

Pathways of organic carbon utilization in small lakes: Results from a whole-lake ^{13}C addition and coupled model

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Abstract

In many small aquatic ecosystems, watershed loading of organic C exceeds autochthonous primary production. Although this allochthonous organic C has long been thought of as refractory, multiple lines of evidence indicate that substantial portions are respired in the receiving aquatic ecosystem. To what extent does this terrestrial C support secondary production of invertebrates and fish? Do current models adequately trace the pathways of allochthonous and autochthonous C through the food web? We evaluated the roles of allochthonous and autochthonous organic C by manipulating ^{13}C content of dissolved inorganic C in a small, softwater, humic lake, thereby labeling autochthonous primary production for about 20 d. To ensure rapid and sufficient uptake of inorganic ^{13}C , we enriched the lake with modest amounts of N and P. We constructed a carbon flow model based on the ambient and manipulated levels of ^{13}C in C compartments in the lake, along with information on key rate processes. Despite the short nature of this experiment, several results emerged. (1) Fractionation of photosynthetically assimilated $^{13}\text{C}\text{-CO}_2$ by phytoplankton (ϵ) is lower ($\sim 6\%$) than physiologic models would estimate ($\sim 20\%$). (2) Bacteria respire, but do not assimilate, a large amount of terrestrially derived dissolved organic C (DOC) and pass little of this C to higher trophic levels. (3) The oxidation of terrestrial DOC is the major source of dissolved inorganic C in the lake. (4) Zooplankton production, a major food of young-of-year fishes, is predominantly derived from current autochthonous carbon sources under the conditions of this experiment.

Aquatic ecosystems receive organic matter from two distinct sources: primary production occurring within the system (autochthonous production) and loading of terrestrial organic matter from the watershed (allochthonous loading). The magnitude and proportion of these sources vary widely among different systems (Wetzel 2001). For small- to moderate-sized lakes, unless they are eutrophied, allochthonous loading and autochthonous primary production tend to be similar, whereas in unproductive lakes, the loading of allochthonous DOC can greatly exceed autochthonous primary production (Caraco and Cole 2002).

The relative importance of within-system primary produc-

tion versus terrestrial organic matter in fueling food webs has been an intriguing and unresolved question in lake ecology for some time. Since Elton (1927) first compiled diagrams of food webs, ecologists have viewed ecosystems as operating primarily via energy derived from photosynthetic carbon fixation occurring within the system. This autotrophically produced carbon is consumed both by direct grazing and subsequent predation and through detrital pathways as formalized by Lindeman (1942). This view either ignores the large load of allochthonous organic matter or assumes that it is relatively refractory to biological use.

Many lakes, however, are “net heterotrophic,” meaning that respiration of the system, R , exceeds gross primary production (GPP). Thus, net heterotrophy implies that net ecosystem production ($\text{NEP} = \text{GPP} - R$) is negative or $\text{GPP}/R < 1$. The evidence for net heterotrophy comes from studies of GPP and R in bottles (del Giorgio and Peters 1994; *but see* Carignan et al. 2000), gas saturation and flux from whole lakes (Kling et al. 1991; Cole et al. 1994; Riera et al. 1999), and continuous measurements of free-water oxygen or CO_2 changes over diel cycles (Sellers et al. 1995; Cole et al. 2000). Net heterotrophy can be maintained only if R is subsidized by the metabolism of allochthonous organic matter. Net heterotrophy depends on the active metab-

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olism of allochthonous organic matter in the lake and is supported by studies of DOC degradation by microorganisms (Tranvik 1992; Wetzel 2001) and whole-lake C budgets (Dillon and Molot 1997; Jansson et al. 2000).

We know that terrestrial organic matter is respired in many lakes, but we are just beginning to learn how important this terrestrial C is to the food webs that lead to the secondary production of invertebrates and fish (e.g., Meili et al. 1996; Grey et al. 2001). An obvious way to trace the flow of terrestrial and autochthonous organic C into the aquatic food web is the use of stable isotopes (Kling et al. 1992; France et al. 1997). If there is a contrast between the $\delta^{13}\text{C}$ of terrestrial and aquatic primary production, it is possible to compare components of the food web to these two extremes (Meili et al. 1996; France et al. 1997; Jones et al. 1999). In many cases, this contrast is small and one cannot easily resolve sources to the food web (Schiff et al. 1990). Another approach is to construct a model of the lake food web and follow the paths of autochthonous and allochthonous C, but calibration and validation of such models is difficult. An alternative approach is to experimentally manipulate the isotopic signature either of the autotrophically fixed C (Hesslein et al. 1980; Bower et al. 1987) or of the allochthonously loaded C and then, using a model, trace the flows of these isotopes.

We have been working in softwater, humic lakes where the DOC and particulate organic C (POC) have $\delta^{13}\text{C}$ isotopic signatures of (-26‰ to -29‰), which overlap those of terrestrial inputs. We reasoned that by manipulating the $\delta^{13}\text{C}$ of dissolved inorganic C (DIC) in the lake, the autochthonous primary production would acquire a distinctive isotopic signature. Coupled with measurements of total C pools and most of the relevant C transformations, the ^{13}C manipulation should create ideal conditions under which to test how ^{13}C is fractionated during initial fixation under field conditions, moves through the food web, and is diluted by the utilization of allochthonous C. Prior experiments with whole-lake ^{14}C additions have been successful for the estimation of gas exchange and as validations for bottle-based primary production estimates (Hesslein et al. 1980; Bower et al. 1987) but have not been extended to evaluate trophic dynamics.

The purpose of this study was to create and experimentally test a C model of a lake ecosystem that would encompass the fates of both allochthonously loaded and autochthonously produced organic matter.

Materials and methods

Site description—East Long Lake is part of the University of Notre Dame Environmental Research Center (UNDERC) near Land o' Lakes, Wisconsin ($89^{\circ}32'\text{W}$; $46^{\circ}13'\text{N}$) and is the eastern basin of Long Lake. A neoprene curtain deployed in 1991 separated this basin from the rest of the lake. East Long Lake has been extensively studied in the context of whole-lake manipulations of nutrients and food web structure (Carpenter et al. 2001). The lake is small (2.3 ha), relatively deep (mean depth = 4.9 m), well stratified, and darkly stained with DOC (1,100 μM ; Fig. 1). The mixed depth of the epilimnion is about 1 m. East Long Lake has modest

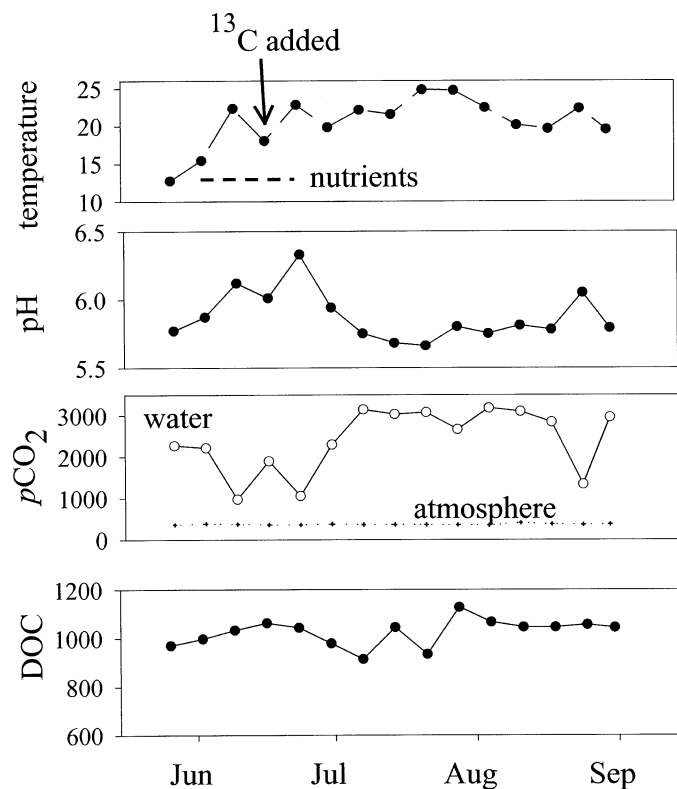


Fig. 1. Time series of selected constituents in the surface water of East Long Lake in 1999. (A) Temperature ($^{\circ}\text{C}$). (B) pH. (C) pCO_2 (μatm) in the surface water (open circles) and in the air 1 m above the lake surface (crosses, dotted line). (D) DOC in surface water (mg C L^{-1}). The arrow shows the point at which ^{13}C -DIC was added; the dotted line shows the extent of the nutrient addition (see text).

fish populations and low rates of zooplanktivory. Zooplankton are generally dominated by large-bodied *Daphnia* (Carpenter et al. 2001). The hydrologic residence time of East Long Lake, measured by whole system LiBr addition, is about 1.5 yr (Cole and Pace 1998). DIC averages $\sim 80 \mu\text{M}$, and the lake is acidic (mean pH 5.2; Fig. 1). Phytoplankton are typical of humic lakes in the region and are dominated by small flagellates, mostly Chrysophytes. Surface water chlorophyll *a* (Chl *a*) concentrations in the absence of fertilization are moderate, averaging 10 to 20 $\mu\text{g L}^{-1}$.

To insure sufficient and rapid uptake of ^{13}C , we also enriched the lake with inorganic N and P. Beginning 2 weeks prior to the addition of ^{13}C (below) we added NH_4NO_3 and H_3PO_4 at a rate of 1.02 and 0.05 $\text{mmol m}^{-2} \text{d}^{-1}$, respectively. These additions, which are in the low end of the range used in previous long-term eutrophication experiments in this lake, were made at a weekly time step and continued for 3 weeks during the 2 weeks prior to and 1 week of ^{13}C addition (Carpenter et al. 2001). The goal was to reduce nutrient limitation and thereby ensure labeling of phytoplankton with ^{13}C . Thus, the nutrient enrichment was modest and brief.

Stable isotope addition—On 23 June 1999 we added 4 mol of $\text{NaH}^{13}\text{CO}_3$ (Isotech; ^{13}C content $> 99\%$) to the epilimnion of East Long Lake. The addition was accomplished

by dissolving aliquots of the isotope in a gas-tight carboy of lake water and feeding this water by gravity into the propeller wash of an electric outboard motor at a depth of 0.25 m. We kept the boat moving to achieve maximal initial mixing into the lake. Prior experience with other tracer additions in this lake demonstrated complete mixing of the epilimnion within 24 h. Furthermore, LiBr added to the epilimnion did not enter the hypolimnion until thermocline deepening occurred 50 d after the addition (Cole and Pace 1998). The stable isotope addition greatly changed the $\delta^{13}\text{C}$ of DIC (*below*) but increased the total DIC concentration by only 0.2% and acid neutralizing capacity by $0.18 \mu\text{eq L}^{-1}$.

Stable isotope samples and analyses—Samples for DIC were collected in two ways and sent to two different laboratories for analysis. Samples were collected at daily and twice-daily intervals during the initial phase of the ^{13}C addition when concentrations changed rapidly. Prior to, and 2 weeks after, the addition samples were taken at weekly intervals. One-liter gas-tight bottles were completely filled with lake water, acidified to pH 2 with H_2SO_4 , and sent to the Marine Biological Laboratory for analysis. Evacuated 100-ml serum vials were filled with 10 ml of N_2 and 90 ml of lake water, acidified to pH 2 with H_2SO_4 , and sent to Cornell University's Stable Isotope Facility for analysis. Replicate samples were sent to both laboratories. Samples for particulate organic C were collected by filtering water through precombusted Whatman GF/F filters that had been rinsed with dilute HCl (0.1%) prior to use. These filters were dried at 40°C and stored in a desiccator. Analysis was accomplished by in-line combustion to CO_2 followed by introduction to the mass spectrometer at Cornell. Samples for DOC were taken from the filtrate of the POC filtration, acidified to pH 2, and dried at 40°C . The dried residue was sent to the University of Alaska or Cornell University for combustion and ^{13}C analysis. Samples of organisms were collected in various ways, sorted, dried (40°C), and sent to the University of Alaska for ^{13}C measurement. Zooplankton were collected by oblique net tows within the epilimnion. Samples were sorted live, by hand, under a dissecting microscope. Two categories are used in this analysis: *Daphnia* (i.e., all species of *Daphnia* combined) and total zooplankton, which included all crustacean zooplankton. Small fish (young of year) samples were entire fish. For larger fish, samples consisted of filets of muscle. Samples for periphyton were collected from submerged logs (epixylic algae) and from clay tiles deployed for 7 d, allowing periphyton to colonize. These samples were taken both 1 week before and again 1 and 2 weeks following the ^{13}C addition.

Other analyses—Sample collection and analysis followed protocols described for prior studies of these lakes (Pace and Cole 2000; Carpenter et al. 2001). Only a few key methods need be detailed here. Dissolved inorganic C and the partial pressure of CO_2 in the water and the atmosphere were measured directly using a Shimadzu GC-8 (*see* Cole et al. 2000). The CO_2 concentration of surface water in equilibrium with the air was calculated from Henry's constant (K_{H}) and the partial pressure of CO_2 in the atmosphere. DOC was measured on Whatman GF/F filtered samples using a Shimadzu

5050 TOC analyzer. POC was measured on samples retained by Whatman GF/F filters using a Carlo-Erba CN analyzer. Each of these carbon pools was measured at weekly intervals.

GPP and respiration (R_{tot}) were estimated from continuous measurement of dissolved oxygen in the mixed layer of the lake using YSI-Endeco UPG-6000 sondes, which employed pulsed oxygen electrodes (*see* Cole et al. 2000). During 1999, sondes were deployed in East Long Lake 4 days of each week, and O_2 and temperature were recorded at 5-min intervals. Electrode drift and exchange with the atmosphere were calculated as in Cole et al. (2000). During night, the diffusion-corrected change in O_2 is a measure of ecosystem R (R_{tot}). During daylight, the diffusion-corrected change in O_2 is a measure of the difference between GPP and R_{tot} ($\text{NEP} = \text{GPP} - R_{\text{tot}}$) for the mixed layer of the lake. For these calculations, we assumed a gas piston velocity (k_{600}) of 0.48 m d^{-1} , which is appropriate for wind speeds measured on these small lakes (Hesslein et al. 1980; Cole and Caraco 1998). Note that the mixed layer includes the water volume and sediment area to the depth of the mixed layer. We assume that R_{tot} in the dark and light are equal, and the respiratory quotient is $1 \text{ mol CO}_2 \text{ mol}^{-1} \text{ O}_2$. We discussed the sensitivity of results to these assumptions in a prior paper (Cole et al. 2000). In the light, photooxidation is a potential component of R_{tot} that we cannot estimate directly. We assume that depth-integrated photooxidation is small in comparison to R_{tot} (Vahatalo et al. 2000) and discuss the effect of this assumption in the Discussion.

Pelagic respiration was measured during 24-h incubations of water in BOD bottles in the dark at ambient temperature at weekly intervals (Pace and Cole 2000). This measurement excludes the respiration of large zooplankton (*below*). Epilimnetic sediment respiration was estimated as the difference between R_{tot} and pelagic respiration. The water budget of East Long Lake was measured by experimentally adding a LiBr tracer in previous years (Cole and Pace 1998). This budget allows us to estimate the outflow coefficient of water. The outflow of any C pool is simply the product of the outflow of water and the concentration of that pool in the water. The input of DIC, POC, and DOC in groundwater were calculated from the water budget and the mass balance of each pool (*below*). The net exchange of DIC with the atmosphere was calculated as

$$\text{Flux} = k(\text{CO}_{2\text{wat}} - \text{CO}_{2\text{air}})$$

where k , the gas piston velocity, was obtained statistically from model fitting (*below*). Because of the low pH in East Long Lake, it was not necessary to consider chemically enhanced diffusion (Wanninkhof and Knox 1996).

Planktonic bacterial production (BP) was estimated using the ^3H -leucine method of Smith and Azam (1993), conducted at weekly intervals. Conditions and assumptions for these measurements are well described for the UNDERC lakes (Pace and Cole 1996). Bacterial respiration (BR) was initially estimated from BP and an estimate of bacterial growth efficiency (del Giorgio and Cole 1998); we adjusted this estimate slightly to achieve mass balance for C. We assumed that DOC is the proximate substrate for pelagic bacteria.

In the model (Tables 1, 2), some of the fluxes are calcu-

Table 1. Mass balance equations and definitions for the carbon model. Other abbreviations are explained in Table 2. In these equations, there are six carbon compartments: DIC = 1, DOC = 2, Bacteria = 3, Phytoplankton = 4, Dead POC = 5, Zooplankton = 6. Units: All pools are mmol C m⁻², and all fluxes are mmol C m⁻² d⁻¹. X_n and x_n are the total C and ¹³C in compartment *n*. P_n is the ratio of ¹³C to total C in compartment *n*. p_{nm} is the proportion of material in pool *m* consumed by consumer *n*. c_{nm} is the consumption coefficient of material *m* by consumer *n* (e.g., flux from *m* to *n* = c_{nm}·X_m). C_n is total consumption of all material by consumer *n*. a_{sn} and r_n are the assimilation and respiratory coefficients for consumer *n* (e.g., respiratory flux = r_n·X_n). sed_n is the sedimentation flux from compartment *n*, and in_n is the input flux to compartment *n*.

Dissolved inorganic carbon

$$dX1/dt = -k[(X1/zmix) - aircon] - GPP + Rtot + in1 - out \cdot X1$$

$$dx1/dt = -k[(X1 \cdot P1/zmix) - (aircon \cdot Patm)] - GPP \cdot fl3 \cdot P1 + in1 \cdot Pgw - out \cdot X1 \cdot P1 + r3 \cdot X3 \cdot P3 + r4 \cdot X4 \cdot P4 + r6 \cdot X6 \cdot P6 + sedresp \cdot Psed$$

Dissolved organic carbon

$$dX2/dt = in2 - out \cdot X2 - c32 \cdot X2 + c24 \cdot X4$$

$$dx2/dt = in2 \cdot Pter - out \cdot X2 \cdot P2 - c32 \cdot X2 \cdot P2 + c24 \cdot X4 \cdot P4$$

Bacteria

$$dX3/dt = c32 \cdot X2 - r3 \cdot X3 - c63 \cdot X3$$

$$dx3/dt = c24 \cdot X4 \cdot P4 + (c32 \cdot X2 - c24 \cdot X4) \cdot P2 - r3 \cdot X3 \cdot P3 - c63 \cdot X3 \cdot P3$$

Phytoplankton

$$dX4/dt = (1 - BPP) \cdot GPP - c24 \cdot X4 - r4 \cdot X4 - c54 \cdot X4 - c64 \cdot X4 - physed \cdot X4$$

$$dx4/dt = (1 - BPP) \cdot GPP \cdot fl3 \cdot p1 - c24 \cdot X4 \cdot P4 - r4 \cdot X4 \cdot P4 - c54 \cdot X4 \cdot P4 - c64 \cdot X4 \cdot P4 - physed \cdot X4 \cdot P4$$

Dead POC

$$dX5/dt = in5 + (1 - fecesed) \cdot (1 - as6) \cdot c64 \cdot X4 + (1 - fecesed) \cdot (1 - as6) \cdot c63 \cdot X3 + (1 - fecesed) \cdot (1 - as6) \cdot c65 \cdot X5 + c54 \cdot X4 - sed5 \cdot X5 - out \cdot X5 - c65 \cdot X5$$

$$dx5/dt = in5 \cdot Pter + (1 - fecesed) \cdot (1 - as6) \cdot c64 \cdot X4 \cdot P4 + (1 - fecesed) \cdot (1 - as6) \cdot c63 \cdot X3 \cdot P3 + (1 - fecesed) \cdot (1 - as6) \cdot c65 \cdot X5 \cdot P5 + c54 \cdot X4 \cdot P4 - sed5 \cdot X5 \cdot P5 - out \cdot X5 \cdot P5 - c65 \cdot X5 \cdot P5$$

Zooplankton

$$dX6/dt = as6 \cdot c63 \cdot X3 + as6 \cdot c64 \cdot X4 + as6 \cdot c65 \cdot X5 - r6 \cdot X6$$

$$dx6/dt = as6 \cdot c63 \cdot X3 \cdot P3 + as6 \cdot c64 \cdot X4 \cdot P4 + as6 \cdot c65 \cdot X5 \cdot P5 - r6 \cdot X6 \cdot P6$$

lated as fractions of the pool size per unit time. Thus, we needed estimates of the biomass for some pools. Bacterial abundance was measured at weekly intervals using epifluorescence microscopy, and bacterial biomass was calculated according to Pace and Cole (1996). Chl *a* was measured at weekly intervals using fluorometry on methanol extracts. Phytoplankton C was calculated assuming a C:Chl *a* ratio of 40, typical of direct measurements on UNDERC lakes in prior years (Carpenter and Levitt 1991). POC in excess of this ratio was assigned to the nonliving POC pool, which is a mixture of autochthonous and allochthonous sources. Zooplankton were collected using weekly vertical net tows; samples were counted and biomass computed as previously described (Carpenter et al. 2001).

Assumed values—In order to construct the C model described below, several additional values had to be assumed or derived from statistical fits of the data. Benthic primary production was estimated from the morphometry of East Long Lake and measurements of benthic primary production in similar lakes at UNDERC conducted by Vadeboncouer et al. (2001), who found that benthic primary production on an areal basis was roughly coequal to that of phytoplankton

primary production. Thus we calculated the area of the rotational torus of sediments that is above the mixed layer depth of the lake (9.4% of the area of East Long Lake) and assumed that benthic GPP equaled pelagic GPP per unit area of sediment.

The loss of phytoplankton C into the DOC (c24) pool was estimated as 10% of phytoplankton C per day and is comparable to literature-based estimates, which are usually normalized to phytoplankton production (Baines and Pace 1991). The ¹³C content of atmospheric CO₂ was assumed to be -7‰. The ¹³C content of DIC in groundwater was assumed to be equal to that of the lake at ice out (-28‰). The ¹³C of terrestrial DOC and POC entering the lake was assumed to be -28‰, a typical value for the C-3 vegetation (maples, birch, aspen, and confers) in the watershed of East Long Lake.

The model also includes some assumed relationships. We assumed that zooplankton feed nonselectively on the particles available to them (nonliving POC, phytoplankton, bacteria) in proportion to the masses of each pool. The total consumption of organic C by zooplankton is derived from the mass balance for zooplankton, assuming steady state. We also assumed that bacterial assimilation depletes all of the

Table 2. Parameters and variables of the carbon model. Shown are the symbol for each parameter as used in the code (Table 1), the value and units of each parameter, a descriptive name for each parameter and the source for each values.

Symbol	Value	Units	Name	Source*
k	0.45	m d ⁻¹	Gas piston velocity	Fitted
Zmix	1	m	Mixed layer depth	Measured
CO _{2(sat)}	13	mmol m ⁻³	CO ₂ concentration in equilibrium with atmosphere	Measured, used mean
GPP	57	mmol m ⁻² d ⁻¹	Gross primary production	Measured
Rtot	82	mmol m ⁻² d ⁻¹	Total ecosystem respiration	Measured
in1	0.83	mmol m ⁻² d ⁻¹	DIC inflow	Estimated from water budget and mass balance
out	0.0027	d ⁻¹	Outflow coefficient	Measured
Patm	-7	δ ¹³ C	Proportion of ¹³ C in atmosphere	Literature
f13	Dynamic	Unitless	Ratio of ¹³ C in photosynthate to ¹³ C in DIC	Calculated from fitted fractionation factor for GPP (5.4 per mil)
Pgw	-28	δ ¹³ C	Proportion of ¹³ C in groundwater DIC	Assumed
sedresp	49	mmol m ⁻² d ⁻¹	Sediment respiration	Calculated by difference
Psed	-31	δ ¹³ C	Proportion of ¹³ C in sediment	Measured
in2	23.3	mmol m ⁻² d ⁻¹	Influx of DOC	Estimated from water budget and mass balance
c32	0.0178	d ⁻¹	DOC consumption coefficient of bacteria	Estimated from measured bacterial production
c24	0.1	d ⁻¹	DOC release coefficient by phytoplankton	Literature
Pter	-28	δ ¹³ C	Proportion of ¹³ C in terrestrial DOC	Literature
r3	6.27	d ⁻¹	Respiration coefficient of bacteria	Estimated based on measured BP; literature
c63	0.054	d ⁻¹	Consumption coefficient of bacteria by zooplankton	Calculated from zooplankton mass balance assuming nonselective grazing
BPP	0.094	Dimensionless	Proportion of GPP due to benthos	Estimated from morphometry and measured benthic PP; literature
r4	0.073	d ⁻¹	Phytoplankton respiration coefficient	Literature
c64	0.19	d ⁻¹	Consumption coefficient of phytoplankton by zooplankton	Calculated from zooplankton mass balance assuming nonselective grazing
physed	0.5	Unitless	Proportion of phytoplankton that sediment directly without entering dead POC pool	Fitted
in5	1.8	mmol m ⁻² d ⁻¹	Input flux of dead POC	Estimated from water budget and mass balance
fecesed	0.9	Unitless	Proportion of zooplankton feces that sediment directly without entering dead POC pool	Fitted
as6	0.7	Unitless	Proportion of zooplankton consumption that is assimilated	Literature
c65	0.054	d ⁻¹	Consumption coefficient of dead POC by zooplankton	Calculated from zooplankton mass balance assuming nonselective grazing
sed5	0.37	d ⁻¹	Sedimentation coefficient of dead POC	Calculated from mass balance
r6	0.3	d ⁻¹	Respiration coefficient for zooplankton	Fitted

* Measured, the value was measured in East Long Lake as part of this study or calculated from one or more measured values (*see text for explanation*); Literature, an estimate was taken from the literature (*see text*); Fitted, the value was arrived at by statistical fit of the model (*see text*).

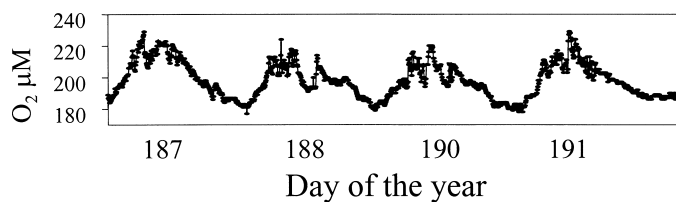


Fig. 2. Continuous measurements of dissolved O_2 in East Long Lake. Four days of measurements are plotted during the ^{13}C addition 1999; the x -axis shows day of year. The top of the scale ($240 \mu\text{M}$) is close to 100% saturation.

DOC of phytoplankton origin before any of the allochthonous DOC pool is used. Thus, bacterial C demand in excess of phytoplankton DOC release is satisfied by the residual allochthonous DOC pool.

The model—Using the observations and assumptions described above, the model calculates C flow for both ^{12}C and ^{13}C between six compartments within the lake and across the external boundaries of the ecosystem (Table 1). The boundaries of the ecosystem are the bottom of the mixed layer, the atmosphere, and the sediments (*below*). Two differential equations (one for mass balance dynamics of each C isotope) describe each of the six components of the model (DIC, DOC, pelagic bacteria, phytoplankton, detrital POC, and zooplankton; Table 1). Thus we have a total of 12 differential equations for the mass balance dynamics of ^{12}C and ^{13}C . Steady-state solutions of these equations were calculated to obtain initial conditions to evaluate the model. Also, steady-state results were compared with measured initial conditions to evaluate the model. We use steady-state calculations to compare the relative importance of allochthonous and autochthonous C for consumers. Dynamic simulations were used (in Matlab, Shampine and Reichelt 1997) to compare model results with observations of isotope dynamics during the experiment.

Most model parameters were measured directly or calculated from mass balance (*see above*; Table 2). Four parameters were estimated by fitting the model to data (Table 2). The gas piston velocity (k) and the fractionation factor for phytoplankton (ϵ , f_{13}) were estimated by comparing steady-state solutions for $\delta^{13}\text{C}$ prior to the addition of isotope. We selected values for these parameters that were both plausible and produced steady-state estimates close to the observed data. Two other parameters (proportion of phytoplankton that sedimented directly [physed]) and zooplankton respiration (r_6) were estimated by least squares. The least squares estimates are the values of these parameters that minimize the sum of squared deviations between simulated and observed $\delta^{13}\text{C}$ during the pulse experiment, with all other parameters fixed at their nominal values. As a measure of goodness of fit, we present the standard error of the deviation between simulated and observed $\delta^{13}\text{C}$ values. This represents the average error of the model projections in the same units as $\delta^{13}\text{C}$ (*see Tables 1, 2*).

Results

Ecosystem metabolism—As in many DOC-rich lakes, whole-lake respiration (R_{tot}) exceeded gross primary produc-

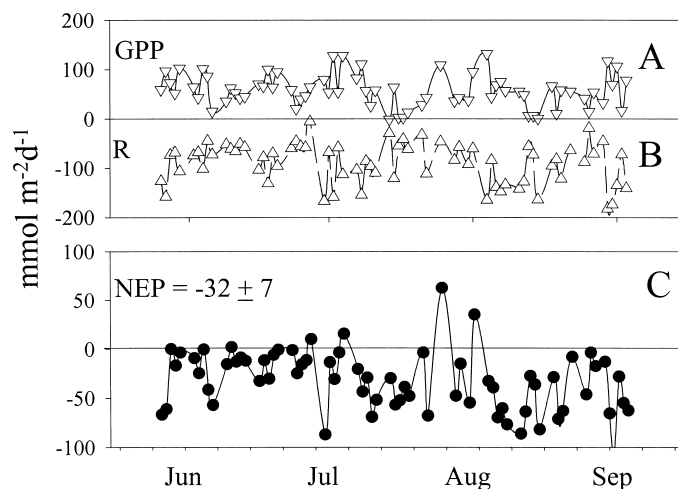


Fig. 3. Estimates of whole system metabolism for East Long Lake during summer 1999. The data are derived from continuous O_2 measurements as in Fig. 2. (A) Gross primary production (GPP). (B) Ecosystem respiration (R_{tot}) plotted as a negative value to aid visualization. (C) Net ecosystem production (NEP, $\text{GPP} - R$). Negative values of NEP mean that respiration exceeds gross photosynthesis (*see text*).

tion and the lake was undersaturated on O_2 (Fig. 2) and was net heterotrophic (Fig. 3). Averaged for the entire season and using the continuous O_2 data from the sondes (Fig. 3), NEP was $-32 \pm 7 \text{ mmol m}^{-2} \text{ d}^{-1}$ (mean \pm SD). Using the weekly CO_2 data (Fig. 1), NEP was $-32 \pm 12 \text{ mmol m}^{-2} \text{ d}^{-1}$. Both estimates were in good agreement with each other and prior measurements in East Long Lake at comparable nutrient loading (Cole et al. 2000). During the period of the ^{13}C addition, NEP was slightly less negative: $-27 \text{ mmol m}^{-2} \text{ d}^{-1}$. Calculated values of GPP and R were 57 ± 4 and $82 \pm 7 \text{ mmol m}^{-2} \text{ d}^{-1}$. The mean ratio of GPP/R was 0.66 ± 0.05 .

Despite the nutrient enrichment, whole-system R was considerably larger than GPP and exceeded our estimate of pelagic R based on dark-bottle O_2 consumption ($22 \text{ mmol m}^{-2} \text{ d}^{-1}$). Thus by difference, benthic R was about 73% of system R ($60 \text{ mmol m}^{-2} \text{ d}^{-1}$).

^{13}C natural abundances—Prior to the addition of the isotope, the ambient levels of $\delta^{13}\text{C}$ in the various C pools were low (moderately depleted in ^{13}C) and typical for values in humic, softwater lakes (Fig. 4). DIC averaged -28.2‰ ; POC, -30.2‰ ; DOC, -29‰ ; and *Daphnia* -28.4‰ . Benthic algae collected from tile recolonization experiments were also highly depleted (-26.5‰), whereas epixylic algae collected from logs in the lake were somewhat less depleted -22‰ . With the exception of epixylic algae, these ^{13}C values all are close to that of terrestrial primary production and soils (about -28‰).

Response to ^{13}C -DIC addition—The addition of ^{13}C -DIC resulted in an immediate, large, and transient increase in several of the major C pools (Fig. 4). At the time of the addition the DIC pool rose to approximately $+197\text{‰}$. Because of rapid atmospheric exchange, by day 3 DIC was at -16‰ , and by 10 d after the addition, DIC was only slightly

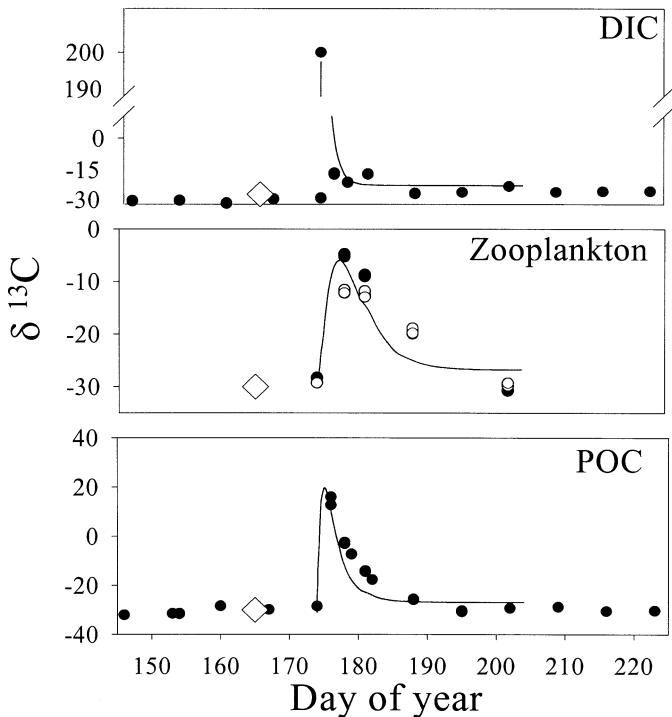


Fig. 4. Measured and modeled (lines) values of $\delta^{13}\text{C}$ in three key carbon pools. (A) Dissolved inorganic carbon. (B) Zooplankton (filled circles are *Daphnia*; clear circles are total zooplankton). (C) Particulate organic C. The open diamonds are the ambient (prespike) values of $\delta^{13}\text{C}$ arrived at by the model. The ^{13}C addition occurred on day 173 on this scale.

elevated over prespike levels. Peak ^{13}C was measured in zooplankton at -5‰ 5 d after the spike. The elevated values in zooplankton persisted much longer than in the DIC and did not decline to prespike levels until about 20 d following the spike. The POC pool had peak values ($+12\text{‰}$) on day 3. Like the zooplankton, POC returned to prespike values within about 20 d. We have too few samples of periphyton to accurately chart the time course, but peak measured values were near -12‰ , 9 d after the spike and were very close to the values in POC on the same day. The ^{13}C of DOC was not changed measurably by the ^{13}C addition.

Model results and analysis—Using these values, along with those in Table 1, the model produced a steady-state solution for C fluxes that agreed well with the baseline (prespike) ^{13}C values in the lake (diamonds in Fig. 4). The fitted parameters agreed reasonably well with estimations of those parameters from other approaches. For example, in order to achieve an overall balance for C and initial ^{13}C in the model, we estimated k , the gas piston velocity, at 0.45 m d^{-1} . Based on the equation in Cole and Caraco (1998), the wind-based calculation estimated k at 0.48 m d^{-1} . Both estimates were very close to that obtained by whole-lake ^{14}C addition to a small lake in Canada (Hesslein et al. 1980). The best fit of the model was obtained when a fairly large proportion of phytoplankton biomass ($50\% \text{ d}^{-1}$) sedimented without being grazed. In the 1-m mixed layer of East Long Lake, this is equivalent to a settling velocity for phytoplankton of 0.5 m

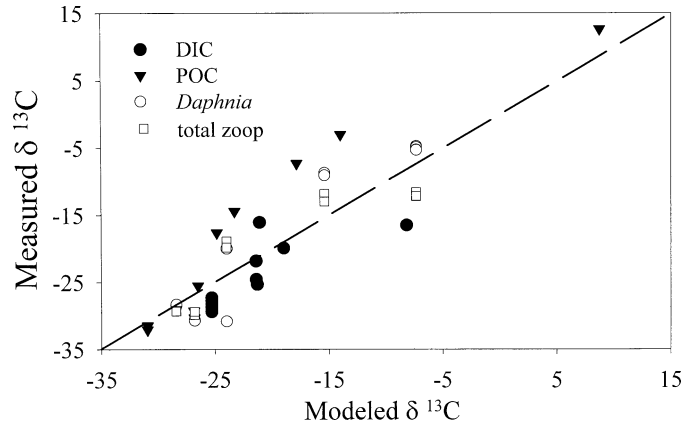


Fig. 5. Measured versus modeled values of $\delta^{13}\text{C}$ in DIC, POC, *Daphnia*, and total zooplankton. The dashed line is $Y = X$. The actual regression is $Y = 1.065 (\pm 0.075) \times X + 1.8 (\pm 1.63)$, $r^2 = 0.81$; $P < 0.0001$; $n = 50$. Excluded from this regression is the initial value of DIC at the time of addition. Including this value ($\delta^{13}\text{C} = 197$) improves the r^2 to 0.98 and does not alter the slope (see Table 3).

d^{-1} , which is reasonable (Baines and Pace 1994). On the other hand, the fitted fractionation factor for phytoplankton was 5.4‰ , which is considerably lower than the 15 to 20‰ in much of the literature (below). The fitted value for the sedimentation of zooplankton feces (0.9 , fecesed variable in Table 2) is equivalent to a sinking rate of 0.9 m d^{-1} . The fit value for zooplankton respiration is 30% of biomass per day (R6 in Table 2), which is within the range of literature values (Lampert 1984).

With these fitted and measured parameters in the model, we then simulated the ^{13}C addition and its distribution into the various C pools. The model reproduced both the dynamics and the magnitudes of change in ^{13}C as it moved into and out of the various pools (Fig. 4). A plot of measured versus modeled ^{13}C in the DIC, POC, and zooplankton pools (Fig. 5) is highly significant ($P < 0.001$) has a slope indistinguishable from 1 (1.065 ± 0.075) and accounts for much of the variance ($r^2 = 0.81$). Looking at the pools independently reveals significant fits for each pool and little evidence of bias (Table 3).

Fractionation of ^{13}C -DIC by phytoplankton—The best fit of the model occurred when the fractionation factor for photosynthesis was low (5.4‰). We varied this parameter from 0 (no fractionation) to 20‰, a value near the high end of fractionation factors from the literature (Laws et al. 1995). As we increased the fractionation factor, the overall fit of the model to the data decreased (i.e., the standard error increased, Fig. 6A). Furthermore, increasing the fractionation factor caused the predicted ambient (prespike) ^{13}C levels to diverge away from measured values (Fig. 6B). With increasing fractionation, the model predicted $\delta^{13}\text{C}$ in DIC that was less depleted than we measured and zooplankton ^{13}C that was more depleted than we measured. Thus, if the fractionation factor was set larger than about 10‰, the model no longer produced plausible predictions.

Table 3. Measured and modeled values of $\delta^{13}\text{C}$ in various categories. Shown are the linear regressions of modeled (as the independent variable) versus measured value of $\delta^{13}\text{C}$ as in Fig. 5.

Data*	n^\dagger	r^2	P	Slope	SE slope
All	51	0.98	<0.001	1.01	0.015
All – initial DIC spike	50	0.81	<0.001	1.07	0.08
All DIC	12	0.99	<0.001	1.00	0.02
All DIC – initial spike	11	0.62	<0.03	0.76	0.20
POC	11	0.89	<0.001	1.20	0.14
<i>Daphnia</i>	15	0.90	<0.001	1.23	0.11
Total zooplankton	13	0.77	<0.001	0.766	0.13

* All, the entire data set, which includes the DIC value at the time of addition; All—initial DIC spike, excludes the DIC value at the time of addition and is the regression shown in Fig. 4. The other four categories of DIC—with and without the initial spike value, POC, *Daphnia*, and total zooplankton—are as in Fig. 4.

$^\dagger n$ is the number of samples in each data set; SE slope is the standard error of the slope.

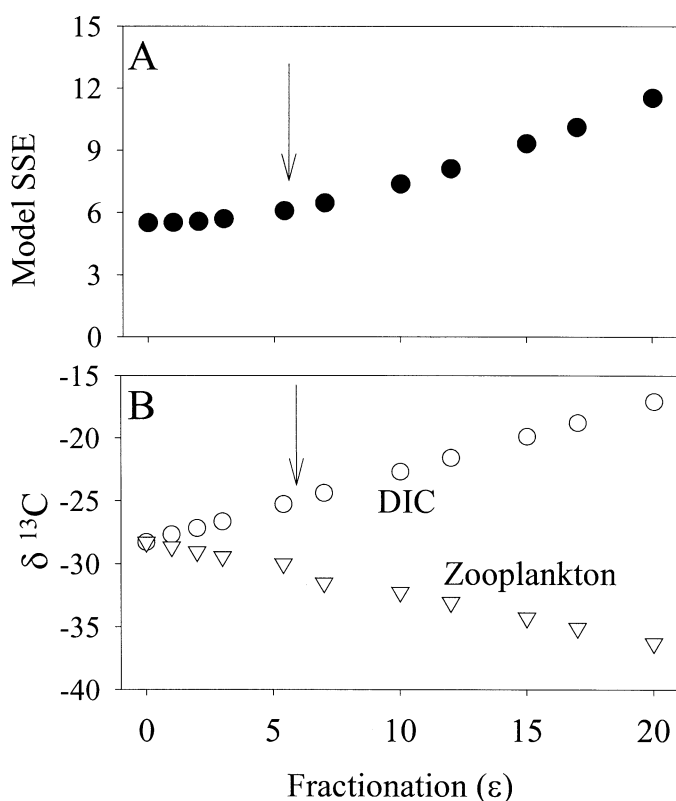


Fig. 6. Response of the model to the fractionation factor for ^{13}C during photosynthesis (ϵ). The X-axis shows values for ϵ from 0 to 20‰. The arrow denotes the value of ϵ (5.4‰) for which the model gave the overall best fit. (A) Standard error (in units of $\delta^{13}\text{C}$) for the model as a function of ϵ ; higher values of the standard error indicate poorer fit between measured and modeled values. (B) Modeled solution for the steady-state (prespike) values of $\delta^{13}\text{C}$ for DIC and zooplankton. The actual measured values (pre- ^{13}C addition) for DIC and zooplankton were both near -28‰ (see text).

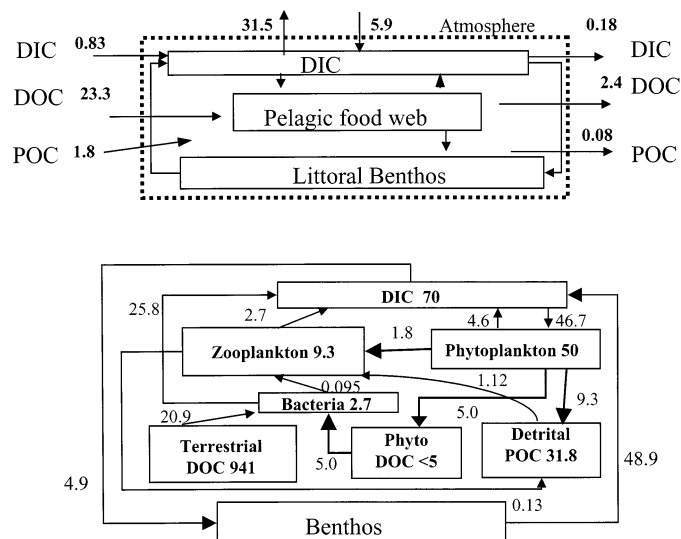


Fig. 7. Results of C model for East Long Lake. (Upper panel) Box and arrow diagram of the C flow model. Shown are the ecosystem boundaries (heavy dashed line) and the C flux (mmol m^{-2} [of lake] d^{-1}) across these boundaries for the steady-state fit of the model. The simplified internal structure of the model is shown by the boxes and arrows within the ecosystem boundaries. The left side shows inputs and the right side outputs of flowing water. Littoral benthos include both autotrophic (periphyton) and heterotrophic (benthic invertebrates, microbes, etc.) components for the torus of sediments that intersects the mixed zone of the water column (see text). The exchange with the atmosphere includes both invasion and evasion terms; this is necessary to calculate the ^{13}C - CO_2 fluxes across the air–water interface. (Lower panel) Detail of some of the internal part of the model to highlight the fluxes of organic C to pelagic bacteria and zooplankton. The bold numbers inside the boxes are standing stocks (mmol C m^{-2}); the numbers associated with arrows are fluxes (mmol $\text{m}^{-2} \text{d}^{-1}$). The values shown are for the best fit of the model (minimum SSE). The fluxes from organisms to DIC denote respiration; the flux of DIC to phytoplankton plus benthos denotes gross primary production. Phyto-DOC is DOC of phytoplankton origin; detrital POC is nonliving POC (see text) and could be of autochthonous or allochthonous origin.

Carbon budget of East Long Lake—The model reproduced the measured distribution of added ^{13}C to East Long Lake with high fidelity. The model is tightly constrained by data that includes total C fluxes and ^{13}C values prior to, during, and following the spike. The overall goodness of fit gives us some confidence that flux estimates are realistic. Carbon flows based on the model are illustrated in Fig. 7. Several general points emerge. First, despite the moderate nutrient addition, East Long Lake remained net heterotrophic and system R exceeded system GPP by large amounts, implying that terrestrial organic matter fuels much of heterotrophic respiration (see upper panel, Fig. 7). Second, DIC in the lake is derived largely from this internal respiration, which is much larger than the external inputs of DIC in groundwater. Third, although externally supplied DOC supports a large part of system R, zooplankton, a key component of pelagic food, is predominantly supported by phytoplankton primary production under the conditions of this experiment (see lower panel, Fig. 7). Finally, pelagic bac-

teria appear to respire large amounts of terrestrially derived DOC but pass very little of this organic C up the food web.

Discussion

Before we interpret the experiment and model results, there are a few caveats. Numerous parameters and the pre-spike conditions were estimated by assuming steady state for total C. Most of the pools and fluxes that we actually measured did not vary greatly during this experimental period (e.g., Fig. 1). Furthermore, that this model is able to reproduce the dynamics of the ^{13}C spike suggests that results were not greatly affected by the steady-state assumption. Second, we assumed in the model that the only way inorganic C could be converted to organic C was by photosynthesis. We have ignored several mechanisms whereby CO_2 can be reduced heterotrophically. Using energy from metabolism, some bacteria can carboxylate the C skeletons of the tricarboxylic acid cycle (anaplerotic reactions), resulting in a net uptake of CO_2 . We can put an upper limit on heterotrophic CO_2 uptake in the pelagic region since dark CO_2 uptake by bacteria has been estimated at 6% of bacterial secondary production (Jordan and Likens 1980). In the surface water of East Long Lake during summer 1999, ^3H -leucine-based estimates of BP averaged $8.1 \pm 5.1 \mu\text{g C L}^{-1} \text{d}^{-1}$ or $0.67 \text{ mmol C m}^{-2} \text{d}^{-1}$. "Dark" CO_2 uptake by pelagic bacteria should be no more than $0.04 \text{ mmol C m}^{-2} \text{d}^{-1}$, which is less than 0.08% of GPP. Therefore, the heterotrophic route for CO_2 entry into the food web can be ignored. Finally, we have attributed CO_2 production and oxygen consumption to respiration. Photooxidation of DOC could be an additional and significant source of CO_2 and a sink for O_2 (e.g., Wetzel et al. 1995; Graneli et al. 1996) that we have not included in the model. Our estimate of ecosystem R, however, comes from night declines in O_2 ; our estimate of pelagic R comes from incubation in dark bottles. In both cases, we might be underestimating the total degradation of DOC to CO_2 in the light, but we are not overestimating biological R. In addition, part of the excess CO_2 in the water (e.g., Fig. 1) could come from photooxidation. Vahatalo et al. (2000) measured and modeled depth-integrated photochemical DIC production in a humic lake that is very similar to East Long Lake in pH and DOC concentration and reported a value of $0.99 \text{ mmol C m}^{-2} \text{d}^{-1}$. This value is <2% of our estimate of biological R_{tot} . Our model correctly predicts both the ^{13}C of DIC and the net gas flux. These results support the assumption we made that photooxidation is minor in comparison with system R in this lake.

Organic C in East Long Lake is primarily of allochthonous origin (Fig. 7). Allochthonously derived DOC accounts for more than 90% of organic C in the water column and is a major substrate for metabolism in the system. R exceeds GPP by large amounts, as indicated by the net gas fluxes (see upper panel, Fig. 7). The model calculates that at least 52% of ecosystem respiration is supported by allochthonous C sources, and most of this respiration is bacterial (see lower panel, Fig. 7). Bacteria process a large amount of terrestrial organic C at low efficiency in this system.

This experiment was too short to provide a quantitative

estimate of the amount of terrestrial C that might support the production of fishes. However, we can estimate the extent to which zooplankton, a major prey of small fishes, is supported by allochthonous and autochthonous sources in East Long Lake (Fig. 7). If zooplankton were supported entirely by terrestrial organic C (via bacterial uptake), there would have been no shift in the $\delta^{13}\text{C}$ of zooplankton. The large increases in $\delta^{13}\text{C}$ for both *Daphnia* and total zooplankton demonstrate unequivocally that newly photosynthesized autochthonous C is an important source for zooplankton under the conditions of this experiment. Using the model, we can calculate the quantity of C and the pathways supporting zooplankton in this experiment. Zooplankton assimilation is dominated by inputs from phytoplankton, with 59% coming from living phytoplankton and 31% from the nonliving POC of autochthonous origin. According to the model, only 8.4% of the C assimilated by zooplankton is of allochthonous origin. (Fig. 7).

The conclusion that allochthonous C is relatively unimportant to zooplankton can be corroborated by an independent calculation. If pelagic bacterial C demand were supported entirely by DOC of allochthonous origin, bacterial secondary production (BP; $0.67 \text{ mmol C m}^{-2} \text{d}^{-1}$) would be a maximum estimate of allochthonous DOC available to zooplankton. This is only about 21% of zooplankton C assimilation. Furthermore, some BP is not directly available to zooplankton because it is grazed by protists and rotifers, and a portion of BP is supported by DOC of autochthonous origin (Fig. 7). Thus, if allochthonous C is available to zooplankton only via bacteria, we would expect that allochthonous organic C should support less than 21% of crustacean zooplankton assimilation, in agreement with the model and the isotope results, which suggest 8.4%. If zooplankton consumed allochthonous POC directly, the importance of allochthonous C could be larger than this estimate suggests.

Because zooplankton were labeled with autochthonous C, can we see this label in fast-growing fish that consume them? Prior to the addition, planktivorous, young of year (YOY) large-mouth bass (*Micropterus salmonoides*) isotopically resembled many of the other C pools in the lake ($\delta^{13}\text{C} \approx -30\text{‰}$; Fig. 8). Following the addition, there was a 6‰ shift to the more positive value of -24‰ , indicating some connection of these fish to autochthonous production. In contrast, a benthivorous fish, adult yellow perch (*Perca flavescens*), remained at -30‰ throughout the study. The experiment did not run long enough for us to make any strong conclusions about fishes. The lack of label in the yellow perch could simply be the result of slower growth rates in these adult fish and the time required for transfer of the label to the next trophic level. Although we cannot yet quantify the importance of autochthonous organic matter to fish, its presence in YOY bass suggests it is important in supporting the highest trophic levels.

Although pelagic bacteria pass relatively little allochthonous organic C up the food web, they assimilate a large amount of DOC of allochthonous origin ($21 \text{ mmol m}^{-2} \text{d}^{-1}$ according to the model). We do not have a way to separately estimate the growth efficiency of bacteria using allochthonous and autochthonous DOC sources, but we can estimate the combined growth efficiency. The model suggests that

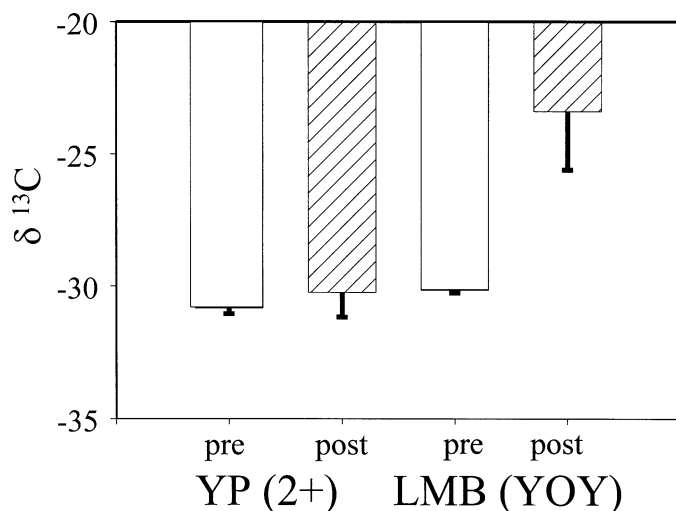


Fig. 8. Values of $\delta^{13}\text{C}$ in adult fish (2+ yellow perch) with broad diets of young planktivorous fish (young-of-year large-mouth bass, LMB). Shown are values prior to (pre-) and following (post-) the ^{13}C spike in East Long Lake.

bacterial respiration is about 25 times larger than BP, or that BGE (= BP/BP + BR) is about 4% (see lower panel, Fig. 7). Using the predictive equation of del Giorgio and Cole (1998), BGE in East Long Lake would be somewhat higher, 11.9%, and BR would be 7.3 times larger than BP. Either way, BR would greatly exceed BP. The very low BGE for pelagic bacteria suggested by the model was required to achieve mass balance for the DOC and to fit the observed ^{13}C distribution. The value is also consistent with other mass balance models of nearby lakes similar to East Long where food web manipulations have been conducted (Vezina and Pace 1994). Also, although measurements of pelagic respiration include that of protists and small metazoans, the model does not explicitly distinguish these groups and lumps them in with bacterial R.

The model and experiment provide some insight as to the origin of POC and DOC. The model suggests that the POC turns over relatively quickly, about once every 3 d, and that phytoplankton production (and phytoplankton egested by zooplankton) are the major inputs to this pool. Allochthonous POC is supplied at about one-sixth the rate of autochthonous POC. The increase in $\delta^{13}\text{C}$ in this pool from -28 to $+12$ ‰ is consistent with this view. The turnover of DOC is much slower and the dominant input is terrestrial DOC in groundwater. The model suggests that DOC turns over about once in 45 d and that allochthonous input is five times greater than the autochthonous input. This is consistent with the lack of increase of $\delta^{13}\text{C}$ in the DOC pool in this short experimental addition and with the results from the whole-lake ^{14}C additions to Lake 224 and 226 N in Canada (Hesslein et al. 1980; Bower et al. 1987). The model also suggests bacterial utilization of DOC is much larger (10-fold) than the outflow of DOC.

The origin of DIC in East Long Lake might be inferred from its $\delta^{13}\text{C}$ value of -28 ‰, which is very similar to terrestrial vegetation. The oxidation of this vegetation in the soil could produce this DIC, as could the oxidation of DOC

in the lake. The model suggests that respiration of allochthonous DOC in the lake is a much larger source (>25 -fold) of DIC than is groundwater input for this softwater lake (Fig. 7). In fact, the isotopic signature of the DIC is a result of a combination of the respiration of allochthonous DOC, primary production, and atmospheric exchange. This view of multiple controls is consistent with the large degree of variation in DIC ^{13}C values among small lakes (Hesslein et al. 1991; Streigl et al. 2001).

The model and experiment lead to an interesting conundrum about our knowledge of C cycling in lakes. If we had not added the ^{13}C tracer, we could have come to the conclusion that the food web in East Long Lake was supported almost exclusively by allochthonously supplied organic C. The fractionation of ^{13}C uptake by phytoplankton has been well studied in marine systems under both field and laboratory conditions (e.g., Laws et al. 1995; Bidigare et al. 2001; Rau et al. 2001). This fractionation is rarely measured for freshwater phytoplankton (see Yoshioka 1997; Jones et al. 1998). In marine systems, phytoplankton fractionation can be estimated from external CO_2 concentration, temperature, and growth rate of the phytoplankton—all known variables in East Long Lake. Applying one well-known estimator (Laws et al. 1995) to East Long Lake yields $\epsilon = -25$ ‰ and an expected $\delta^{13}\text{C}$ value for phytoplankton of -50 ‰ if the growth rate of phytoplankton (μ) is 1 d^{-1} . Letting the growth rate range from 0.25 to 8 d^{-1} has only a small effect on ϵ , and the expected $\delta^{13}\text{C}$ would range from -44 to -51 ‰. Intriguingly, there are no measured components in the food web of East Long Lake that are this depleted in ^{13}C (Figs. 4, 8), so in the absence of the manipulation, we might have erroneously concluded that the major C source for zooplankton was terrestrial organic matter and not phytoplankton production. Similarly, assuming $\epsilon = 20$ ‰, one would conclude that POC could not be largely of autochthonous origin. The large shifts in the $\delta^{13}\text{C}$ values for POC and zooplankton clearly indicate a strong connection to the autochthonous food web. Our model suggests that the fractionation for phytoplankton in situ in this softwater lake is unexpectedly low and not well predicted by generic models largely developed for marine phytoplankton growing in bicarbonate-rich culture media or in marine systems in the field (Bidigare et al. 2001). Our values of ϵ , while low, are within the published range (Laws et al. 1995; Rau et al. 2001) but surprising in this CO_2 -rich softwater lake.

Although we cannot yet explain the physiologic or species-specific basis for the low ϵ implied by the model, we can constrain the bounds with a simple calculation. If all sestonic POC were of phytoplankton origin, then the contrast between the $\delta^{13}\text{C}$ of DIC and POC would be a direct measure of ϵ . The prespike values would produce $\epsilon = 2.6$ ‰; the postspike values 6.3 ‰. Clearly this approach ignores the portion of the POC that is of allochthonous origin. We can approach this portion in several ways. A maximum estimate of this fraction would be to assume that all nonliving POC is allochthonous, thus ignoring the contribution of formerly living phytoplankton to nonliving POC. We can calculate the algal fraction of POC from the C:Chl *a* ratio and measured chlorophyll. The C:Chl *a* ratio in UNDERC lakes was measured directly by Carpenter and Leavitt (1991) and averaged

about 40:1—close to frequently assumed values in the literature. Because we know the ^{13}C of POC, and assuming that the allochthonous portion has $\delta = -28\text{‰}$, we can solve algebraically for ϵ , which is -6‰ . These two independent assessments of ϵ produce similarly low values, in good agreement with the value obtained by best fit of the model.

Our model and experiment suggest that the zooplankton and, by extension, the YOY fish that prey on them are components of a food web that is not very dependent on the vast amount of allochthonous C loading to East Long Lake. The metabolism of allochthonous organic C by pelagic bacteria is either a direct respiratory sink for the allochthonous C or supports a microbial loop that does not strongly interact with the food chain supported by phytoplankton production (Ducklow et al. 1986).

The conclusions we can draw here about carbon cycling, although intriguing, pertain to this one lake and under a regime of N and P enrichment. We enriched the lake with nutrients, so that we would be assured of measurable assimilation of the added ^{13}C into the autotrophic part of the food web, and used this enrichment to calibrate the model. Without this enrichment, it is possible that the autochthonous pathways would be less important than we estimated. Furthermore, we caution that our pulse experiment was too brief to effectively label DOC, benthic organisms, or longer lived fish. The large amount of benthic R derived from our model could suggest that allochthonously loaded C is important to benthic invertebrates and the larger fishes that prey on them. Finally, our experiment occurred during the summer growing season and with a particular zooplankton community. For example, using ambient stable isotopes in Loch Ness, Grey et al. (2001) found that the ^{13}C content of *Daphnia* during summer was consistent with a phytoplankton origin. However, over the entire annual period, Loch Ness zooplankton were supported about 40% by terrestrial organic C. These unknowns might be resolved by a sustained ^{13}C addition without the addition of nutrients. A sustained experiment would both shed light on benthic connections and help determine the roles of allochthonous and autochthonous C under ambient, nutrient-limited conditions.

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