

Bacterial Growth on Allochthonous Carbon in Humic and Nutrient-enriched Lakes: Results from Whole-Lake ^{13}C Addition Experiments

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ABSTRACT

Organic carbon (C) in lakes originates from two distinct sources—primary production from within the lake itself (autochthonous supply) and importation of organic matter from the terrestrial watershed (allochthonous supply). By manipulating the ^{13}C of dissolved inorganic C, thereby labeling within-lake primary production, we examined the relative importance of autochthonous and allochthonous C in supporting bacterial production. For 35 days, $\text{NaH}^{13}\text{CO}_3$ was added daily to two small, forested lakes. One of the lakes (Peter) was fertilized so that primary production exceeded total respiration in the epilimnion. The other lake (Tuesday), in contrast, was low in productivity and had high levels of colored dissolved organic C (DOC). To obtain bacterial C isotopes, bacteria were regrown in situ in particle-free lake water in dialysis tubes. The contribution of allochthonous C to bacterial biomass was calculated by applying a two-member mixing model. In the absence of a direct measurement, the iso-

topic signature of the autochthonous end-member was estimated indirectly by three different approaches. Although there was excess primary production in Peter Lake, bacterial biomass consisted of 43–46% allochthonous C. In Tuesday Lake more than 75% of bacterial growth was supported by allochthonous C. Although bacteria used autochthonous C preferentially over allochthonous C, DOC from the watershed contributed significantly to bacterial production. In combination with results from similar experiments in different lakes, our findings suggest that the contribution of allochthonous C to bacterial production can be predicted from ratios of chromophoric dissolved organic matter (a surrogate for allochthonous supply) and chlorophyll *a* (a surrogate for autochthonous supply).

Key words: lakes; bacteria; dissolved organic carbon; allochthonous carbon; autochthonous carbon; stable isotope.

INTRODUCTION

In many lakes, respiration (R) exceeds gross primary production (GPP, Cole and others 1994, 2000; del Giorgio and Peters 1994; Riera and others 1999;

Received 15 September 2004; accepted 19 May 2005; published online 13 April 2006.

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Hanson and others 2003). Negative net ecosystem production ($NEP = GPP - R$) implies the presence of an important carbon (C) source in addition to that supplied by within-lake primary producers. Metabolism in many lakes is significantly subsidized by terrestrial (allochthonous) sources of organic C based on studies both of relative rates of GPP and R (above) and whole-lake C budgets (see, for example, Dillon and Molot 1994; Wetzel 1995; del Giorgio and others 1999). Less information is available on the fate of the allochthonous C and its importance to the secondary production of pelagic food webs.

Because most terrestrial C enters lakes as dissolved organic C (DOC), bacteria are a possible link transferring allochthonous C to phagotrophic organisms. Although there is some direct evidence that planktonic bacteria use allochthonous C for the production of new biomass (Coffin and Cifuentes 1999; Kritzberg and others 2004), other studies suggest that bacteria respire large amounts of terrestrially derived DOC but pass very little of this organic C up the food web (Ducklow and others 1986; Cole and others 2002; Kritzberg and others 2005). Thus, pelagic bacteria may be more of a respiratory sink for this C than a link between terrestrial DOC and organisms higher on the aquatic food web (Pomeroy 1974). Our goal is to understand the role that pelagic bacteria play in both respiring and transferring DOC of autochthonous and allochthonous origin to organisms higher on the food web. To accomplish this goal, we need to determine the extent to which C from each source (allochthonous or autochthonous) is actually used by bacteria.

By adding inorganic ^{13}C to two small, unproductive, low humic forest lakes and tracing its subsequent uptake by phytoplankton and on to bacteria, Kritzberg and others (2004) found that pelagic bacteria to a large extent derive their biomass from allochthonous C. Yet autochthonous DOC was used preferentially relative to terrestrial DOC. In theory, the terrestrial contribution of organic C to bacterial biomass (allochthony) should be lower in a lake with similar terrestrial loading but higher internal primary productivity. Correspondingly, in a lake with high terrestrial loading and relatively low primary productivity, bacteria should derive more of their biomass from allochthonous C.

We tested this theory by adding nutrients to one of the lakes used in the previous experiment (Kritzberg and others 2004). The aim was to make the mixed layer of the lake net autotrophic (GPP greater than R) to see if this would make bacteria independent of allochthonous C. As a contrast, we

compared the relative importance of autochthonous and allochthonous C to bacteria in an oligotrophic humic lake. Would the comparatively large pool size of terrestrial DOC uncouple bacteria from autochthonous DOC completely?

To produce a marked contrast between the ^{13}C of terrestrial and aquatic primary production, we made sustained, whole-lake additions of $\text{NaH}^{13}\text{CO}_3$. The design of the experiment largely followed that described by Kritzberg and others (2004) and Pace and others (2004). In addition, one of the lakes was fertilized by daily additions of nitrogen (N) and phosphorus (P) to make the epilimnion of the lake strongly net autotrophic for the duration of the experiment. The isotopic signature of pelagic bacteria was obtained by in situ bacterial regrowth experiments in dialysis tubes (Kritzberg and others 2004; Herndl and others 1993) and in vitro bacterial regrowth cultures in particle-free water (see, for example, Coffin and others 1989).

METHODS

Lake Description

The experiments were conducted in Peter and Tuesday lakes at the University of Notre Dame Environmental Research Center near Land O' Lakes, Wisconsin, USA ($89^{\circ}32'W$, $46^{\circ}13'N$). Peter and Tuesday are small kettle lakes (surface areas of 2.5 and 0.9 ha, respectively) with forested watersheds dominated by sugar maple (*Acer saccharum*), yellow birch (*Betula lutea*), and balsam fir (*Abies balsamea*) and wetlands dominated by *Sphagnum* spp., ericaceous shrubs, and sedges (Carpenter and Kitchell 1993). When unfertilized, both lakes are moderately unproductive and similar with regard to epilimnetic total phosphorus (TP) concentration (surface means of TP from May through August of 2001 were 8 and 10 mg P m^{-3}) and chlorophyll *a* (Chl *a*) (4.1 and 5.3 mg m^{-3}). Also, the pelagic food webs are similar, with extensive minnow populations [for example, fathead minnows (*Pimephales promelas*), finescale dace (*Phoxinus neogaussey*)] as well as sticklebacks (*Gasterosteus aculeatus*). Neither lake has significant populations of piscivorous fish. Peter and Tuesday lakes differ, however, with regard to DOC (4.5 and 8.4 g C m^{-3}) and color (1.3 and 3.5 m^{-1} ; absorbance at 440 nm) consistent with the area of wetlands surrounding Tuesday Lake being greater than Peter Lake. As a reference lake, to check for treatment effects between 2001 and 2002 in Peter Lake, we used Paul Lake. Paul and Peter lakes are separated by a dike; when unfertilized, the two lakes have very

similar lake characteristics (Table 1; data for 2001), although Paul Lake has piscivorous fish [largemouth bass (*Micropterus salmoides*)], and few minnows, and zooplankton are dominated by large-bodied cladocerans.

Whole-Lake ^{13}C Experiment

The present experiment was performed in 2002 and followed the protocols and methods in a whole-lake ^{13}C addition in 2001 (Kritzberg and others 2004; Pace and others 2004). The main difference was the enrichment of Peter Lake by additions of liquid fertilizer containing NH_4 , NO_3 , and PO_4 at an atomic N:P ratio of 25. We made an initial addition of 23 and 265 mg N m^{-2} on 3 June. From 10 June to 25 August, additions corresponding to a P-loading rate of 3.3 mg P $\text{m}^{-2} \text{d}^{-1}$ were made daily from a moving boat. From 17 June through 21 July, we increased the ^{13}C of epilimnetic dissolved inorganic C (DIC) by daily additions of $\text{NaH}^{13}\text{CO}_3$ (more than 99 ^{13}C atom percent; ISOTEC). At early morning (6–8 A.M.), $\text{NaH}^{13}\text{CO}_3$ dissolved in lake water was discharged at 0.5-m depth by continuous pumping into the epilimnion of each lake from a moving boat to enhance spatial mixing. Tuesday Lake received 28 $\mu\text{mol} \text{m}^{-2} \text{d}^{-1}$ and Peter Lake received 24 $\mu\text{mol} \text{m}^{-2} \text{d}^{-1}$. Samples for ^{13}C of major C pools were taken from 28 May through 4 September. During the period of isotope enrichment, sampling for DI^{13}C , DO^{13}C , and particulate organic ^{13}C (PO^{13}C) was done prior to the daily $\text{NaH}^{13}\text{CO}_3$ addition. Although a wide variety of stable isotope pools were followed over the course of this experiment, only bacteria and data from weekly sampling of DIC, DOC, particulate organic C (POC), and periphyton are included here. Periphyton was scraped off at midday from acid-cleaned tiles that were suspended at 0.5 m for 1-week colonization periods. For details on collection and treatment of samples (see Kritzberg and others 2004).

Bacterial Growth Experiments

Dialysis Cultures. To separate bacteria for isotope analyses, bacteria were cultured in dialysis tubes in situ on four occasions in each lake. The initial incubations were done right before the onset of ^{13}C additions; the other three were done during the course of ^{13}C additions. Procedures for these incubations have previously been described in detail (Kritzberg and others 2004) and will be summarized only briefly here. Three replicate dialysis tubes (Spectra Por 2, cutoff 12,000–14,000 D, 45-mm flat width, $\delta^{13}\text{C}$ of the membrane—24.8‰)

were filled with 1,080 ml of 0.2- μm filtered (Supor-200, PALL Life Sciences, NY, USA) and 120 ml of a grazer free inoculum (9:1 vol:vol), which was made by filtering lake water through Whatman GF/D filters (nominal pore size, 2.7 μm). The sealed tubes were incubated in situ and suspended at 0.5-m depth. After 48 h of in situ incubation, bacteria were harvested by filtering the water through 25-mm Whatman GF/F filters (nominal pore size, 0.7 μm). The filters were dried at 60°C for 24 h and stored in desiccators for subsequent analysis of $\delta^{13}\text{C}$. To confirm the growth of bacteria, we sampled for bacterial abundance and organic C. The dialysis tubes contained, on average, 400% more bacterial cells and 450% more organic C at the end of the incubation than at the start.

One additional 0.5-m dialysis tube, with water filtered as for the dialysis cultures, was incubated to evaluate the possibility that phytoplankton growth occurred in the tubes. Filtration of the water used for incubations reduced the Chl *a* concentration to less than 1% of the concentration in the unfiltered lake water. During the course of incubation, Chl *a* decreased, on average, 58%, to an average of 0.06 $\mu\text{g} \text{L}^{-1}$ (SD = 0.07; $n = 8$). Microscopic inspection revealed no presence of cyanobacteria or small eukaryotic algae in either the inocula or the incubated cultures. Thus, growth of phytoplankton in the tubes was insignificant, and phytoplankton POC did not significantly contaminate the bacterial regrowth cultures.

Batch Cultures. For comparison with the dialysis incubations, synchronous batch cultures were also run. The batch cultures were made following a modified version of the method described by Coffin and others (1989) using water collected for dialysis cultures. Lake water filtered through 0.2- μm and GF/D filters (9:1 vol:vol) was incubated in triplicate glass bottles (1.2 L), in darkness at in situ temperature for 48 h. Bacterial $\delta^{13}\text{C}$ from dialysis tubes and batch cultures were similar and overlapping both in Peter Lake ($r = 0.97$, $P < 0.05$; paired t -test $P = 0.40$, $n = 4$) and Tuesday Lake ($r = 0.995$, $P < 0.01$; paired t -test $P = 0.66$, $n = 4$).

Analytical Methods

Gross primary production (GPP) and respiration (R) were estimated from continuous measurements of dissolved oxygen in the mixed layer using an instrument with pulsed oxygen electrodes and a thermistor recording data at 5-min intervals (YSI-Endeco sondes model 6000, MA, USA) (see, Cole and others 2000, 2002; Hanson and others 2003). Respiration was estimated as the diffusion cor-

rected change in oxygen during night. During day, the diffusion-corrected change in oxygen is a measure of net ecosystem production (GPP-R) in the mixed layer, and GPP was calculated assuming that R in the dark and light were equal. Diffusion was estimated from measurements of gas piston velocity (k_{600}) based on whole-lake sulfur hexafluoride additions and continuous measurements of lakeside wind (Wanninkhof and others 1985). Oxygen flux was then calculated for each temperature from the estimate of k_{600} and the ratio of the Schmidt numbers (Jahne and others 1987). The oxygen in the mixed layer is affected by both pelagic and benthic processes to the depth of the mixed layer; hence our data represent an integrated measure of pelagic and benthic processes in these lakes. To enable comparison with bacterial production, oxygen was converted to C, assuming respiratory and photosynthetic quotients of 1.

Using microcentrifuge tubes as described by Smith and Azam (1992), bacterial production (BP) was estimated for four depths (from 0.5 to 5 m) in the upper layer of each lake by measuring the incorporation of ^3H -leucine (Kirchman 1993). Water samples (1.5 mL; four replicates and one killed control) were incubated with 35 nM final concentration of ^3H -leucine (42.5 Ci/mmol) at in situ temperature for 45 min. The incubation was terminated with 30 μL of 50% TCA. The samples were then centrifuged at 17,000g for 10 min, and the pellet was rinsed with 1.5 mL of 5% TCA. After addition of 0.5 mL scintillation cocktail (Scintiverse BD, Fisher Scientific, New Jersey, US), samples were mixed vigorously and ^3H -activity was measured with a Beckman LS, Beckman Instruments Inc., CA, USA 6500 scintillation counter. We calculated BP according to Smith and Azam (1992). Areal values were calculated by trapezoidal integration and included only measurements from the epilimnion.

Bacterial abundance in the dialysis tubes was measured using the acridine orange direct count method with 1% formalin preservation (Hobbie and others 1977). Ten grids with a minimum of 40 cells were counted from duplicate slides. After Chl *a* samples were filtered onto Whatman GF/F filters, they were frozen and extracted in methanol, and the chlorophyll was determined fluorometrically (Carpenter and others 1996). After prefiltration through 153 μm to remove large zooplankton, POC was collected on GF/F filters and analyzed using a Carlo Erba CN analyzer. We measured DOC (Pt-catalyzed high-temperature combustion method) on GF/F filtered samples using a Shimadzu TOC-5000 carbon analyzer, Shimadzu Corpora-

tion, Tokyo, Japan. We measured TP on a Lachat AE, Lachat Instruments, WI, USA autoanalyzer after persulfate digestion using the molybdate blue method. For TN, unfiltered water was digested in basic persulfate (D'Elia and others 1977), and the resulting nitrate was measured by the sulfanilamide method modified for an Alpkem Autoanalyzer, Technicon Instruments, SK, Canada.

Carbon isotopes were measured using elemental analysis-isotope ratio mass spectrometry (EA-IRMS), (Fry and others 1992). We analyzed DI^{13}C using a Micromass Isochrome GC-C-IRMS, GV Instruments, Manchester, UK at the University of Waterloo, OT, Canada. Analysis of $\delta^{13}\text{C}$ for bacteria was carried out using an ANCA-NT system and a 20–20 Stable Isotope Analyzer (PDZ Europa) at the Ecology Department, University of Lund, Sweden. Dissolved organic carbon (after acidification and drying), POC, and periphyton were analyzed for isotope content at the University of Alaska-Fairbanks stable isotope facility, using a Carlo Erba Elemental Analyzer (NC2500) and a Finnigan MAT Conflo II/III interface with a Delta+ Mass Spectrometer.

To reduce the potential of organic C contamination, all glass fiber filters were precombusted at 450°C, and all membrane filters were prerinsed with deionized water.

RESULTS

Metabolic Measurements

Table 1 shows selected physical, chemical, and biological parameters measured in the three lakes in 2001 and 2002. The nutrient additions to Peter Lake resulted in significant increases in TP (Mann-Whitney *U* test $P < 0.001$, $n = 14\text{--}15$) and TN ($P < 0.001$, $n = 14$) that in turn caused a large increase in whole-system gross primary production (Mann-Whitney *U* test $P < 0.001$, $n = 92$) and Chl *a* concentrations (Mann-Whitney *U* test $P < 0.001$, $n = 14\text{--}15$) as compared to 2001. Consequently, the upper mixed layer (benthic plus pelagic components) of Peter Lake changed from a net heterotrophic system in 2001 to a net autotrophic system in 2002 (NEP > 0 ; one-sample *t*-test $P < 0.001$, $n = 92$). Also, pelagic BP in the mixed layer increased from 1.0 to 4.7 mmol C $\text{m}^{-2} \text{d}^{-1}$ compared to the previous year (Mann-Whitney *U* test $P < 0.001$, $n = 13\text{--}14$). Gross primary production increased from 33.2 to 92.5 mmol C $\text{m}^{-2} \text{d}^{-1}$. Thus, BP showed a greater response (4.8-fold) to the nutrient additions than did GPP (2.8-fold). Water temperature between the 2 years was similar

Table 1. Physical, Chemical, and Biological Parameters Measured in the Three Lakes

	Tuesday (2002)	Peter (2001)	Peter (2001)	Paul (2001)	Paul (2002)
Nutrient status	Ambient	Ambient	Amended	Ambient	Ambient
TP (mg P m ⁻³)	12.1 (2.5)	8.1 (1.2)	26.4 (8.1)	9.6 (1.9)	9.6 (2.5)
TN (mg N m ⁻³)	399.0 (60.2)	424.2 (32.2)	653.8 (98.0)	376.6 (50.4)	232.4 (37.8)
DOC (g C m ⁻³)	8.5 (1.1)	4.5 (0.2)	5.8 (0.8)	3.8 (0.4)	4.6 (0.7)
Chl <i>a</i> (mg m ⁻³)	6.8 (3.2)	3.1 (1.5)	42.3 (30.4)	3.9 (1.4)	5.9 (5.2)
BP (mmol C m ⁻² d ⁻¹)	1.5 (0.7)	1.0 (0.4)	4.7 (2.6)	1.2 (0.6)	1.1 (0.5)
GPP (mmol C m ⁻² d ⁻¹)	51.2 (26.5)	33.2 (19.8)	92.5 (45.2)	47.0 (21.6)	—
R (mmol C m ⁻² d ⁻¹)	53.2 (24.5)	43.2 (22.6)	65.8 (47.3)	61.9 (21.0)	—
NEP (mmol C m ⁻² d ⁻¹)	-2.0 (16.5)	-10.0 (10.4)	26.6 (26.4)	-14.8 (11.6)	—
Temperature (°C)	23.7 (2.5)	22.7 (3.0)	23.1 (3.0)	22.6 (3.2)	—

TP, total phosphorus; TN, total nitrogen; DOC, dissolved organic carbon; Chl *a*, chlorophyll *a*; BP, bacterial production; GPP, gross primary production; R, respiration; NEP, net ecosystem production.

Bacterial production (BP) was measured in the pelagic of the mixed layer; GPP, R, and NEP were measured in the mixed layer, including the benthic zone.

Values in parentheses are standard deviation.

(ANOVA $P = 0.39$, $df = 182$), so temperature does not account for differences in GPP or BP. In Paul Lake, the only measured parameter that showed a significant difference between the 2 years was TN, which declined in 2002 relative to 2001 (ANOVA TN $P < 0.01$, $df = 26$; TP $P = 0.85$, $df = 26$; Chl *a* $P = 0.173$, $df = 26$; BP $P = 0.79$, $df = 25$). The general similarity of Paul Lake in 2002 relative to 2001 indicates that the differences in Peter Lake during the 2 years were mainly a result of the nutrient additions rather than climatic variation or other changes.

Tuesday Lake was more productive than Peter or Paul under ambient conditions (Table 1). The average of daily NEP was negative, suggesting that Tuesday Lake tended to be net heterotrophic. Daily variability in NEP was, however, large enough that the 95% confidence interval on daily NEP includes NEP = 0. Thus, Tuesday Lake was not as strongly net heterotrophic as Peter Lake or Paul Lake in 2001 and was probably close to balance in GPP and R.

$\delta^{13}\text{C}$ Dynamics of Major C Pools

The $\text{NaH}^{13}\text{CO}_3$ additions greatly increased the $\delta^{13}\text{C}$ of DIC (preaddition and maximum values were -16 and $+21\text{‰}$ in Peter Lake and -19 and $+22\text{‰}$ in Tuesday Lake), but they increased the total DIC by less than 1% and did not measurably change pH. The DI^{13}C enrichment resulted in noticeable changes in the PO^{13}C , indicating that phytoplankton became enriched in response to the ^{13}C manipulations (Figure 1). Although the absolute increase in PO^{13}C was similar to that in DI^{13}C in Peter Lake, in Tuesday Lake POC became only about half as ^{13}C -enriched as did DIC.

Periphyton were also labeled as a result of the $\text{NaH}^{13}\text{CO}_3$ additions (Figure 1). In Peter Lake, the $\delta^{13}\text{C}$ of the periphyton dynamics was very similar to that of POC ($\text{PO}^{13}\text{C} = 0.935 \times ^{13}\text{C}$ -periphyton -4.5 , $r = 0.93$, $P < 0.001$); whereas in Tuesday Lake, the periphyton responded more strongly to the enrichment than POC ($\text{PO}^{13}\text{C} = 0.37 \times ^{13}\text{C}$ -periphyton -20.4 , $r = 0.97$, $P < 0.001$).

The pre-addition $\delta^{13}\text{C}$ values of the DOC were -27.3 and -28.6‰ in Peter and Tuesday lakes, respectively, similar to reported values for terrestrially produced organic C (Lajtha and Michener 1994). The marked ^{13}C -enrichment of the DOC in Peter Lake (9.5‰) and the slight enrichment in Tuesday Lake (1.5‰) reflect inputs of C from internal autotrophic pathways such as losses of labeled DOC by algae and heterotrophs.

Pre-addition $\delta^{13}\text{C}$ values of bacteria were similar to PO^{13}C . Measurements of bacterial $\delta^{13}\text{C}$ made during the course of additions followed the same pattern as PO^{13}C , but with less enrichment (Figure 1). The relative contribution of allochthonous C to bacterial biomass was calculated by using a two end-member mixing model:

$$\% \text{ allochthonous C} = (\delta^{13}\text{C}_{\text{bacteria}} - \delta^{13}\text{C}_{\text{autochthonous}}) / (\delta^{13}\text{C}_{\text{allochthonous}} - \delta^{13}\text{C}_{\text{autochthonous}}) \times 100$$

For $\delta^{13}\text{C}_{\text{bacteria}}$, we used values from the dialysis incubations, and the allochthonous end-member was estimated at -28‰ , after Lajtha and Michener (1994), and similar to measurements of terrestrial vegetation in the area (mean = -29‰ , SD = 1.7). We estimated the autochthonous end-member

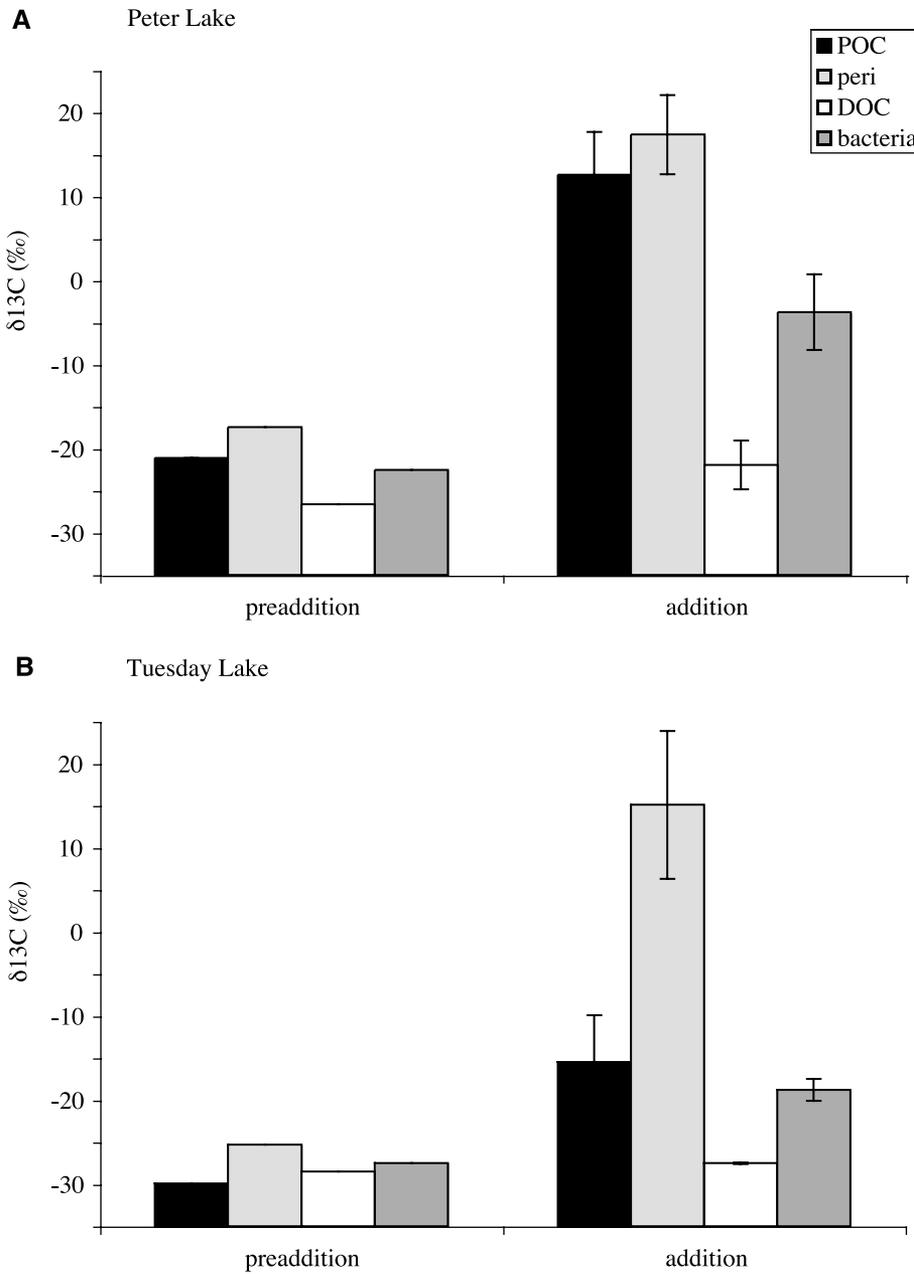


Figure 1. $\delta^{13}\text{C}$ of particulate organic carbon (POC), periphyton (peri), dissolved organic carbon (DOC), and bacteria in (A) Peter and (B) Tuesday lakes. The first set of bars show values prior to isotope additions; the second set show averages after enrichment. Error bars represent SD ($n = 5$ except for bacteria, where $n = 3$).

indirectly by three approaches. The first approach assumed that POC was 100% autochthonous and that the PO^{13}C value was the same as that of phytoplankton. To the extent that POC was of terrestrial origin, this approach underestimates the ^{13}C content of phytoplankton. This approach yielded the lowest contribution of allochthonous C to bacterial biomass. The means and range (in parentheses) for three dates in each lake were 43% (35–45%) and 20% (0–43%) in Peter and Tuesday lakes, respectively (Figure 2).

The second approach assumed that the algal signature could be derived by calculating the algal

proportion of POC from a fixed POC:Chl *a* ratio of 40. We presumed that the algal fraction of POC had an autochthonous signature and that the rest was allochthonous. The bacterial biomass would then be 44% (39–49%) and 76% (70–83%) allochthonous in Peter and Tuesday lakes, respectively (Figure 2).

The third approach assumed that periphyton collected from tile recolonization experiments had the $\delta^{13}\text{C}$ of phytoplankton. Because periphyton tend to deplete local carbon dioxide (CO_2) concentrations, isotopic fractionation tends to be lower than that of phytoplankton, so this approach

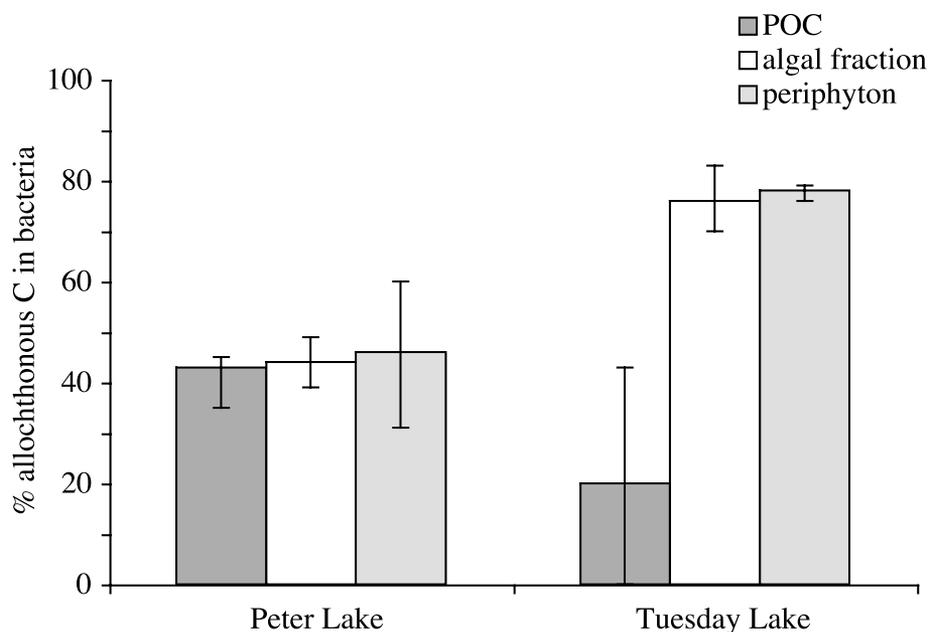


Figure 2. Different estimates of the relative contribution of allochthonous carbon (C) to bacterial biomass. PO^{13}C , a value derived from the algal fraction of the particulate organic carbon (POC), and $\delta^{13}\text{C}$ of periphyton were used as the isotope signature of the autochthonous end-member. The bars show mean values; error bars indicate the range from three sampling dates.

may overestimate the $\delta^{13}\text{C}$ of phytoplankton. This approach gave an estimated allochthonous contribution to bacterial C of 46% (31–60%) and 78% (76–79%) in Peter and Tuesday lakes, respectively.

DISCUSSION

If pelagic bacteria were entirely supported by DOC of allochthonous origin, their $\delta^{13}\text{C}$ would not have changed in response to the whole-lake ^{13}C additions, except to the minor extent that bacteria take up some CO_2 in the anapleurotic reactions of the tricarboxylic acid cycle. If bacteria were supported entirely by autochthonous C, their labeling would have been equal to that of phytoplankton. In both Peter and Tuesday lakes, the dynamics of bacterial $\delta^{13}\text{C}$ changed markedly and paralleled those of PO^{13}C and periphyton $\delta^{13}\text{C}$, but with significantly less enrichment. Thus bacteria must have utilized both allochthonous and autochthonous C sources.

The pattern of bacterial signatures in relation to the PO^{13}C and DO^{13}C pools confirms the main conclusions from previous whole-lake experiments (Kritzberg and others 2004). First, bacteria in both Peter and Tuesday lakes grew on a mixture of autochthonous and allochthonous C. Second, bacteria were consistently enriched compared to DOC, suggesting that although DOC of recent autochthonous origin was a small part of the total DOC pool, it was used preferentially by bacteria over DOC of terrestrial origin. Finally, part of phytoplankton C remained in the DOC pool, as shown by

the increasing $\delta^{13}\text{C}$ signature of DOC. Thus, bacteria did not deplete the entire supply of DOC of phytoplankton origin (see Bade 2004).

In our earlier work, we estimated the contribution of allochthonous and autochthonous C to bacteria in two lakes with very similar GPP and DOC (Kritzberg and others 2004). The present study extends the range of observations to a more humic and a more eutrophic lake. Because there is uncertainty in the $\delta^{13}\text{C}$ of phytoplankton, the autochthonous end-member, we erected three models to calculate the allochthonous and autochthonous C used by bacteria. Although all three approaches have their limitations, estimates based on the first (POC) and third (periphyton) approaches represent the theoretically least and largest contributions of allochthonous C to bacteria. Moreover, the reasonableness of using POC as the autochthonous end-member can be evaluated (see below). All three approaches indicate very substantial allochthonous subsidies to the bacteria of both eutrophic Peter Lake and humic Tuesday Lake. Because of the time and effort involved in making the dialysis incubations, the analysis of bacterial C use is based on measurements of the $\delta^{13}\text{C}$ of bacteria on only four dates in each lake. Hence, there were too few observations to enable comparison of dynamic models with $\delta^{13}\text{C}$ time series of bacteria as used for other ecosystem constituents (see, for example, Pace and others 2004). However, an ecosystem C flow model that estimates the utilization of allochthonous C by all constituents of the food web was developed (see

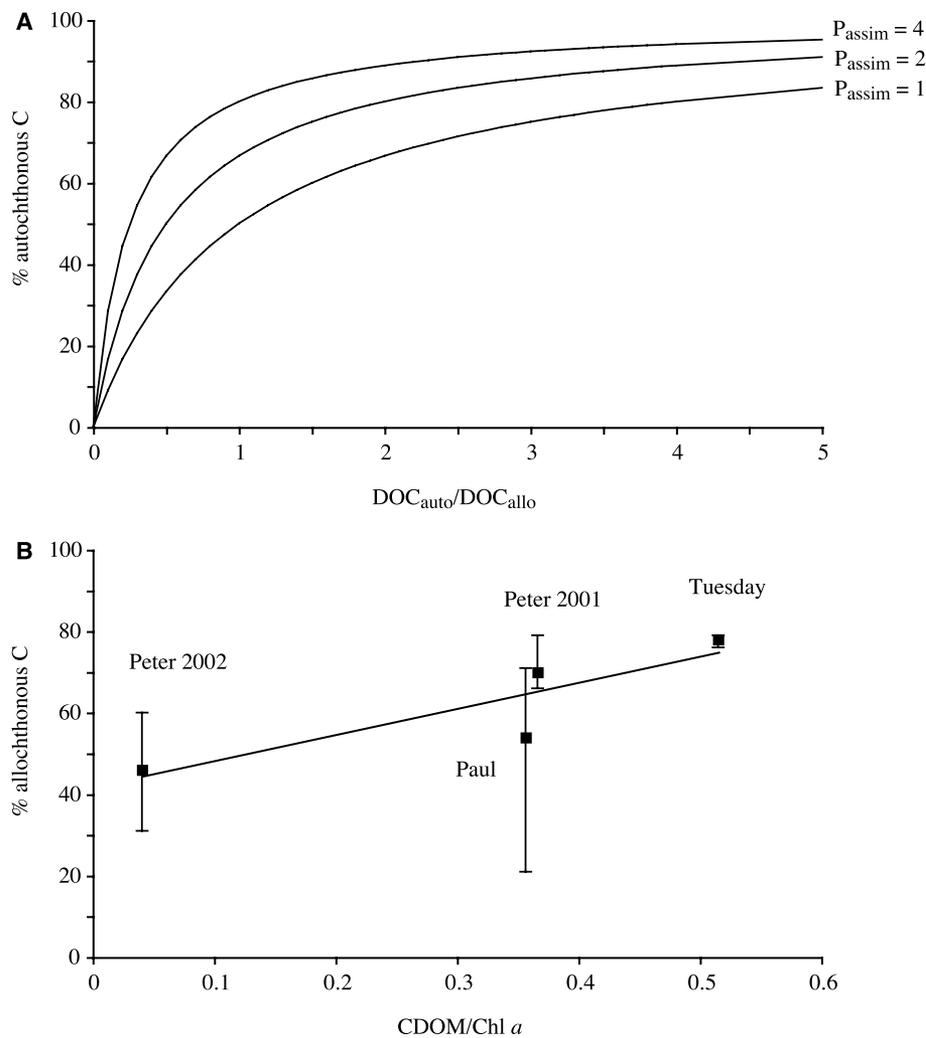


Figure 3. **A** The relative contribution of autochthonous carbon (C) to bacterial C assimilation at different ratios of autochthonous and allochthonous dissolved organic carbon (DOC). **B** The relative contribution of allochthonous C to bacterial biomass in Peter and Tuesday lakes in 2002 (this study) and 2001 (data from Kritzberg and others 2004) against chromophoric dissolved organic matter (CDOM) to chlorophyll *a* (Chl *a*). $\delta^{13}C$ of periphyton was used as the isotope signature of the autochthonous end-member.

Carpenter and others 2005), and this model produced estimates consistent with the analysis of this paper.

The first approach assumed that POC was entirely composed of algae so that $PO^{13}C$ could replace a measured phytoplankton signature. In Peter Lake, $PO^{13}C$ was a good measure of the autochthonous end-member, as POC:Chl *a* was low (43) and $PO^{13}C$ showed a strong response to the isotope additions. Also, $\delta^{13}C$ of physically separated algal material and bulk POC were similar (Bade 2004). The second approach, deriving the algal signature from the estimated algal proportion of the POC pool, had a similar outcome (Figure 2). These estimates were further supported by the third approach, which assumed that periphyton had the $\delta^{13}C$ of phytoplankton (Figure 2). In conclusion, the allochthonous contribution to bacterial biomass could be well constrained to be between 43 and 46%. Thus, even though nutrient additions resulted in substantially increased primary produc-

tion, which exceeded total respiration (seasonal mean GPP:R = 1.4:1), bacterial autochthony only increased from 30% in 2001 to 54% in 2002 (using periphyton as the autochthonous end-member). Compared to 2001, the absolute increase in GPP (approximately $60 \text{ mmol C m}^{-2} \text{ d}^{-1}$) was considerably larger than that of BP (approximately $4 \text{ mmol C m}^{-2} \text{ d}^{-1}$). However, if we assume that 10% of GPP was respired by the phytoplankton, and that 13% of net primary production entered the DOC pool (Baines and Pace 1991; Bade 2004), only $7 \text{ mmol C m}^{-2} \text{ d}^{-1}$ became available as a potential substrate to bacteria. Thus, although this is a rough calculation, the amount of phytoplankton-derived DOC available to bacteria was not as large as it appears by looking at the absolute increase in GPP and BP. Moreover, the autochthonous portion of DOC inputs to Peter Lake was estimated at 17 and 41% of total DOC input in 2001 and in 2002, respectively (Bade 2004). So although there was more algal C produced (GPP) than was consumed

(R) in 2002, bacteria would have to show very strong selectivity to grow on algal DOC only.

It is often assumed that bacteria deplete all autochthonous DOC present and then use allochthonous DOC to support the residual biomass production (Cole and others 2002; Jansson and others 2003). In mechanistic terms, this is an unrealistic assumption. More probably, bacteria take up autochthonous DOC preferentially, but the uptake is confined by its relative contribution to the DOC pool (Kritzberg and others 2005). A selectivity coefficient that denotes differing lability of autochthonous and allochthonous DOC was defined as $P_{\text{assim}} = (\text{autofrac}_{\text{bacteria}}/\text{allofrac}_{\text{bacteria}})/(\text{autofrac}_{\text{DOC}}/\text{allofrac}_{\text{DOC}})$. Autofrac is the fraction of bacterial C assimilation and instantaneous mass of DOC that is autochthonous; allofrac is the fraction that is allochthonous. Figure 3A is a graphical illustration of the selectivity model, where the fraction of bacterial C assimilation that is autochthonous is calculated for a range of theoretical values of the relative contribution of autochthonous C to the DOC pool under the assumption that autochthonous DOC is not ($P_{\text{assim}} = 1$), two times ($P_{\text{assim}} = 2$), or four times ($P_{\text{assim}} = 4$) more available to bacteria than allochthonous C. This model predicts that the input of autochthonous DOC needs to be very large compared to that of allochthonous DOC for bacteria to use only autochthonous C.

When we compared the field seasons of 2001 (unfertilized) and 2002 (fertilized) for Peter Lake, we found that BP increased more (4.8-fold) than primary production (2.8-fold). Considering the lack of differences in Paul Lake during the same years, we conclude that the increase in primary and secondary productivity in Peter Lake was a result of the nutrient additions in 2002. From the measured rates of BP and fractions of BP that derived from allochthonous C in the respective years (70% of BP in 2001 according to Kritzberg and others 2004; 46% of BP in 2002), we could estimate the fraction of the BP increase that was based on allochthonous C to be 40%. Hence, the nutrient additions enhanced bacterial production both via increased production of autochthonous DOC and increased utilization of ambient terrestrial C. Bacterial growth on allochthonous C may have been more efficient as a result of increased nutrient availability. If the low nutritional value of allochthonous dissolved organic matter (DOM) compared to autochthonous DOM (Wetzel 2001) is reflected in the bacterial growth efficiency (Kroer 1993; Cimberlis and Kalff 1998), bacterial utilization of allochthonous C may become more efficient after nutrient additions.

Accordingly, BP is often reported to increase in response to P enrichment regardless of the response by phytoplankton (Pace and Cole 1996; Vrede and others 1999).

In Tuesday Lake, high POC:Chl *a* (mean, 135) suggests that not all POC was the result of recent photosynthesis by phytoplankton, and the low response in PO^{13}C compared to DI^{13}C indicates that POC from this lake had a large terrestrial component. In addition, physically separated algal material was more enriched in ^{13}C than bulk POC (Bade 2004). Thus, PO^{13}C was a poor estimate of the phytoplankton signature in Tuesday Lake and underestimated the bacterial dependence on allochthonous C. Accordingly, deriving the algal signature from the estimated algal proportion of the POC pool resulted in a drastically higher contribution of allochthonous C to bacterial biomass (Figure 2). These values agree well with the approach in which $\delta^{13}\text{C}$ of periphyton was used as the autochthonous isotope signature. In conclusion, the average allochthonous contribution to bacterial C was estimated at 76–78%. Although bacteria in Tuesday Lake were less dependent on phytoplankton-derived C than bacteria in low humic Paul and Peter Lakes in 2001 (Figure 3B), (Kritzberg and others 2004), they did not rely solely on allochthonous inputs, which is in agreement with a suggested bacterial preference for recently produced autochthonous DOC (Kritzberg and others 2004, 2005).

Although the sample size is small, the extent to which bacteria grew on allochthonous C was related to the ratio of chromophoric dissolved organic matter (CDOM) to Chl *a* in the lakes ($r = 0.8$, $P < 0.05$) (Figure 3B). Because CDOM (measured as light absorbance at 440 nm) can be considered an indication of the terrestrial C load (Rasmussen and others 1989) and Chl *a* is proportional to within-lake primary production, CDOM:Chl *a* may be a reasonable proxy for terrestrial C loading in relation to internal C loading. The shallow slope of this relationship indicates that significant differences in CDOM or Chl *a* result in relatively small differences with regard to the bacterial C source, which is in agreement with the predictions from Figure 3A.

Obviously, more lakes and more types of lakes need to be investigated to see if the CDOM:Chl *a* ratio will predict allochthony in bacteria in a general way. Our experiments now cover a large range in DOC, CDOM, Chl *a*, and rates of epilimnetic primary production. Both our model (Figure 3A) and whole-lake ^{13}C experiments suggest that bacterial allochthony is likely to range between about

40 and 80%. Because they are the primary organisms that use DOC, bacteria can potentially link consumers with this otherwise "lost" pool of organic matter (Pomeroy 1974; Paerl 1978). Our study suggests that bacteria would link their consumers to C of both terrestrial and aquatic origin.

ACKNOWLEDGEMENTS

We are grateful for financial support from the National Science Foundation (DEB 9509595), the Swedish Research Council (VR; contract B 5103-649), and the Crafoord Foundation. We thank M. Van de Bogert, D. Bade, G. Birgersson, and C. Fankhauser for technical assistance. Work at the University of Notre Dame Environmental Research Center was facilitated by R. Hellenthal, J. Runde, G. Belovsky, and J. Caudel, and we thank B. Montgomery at ISOTEC for assistance with the purchase of the large quantity of ^{13}C required for these experiments. Finally, we thank Robert Wetzel, Erik Jeppesen, and an anonymous reviewer for greatly improving a previous version of this paper. This is a contribution to the Department of Ecology/Limnology at Lund University and the Institute of Ecosystem Studies.

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