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Experimental evidence that terrestrial carbon subsidies increase CO₂ flux from lake ecosystems

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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 CO₂. I experimentally tested this hypothesis using a series of mesocosm experiments in New England lakes. In the first experiment, I observed that CO₂ flux increased by 160% 4 days following a 1,000 μM C addition in the form of DOM. However, this response was relatively short lived, as there was no effect of DOM enrichment on CO₂ flux beyond 8 days. In a second experiment, I demonstrated that peak CO₂ flux from mesocosms in two lakes increased linearly over a broad DOM gradient (slope for both lakes=0.02±0.001 mM CO₂·m⁻² day⁻¹ per μM DOC, mean±SE). Concomitant changes in bacterial productivity and dissolved oxygen strengthen the inference that increasing CO₂ 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 CO₂ flux. Second, organically bound nitrogen (N) and phosphorus (P) accompanied DOM inputs, but experimental nutrient additions (without organic C) caused mesocosms to be saturated with CO₂. 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 CO₂.

Keywords Bacteria · DOC · DOM · Plankton · Subsidy

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 (e.g., Polis et al. 1997; Jefferies 2000; 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 composed 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 (1,000–4,000 years; see Aiken et al. 1996; Raymond and Bauer 2001), suggesting that some DOM fractions are quite

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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; 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 CO₂ (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 CO₂. First, other sources of allochthonous C subsidize lakes and may contribute to CO₂ 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 CO₂ because they receive groundwater or surface-water inputs that are supersaturated with CO₂ (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 bacteria 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 CO₂ efflux from lake ecosystems. Specifically, experiments were designed to: (1) examine the temporal dynamics of CO₂ flux under contrasting DOM regimes, (2) describe the functional relationship between CO₂ 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 CO₂ 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 they 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., Cole et al. 2000; Prairie et al. 2002; Sobock et al. 2003). In the summer of 2001, I deployed mesocosms in Childs Pond, Vt. (Orange Co.) and Storrs Pond, N.H. (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 that of Storrs Pond (7.5) and this is reflected by differences in alkalinity (136 and 1,395 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 m²) 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 2 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 (2,500 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 Laboratories) 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 dissolved organic carbon (DOC) with a Tekmar-Dohrmann TIC/TOC analyzer after H₂SO₄ 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 (NO₃+NO₂) plus ammonium-N using a Lachat Quick-Chem 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 Ramirez-Ramirez 1996). I quantified humic acids as precipitated DOC after acidifying the leachate to pH 2 with H₃PO₄. I measured protein content using the BCA method (Walker 1996) with a Compat-Able Protein Assay Preparation kit (Pierce Biotechnology, Rockford, Ill.). I measured high molecular weight DOM as DOC retained in 12,000–14,000 D dialysis tubes.

Experimental designs

Two main experiments were conducted in this study. First, I monitored the temporal dynamics of CO₂ 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% (1,650 µM DOC). I then measured CO₂ flux daily for the following 10 days. I used univariate one-way repeated measures ANOVA (RM-ANOVA) to test for the main effect of DOM enrichment on CO₂ flux over the duration of the experiment. I used trapezoidal integration to determine how much terrestrial C was lost to the atmosphere via CO₂ flux between the two DOM treatments.

In the second experiment, I described the relationship between peak CO₂ flux and DOC concentration. Based on results from the temporal dynamics experiment, I defined peak flux as the point of maximum CO₂ flux following DOM enrichment. I used 18 mesocosms in each lake to construct a DOM gradient ranging from ambient concentrations to approximately 2,500 µM DOC. I used multiple regression to determine whether DOC (continuous variable) and lake identity (dummy variable) had a significant effect on CO₂ 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, color (*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 CO₂ flux along the DOM gradient. First, I ran an experiment to evaluate how DOM-mediated light attenuation affected CO₂ flux. I entirely covered two mesocosms having ambient DOM concentrations, with black polyethylene bags. I then compared CO₂ 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 CO₂ flux along the DOM gradient. I tested this by measuring CO₂ 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 (KH₂PO₄) and the remaining 9 mesocosms received both inorganic P and N (KH₂PO₄ and NH₄NO₃, respectively). The inorganic-nutrient experiment was conducted in Childs Pond during the peak flux experiment.

Measurements

I measured CO₂ flux using the headspace equilibrium method (Kling et al. 1992). In this procedure, 100 ml air was left in a 2,000-ml bottle filled with 1,900 ml lake water. I obtained $p\text{CO}_2(\text{water})$ by sampling the headspace with a gas-tight syringe, assuming equilibrium had been reached after shaking the bottle vigorously for 2 min. I obtained $p\text{CO}_2(\text{atmosphere})$ from the air approximately 2 m above the lake. $p\text{CO}_2$ was measured with a calibrated CID (CI-301) infrared gas analyzer (IRGA). An index of CO₂ flux was then calculated as:

$$\text{CO}_2 \text{ flux} = k [\text{CO}_2(\text{water}) - \text{Kh} \times \text{CO}_2(\text{eq})] \quad (1)$$

where k is the gas piston velocity, $\text{CO}_2(\text{water})$ is CO₂ in the lake water, Kh is Henry's constant at ambient temperature, and $\text{CO}_2(\text{eq})$ is CO₂ 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

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	

study, I used a gas piston velocity of 0.63 m day⁻¹, which is an empirically derived estimate based on whole lake additions of SF₆ gas to Mirror Lake, N.H. (Cole and Caraco 1998). This parameter value is consistent with estimates of k found in lakes in different geographic locations using a similar SF₆ approach (Crusius and Wanninkhof 2003).

$p\text{CO}_2$ measurements were taken from mesocosms in random order once per day between 1000 and 1200 hours. Reasonable estimates of CO₂ flux can be obtained from this type of sampling because $p\text{CO}_2$ in the atmosphere and water only exhibit a small amount of diel variation. For example, $p\text{CO}_2(\text{atmosphere})$ near Childs Pond during the time of the mesocosm experiments ranged between 350 and 380 μatm over a 24-h period ($n=1,093$, data not shown). Likewise, Cole and Caraco (1998) found no temporal trend in $p\text{CO}_2(\text{water})$ over a 24-h period, but there was a slight (2 μM) difference in $\text{CO}_2(\text{water})$ when dawn and dusk samples were pooled. The magnitude of these differences is small however, when compared to the range of $p\text{CO}_2(\text{water})$ reported in this mesocosm study (30–2,200 μatm) and among natural lakes (e.g., 300–4,000 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 0.7 μm 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 CO₂ in the headspace following a 2 ml addition of concentrated H₂SO₄ to 100 ml lake water.

Results

Temporal dynamics of CO₂ flux

CO₂ 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). CO₂ flux increased 100% 24 h after the DOM addition (day 2) and peaked 48 h later (day 4) at $27.5 \pm 1.24 \text{ mm m}^{-2} \text{ day}^{-1}$. CO₂ 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 an $\sim 33 \mu\text{M}$ (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).

CO₂ flux along DOM gradients

Peak CO₂ 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

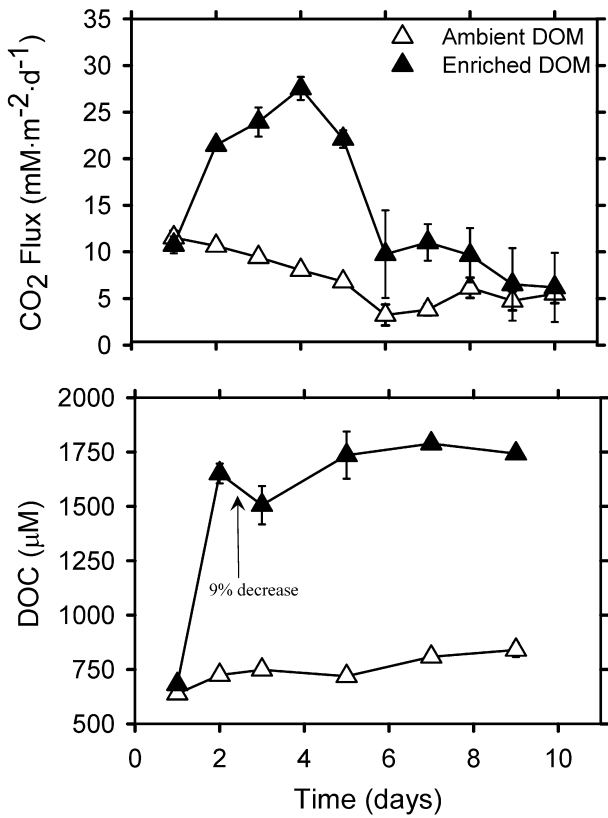


Fig. 1 Temporal dynamics of CO₂ flux (*upper panel*) and DOC (*lower panel*) from in-situ aquatic mesocosms in Childs Pond, Vermont with ambient (△, 650 µM DOC) and enriched (▲, 1,650) levels of terrestrial-derived DOM. DOM enrichment had a significant effect on CO₂ flux over the 10-day experiment (RM-ANOVA, $P < 0.0001$, $F_{9, 90} = 24.4$). Data points represent mean ± SE

Childs Pond compared to Storrs Pond (DOM × Lake interaction, Table 2). Bacterial productivity increased with DOM enrichment, but at a lower rate in Childs Pond (significant DOM × Lake interaction, Table 2). DIC remained constant (340–350 µM) in Storrs Pond along the DOM gradient, but increased with DOM in Childs Pond (33–72 µM, Table 2).

Table 2 Parameter estimates (intercept and 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 = parameter estimate was not significant). A significant B_0 means that

Response variable	Intercept (B_0)	DOC (B_1)	Lake (B_2)	DOC × Lake (B_3)	R^2	Partial r (DOC)	Partial r (lake)
CO ₂ flux (mm m ⁻² day ⁻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 day ⁻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 ± 1.8E ⁻⁵	-0.4 ± 0.038	ns	0.96	-0.75	-0.97
LOG ₁₀ chlorophyll <i>a</i> (µg l ⁻¹)	1.8 ± 0.19	2E ⁻⁴ ± 1.2E ⁻⁴ *	-0.56 ± 0.240	ns	0.50	0.53	-0.47
Color (a_{440})	-1.3 ± 0.27	9E ⁻³ ± 2E ⁻⁴	-2.9 ± 0.42	ns	0.99	0.99	-0.95

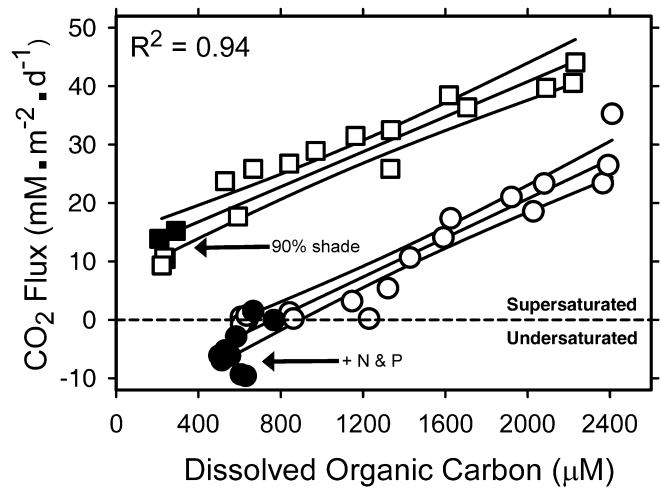


Fig. 2 Peak CO₂ flux from aquatic mesocosms 4 days after creating a terrestrial DOM gradient. Multiple regression revealed that mesocosms in both Childs Pond (○) and Storrs Pond (□) responded identically (i.e., same slopes) to DOM enrichment (Table 2). DOM colored the water (Table 2), but independent experiments showed that the increased CO₂ flux was not due to manipulated light reduction (■). I tested whether mineralization of organically bound nutrients may have increased CO₂ flux by adding inorganic N and P to mesocosms (no organic C) 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 CO₂ (●, one-sample t -test, $t = 3.89$, $df = 8$, $P = 0.005$). Lines are from least squares regression and calculations of 95% confidence intervals

Effects of shading on CO₂ flux

DOM additions attenuated light (a_{440} ; Table 2), but light was affected more by experimental shading (90% reduction). Experimental shading increased CO₂ 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

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 × 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

within the 95% confidence intervals generated by the DOM gradient analysis in Storrs Pond (see Fig. 2).

Effects of inorganic nutrient enrichment on CO₂ flux

Terrestrial DOM contains other elements besides C that may have influenced CO₂ flux (Table 1). Inorganic P additions alone did not cause CO₂ in the mesocosms to deviate from atmospheric equilibrium ($-0.3 \pm 0.58 \text{ mM CO}_2 \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ [mean \pm 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 CO₂ ($-5.0 \pm 1.28 \text{ mM CO}_2 \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ [mean \pm 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 CO₂. 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 CO₂ 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 CO₂ flux

DOM enrichment had a strong, but short-lived, effect on CO₂ 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 CO₂ 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 5-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 CO₂ flux is highly variable through time. In temperate lakes, most variability occurs during spring and autumn mixing events, where there are pronounced peaks in CO₂ flux (e.g., Striegl et al. 2001). CO₂ flux is more stable during periods of summer stratification (Kelly et al. 2001), but this is not true for all lakes. For example, $p\text{CO}_{2(\text{water})}$

fluctuated more through time in high versus low DOC lakes (Hope et al. 1996), suggesting that terrestrial DOM loading rates influence temporal trends in CO₂ 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 CO₂ flux for approximately 8 days (Fig. 1). This duration is longer than the frequency of growing-season rain events in Hanover, N.H. (3.8 ± 0.13 days, <http://lwf.ncdc.noaa.gov>), suggesting that the temporal variability of CO₂ 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 CO₂ flux. Such information would provide insight into the importance of terrestrial subsidies for lake metabolism.

CO₂ flux along DOM gradients

Many studies have documented that lakes are supersaturated with CO₂. Fewer studies have attempted to link patterns of CO₂ flux and DOC concentrations. I found that peak CO₂ flux increased linearly over a range of DOC that captures most of the natural variation found for lakes worldwide (Fig. 2). These results agree qualitatively with a number of recent comparative lake surveys. First, in North America, Hope et al. (1996) found that $p\text{CO}_2$ increased linearly with DOC (150–1,600 μM) in Wisconsin lakes. Likewise, Prairie et al. (2002) found that CO₂ flux increased linearly over a smaller range of DOC (167–933 μM) in Quebec lakes. A positive DOC-CO₂ flux relationship has also been reported for Swedish lakes (Jonsson et al. 2003), even for systems up to 2,700 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 CO₂. Moreover, there is currently no evidence that CO₂ 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 CO₂. 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 CO₂ 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 CO₂. In Childs Pond, $p\text{CO}_{2(\text{water})}$ was at equilibrium with $p\text{CO}_{2(\text{atmosphere})}$ 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 CO₂, as indicated by the positive intercept of the DOC-CO₂ flux relationship (Table 2, Fig. 2). This result indicates that benthic respiration and CO₂-supersaturated water inputs may be important sources of DIC in Storrs Pond (see Kling et al. 1992; Jones et al. 2001).

Shading and CO₂ 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 CO₂ supersaturation. At first glance, the results suggest that the light-attenuating properties of DOM be an important driver of CO₂ flux. For example, shading increased CO₂ flux and decreased dissolved oxygen concentrations. However, CO₂ 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 CO₂ 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 CO₂ 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, 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 CO₂ flux along the DOM gradient (Fig. 2). Inputs of inorganic P alone had no effect on CO₂ flux, whereas inputs of both inorganic N and P caused mesocosms to be undersaturated with CO₂ (Fig. 2). These results highlight how lakes respond differently to inorganic versus 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 CO₂ flux may be less responsive to inorganic nutrients than organic material. For example, maximum CO₂ efflux was 38 mm CO₂·m⁻²·day⁻¹ in this study and can be as high as 50 mm CO₂·m⁻²·day⁻¹ in natural lakes (Kling et al. 1992; Cole et al. 2000). However, even under high levels of inorganic nutrient loading, CO₂ influx was -5 mm CO₂·m⁻²·day⁻¹ in this study and is rarely less than -10 mm CO₂·m⁻²·day⁻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 CO₂.

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 CO₂ flux. Two chemical attributes that affect bacterial DOM consumption are size and stoichiometry. It has traditionally been 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 alternative 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 the 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=14–4,000; Sun et al. 1997; Anesio et al. 2000). It is possible that different patterns of CO₂ 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 (J.T. Lennon and L.E. Pfaff, in preparation). Future comparative studies might explain more variation in DOC-CO₂ 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 CO₂. 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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