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Temperature and nutrient supply interact to control nitrogen fixation in oligotrophic streams: An experimental examination

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Abstract

We performed two experiments to examine how temperature and nutrients interact to control dinitrogen (N_2) fixation, chlorophyll *a* (Chl *a*) biomass, and community composition of periphyton in subalpine oligotrophic streams in the Sawtooth Mountains of Idaho. We grew periphyton on nutrient-diffusing substrata (NDS) in a cold lake inlet (7°C) and a warm lake outlet (18°C). We then switched substrata between the two stream sites to test the effect of incubation temperature on N_2-fixation rates. Periphyton on substrata grown at both sites exhibited greater N_2-fixation rates when incubated in the warm outlet, which indicates physiologic temperature control. Periphyton on P-enriched NDS grown in the warm outlet had the greatest N_2-fixation rates, largest Chl *a* biomass, and largest percentage of N_2-fixing taxa of any treatment, which indicates that temperature and P interact to influence the community. In the second experiment, colonized rocks and uncolonized NDS were placed in cold (13°C) and warm (18°C) mesocosms. Within 2 days, warm temperature stimulated N_2 fixation by the rock periphyton community two times above cold temperatures, which indicates physiologic temperature control. After 45 days, warm temperatures and P enrichment led to *Anabaena* sp. in the periphyton community and the greatest rates of N_2 fixation observed in the experiment, which also indicates temperature and nutrient control at the community level. This study indicates that N_2 fixation and periphyton community composition in oligotrophic streams are controlled by both temperature and P supply, with temperature modulating the response to P.

A dominant paradigm of aquatic ecology is that phosphorus (P) most often limits algal growth and production in freshwater ecosystems (Schindler 1977). This paradigm is rooted in the hypothesis that dinitrogen (N_2) fixation by cyanobacteria should contribute sufficient nitrogen (N) to aquatic ecosystems, which, thus, perpetuates P limitation (Redfield 1958; Schindler 1977). However, other studies have indicated that growth of stream algal communities is as frequently limited by N as by P, and many streams are colimited by N and P (Francoeur 2001), which indicates that some factors must be limiting N_2 fixation and maintaining N limitation in some streams.

N_2-fixation rates in streams are potentially controlled by availability of macronutrients and micronutrients. N additions have been widely demonstrated to suppress N_2 fixation by cyanobacteria because N_2 fixation is a costly process that will not occur when cyanobacteria have access to environmental N (Howarth et al. 1988). Stimulation of N_2 fixation by P additions has been demonstrated in many marine and lake systems (Howarth et al. 1988). Cyanobacterial N_2 fixation can also be limited by trace metals such as iron or molybdenum (Wurtsbaugh and Horne 1983; Howarth et al. 1988). No study has directly examined the effects of nutrients on periphyton N_2-fixation rates in streams but several have identified responses of cyanobacteria abundance to nutrient availability. For example, cyanobacteria or diatoms with N_2-fixing endosymbionts appear to be at a competitive disadvantage compared with other algae, and, thus, have lower abundances at high stream N concentrations in both experimental enrichments (Peterson and Grimm 1992) and under natural conditions (Henry and Fisher 2003).

Temperature can control N_2-fixation rates in a variety of settings by affecting enzymatic activity and, in turn, the physiologic ability of cells to fix N_2 (DeNicola 1996). Reuter et al. (1983) found that N_2-fixation by benthic periphyton from an oligotrophic lake increased linearly between 5°C and 25°C in short-term laboratory measurements. More recently, Staal et al. (2003) showed that N_2-fixation rates of four marine cyanobacteria increased linearly as temperatures increased from 10°C to 35°C in laboratory cultures.

Temperature also has profound influences on periphyton community composition. Cairns (1956) demonstrated that algal communities from a nonpolluted temperate stream shifted from dominance by diatoms at 20°C, to green algae between 30°C and 35°C, and finally to cyanobacteria at temperatures above 35°C. In experimental streams, Wilde and Tilly (1981) found that the cyanobacteria *Schizothrix*

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sp. dominated at temperatures higher than 30°C, and when experimental temperatures were decreased by 12.5°C, *Schizothrix* sp. were replaced by a yellow-green algae within 2 months (Wilde 1982). Collectively, these studies indicate that temperature could control N_2 fixation in streams at the community level.

Nitrogen cycling is currently a pervasive focus of stream ecology (e.g., Peterson et al. 2001), and much work in the field of stream biogeochemistry has recently focused on quantifying denitrification, which represents a potential loss of N (Seitzinger 1988). Yet, we know very little about how N_2 fixation functions in stream systems, and this process rarely has been measured in streams. However, the few studies that have measured N_2 fixation in streams indicate that rates by cyanobacteria may be high (Horne and Carmiggelt 1975; Grimm and Petrone 1997). Additionally, N_2 fixation in oligotrophic aquatic systems has been largely unstudied; available measurements have been focused almost entirely on lakes (Loeb and Reuter 1981).

Understanding how temperature and nutrient supply affect N_2-fixation rates in streams is particularly important because global climate change may lead to increased surface-water temperatures, and anthropogenic disturbances alter watershed nutrient loads. The goal of our study was to examine the impacts of nutrient supply and temperature on N_2 fixation, periphyton biomass, and community composition in oligotrophic streams. We investigated whether N_2-fixation rates were controlled by physiologic stimulation of existing periphyton communities or by community-level shifts that favor N_2-fixing cyanobacteria. Furthermore, we wanted to examine how temperature interacted with the supply of N and P to control N_2-fixation rates. To answer these questions, we used two different experimental approaches. First, we grew periphyton on nutrient-diffusing substrata (NDS) under two different temperature regimes in a cold lake inlet and a warm lake outlet, and then we performed a reciprocal-transplant experiment to test the effects of short-term changes in incubation temperature on N_2-fixation rates. The second experiment tested the effects of temperature and nutrients on N_2-fixation rates of a periphyton community in a streamside mesocosm experiment that lasted 45 d. Together, the two studies were designed to illuminate the interacting effects of temperature and nutrients on stream periphyton at both the physiologic and community levels.

Methods

Study area—This study was conducted in the Sawtooth Lake–Stream District, located in central Idaho in the Sawtooth National Recreation Area and Boise National Forest. This area is a granitic, glaciated landscape with a high density of lakes separated by average stream lengths of 2.8 km. Very little development has occurred in the area, and land use is predominately outdoor recreation and wilderness, with cattle grazing in the valley bottoms. Streams in the northwestern part of the lake–stream district drain to the Payette River, and those in the southern part drain to the Salmon River. The annual hydrograph in these high-elevation (ca. 2000 m) watersheds is dominated by a spring snowmelt runoff pulse that peaks in early June, followed by a prolonged base-flow period that begins in middle to late July. Lakes are typically covered by ice from mid-November through late May or early June. The waters in this area have very low conductivity (20–100 μS cm^{-1}) and low alkalinity and are relatively pristine (Emmett 1975), with low atmospheric N deposition (ca. 130 kg N km^{-2} yr^{-1}, NADP site ID15). Stream nitrate concentrations range from approximately 10 μg N L^{-1} during base flow to 35–40 μg N L^{-1} during spring runoff; phosphate and ammonium are frequently below analytical detection limits. Nutrient-limitation bioassays in both lakes and streams indicated colimitation of algal communities by N and P (Wurtsbaugh et al. 1997; Marcarelli unpubl. data). A bioassay experiment conducted at 19 sites showed that N_2-fixation rates can be high in streams, and N_2 fixation is sometimes limited by P. No difference in N_2-fixation response between N and N+P treatments was observed (Marcarelli unpubl. data), and we consequently chose not to use N+P combination treatments in the current study.

This study was based in two locations within the lake–stream district. The reciprocal transplant experiment was conducted at Warm Spring Creek, a second-order stream that flows through Bull Trout Lake and eventually drains to the Payette River. The inlet site was located approximately 300 m above the lake (UTM Zone 11, 639259 Easting, 4906167 Northing), and the outlet site was approximately 1,400 m below the lake (UTM Zone 11, 638171 Easting, 4907320 Northing). The inlet site has a shallower slope, faster flow, cooler temperatures, and higher concentrations of nitrate than does the outlet site during base flow (Table 1).

The streamside mesocosm experiment was conducted with water from the Salmon River at the Sawtooth Fish Hatchery outside Stanley, Idaho, 50 km SE of Bull Trout Lake (UTM Zone 11, 669493 Easting, 4890293 Northing). The Salmon is a fifth-order stream that had a mean discharge of 7.4 m^3 s^{-1} during the experiment. The site was selected because of proximity to electricity and water sources to run the experiment. Rocks for the streamside experiment were collected from Stanley Creek, a lake outlet that is a tributary of the Salmon River in close proximity to the experiment site (UTM Zone 11, 658581 Easting, 4902106 Northing). Previous sampling showed that the periphyton community in Stanley Creek fixed N_2. Rocks from Warm Spring Creek were not possible to use because Spring Creek is located in a different drainage basin than the Salmon River, and interbasin transfers of biological material are not permitted by the Sawtooth Fish Hatchery.

Reciprocal-transplant experiment—The experiment was conducted between 19 July and 26 August 2004. NDS were constructed from 35-mL polyethylene canisters and lids with holes, on the basis of the design by Gibeau and Miller (1989). The canisters were filled with nutrient-enriched agar, and a 2.5-cm fritted-glass disk was placed on top of the agar to serve as a site for periphyton growth. Three nutrient treatments—control, N-enriched, and P-enriched—were organized into blocks that contained two

Table 1. Environmental variables in the inlet and outlet stream of Bull Trout Lake during the reciprocal-transplant experiment.

Factor	Inlet	Outlet
Mean temperature and range (C)	7.1 (3.5–12.5)	18.0 (12.5–23.5)
Mean daily discharge ($m^3\ s^{-1}$)	0.18	0.21
Median sediment size (D_{50}, mm)	10.5	10.0
Stream gradient (%)	0.25	0.74
Width : depth ratio	4.44	9.46
Mean water velocity ($m\ s^{-1}$)	0.58	0.27
Nitrate–nitrogen ($\mu g\ L^{-1}$)	4.7–5.1	2.2–3.8
Total dissolved nitrogen ($\mu g\ L^{-1}$)	26–30	64–89
Total nitrogen ($\mu g\ L^{-1}$)	32–38	86–97
Phosphate–phosphorus* ($\mu g\ L^{-1}$)	<2	<2
Total dissolved phosphorus† ($\mu g\ L^{-1}$)	<1.9–2.3	1.9–2.6
Total phosphorus ($\mu g\ L^{-1}$)	2.1–2.7	3.6–4.7
Light exposure (%)	95	100

* Detection limit for phosphate–phosphorus was 2 μg PO_3–P L^{-1}; all samples were below detection limit.

† Detection limit for total-dissolved phosphorus was 1.9 μg P L^{-1}; concentrations started the experiment at 2.3 and decreased to below detection limit by the end of the experiment.

Discharge, sediment size, stream gradient, and width : depth ratio data are from C. Arp (unpubl. data). Mean flow is the average of five measurements taken at each site in mid-August. Nutrient concentrations are ranges observed for the three measurements taken at the start, middle, and end of the experiment and varied little for the duration of the experiment. Light values were measured by use of a Solar Pathfinder (Solar Pathfinder Corp., Linden Tennessee) and represent the percent exposure of each stream site throughout the experiment.

replicates of each treatment. N and P were added to the agar as either 0.8 mol L^{-1} $NaNO_3$ or 0.05 mol L^{-1} KH_2PO_4, respectively. Five blocks of NDS were placed in both the inlet and the outlet site between 15-cm and 22-cm depth (Fig. 1). No canopy was present at either site, and NDS were installed near the middle of the thalweg where bank shading was minimized. After 16 d, the agar canisters below the fritted disks were replaced, as nutrients are mostly depleted after 23 d of exposure (Fairchild et al. 1985). Release rates of nutrients from the NDS change as nutrients are depleted, and we estimated the following release rates on day 1 and day 16 of substrate exposure by use of the equations given by Fairchild et al. (1985) and corrected for our smaller diffusion area: N day 1, 229 $\mu g\ h^{-1}$, N day 16, 74 $\mu g\ h^{-1}$; P day 1, 15 $\mu g\ h^{-1}$, P day 16, 8.3 $\mu g\ h^{-1}$. These releases are high compared with the nutrient concentrations in our study streams (Table 1), but determination of how quickly these nutrients are diluted at the substrate surface by stream flow and, therefore, how much is available for periphyton uptake is difficult. Communities on the substrata were grown for 37 d before manipulation. Daily temperatures were measured with HOBOTEMP data loggers at 90-min intervals for the duration of the experiment. Water samples for dissolved and total-nutrient analyses were collected on days 0, 16, and 38 of the experiment.

On day 37, one NDS of each nutrient treatment from each block at each growth site was transplanted from the inlet to the outlet, and vice versa. This transplantation resulted in an experimental design with three factors of interest: nutrient, growth site, and incubation site. Incubation site was manipulated to examine short-term physiologic temperature effects on N_2 fixation, and growth site was manipulated to look at long-term community-level temperature effects. The transplanted periphyton communities equilibrated to the incubation site characteristics for 18 h. This period was chosen to allow physiologic responses to temperature, while community-level adjustments to incubation-site conditions were minimized. Colonization rates reported by Korte and Blinn (1983) indicated that newly colonizing diatoms could have accounted for only 0.6–5.6% of the periphyton community after 18 h. After the equilibration period, N_2 fixation, chlorophyll (Chl) *a* biomass, and community composition were measured as described below.

Streamside experiment—This experiment was conducted from 3 August to 17 September 2004. Nine mesocosms were constructed from 99 cm × 67 cm × 25 cm polyethylene containers that were filled to a depth of 20 cm and contained 113 liters. Water for the experiment was pumped from the Salmon River by use of a centrifugal pump and filtered to remove coarse particulate organic matter and macroinvertebrates, while adequate water flow to the

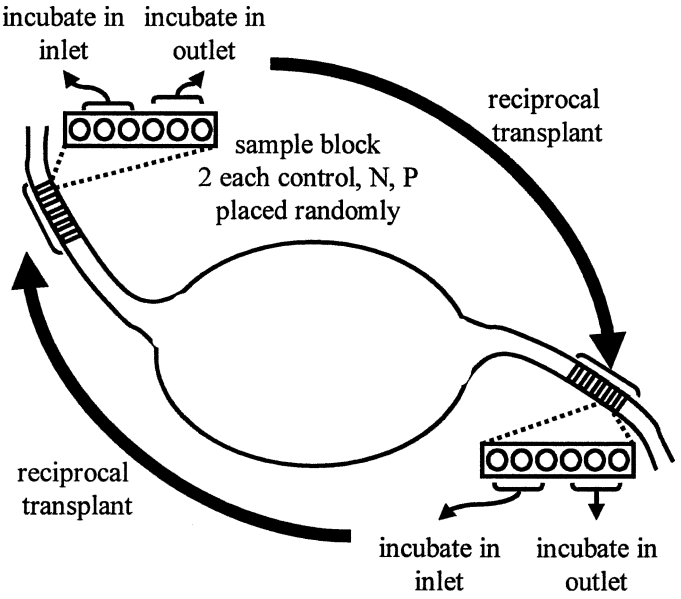

Fig. 1. Explanation of the reciprocal-transplant experiment. Substrata enriched with nitrogen (N), or phosphorus (P) and the control were arranged in 5 blocks that contained two of each nutrient treatment and grown for 37 d in the inlet (7°C) and outlet (18°C) streams. After 37 d, a reciprocal transplant was performed in which half of the substrata from each site were switched to the other study stream and equilibrated for 18 h to the new stream site, and nitrogen fixation was measured.

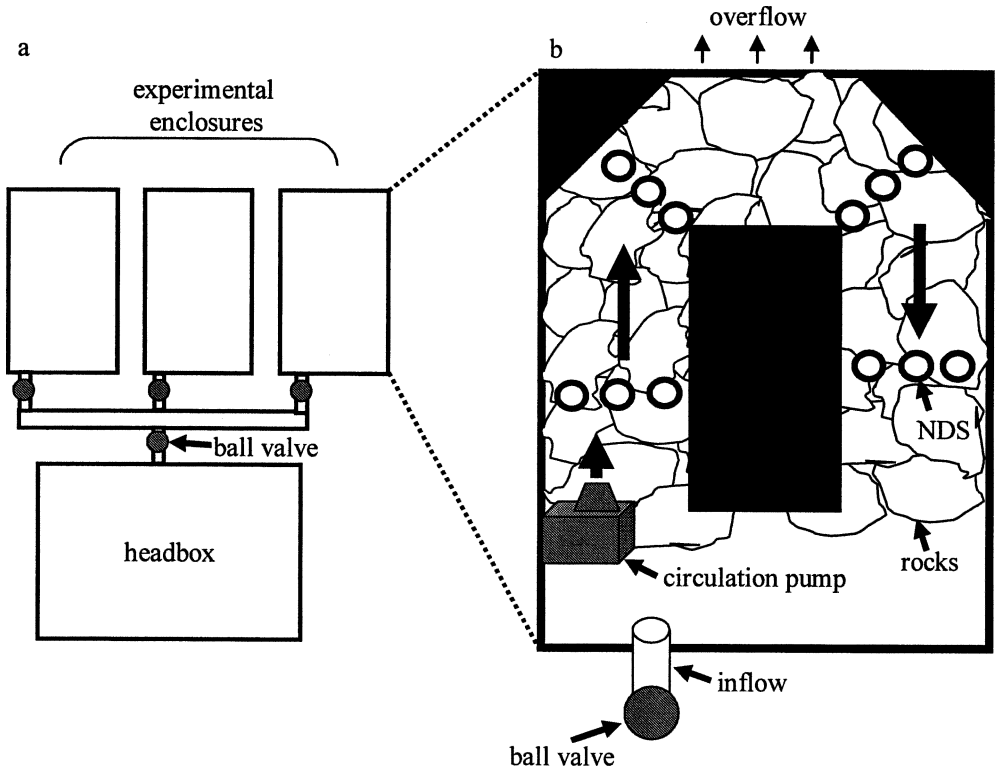

Fig. 2. Layout of the streamside temperature experiment. (a) This diagram represents one temperature manipulation unit; this setup was repeated three times to include 9 experimental enclosures in the full experiment. (b) Setup of one experimental enclosure that shows the layout of rock substrata and nutrient-diffusing substrata (NDS). Black areas were blocked off and had no water flowing through them. Water velocity in the enclosures was maintained at 0.35 m s^{-1}.

enclosures was maintained. We chose to eliminate macroinvertebrates for this experiment to facilitate colonization of NDS by algae and to minimize selective-grazing effects of macroinvertebrates on periphyton communities (Rosemond et al. 1993). The water flowed into three 270-liter plastic head boxes, where temperature manipulations occurred. The temperature in the cold head boxes were maintained with an in-line chiller. The warm head box was equipped with titanium aquarium heaters. Gravity flow of water from each head box into three experiment enclosures was regulated at 1.5 L min^{-1} by ball valves (Fig. 2). This flow replaced the volume in the experimental enclosures 19 times d^{-1}; water left the enclosures via overflow holes at the opposite end of the enclosure from the inlet. Aquarium pumps maintained water velocity within each experimental enclosure at 0.35 m s^{-1}, which was similar to the velocity in the study streams. Screens made of 30% shade cloth were placed on each experimental enclosure.

Two temperature regimes were used in the experiment: cold (12.9°C, $n = 6$) and warm (17.5°C; $n = 3$) (Fig. 3). Temperature in each enclosure was measured at 1-min intervals by HOBOTEMP data loggers. The temperature separation achieved between the cold and warm treatments was good, and the two treatments were in the normal range of temperatures observed in lake inlets and outlets in the Sawtooth Lake–Stream District (Fig. 3).

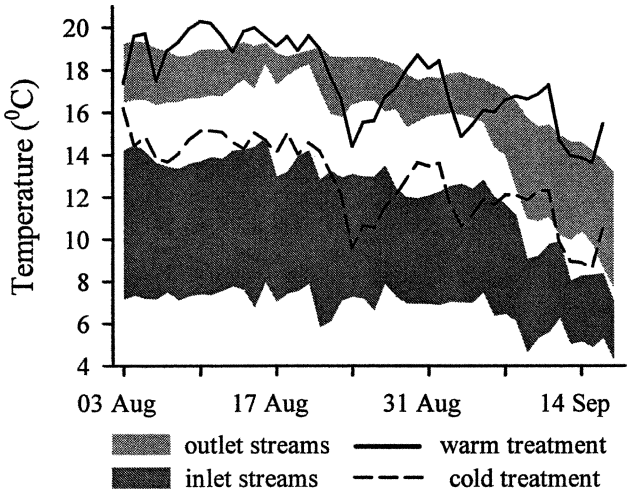

Fig. 3. Daily mean temperatures in the cold and warm treatments of the streamside mesocosm experiment, compared with temperatures measured in seven inlet and six outlet streams. Note that the streamside experiments were conducted in 2004, but the temperatures were measured in the streams in 2003, which may account for the difference between the experimental temperatures and the stream temperatures in early September. However, temperature data from streams were unavailable from 2004, so we were unable to make a better comparison.

Both natural rock substrata and NDS (as described above) were placed inside each experimental enclosure. This design allowed examination of how temperature affects periphyton communities growing on natural substrata, as well as the potential interacting effects of nutrients and temperature on N_2 fixation and periphyton in colonizing communities. We filled 280-liter coolers with rocks and stream water at Stanley Creek and allowed them to stand for approximately 4 h in the dark to asphyxiate macroinvertebrates, which were then removed by hand from each rock. Rocks were placed randomly into the enclosures until the bottom was covered with an even layer. Four sets of NDS identical to those used in the reciprocal-transplant experiment were nestled within the rocks in each enclosure (Fig. 2). This procedure resulted in four subsamples for each of three nutrient treatments in each enclosure, as above: control, N, and P. Dissolved-nutrient and total-nutrient water samples were collected from each enclosure on days 0, 25, and 45 to monitor potential nutrient enrichment of the enclosures by nutrients released from the NDS.

One NDS subsample of each nutrient treatment from each enclosure was collected on experiment days 15, 25, 35, and 45. On experiment days 0, 2, 15, 25, 35, and 45, two periphyton samples from rocks within each enclosure were collected by use of a soft brush and a 9.8-cm^2 plastic frame. The day 0 and day 2 samples were collected to determine short-term physiologic responses by the rock communities to temperature, whereas the day 15, 25, 35, and 45 samples were to look at community-level responses. Slurry samples and NDS were analyzed as described below.

Analytical methods—N_2 fixation was measured by use of an acetylene-reduction assay (Flett et al. 1976). Glass disks were placed into 60-mL centrifuge tubes with 45-mL of stream water and sealed. Periphyton slurry samples were placed into 62-mL glass serum vials and sealed. Tubes and vials were injected with 4 mL of acetylene gas generated from calcium carbide to achieve a headspace of approximately 20% acetylene gas, shaken for 1 min to assure equal partitioning of acetylene between the liquid and gas phase (Flett et al. 1976), and incubated in situ. Standards (in both tubes and vials) that contained known concentrations of ethylene and blanks to control for nonbiological production of ethylene were also run in the field. After 2 h, samples and standards were again shaken for 1 min, and gas samples were collected in cleaned, reevacuated, 3-mL Vacutainer serum vials and returned to the lab. Ethylene and acetylene in each sample were measured within a few weeks by use of a SRI 8610C gas chromatograph equipped with a Poropak T column and a flame-ionization detector. Concentrations of ethylene in the samples were compared with the known concentrations in the standards and then converted to the amount of N_2 fixed by use of an assumed 3:1 ethylene:N_2 conversion ratio (Capone 1993).

We chose to estimate periphyton biomass as Chl *a*, despite the fact that pigment concentrations can vary depending on light, temperature, and species composition of the algal assemblage, because Chl *a* is generally correlated with algal biomass and is easier to measure (Wetzel and Likens 2000). Community composition was also analyzed on each glass disk. Subsamples (~10%) of the community growing on the substrate were preserved for taxonomic analysis by scraping of a 2-mm-wide metal probe down the center of each disk, and then suspension of the scraped algae in deionized water and preservation with Lugol's solution. After the scraping, the glass disk was frozen for Chl *a* biomass analysis, which occurred within 1–2 weeks. For periphyton slurries, a subsample of each slurry was filtered through a 25-mm GF/F filter and frozen for Chl *a* biomass. A second subsample of each slurry was preserved as above for taxonomic analyses.

To measure Chl *a*, frozen disks and filters were extracted in 95% ethanol overnight, and Chl *a* in the extract was measured by application of a nonacidification method (Welschmeyer 1994) that utilized a Turner 10-AU fluorometer. Relative community composition was determined by settling subsamples of the community sample in Utermöhl settling chambers, and then enumerating cells by use of an Olympus inverted microscope at 1,000X. A total of 1,000 cells were classified to genus (species where possible) by application of the method of either Wehr and Sheath (2003) or Prescott (1978). Species identification of the order Rhopalodiales was done by R. Lowe at Bowling Green State University. On all substrata at all sites, a large portion of the periphyton community was composed of small, unicellular picobacteria. This taxon showed no difference between nutrient treatments, and its trophic status was unclear. Because we were uncertain whether these picobacteria were heterotrophic, and because inclusion of this taxon in our analysis masked important changes in N_2-fixing taxa, we chose to exclude it from our community analyses.

Nitrate (NO_3) and phosphate (PO_4) were analyzed by use of ion chromatography on a Dionex DX500. Total nitrogen (TN), total phosphorus (TP), total-dissolved nitrogen (TDN), and total-dissolved phosphorus (TDP) were analyzed by use of persulfate digestion, followed by a spectroscopic analysis of N (Crumpton et al. 1992) and a malachite-green analysis for P (Motomizu et al. 1983).

Statistical methods—For the reciprocal-transplant experiment, Chl *a* biomass and N_2-fixation responses were analyzed as a three-way analysis of variance (ANOVA; factors = incubation site, growth site, and nutrient). For the streamside experiment, N_2 fixation and Chl *a* biomass responses on NDS were analyzed by use of a two-way ANOVA on experiment day 45 to examine long-term community-level responses (factors = temperature and nutrient). N_2 fixation and Chl *a* biomass responses on rocks were analyzed with a one-way ANOVA (factor = temperature) on experiment day 2 to examine short-term physiologic responses and on day 45 to examine long-term community-level responses of Chl *a* biomass and N_2 fixation. All analyses were done by PROC GLM in SAS version 8e. Post-hoc differences were determined by use of LSMEANS and a Tukey adjustment, which performs pairwise comparisons of all treatment-interaction means to each other (e.g., warm control vs. warm N vs. cold control, etc.).

Table 2. Results of a three-way ANOVA for the reciprocal-transplant experiment that considers the following factors: growth site (inlet vs. outlet), incubation site (inlet vs. outlet), and nutrient supply (control, nitrogen, and phosphorus).

Factor	Chlorophyll *a* biomass		Nitrogen fixation	
	F	*p*	*F*	*p*
Growth site (G)	$2.39_{1,48}$	0.13	$48.11_{1,45}$	**0.01**
Incubation site (I)	$7.76_{1,48}$	**0.01**	$15.62_{1,45}$	**0.01**
Nutrient supply (Nut)	$44.12_{2,48}$	**0.01**	$125.61_{2,45}$	**0.01**
G×I	$7.31_{1,48}$	**0.01**	$4.56_{1,45}$	**0.04**
G×Nut	$28.82_{2,48}$	**0.01**	$32.64_{2,45}$	**0.01**
I×Nut	$0.53_{2,48}$	0.59	$3.31_{2,45}$	**0.04**
G×I×Nut	$0.86_{2,48}$	0.43	$1.10_{2,45}$	0.34

The two response variables measured were chlorophyll *a* biomass (μg cm^{-2}) and nitrogen fixation (μg N_2 m^{-2} h^{-1}). *F*-ratios with degrees of freedom are shown; $p<0.05$ is indicated by bold type. Overall ANOVA results were chlorophyll *a* biomass $F_{11,48} = 15.1$, $p<0.01$ and nitrogen fixation $F_{11,45} = 35.8$, $p<0.01$.

Results

Reciprocal-transplant experiment—Short-term temperature changes clearly influenced N_2-fixation rates in this experiment, as indicated by a significant interaction between incubation site and nutrient treatment (Table 2). Communities grown on P-enriched substrata had the greatest rates of N_2 fixation compared with the other nutrient treatments, but those incubated in the outlet had rates 330% greater than those incubated in the inlet. Periphyton on substrata grown in the outlet and incubated in the outlet where water temperature was 11°C higher had the greatest N_2-fixation rates, followed by that grown in the outlet and incubated in the inlet, and that grown in the inlet but incubated in the outlet (Fig. 4a,b). The periphyton on NDS grown in the inlet and incubated in the inlet had the significantly lowest N_2-fixation rates. Chl *a* biomass was significantly greatest on P-enriched NDS grown and incubated in the outlet, with all other treatments the same. Surprisingly, incubation site and growth site also had a significant interaction for Chl *a* biomass (Table 2). Substrata grown in the outlet but incubated in the inlet had 25–43% lower Chl *a* biomass values than those grown and incubated in the outlet (Fig. 4d).

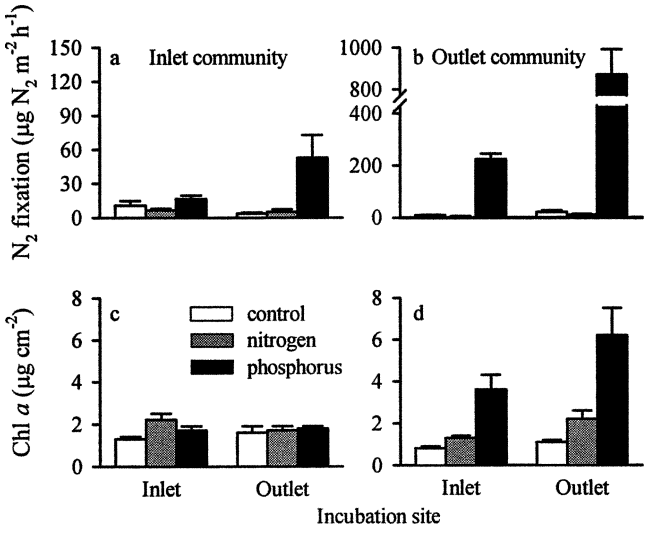

Fig. 4. (a, b) N_2 fixation and (c, d) Chl *a* responses of inlet and outlet communities incubated at both sites in the reciprocal-transplant experiment. Note the difference between the *y*-axes of (a) and (b). Error bars are ± 1 SE.

Long-term, community-level responses in this experiment indicated that N_2-fixation rates were significantly affected by interactions between growth site and P supply. N_2-fixation rates on the NDS grown in the outlet were 10–12 times greater than on those grown in the inlet and were significantly different between the two sites (Fig. 4a,b). A significant interaction occurred between nutrient and growth site (Table 2), such that P additions stimulated N_2 fixation compared with control treatments at both growth sites, but mean stimulation in the inlet was 1.5 times greater than the control compared with 43 times greater in the outlet (Fig. 4a,b). N_2-fixation rates on control and N substrata were significantly lower than those on P substrata and were not significantly different from each other.

The Chl *a* biomass and composition of periphyton communities also differed because of the long-term interaction between growth site and nutrient supply (Table 2). Chl *a* biomass was greatest on P-enriched NDS grown in the outlet, significantly lowest on the outlet control NDS, and indistinguishable among all other nutrient substrata from both the inlet and outlet (Fig. 4c,d). At the end of the experiment, the inlet community was primarily composed of 63–72% diatoms and 18–29% chlorophytes, and the rest of the community was cyanobacteria, irrespective of nutrient treatment (Fig. 5). The main diatom species observed on these substrata were *Navicula* sp., *Achnanthidium* sp., *Synedra* sp., and *Hannaea* sp. The main cyanobacteria observed were *Aphanocapsa* sp. and *Pseudanabaena* sp. Only 1% of the periphyton community on the control and P-enriched substrata was composed of N_2-fixing taxa, specifically, the diatoms *Epithemia* sp. and *Rhopalodia gibba*, which contain cyanobacterial endosymbionts (Prechtl et al. 2004). None of the taxa found on the N-enriched substrata in the inlet were capable of N_2 fixation. In the outlet, the periphyton community was composed of 41–44% diatoms and 37–42% non–N_2-fixing cyanobacteria on the control and N-enriched treatments. On all three nutrient treatments, *Navicula* sp., *Achnanthidium* sp., *Synedra* sp., *Aphanocapsa* sp., and *Pseudanabaena* sp. were common. However, the

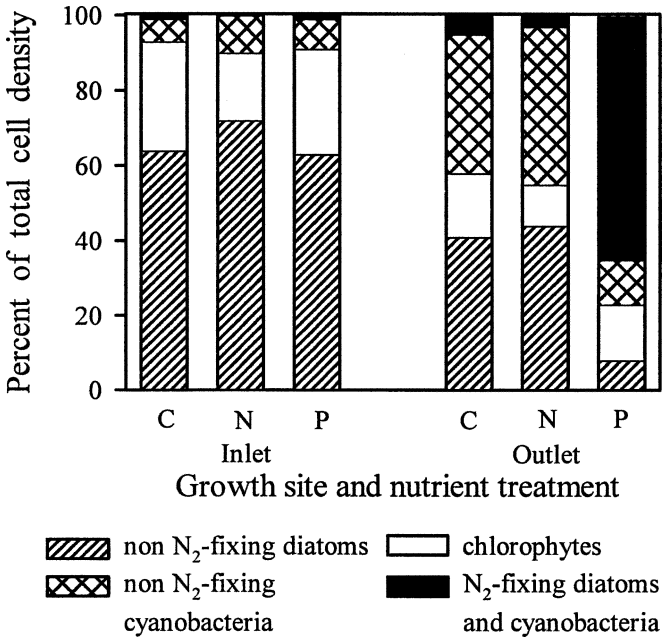

Fig. 5. Relative proportions of non–N_2-fixing diatoms, chlorophytes, non–N_2-fixing cyanobacteria, and N_2-fixing cyanobacteria and diatoms on the nutrient-diffusing substrata at the end of the reciprocal-transplant experiment. The nutrient treatments were control (C), nitrogen (N), and phosphorus (P).

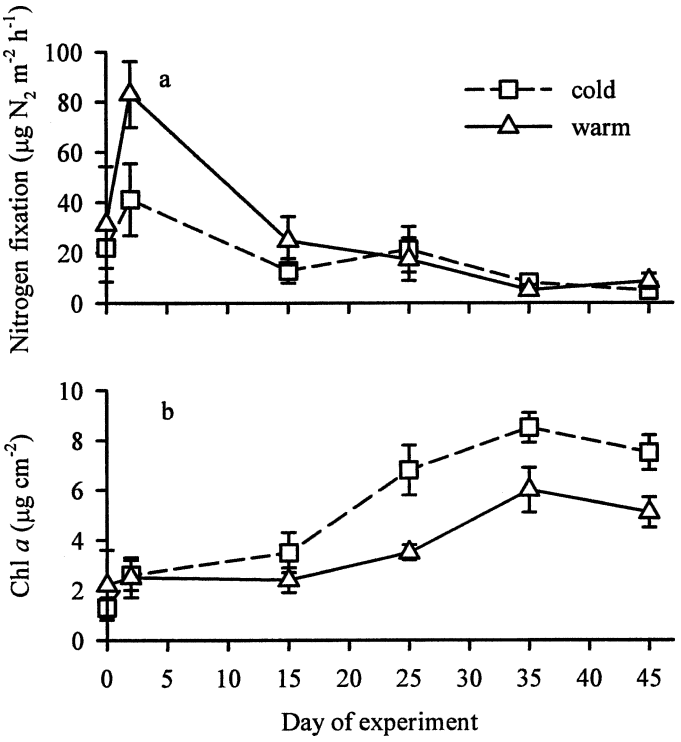

Fig. 6. Responses by (a) N_2 fixation and (b) Chl *a* on rock substrata in the streamside temperature experiments. Error bars are ± 1 SE.

community on the P-enriched substrata was composed of 66% N_2-fixing taxa, including the diatoms *Epithemia* sp. and *Rhopalodia gibba* and high densities of the heterocystous cyanobacterium *Anabaena* sp., which indicated an important community-level interaction between growth site and P enrichment.

Streamside experiment—Short-term responses of N_2 fixation and temperature were observed on the rock substrata in the streamside experiment, where the effects of temperature and nutrients were tested simultaneously in temperature-controlled mesocosms. N_2 fixation showed an initial boost of 260% after 2 d in the warm treatment (Fig. 6), although this increase was not significant (Table 3). The periphyton community on the rocks was initially composed of 55% non–N_2-fixing cyanobacteria (Fig. 7), including *Aphanocapsa* sp., *Oscillatoria* sp., and *Pseudanabaena* sp. An additional 9% of the initial community was composed of the heterocystous cyanobacterium *Calothrix* sp. and the diatom *Epithemia* sp.

By day 45 of the experiment, the effects of temperature on N_2 fixation by the rock periphyton disappeared. After the initial boost in N_2 fixation, an exponential decline occurred until rates were near detection levels on days 35 and 45 in both temperature treatments (Fig. 6a). Chl *a* biomass was greater in the cold treatment then in the warm treatment on days 25 through 45 (Fig. 6b), but the difference was marginally nonsignificant on day 45 (Table 3). At the end of the experiment, the periphyton community on the rocks shifted to 74% diatoms in the cold treatment and 51% diatoms in the warm treatment, whereas cyanobacteria declined to 17% and 34%, respectively (Fig. 7). More importantly, N_2-fixing taxa

Table 3. Results of one-way and two-way ANOVA for the streamside temperature experiment.

Substrate	Day of experiment	Factor	Chlorophyll *a* biomass		Nitrogen fixation	
			F	*p*	*F*	*p*
Rock	2	T	$0.00_{7,1}$	0.95	$2.29_{6,1}$	0.18
	45	T	$4.76_{7,1}$	0.06	$2.36_{7,1}$	0.17
NDS	45	T	$0.11_{1,21}$	0.75	$14.2_{1,21}$	**0.01**
		Nut	$5.29_{2,21}$	**0.01**	$42.7_{2,21}$	**0.01**
		T × Nut	$4.24_{2,21}$	**0.03**	$6.2_{2,21}$	**0.01**

Tests were run on single days of the experiment and separately for rock and nutrient-diffusing substrata (NDS). Factors were temperature (T; warm vs. cold) and nutrient supply (Nut; control, nitrogen, and phosphorus, for NDS only). The two response variables measured were chlorophyll *a* biomass (μg cm^{-2}) and nitrogen fixation (μg N_2 m^{-2} h^{-1}). *F*-ratios with degrees of freedom are shown; $p<0.05$ is indicated by bold type. Overall results for the NDS two-way ANOVA were chlorophyll *a* biomass $F_{5,21} = 5.0$, $p<0.01$ and nitrogen fixation $F_{5,21} = 20.3$, $p<0.01$.

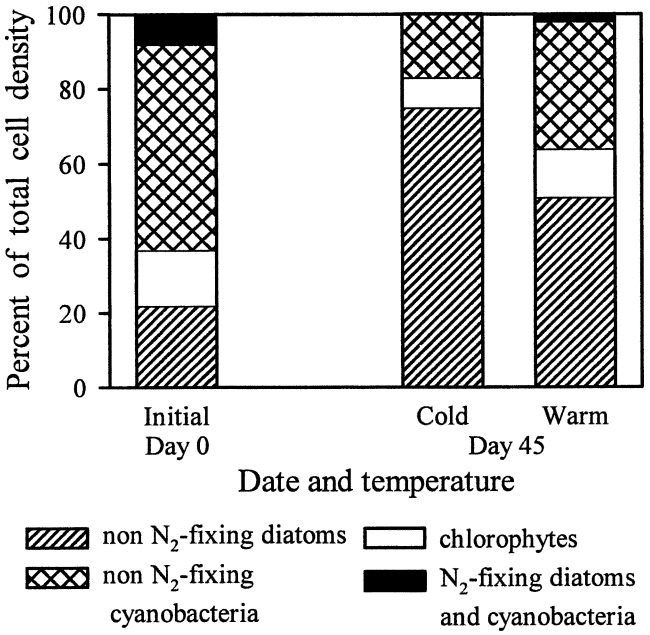

Fig. 7. Relative proportions of non–N_2-fixing diatoms, chlorophytes, non–N_2-fixing cyanobacteria, and N_2-fixing cyanobacteria and diatoms on the rock substrata in the streamside temperature experiment at the beginning (day 0) and end (day 45) of the experiment.

largely disappeared from the final periphyton communities in both temperature treatments.

Day 45 responses on NDS indicated that N_2 fixation was controlled by interactions between nutrient availability and temperature (Table 3). P stimulated N_2 fixation in both temperature treatments; however, P stimulated N_2 fixation only 3-fold in the cold treatment compared with 23-fold above control levels in the warm treatment (Fig. 8a,b). The greatest N_2-fixation rates were on the warm P treatment. N_2-fixation rates were significantly lowest on warm and cold N treatments, which were not different from each other. N_2-fixation rates were statistically indistinguishable on the warm control, cold P, and cold control treatments (Fig. 8a,b).

Increased rates of N_2 fixation on P-enriched NDS in the warm treatment were not driven by simple increases in Chl *a* biomass, but were related to changes in community composition. On day 45, Chl *a* biomass was greater on the cold P treatment than on the cold control, cold N, and warm control treatments, and these three treatments were not statistically different from one another (Fig. 8c,d). The communities on all NDS were largely dominated by diatoms (mean 60%), followed by non–N_2-fixing cyanobacteria (27%), and finally by chlorophytes (12%) (Fig. 9). The most-common diatom species observed were *Achnanthidium* sp., *Cymbella* sp., *Gomphonema* sp., *Navicula*

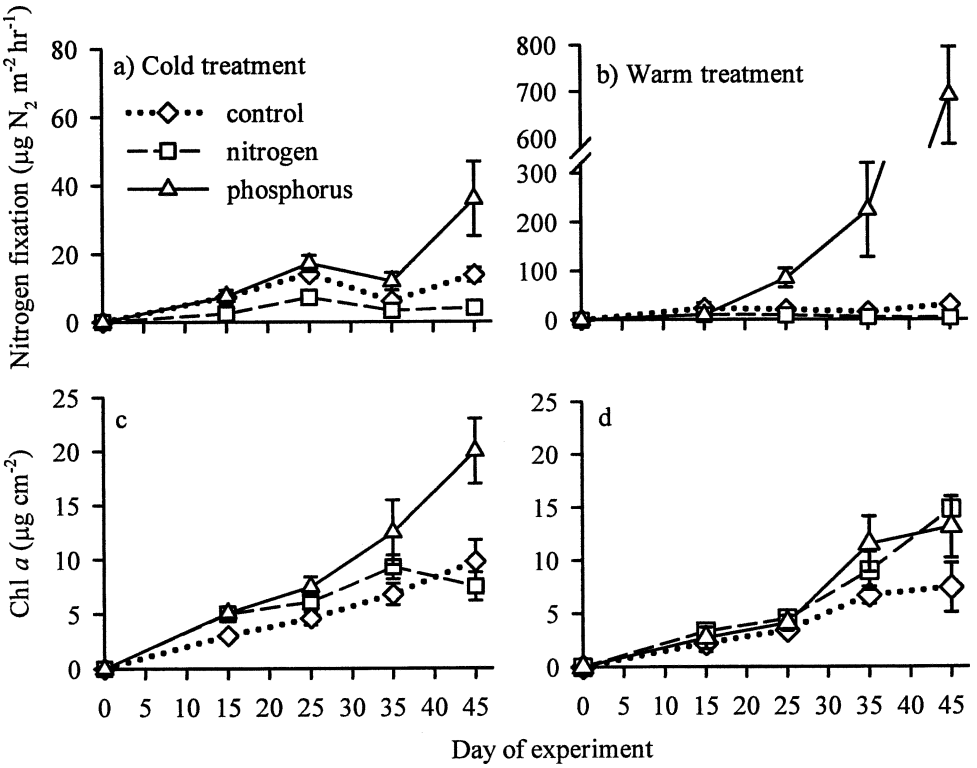

Fig. 8. (a, b) N_2-fixation rates and (c, d) Chl *a* on nutrient-diffusing substrata in the cold and warm treatments in the streamside temperature experiment. Note that the N_2 fixation *y*-axis scale on the right panel is 10 times greater than on the left panel. Error bars are ± 1 SE.

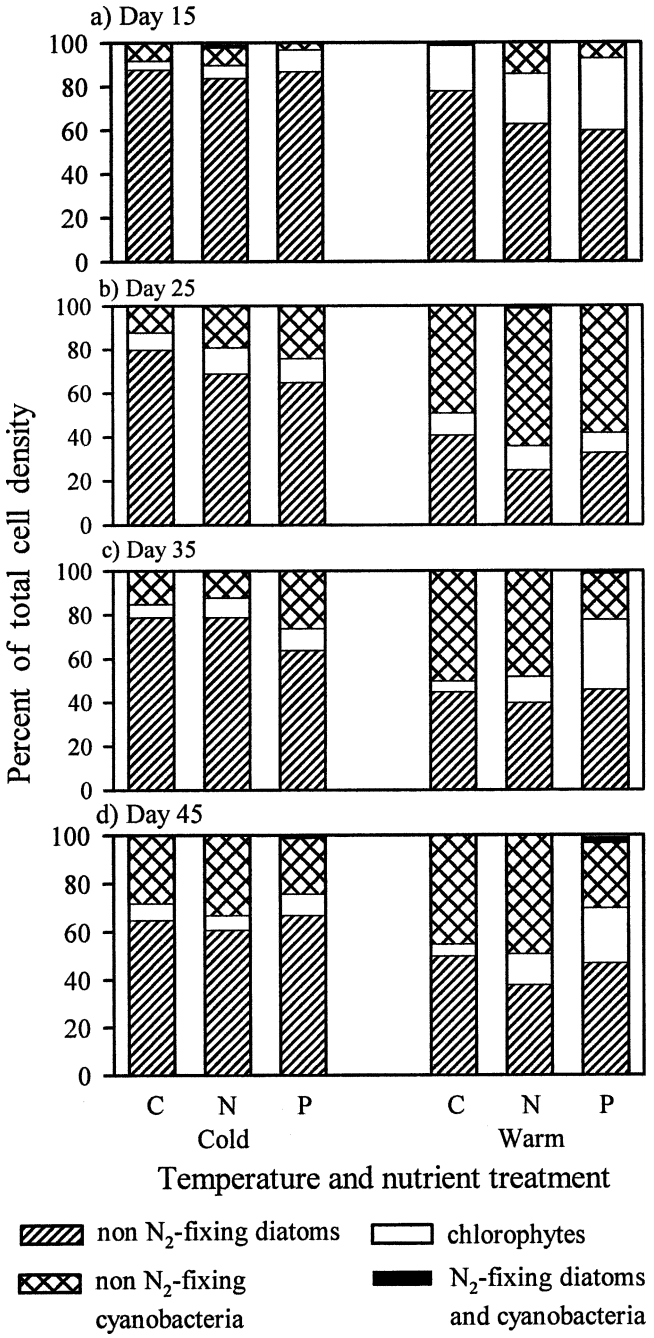

Fig. 9. Relative proportions of non–N_2-fixing diatoms, chlorophytes, non–N_2-fixing cyanobacteria, and N_2-fixing cyanobacteria and diatoms on the nutrient-diffusing substrata in the streamside temperature experiment. Each panel is a different day of the study. The nutrient treatments are control (C), nitrogen (N), and phosphorus (P).

sp., and *Synedra* sp. The two most-common chlorophyte species were *Coccochlorus* sp. and *Oocystis* sp. The diatoms *Hannaea arcus* and *Diatoma hiemale* were abundant on all nutrient treatments in the cold temperature on days 35 and 45. N_2-fixing taxa, including *Rhopalodia gibba, Epithemia sorex*, and *Epithemia adnata* with cyanobacterial endosymbionts, comprised 2–4% of the periphyton community on the P-enriched substrata in the warm-temperature treatment on days 35 and 45 and in the cold-temperature treatment on day 45 (Fig. 9c,d). The heterocystous *Anabaena* sp. was observed only on the warm P-enriched substrata on day 45, which, again, indicates an important community-level interaction between temperature and P supply.

Nutrient concentrations were relatively constant for the duration of the experiment. NO_3–N concentrations ranged from 22 to 46 μg N L^{-1}, and TDN ranged from 88 to 113 μg N L^{-1}. These values were 3–5 times greater for NO_3–N and twofold greater for TDN than those typically observed in Stanley Creek during the summer (Table 4). PO_4–P was routinely below the detection limit of 2 μg L^{-1}, but TDP ranged from 2.0 to 4.5 μg P L^{-1} and was also greater than that typically found in Stanley Creek (Table 4).

Discussion

In our experiments, the occurrence of N_2 fixation was controlled by P availability, and the magnitude of response to P was modulated by temperature. The effect was most notable in the streamside experiment, where P stimulated N_2 fixation 260% above controls in the cold treatment but stimulated N_2 fixation 2,300% above controls in the warm treatment. Other researchers have suggested that P limitation of N_2 fixation may be difficult to detect because many algae can store P via luxury uptake (Howarth et al. 1988) or because of colimitation of N_2 fixation by P and trace metals such as iron (Mills et al. 2004). The fact that we demonstrated high levels of N_2 fixation on our P substrata may indicate that no trace-metal limitation of N_2 fixation occurred in our streams. However, we had no data to test this interaction. N suppression of N_2 fixation is a very commonly reported cyanobacterial response because N_2 fixation is an energetically expensive process that cells will cease when ample N is available for growth (Howarth et al. 1988). This effect likely caused *Calothrix* sp. to disappear from the rock community by day 45 in the streamside temperature experiment. Nitrate concentrations were two times greater in the experimental enclosures than in the rock source stream, and perhaps as a result, N_2 fixation decreased over time.

Temperature controlled N_2-fixation rates at the physiologic level in both of our experiments. In the reciprocal-transplant experiment, N_2-fixation rates were higher on P-diffusing substrata from both inlet and outlet growth sites when incubated in the warmer lake outlet. This response indicates that even communities with small numbers of N_2 fixers, such as the inlet community, can exhibit physiologic stimulation of N_2 fixation when exposed to higher temperatures. This finding also agrees with laboratory studies that have shown that higher temperatures immediately stimulate N_2-fixation rates of cyanobacterial cultures (Staal et al. 2003), plankton communities (Goering and Neess 1964), and benthic lake communities (Reuter et al. 1983). In the streamside experiments, the warm-temperature treatment had an immediate stimulatory effect on N_2 fixation by the rock community, which was likely caused by physiologic

Table 4. Chemical characteristics of water in the streamside temperature experiments and in Stanley Creek, where the rocks for the experiment were collected.

Factor	Day and temperature treatment						Stanley Creek	
	Day 0		Day 25		Day 45			
	Cold	Warm	Cold	Warm	Cold	Warm	Aug 2002	Aug 2003
Dissolved organic carbon (mg C L^{-1})	0.7	0.8	0.8	0.8	0.8	0.8	NA	1.0
Nitrate–nitrogen (μg NO_3–N L^{-1})	46	54	22	52	41	35	7	NA
Total-dissolved nitrogen (μg N L^{-1})	99	113	88	110	97	88	NA	66
Total nitrogen (μg N L^{-1})	151	141	97	135	111	95	61	77
Total-dissolved phosphorus (μg P L^{-1})	2.1	2.0	4.0	4.5	3.7	3.8	NA*	BDL*
Total phosphorus (μg P L^{-1})	4.3	5.4	5.9	5.9	5.0	4.5	4.1	2.6

* BDL, below detection limit for the nutrient analysis used; NA, data not available.

Detection limit for total-dissolved phosphorus was 1.9 μg P L^{-1}. Nitrate–nitrogen and total-nitrogen concentrations were higher in the experimental enclosures that received water from the Salmon River than those observed in Stanley Creek. Phosphate concentrations in all samples were <2 μg P L^{-1}.

stimulation of N_2 fixation by *Calothrix* sp. However, this physiologic increase was not significant and was not maintained for the duration of the experiment, likely because of the overriding effects of adequate N supplies.

Temperature and nutrient supply also interacted to control N_2-fixation rates by alteration of the periphyton Chl *a* biomass and particularly the community composition. By day 45 in the streamside experiment, P additions had stimulated Chl *a* biomass 2-fold in the warm treatment but stimulated N_2-fixation rates 23-fold, which indicated that N_2-fixing organisms increased more than other taxa. In the reciprocal-transplant experiment, P limitation of Chl *a* was observed in the warm outlet, but in the cold inlet, no significant response of Chl *a* biomass or N_2 fixation to either N or P was seen. In both experiments, additions of P and increased temperatures stimulated increased abundance of diatoms of the order Rhopalodiales (*Epithemia* sp. and *Rhopalodia* sp.) with cyanobacterial endosymbionts. Prechtl et al. (2004) demonstrated that endosymbionts within *Rhopalodia gibba* are able to fix N_2 and confirmed that the endosymbionts within *R. gibba* are of cyanobacterial origin and are closely related to two strains of the N_2-fixing cyanobacterium *Cyanothece* sp. DeYoe et al. (1992) demonstrated that Rhopalodiales can alter their endosymbiont load in response to nutrient supply in cultures, so that the number of endosymbionts per diatom increased as N became limiting. Additionally, in our experiments, *Anabaena* sp. was observed only in warm-temperature and P-enriched treatments, where the highest N_2-fixation rates were measured. Other studies have also shown community dominance by heterocystous cyanobacteria or Rhopalodiales with P additions in freshwater lakes (Fairchild et al. 1995), N-poor streams (Peterson and Grimm 1992), and N-depleted wetlands (Scott et al. 2005). Finally, several cold-water, non–N_2-fixing species of diatoms were observed in the cold inlet community and in the cold treatment of the experiments, most notably *Hannaea* sp. Antoniades and Douglas (2002) showed that the abundance of *Hannaea arcus* in epilithic habitats was strongly negatively correlated with water temperature.

In contrast to other studies, the Chl *a* biomass on rocks in the streamside experiment was greater in the cold treatment compared with the warm treatment. In general, increased temperature has increased algal biomass in other experiments (DeNicola 1996). For example, Lamberti and Resh (1983) found that elevating temperature by 7.5°C increased benthic Chl *a* biomass by 40 times in experimental stream enclosures. The greater Chl *a* biomass observed in the cold treatments in our experiment could be caused by different grazing rates of meiofauna. Although we removed all macroinvertebrates in the experiments, we did not remove meiofauna associated with the periphyton biofilm, and these organisms can have consumption rates as high as 1.6% of diatom abundance h^{-1} (Bott and Borchardt 1999). The elimination of grazing pressure on meiofauna by macroinvertebrates (Schmid-Araya and Schmid 2000) and increased temperature in the warm treatment possibly led to increased meiofaunal metabolism and population size, and, in turn, greater meiofaunal grazing rates than in the cold treatments.

N_2-fixing taxa never made up more than 10% of the periphyton community in the streamside experiment, and as a result, N_2-fixation rates in this experiment were much lower than those measured in other stream studies in which cyanobacteria dominate the periphyton community. Grimm and Petrone (1997) measured a wide range of N_2-fixation rates in a desert stream with a maximum rate of 51,000 μg N_2 m^{-2} h^{-1} on a dense cyanobacterial mat composed mostly of *Anabaena* sp.; the average of all rates measured in their study was 5,820 μg N_2 m^{-2} h^{-1}. This average is five times greater than the maximum N_2-fixation rate observed in either of our experiments. Maximum rates of N_2 fixation by *Nostoc* sp. in a small California stream were 11,000 μg N_2 m^{-2} h^{-1} (Horne and Carmiggelt 1975), or 10 times greater than the maximum rate reported in our study. Our rates are similar to those measured by the epilithic communities of five oligotrophic western lakes, where rates ranged from 41 to 140 μg N_2 m^{-2} h^{-1} (Loeb and Reuter 1981). The N_2-fixation rates measured in our study are more reflective of those by natural mixed communities of algae in oligotrophic mountain streams.

The interaction between nutrients and temperature that controlled N_2 fixation in our study was not likely a simple interaction of limiting factors, but rather an interaction of a controlling and a limiting factor. Fry (1947) suggested that temperature should be considered a controlling factor,

which he defined as a factor that controls a metabolic process by conditioning the environment in which that process occurs. Controlling factors do not operate in relation to Leibig's law of the minimum or the single-nutrient limitation paradigm, which states that the rate of a process will be limited by the resource in least supply (Francoeur 2001). Rather, controlling factors govern the range of rates possible within a system. A major difference between limiting and controlling factors is that limiting factors operate consecutively (e.g., once N limitation is alleviated, then P becomes limiting), whereas controlling factors act concurrently on a process such as growth. Under this framework, we can imagine that temperature will dictate the sites where N_2 fixation is possible within a stream network, as well as the range of rates that are possible in any area, but at any site, the actual rates of N_2 fixation will be limited by concentrations of N, P, or micronutrients, which can be considered limiting factors.

The interacting influence of temperature and nutrients on N_2-fixation rates in these experiments has important implications for understanding the spatial distribution of N_2 fixation in oligotrophic streams. In the Sawtooth Mountain Lake–Stream District, we see N_2-fixation rates in lake outlets that are 2–3 times greater than those observed in inlets (Marcarelli unpubl. data). This modest, but consistently significant, difference may reflect the fact that temperatures are much greater in lake outlets than in lake inlets because of surface warming of the lake water (Wotton 1995), but dissolved nutrient supplies are generally greater in lake inlets, as lakes tend to convert inorganic nutrients to particulate organic material (Kling et al. 2000). In this situation, temperature controls N_2-fixation rates such that much greater rates are observed in warmer outlet sites. However, in the outlets, N_2-fixation rates should be consistently limited by the supply of dissolved P, which indicates that nutrients limit the actual rates of N_2 fixation in these sites. The fact that higher P concentrations favor N_2 fixation in the inlet, whereas higher temperature favors N_2 fixation in the outlet, levels the differences between N_2 fixation rates at the two different sites and leads to lower rates in the outlets than would be possible with greater P supplies. In addition, many other factors that may affect algal communities, such as sediment size, flow velocity, and susceptibility to spates, differ between lake inlets and lake outlets and may affect observed N_2-fixation rates in these two types of streams.

The joint effect of temperature and nutrients on N_2 fixation is also important in consideration of the effects of disturbances on aquatic systems because many perturbations affect multiple stream characteristics. Mountain-stream temperatures are most directly controlled by basin elevation, with smaller controls by riparian tree abundance (Issak and Hubert 2001). Land-use practices that reduce riparian shading, such as logging and cattle grazing (Davies and Nelson 1994; Belsky et al. 1999), also alter nutrient loads to streams. Global climate change may also have profound effects on aquatic ecosystems by increasing water temperature, but this change rarely occurs without a simultaneous change in nutrient loads, whether directly because of the effects of climate change (e.g., O'Reilly et al. 2003) or because of other disturbances. Our results predict that N_2-fixation rates will increase in subalpine watersheds if stream temperatures increase, especially if these increases are accompanied by an increase in P supply. However, anthropogenic activities may also increase N supplies to these subalpine watersheds as we increase the amount of N in atmospheric circulation (Vitousek et al. 1997), and this increased N supply would likely cause N_2 fixation to decrease. Therefore, the future course of change in these freshwater ecosystems is difficult to predict.

In conclusion, we found that N_2 fixation in oligotrophic streams is primarily limited by the supply of P and simultaneously controlled by water temperature. Responses to increased temperature occur at both the individual physiologic level and the community level by favoring the inclusion of cyanobacteria and diatoms with cyanobacterial endosymbionts in algal communities. To understand where and when N_2 fixation can be an important source of N to stream communities, we must understand more about how other environmental factors, such as discharge, substrate composition, and light exposure, interact to control N_2-fixation rates of the periphyton community. This work is particularly important as global warming and alteration of the N cycle potentially alter the controls on N_2 fixation. Understanding N_2 fixation in oligotrophic, undisturbed systems can provide a baseline of knowledge for understanding changes in other ecosystems.

References

Antoniades, D., and M. S. V. Douglas. 2002. Characterization of high arctic stream diatom assemblages from Cornwallis Island, Nunavut, Canada. Can. J. Bot. **80:** 50–58.

Belsky, A. J., A. Matzke, and S. Uselman. 1999. Survey of livestock influences on stream and riparian ecosystems in the western United States. J. Soil Water Cons. **54:** 419–431.

Bott, T. L., and M. A. Borchardt. 1999. Grazing of protozoa, bacteria, and diatoms by meiofauna in lotic epibenthic communities. J. North Am. Benthol. Soc. **18:** 499–513.

Cairns, Jr., J. 1956. Effects of increased temperature on aquatic organisms. Ind. Waste **1:** 150–152.

Capone, D. G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure, p. 621–631. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in microbial ecology. Lewis.

Crumpton, W. G., T. M. Isenhart, and P. D. Mitchell. 1992. Nitrate and organic N analyses with 2nd-derivative spectroscopy. Limnol. Oceanogr. **37:** 907–913.

Davies, P. E., and M. Nelson. 1994. Relationships between riparian buffer widths and the effects of logging on stream habitat, invertebrate community composition and fish abundance. Aust. J. Mar. Fish. Res. **45:** 1289–1305.

DeNicola, D. M. 1996. Periphyton responses to temperature at different ecological levels, p. 147–181. *In* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe [eds.], Algal Ecology: Freshwater benthic ecosystems. Academic.

DeYoe, H. R., R. L. Lowe, and J. C. Marks. 1992. Effects of nitrogen and phosphorus on the endosymbiont load of *Rhopalodia gibba* and *Epithemia turgida* (Bacillariophyceae). J. Phycol. **28:** 773–777.

Emmett, W. W. 1975. The channels and water of the Upper Salmon River area, Idaho. U.S. Geological Survey Professional Paper 870-A.

FAIRCHILD, G. W., R. L. LOWE, AND W. B. RICHARDSON. 1985. Algal periphyton growth on nutrient-diffusing substrata: An in situ bioassay. Ecology **66:** 465–472.

FLETT, R. J., R. D. HAMILTON, AND N. E. R. CAMPBELL. 1976. Aquatic acetylene-reduction techniques: Solutions to several problems. Can. J. Microbiol. **22:** 43–51.

FRANCOEUR, S. N. 2001. Meta-analysis of lotic nutrient amendment experiments: Detecting and quantifying subtle responses. J. North Am. Benthol. Soc. **20:** 358–368.

FRY, F. E. J. 1947. Effects of the environment on animal activity. Univ. Toronto Press.

GIBEAU, JR., G. G., AND M. C. MILLER. 1989. A micro-bioassay for epilithon using nutrient-diffusing artificial substrata. J. Freshw. Ecol. **5:** 171–176.

GOERING, J. J., AND J. C. NEESS. 1964. Nitrogen fixation in two Wisconsin lakes. Limnol. Oceanogr. **9:** 530–539.

GRIMM, N. B., AND K. C. PETRONE. 1997. Nitrogen fixation in a desert stream ecosystem. Biogeochemistry **37:** 33–61.

HENRY, J. C., AND S. G. FISHER. 2003. Spatial segregation of periphyton communities in a desert stream: Causes and consequences for N cycling. J. North Am. Benthol. Soc. **22:** 511–527.

HORNE, A. J., AND C. J. W. CARMIGGELT. 1975. Algal nitrogen fixation in California streams: Seasonal cycles. Freshw. Biol. **5:** 461–470.

HOWARTH, R. W., R. MARINO, AND J. J. COLE. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. Limnol. Oceanogr. **33:** 688–701.

ISSAK, D. J., AND W. A. HUBERT. 2001. A hypothesis about factors that affect maximum summer stream temperatures across montane landscapes. J. Am. Water Resour. Assoc. **37:** 351–366.

KLING, G. W., G. W. KIPPHUT, M. M. MILLER, AND W. J. O'BRIEN. 2000. Integration of lakes and streams in a landscape perspective: The importance of material processing on spatial patterns and temporal coherence. Freshw. Biol. **43:** 477–497.

KORTE, V. L., AND D. W. BLINN. 1983. Diatom colonization on artificial substrata in pool and riffle zones studied by light and scanning electron microscopy. J. Phycol. **19:** 332–341.

LAMBERTI, G. A., AND V. H. RESH. 1983. Geothermal effects on stream benthos: Separate influences of thermal and chemical components on periphyton and macroinvertebrates. Can. J. Fish. Aquat. Sci. **40:** 1995–2009.

LOEB, S. L., AND J. E. REUTER. 1981. The epilithic periphyton community: A five-lake comparative study of community productivity, nitrogen metabolism and depth-distribution of standing crop. Verh. Internat. Verein. Limnol. **21:** 346–352.

MILLS, M. M., C. RIDAME, M. DAVEY, J. LA ROCHE, AND R. J. GELDER. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature **429:** 292–294.

MOTOMIZU, S., W. TOSHIAKI, AND K. TOEI. 1983. Spectrophotometric determination of phosphate in river waters with molybdate and malachite green. Analyst **108:** 361–367.

O'REILLY, C. M., S. R. ALIN, P.-D. PLISNIER, A. S. COHEN, AND B. A. MCKEE. 2003. Climate change decreases productivity of Lake Tanganyika, Africa. Nature **424:** 766–768.

PETERSON, C. G., AND N. B. GRIMM. 1992. Temporal variation in enrichment effects during periphyton succession in a nitrogen-limited desert stream ecosystem. J. North Am. Benthol. Soc. **11:** 20–36.

PETERSON, B. J., AND OTHERS. 2001. Control of nitrogen export from watersheds by headwater streams. Science **292:** 86–90.

PRECHTL, J., C. KNEIP, P. LOCKHART, K. WENDEROTH, AND U-G. MAIER. 2004. Intracellular spheroid bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin. Mol. Biol. Evol. **21:** 1477–1481.

PRESCOTT, G. W. 1978. How to know the freshwater algae. Brown.

REDFIELD, A. C. 1958. The biological control of chemical factors in the environment. Am. Sci. **46:** 205–221.

REUTER, J. E., S. L. LOEB, AND C. R. GOLDMAN. 1983. Nitrogen fixation in periphyton of oligotrophic Lake Tahoe, p. 101–109. *In* R. G. Wetzel [ed.], Periphyton of freshwater ecosystems. Junk.

ROSEMOND, A. D., P. J. MULHOLLAND, AND J. W. ELWOOD. 1993. Top-down and bottom-up control of stream periphyton: Effects of nutrient and herbivores. Ecology **74:** 1264–1280.

SCHINDLER, D. W. 1977. Evolution of phosphorus limitation in lakes. Science **195:** 260–262.

SCHMID-ARAYA, J. M., AND P. E. SCHMID. 2000. Trophic relationships: Integrating meiofauna into a realistic benthic food web. Freshw. Biol. **44:** 149–163.

SCOTT, J. T., R. D. DOYLE, AND C. T. FILSTRUP. 2005. Periphyton nutrient limitation and nitrogen fixation potential along a wetland nutrient-depletion gradient. Wetlands **25:** 439–448.

SEITZINGER, S. P. 1988. Denitrification in fresh-water and coastal marine ecosystems—ecological and geochemical significance. Limnol. Oceanogr. **33:** 702–724.

STAAL, M., F. J. R. MEYSMAN, AND L. J. STAL. 2003. Temperature excludes nitrogen-fixing heterocystous cyanobacteria in the tropical oceans. Nature **425:** 504–507.

VITOUSEK, P. M., AND OTHERS. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol. Appl. **7:** 737–750.

WEHR, J. D., AND R. G. SHEATH. 2003. Freshwater algae of North America. Academic.

WELSCHMEYER, N. A. 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and phaeopigments. Limnol. Oceanogr. **39:** 1985–1992.

WETZEL, R. G., AND G. E. LIKENS. 2000. Limnological analyses, 3rd ed. Springer.

WILDE, E. W. 1982. Responses of attached algal communities to termination of thermal pollution. Hydrobiologia **94:** 135–138.

———, AND L. J. TILLY. 1981. Structural characteristics of algal communities in thermally altered artificial streams. Hydrobiologia **76:** 57–63.

WOTTON, R. S. 1995. Temperature and lake-outlet communities. J. Therm. Biol. **20:** 121–125.

WURTSBAUGH, W. A., AND A. HORNE. 1983. Iron in eutrophic Clear Lake, California: Its importance for algal nitrogen fixation and growth. Can. J. Fish. Aquat. Sci. **40:** 1419–1429.

———, H. P. GROSS, C. LUECKE, AND P. BUDY. 1997. Nutrient limitation of oligotrophic sockeye salmon lakes of Idaho (USA). Verh. Int. Ver. Limnol. **26:** 413–419.

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