

The influence of dissolved organic carbon on bacterial phosphorus uptake and bacteria–phytoplankton dynamics in two Minnesota lakes

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Abstract

The balance of production in any ecosystem is dependent on the flow of limiting nutrients into either the autotrophic or heterotrophic components of the food web. To understand one of the important controls on the flow of inorganic nutrients between phytoplankton and bacterioplankton in lakes, we manipulated dissolved organic carbon (DOC) in two lakes of different trophic status. We hypothesized that labile DOC additions would increase bacterial phosphorus (P) uptake and decrease the response of phytoplankton to nutrient additions. Supplemental nutrients and carbon (C), nitrogen (N, $1.6 \mu\text{mol NH}_4\text{Cl L}^{-1} \text{d}^{-1}$), P ($0.1 \mu\text{mol KH}_2\text{PO}_4 \text{L}^{-1} \text{d}^{-1}$), and DOC (glucose, $15 \mu\text{mol C L}^{-1} \text{d}^{-1}$) were added twice daily to 8-liter experimental units. We tested the effect of added DOC on chlorophyll concentration, bacterial production, biomass, and P uptake using size-fractionated ³³P-PO₄ uptake. In the oligotrophic lake, DOC additions stimulated bacterial production and increased bacterial biomass-specific P uptake. Bacteria consumed added DOC, and chlorophyll concentrations were significantly lower in carboys receiving DOC additions. In the eutrophic lake, DOC additions had less of a stimulatory effect on bacterial production and biomass-specific P uptake. DOC accumulated over the time period, and there was little evidence for a DOC-induced decrease in phytoplankton biomass. Bacterial growth approached the calculated μ_{max} and yet did not accumulate biomass, indicating significant biomass losses, which may have constrained bacterial DOC consumption. Excess bacterial DOC consumption in oligotrophic lakes may result in greater bacterial P affinity and enhanced nutrient uptake by the heterotrophic compartment of the food web. On the other hand, constraints on bacterial biomass accumulation in eutrophic lakes, from either viral lysis or bacterial grazing, can allow labile DOC to accumulate, thereby negating the effect of excess DOC on the planktonic food web.

Dissolved organic carbon (DOC) influences the physical, chemical, and biological characteristics of aquatic habitats (Wetzel 1992). DOC inputs can affect overall lake metabolism by either reducing light availability to autotrophs, as is the case in humic lakes (Carpenter et al. 1998; Kankaala et al. 1996), or by stimulating bacterial growth, as is demonstrated by whole-lake DOC additions (Blomqvist et al. 2001). Relief from carbon limitation presumably makes heterotrophic bacteria more competitive for other nutrients, such as phosphorus (P), which are often limiting to phytoplankton primary production as well.

Heterotrophic bacteria have a greater affinity for phosphorus than phytoplankton (Currie and Kalff 1984; Cotner and Wetzel 1992; Jansson 1993) and can out-compete phytoplankton for phosphorus at low nutrient concentrations (Rhee 1972; Jansson 1993). The failure of bacteria to displace phytoplankton in P-limited aquatic ecosystems was initially attributed to bacterial DOC limitation (Currie and Kalff 1984). However, inorganic nutrients limit bacterial production in a variety of aquatic

habitats (Cotner et al. 1997; Thingstad et al. 1998; Caron et al. 2000), casting doubt on the universality of bacterial DOC limitation. There is a growing recognition of the importance of food-web structure, including grazer control of bacterial metabolism (Thingstad et al. 1997) and the ability of some planktonic autotrophs to compete with bacteria for inorganic nutrients (Havskum et al. 2003). Additionally, the finding that bacteria can be simultaneously limited by more than one substrate (Rivkin and Andersen 1997) suggests that traditional resource competition theory does not adequately describe bacterial nutrient requirements.

Microbiological studies provide evidence of a biochemical link between DOC metabolism and bacterial inorganic nutrient uptake. Some high-affinity nutrient-uptake systems are energetically expensive and require excess DOC metabolism to become fully operational (Teixeira de Mattos and Neijssel 1997). Jansson (1993) found that the high-affinity phosphorus uptake system in *Pseudomonas* only functioned in the presence of excess glucose. According to this evidence, DOC additions would not only drive bacteria toward inorganic nutrient limitation, but would enhance bacterial nutrient affinity.

Despite the evidence for a link between DOC metabolism and bacterial nutrient uptake, the ecological significance of these findings is not clear. Published studies lack consensus on whether DOC additions encourage bacterial dominance in aquatic ecosystems. Several studies confirm the prediction that DOC additions will cause a coincident increase in bacterial biomass and decrease in phytoplank-

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ton biomass (Joint et al. 2002; Hasegawa et al. 2005; Klug 2005). Although nutrient competition is often cited as the mechanism promoting heterotrophic dominance in these field experiments, nutrient uptake has rarely been measured. One exception is a study by Hasegawa et al. (2005), which found that glucose additions to Sagami Bay water promoted bacterial NH_4^+ uptake.

The results of other similar experiments have been less clear, and they emphasize the importance of food-web interactions in determining the effect of DOC additions on bacteria–phytoplankton interactions (Thingstad et al. 1999; Blomqvist et al. 2001; Havskum et al. 2003). Furthermore, DOC additions can complicate interpretations of heterotrophic metabolism in at least two ways: (1) high concentrations of glucose stimulate non-growth-related metabolism in bacteria (Teixeira de Mattos and Neijssel 1997); and (2) facultatively heterotrophic phytoplankton can consume DOC when concentrations are high in the growth medium (Wright and Hobbie 1966; Bouarab et al. 2004; Kang et al. 2004). Either mechanism would result in DOC consumption in excess of bacterial growth and therefore would decrease the overall effect. Given the conflicting results of previous DOC addition experiments, we carried out a study of DOC effects on the competitive ability of bacteria for nutrients, with an emphasis on bacteria–phytoplankton dynamics.

We used laboratory incubations of unfiltered water from two Minnesota lakes to test the hypothesis that DOC additions would promote bacterial P uptake and decrease the phytoplankton response to nutrient additions. Food-web (Thingstad et al. 1997) and nutrient-uptake models (Currie and Kalff 1984; Cotner and Wetzel 1992; Jansson 1993) predict that phytoplankton will dominate ecosystems with high nutrient-supply rates. Therefore, we chose lakes with contrasting trophic states to test the additional hypothesis that the effect of DOC additions would be greater in an oligotrophic lake than in a eutrophic lake. We observed the response of bacteria and phytoplankton to DOC additions by measuring bacterial biomass and production, P uptake, and chlorophyll concentrations in experimental incubations.

Methods

Experimental design—This study consisted of laboratory incubations of unfiltered lake surface water under controlled light and temperature conditions for approximately 8 d. The effects of nutrients and DOC addition on phytoplankton–bacteria interactions were studied by adding either nutrients (N and P) alone or nutrients and glucose together. The treatments were designed to encourage a phytoplankton bloom through inorganic nutrient additions (NH_4Cl and KH_2PO_4). Our questions focused on the effect of DOC on the interaction between phytoplankton and bacteria; therefore, we did not include a “DOC only” treatment, since the nutrient additions were necessary to observe the response of phytoplankton during the incubation. Each treatment was carried out in duplicate 8-liter polycarbonate carboys, for a total of 8 carboys for each experiment. In late summer 2005, experiments were

Table 1. Basic limnological characteristics of Christmas Lake and Lake Owasso.

	Christmas Lake	Lake Owasso
Area (km^2)	1.12	1.55
Chlorophyll ($\mu\text{g L}^{-1}$)	2.7	30.9
DOC ($\mu\text{mol L}^{-1}$)	461	768
Total phosphorus ($\mu\text{mol L}^{-1}$)	0.7	1.3

performed using water from Christmas Lake, a small oligotrophic kettle lake located in Hennepin County, Minnesota (Table 1), and Lake Owasso, a small eutrophic kettle lake located in Ramsey County, Minnesota (Table 1). The lake surface-water temperature for both lakes was approximately 24°C at the time of water collection.

Surface water was collected into three 20-liter plastic carboys that were then placed in a dark cooler and returned to the laboratory for processing. In the laboratory, each carboy was mixed by inverting ~ 20 times, after which it was placed on a magnetic stir plate and gently stirred with a Teflon-coated stir bar. Each 8-liter experimental carboy was filled by siphoning through 0.95-cm (outside diameter) silicone tubing from the three lake-water carboys simultaneously. Carboys were randomly assigned to experimental treatments and then placed in a walk-in environmental chamber set to 24°C and a 14 h : 10 h light : dark cycle ($\sim 75\%$ of full sun, negligible absorbance of photosynthetically active radiation by the carboy) for the duration of the experiment.

Nutrient and glucose treatments—Each experiment consisted of four treatments designed to test the combined effects of nutrients and DOC on phytoplankton–bacteria interactions in the two lakes. The treatments included a control (no treatment), which was simply unfiltered lake water, a nutrients-only (+N&P) press treatment, which consisted of daily additions of NH_4Cl ($1.6 \mu\text{mol N L}^{-1} \text{d}^{-1}$) and KH_2PO_4 ($0.1 \mu\text{mol P L}^{-1} \text{d}^{-1}$), a glucose press (+Glu Press) treatment, which was identical to the +N&P treatment except glucose was added at a rate of $15 \mu\text{mol C L}^{-1} \text{d}^{-1}$, and a glucose pulse (+Glu Pulse) treatment, which was identical to the +N&P treatment except $120 \mu\text{mol C L}^{-1}$, as glucose, added as a single pulse at the beginning of the incubation. All nutrient and glucose press amendments were added twice daily: stock solutions were pipetted into the appropriate carboy, and mixing was accomplished by gently inverting the carboy ~ 20 times.

Stock solutions of NH_4Cl , KH_2PO_4 , and glucose were made fresh at the beginning of each experiment, divided into 16 separate aliquots (two per day), and kept refrigerated until added to the carboy.

Measurement of chemical and biological responses—The carboys were sampled each day of the experiment by filling 250-mL polycarbonate bottles with sample water by pumping it through silicone tubing by means of a peristaltic pump. The polycarbonate bottles were allowed to overflow with approximately 100 mL of sample water, capped tightly, and then used for routine measurement of the response parameters. Daily measurements were made of

chlorophyll concentration and bacterial abundance. Bacterial production (^3H -leucine uptake) and DOC concentration were measured on days 0, 2, 4, and 6 of the experiment. Size-fractionated ^{33}P - PO_4 uptake was measured on days 1, 3, 5, and 7 of the experiment.

Chlorophyll *a* concentrations were determined by fluorometric analysis of experimental water samples. For each carboy, duplicate GF/F filters were prepared for chlorophyll analysis by gently filtering 50 mL of sample water. Filters were extracted by soaking them in 10 mL of 90% acetone at 4°C for approximately 24 h, and the results were analyzed within two days using a Turner Designs 10-AU fluorometer. DOC concentration was measured on samples filtered through a combusted GF/F filter by high-temperature Pt-catalyzed combustion (Shimadzu TOC-5000A). All filters and glassware used in DOC collection were combusted (450°C, >6 h) prior to use. Samples were preserved by adding 250 μL of 10% HCl and keeping them refrigerated until analysis, approximately 90 d.

Bacterial production was measured by ^3H -leucine incorporation into the trichloroacetic acid (TCA)-insoluble fraction of bacterial cells (Kirchman et al. 1985). Ten milliliters of unfiltered lake water were pipetted into four plastic test tubes. One test tube served as a killed control by addition of 1 mL of 50% TCA and incubation for 5 min. The remaining three tubes were used for live incubation. A mixture of nonradioactive and ^3H -labeled (2.49 GBq mmol^{-1}) leucine was added to the tubes (final concentration: 70 nmol L^{-1}), which were then allowed to incubate for 1–2 h in the dark. Extraction of cellular protein was carried out by adding 1 mL of 50% TCA to the lake-water samples and filtering them through 25-mm-diameter Supor 200 (GE Osmonics) polyethersulfone filters. Total disintegrations per minute (DPM) on the filters was counted on a Beckman–Coulter 6000 Scintillation Counter. After correcting for the killed control (always <0.5% of total DPM), DPM was converted into bacterial carbon production using the protocol outlined by Simon and Azam (1989) and assuming an isotope dilution of 1 (E.K. Hall pers. comm.). The bacterial production rates were expressed as $\mu\text{g C L}^{-1} \text{d}^{-1}$.

Bacterial cell counts and biomass calculations—Samples (5 mL) for bacterial abundance were preserved with filtered formalin (4% final concentration) and stored at 4°C until bacterial count slides were prepared, within 2 weeks of sample collection. Slides were kept frozen until analysis by epifluorescence microscopy. Small rod, coccoid, and crescent-shaped cells were counted manually under 1,000 \times magnification. Larger filamentous bacteria biovolume was determined by image analysis under 900 \times magnification using the imaging software Image Pro. Bacterial cells that were greater than 3 μm in length and that had a length:width ratio >10 were defined as filamentous bacteria. The biovolume of filaments was calculated by assuming a cylindrical shape, $V = \pi \times \text{radius}^2 \times \text{length}$. Average volume of smaller cells was also estimated by assuming an ellipsoid shape, $V = (\pi \times \text{length} \times \text{width}^2)/6$. The total biovolume of smaller cells was then estimated by multiplying the average volume by the cell abundance. Bacterial biomass was then estimated

by applying a volume-specific conversion from bacterial biovolume to carbon mass, $\text{pg C } \mu\text{m}^{-3} = 3.05 \times 10^{(-4.952 \times \mu\text{m}^3)}$ (Kroer 1994). The total bacterial mass was then calculated as the sum of the biomass of each individual cell, expressed as $\mu\text{g C L}^{-1}$.

Phosphorus uptake—Phosphorus uptake was measured as the incorporation of ^{33}P - PO_4 into particles. Prior to adding radioactive phosphorus, the samples were separated into size fractions by gravity filtration of approximately 100 mL of sample water through a 47-mm Whatman GF/D filter (nominal pore size: 2.7 μm). Phosphorus uptake was then measured on both the unfiltered experimental water (whole-lake water, WLW) and the GF/D filtrate (assumed to be the bacterial size fraction). We used GF/D filters to minimize artifacts associated with vacuum filtration, such as perturbation of the dissolved nutrient pool. The nominal pore size of GF/D filters, 2.7 μm , is close to the cutoff of 1.0 μm that is typically considered to be the “bacterial” size fraction in limnological experiments and allowed gravity filtration. Microscopic analysis of unfiltered lake water showed that >90% of the free-living bacterial cells had a diameter less than 2.7 μm .

We performed the P-uptake experiments in the presence of a low concentration of nonradioactive PO_4 . Earlier tests showed that this technique improved repeatability without substantially altering either the measured P-uptake rate or the amount of P absorbed through abiotic binding. At the beginning of each experiment, a series of 2.8 $\mu\text{mol KH}_2\text{PO}_4 \text{ L}^{-1}$ primary stocks were made and frozen until used for P-uptake measurements. Each day that P-uptake measurements were made, a primary stock vial was thawed, and carrier-free ^{33}P - PO_4 (37 MBq mL^{-1} , MP Biomedicals) was added to the primary stock. For each experimental carboy, triplicate plastic 15-mL test tubes with snap-on caps were filled with 10 mL of sample water. The reaction was begun by pipetting 100 μL of the ^{33}P stock into each test tube (final KH_2PO_4 concentration: 28 nmol L^{-1} , ~20,000 DPM), capping the test tube, and inverting it three times. Sample water was allowed to react with the radioactive phosphorus for 3–4 min, and the reaction was stopped by adding 1 mL of nonradioactive 10 $\text{mmol KH}_2\text{PO}_4 \text{ L}^{-1}$ to the test tube, which stops apparent uptake by greatly diluting radiolabeled P in the dissolved pool. Prior tests showed that DPM measured on filters was stable for at least 45 min after the addition of the unlabeled KH_2PO_4 . After stopping the reaction, water samples were vacuum-filtered through 25-mm-diameter Supor 200 (GE Osmonics) polyethersulfone filters at low (<100 mm Hg) vacuum pressure. Filters were presoaked in 1 $\text{mmol KH}_2\text{PO}_4 \text{ L}^{-1}$ to minimize adsorption of ^{33}P - PO_4 to the filter. After filtration, the filters were rinsed with ~1 mL of 1 $\text{mmol KH}_2\text{PO}_4 \text{ L}^{-1}$ and placed in 7-mL borosilicate scintillation vials. One-hundred-microliter aliquots of the ^{33}P - PO_4 stock were also added to two scintillation vials to determine the total activity added to each sample. Scintillation fluid was added within 4 h, and samples were counted on a Beckman–Coulter 6000 Scintillation Counter on an open channel. Killed samples (boiled for 10 min) averaged ~2% of total DPM uptake in previous experiments in these lakes (Stets

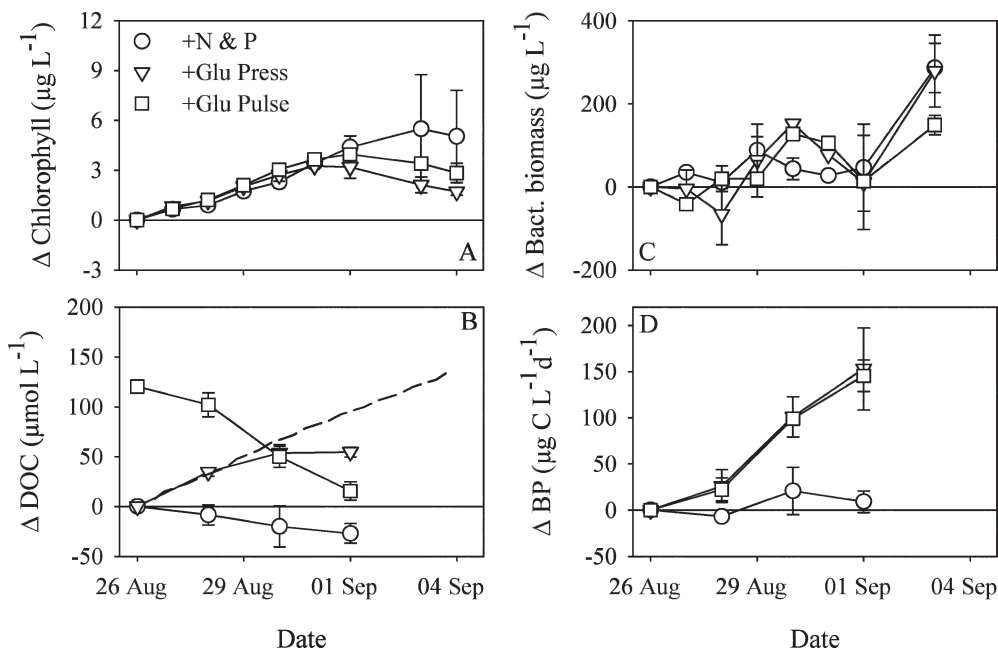


Fig. 1. Christmas Lake experiment. Results are expressed as difference from no-treatment carboys. A negative value indicates that the observation was lower than the no-treatment carboys. (A) Chlorophyll *a* concentration ($\mu\text{g L}^{-1}$). (B) Dissolved organic carbon (DOC) concentration ($\mu\text{mol C L}^{-1}$). The dashed line shows the calculated DOC concentration, assuming that DOC is conserved during addition in the +Glu Press treatment. (C) Bacterial biomass ($\mu\text{g C L}^{-1}$). (D) Bacterial production ($\mu\text{g C L}^{-1} \text{d}^{-1}$).

unpubl. data); therefore, we subtracted 2% of measured DPM incorporated in the present experiment in lieu of preparing killed samples.

The P-uptake rate constant was calculated by applying a concentration-dependent exponential uptake function

$$r_{\text{PO}_4} = -\ln(1 - [\text{Uptake}/\text{Added}])/t$$

where r_{PO_4} is the P-uptake rate constant (min^{-1}), Uptake is the DPM measured on the filter, Added is DPM of $^{33}\text{P-PO}_4$ added, and t is the incubation time (min). The chief assumption of this model is that samples are capable of taking up all of the added $^{33}\text{P-PO}_4$, so that the theoretical maximum uptake is equal to the total DPM added. Prior experiments using lake water in Minnesota showed that this model provided the best fit to time-series $^{33}\text{P-PO}_4$ uptake data. A simple linear uptake model would greatly underestimate the uptake rate constant in samples taking up a large percentage of the added $^{33}\text{P-PO}_4$. The model used is extremely sensitive when $>90\%$ of the label is incorporated. In the present study, no samples incorporated more than 70% of the added label.

Statistical analysis—We report all error estimates as one standard deviation unless otherwise noted. For calculated values, we used error propagation techniques to calculate total error. For example, the values presented in Figs. 1 and 2 are differences between each treatment and the no-treatment experimental carboy. The error bars reflect propagated error in the difference.

The phytoplankton biomass response to experimental treatments was tested using one-way analysis of variance

(ANOVA) on chlorophyll data from the final two days. The number of experimental units (carboys) in this experiment did not provide enough degrees of freedom for repeated measures ANOVA to be used in this analysis.

The hypothesis that DOC additions would increase bacterial competitive ability for P uptake was tested using analysis of covariance (ANCOVA) for each experiment. We used ANCOVA as a way to control for the effect of bacterial biomass on bacterial P uptake and to focus instead on the effect of glucose additions on bacterial competitive ability for P. Our model included log-transformed bacterial biomass as a continuous variable and a two-level categorical variable, “DOC,” which was designed to test for the effect of glucose on bacterial P uptake by combining +Glu Pulse and +Glu Press treatments in one level and the NT and +N&P treatments in the other level. The interaction term was used to test for heterogeneity of slopes. A significant DOC term in the model means that differences in bacterial P uptake were not simply due to higher bacterial biomass, but rather, they were due to a shift in the ability of the community to acquire P at a given biomass. All statistical analyses were performed using the statistical program JMP IN 5.1.2 (SAS Institute).

Results

Christmas Lake experiment—Results from the Christmas Lake experiment support the hypothesis that DOC additions stimulated heterotrophic bacterial production at the expense of phytoplankton biomass. The initial chlorophyll

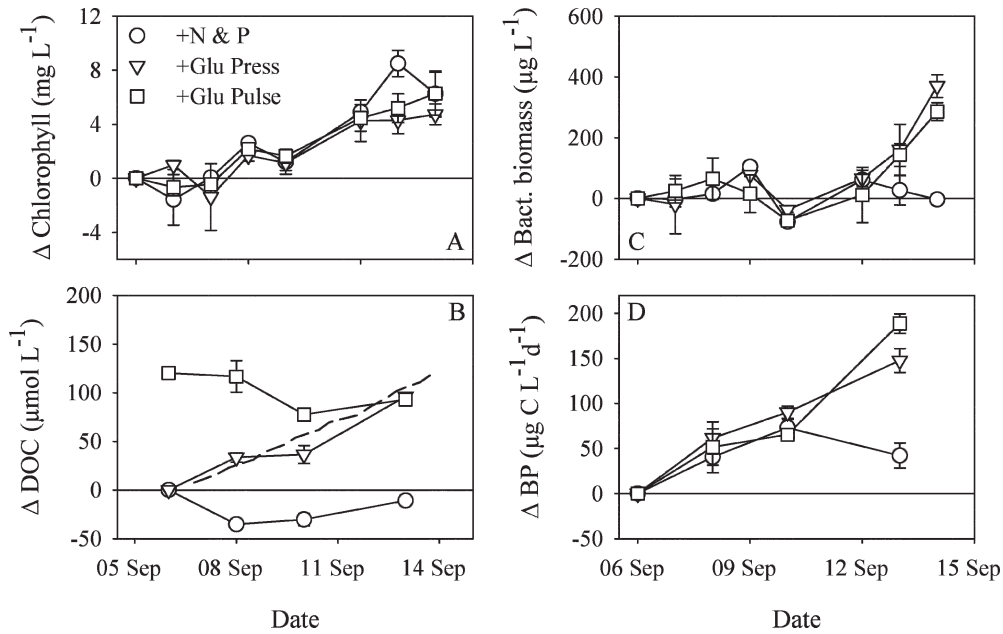


Fig. 2. Lake Owasso experiment. See caption for Fig. 1.

concentration of Christmas Lake was $2.7 \mu\text{g L}^{-1}$ (Table 1), and it remained relatively constant in the no-treatment carboys throughout the course of the experiment (not shown). In all other treatments, phytoplankton biomass increased, as evidenced by elevated chlorophyll concentrations (Fig. 1A). Glucose additions attenuated the increase in phytoplankton biomass starting on approximately day 6 of the experiment; the final chlorophyll concentrations were highest in the +N&P carboys, lowest in the no-treatment carboys, and intermediate in the +Glu Press and +Glu Pulse carboys (Fig. 1A; Table 2).

The DOC data confirm that the planktonic community was capable of using the glucose added in Christmas Lake (Fig. 1B). By day 6 of the experiment, virtually all of the added glucose was consumed in the +Glu Pulse treatment, and $\sim 50\%$ of the added glucose was consumed in the +Glu Press treatment (Fig. 1B).

At the beginning of the experiment, bacterial biomass was $400 \mu\text{g C L}^{-1}$, and it declined to $\sim 200 \mu\text{g C L}^{-1}$ in all treatments. Bacterial biomass was similar in all treatments until the final day of the experiment, when it increased in all amended carboys (Fig. 1C).

Stimulation of heterotrophic metabolism in the +DOC Press and +DOC Pulse experimental carboys was also evident in bacterial production data (Fig. 1D). By day 6 of the experiment, bacterial production was nearly an order of magnitude greater in the +Glu Press and +Glu Pulse experimental carboys relative to the no-treatment carboys (Fig. 1D). In contrast, the +N&P carboys had only modestly elevated bacterial production relative to the no-treatment carboys: 26 ± 2 versus $17 \pm 2 \mu\text{g C L}^{-1} \text{d}^{-1}$, respectively, by day 6 of the experiment (Fig. 1D).

Lake Owasso experiment—There was very little support for our hypotheses concerning the influence of DOC on bacteria–phytoplankton interactions from experiments in eutrophic Lake Owasso. There was only weak evidence for a negative effect of DOC on phytoplankton biomass production. Initial chlorophyll concentrations were $31 \mu\text{g L}^{-1}$, and they decreased to $17 \mu\text{g L}^{-1}$ at a linear rate of $1.9 \mu\text{g L}^{-1} \text{d}^{-1}$ in the no-treatment carboys (not shown). Chlorophyll concentrations also decreased in the other carboys, but final concentrations were $4\text{--}6 \mu\text{g L}^{-1}$ higher than the no-treatment carboys (Fig. 2A). There was

Table 2. Results for one-way ANOVA and post-hoc Student’s *t*-test of chlorophyll data averaged from the final two days of each experiment.

Lake	Treatment	Avg	Student’s <i>t</i> -test
Christmas	+N&P	7.2	A
	+DOC Pulse	5.1	B
	+DOC Press	3.8	B
	No treatment	1.9	C
Owasso	+N&P	20.0	A
	+DOC Pulse	19.2	AB
	+DOC Press	18.1	B
	No treatment	14.4	C

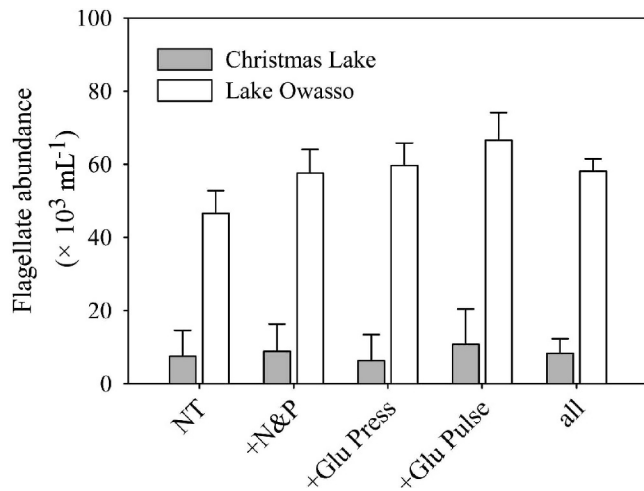


Fig. 3. Flagellate abundances expressed as experimental mean for each treatment in the Christmas Lake (dark bars) and Lake Owasso (light bars) experiment.

a statistically significant difference between the +Glu Press and +N&P treatments (Fig. 2A; Table 2).

DOC data suggested that the planktonic community was not able to consume the excess glucose added to the Lake Owasso experimental carboys. There were only minor variations in DOC concentrations in all experimental treatments, except the +Glu Press carboys (Fig. 2B), in which DOC accumulation was nearly identical to the glucose addition in this treatment (Fig. 2B).

Similar to the Christmas Lake experiment, initial bacterial biomass was $400 \mu\text{g C L}^{-1}$, and it declined to $\sim 200 \mu\text{g C L}^{-1}$. All treatments had similar bacterial biomass until the final two days of the experiment, when bacterial biomass increased in the +Glu Press and +Glu Pulse treatments (Fig. 2C), possibly indicating that the bacterial community became C-limited late in the experiment.

Bacterial production decreased from 117 to $30 \mu\text{g C L}^{-1} \text{d}^{-1}$ throughout the course of the experiment in the no-treatment carboys. All other treatments stimulated bacterial production relative to the no-treatment carboys, and the highest rates, $257\text{--}321 \mu\text{g C L}^{-1} \text{d}^{-1}$, occurred in the +DOC Press and +DOC Pulse treatments (Fig. 2D).

Bacterial community—Bacterial biomass was similar in the two lakes despite the fact that eutrophic Lake Owasso

had a much higher rate of bacterial production, suggesting that bacterial biomass turnover was greater in the eutrophic lake. Although we did not directly measure viral and grazer-mediated causes of bacterial biomass turnover, flagellates were much more abundant in Lake Owasso than in Christmas Lake (Fig. 3), suggesting that grazers were responsible for this difference.

Grazing pressure can induce formation of filamentous bacteria (Pernthaler et al. 1997). In the present study, filamentous bacteria were initially scarce in both lakes and increased during the incubation. Extreme variation in the measurement of filamentous bacteria obscured many possible treatment effects, although the +Glu treatments appeared to have a greater abundance of filaments in Christmas Lake (Table 3). In Lake Owasso, the initial biovolume of filamentous bacteria was low, $70 \times 10^3 \mu\text{m}^3 \text{mL}^{-1}$, and it increased in all carboys (Table 3). The prevalence of filaments in these experiments is consistent with high grazing pressure.

Bacteria comprise a larger proportion of total planktonic biomass in oligotrophic lakes than in eutrophic lakes (Simon et al. 1992; Biddanda et al. 2001; Cotner and Biddanda 2002). A similar pattern was evident in our study—a much higher bacterial biomass-to-chlorophyll ratio in oligotrophic Christmas Lake, 106 ± 46 , than eutrophic Lake Owasso, 11 ± 5 ($t = 11.3$, $df = 57$, $p < 0.001$), indicating that bacteria were a smaller proportion of total planktonic biomass in the more eutrophic lake. The relatively mild effect of glucose additions on phytoplankton biomass in Lake Owasso may have been because bacteria comprised a lower proportion of total planktonic biomass, which would have minimized both the amount of labile DOC consumed by the bacterial community as well as the mass flux of P into the heterotrophic bacterial biomass pool.

DOC effects on bacterial P uptake—Our hypothesis stated that DOC additions would result in greater bacterial competitive ability for P. Initial measurements from Christmas Lake water showed that 40% of the $^{33}\text{P-PO}_4$ label was incorporated into particles in the <GF/D, “bacterial” size fraction. DOC additions stimulated bacterial P uptake, and the peak response occurred on day 5 of the incubation (Fig. 4A). The appearance of filaments in the +Glu Press and +Glu Pulse experimental units (Table 3)

Table 3. Biovolume of filamentous bacteria in experimental carboys estimated from epifluorescence microscopy. For each treatment, we present the incubation mean and the maximum observed value.

Lake	Treatment	Filamentous bacteria biovolume ($\times 10^3 \mu\text{m}^3 \text{mL}^{-1}$)	
		Mean	Maximum
Christmas	No treatment	62	270 ± 25
	+N&P	63	270 ± 25
	+Glu Press	546	$2,053 \pm 750$
	+Glu Pulse	675	$2,398 \pm 1,938$
Owasso	No treatment	659	$1,145 \pm 1,023$
	+N&P	1,202	$2,408 \pm 575$
	+Glu Press	458	$1,060 \pm 853$
	+Glu Pulse	871	$1,828 \pm 1,346$

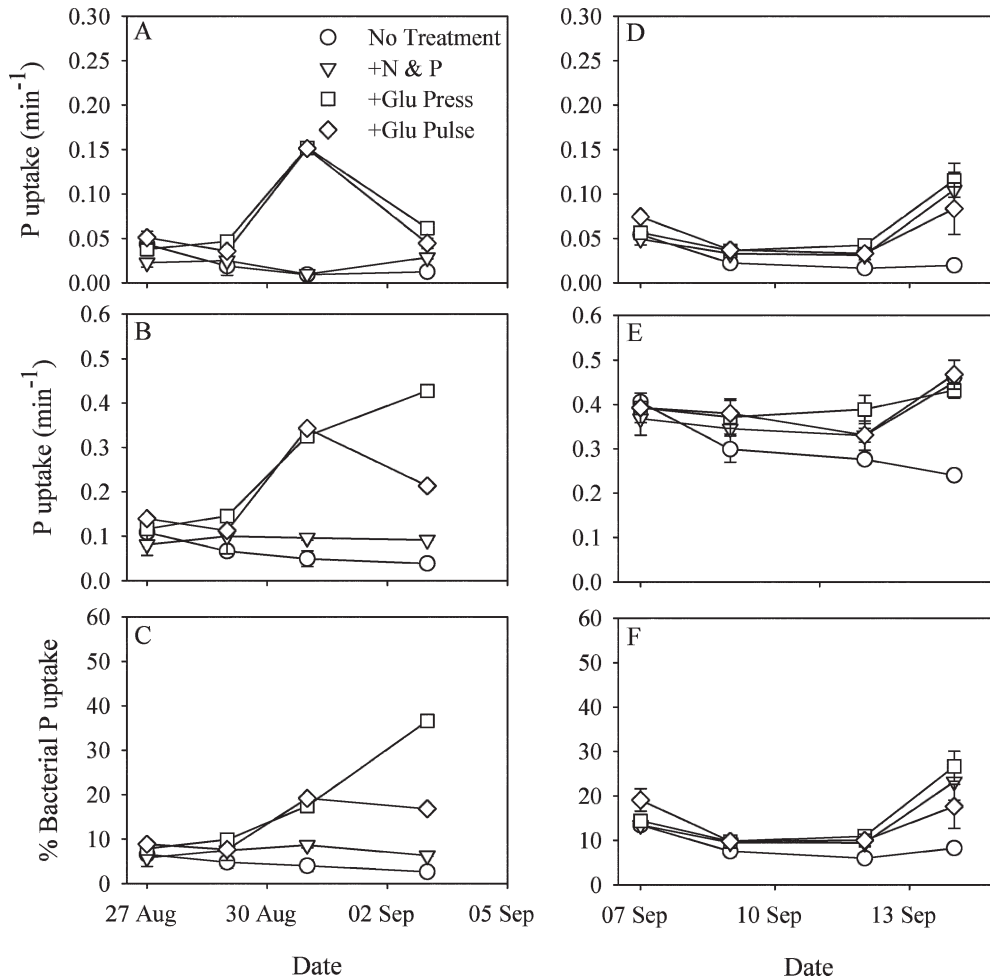


Fig. 4. P-uptake rate constants (min^{-1}). Christmas Lake results (A–C), Lake Owasso results (D–F). (A, D) Bacterial P uptake. (B, E) Total planktonic P uptake. (C, F) Percent of planktonic P uptake by bacteria.

may have complicated size fractionation and resulted in underestimations of bacterial P uptake, particularly on day 7 of the incubation. Data from the unfiltered water samples showed that total P uptake was stimulated by DOC additions (Fig. 4B). Total P uptake in the +Glu Pulse carboys was lower on day 7 than day 5, possibly due to the depletion of added DOC in these carboys (Fig. 4B).

In the Lake Owasso experiment, all treatments stimulated P uptake relative to the no-treatment experimental carboys in both the unfiltered and bacterial size fractions. Bacterial P-uptake rate constants in Lake Owasso were similar to Christmas Lake, ranging from 0.02 to 0.12 min^{-1} (Fig. 4D), while the rate constants from the unfiltered lake water were much higher in Owasso, 0.22 to 0.38 min^{-1} (Fig. 4E). As a result, the percentage of P taken up by the bacterial size fraction was much lower in Lake Owasso, beginning at 13% \pm 1% and peaking at 27% \pm 4% in the +Glu Press experimental carboys on day 7 of the experiment (Fig. 4F).

Glucose additions stimulated bacterial biomass-specific P uptake in Christmas Lake but not in Lake Owasso (Fig. 5A,B; Table 4). The initial ANCOVA model results

for Christmas Lake showed that bacterial biomass was not a significant factor controlling bacterial P uptake ($F_{3,28} = 2.34$, $p = 0.14$, not shown), making ANCOVA an inappropriate method to test our hypothesis. However, DOC additions resulted in significantly greater bacterial P uptake according to a two-tailed t -test ($t = 5.99$, $df = 30$, $p < 0.0001$, not shown). Because of the unusually high bacterial P uptake observed on day 5 (31 Aug) in Christmas Lake (Fig. 4A), we repeated the ANCOVA excluding data from that day; our overall conclusions did not change, since the DOC effect was still highly significant (Fig. 5A; Table 4). In contrast, the observed stimulation of P uptake in Lake Owasso was entirely due to increases in bacterial biomass (Fig. 5B; Table 4).

Discussion

Glucose additions stimulated biomass-specific bacterial P uptake and attenuated the phytoplankton bloom in the Christmas Lake +Glu Press and +Glu Pulse carboys. In Lake Owasso, bacterial biomass turnover rates were high, most likely due to grazing pressure, and glucose additions

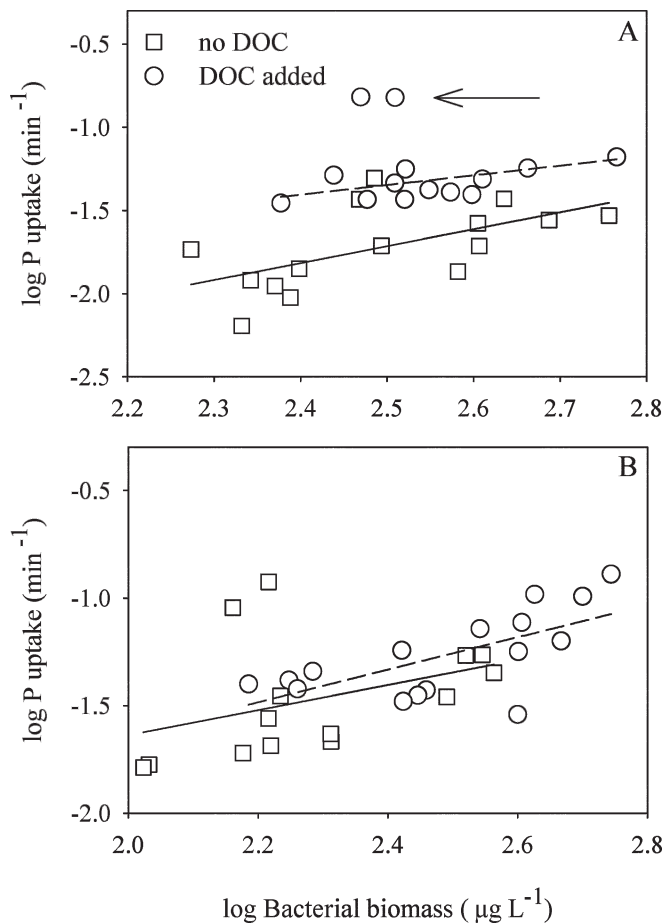


Fig. 5. Bacterial biomass-specific P uptake in (A) Christmas Lake and (B) Lake Owasso. The data were analyzed using ANCOVA (see Methods), and statistical results are summarized in Table 4. The arrow indicates the two data points from day 5 of the Christmas Lake experiment, which were not included in the ANCOVA analysis.

had very little effect on biomass-specific bacterial P uptake. Saturation of bacterial C consumption was evident from the persistence of excess DOC in the Lake Owasso carboys, although glucose additions stimulated bacterial production and biomass on the final two days of the experiment, possibly indicating development of C limitation late in the experiment. Regardless of the ultimate cause, glucose had a much smaller effect on bacterial P uptake and phytoplankton biomass accumulation in the Lake Owasso

experiment. The results of this study suggest that excess DOC can encourage heterotrophic dominance of lake plankton by increasing bacterial nutrient acquisition and decreasing phytoplankton biomass accumulation. In eutrophic lakes, constraints on bacterial biomass production, most likely due to grazing or viral lysis, may negate this effect, at least on short timescales.

The effect of DOC on overall ecosystem functioning was most directly measured by variation in chlorophyll concentration in these two experiments. Several other studies have found convincing evidence for a negative effect of DOC on autotrophic phytoplankton biomass and production (Parsons et al. 1981; Joint et al. 2002; Klug 2005). The results from our experiments were less pronounced, although they were statistically significant (Table 2). As pointed out by Blomqvist et al. (2001), phytoplankton growth on internal nutrient stores can cause a delay in the observable response of phytoplankton to DOC additions. However, in other experiments, a delay in the phytoplankton response was either absent (Klug 2005) or was limited to a few days (Joint et al. 2002). The results from Christmas Lake are consistent with phytoplankton growth on internal nutrient stores with effects of glucose addition becoming manifest only after six days of incubation (Fig. 1A). In Lake Owasso, chlorophyll concentrations declined throughout the experiment, most likely due to either zooplankton grazing or photoacclimation of the phytoplankton community (Rhee and Gotham 1981). Alkalinity in Lake Owasso was 110 mg L^{-1} , which makes inorganic C limitation of phytoplankton unlikely (Hein 1997). Although we predicted less of a response by the plankton community to glucose additions in Lake Owasso, our prediction was based on the logic that nutrient competition would be less severe in this eutrophic lake. The reasons for a decreased response in the Lake Owasso experiment are not completely clear, but the data are most consistent with grazer control of bacterial community metabolism.

The DOC data from Lake Owasso indicate that there was very little net DOC consumption. The DOC supply rate from phytoplankton biomass turnover was likely to be high given the high initial chlorophyll concentration. Production of slowly degrading DOC from phytoplankton biomass turnover may have contributed to DOC accumulation (Fry et al. 1996; Kragh and Søndergaard 2004), and this is consistent with the slight increase in DOC concentrations in the +N&P treatment (Fig. 2B). However,

Table 4. Results of ANCOVA test for DOC effects on biomass-specific P uptake. DOC includes +DOC Press and +DOC Pulse experimental treatments. The response measured is log (bacterial P uptake) for both lakes. The Christmas Lake analysis excludes data from day 5 (31 Aug) of the experiment.

Lake	Source	
Christmas Lake	DOC	$F_{1,22}=26.2242, p<0.0001$
	log biomass	$F_{1,22}=7.6388, p=0.01$
	log biomass×DOC	$F_{1,22}=0.6744, p=0.42$
Lake Owasso	DOC	$F_{1,26}=2.2160, p=0.15$
	log biomass	$F_{1,26}=10.2376, p=0.04$
	log biomass×DOC	$F_{1,26}=0.0034, p=0.95$

it is likely that bacterial consumption of labile DOC was saturated in the +Glu Press and +Glu Pulse treatments. High growth efficiency and biomass turnover may have limited bacterial DOC consumption. Bacterial growth efficiency tends to be high in eutrophic systems (Del Giorgio and Cole 1998; Biddanda et al. 2001; Smith and Prairie 2004), decreasing the amount of C consumed by bacteria. High bacterial turnover rates in Lake Owasso may have also limited biomass accumulation and thereby constrained the total amount of DOC consumed by the bacterial community. In the amended carboys, μ peaked at 1.46–1.82 d⁻¹, which is close to the μ_{\max} of 2.3 d⁻¹ calculated as a function of temperature (Morris and Lewis 1992), implying that bacterial biomass did not increase even though bacterial μ approached μ_{\max} .

Viewed another way, the bacterial biomass-to-chlorophyll ratio was nearly an order of magnitude lower in Lake Owasso than Christmas Lake (*see* Results). While we cannot rule out a role of viruses in controlling bacterial biomass in Lake Owasso, the high abundance of flagellates (Fig. 3) and prevalence of bacterial filaments (Table 3) are consistent with heightened grazing on bacteria (Pernthaler et al. 1997; Simek et al. 1997). Therefore, we conclude that the failure of bacteria to consume the added DOC in Lake Owasso was a result of controls on bacterial biomass, most likely grazing, combined with a saturating DOC supply rate from phytoplankton biomass turnover. Also, while the gross DOC consumption rate was probably quite high, as evidenced by the high bacterial production rates (Fig. 2C), the DOC supply rate was also high, which led to DOC accumulation in the experimental carboys.

Food-web constraints on net labile DOC consumption could cause a decoupling between autotrophic production and heterotrophic metabolism in aquatic ecosystems and allow the accumulation of otherwise degradable organic material. Such a mechanism was predicted by Thingstad et al. (1997) and observed in laboratory experiments (Vadstein et al. 2003). Because bacteria are unable to consume some of the phytoplankton-derived organic material, this line of reasoning is also consistent with net autotrophy (Del Giorgio and Peters 1994; Del Giorgio et al. 1997; Cole et al. 2000) and net CO₂ invasion (Schindler et al. 1997) in eutrophic lakes. Nevertheless, the limits of inferences from this experiment must be acknowledged. For example, bacterial biomass increased dramatically in the +Glu Press and +Glu Pulse carboys on the final days of the Lake Owasso experiment (Fig. 2C), perhaps indicating a relaxation of grazer control. Therefore, the temporal and spatial scales over which biodegradable DOC might accumulate in eutrophic lakes are uncertain.

DOC additions stimulated P uptake in both lakes, but while the increase in Lake Owasso was entirely due to increased bacterial biomass, the biomass-specific P uptake was greater in Christmas Lake (Fig. 5A,B; Table 4). Since P-uptake measurements were expressed as rate constants, increased P uptake could have resulted from either greater P mass flux into bacteria or from lowered ambient PO₄ concentrations. In Lake Owasso, increases in P uptake were associated with increases in bacterial biomass (Fig. 5B; Table 4), suggesting that there was greater P mass flux into

bacteria, which could have been achieved without increasing either P affinity or bacterial P content. In this case, increases in bacterial P uptake would depend entirely upon increases in bacterial biomass. As discussed already, high bacterial biomass turnover rate prevented substantial bacterial biomass accumulation in Lake Owasso and minimized the effect of bacterial P uptake on phytoplankton. On the other hand, the increased P uptake in Christmas Lake occurred independently of bacterial biomass, suggesting that either ambient PO₄ concentrations decreased or that bacterial P content increased. While the distinction between these two mechanisms is important physiologically, either explanation suggests that glucose additions resulted in a greater capacity for P uptake by bacteria independent of biomass effects in Christmas Lake. Overall, the results are consistent with the idea that bacteria are energy-limited in their ability to acquire dissolved inorganic nutrients (Kirchman et al. 1990) and point to an ecologically important role for excess bacterial DOC consumption.

It is important to note that the bacterial P-uptake measurements were performed on filtered-water samples typically within 20 min of filtration, so that most grazers were removed, thereby limiting P regeneration due to bacterial grazing. If a significant number of bacterial grazers were included in the bacterial P-uptake samples, the differences between the lakes may have been artificially inflated, since Lake Owasso did have greater flagellate abundance (Fig. 3). Also, the development of bacterial filaments may have interfered with our measurement of bacterial P uptake by reducing the efficiency of our size-fractionation experiments. Direct P uptake by filaments was likely to be low given that grazing-induced filaments grow very slowly (Pernthaler et al. 1997; Simek et al. 1997), but the presence of filaments could have interfered with the mechanics of size fractionation by clogging the filter.

Our hypothesis concerning DOC stimulation of P uptake was largely confirmed, and the caveats limiting our conclusions are instructive. First, DOC additions had no effect in eutrophic Lake Owasso because the bacterial community was already saturated in its ability to consume DOC. The consequent effects of DOC additions on phytoplankton biomass dynamics were also considerably weaker than in Christmas Lake. The results of this experiment also confirm that labile DOC can accumulate in eutrophic systems, most likely as a result of food-web constraints on bacterial DOC consumption, although the generality of this conclusion is still unclear. In contrast, oligotrophic Christmas Lake showed a stronger response to DOC additions, which stimulated bacterial growth and biomass-specific P-uptake rates. The bacteria in Christmas Lake were able to consume added labile DOC, which apparently increased their competitive ability for inorganic nutrients and resulted in an attenuation of the phytoplankton bloom in this experiment. So while increasing DOC inputs to oligotrophic lakes can influence bacteria–phytoplankton dynamics by favoring bacterial P uptake, this mechanism has a diminished importance in eutrophic systems due to food-web constraints on the bacterial community.

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