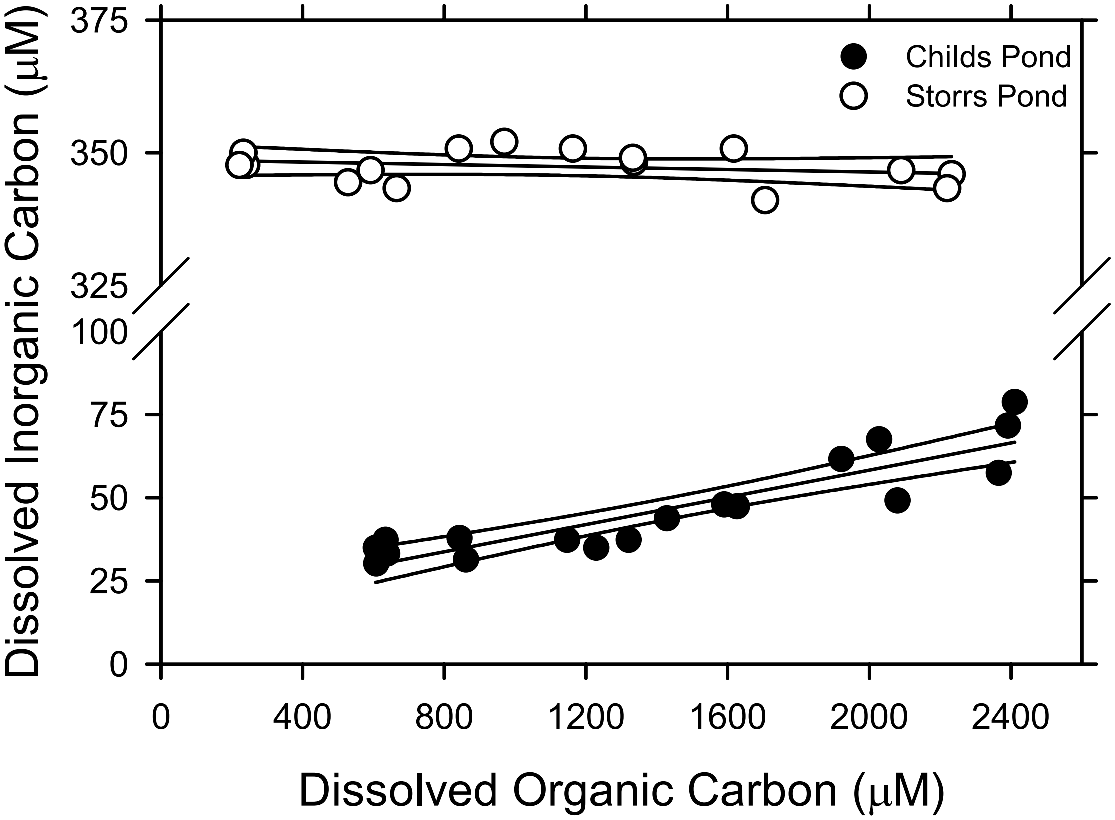


Childs Pond
Storrs Pond
Dissolved Oxygen (μM)
200
220
240
Dissolved Organic Carbon (μM)
0
400
800
1200
1600
2000
2400



● Childs Pond
○ Storrs Pond
Absorbance (440 nm)
Dissolved Organic Carbon (μM)
21
18
15
12
9
6
3
0
400
800
1200
1600
2000
2400


7.8
7.6
7.4
7.2
7.0
6.8
pH
0
400
800
1200
1600
2000
2400
Dissolved Organic Carbon (μM)
● Childs Pond
○ Storrs Pond



375
350
325
100
75
50
25
0
0
400
800
1200
1600
2000
2400
Dissolved Inorganic Carbon (μM)
Dissolved Organic Carbon (μM)
● Childs Pond
○ Storrs Pond

CHAPTER 4: LAKE CARBON FLOW IS MODIFIED BY THE CONCENTRATION OF TERRESTRIAL-DERIVED DISSOLVED ORGANIC MATTER

ABSTRACT

Terrestrial ecosystems export large quantities of dissolved organic matter (DOM) to aquatic ecosystems. Terrestrial-derived DOM can serve as a supplemental resource for heterotrophic bacteria and thus influence whether lakes function as sources or sinks of atmospheric CO_2. However, the degree to which terrestrial DOM subsidizes plankton food webs remains unclear. We conducted a comparative survey of 70 lakes in the northeastern U.S. along a gradient of terrestrial-derived DOM. We used naturally occurring stable isotopes of CO_2, particulate organic matter (POM), and crustacean zooplankton to evaluate the efficiency of terrestrial carbon flow in lake food webs. We found that the concentration of epilimnetic CO_2 increased with dissolved organic carbon (DOC), suggesting that terrestrial-derived DOM is assimilated and respired by lake biota. Moreover, stable isotope ratios of CO_2 became progressively depleted in ^{13}C relative to the atmosphere, suggesting that phytoplankton rely more on heterotrophically-respired CO_2 with increasing concentrations of terrestrial DOM. Stable isotope ratios of POM and zooplankton also declined with dissolved organic carbon (DOC) at the same rate as CO_2. However, based on estimates of phytoplankton stable isotope ratios, we found that the POM pool was diluted with another carbon source, most likely terrestrial organic matter. Although a substantial fraction of the assimilated terrestrial-carbon was used inefficiently and recycled as CO_2, our evidence suggests that both POM and zooplankton are subsidized by terrestrial carbon in lakes with low and high concentrations of DOC.

INTRODUCTION

Ecologists have long recognized that spatially separated ecosystems are connected by the movement of nutrients and organic matter (Odum 1971; Likens 2001). Recently, however, there has been realization that these allochthonous materials has important implications for food web dynamics and ecosystem processes (e.g., Polis et al. 1997; Kerfoot and Kirk 1991; Menge et al. 2003; Grimm et al. 2003). For example, freshwater, estuarine, and coastal marine ecosystems all receive large inputs of allochthonous material as dissolved organic matter (DOM) from terrestrial ecosystems (Goni et al. 1997; Neff and Asner 2001; Raymond and Bauer 2001; Aitkenhead-Peterson et al. 2003). Although historically overlooked (see Wetzel 1995; Hessen and Tranvik 1998), there has been a recent shift in the perceived role of terrestrial DOM in aquatic ecosystems (Williamson et al. 1999). In addition to altering numerous physical and chemical features (e.g., Jones 1992, Nürnberg and Shaw 1998), DOM appears to affect the metabolism of aquatic ecosystems. For example, almost 90% of the lakes in a world-wide survey were supersaturated with CO_2 (Cole et al. 1994). This pattern is consistent with the phenomenon of net heterotrophy, in which community respiration (R) exceeds gross primary productivity (GPP). Ecosystems must receive an external energy source in order to be persistently net heterotrophic (Cole et al. 2000). Accumulating evidence now suggests net heterotrophy in aquatic ecosystems is supported in part by the metabolism of terrestrial-derived DOM (Prairie et al. 2002; Hanson et al. 2003; Jonsson et al. 2003; Lennon 2004).

The importance of DOM as an energetic subsidy for lake food webs, however, remains unclear partly because bacteria, the primary consumers of terrestrial DOM, often have low growth efficiencies. For example, in some oligotrophic waterbodies, up to 99% of assimilated carbon is lost from the planktonic food web as CO_2 respired by bacteria (del Giorgio and Cole 1998). In these unproductive lakes, the assimilation of terrestrial carbon helps bacteria meet their energetic demands, but the subsequent transfer of allochthonous matter to higher trophic levels is negligible. In productive waterbodies, bacteria respire far less assimilated carbon (~50%; del Giorgio and Cole 1998) and the remaining organic matter is available for bacterial biomass production. Under productive conditions, therefore, more terrestrial carbon may be transferred to higher trophic levels, depending on zooplankton community structure. For example, the trophic transfer of terrestrial carbon in copepod-dominated lakes should be relatively inefficient because these zooplankton feed selectively upon phytoplankton or smaller zooplankton, not bacteria (Jürgens et al. 1997; Zollner et al. 2003). In contrast, the trophic transfer of terrestrial carbon in cladoceran-dominated lakes should be more efficient because these generalist grazers are often capable of feeding on bacteria (Pace and Funke 1991; Jürgens et al. 1994), including those subsidized by terrestrial DOM. Therefore, the energetic importance of terrestrial DOM may vary among lakes depending on lake productivity, bacterial growth efficiencies, and food web structure.

Studying how lakes use terrestrial-derived DOM lakes is complicated by the logistical difficulty of distinguishing between locally-produced and terrestrial-derived carbon within plankton food webs. Stable isotope analysis is an effective approach for tracking carbon flow and is often used to infer trophic relationships within food webs (e.g., Hart et al. 2000; Vander Zanden et al. 2003; Simon et al. 2003). Recently, the question of whether terrestrial carbon supports aquatic food webs was addressed in studies that experimentally manipulated the isotopic signature of phytoplankton in three lakes (Cole et al. 2002; Pace et al. 2004). Cole et al. (2002) showed that bacteria respired large amounts of terrestrial carbon, but that these subsidies made only minor contribution to the biomass of invertebrates and fish. In contrast, Pace et al. (2004) demonstrated that 40-55% of the particulate organic matter (POM) and 22-50% of the zooplankton carbon was derived from terrestrial ecosystems. Although possibly due to variations in experimental design (Pace et al. 2004), these results suggest that there may be important among-lake differences determining the flow of terrestrial carbon in aquatic food webs.

We tested hypotheses about the efficiency of terrestrial carbon flow in lakes using naturally occurring stable isotope ratios of CO_2, POM, and crustacean zooplankton. If terrestrial carbon is used efficiently (Fig. 1a), we expect phytoplankton to be a less important resource for crustacean zooplankton as DOM concentrations increase. As a consequence, the isotope ratios of POM and cladoceran zooplankton should converge upon the isotopic signature of terrestrial organic matter with increasing DOM (*sensu* Jones et al. 1999). In contrast, if terrestrial carbon is used inefficiently (Fig. 1b), we expect phytoplankton-derived carbon to dominate the POM pool along the entire DOM gradient. As such, any shift in the isotopic signature of POM over the DOM gradient should be due to changes in the isotopic ratio of CO_2 (*sensu* France et al. 1997). Furthermore, there should be no differences in the stable isotope ratios of cladoceran and copepod zooplankton when there is inefficient terrestrial carbon flow.

METHODS

During the summers of 2002 and 2003, we sampled lakes in the northeastern US in attempt to capture a natural gradient of terrestrial-derived dissolved organic matter (DOM). We selected lakes based on recommendations from regional limnologists and by consulting public databases that contained information on the color or dissolved organic carbon (DOC) concentration of lake water. In total, we visited 70 lakes in New Hampshire (39), Vermont (18), Maine (8), New York (4), and Connecticut (1) (see Fig. 2). Eight of these lakes were sampled in both years, but we have treated the data from these samples as independent following Carpenter et al. (1991).

This comparative study comprises two parts. First, we conducted an among-lake survey to test hypotheses about epilimnetic carbon cycling along a gradient of terrestrial DOM. Second, since epilimnetic isotope ratios may be influenced by hypolimnetic conditions (del Giorgio and France 1996), we conducted an intensive within-lake survey of three lakes having low, medium, and high DOM to assess how stable isotope ratios varied with depth.

Sample collection— For both parts of our study, we restricted our sampling to a 6 wk period from late July through early September. We sampled all lakes at a central location with the exception of impoundments, which were sampled near dams. All data from the among-lake survey represent epilimnetic samples. We determined the depth of the epilimnion based on temperature and dissolved oxygen (O_2) measurements taken at 0.5 m intervals with a Quanta Hydrolab water system. We obtained depth integrated water samples with a column sampler constructed from PVC tubing with an attached swing-flap check valve. We obtained depth integrated zooplankton samples with an 80 μm net. For the within-lake survey, we obtained water samples and zooplankton samples from discrete depths with a horizontal Van Dorn sampler and a Schindler-Patalas trap (80 μm net), respectively. In addition, we collected DIC samples from the inlet streams of each waterbody in the within-lake survey.

Estimation of terrestrial DOM— We used DOC and color as surrogate measures of terrestrial-derived DOM. We measured dissolved organic carbon (DOC) on 0.7 μm-filtered (Whatman GF/F) samples with a Schimadzu TOC-5000 total carbon analyzer. We measured color on 0.7 μm-filtered (Whatman GF/F) water samples at 440 nm using a 10-cm quartz cuvette. We expressed color as an absorbance coefficient: $a_{440} = 2.303 \times$ (absorbance at 440 nm / 0.1 m) (Cuthbert and Delgiorgio 1992).

Stable isotope ratios of inorganic carbon pools— In 2003, we measured the concentrations and stable isotope ratios of dissolved inorganic carbon (DIC) in 36 lakes. First, we prepared sample vials by injecting 150 μL of H_3PO_4 into a 10 mL vial. We then sealed the vial with a septum cap and flushed it for 5 min with He gas using a double-holed needle. In the field, we injected 5 mL of lake water with a 10 mL gas-tight syringe into the sample vial. In the laboratory, we forced a small stream of He into the sample and the displaced gas was fed through capillary tubing and a water removal system (The Gas Bench, Thermo Finnigan). The displaced gas was then passed through a 2 m HayeSep D micro-packed stainless steel column kept at a constant 50°C for separation of CO_2 from other gases. We measured the concentration and $\delta^{13}C$ of DIC on a Thermo-

Finnigan Delta Plus XL mass spectrometer. We then calculated the concentrations and $\delta^{13}C$ of aqueous carbon dioxide ($CO_{2(aq)}$) based on DIC, temperature, pH, and literature-reported fractionation factors (Mook et al. 1974).

Estimation of phytoplankton staple isotope ratios— Estimates of the phytoplankton $\delta^{13}C$ ($\delta^{13}C_{PHYTO}$) are important for understanding the contribution of allochthonous material to the pool of particulate organic matter (POM). Unfortunately, it is difficult to isolate phytoplankton from the heterotrophic organisms and detritus in the POM size fractions (e.g., Jones et al. 1998; Pel et al. 2003). Therefore, we approximated $\delta^{13}C_{PHYTO}$ by estimating the photosynthetic fractionation factor (α) based on $CO_{2(aq)}$ concentrations. Although α is influenced by other factors including phytoplankton growth rates, it is well documented that discrimination against ^{13}C increases with $CO_{2(aq)}$ concentrations (Yoshioka 1997). As such, we used an empirical equation derived from marine systems (Hinga et al. 1994) to estimate α based on our measurements of epilimnetic $CO_{2(aq)}$:

$$\alpha = 1.0007 \ + \ 0.0019 \ \times \ \left(\frac{CO_{2(aq)} - 5.6}{CO_{2(aq)}} \right) \qquad \text{(eq. 1)}$$

We then calculated $\delta^{13}C_{PHYTO}$ as ($\delta^{13}C_{CO2}$ - ε), where $\varepsilon = 1000 \ln(\alpha)$ (Yoshioka 1997).

Stable isotopes of organic carbon pools— In 2002 and 2003, we measured the stable isotope ratios of POM and zooplankton. To obtain the $\delta^{13}C$ of POM ($\delta^{13}C_{POM}$), we dried samples at 60°C after sieved (80 μm) water was retained on precombusted 0.7 μm filters (Whatman GF/F). For zooplankton, we isolated animals from samples under a dissecting microscope. We separated cladocerans from copepods when they co-occurred to obtain $\delta^{13}C$ estimates for each group of zooplankton then filtered the isolated zooplankton onto precombusted 0.7 μm filters (Whatman GF/F) and dried them at 60°C. The total zooplankton isotope ratio ($\delta^{13}C_{ZP}$) represents the isotope ratio of the numerically abundant taxa (>100 animals per sample). When there was more than one taxonomic group of abundant zooplankton in a sample, $\delta^{13}C_{ZP}$ represents the mean $\delta^{13}C$ of these populations.

We also measured the $\delta^{13}C$ of DOM and sediments for the three lakes that we intensively sample in 2003. We measured the $\delta^{13}C$ of DOM ($\delta^{13}C_{DOM}$) by first acidifying 1 L of filtered (Whatman GF/F) water with HCl to inhibit microbial activity and remove carbonate. We then recovered the dried organic matter after the sample had been evaporated in a glass beaker at 60°C (Darren Bade, Univ. Wisconsin, personal communication). For the $\delta^{13}C$ of sediments, we took replicate samples within each of the three lakes using an Eckman dredge. We dried the sediment at 60°C and treated the samples with 1 N HCl to remove any carbonate isotope signal (Midwood and Boutton 1998).

All of the organic carbon samples were analyzed for $\delta^{13}C$ at the UC Davis Stable Isotope Facility using a PDZ Europa trace gas analyzer and a continuous-flow Europa 20/20 isotope ratio mass spectrometer (IRMS). All inorganic and organic carbon isotope values are expressed in delta notation: $\delta = [(R_{\text{sample}} / R_{\text{reference}}) - 1] \times 1000$, where R is the ratio of ^{13}C to ^{12}C for samples and reference material (Vienna Peedee Belemnite).

***Statistical analyses*—** We used several types of linear regression to analyze data from the among-lake survey. We used indicator variables multiple regression (Neter et al. 1996) to test hypotheses about lake carbon cycling by contrasting the responses of different $\delta^{13}C$ pools (CO_2, POM, and zooplankton were coded with categorical indicator variables) to DOC (continuous predictor). Like analysis of covariance (ANCOVA), we centered the DOC data by subtracting each observation from the mean value. To test for statistical differences in $\delta^{13}C$, we constructed 95% confidence limits around the difference of parameter estimates for the carbon pools (see Neter et al. 1996, pp 455-490). We concluded that there was no difference between different carbon pools if the confidence limits for the difference between the parameter values contained zero.

RESULTS

***Terrestrial DOM gradient*—** The lake survey data suggest that we captured a gradient of terrestrial-derived DOM. DOC concentrations ranged from 120 – 2955 µmol/L and increased as a power function of water color (a_{440}; see Fig. 3):

$$\text{DOC} = 144 + 182\,(a_{440}{}^{0.63}),\ r^2 = 0.83,\ P < 0.0001 \qquad \text{(eq. 2)}$$

We removed two outliers prior to evaluating any of the stable isotope data. The two lakes with the highest DOC had much lower color than predicted by eq. 2. This suggests that a substantial portion of the measured DOC in these lakes may have been due to phytoplankton production and not terrestrial DOM. In addition, the removal of these lakes from our analyses eliminated a 1200 µmol/L gap in our DOC gradient.

Epilimnetic $CO_{2(aq)}$ along the DOC gradient— Concentrations of epilimnetic $CO_{2(aq)}$ increased logarithmically along the DOC gradient:

$$\mathrm{Log}_{10}\ CO_{2(aq)} = 0.91 + 0.0011\ \mathrm{DOC};\ r^2 = 0.55,\ P < 0.0001 \qquad \text{(eq. 3)}$$

Using Henry's constant and epilimnetic water temperatures, we calculated equilibrium CO_2 concentrations for each lake assuming a mean atmospheric pCO_2 of 380 µatm. Equilibrium CO_2 concentrations ranged from 11.8 – 16.0 µmol/L. Based on these estimates, 30 of 36 lakes (83%) were supersaturated with CO_2 relative to the atmosphere (Fig. 4). Using eq. 3 and the range of equilibrium CO_2 values, we estimated that the lakes in our study became supersaturated with CO_2 when DOC concentrations exceeded 147 – 267 µmol/L.

Epilimnetic $\delta^{13}C$ along the DOC gradient— DOC had a strong influence on the $\delta^{13}C$ of all carbon pools in the epilimnia of our sampled lakes (Fig. 5). The multiple regression model used to test predictions about the efficiency of terrestrial carbon flow was highly significant and explained a substantial amount of the variability in epilimnetic $\delta^{13}C$ ($R^2 = 0.91$, $P < 0.0001$). $\delta^{13}C_{CO2}$, $\delta^{13}C_{POM}$, and $\delta^{13}C_{ZP}$ all declined significantly over the DOC gradient ($P < 0.0001$) at the same rate (Table 1). However, there were significant differences in the $\delta^{13}C$ signatures of the different carbon pools (Table 1): the DOC-standardized intercept for $\delta^{13}C_{POM}$ was 11.9‰ lower than $\delta^{13}C_{CO2}$, and the DOC-standardized intercept for $\delta^{13}C_{ZP}$ was 4.6‰ lower than $\delta^{13}C_{POM}$. There was no difference in δ^{13}C of cladocerans and copepods along the DOC gradient (paired t-test, $t_{44} = 1.5$, $P = 0.13$; Fig. 6).

Based on eq. 1, the phytoplankton fraction factor (ε) ranged between -19.7 and -4.9 ‰. We did not include the estimates of $\delta^{13}C_{PHYTO}$ in the multiple regression analysis because they were not independent from the $\delta^{13}C_{CO2}$ measurements. Considered separately, $\delta^{13}C_{PHYTO}$ ranged from -42.0 to -17.6‰ and declined significantly along the DOC gradient:

$$\delta^{13}C_{PHYTO} = -23.1 - 0.018\ \mathrm{DOC},\ r^2 = 0.55,\ P < 0.0001; \qquad \text{(eq. 4)}$$

We qualitatively evaluated the degree to which the POM pool was diluted by non-phytoplankton carbon source by plotting $\delta^{13}C_{POM}$ against $\delta^{13}C_{PHYTO}$. Deviations of $\delta^{13}C_{POM}$ from the 1:1 line were negatively correlated with DOC ($r = -0.58$, $P = 0.0002$) indicating the POM pool was isotopically enriched with ^{13}C relative to $\delta^{13}C_{PHYTO}$ in high DOC lakes, but depleted in ^{13}C relative to $\delta^{13}C_{PHYTO}$ in low DOC lakes (see Fig. 7a).

We used a similar approach to evaluate the relative contribution of phytoplankton carbon to zooplankton diets. We plotted $\delta^{13}C_{ZP}$ against $\delta^{13}C_{PHYTO}$ and found that the deviations from the 1:1 line were also negatively correlated with DOC ($r = -0.49$, $P = 0.006$), indicating that zooplankton were enriched with ^{13}C relative to phytoplankton in high DOC lakes and depleted in ^{13}C relative to phytoplankton in low DOC lakes (Fig. 7b).

Vertical distributions of $\delta^{13}C$— DOC had a large effect on the vertical distribution of physical and chemical attributes in the three intensively sampled lakes (Fig. 8). In general, O_2, temperature, and DIC became less uniformly distributed with depth as DOC concentration increased. In addition, the stable isotopic ratios of DIC were affected by depth and DOC concentrations. For example, $\delta^{13}C_{DIC}$ values were uniformly distributed with depth in the low DOC lake, but were more variable with depth in the medium and high DOC lakes. In particular, we observed large fluctuations in $\delta^{13}C_{DIC}$ at the oxycline (Fig. 9).

The isotopic ratios of the organic carbon pool were less affected by depth and DOC. For example, the $\delta^{13}C_{DOM}$ and $\delta^{13}C_{ZP}$ were uniformly distributed with depth in all three lakes (Fig. 9). $\delta^{13}C_{POM}$ was uniformly distributed in the low DOC lake, but had more variable distributions in the medium and high DOC lakes (Fig. 9). Despite these differences zooplankton were generally depleted in $\delta^{13}C$ relative to POM through most of the water column (Fig. 9). The maximum differences between the highest $\delta^{13}C_{ZP}$ and the lowest $\delta^{13}C_{POM}$ were -0.4, 2.8, and 1.2‰ for the low, medium, and high DOC lakes, respectively.

DISCUSSION

This comparative study used natural occurring carbon stable isotopes to test hypotheses about the flow of terrestrial carbon in lakes along a natural gradient of terrestrial DOM. Our estimates of terrestrial DOM served as a strong predictor of all measured $\delta^{13}C$ pools (see Fig. 5). Our results suggest that terrestrial DOM was respired in lakes and that phytoplankton used the resulting CO_2 in photosynthesis. The isotopic signature of respired carbon was recorded in the POM and zooplankton, consistent with the prediction that terrestrial carbon flow was relatively inefficient (Fig. 1b). However, the phytoplankton isotopic signal was diluted by another carbon source, and zooplankton supplemented their diets with terrestrial carbon in lakes with low and high DOM concentrations.

$CO_{2(aq)}$ along the DOC gradient— Our results are consistent with other studies that have found a positive relationship between epilimnetic $CO_{2(aq)}$ and DOC (Hope et al. 1996; Jonsson et al. 2003; Sobek et al. 2003). One explanation for this trend is that lakes are the recipients of stream water or groundwater that is supersaturated with CO_2 (Kling et al. 1992; Dillon and Molot 1997; Jones et al. 2001). However, results from our intensively sampled lakes are not consistent with this explanation. For example, the low DOC lake was fed by an inlet that had high DIC concentrations (1550 μmol/L, Fig. 8), yet $CO_{2(aq)}$ in the lake was near equilibrium with the atmosphere. Similarly, it is unlikely that the high DIC concentrations in the medium and high DOC lakes were supported by the relatively low DIC concentrations found in the stream inlets (Fig. 8). Our findings are more consistent with evidence from mesocosm experiments (Lennon 2004), diel oxygen sampling (Hanson et al. 2003), and whole-ecosystem studies (Cole et al. 2002; Pace et al. 2004), which suggests that terrestrial DOM is respired by organisms within lake food webs. The following section assesses whether the terrestrial carbon was respired predominantly by bacteria (inefficient flow, Fig. 1b) or by bacteria and crustacean zooplankton (efficient flow, Fig. 1a).

Terrestrial carbon flow in lake food webs— We predicted that $\delta^{13}C_{POM}$ and $\delta^{13}C_{ZP}$ would converge upon the isotopic signature of terrestrial organic matter (-28‰; Jones et al. 1999) along the DOM gradient if terrestrial carbon was transferred efficiently within plankton food webs. Moreover, we predicted that the isotopic ratios for different zooplankton functional groups would reflect their ability to consume DOM-subsidized bacteria. Results from our among-lake survey did not support either of these predictions. First, we found that $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$ decreased with DOC at the same rate and approached isotopic values that were depleted in ^{13}C relative to terrestrial organic matter (-33‰ and -38‰ for POM and zooplankton, respectively). Second, we found no difference between the $\delta^{13}C$ of cladoceran and copepod zooplankton along the DOC gradient (Fig. 6). Together, these results do not support the predictions for the efficient flow of terrestrial carbon in plankton food webs.

Nevertheless, carbon stable isotope ratios were strongly influenced by DOC. Our results indicate that changes in the isotopic composition of different carbon pools may have been due to increased utilization of respiratory CO_2 by phytoplankton over the DOC gradient (i.e., CO_2 recycling, France et al. 1997). For example, in low DOC lakes, $CO_{2(aq)}$ concentrations were close to atmospheric equilibrium and stable isotope data approximated expected values based on the diffusion and dissolution of atmospheric CO_2 into water at ambient temperatures (Mook et al. 1974; Fig. 5). High DOC lakes, were supersaturated with $CO_{2(aq)}$ and had stable isotope ratios of $CO_{2(aq)}$ that were highly depleted in ^{13}C relative to the atmosphere. We contend that phytoplankton rely less on atmospheric-derived CO_2 and more on CO_2 from respired terrestrial carbon with increasing concentration of DOM.

Assuming that isotopic composition of $CO_{2(aq)}$ was not influenced by additional factors (i.e., groundwater, calcite, stream inlets, hypolimnetic processes, see Fig. 1), we can use mass balance to make a crude estimate of how the internal respiration of terrestrial carbon contributed to the $CO_{2(aq)}$ pool along our DOC gradient:

$$\mathit{Terrestrial\ CO_2} = 1 - \left(\frac{\delta^{13}\mathrm{CO}_{2\,(\mathrm{obs})} - \delta^{13}\mathrm{CO}_{2\,(\mathrm{terrestrial})}}{-\delta^{13}\mathrm{CO}_{2\,(\mathrm{terrestrial})} + \delta^{13}\mathrm{CO}_{2(\mathrm{atmosphere})}} \right) \qquad \text{(eq. 5)}$$

where Terrestrial CO_2 is the fraction of CO_2 in the epilimnion that is respired carbon of terrestrial origin, $\delta^{13}C_{CO2(obs)}$ is the observed isotopic ratio of CO_2, $\delta^{13}C_{CO2(terrestrial)}$ is the isotopic ratio of terrestrial organic carbon, which we assume to be -28‰, and $\delta^{13}C_{CO2(atmosphere)}$ is the isotopic ratio of atmospheric CO_2, which we assume to be -10‰ (Mook et al. 1974). Based on this simple model and set of assumptions, we estimated that terrestrial-derived CO_2 increased from ~25% in low DOC lakes (200 µmol/L) to ~75% in high DOC lakes (1200 µmol/L). These approximations are consistent with other studies that have attempted to identify DIC sources in lake ecosystems. For example, Cole et al. (2002) found that 90% of the epilimnetic DIC in a high DOC lake (1100 µmol/L) was supplied by internal heterotrophic respiration. Similarly, Wachniew and Rozanski (1997) reported that 66% of the DIC in a predominantly groundwater-influenced lake was derived from the decomposition of organic matter, although it was not clear whether the organic matter was terrestrial-derived or locally-produced.

The isotope evidence for strong CO_2 recycling implies that terrestrial carbon was used rather inefficiently in our study of lake food webs (France et al. 1997). As a consequence, we predicted that phytoplankton-derived carbon would comprise a major portion of the POM pool (Fig. 1b). At first glance, this prediction is supported by the observation that $\delta^{13}C_{POM}$ is correlated with $\delta^{13}C_{CO2}$ (Fig. 5). The most parsimonious interpretation of this trend is that the ratio of heterotrophic to autotrophic biomass remained constant along the DOC gradient. However, this is probably not a realistic or safe assumption for two reasons. First, inputs of allochthonous DOC have been shown to influence the relative abundance of heterotrophic and autotrophic biomass (del Giorgio and Gasol 1995; del Giorgio et al. 1999). Second, it is unlikely that $\delta^{13}C_{PHYTO}$ remained constant along the DOC gradient since phytoplankton isotopic fraction is dependent on $CO_{2(aq)}$ concentrations (Hinga 1994; Rau et al. 1997; Yoshioka 1997).

We used estimates of $\delta^{13}C_{PHYTO}$, which took into account CO_2-dependent fractionation, to qualitatively assess how phytoplankton carbon contributed to the total POM pool. $\delta^{13}C_{PHYTO}$ did not fall on the 1:1 line with $\delta^{13}C_{POM}$ suggesting that isotopic signature of phytoplankton in the POM pool was diluted by another carbon source, possibly terrestrial organic matter. Our results indicated that the direction of the dilution was influenced by DOC concentration. Specifically, the POM pool was isotopically enriched with ^{13}C relative to $\delta^{13}C_{PHYTO}$ in high DOC lakes, but depleted in ^{13}C relative to $\delta^{13}C_{PHYTO}$ in low DOC lakes (see Fig. 7a). Together, these results suggest that terrestrial-derived organic carbon may have contributed to POM pool and therefore had the potential to support zooplankton biomass in both low and high DOC lakes (Fig. 7b). However, this interpretation must be approached with caution due to the uncertainty of our $\delta^{13}C_{PHYTO}$ estimates. Our estimates are based upon a single empirical model that was developed for marine phytoplankton and assumes that $\delta^{13}C_{PHYTO}$ is influenced solely by $\delta^{13}C_{CO2}$ and $CO_{2(aq)}$. Although the mechanisms for photosynthetic fractionation should hold across systems, there may be taxa- or system-specific attributes of lake ecosystems that modify the parameters of the fractionation model used in this study.

Zooplankton diets along a DOC gradient— Bulk POM did not accurately represent the diet of crustacean zooplankton in our survey of northeastern lakes. As with other studies (Zohary et al. 1994, del Giorgio and France 1996; Jones et al. 1999; Karlsson et al. 2003), zooplankton were depleted in ^{13}C relative to their putative food source (i.e., POM). The average difference between $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$ was 4.6‰ and was consistent across the entire DOC gradient (Table 1). Since consumers should be ≤ 1‰ heavier than their food source (Peterson and Fry 1987), our results raise questions regarding the diets and feeding behavior of crustacean zooplankton in lake ecosystems.

One hypothesis for the discrepancy between $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$ is that zooplankton consume isotopically light POM during diel vertical migration (del Giorgio and France 1996; Jones et al. 1999; Bastviken et al. 2003). This hypothesis arose because hypolimnetic DIC is often depleted in ^{13}C (e.g., Rau 1978; Stiller and Nissenbaum 1999; Fig. 9) and metalimnetic $\delta^{13}C_{POM}$ was reported to be 5‰ lighter than epilimnetic $\delta^{13}C_{POM}$ in a subset of unproductive Quebec lakes (France et al. 1997). In addition, vertically migrating zooplankton may have access to isotopically light food items such as sulfur oxidizing bacteria (Hadas et al. 2001) and methanotrophic bacteria (Bastviken et al. 2003).

However, diel vertical migration does not seem to explain the large discrepancy between $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$ in our survey. First, not all zooplankton exhibit vertical migration behavior (e.g., Gilbert and Burns 2000). Often, larger-bodied zooplankton (e.g., *Daphnia* spp.) migrate more than smaller-bodied zooplankton (e.g., copepods) due to their probability of being detected by visual-feeding predators (Lampert and Sommer 1997). Thus, we might expect that our cladoceran samples, which consisted mostly of *Daphnia* spp., to be more depleted in ^{13}C than copepods, but the data do no support this expectation. Second, zooplankton should migrate less in high DOC lakes because of reduced predation risk (Wissel et al. 2003) and/or reduced UV stress (Boeing et al. 2004). As a consequence, we would predict zooplankton to become increasingly enriched in ^{13}C relative to POM along a DOC gradient, but again the data are not consistent with this expectation (Fig. 5). Finally, observations from our within-lake survey do not support the vertical migration hypothesis. In order for the vertical migration hypothesis to be plausible in our study, there would have needed to be at least a -4.6‰ difference between zooplankton and POM at some location within the water column. At no depth did we find such a difference (Fig. 9), although it is possible that small, isotopically-light bacteria were not retained in our POM samples.

An alternate hypothesis for the discrepancy between $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$ is related to the selective feeding habits of crustacean zooplankton. Pel et al. (2003) used a combination of flow cytometry and isotope-ratio mass spectrometry to show that there was a 6 – 10‰ difference in δ^{13}C between populations of phytoplankton in a small Dutch lake. Moreover, although the lake was dominated by cyanobacteria and detritus, zooplankton fed on less abundant eukaryotic algae (Pel et al. 2003). Therefore, if zooplankton are selective feeders, isotope heterogeneity within the phytoplankton community might be sufficient to explain the commonly observed discrepancy between $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$. It is not difficult to meet this assumption for copepods because it is well documented that they are specialist consumers (e.g., Demott 1988; Kerfoot and Kirk 1991). In contrast, cladocerans are typically perceived as generalists that consume detritus, bacteria, and phytoplankton (Dodson and Frey 1991; Jürgens 1994). However, there are a number of active and passive mechanisms that allow cladocerans to selectively feed on diet items within the POM pool. First, some cladocerans "taste-test" food items before they are ingested (Kerfoot and Kirk 1991). Second, the filtering apparatus of cladocerans is plastic and responds to changes in food quality, food quantity, and possibly dissolved chemicals (Lampert 1994; Machacek 2001; Ghadouani and Pinel-Alloul 2002). Finally, morphologically protected algae may comprise a large percentage of the phytoplankton community, but are not heavily grazed by cladocerans due to size constraints of their mandibles and carapace gape (e.g., Leibold 1989).

Conclusion— This study demonstrates that terrestrial-derived DOM has large effects on the carbon flow of lake ecosystems. Our results support the idea that terrestrial DOM is assimilated, and as a result has a large influence on the metabolism of lake ecosystems. In addition to documenting changes in ecosystem processes, a major aim of this study was to use stable isotopes to evaluate whether inputs of terrestrial carbon subsidize plankton food webs. For example, a recent study by Pace et al. (2004) demonstrated that a significant fraction of POM and zooplankton in two Wisconsin lakes was supported by terrestrial-derived carbon. Karlsson et al. (2003) came to similar conclusion regarding Swedish lakes, but stated that the dependence of zooplankton on allochthonous carbon was not related to DOC concentrations, but instead to factors that controlled the metabolism of bacterioplankton. Our results are consistent with those of Karlsson et al.

(2003) in that POM and zooplankton appeared to be subsidized in lakes with both low and high concentrations of terrestrial DOM. Together, this study and others (e.g., Jones et al. 1999; Karlsson et al. 2003; Pace et al. 2004) reveal that terrestrial-derived DOM needs to be taken into consideration when investigating the food web dynamics and energetics of lake ecosystems.

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Table 1. Parameter estimates (with errors) from the multiple regression analysis for the effects of DOC on epilimnetic $\delta^{13}C_{CO2}$, $\delta^{13}C_{POM}$, and $\delta^{13}C_{ZP}$. We used DOC as a continuous predictor variable (standardized to the mean values; 532 μmol/L) and indicator variables to code for the different $\delta^{13}C$ samples.

$\delta^{13}C$	Intercept		Slope	
	mean	SE	mean	SE
CO_2	-17.2	0.42	-0.009	0.0016
POM	-29.1	0.34	-0.006	0.0013
Zooplankton	-33.6	0.25	-0.008	0.0010

FIGURE CAPTIONS

Fig. 1. Factors expected to influence the efficiency of terrestrial carbon flow in the epilimnia of lake ecosystems. Dark lines represent the dominant terrestrial carbon pathways and dashed lines represent respiration. The stable isotope ratio ($\delta^{13}C$) of $CO_{2(aq)}$ is influenced by atmospheric CO_2, ground- and stream-water inputs, and heterotrophic respiration within the lake. The putative food source for crustacean zooplankton is measured as the $\delta^{13}C$ of particulate organic matter (POM). POM is comprised of autotrophic biomass (POM_A) and heterotrophic microorganisms (POM_H), which are capable of using terrestrially-derived dissolved organic carbon (DOM). Different types of zooplankton (copepods and cladocerans) selectively feed on different amounts of POM_A and POM_T.

Fig. 2. Map depicting the 70 lakes sampled during the summers of 2002 and 2003.

Fig. 3. The relationship between dissolved organic carbon (DOC) and water color (a_{440}) in samples from the among-lake survey. Line represents a power function from a non-linear regression analysis.

Fig. 4. The relationship between $CO_{2(aq)}$ and DOC in the epilimnia of samples from the among-lake survey . The horizontal lines represent the range of equilibrium CO_2 concentrations based on Henry's Law, temperature, and the assumption of atmospheric CO_2 (380 μatm). Data were fit with simple linear regression and 95% confidence intervals.

Fig. 5. The relationship between $\delta^{13}C$ and DOC for multiple carbon compartments (CO_2, POM, and zooplankton) obtained from the epilimnia of the among-lake survey. The $\delta^{13}C$-DOC slopes for CO_2, POM, and zooplankton (ZP) are identical, though intercepts were all significantly different from one another (Table 1). Lines represent the predicted values from the multiple regression analysis.

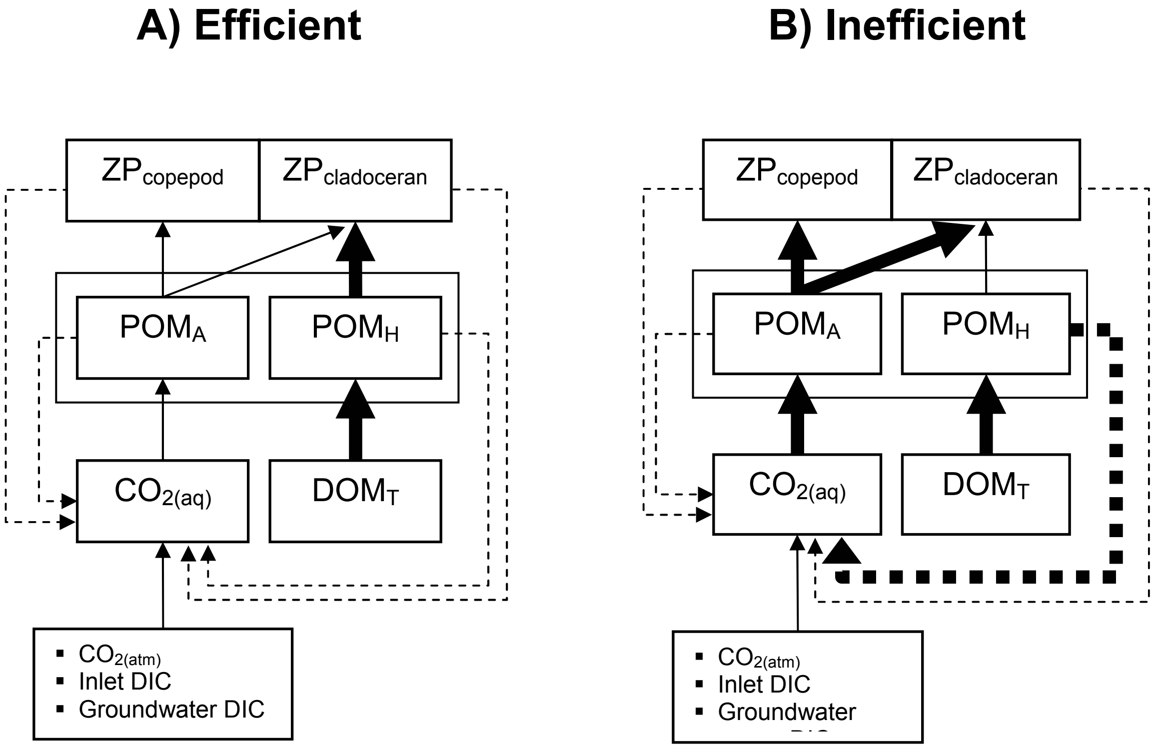
Fig. 6. Distribution of paired differences in $\delta^{13}C$ between cladoceran and copepod zooplankton in 44 lakes.

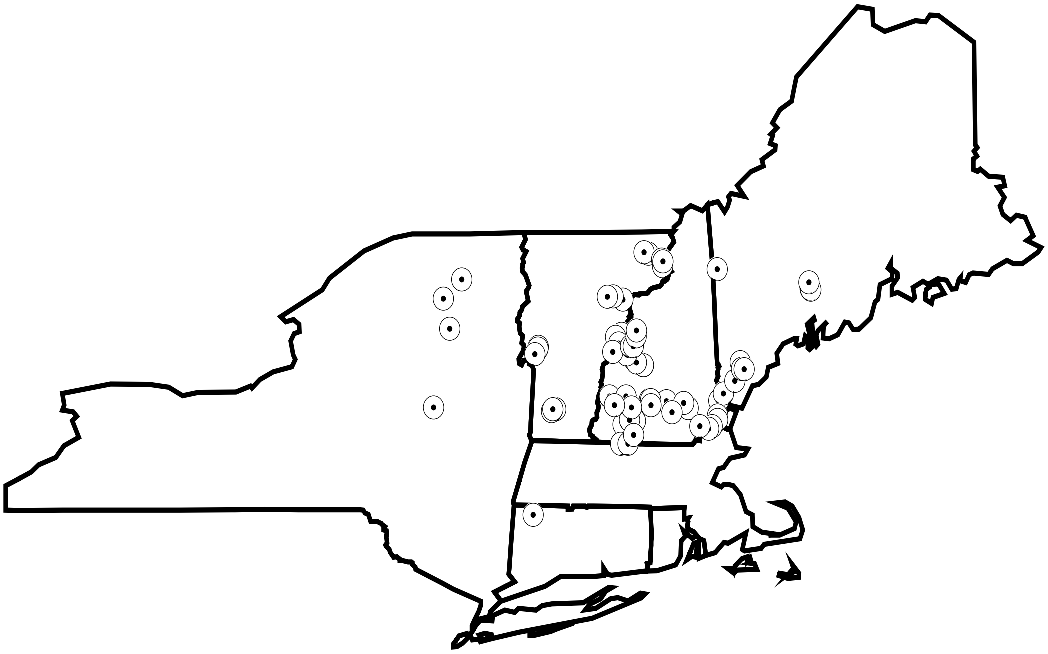
Fig. 7. The relationship between A) $\delta^{13}C_{POM}$ and estimated $\delta^{13}C_{PHYTO}$ and B) $\delta^{13}C_{ZP}$ and estimated $\delta^{13}C_{PHYTO}$. Symbol size is positively correlated with the concentration of DOC in each sample. Dashed lines represent the 1 : 1 lines.

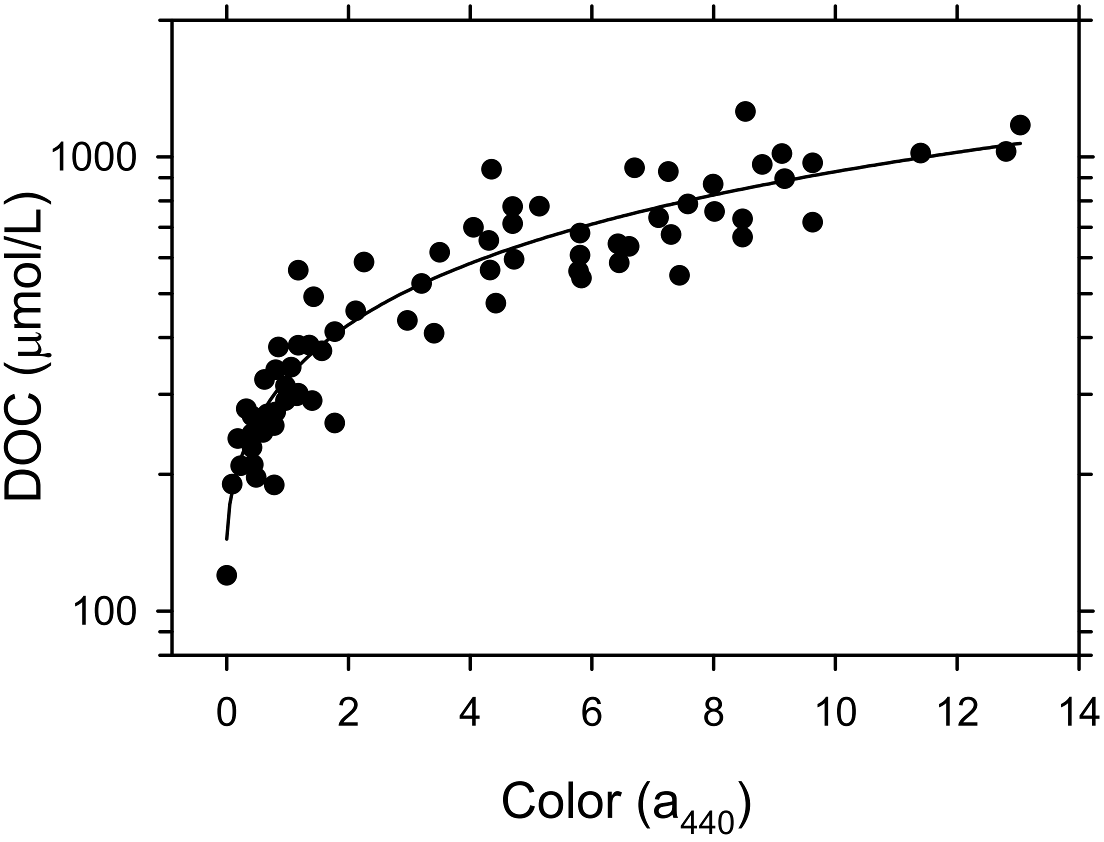
Fig. 8. Vertical distribution of dissolved oxygen (O_2), temperature (°C), and dissolved inorganic carbon (DIC) in three lakes with varying DOC concentrations (Low DOC = 229 μmol/L; medium DOC = 586 μmol/L; high DOC = 962 μmol/L). Downward-pointing arrows represent DIC concentrations in the stream inlet of each lake.

Fig. 9. Vertical distributions of $\delta^{13}C_{ZP}$, $\delta^{13}C_{POM}$, $\delta^{13}C_{DOM}$, and $\delta^{13}C_{DIC}$ in the three lakes with varying DOC (Low DOC = 229 μmol/L; medium DOC = 586 μmol/L; high DOC = 962 μmol/L). The horizontal dashed line represents the oxycline (anoxic-oxic boundary layer); upward-pointing white arrows represent $\delta^{13}C$ of the sediments in each lake;

downward-pointing black arrows represent the $\delta^{13}C$ of the stream inlet DIC for each lake.


A) Efficient
B) Inefficient
ZPcopepod
ZPcladoceran
POMA
POMH
CO2(aq)
DOMT
CO2(atm)
Inlet DIC
Groundwater DIC
ZPcopepod
ZPcladoceran
POMA
POMH
CO2(aq)
DOMT
CO2(atm)
Inlet DIC
Groundwater

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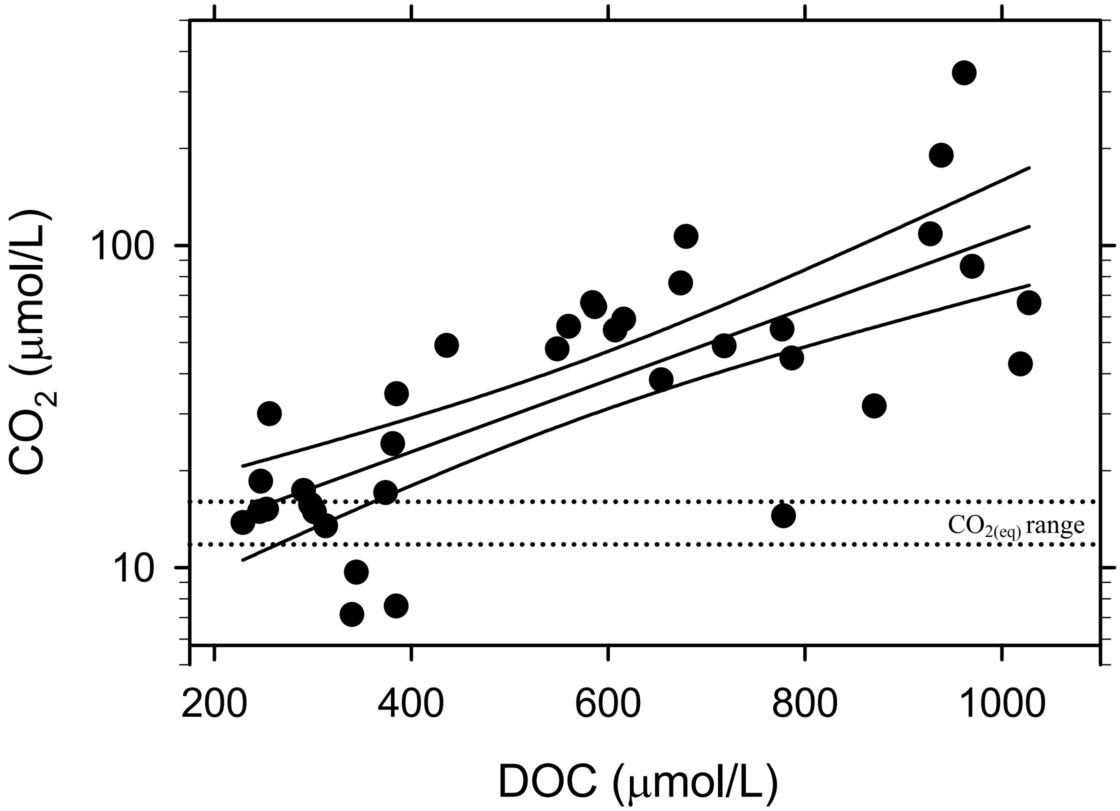
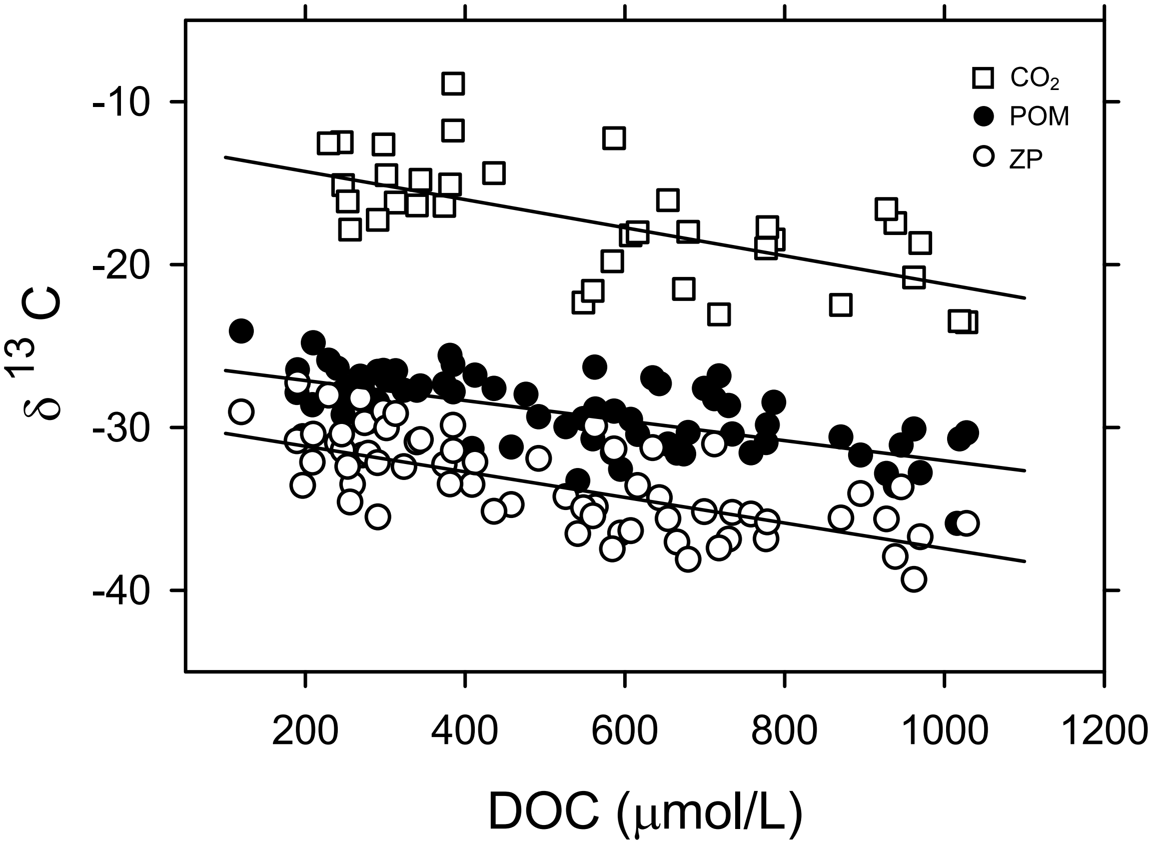
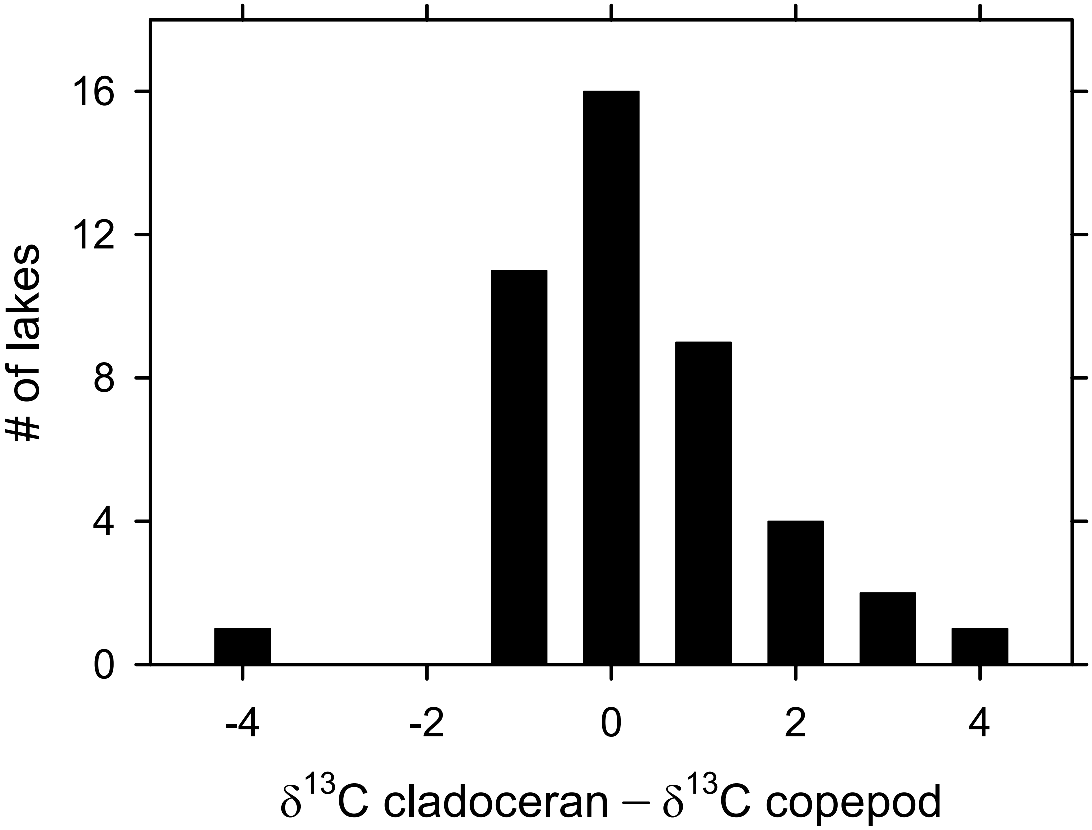
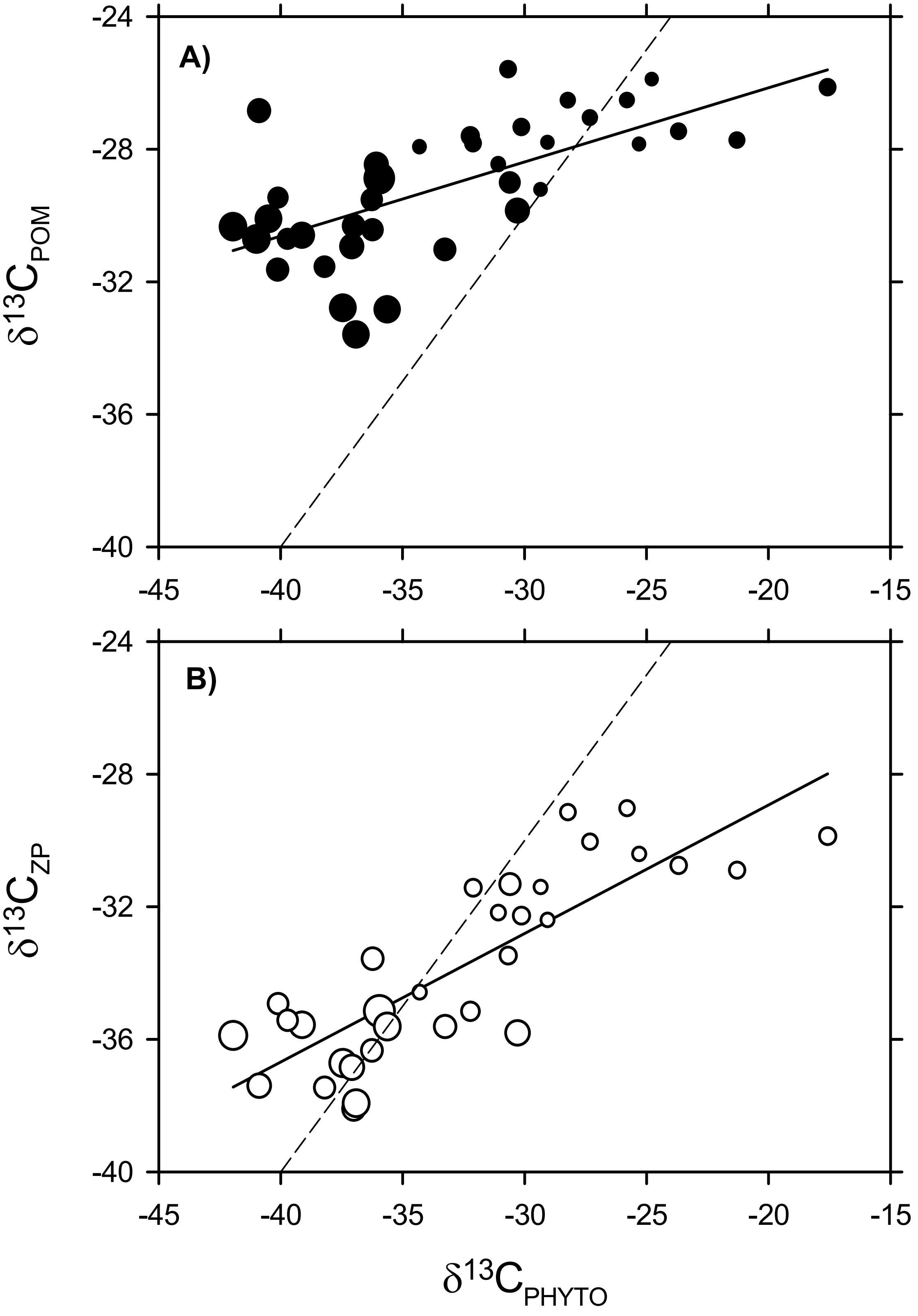
100
0
2
4
6
8
10
12
14
Color (a_{440})


CO2 (μmol/L)
100
10
200
400
600
800
1000
DOC (μmol/L)
CO2(eq) range


δ ¹³ C
-10
-20
-30
-40
200
400
600
800
1000
1200
DOC (μmol/L)
□ CO₂
● POM
○ ZP



16
12
8
4
0
# of lakes
-4
-2
0
2
4
δ13C cladoceran – δ13C copepod


A)
B)
δ13C POM
δ13C ZP
δ13C PHYTO
-24
-28
-32
-36
-40
-45
-40
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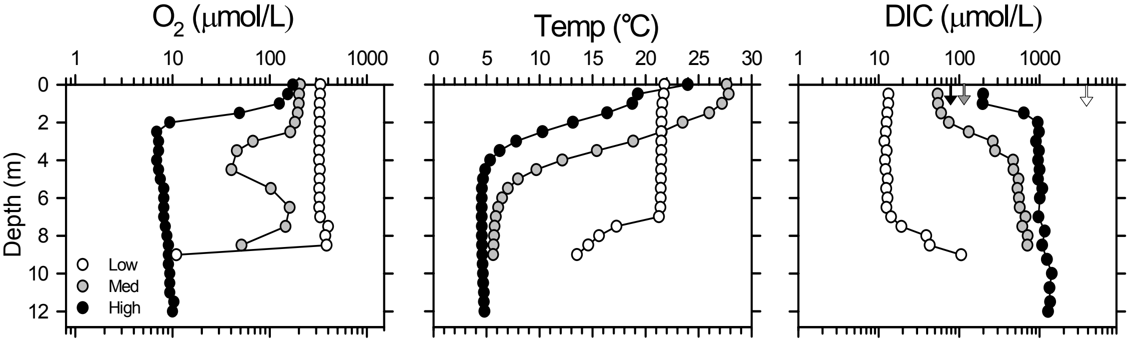
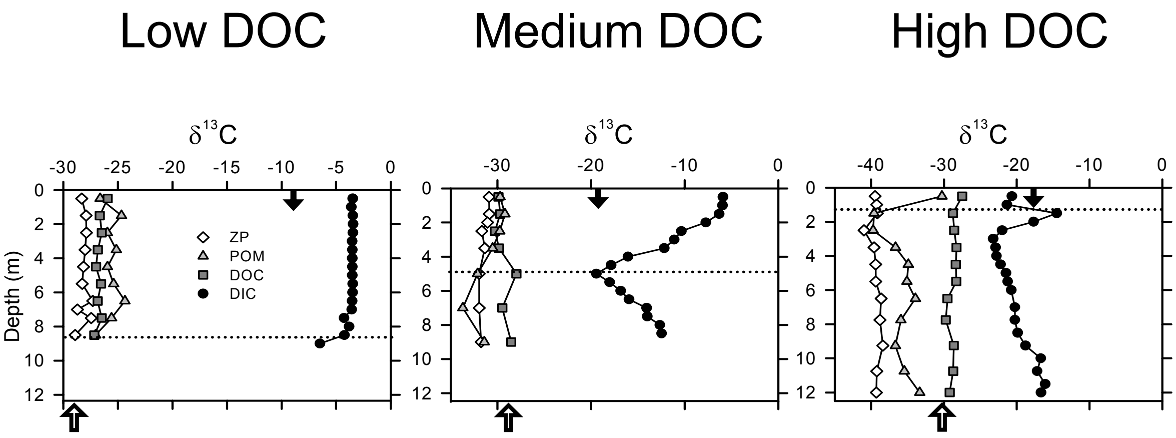
-30
-25
-20
-15



O$_2$ (μmol/L)
1
10
100
1000
Temp (℃)
0
5
10
15
20
25
30
DIC (μmol/L)
1
10
100
1000
Depth (m)
0
2
4
6
8
10
12
Low
Med
High


Low DOC
Medium DOC
High DOC
$\delta^{13}C$
-30
-25
-20
-15
-10
-5
0
Depth (m)
0
2
4
6
8
10
12
ZP
POM
DOC
DIC
$\delta^{13}C$
-30
-20
-10
0
$\delta^{13}C$
-40
-30
-20
-10
0