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Relative importance of CO_2 recycling and CH_4 pathways in lake food webs along a dissolved organic carbon gradient

Jay T. Lennon[1]
Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

Anthony M. Faiia and Xiahong Feng
Department of Earth Sciences, Dartmouth College, Hanover, New Hampshire 03755

Kathryn L. Cottingham
Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

Abstract

Terrestrial ecosystems export large quantities of dissolved organic carbon (DOC) to aquatic ecosystems. This DOC can serve as a resource for heterotrophic bacteria and influence whether lakes function as sources or sinks of atmospheric CO_2. However, it remains unclear as to how terrestrial carbon moves through lake food webs. We addressed this topic by conducting a comparative lake survey in the northeastern U.S. along a gradient of terrestrial-derived DOC. We used naturally occurring carbon stable isotopes of CO_2, particulate organic matter (POM), and crustacean zooplankton, as well as gas measurements and culture-independent assessments of microbial community composition to make inferences about the flow of terrestrial carbon in lake food webs. Stable isotope ratios of POM and zooplankton decreased with DOC and were often depleted in ^{13}C relative to terrestrial carbon, suggesting the importance of an isotopically light carbon source. It has been proposed that the incorporation of biogenic methane (CH_4) into plankton food webs would account for such trends in stable isotope ratios, but we found weak evidence for this hypothesis, on the basis of relationships of CH_4, methanogenic archaebacteria, and methanotrophic bacteria in our lakes. Instead, our results are consistent with the view that phytoplankton increase their use of heterotrophically respired CO_2 with increasing concentrations of terrestrial-derived DOC. The effect of this CO_2 recycling can be detected in the stable isotope composition of crustacean zooplankton, suggesting that the direct transfer of terrestrial DOC inputs to higher trophic levels may be relatively inefficient.

Freshwater, estuarine, and marine ecosystems receive large inputs of terrestrial carbon in the form of dissolved organic carbon (DOC). In addition to altering numerous physical and chemical attributes, DOC has a strong influence on the metabolic functioning of aquatic ecosystems (Hanson et al. 2003). However, for a number of reasons, the flow of terrestrial-derived DOC in lake food webs is less clear. First, a large percentage (85–90%) of the total DOC pool is biologically recalcitrant (Søndergaard and Middelboe 1995), whereas the remaining percentage is restricted to consumption primarily by aquatic bacteria. Second, planktonic bacteria have relatively low growth efficiencies and respire 35–99% of consumed DOC as CO_2 (del Giorgio and Cole 1998). Finally, terrestrial carbon flow in lake food webs may be influenced by the limited ability of some zooplankton functional groups to graze on DOC-subsidized bacteria (Jürgens 1994). Despite these trophic constraints, some studies report that >50% of the carbon in particulate organic matter (POM), zooplankton, and fish may ultimately be derived from terrestrial ecosystems (Grey et al. 2001; Pace et al. 2004; Carpenter et al. 2005).

The DOC concentration in lakes varies across the landscape (Canham et al. 2004) and may influence the degree to which aquatic food webs are subsidized by terrestrial carbon. This hypothesis has been addressed using naturally occurring stable isotope ratios, with the prediction that zooplankton should converge upon the isotopic signature of terrestrial carbon with increasing concentrations of DOC (Jones et al. 1999). Zooplankton stable isotope ratios decrease with DOC concentration and also tend to be depleted in ^{13}C relative to both POM and terrestrial-derived carbon (Jones et al. 1999; Karlsson et al. 2003). Although these observations reveal that DOC inputs

[1] To whom correspondence should be addressed. Present address: W.K. Kellogg Biological Station and Department of Microbiology & Molecular Genetics, Michigan State University, Hickory Corners, MI 49060 (lennonja@msu.edu).

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alter carbon flow, they also suggest the importance of other processes that modify the carbon stable isotope composition of lake food webs.

One explanation for the stable isotope trends along DOC gradients is that methane (CH_4) may be an important, but overlooked, source of carbon in lake food webs (Bastviken et al. 2003). In anaerobic lake sediments, methanogenic archaebacteria produce CH_4 either through acetate fermentation or CO_2 reduction. This CH_4 is depleted in ^{13}C with carbon isotope signatures ranging from −110 to −50‰ (Whiticar et al. 1986). CH_4 is typically oxidized by methanotrophic bacteria at the anoxic-oxic boundary layer in lakes (Rudd and Taylor 1980). Recently, it has been hypothesized that ^{13}C-depleted isotope signatures of consumer populations in a diversity of aquatic ecosystems are due to the ingestion of methanotrophic bacteria (Grey et al. 2004; Kohzu et al. 2004). Furthermore, it has been hypothesized that this CH_4-consumer link may be more important in lakes with high DOC concentrations (Jones et al. 1999), because inputs of terrestrial-derived organic material may create conditions that favor CH_4 production (Casper et al. 2003; Houser et al. 2003).

Alternatively, trends in carbon stable isotopes among lakes may be due to CO_2 recycling (France et al. 1997), which is defined as the refixation of respired CO_2 before it leaves an ecosystem (Yakir and Sternberg 2000). For example, the DIC in lakes with low DOC concentrations is expected to consist mainly of atmospheric or geogenic sources and, thus, have a relatively ^{13}C-enriched isotopic signature. In contrast, the DIC in lakes with high DOC concentrations may have a ^{13}C-depleted isotopic signature if the contribution of atmospheric or geogenic derived CO_2 becomes diluted by heterotrophically respired terrestrial carbon. Phytoplankton will reflect such trends in DIC isotope signatures but may be further affected by changes in CO_2 concentrations resulting from the metabolism of terrestrial carbon inputs. For example, in marine ecosystems, photosynthetic isotope fractionation often increases with the concentration of CO_2, resulting in phytoplankton biomass that is depleted in ^{13}C (Hayes 1993). Such changes in the source and concentration of CO_2 should influence the stable isotope compositions of lake food webs, especially when phytoplankton-derived carbon is the dominant source of energy for higher trophic levels.

We tested two competing hypotheses about the flow of terrestrial carbon in planktonic food webs in a comparative study of 68 northeastern U.S. lakes varying in DOC concentration. First, we predicted that CH_4 concentrations, methanogenic archaebacteria, and methanotrophic bacteria would increase with DOC if terrestrial carbon loading is responsible for increasing CH_4 contributions to planktonic food webs. We also tested whether the stable isotope ratios of carbon pools decreased with increasing CH_4 concentrations. Second, we predicted that, if stable isotope trends in lakes are due to recycling of heterotrophically respired terrestrial carbon, then the stable isotope ratios of CO_2 and phytoplankton biomass would decrease with DOC and explain variation in zooplankton stable isotope ratios among our sampled lakes.

Methods

Lake survey and sample collection—During the summers of 2002 and 2003, we obtained samples from lakes in the northeastern U.S. to capture a natural gradient of terrestrial-derived DOC. In total, we sampled 68 lakes: in New Hampshire (39 lakes), Vermont (18), Maine (8), New York (4), and Connecticut (1). Seven lakes were sampled in both years, but we have treated these data as independent because the interannual variability of resampled lakes was equal to the between-lake variability for lakes sampled only once. We collected samples for POM and zooplankton isotope analysis from all 68 lakes. We collected samples for analysis of CO_2 (concentrations and stable isotopes), CH_4 and for detection of microorganisms from all 37 lakes visited in 2003. In addition, we intensively collected samples from three lakes along the DOC gradient (low, medium, and high DOC concentrations) to better evaluate how metalimnetic and hypolimnetic conditions influence epilimnetic carbon stable isotope ratios. The geographic locations and limnological characteristics of the lakes from which samples were obtained can be found in Web Appendix 1 (http://www.aslo.org/lo/toc/vol_51/issue_4/1602a1.pdf).

We restricted our sampling to a 6-week 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. The depths of the epilimnia and oxyclines (oxic-anoxic interface) were determined by measuring the temperature and the dissolved oxygen (O_2) concentration, respectively, at 0.5-m intervals with a Quanta Hydrolab water system. The oxycline was defined as the depth where O_2 decreased to <30 μmol L^{-1}; depending on the lake, this transition occurred in the water column or at the sediment-water interface. When an oxycline was detected in the water column, the lake was considered to have an anoxic hypolimnion. We obtained epilimnetic water samples with a depth-integrated column sampler constructed from PVC tubing and a swing-flap check valve. Epilimnetic zooplankton samples were taken with an 80-μm net. For sampling of discrete depths, we obtained water with a Van Dorn sampler and zooplankton with a Schindler-Patalas trap (80-μm net). We also collected grab samples from the inlet streams of the three intensively surveyed lakes to evaluate similarities between stream and lake water chemistry.

Estimation of terrestrial DOC concentration—We used DOC and color as estimates of terrestrial-derived DOC in our lakes. DOC was measured on 0.7 μm-filtered (Whatman GF/F) samples with a Shimadzu TOC-5000 total carbon analyzer. Color was measured on 0.7-μm filtered (Whatman GF/F) water samples at 440 nm with a 10-cm quartz cuvette. We expressed color as the following absorbance coefficient: $a_{440} = 2.303$ (absorbance at 440 nm/0.1 m) (Cuthbert and del Giorgio 1992).

Methane, methanogens, and methanotrophic bacteria—Water samples for analysis of CH_4 were collected with a Van Dorn sampler from the water column (for the three

intensively sampled lakes) and at the sediment-water interface. On the boat, we transferred 50 mL of lake water with a syringe into gas-evacuated 150 mL septum-sealed jars. We then injected 50 mL of supersaturated NaCl into the sample jar to reduce CH_4 solubility (Yamamoto et al. 1976), so the gas could be quantitatively measured in the headspace (Casper et al. 2003). CH_4 was measured with an FID detector on a Shimadzu GC-9A fit with a Carboxen 1000 column (Supelco, Bellefonte, PA). We set the furnace temperature of the GC to 200°C and adjusted the flow rates of N_2 (carrier) to 60 mL min^{-1}, H_2 to 50 mL min^{-1}, and air to 400 mL min^{-1}. The CH_4 concentration for each sample was expressed as the mean of 10 replicate injections by using a standard curve built from a known standard (Scott Specialty Gases).

We quantified the abundance and relative abundance of methanogens from the sediment-water interface with epifluorescence microscopy. Sediment microorganisms were extracted via centrifugation at 750 × g for 10 min (Furtado and Casper 2000) and preserved in 4% formalin. We filtered samples onto 0.2-μm white polycarbonate filters, which were then mounted with 40 μL of Citifluor antifading solution (AF1; Citifluor). We enumerated methanogens with a filter set (11005V2, with 405-nm excitation and 455-nm emission; Chroma Technology) that detected the autofluorescence of coenzyme F_{420} (Doddeman and Vogels 1978). Although some other microorganisms possess F_{420}, this potential bias is probably negligible for our application (Casper et al. 2003). The concentration of total sediment bacteria was quantified via DAPI staining (1 μg mL^{-1} of DAPI solution to 2 mL of sample). In duplicate, we counted all cells in 10 random fields for each sample using a Nikon TE2000-U inverted microscope with Compix imaging software. We defined the relative abundance of methanogens as the concentration of F_{420} autofluorescent cells divided by the concentration of DAPI-stained cells.

We quantified the abundance and relative abundance of methanotrophic bacteria with fluorescent in situ hybridization (FISH). We used fluorescently labeled DNA probes (MWG Biotech) targeting the 16S rRNA of type I methanotrophs within the γ-proteobacteria (probe M-84, Cy3-labeled) and type II methanotrophs within the α-proteobacteria (probe M-450, Cy5-labeled) (Dedysh et al. 2001). We collected water samples for detection of methanotrophs at the oxycline of each lake, where there are typically high rates of CH_4 oxidation (Rudd and Taylor 1980). Samples were preserved in 2% paraformaldehyde (PFA) and filtered onto white 0.22-μm polycarbonate filters. Hybridizations were initiated by placing filters facedown on a parafilm-covered slide with 30 μL of probe solution. The probe solution consisted of eight parts hybridization buffer (0.9 mol L^{-1} NaCl, 20 mmol L^{-1} Tris, 25% formamide, and 0.01% SDS) and one part of each probe stock (50 ng of dried probe in 1 μL of nuclease-free water). Samples were incubated in 50-mL hybridization chambers for 6 h at 42°C. We washed the filters in buffer (225 nmol L^{-1} NaCl, 20 mmol L^{-1} Tris, and 0.01% SDS) for 30 min at 48°C, counterstained them with 100 μL of DAPI (1 μg mL^{-1}) for 3 min, and rinsed them with 80% ethanol. Dry filters were mounted with a 4 : 1 mixture of Citifluor and Vectashield (Vector Laboratories). We also hybridized known populations of bacteria with our labeled probes. We used *Methylomicrobium album* BG8 (type I), *Methylococcus capsulatus* Bath (type I), and *Methylosinus trichosporium* OB3b (type II) as positive controls, and *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* as negative controls. We counted all cells in 10 random fields for each sample with a Nikon TE2000-U inverted microscope (equipped with Cy3, Cy5, and ultraviolet [UV] filter sets) and Compix imaging software.

Inorganic carbon concentrations and stable isotopes—We measured the concentrations and stable isotope ratios of DIC for 37 lakes in 2003. Sample vials were prepared by injecting 150 μL of H_3PO_4 into a 10-mL vial. We then sealed each vial with a septum cap and flushed it for 5 min with He gas using a double-holed needle. On the boat, we injected 5 mL of lake water into the sample vial with a 10-mL gas-tight syringe. In the laboratory, a small stream of He was forced into the sample, and the displaced gas was fed through capillary tubing and a water removal system (Gas Bench; Thermo Finnigan). The displaced gas was then passed through a 2-m HayeSep D micropacked stainless steel column kept at a constant temperature of 50°C for separation of CO_2 from other gases. We measured the concentration and stable isotope ratio of the evolved CO_2 on a Thermo-Finnigan Delta Plus XL mass spectrometer. We calculated the concentrations of carbonate species using DIC, pH, and the equilibrium constants provided by Wetzel and Likens (2000). To evaluate the relationship between CO_2 supersaturation and DOC, we used Henry's constant and epilimnetic water temperatures to calculate equilibrium CO_2 concentrations [$CO_{2(eq)}$] for each lake, assuming an atmospheric CO_2 partial pressure of 38 Pa.

To determine the isotopic ratio of $CO_{2(aq)}$, we first calculated the isotopic fractionation between $CO_{2(aq)}$ and HCO_3^- at our measured temperatures by using the equation $\varepsilon = (-9866/T) + 24.12$, where ε is defined as $[(R_{CO_2}/R_{HCO_3}) - 1] \times 1000$, R is the ratio of ^{13}C to ^{12}C, and T is temperature in degrees Kelvin (Mook et al. 1974). We then solved for the equilibrium partitioning, by treating $CO_{2(aq)}$ and H_2CO_3 as one species and assuming HCO_3^- and CO_3^{2-} have the same isotopic ratio, according to the equation

$$\delta^{13}C_{DIC} \times [DIC] = \delta^{13}C_{CO_2} \times [CO_2 + H_2CO_3] + \delta^{13}C_{HCO_3-} \times [HCO_3^- + CO_3^{2-}] \quad (1)$$

where $\delta^{13}C = [(R_{sample}/R_{reference}) - 1] \times 1000$, and R is the ratio of ^{13}C to ^{12}C for samples and reference material (Vienna Peedee Belemnite).

Organic carbon stable isotopes—We processed samples for $\delta^{13}C$ of POM ($\delta^{13}C_{POM}$) by drying organic matter (60°C) in sieved lake water (80 μm) that was retained on precombusted 0.7-μm filters (Whatman GF/F). For zooplankton, we separated cladocerans from copepods with a dissecting microscope, filtered the isolated animals onto precombusted 0.7-μm filters (Whatman GF/F), and dried

them at 60°C. Occasionally, cladocerans and copepods did not co-occur at high densities within a lake (>100 animals per taxa per sample). In these cases, $\delta^{13}C_{ZP}$ represents the isotope value of either copepods or cladocerans. When cladocerans and copepods co-occurred at high densities, $\delta^{13}C_{ZP}$ represents the mean isotope value of cladocerans and copepods.

We measured $\delta^{13}C$ of DOC and sediments for the three intensively sampled lakes. We measured $\delta^{13}C$ of DOC ($\delta^{13}C_{DOC}$) by first acidifying 1 L of filtered (Whatman GF/F) water with HCl (1 mol L^{-1}) to inhibit microbial activity and remove inorganic carbon. Dried organic matter was then recovered after the sample had evaporated at 60°C (Darren Bade, personal communication). For the $\delta^{13}C$ of sediments, we obtained replicate samples within each of the lakes using an Ekman dredge. We dried the sediment at 60°C and treated the samples with 1 mol L^{-1} HCl to remove carbonate.

All organic carbon samples were analyzed for $\delta^{13}C$ at the University of California–Davis Stable Isotope Facility with a PDZ Europa trace gas analyzer and a continuous-flow Europa 20/20 isotope ratio mass spectrometer (IRMS).

Phytoplankton stable isotope estimates—We estimated phytoplankton stable isotope composition as $\delta^{13}C_{PHYTO} = \delta^{13}C_{CO_2} + \varepsilon_p[1 + (\delta^{13}C_{CO_2}/1000)]$. We defined the photosynthetic fractionation factor (ε_p) as $(R_{Phyto}/R_{CO_2} - 1) \times 1000$, where R is the ratio of ^{13}C to ^{12}C; ε_p values are negative in this formulation. A number of marine studies have documented that photosynthetic fractionation increases with increasing $CO_{2(aq)}$ (Hayes et al. 1993). Therefore, we estimated ε_p as a function of $CO_{2(aq)}$ using two empirical models from the literature. The first model comes from Hinga et al. (1994) and describes ε_p for systems at 25°C with a $CO_{2(aq)}$ of 5–125 μmol L^{-1} as follows:

$$\varepsilon_p = -\left(1.89 + 9.09 \times \log_{10}CO_{2(aq)}\right) \quad (2)$$

Second, using data from Table 2 in Bidigare et al. (1997), we described ε_p as a hyperbolic function of $CO_{2(aq)}$ (10–275 μmol L^{-1} [$R^2 = 0.84$; $p = 0.0005$]).

$$\varepsilon_p = -\left(\frac{25.4 \times CO_{2(aq)}}{3.7 + CO_{2(aq)}}\right) \quad (3)$$

There are far fewer estimates of ε_p for freshwater ecosystems, and we are unaware of any studies that describe a functional relationship between ε_p and $CO_{2(aq)}$. Therefore, we relied on two constant estimates of ε_p (−11.4 and −5.6), which were derived from whole ecosystem isotope manipulations (Pace et al. 2004 and Cole et al. 2002, respectively) in Wisconsin lakes with $CO_{2(aq)}$ concentrations of ~50 μmol L^{-1}. Taken together, these four estimates of ε_p allowed us to generate a potential $\delta^{13}C_{PHYTO}$ range for a given lake sample.

Statistical analyses—We used linear, nonlinear, and logistic regression analyses to determine whether there were relationships between our response variables and epilimnetic DOC. When necessary, we $\log_{10}$-transformed the response variables in order to meet the assumptions of equal variance and normality. We described the relationship between DOC and color with a power function, although other nonlinear models fit these data equally well. We tested whether the occurrence of hypolimnetic anoxia increased with epilimnetic DOC with logistic regression. We used indicator variables multiple regression (Neter et al. 1996) to test hypotheses about lake carbon flow by contrasting how the $\delta^{13}C$ of different carbon pools (CO_2, POM, and zooplankton were coded with categorical indicator variables) varied with DOC (continuous predictor). We also used color as a predictor variable in a similar multiple regression analysis to assess whether $\delta^{13}C$ responded differently to our estimates of terrestrial DOC. Finally, we used the indicator variables multiple regression to test whether epilimnetic $\delta^{13}C$ was related to CH_4 concentrations measured at the sediment-water interface. Like analysis of covariance (ANCOVA), the predictor variables were centered by subtracting each observation from the mean value. We did not include estimates of $\delta^{13}C_{PHYTO}$ in the multiple regression analyses because they were not independent from the $\delta^{13}C_{CO2}$ measurements. To test for statistical differences in $\delta^{13}C$ for two carbon pools, 95% confidence limits were constructed around the difference between the estimated parameters for each pool (Neter et al. 1996). We concluded that there was no statistical difference between carbon pools if the confidence limits for the difference between the parameter values contained zero. We used a paired t-test to determine differences between the $\delta^{13}C$ of cladoceran and copepod zooplankton along the DOC gradient.

Results

Terrestrial DOC gradient—Our data suggest that we captured a broad gradient of terrestrial-derived DOC in our lake survey. DOC ranged from 120 to 1174 μmol L^{-1}, and color ranged from 0.3 to 11.4 m^{-1}. DOC increased as a power function of water color (a_{440}) as follows:

$$DOC = 144 + 182\left(a_{440}{}^{0.63}\right) \left(R^2 = 0.83;\ p < 0.0001\right) \quad (4)$$

Methane, methanogens, and methanotrophic bacteria—CH_4 at the sediment-water interface increased marginally with DOC as follows (Fig. 1a):

$$\text{Log}_{10}\, CH_4 = 0.46 + 0.001(DOC) \left(r^2 = 0.11;\ p = 0.052\right) (5)$$

In contrast to our original prediction, methanogens decreased with DOC (linear regression, $r^2 = 0.12$; $p = 0.033$). The occurrence of anoxic hypolimnia, which is considered to be a prerequisite for methanogenesis, was not related to DOC (logistic regression, $p = 0.83$). The density of total bacteria at the sediment-water interface was not related to DOC (linear regression, $r^2 = 0.03$; $p = 0.31$), and thus the relative abundance of methanogens also decreased

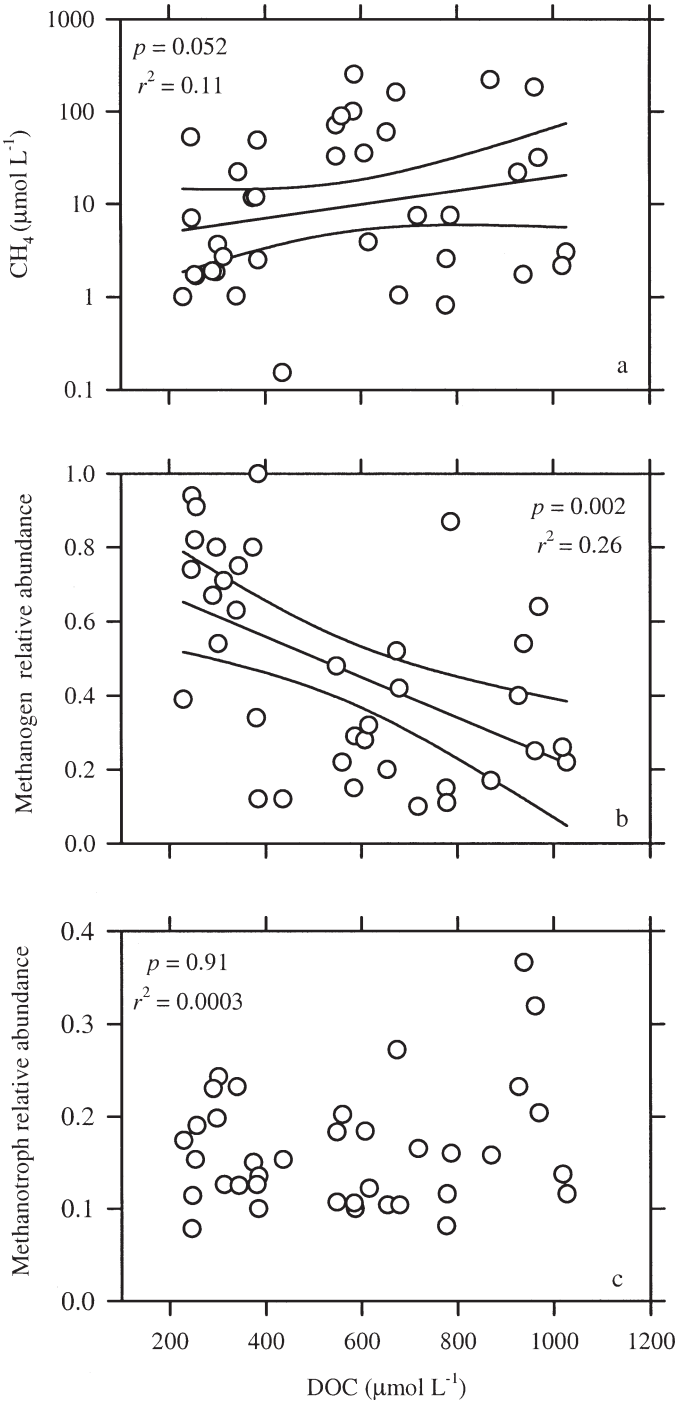

Fig. 1. The relationship between (a) CH_4 at the sediment-water interface, (b) the relative abundance of methanogenic archaebacteria, and (c) the relative abundance of methanotrophic bacteria as a function of DOC in our lake survey. Methanogens were sampled from surface sediments and methanotrophs were sampled from the oxycline. Lines are predicted values and 95% confidence intervals from simple linear regression.

with DOC as follows (Fig. 1b):

$$\text{Methanogen relative abundance} = 0.78 - 0.00055\,(\text{DOC})\ \left(r^2 = 0.29;\ p = 0.0005\right) \quad (6)$$

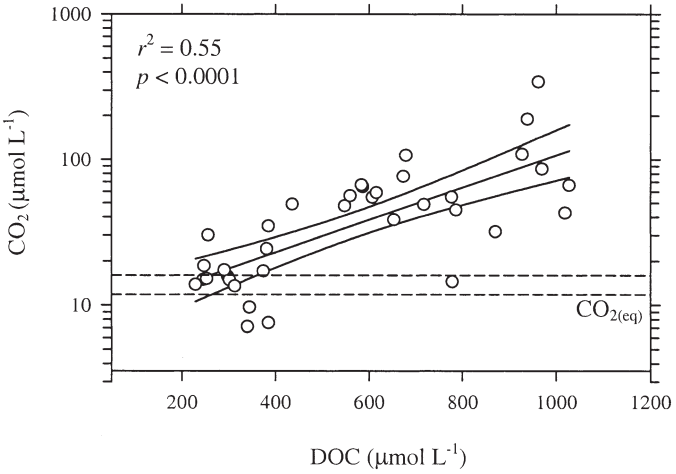

Fig. 2. The relationship between epilimnetic $CO_{2(aq)}$ and DOC from our lake survey. The dashed horizontal lines represent the range of equilibrium CO_2 concentrations based on Henry's law, temperature, and an assumed 38 Pa atmospheric CO_2 partial pressure. Other lines are predicted values and 95% confidence intervals from simple linear regression.

The abundance and relative abundance of methanotrophic bacteria (type I, type II, and the sum of these) were not correlated with DOC (linear regression, $p > 0.05$) (Fig. 1c). This lack of relationship held when we examined the different types of bacteria separately (types I and type II). On average, type I methanotrophic bacteria were 5 times more abundant than type II methanotrophic bacteria (mean cell counts [±SEM], $2.2 \times 10^5 \pm 0.20 \times 10^5$ cells mL^{-1} vs. $4.3 \times 10^4 \pm 0.63 \times 10^4$ cells mL^{-1}).

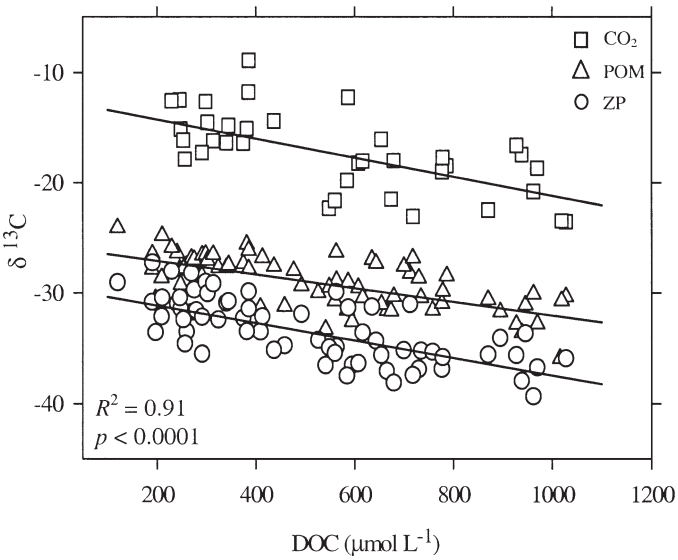

Fig. 3. The relationship between $\delta^{13}C$ and DOC for multiple carbon compartments (CO_2, POM, and zooplankton) obtained from the epilimnia of our lake survey. The $\delta^{13}C$-DOC slopes for CO_2, POM, and zooplankton (ZP) are the same, although intercepts were all significantly different from one another (Table 1). Lines are the predicted values from multiple regression analysis.

Table 1. Parameter estimates (means and SEs) from a multiple regression analysis to test 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 value; 532 μmol L^{-1}) and indicator variables to code for the different $\delta^{13}C$ samples. The slopes of the different carbon pools were not statistically different from one another. In contrast, the intercepts of the different carbon pools were all statistically different from one another. Subtracting the intercept values from one another provides an estimate for the average $\delta^{13}C$ difference between different carbon pools.

	Intercept		Slope	
$\delta^{13}C$	Mean	SE	Mean	SE
CO_2	−12.6	0.98	−0.009	0.0016
POM	−25.7	0.77	−0.006	0.0013
Zooplankton	−29.3	0.56	−0.008	0.0010

Together, methanotrophs comprised 16 ± 1.1% of the bacteria at the oxycline of the sampled lakes.

All of the regression results above were qualitatively similar when we used color instead of DOC as a predictor variable.

Epilimnetic $CO_{2(aq)}$ along the DOC gradient—Epilimnetic $CO_{2(aq)}$ increased with DOC as follows:

$$\text{Log}_{10}\, CO_{2(aq)} = 0.91 + 0.0011\ (\text{DOC})\ (r^2 = 0.55;\ p < 0.0001) \quad (7)$$

The $CO_{2(eq)}$ was 11.8–16.0 μmol L^{-1}, and on the basis of these estimates, 30 (~80%) of 37 lakes were supersaturated with CO_2 relative to the atmosphere (Fig. 2). Using Eq. 7 and $CO_{2(eq)}$, we estimated that lakes would become CO_2-supersaturated when the DOC exceeded 147–267 μmol L^{-1}. These relationships held when we used color instead of DOC as a predictor variable.

Epilimnetic $\delta^{13}C$ along the DOC gradient—There was a strong inverse relationship between $\delta^{13}C$ of epilimnetic carbon pools and DOC (Fig. 3). The indicator variables regression model was highly significant and explained a large fraction of the variability in $\delta^{13}C$ ($R^2 = 0.91$; $p < 0.0001$). $\delta^{13}C_{CO_2}$, $\delta^{13}C_{POM}$, and $\delta^{13}C_{ZP}$ significantly decreased ($p < 0.0001$) at similar rates with increasing DOC (Table 1; Fig. 3). However, there were significant differences in the $\delta^{13}C$ of the different carbon pools: the DOC-standardized intercept for $\delta^{13}C_{POM}$ was 11.9‰ lower than that for $\delta^{13}C_{CO_2}$, and the DOC-standardized intercept for $\delta^{13}C_{ZP}$ was 4.6‰ lower than than that for $\delta^{13}C_{POM}$ (Table 1). The statistical outcomes were qualitatively similar when we used color as a predictor of $\delta^{13}C$ ($R^2 = 0.92$; $p < 0.0001$). In contrast, $\delta^{13}C$ of the different carbon pools did not change as a function of CH_4 concentration measured at the sediment-water interface ($p = 0.32$).

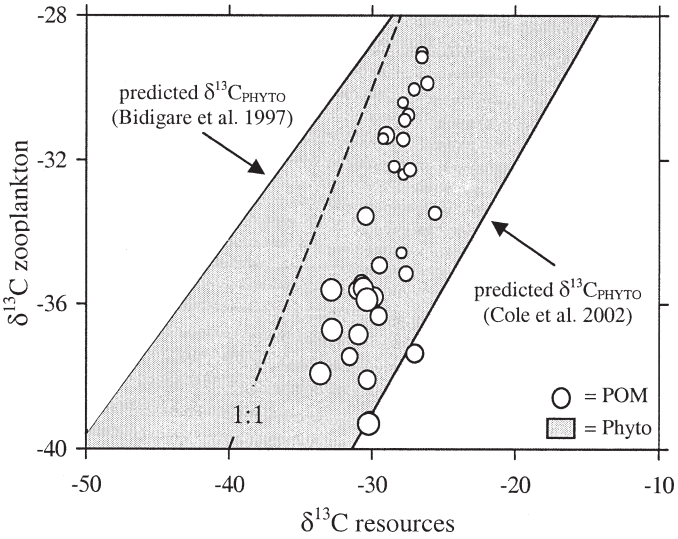

Fig. 4. The stable isotope relationship between zooplankton and two potential resources: POM and phytoplankton biomass. $\delta^{13}C_{POM}$ was measured directly and is represented by white symbols, the size of which is positively correlated with the epilimnetic DOC in each sample. The shaded grey region delineates the predicted range of potential $\delta^{13}C_{PHYTO}$ generated from our data and four literature-based estimates of photosynthetic fractionation (Table 2). The 1 : 1 line represents the assumption that consumer populations closely reflect the isotopic composition of their assimilated food source. $\delta^{13}C_{POM}$ is an inaccurate predictor of $\delta^{13}C_{ZP}$ as evidenced by deviations from the 1 : 1 line. Although broad, the range of $\delta^{13}C_{PHYTO}$ includes almost all of the $\delta^{13}C_{ZP}$ data points, suggesting that phytoplankton biomass may explain the isotopic composition of lake zooplankton.

Cladocerans and copepods co-occurred at high densities in ~70% of the sampled lakes. This pattern of co-occurrence was not related to DOC (logistic regression, $p = 0.68$). There was no significant difference in $\delta^{13}C$ of cladocerans and copepods (mean differences ± SEM = −0.3 ± 0.20‰; paired t-test, $t_{44} = 1.5$; $p = 0.13$). Furthermore, pairwise differences in $\delta^{13}C$ of cladocerans and copepods were not significantly related to DOC (linear regression, $p = 0.22$).

Table 2. Results from simple linear regression analyses using different estimates of $\delta^{13}C_{PHYTO}$ as predictors of $\delta^{13}C_{ZP}$. Estimates of $\delta^{13}C_{PHYTO}$ were calculated from our data ($\delta^{13}C_{CO_2}$ and $CO_{2(aq)}$) and four literature-based equations for phytoplankton fractionation (ε_p).

	Intercept		Slope			
Model	Mean	SE	Mean	SE	r^2	p
Cole et al. (2002)	−18.1	2.64	0.70	0.116	0.54	<0.0001
Pace et al. (2004)	−13.9	3.33	0.70	0.116	0.54	<0.0001
Hinga et al. (1994)	−21.0	1.33	0.41	0.004	0.76	<0.0001
Bidigare et al. (1997)	−13.2	2.41	0.54	0.060	0.71	<0.0001

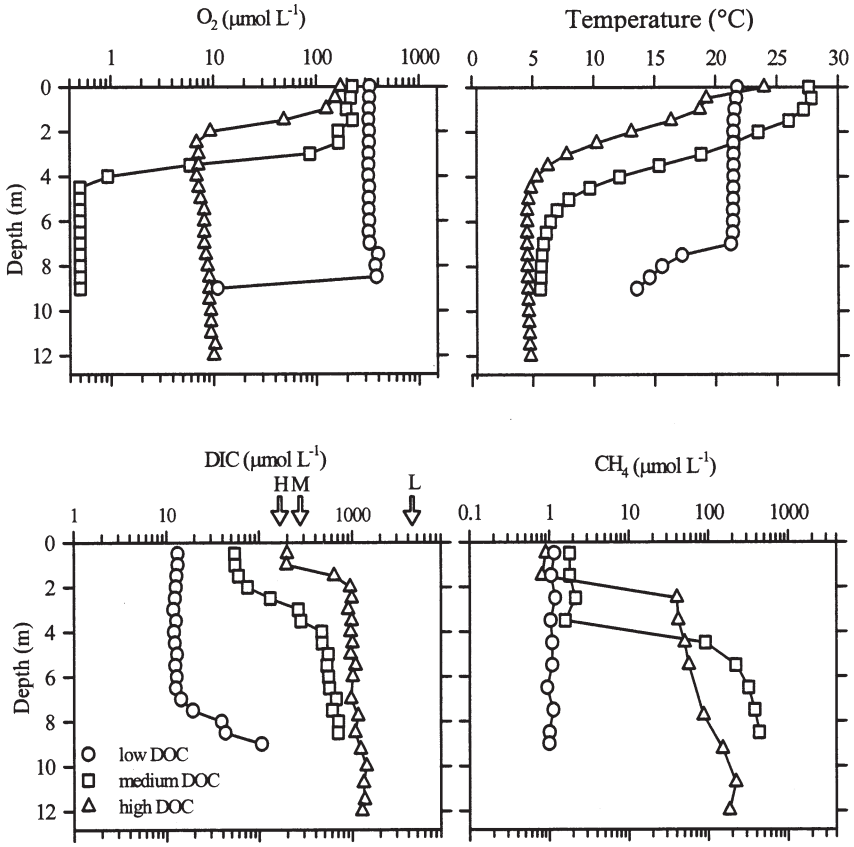

Fig. 5. Vertical distribution of dissolved oxygen (O_2), temperature (°C), dissolved inorganic carbon (DIC), and CH_4 in three lakes with varying DOC concentrations (low DOC = 229 μmol L^{-1}, medium DOC = 586 μmol L^{-1}, and high DOC = 962 μmol L^{-1}). Downward-pointing arrows and letters (L = low DOC, M = medium DOC, and H = high DOC) represent the DIC concentrations in the stream inlet of each lake.

In general, the two freshwater ε_p models had less photosynthetic fractionation and thus generated more positive $\delta^{13}C_{PHYTO}$ than the CO_2-dependent marine models. Considering all four fractionation models, ε_p ranged from −25.1 to −5.4. These estimates produced $\delta^{13}C_{PHYTO}$ ranging from −46.9 to −14.2‰ (Table 2), which in almost all cases could account for the observed variation in $^{13}C_{ZP}$ (Fig. 4).

Vertical profiles from lakes with varying DOC—There were differences in the vertical distribution of gases and $\delta^{13}C$ in the intensively sampled low, medium, and high DOC lakes. In general, gas concentrations (i.e., O_2, DIC, and CH_4) became less uniformly distributed with depth as DOC concentration increased (Fig. 5). Similarly, $\delta^{13}C_{DIC}$ was uniformly distributed with depth in the low DOC lake but was more variable in the medium and high DOC lakes (Fig. 6).

The isotopic ratios of the organic carbon pools were less affected by depth and DOC. For example, $\delta^{13}C_{DOC}$ and $\delta^{13}C_{ZP}$ were uniformly distributed with depth in all three lakes (Fig. 6). In contrast, $\delta^{13}C_{POM}$ was uniformly distributed in the low DOC lake but had more variable distributions in the medium and high DOC lakes (Fig. 6). Zooplankton were generally depleted in ^{13}C relative to POM through most of the water column (Fig. 6). The maximum differences between the $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$ for any combination of depths within the lakes were −0.4, 2.8, and 1.2‰ for the low, medium, and high DOC lakes, respectively.

Discussion

The relatively strong relationship between $\delta^{13}C$ and DOC observed in this study indicates that lake carbon flow is strongly influenced by landscape variation in DOC concentration. However, in lakes with high DOC, $\delta^{13}C_{POM}$ and $\delta^{13}C_{ZP}$ approached values that were depleted in ^{13}C (−33‰ and −38‰, respectively) relative to the stable isotope signature of terrestrial carbon in the surrounding watersheds (approximately −28‰). Therefore, the dominant flow path of terrestrial carbon in lake food webs does not seem to be best represented by a simple DOC-bacteria-zooplankton food chain. Our results suggest an increased importance of an isotopically light carbon source with increasing concentrations of DOC. Similar trends in other studies (Jones et al. 1999) have led to the hypothesis that methanotrophic bacteria may be an important food resource fueling plankton food webs (Bastviken et al. 2003). However, our measurements do not support this

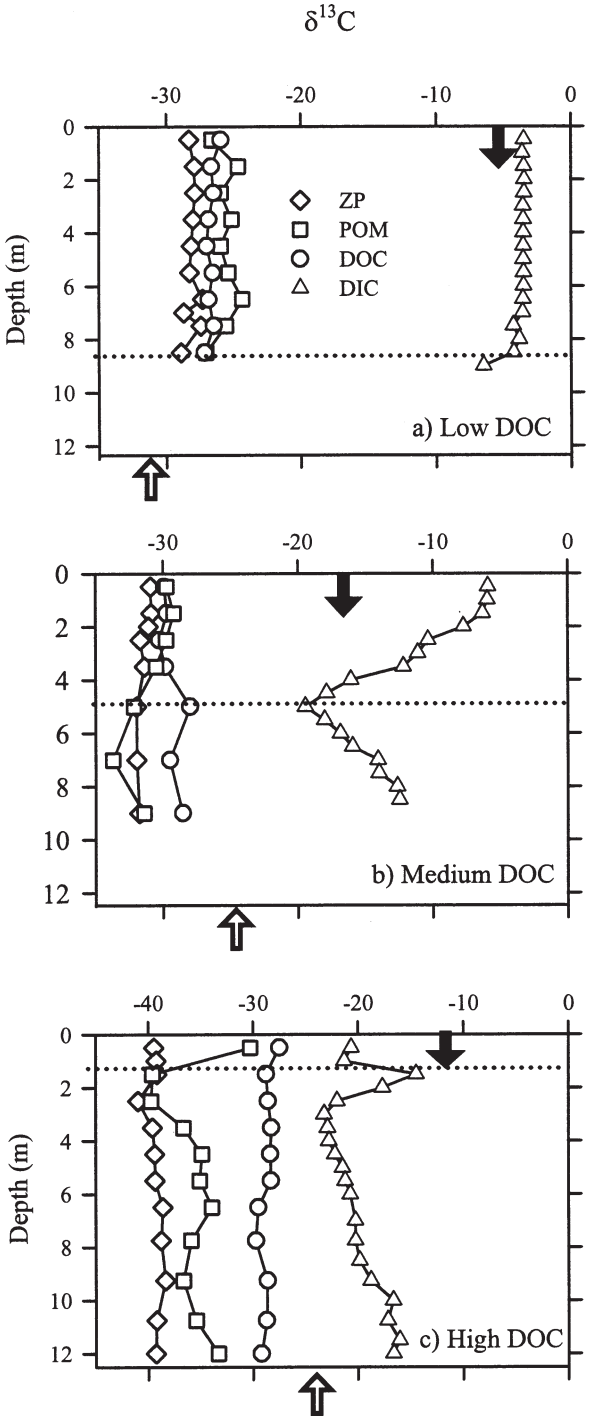

Fig. 6. Vertical distributions of $\delta^{13}C_{ZP}$, $\delta^{13}C_{POM}$, $\delta^{13}C_{DOC}$, and $\delta^{13}C_{DIC}$ in the three lakes with low, medium, and high DOC (see Fig. 5 for concentrations). The horizontal dotted lines represent the oxyclines. Upward-pointing arrows represent $\delta^{13}C$ of the sediments in each lake; downward-pointing arrows represent the $\delta^{13}C$ of the stream inlet DIC for each lake. Note different $\delta^{13}C$ scales for low, medium, and high DOC lakes.

hypothesis. In particular, CH_4, methanogenic archaebacteria, and methanotrophic bacteria did not increase with DOC. Furthermore, CH_4 itself was a poor predictor of epilimnetic $\delta^{13}C$. Our results are more in line with the CO_2 recycling hypothesis, which states that plankton food webs become progressively lighter with increasing DOC because of the dilution of atmospheric or geogenic CO_2 by heterotrophically respired terrestrial carbon (i.e., CO_2 recycling).

Methane hypothesis—We found weak support for the CH_4 hypothesis. CH_4 concentrations at the sediment-water interface increased only marginally along the DOC gradient (Fig. 1a). In a previous study of Wisconsin lakes, hypolimnetic CH_4 was also not correlated with surface DOC, although CH_4 accumulation increased over time with DOC in the same systems (Houser et al. 2003). In another comparative study of 79 lakes, water column CH_4 was inversely related to DOC, but lake size and the fraction of anoxic volume were better predictors of CH_4 concentrations (Bastviken et al. 2004). On the basis of this limited number of studies, there seems to be neither a strong nor a consistent relationship between the concentrations of DOC and CH_4 in lake ecosystems.

Contrary to our original prediction, the relative abundance of methanogens in the upper sediments significantly decreased with DOC concentration (Fig. 1b). One potential explanation for this relationship is that methanogens responded to conditions that covaried with DOC. The occurrence of anoxic hypolimnia was unaffected by DOC and therefore could not account for the compositional changes in these obligately anaerobic microbes. However, the relative abundance of methanogens increased with pH ($r^2 = 0.25$; $p = 0.0015$), consistent with reports that methanogens are potentially sensitive to acidic conditions (Garcia et al. 2000). High DOC lakes also have high iron concentrations (Maranger and Pullin 2003), which may favor Fe^{3+}-reducing bacteria over methanogens under anoxic conditions (van Bodegom et al. 2004). Finally, the humic substances in high DOC lakes may have inhibited methanogenesis because they were used as terminal electron acceptors by other anaerobic microorganisms (Lovley et al. 1996).

The relative abundance of methanotrophic bacteria did not change along the DOC gradient (Fig. 1c). This pattern is not surprising, given the weak DOC-CH_4 relationship (Fig. 1a). Nevertheless, our results are generally consistent with the few studies that have used culture independent approaches to characterize the distribution and abundance of freshwater bacterioplankton capable of using single-carbon compounds (i.e., methylotrophs). Methanotrophs (type I + type II) comprised 16% of the total bacterioplankton in our sampled lakes, whereas methylotrophs made up 10–46% of the bacteria in the water column of a shallow floodplain lake (Ross et al. 1997). On the basis of phospholipid fatty acid analyses, methanotrophs accounted for 10–11% of the total bacterial biomass in Swedish lakes with low DOC but only contributed 3% to the total bacterial biomass in a high DOC lake (Bastviken et al. 2003). Similarly, a recent comparative study of two German lakes found that methanotroph abundance and rates of CH_4 oxidation were influenced more by lake mixing regimes than DOC concentration.

One implicit, but critical, assumption of the CH_4 hypothesis is that epilimnetic zooplankton graze upon

methanotrophic bacteria in deeper waters during diel vertical migrations (Bastviken et al. 2003). Such behavior might also explain the commonly observed discrepancy between epilimnetic $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$ (Fig. 3) (del Giorgio and France 1996). Our results, however, are not consistent with the view that zooplankton were encountering ^{13}C-depeleted bulk POM during diel vertical migration. First, larger-bodied zooplankton tend to migrate more than smaller-bodied zooplankton because of their greater probability of being detected by visual-feeding predators (Lampert and Sommer 1997). As such, we would expect our cladoceran samples, which consisted primarily of large *Daphnia* spp., to be more depleted in ^{13}C relative to our smaller-bodied copepod samples, but this was not the case. Second, it has been shown that zooplankton migrate less in high DOC lakes because of reduced predation risk (Wissel et al. 2003) and/or reduced UV stress (Boeing et al. 2004). On the basis of these observations, we would not have expected $\delta^{13}C_{ZP}$ to decrease with increasing DOC as it did in our study (Fig. 3). Lastly, there would need to be at least a $-4.6‰$ difference between $\delta^{13}C_{ZP}$ and $\delta^{13}C_{POM}$ at different depths within the water column of a given lake if diel vertical migration were to explain the isotope trends in our study (Table 2; Fig. 3). We found no such difference from our three intensively sampled lakes (Fig. 6), although it is possible that some small (<0.7 μm), isotopically light bacteria were not retained in our hypolimnetic POM samples.

In sum, our results suggest a weak link between DOC, CH_4, and planktonic food webs in lake ecosystems. This does not however, preclude the importance of CH_4-derived carbon for consumer populations in some aquatic food webs. For example, beetle larvae in backwater pools of a Japanese stream were reported to have $\delta^{13}C$ values of $-68‰$ (Kohzu et al. 2004), and some chironomid species from the sediments of a productive German lake were $-65‰$ (Grey et al. 2004). Arguably, such low isotopic values present solid evidence for the incorporation of biogenic CH_4 into consumer biomass. Indeed, fatty acid analyses indicated the presence of type I methanotrophs in chironomids with low $\delta^{13}C$ signatures (-62 to $-55‰$; Kiyashko et al. 2004). However, it is possible the studies above reflect metazoan-methanotroph symbioses and not the direct consumption of methanotrophic biomass. Importantly, in cases where the $\delta^{13}C$ of a consumer is $-56‰$ or higher, it may be inappropriate to invoke the consumption of methanotrophic bacteria on the basis of stable isotope data alone, because the metabolic fractionation factors of some sulfur-oxidizing bacteria and oxygenic photoautotrophs can be as much as $-25‰$ (Ruby et al. 1987 and Hayes 1993, respectively) and because $\delta^{13}C_{DIC}$ in the epilimnia of some lakes can be as low as $-31‰$ (Bade et al. 2004). Finally, consumer population may be ^{13}C-depleted, not because they ingest methanotrophic biomass, but because they ingest organisms that use the CO_2 byproduct of methane oxidation.

CO_2 recycling hypothesis—Our results are consistent with the hypothesis that variability in planktonic $\delta^{13}C$ is due to increased CO_2 recycling with increasing DOC concentration. We observed changes in both the concentration and isotopic composition of CO_2, which most likely reflected heterotrophic respiration of terrestrial DOC. We contend that as DOC increased, respired terrestrial carbon diluted the relative contribution of atmospheric and geogenic CO_2 to the DIC pool. By our estimates, these changes in the isotopic composition and concentration of CO_2 could have led to the growth of ^{13}C-depleted phytoplankton, which in turn could explain the observed trends in $\delta^{13}C_{ZP}$.

Most lakes in our survey were supersaturated with CO_2, and as with other studies (Sobek et al. 2003), epilimnetic $CO_{2(aq)}$ was positively correlated with DOC. One explanation for this trend is that lakes are strongly influenced by inputs of CO_2-supersaturated stream water or groundwater. Results from our three intensively sampled lakes do not support this hypothesis. For example, the low DOC lake was fed by a stream that had a high DIC concentration (1550 μmol L^{-1}) (Fig. 5), yet $CO_{2(aq)}$ in the lake was near equilibrium with the atmosphere (14 μmol L^{-1}). Similarly, it is unlikely that the high DIC concentrations in the medium and high DOC lakes were sustained by the relatively low DIC concentrations found in their respective stream inlets (Fig. 5). It is important to note, however, that our snapshot samples may not accurately reflect the importance of watershed DIC contributions to lakes on an annual scale. Nevertheless, our findings are consistent with evidence from mesocosm experiments (Lennon 2004), diel oxygen sampling (Hanson et al. 2003), and whole-ecosystem studies (Cole et al. 2002), all of which suggest that CO_2 supersaturation arises from in-lake respiration of terrestrial carbon inputs.

Although influenced by geochemical factors, our results support the view that the isotopic composition of DIC is modified by in-lake metabolism of DOC. In low DOC lakes with $CO_{2(aq)}$ concentrations close to atmospheric equilibrium (Fig. 2), $\delta^{13}C_{CO2}$ was $-14‰$ and approximated expected isotope values based on the diffusion and dissolution of atmospheric CO_2 into water at ambient temperatures (Fig. 3). In contrast, high DOC lakes that were supersaturated with CO_2 had $\delta^{13}C_{CO_2}$ values of approximately $-22‰$. Moreover, epilimnetic $\delta^{13}C_{DIC}$ from our intensively sampled lakes was different from the $\delta^{13}C_{DIC}$ of its respective stream inlets (Fig. 6). These results are consistent with findings from comparative surveys and process-based simulations where $\delta^{13}C_{DIC}$ increases with the ratio of gross primary productivity to community respiration and decreases as a function of DOC (Bade et al. 2004). Similar results have been found in other studies, in which ~90% of the epilimnetic DIC in a high DOC lake (1,100 μmol L^{-1}) was attributed to internal heterotrophic respiration (Cole et al. 2002), and ~66% of the DIC in a predominantly groundwater-influenced lake was derived from the decomposition of organic matter (Wachniew and Rozanski 1997). Clearly, in-lake metabolism of terrestrial organic matter has the potential to alter the isotopic composition of DIC that is used by phytoplankton and other autotrophic organisms.

Phytoplankton biomass is generally considered a dominant source of carbon-based energy in lake ecosystems. Unfortunately, it is difficult to isolate phytoplankton from

heterotrophic organisms and detritus within the POM size fraction used for stable isotope analysis. Nevertheless, there are a variety of means for obtaining $\delta^{13}C_{PHYTO}$. Some groups of phytoplankton may be separated from other components of POM by sedimentation (Jones et al. 1999), cell sorting (Pel et al. 2003), or centrifugation (Hamilton et al. 2005). Alternatively, $\delta^{13}C_{PHYTO}$ has been estimated from whole lake isotope manipulations and model fitting of time series data (Pace et al. 2004), although this approach is not practical for large comparative studies such as ours. A final strategy, and the one that we adopted, is to calculate $\delta^{13}C_{PHYTO}$ by by use of literature-based estimates of ε_p (Karlsson et al. 2003) from studies in which $\delta^{13}C_{PHYTO}$ and $\delta^{13}C_{CO2}$ were both directly measured.

In theory, $\delta^{13}C_{PHYTO}$ is influenced predominantly by the isotopic composition of the DIC source (i.e., $\delta^{13}C_{CO_2}$). As such, we can be reasonably confident that $\delta^{13}C_{PHYTO}$ decreased with DOC concentration in our lakes (Fig. 3). However, $\delta^{13}C_{PHYTO}$ becomes more depleted in ^{13}C via photosynthetic fractionation associated with carbon assimilation (Hayes et al. 1993). The magnitude of ε_p is thought to be influenced mainly by variability in phytoplankton growth rates and $CO_{2(aq)}$. In particular, photosynthetic fractionation for some marine phytoplankton increases with $CO_{2(aq)}$ because of changes in diffusion rates or active transport mechanisms (Hayes 1993). One of the CO_2-dependent fractionation models (Eq. 3) generated low ε_p values for some lakes (-25), which in turn yielded $\delta^{13}C_{PHYTO}$ of -46.9‰. However, to our knowledge, a CO_2-dependent isotope effect has not been documented for freshwater phytoplankton. The $CO_{2(aq)}$-independent freshwater models generated much smaller ε_p values and thus more positive $\delta^{13}C_{PHYTO}$ (-35 to -18‰) than the CO_2-dependent marine models (Table 2). The apparent discrepancy between freshwater and marine ε_p may reflect differences in experimental approaches, environmental conditions, and/or phytoplankton physiology. For example, lakes typically have higher $CO_{2(aq)}$ than marine ecosystems, although this should lead to enhanced photosynthetic fractionation for freshwater phytoplankton. In addition, some marine phytoplankton use carbon concentrating mechanisms (CCM) for active CO_2 and bicarbonate uptake, which yield low ε_p values (-7 to -5‰) despite variation in $CO_{2(aq)}$ (Tortell et al. 2000). Much less is known about the isotope effects of CCM for freshwater phytoplankton.

In almost all our sampled lakes, the estimated range of $\delta^{13}C_{PHYTO}$ could account for the variation in $\delta^{13}C_{ZP}$ (Fig. 4). However, the use of $\delta^{13}C_{PHYTO}$ as a mechanistic predictor for trends in our $\delta^{13}C_{ZP}$ rests on the assumption that zooplankton selectively fed upon ^{13}C-depleted phytoplankton over other potential resources in the POM size fraction. It is not difficult to assert selective feeding for some zooplankton functional groups. For example, rotifers in a Dutch lake preferentially fed upon ^{13}C-depleted algae over numerically dominant cyanobacteria (Pel et al. 2003), and it is well-documented that many copepods are specialist consumers of phytoplankton (DeMott 1988). In contrast, cladocerans are typically perceived as generalists that ingest detritus, bacteria, and phytoplankton (Jürgens 1994). There are, however, a number of active and passive mechanisms by which cladocerans can act as semiselective consumers. For example, some cladocerans ''taste-test'' their food before ingestion (Kerfoot and Kirk 1991), and the morphology of the cladoceran filtering apparatus can change in response to resource availability (Lampert 1994). Lastly, many phytoplankton populations are not incorporated into cladoceran biomass, because of ingestion- and/or digestion-resistance traits (DeMott and Tessier 2002). Together, findings of the studies above demonstrate the capacity for preferential ingestion and/or assimilation of isotopically light phytoplankton by copepod and cladoceran zooplankton.

Understanding the flow of energy and materials in food webs is a fundamental goal of aquatic community and ecosystem ecology. We used naturally occurring carbon stable isotopes to make inferences about how terrestrial carbon flows in lake food webs. Results from our comparative study revealed that $^{13}C_{POM}$ and $\delta^{13}C_{ZP}$ decrease at the same rate with increasing concentrations of DOC. Our study also documented a decrease in $\delta^{13}C_{CO2}$ with DOC, providing evidence for the refixation of heterotrophically respired terrestrial carbon (i.e., CO_2 recycling).

Our results have implications for understanding the energetics of lake food webs. We expected that $^{13}C_{ZP}$ would converge upon an isotopic composition of approximately -28‰ if zooplankton were directly subsidized by terrestrial carbon inputs. In contrast, zooplankton were often depleted in ^{13}C relative to terrestrial carbon. CH_4 is an isotopically light source of carbon and energy that may influence the isotopic composition of consumer populations in some systems. However, our gas measurements and assessments of microbial composition did not vary with DOC in ways that were consistent with the CH_4 hypothesis. Instead, our results are in agreement with the view that many aquatic consumer populations acquire a large fraction of their energy via primary production, even in systems receiving high loads of terrestrial-derived resources (Sobczak et al. 2000). Because of the uncertainties associated with photosynthetic fractionation, however, it is still difficult to quantitatively estimate the relative contribution of terrestrial versus algal carbon in lake food webs.

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