

## The effect of temperature on the coupling between phosphorus and growth in lacustrine bacterioplankton communities

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### *Abstract*

Phosphorus (P) routinely limits microbial growth in freshwater ecosystems. The growth rate hypothesis (GRH) relates growth to biomass P content mechanistically through changes in ribosomal ribonucleic acid (rRNA). Although the GRH has been shown in cultured bacteria, less well understood is how GRH relationships are affected by interactions with environmental parameters such as temperature. To address this, we evaluated the relationship between bacterial biomass P, RNA:deoxyribonucleic acid (DNA) ratio, and growth rate in 47 northern temperate lakes. Although RNA:DNA ratio was positively correlated with bacterial biomass P:carbon (C), we found no correlation between growth rate and RNA:DNA or between growth rate and biomass P:C. There was, however, a significant effect of temperature on biomass P:C. We investigated the effect of temperature more directly using filter-separated bacterial communities from two lakes during two seasons while experimentally manipulating temperature and resources. For the summer communities, bacterial production (BP) and biomass P were positively correlated, and the slope of that relationship increased with increasing temperature. In winter communities, BP and biomass P were again positively correlated; however, the slope of that relationship decreased with increasing temperature. The slope of the relationship between BP and biomass P is a metric of nutrient use efficiency and was strongly influenced by temperature. More importantly, bacterioplankton demonstrated seasonal acclimation or adaptation to in situ temperature, suggesting that studies evaluating multiple communities in time and space fail to find a clear relationship between temperature and bacterial metabolism because bacterial communities are locally adapted to in situ temperature.

It is well known that pelagic heterotrophic bacteria (hereafter bacterioplankton) play a fundamental role in processing elements in aquatic ecosystems. It is, however, unclear how changing lake temperature caused by climate change will affect the bacterial contribution to important ecosystem processes, e.g., carbon (C) and phosphorus (P) cycling. One reason for this uncertainty is that the effect of temperature on bacterial metabolism is dependent on the in situ resource pool (Pomeroy and Wiebe 2001). Because phosphorus is commonly the limiting nutrient to bacterial growth in freshwater (Carlsson and Caron 2001; Graneli et al. 2004; Smith and Prairie 2004) and marine ecosystems (Cotner et al. 1997; Zohary and Robarts 1998; Cotner 2000), it is expected to play an integral role in regulating the bacterial community metabolic response to temperature.

Here we use the conceptual framework of the growth rate hypothesis (GRH) to evaluate how temperature affects P–growth relationships in natural bacterioplankton communities. The GRH relates growth rate to biomass P content through changes in ribosomal ribonucleic acid (rRNA) content. RNA is rich in P and a major component of total

biomass P (Elser et al. 1996). Therefore, increases in rRNA content, concomitant with growth, result in increased biomass P and a positive correlation between biomass P:C and growth rate (Sterner and Elser 2002; Makino et al. 2003).

In addition, we evaluated the effect of temperature on the nutrient use efficiency (NUE) of bacteria. When bacterial production (BP, e.g.,  $\mu\text{g C L}^{-1} \text{h}^{-1}$ ) is measured instead of specific growth rate ( $\text{h}^{-1}$ ), then measuring the amount of bacterial biomass added per unit phosphorus ( $\text{C P}^{-1} \text{h}^{-1}$ ) is analogous to NUE, a metric commonly used to evaluate the physiology of terrestrial autotrophs (Vitousek 1982). Although there are several ways to define NUE (Sterner and Elser 2002, chapter 8), each approach evaluates the amount of nutrient used per unit biomass or C acquired. Here we define NUE as biomass added (measured as leucine synthesis) per unit P per hour (i.e., leucine  $\text{P}^{-1} \text{h}^{-1}$ ). Evaluating NUE allows for direct linkages between phosphorus dynamics and important biogeochemical processes, e.g., C flux through the bacterioplankton, in the context of a well-developed literature from other disciplines.

We conducted an observational study of temperate lakes in the upper Midwest of the U.S.A. and two experimental microcosm studies, one in summer and one in winter, designed to evaluate how temperature affects the relationship between P and growth rate in natural bacterial communities from different in situ temperatures. We also evaluated the relationship between biomass P and growth in bacterial communities at three in situ temperatures in 10,000-liter mesocosms wherein resource P was experimentally manipulated. These studies were designed to test two

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specific hypotheses. First, we hypothesized that RNA:deoxyribonucleic acid (DNA), biomass P, and growth rate would be positively correlated in natural bacterial communities. Second, we hypothesized that per unit cellular P, bacterial communities would have higher biomass production at higher temperatures. That is, we hypothesized that bacterial communities would have increasing NUE with increasing temperature because of increased kinetic energy of ribosomes and other cellular “machinery” involved in biomass synthesis.

## Methods

*Sampling and experimental design*—Lake survey: During the summers of 2000 and 2001, we sampled 128 lakes in the upper Midwest of the U.S.A. Of these 128 lakes, 47 contained the complete set of parameters to be evaluated for this current study (e.g., RNA was not measured in many of the lakes). All 47 lakes were located within ca. 50 km of four different biological field station sites (Itasca, Minnesota, Itasca Biological Station, University of Minnesota [Itasca]; Twin Cities, Minnesota, University of Minnesota [Metro]; Hickory Corners, Michigan, Kellogg Biological Station, Michigan State University [MI]; and Oak Lake, South Dakota, Oak Lake Biological Research Station, South Dakota State University [SD]). This analysis includes 15 lakes each from MI and Itasca, 8 lakes from the Metro area, and 9 lakes from SD. All lakes were sampled at the deepest point in the lake or, when maximum depth was not known, in the approximate center. Samples were taken from the mixed layer when the system was stratified or from the upper third of the water column when the system was unstratified. Stratification was determined by vertical temperature and oxygen profiles taken at each sampling site using a sonde (model 6000-B-D-O, YSI). All water samples were collected with a Van Dorn bottle, placed into 20-liter carboys, and kept in a dark cooler while traveling between the lake and the laboratory, where they were processed and preserved for analysis.

*Microcosm experiments*: Bacterial communities were separated by filtration from two neighboring lakes in Clearwater County, Minnesota, in August 2003 and January 2004. Long Lake is relatively small (surface area  $5.9 \times 10^5$  m<sup>2</sup>, mean depth = ~8 m), cold, and oligotrophic (chlorophyll *a* [Chl *a*] ~ 1.2  $\mu\text{g L}^{-1}$ ). Lake Itasca, the “headwaters of the Mississippi,” is larger (surface area =  $4.4 \times 10^6$  m<sup>2</sup>), warmer, shallower (mean depth = ~2 m), and mesotrophic (Chl *a* ~ 10.6  $\mu\text{g L}^{-1}$ ). During sampling both lakes were 3°C ( $\pm 1^\circ\text{C}$ ) in January, whereas in August epilimnion temperatures were 23°C ( $\pm 2^\circ\text{C}$ ) in Long Lake and 26°C ( $\pm 2^\circ\text{C}$ ) in Lake Itasca.

After filtration (1- $\mu\text{m}$  pore size), water was placed in 1-liter microcosms at one of the three (4°C, 14°C, or 24°C) experimental temperatures. Once all water was filtered and each microcosm was at the desired temperature, nutrients were added as a combination of C, nitrogen (N), and P at one of three resource levels. In summer, C (equal parts acetate and glucose), N (NH<sub>4</sub>Cl), and P (KH<sub>2</sub>PO<sub>4</sub>) were added to the 2 $\times$  treatment to final concentrations of 80, 12,

and 0.75  $\mu\text{mol L}^{-1}$ , respectively, and were increased proportionally for the 5 $\times$  and 10 $\times$  treatments. In January, because our previous work indicated that bacteria have higher P requirements at colder temperatures (Cotner et al. 2006), P amendments to each treatment were increased fourfold whereas C and N amendments remained the same, thus changing the ratios of C:N:P of the nutrient treatment between seasons but not between lakes within a season. No nutrients were added to the ambient treatment in either season.

To ensure that each community had sufficient time to respond to resource additions at each temperature, the week prior to each experiment we placed filter-separated bacterial communities from each lake into 20-liter carboys at two of the four resource levels (ambient and 5 $\times$ ) for each experimental temperature. We measured BP every 24 h for 8 d, and at the highest temperature (24°C) we measured BP at 12 h, at 24 h, and in 24-h increments thereafter. We used the first peak in BP at each experimental temperature as the maximum response of each community for that treatment. Experimental incubation times across all temperatures and seasons ranged from ~15 to 150 h. The time until the maximum response was then used in the subsequent experiments as the predetermined stop time for each experimental temperature (Hall and Cotner 2007). Microcosms were sampled at the predetermined stop time for BP, bacterial abundance (BA), RNA:DNA, and biomass P and C.

*Mesocosm experiments*: In order to evaluate whether the results from our microcosm experiments were representative of more complex systems, i.e., systems with multiple trophic levels, we investigated bacterial communities in larger mesocosms at three dates with seasonal temperatures that corresponded to the temperatures in our microcosm experiment. A series of twenty 10,000-liter mesocosms (2.4-m depth; 4.8-m diameter) located at the University of Kansas Field Station and Reserves were inoculated with water from a nearby artificial pond and administered approximately biweekly phosphorus additions at five different levels to create a trophic gradient between mesocosms. The ambient P concentration in the source water (15  $\mu\text{g L}^{-1}$ ) was used as the base P level, and four additional P treatments were established at 3 $\times$ , 5 $\times$ , 7 $\times$ , and 9 $\times$ . These target concentrations were maintained for more than 3 months prior to data collection.

Mesocosms were sampled on 30 August 2003, 31 October 2003, and 05 March 2004, with water temperatures of 24°C, 14°C, and 4°C, respectively. This allowed us to address the quantitative and qualitative validity of the relationship we found in the microcosm systems at a level of complexity that was intermediate between the highly reduced microcosm systems and the uncontrolled lake ecosystems. Water was collected from multiple locations within each mesocosm using a depth-integrated polyvinyl chloride tube sampler. The water was then returned to the lab, where bacterial communities were filter-separated and BP and biomass P were analyzed following the same methods as in the microcosm work.

*Analytical methods*—Biomass stoichiometry and filtration: To examine bacterial biomass C and P, lake water samples were pre-filtered through a 147-mm 1- $\mu\text{m}$  polycarbonate filter at low pressure (<100 mm Hg) to remove the larger plankton. Bacterial carbon and phosphorus samples were collected by filtering between 100 and 300 mL of pre-filtered lake water onto GF/F filters using a vacuum pump at low pressure ( $\leq 5$  mm Hg). All GF/F filters were precombusted in a muffle furnace at 450°C for 3–4 h, and filters used for P analysis were acid-rinsed (1% hydrochloric acid) and then washed with nanopure water before filtration. For systems with high carbonate (i.e., MI and SD), filters used for CHN analysis also received a post-filter acid rinse (1% HCL) to remove any inorganic C adhered to the filter. GF/F filters were then placed in a drying oven ( $\sim 60^\circ\text{C}$ ) overnight and then kept in desiccators in individual tinfoil envelopes until analysis. CHN samples were analyzed using a Perkin Elmer CHN analyzer or from a calibrated algorithm developed for use with a separate near-infrared spectrum system (Hood et al. 2006).

Phosphorus samples were first digested using acid persulfate for particulate P or directly analyzed without digestion for soluble reactive phosphate (SRP) spectrophotometrically (APHA 1992). All dissolved nutrient samples were filtered through a 47-mm glass fiber (GF/F) filter (0.7  $\mu\text{m}$  nominal cutoff) using a hand pump at low pressure (<100 mm Hg) and maintained frozen until analysis. Dissolved organic C (DOC) and total dissolved N (TDN) were analyzed using a Shimadzu T5000. Water samples for Chl *a* were filtered onto GF/F filters, and the filters were kept frozen in the dark until analysis. Pigments were extracted in 90% acetone and analyzed against Chl *a* standards in a Turner Designs 10-AU fluorometer (Parsons et al. 1984).

Separating the bacterial community by size-fraction filtration for elemental analysis presents two important methodological issues that deserve mention here. Pre-filtering through a 1- $\mu\text{m}$  filter in order to exclude eukaryotic cells from our analysis may have also excluded a portion of the bacterioplankton, especially the bacteria attached to detrital particles or fecal pellets. Previous work conducted on a subset of the same lakes included in this analysis showed that typically more than 95% of the total bacterial community passes the 1- $\mu\text{m}$  pore size (Biddanda et al. 2001). At the other end of the size spectrum, GF/F (nominal pore size of  $\sim 0.7$   $\mu\text{m}$ ) filters have been shown to allow a fraction of a marine bacterial community to pass through the filter (Lee et al. 1995). Although we assume that we lost a portion of the smallest cells from the bacterial community through the GF/F filters, the lakes surveyed in this study were consistently more nutrient-rich than the environments assessed in the marine study cited above and therefore presumably had larger cells, allowing more to be retained on the filter for analysis. We did not directly evaluate the filtrate to determine the portion of the community that passed through the GF/F filter.

We evaluated our samples to ensure that the organic matter we analyzed was composed primarily of bacterial biomass. Visual analysis of the bacterial size fraction did

not routinely show any extracellular polymeric substance (EPS). The highly homeostatic C:N of all our samples (biomass C [ $\mu\text{mol L}^{-1}$ ] = 8.04  $\times$  biomass N [ $\mu\text{mol L}^{-1}$ ] + 0.32;  $n = 47$ ,  $p < 0.0001$ ,  $R^2 = 0.92$ ) is unlikely if the biomass being measured was detrital. In addition, a type II regression of C vs. N indicated the absence of a significant positive intercept ( $p = 0.80$ ) for C, which would be expected if a significant portion of the biomass was composed of EPS, colloids, or other C-rich detrital material. For these reasons, we contend that our samples represented a significant fraction of the bacterioplankton community and also that bacterial biomass dominated the organic matter in our samples.

*Nucleic acid analysis*: RNA and DNA content was measured by filtering 25–50 mL of pre-filtered (<1  $\mu\text{m}$ ) water onto 0.2- $\mu\text{m}$  white polycarbonate filters, which were flash-frozen in liquid N (in 2-mL cryovials) in the field, and transferred to a  $-70^\circ\text{C}$  freezer in the laboratory. Analyses were performed using a BioTek FL600 microplate reader and the nucleic acid stain Ribogreen following the methods of Makino et al. (2003). RNA content is reported here as RNA : DNA.

*Growth and production measurements*: Bacterial growth rate (SGR,  $\text{h}^{-1}$ ) was measured using dilution cultures run in triplicate similarly to Ammerman et al. (1984) for the lake survey, or in the microcosms by first measuring BP using leucine uptake and then correcting for differences in biomass pool size by dividing by C-converted BA using a literature-derived value of 100 fg cell $^{-1}$  (Vadstein and Olsen 1989). For dilution cultures, 20 mL of <1- $\mu\text{m}$ -filtered water was added to 180 mL of <0.2- $\mu\text{m}$ -filtered water in 250-mL polycarbonate bottles. Bottles were kept in the dark at in situ temperature ( $\pm 2^\circ\text{C}$ ) and incubated for  $\sim 24$  h. Subsamples for abundance were taken at approximately 0, 12, and 24 h and preserved in formalin (5% final concentration) and placed in the dark at 4°C until slides were made within 4–5 d at most. For enumeration, samples were stained with acridine orange, filtered onto 0.2- $\mu\text{m}$  black polycarbonate filters, and counted on an Olympus BX40 epifluorescence microscope (Hobbie et al. 1977), with a minimum of 300 cells counted for each sample. SGR was calculated as

$$\mu_{\text{max}} = \left( \frac{\left( \frac{\ln N_t}{\ln N_{t-1}} \right)}{t} \right)$$

where  $t$  is time and  $N_t$  is bacterioplankton population size (cell number) at time  $t$ .

To measure BP, four 10-mL aliquots (one control, killed with 50% trichloroacetic acid [TCA], and three samples) were taken from each microcosm and incubated with a mixture of “cold” leucine (Aldrich L602500) and  $^3\text{H}$ -labeled leucine (1.9 mmol MBq $^{-1}$ , Amersham Bioscience RK170) to a final concentration of 30 nmol L $^{-1}$  leucine for the summer experiment and 45 nmol L $^{-1}$  leucine for the winter experiment for 2 h. For each season separate experiments confirmed that the concentration of leucine used was

Table 1. Simple linear regressions of the predicted GRH relationships for the experimental microcosms using both bacterial production (BP) and bacterial production normalized for BA (SGR) as indices of growth. All data were log transformed before analysis.\*

y	x	Intercept	Slope	$R^2$	$p$ value	$n$
BP	RNA : DNA	-0.80	0.96	0.31	<0.0001	96
P : C	BP	-1.89	0.32	0.31	<0.0001	92
P : C	RNA : DNA	-2.11	0.26	0.07	0.0091	92
SGR	RNA : DNA	-1.70	0.48	0.14	<0.0003	91
P : C	SGR	-1.58	0.26	0.13	0.0011	87

\* P, phosphorus; C, carbon; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; SGR, bacterial growth rate.

sufficient to saturate uptake (data not shown). Incubations were stopped by adding 1 mL of 50% TCA to the three additional “non-kill” aliquots. Samples were filtered onto 0.22- $\mu$ m nitrocellulose filters (Millipore) and rinsed twice with 1 mL of cold TCA. Filters were then placed in 7-mL scintillation vials, suspended in  $\sim$ 4 mL of scintillation cocktail, and counted on a scintillation counter (Coulter-Beckman). Samples for BA, nucleic acid content, and particulate C and P were sampled, preserved, and measured as described above.

We evaluated the relationship between growth and phosphorus using either SGR or a combination of SGR and BP as indicators of bacterial growth. Because of the experimental design of the microcosm experiments, i.e., because for the same community all treatments began with approximately the same population density, differences in BP were attributable to accumulated differences in SGR integrated over time. With samples from the natural environment, this was not the case, and BP and SGR represent two independent, although related, measures of bacterial growth. When possible, we tried to use the least derived response variable, i.e., we report the response variable closest to what was directly measured. Therefore, in the lake survey, in which growth was determined by dilution culture, we report SGR, whereas in the microcosm and mesocosm studies, in which tritiated leucine uptake was measured, we report BP as nmol leucine  $L^{-1} h^{-1}$ . In some instances, for comparison purposes, we calculate

SGR from BP by dividing by BA and converting to units C using literature-derived C values as noted above (e.g., Table 1).

All data were analyzed using either simple linear regression or multiple linear regression models unless otherwise stated. Variables that were not normally distributed were first log transformed and tested for normality before statistical analysis in order to meet the assumptions of linear regression. All statistical analyses were performed using JMP<sup>®</sup> statistical software.

## Results

In the 47 temperate lake ecosystems that we analyzed, biomass P:C and RNA:DNA were significantly and positively correlated (Fig. 1a), consistent with the GRH. However, RNA:DNA was not correlated with growth rate ( $p = 0.89$ ), and therefore we found no significant relationship between biomass P:C and growth rate ( $p = 0.95$ ), suggesting that other factors influence the relationship between growth and RNA:DNA in the natural environment. A multiple regression model including seven additional environmental variables (Chl *a*, geo-region, TDN, Secchi depth, temperature, SRP, and DOC) explained 84% of the variation in biomass P:C; however, only the effects of temperature and geographic region were significant ( $p < 0.0001$  for both). Temperature alone was able to explain 31% of the variation in P:C ratio ( $R^2 =$

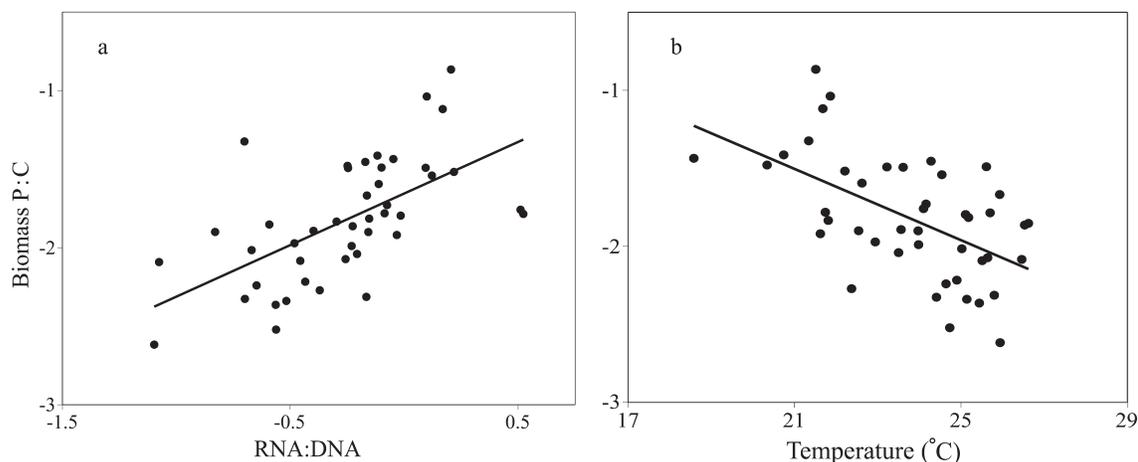


Fig. 1. The relationship between (a) bacterial biomass P:C and RNA:DNA for 47 lakes in the upper Midwest, U.S.A., and (b) bacterial biomass P:C and temperature. The complete regression equations for each are: (a) biomass P:C = 0.67 (RNA:DNA) - 1.67 ( $n = 47$ ,  $p < 0.0001$ ); and (b) biomass P:C = -0.11 temperature + 0.89 ( $n = 47$ ,  $p = 0.0001$ ).

Table 2. The reduced multiple regression model for the lake survey data. In a full factorial model including these predictors and their interaction terms, none of the interaction terms were significant. This model is the simplest model that explained the greatest amount of variation in P:C of the  $>1\text{-}\mu\text{m}$  biomass.  $R^2=0.72$ ,  $n=44$ ,  $df=39$ , LOG P:C is the response variable.\*

Predictor variable	Estimate	SE	F ratio	p value
Temperature	-0.081	0.018	9.37	0.004
RNA:DNA	0.242	0.111	4.11	0.04
Geo-region	0.181	0.035	9.27	<0.0001

\* RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

0.31,  $p < 0.0001$ ,  $n = 47$ ; Fig. 1b). There was no significant relationship between geographic region and any of the other variables in the model (full model  $p = 0.09$ ), indicating that geographic region provided information in addition to the environmental parameters that were measured. A sub-model that included only temperature, RNA:DNA ratio, and geographic region explained 72% of the variance in P:C biomass (Table 2). Therefore, in temperate lake ecosystems, whereas a significant fraction of cellular P is contained in RNA, temperature plays an important role in coupling P to growth, and regional differences (Fig. 2), attributable perhaps to differences in community composition, also affect P-growth relationships.

To more explicitly evaluate how temperature affected the relationship between biomass P and growth rate, we experimentally manipulated temperature and resources in 1-liter microcosms and grew filter-separated communities from two lakes in batch culture. In the microcosm experiments, RNA:DNA and biomass P:C were again significantly correlated; however, unlike in the field

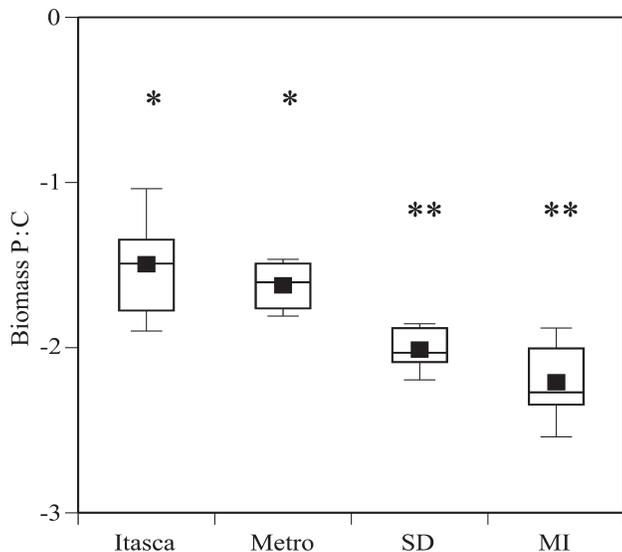


Fig. 2. The influence of geographic region on P:C. Sites with a different number of asterisks are significantly different from each other as determined by a least square means Tukey honestly significant differences (HSD) test. Geographic region alone had a significant effect on P:C ( $p < 0.0001$ ) as indicated by a full model ANOVA.

Table 3. Reduced model of the microcosm experiments explains 77% of the variance in BP when all microcosm experiments are combined. Note that although season was not a significant predictor of BP, the interactions between season and temperature and between season and biomass P:C were both highly significant ( $n=92$ ,  $df=86$ ).\*

Term	Estimate	SE	F ratio	p value
Temperature	0.01	0.01	2.07	0.15
RNA:DNA	0.41	0.11	14.69	0.0002
P:C	1.04	0.13	68.3	<0.0001
RNA:DNA×P:C	-1.62	0.32	25.55	<0.0001
Season×temperature	-0.03	0.01	61.07	<0.0001
Season×P:C	0.51	0.14	13.01	0.0005

\* RNA, ribonucleic acid; DNA, deoxyribonucleic acid; P, phosphorus; C, carbon.

observations, both RNA:DNA and biomass P:C were also significantly correlated with growth rate, although no single predictor had a high level of explanatory power (Table 1). A multiple regression model including all predictor variables (biomass P:C, RNA:DNA, temperature, lake, and season, with two-way interaction terms) explained 81% of the variation in BP across the complete temperature and resource landscape ( $p < 0.0001$ ,  $R^2 = 0.81$ ), whereas a sub-model including only temperature, RNA:DNA, biomass P:C, and interactions with season explained a similar amount of variation in BP ( $p < 0.0001$ ,  $R^2 = 0.77$ ; Table 3).

Season alone was not a significant predictor variable; however, interaction terms containing season were significant. Therefore, to evaluate how temperature influenced the relationship between bacterial growth and biomass P content without the confounding influence of seasonality, we separated experiments by season. In the summer, BP and biomass P were positively correlated at each incubation temperature, and the slope of the relationship between BP and biomass P increased with increasing temperature (Fig. 3), i.e., NUE increased with increasing temperature, consistent with our second hypothesis. For the winter communities BP and biomass P were again significantly and positively correlated; however, at each incubation temperature NUE decreased with increasing temperature (Fig. 3).

Both the significant effect of seasonality on BP (Table 3) and the change in the sign of the slope of the relationship between NUE and temperature in winter vs. summer (Fig. 3) indicated functional differences between summer and winter communities. However, our nutrient amendments were confounded with season (i.e., winter experiments received more P than summer experiments); therefore, to address this we plotted the total amount of P consumed (measured as the difference between initial and final SRP in each microcosm) vs. BP for each season × lake community. This allowed us to evaluate whether differences in BP between seasons were attributable to insufficient P amendments in any of the treatments or whether all treatments received P in excess. For each community at each incubation temperature, the community with the lower BP was consistently “saturated” with respect to P,

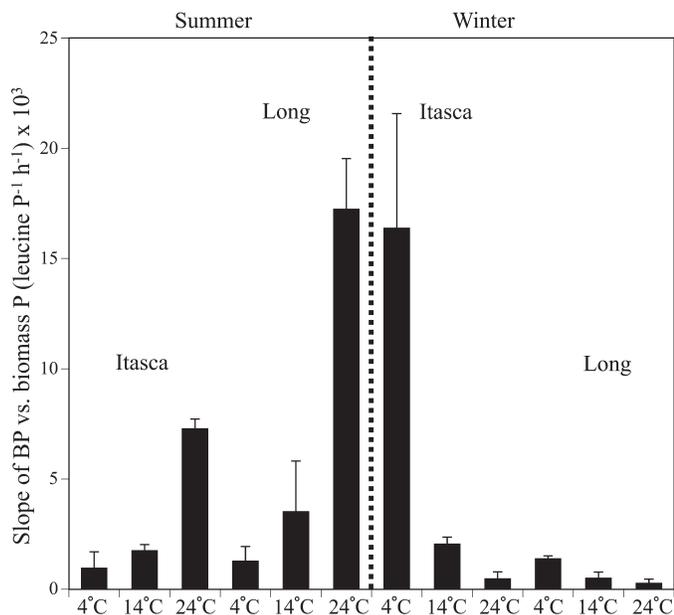


Fig. 3. The slope of BP vs. biomass P for bacterial communities from each lake during each season. Experimental microcosm temperatures are listed on the x axis while each lake is listed above the set of corresponding bars. Error bars are  $\pm 1$  SE of the estimated slope.

indicating that P amendments were in excess (Fig. 4). BP per unit P consumed was higher for the winter communities than the summer communities in the 4°C and 14°C incubations, whereas at 24°C the summer communities had higher BP per unit P consumed than the winter communities (Fig. 4). In general, bacterial communities had higher BP per unit P consumed at temperatures closer to their in situ temperatures, again suggesting that they were acclimated or adapted to in situ temperature.

The microcosm systems were by design highly simplified ecosystems, e.g., absence of predation and interactions with

autotrophs. Therefore, we evaluated the relationship between biomass P and BP in larger mesocosms with more complex planktonic communities (Lennon et al. 2003) at three times of the year when the ambient water temperature corresponded to our experimental microcosm temperatures. In the mesocosms the slopes of the relationship between BP and biomass P were again positively correlated at each sampling date, with different slopes at each temperature, although the relationship was significant only at 24°C (Fig. 5). Perhaps more interesting, though, is that the slopes of the BP vs. P biomass relationship were an order of magnitude lower in the mesocosm communities than in the microcosm communities. So although BP increased with increasing biomass P at all three temperatures, the mesocosm communities had consistently lower NUE than did the microcosm communities (Fig. 5), indicating that vertical and horizontal community dynamics might be an important factor in determining NUE.

## Discussion

In the 47 temperate lakes we evaluated, we found a strong relationship between bacterial P:C content and the RNA:DNA ratio (Fig. 1a). An obvious factor that influences the RNA:DNA ratio is growth rate, and previous studies have demonstrated the relationship between RNA:DNA and growth rate in bacteria both in monoculture and in mixed culture (Kemp et al. 1993; Makino and Cotner 2003). However, when multiple bacterial communities were combined in a single analysis, we found no evidence of coupling between growth and RNA:DNA or between growth and biomass P:C. Although multiple environmental parameters might affect the relationship between growth and RNA content, the pronounced effect of temperature on biomass P:C that we observed suggested that in temperate lake ecosystems temperature plays an important role in P-growth couplings.

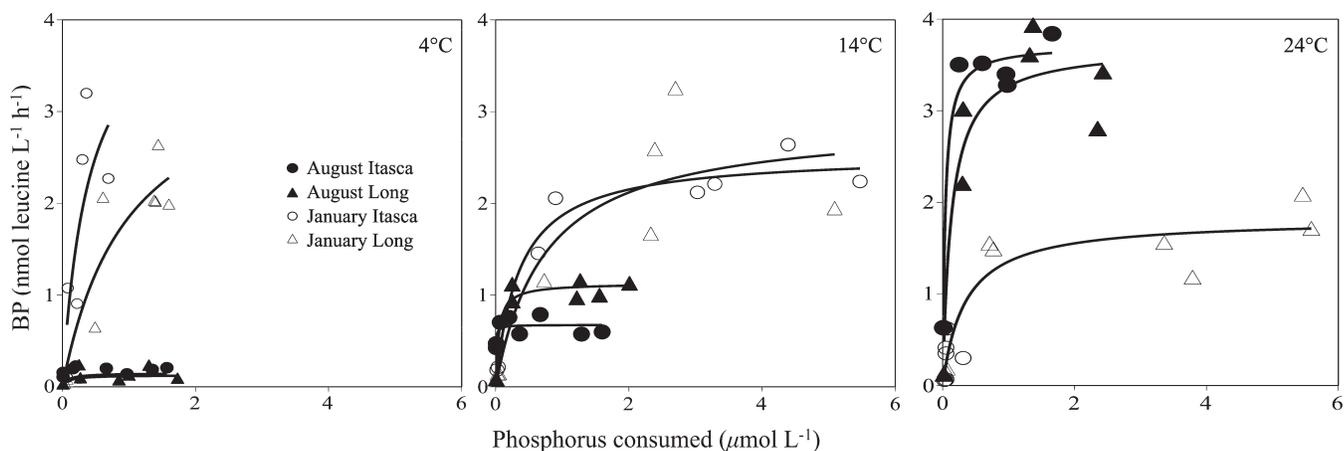


Fig. 4. BP vs. P consumed for winter and summer communities from each lake at the three experimental temperatures. At 4°C and 14°C the winter communities from each lake have higher BP for a given amount of P consumed than those sampled in summer. At 24°C the summer communities from each lake have higher BP per unit P consumed than the communities sampled in winter. For Lake Itasca in January there are no data points for the greater level of phosphorus consumed, and therefore only a single regression line is plotted for both lakes.

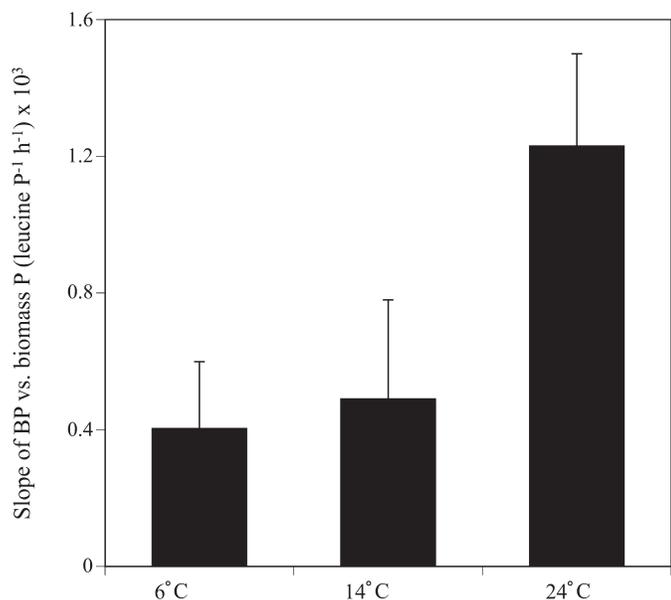
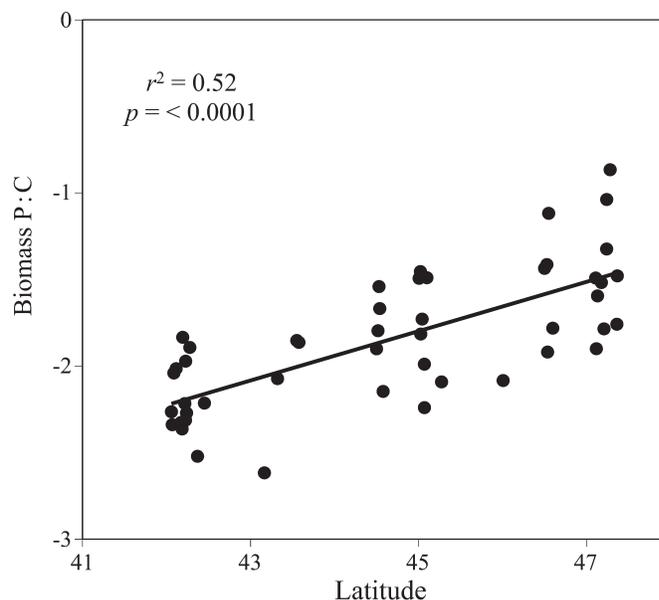


Fig. 5. The slope of the relationship between BP and biomass P for each of the three sampling dates for the experimental mesocosms. The full regression equation for each sampling date and corresponding in situ temperature are: for 6°C,  $BP = 0.40 \times \text{biomass P} - 0.58$  ( $n = 15$ ,  $p = 0.06$ ,  $R^2 = 0.25$ ); for 14°C,  $BP = 0.50 \times \text{biomass P} - 0.51$  ( $n = 15$ ,  $p = 0.11$ ,  $R^2 = 0.18$ ); and for 24°C,  $BP = 1.23 \times \text{biomass P} - 0.29$  ( $n = 11$ ,  $p = 0.0014$ ,  $R^2 = 0.70$ ). All data were log transformed prior to analysis.

For the two summertime communities (Long Lake and Lake Itasca) where we experimentally manipulated temperature, NUE, measured as the slope of the relationship between BP and biomass P, increased with increasing temperature as hypothesized (Fig. 3). We interpret this result as the direct effect of temperature on the kinetic energy of ribosome-driven protein synthesis (i.e., similar growth rates can be achieved with fewer ribosomes if the kinetic energy of the system is higher). Such changes in NUE with temperature could in part mask a clear relationship between RNA and growth rate. In the mesocosm study, we also observed increasing mean NUE with increasing seasonal temperature (Fig. 5). Although the NUE in the mesocosm study was consistently lower relative to the microcosm study, these results suggest that temperature exerts significant influence on NUE even in environments with more complex community dynamics.

Increasing NUE with increasing temperature has also been reported in terrestrial autotrophs. For example, a simulation model of a terrestrial tussock community found that increasing temperature selected for high NUE organisms even when increased mineralization of N, the limiting nutrient, was accounted for (Herbert et al. 1999). Similarly, broad biogeographical patterns of biomass stoichiometry show that both N and P content of tree leaves decreases with warmer mean annual temperatures across a latitudinal gradient, in part because of changes in temperature-dependent physiology of the organisms (Reich and Oleksyn 2004). In our lake survey, additional variation in bacterial biomass P:C was explained by geographic region. Although geographic region did not correlate with



acclimated to in situ temperature, primarily through their resource use efficiency.

Other studies that address how temperature and resources control bacterial growth have proposed a threshold temperature (e.g., 12°C), below which growth is controlled by temperature and above which it is controlled by resources (Felip et al. 1996). The prevalence of resource limitation at warmer temperature is consistent with the selection for organisms that increase NUE with increasing temperature when resource limitation is common. However, Felip et al. (1996) found no evidence for temperature adaptation or acclimation by winter bacterial communities, i.e., winter communities were consistently temperature-limited even in the presence of resource amendments. This may in part be because of the short length of their incubations. Experiments with winter communities were incubated for 60 h, at which point we also had not seen a metabolic response to resource amendments. Instead, winter communities at the coldest temperature in our study took more than twice as long to demonstrate a pronounced metabolic response to resource amendments (~150 h; table 1 in Hall and Cotner 2007).

Multiple other studies have also found a threshold effect of temperature limitation, with the “threshold temperature” ranging from 6°C to 14°C (reviewed in Felip et al. 1996). These differences in temperature threshold suggest differences in the level of community adaptation or acclimation of in situ temperature and support our contention that thermal adaptation or acclimation is a function of in situ temperature as well as the annual range and frequency distribution of the in situ temperature in their environment. For example, we saw a significant interaction between lake and temperature in a full model predicting biomass P:C (lake  $\times$  temperature,  $p = 0.05$ ), indicating that communities from distinct lakes responded differently to temperature, most likely because of differences in the thermal environment of each lake, i.e., Long Lake is deeper and on average colder than Lake Itasca. Further evidence supporting bacterial community adaptation or acclimation to in situ temperature can be found in marine systems in which differences in bacterial community metabolism to changes in incubation temperature have also been shown to correlate to in situ temperature when communities were sampled along spatial (i.e., depth and latitudinal) temperature gradients (Fuhrman and Azam 1983; Simon et al. 1999).

These functional changes in bacterial community metabolism over temporal and spatial temperature gradients are supported by emerging evidence of seasonally patterned changes in bacterial community composition. Multiple studies show that temperature is an important environmental predictor of seasonal community composition in freshwater, both lentic (Shade et al. 2007) and lotic (Crump and Hobbie 2005; Hullar et al. 2006), and marine (Fuhrman et al. 2006) ecosystems. Our data suggest that these seasonal taxonomic differences might be because of selection for bacterial communities that maximize NUE near the in situ temperature. In ecosystems where P limitation is prevalent (Carlsson and Caron 2001; Graneli et al. 2004; Smith and Prairie 2004), it is perhaps not surprising that selection pressure for temperature-depend-

ent P use efficiency might be an important determinant of community composition.

The strong influence of season and the subtler but significant influence of habitat on community metabolic response to temperature, and community composition, suggests that changes in bacterially mediated biogeochemical cycling in aquatic ecosystems because of increasing global temperatures depend on the level of acclimation or adaptation of each bacterial community to the local temperature regime. Therefore, in the context of global change it is also important to understand how increased thermal forcing because of climate change will influence the thermal habitat of temperate lakes.

Temperate lake environments provide a special habitat with respect to temperature. Although most organisms at temperate latitudes experience temperatures in the high 20°C to mid-30°C range during the mid-late summer months, organisms in the hypolimnion of lakes routinely experience temperatures between 4°C and 8°C year round. Thus lakes are thermally unique ecosystems in that they provide consistent cold refugia, where psychrotrophic organisms can maintain metabolic activity and are not competitively excluded, even during the warmest months of the year. As climate warms, physical models suggest temperate lakes will become more strongly stratified and experience warmer epilimnetic temperatures for extended periods of time. This projection has been supported by the effects of a recent European heat wave on two Swiss lakes (Jankowski et al. 2006). Specific to the geographic region studied here, effects of atmospheric warming on lake surface temperatures suggest that in inland lakes in the upper Midwest surface temperatures will increase somewhere in the range of 1–7°C in the next 50–100 yr (Hondzo and Stefan 1991, 1993; Stefan et al. 1996). Effects on hypolimnetic temperatures are harder to predict because of increased variability in the onset of stratification in dimictic lakes. Despite this variability, it is estimated that the mean volume-integrated temperature for lakes in this region will increase by a few degrees (Hondzo and Stefan 1991, 1993; Stefan et al. 1996). Globally, lakes are experiencing decreasing annual ice cover (Magnuson et al. 2000), so over annual periods, temperate lakes will also receive increased solar radiation to the water column. Therefore, in addition to the direct effect of temperature on the lake environment, extended periods of warmer temperatures and stratification because of increased solar inputs can be expected to decrease transfer of mineral nutrients from the hypolimnion to the epilimnion, ultimately prolonging exposure of bacterial communities to warmer epilimnetic temperatures and lower nutrient availability.

Our results suggest that these changes in the physical and chemical environment of temperate lakes should lead to selection for organisms with high NUE. If increasing NUE with increasing temperature, as we saw in the summer communities (Figs. 3, 4), results in decreasing biomass P:C of the bacterioplankton community, as we saw in the lake survey (Fig. 1b), ecological theory suggests that this would allow bacterial communities to become superior competitors for P (Hall et al. 2008), resulting in shifting bacterial community composition and perhaps in changes in the

relative biomass between heterotrophic prokaryotes and other members of the plankton, e.g., algae. Recent empirical work in the Mediterranean Sea projects that C flow through the bacterial pool will increase with increasing temperature, resulting in increasing C flux to the atmosphere because of a maintenance of low bacterial growth efficiency (Vazquez-Dominguez et al. 2007). The magnitude of such an effect is at least partially dependent on whether or not increasing temperatures are accompanied by increasing NUE of the bacterioplankton as we saw with summertime communities.

In conclusion, our results point to two important implications with respect to how temperature affects bacterially mediated biogeochemistry. First, warm-adapted or warm-acclimated bacterial communities respond to increasing temperatures, in a similar manner to that of other organisms, by increasing NUE, most likely because of an increase in biochemical kinetics that accompanies increasing temperatures. Second, and perhaps the more important result with respect to interpreting the effects of global warming on natural bacterial community metabolism, we saw a relatively rapid, i.e., seasonal, acclimation or adaptation of the bacterial community to in situ temperature. Such an adaptation of bacterial communities to local thermal regimes, in time and space, is consistent with seasonally changing community composition and would explain why an unambiguous response of bacterial metabolism to temperature when multiple bacterial communities are considered in a single analysis is difficult to elucidate, as seen in our lake survey and reported elsewhere (Rivkin et al. 1996; Del Giorgio and Cole 2000). The ability of bacterial communities to functionally adapt or acclimate to such a broad range of in situ temperatures on intra-annual timescales is one example in which, because of high genotypic and phenotypic diversity and rapid generation times, microbial ecological theory should differ from "macroalgal" ecological theory. In the context of global climate change, this difference has important implications for how predictive models account for shifts of bacterially mediated global element cycling. Therefore, in order to better understand how temperature forcing will affect bacterially mediated biogeochemical cycles, studies should focus on how changing thermal regimes will shift relative temperature acclimation or adaptation of bacterial communities and how that in turn will affect microbial dynamics, especially NUE. Ignoring adaptation to the local temperature environment and assuming a similar response of bacterial metabolism to changing temperatures for a range of habitats will result in broad generalizations between temperature and bacterial metabolism, with very low spatial and temporal resolution, and therefore generally poor predictability for global models.

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