Periphyton response to long-term nutrient enrichment in a shaded headwater stream

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Abstract: We maintained elevated but moderate concentrations of nitrogen and phosphorus continuously for 2 years in a heavily shaded headwater stream and compared effects on stream periphyton with a reference stream. Both streams were sampled for 1 year before treatment. Some measures of periphyton biomass (ash-free dry mass and chlorophyll *a*) responded positively to enrichment. Increased chlorophyll *a* was likely a result of higher chlorophyll per cell, as total algal biovolume did not change with enrichment. These differences were greatest during high-light months (November–May), when cellular growth rates (a proxy for production) were also highest with enrichment. Algal assemblages were dominated by diatoms and remained similar between the treatment and reference streams throughout the enrichment period. Although nutrients stimulated algal growth rates, the long-term effects of nutrient addition on periphyton biomass were small in magnitude compared with other published values and were potentially suppressed by light availability and invertebrate consumption. These and other factors may have also been important in limiting the algal species pool and thus a taxonomic response to enrichment. Our results indicate that in headwater streams with intact tree canopies, chronic nutrient enrichment at moderate concentrations may have little detectable effect on benthic algal composition or periphyton biomass. Although nutrients stimulated algal growth rates, the long-term effects of nutrient addition on periphyton biomass were small in magnitude compared with other published values and were potentially suppressed by light availability and invertebrate consumption. These and other factors may have also been important in limiting the algal species pool and thus a taxonomic response to enrichment. Our results indicate that in headwater streams with intact tree canopies, chronic nutrient enrichment at moderate concentrations may have little detectable effect on benthic algal composition or periphyton biomass.

Résumé : Pendant deux années complètes, nous avons maintenu modérément élevées les concentrations d'azote et de phosphore d'un ruisseau de tête de bassin fortement ombragé; nous avons comparé les effets sur le périphyton de ce ruisseau aux conditions observées dans un ruisseau témoin. Les deux ruisseaux ont aussi été échantillonnés pendant un an avant le traitement expérimental. Certaines valeurs de biomasse du périphyton (masse sèche sans les cendres et chlorophylle *a*) augmentent après l'enrichissement. L'augmentation de chlorophylle *a* est vraisemblablement due à un contenu plus élevé de chlorophylle par cellule, car le biovolume total des algues ne change pas avec l'enrichissement. Ces différences sont plus marquées durant les mois de forte lumière (novembre à mai) pendant lesquels les taux de croissance cellulaire (une valeur de remplacement de la productivité) sont aussi maximaux dans les conditions d'enrichissement. Les peuplements d'algues sont dominés par les diatomées et sont restés semblables dans le ruisseau expérimental et le ruisseau témoin durant la durée de l'enrichissement. Bien que les nutriments stimulent les taux de croissance des algues, les effets à long terme de l'addition de nutriments sur le périphyton restent de faible amplitude par comparaison à d'autres valeurs trouvées dans la littérature; il y a donc potentiellement une suppression des effets par la disponibilité de la lumière et la consommation des invertébrés. Ces facteurs et d'autres peuvent aussi être importants pour limiter le pool d'espèces d'algues et ainsi réduire la réponse taxonomique à l'enrichissement. Nos résultats indiquent que, dans les ruisseaux en tête de bassin où la couverture des arbres est intacte, un enrichissement chronique mais modéré en nutriments peut avoir peu d'effets décelables sur la composition des algues benthiques ou sur la biomasse du périphyton.

[Traduit par la Rédaction]

Introduction

Rivers play critical roles on the landscape in providing essential services to humans. Intact headwater streams are crucial to the functioning of river systems (Meyer and Wallace 2001) and have been shown to be critical sites in river networks for processes such as nutrient uptake and retention (Peterson et al. 2001). However, headwater streams are also particularly vulnerable to changing land use and non-point-source pollutants. In many cases, these streams are not pro-

Received 6 July 2004. Accepted 24 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 6 September 2005.
J18207

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 doi: 10.1139/F05-117

tected simply because they do not appear on maps or are not adequately protected by law (Meyer and Wallace 2001). Small-order streams also have a greater contribution of watershed area to stream area compared with larger streams (Selby 1985). Thus, first-order streams may experience greater nutrient inputs than larger streams owing to atmospheric deposition, saturation of terrestrial ecosystems, or mobilization from soils from the surrounding catchment. However, the effects of long-term nutrient input to forested headwater streams are essentially unknown.

Most experimental work examining effects of enrichment on periphyton has been short term in nature (less than ~8 weeks; Francoeur 2001) and thus demonstrates potential or transient responses rather than ultimate effects. The importance of long-term studies is evident from a 16-year study in the Kuparak River (Alaska, USA), where nutrient addition during the growing season each year resulted in consistently higher algal biomass in a fertilized versus reference reach of stream (Slavik et al. 2004). The effects of enrichment were greatest during the first 2 years of enrichment but were suppressed by grazers in subsequent years (Miller et al. 1992; Peterson et al. 1993). Further, a dramatic increase in bryophyte cover after 8 years of enrichment reduced the magnitude of enrichment effects on epilithic algal biomass, but epiphytic algae associated with bryophytes exhibited a strong positive response to enrichment (Slavik et al. 2004). The high-light environment of a tundra stream like the Kuparak contrasts with many headwater streams that are heavily shaded. Short-term experimental work in forested headwater streams indicates that the response of algal biomass to nutrient enrichment can be limited by light availability and invertebrate consumption (e.g., Lowe et al. 1986; Rosemond 1993; Bernhardt and Likens 2004). In environments where response to enrichment is predicted to be subtle or suppressed by other factors (systems with heavy shade or where algal biomass is controlled by grazers), longer-term studies are critical for predicting the potential ultimate effects of nutrients on periphyton structure and function.

Fewer data exist to make clear predictions regarding the expected response of algal assemblages to nutrient enrichment. Algal assemblages are often sensitive to changes in water chemistry because of different tolerance optima among individual populations (Lowe 1974) or differences in competitive ability among species (Tilman 1977), and the great majority of studies that have examined species assemblage response to elevated nutrient concentrations have found significant shifts in taxonomic composition (e.g., Fairchild et al. 1985; Marks and Lowe 1989; Mulholland and Rosemond 1992). However, community shifts are difficult to predict, since they do not always change with nutrient enrichment, regardless of the biomass response (Shortreed et al. 1984; Lohman et al. 1991; Peterson et al. 1993). Specifically, in some situations, other factors (i.e., stressful or poor growing conditions, intense grazing) can be more important than nutrients in driving variation in assemblage composition and can minimize the capacity of species assemblages to respond to nutrient enrichment (Hill et al. 1992; Rosemond et al. 2000).

Our study examined periphyton response to long-term nutrient addition in a shaded headwater stream. We determined the effects of a 2-year continuous enrichment of nitrogen and phosphorus on several characteristics of epilithic periphyton assemblages. We measured algal biomass as ash-free dry mass (AFDM) and chlorophyll *a*, determined algal species composition, and assessed algal growth rates as a proxy measure of productivity. We hypothesized that because of light limitation, nutrient enrichment would have little overall effect on algal biomass and cellular growth rates of benthic algae would be constrained. However, because of variation in nutrient optima among algal taxa, we predicted that algal assemblage structure would be altered with changes in the relative abundance of common species.

Materials and methods

Study site

Two headwater streams, one enriched and one serving as a reference, were examined for 3 years (July 1999 – July 2002) at the Coweeta Hydrologic Laboratory, a USDA Forest Service research facility located in the Blue Ridge Mountain physiographic province in the southern Appalachian Mountains (North Carolina, USA). The reference stream (Catchment 53) and the treatment stream (Catchment 54) have similar physical and chemical characteristics (Table 1). Dominant vegetation includes tulip poplar (*Liriodendron tulipifera*), white oak (*Quercus alba*), red oak (*Quercus rubra*), red maple (*Acer rubrum*), and dogwood (*Cornus florida*) (Swank and Crossley 1988). Rhododendron (*Rhododendron maximum*), an evergreen, grows as a dense understory in the riparian zone. Thus, a double canopy of deciduous trees and rhododendron shades the study streams to some degree during all seasons.

Nutrient enrichment

Pretreatment data were collected from both streams from July 1999 to July 2000. Beginning 11 July 2000, the treatment stream was continuously enriched with NH_4NO_3, KH_2PO_4, and K_2HPO_4 along the entire 150-m length of the study reach for 2 years. A dissolved nutrient salt solution was pumped into an irrigation line (~2 cm in diameter) that was fed with stream water from an upstream head tank. The line ran the entire length of study reach adjacent to the streambed with nutrient solution delivered from multiple spigots. A metering pump (Liquid Metronics, Inc., Acton, Mass.) was electronically linked with a Campbell data logger to an Isco (Teledyne Isco, Los Angeles, Calif.) flow measurement device at the downstream end of the stream reach to deliver nutrients in a discharge-dependent manner. Stream water nutrient concentrations were measured during the pretreatment and experimental periods. During the pretreatment period, one to four samples were taken from the reference and treatment streams on five sampling dates in the reference stream and 12 sampling dates in the treatment stream. During the enrichment period, one sample was taken from the reference stream and five samples were taken along the length of the treatment stream approximately every 2 weeks to confirm that nutrients were elevated in an even distribution within the study reach. Stream water samples were filtered with Millipore HA filters (Millipore Corp., Billerica, Mass.) into acid-washed bottles and frozen until analysis. Concentrations of NO_2^--N, NO_3^--N, NH_4^+-N, and soluble reactive phosphorus (SRP) were determined with an

Table 1. Physical and chemical characteristics of the reference stream and enriched stream from the Coweeta Hydrologic Laboratory.

		Reference	Treatment
Catchment	Area (ha)	5.2	5.5
	Elevation (m above sea level)	820	841
Channel	Gradient ($cm \cdot m^{-1}$)	27	33
	Length (m)	145	282
	Bankfull area (m^2)	327	443
Temperature (°C)	Daily mean (*n*)	12.0 (336)	12.0 (336)
	Range	2.6–18.6	4.8–16.7
Discharge ($L \cdot s^{-1}$)	Daily mean (*n*)	0.32 (1114)	0.53 (1114)
	Range	0.006–3.8	0.06–4.8
pH	Mean (*n*)	6.59 (24)	6.87 (18)
	Range	6.2–7.0	6.6–7.9
NO_3-N ($\mu g \cdot L^{-1}$)	Mean (*n*)	15.4 (5)	18.8 (12)
	Range	9.4–25.8	4.0–39.5
NH_4-N ($\mu g \cdot L^{-1}$)	Mean (*n*)	9.4 (4)	9.9 (12)
	Range	0–30.4	0–24.9
SRP ($\mu g \cdot L^{-1}$)	Mean (*n*)	7.6 (5)	8.8 (12)
	Range	0–20.3	0–22.1

Note: Discharge and temperature are from July 1999 to July 2002 and nutrient and pH data are from the pretreatment period July 1999 – July 2000. SRP, soluble reactive phosphorus.

Alpkem Rapid Flow Analyzer 300 (Alpkem, College Station, Texas) at the University of Georgia Chemical Analysis Laboratory (Athens, Georgia).

Biomass

Epilithic algae were sampled from unglazed ceramic tiles (5.3 cm^2) every 2 months on approximately the 15th of the month from July 1999 through July 2002. Tiles were colonized for 2 months prior to collection (i.e., when tiles were collected, they were replaced with uncolonized tiles that were collected 2 months later). Five sets of two tiles were secured to the streambed along the length of each stream. Accumulated debris (particularly allochthonous leaf material) was cleared from tiles at least once per week. One tile was used for determination of AFDM and the other for chlorophyll *a*. Periphyton used for AFDM samples was brushed from the tile with a toothbrush, rinsed, and the resulting slurry filtered onto a preashed Gelman A/E glass fiber filter (Pall Life Sciences, Ann Arbor, Mich.). The filters were weighed before and after combusting at 500 °C to determine AFDM. Chlorophyll *a* was measured by extracting colonized tiles directly in 20 mL of 90% alkaline (with NH_4OH) acetone solution in a freezer for 24 h. Chlorophyll *a* content was measured with a Turner model 112 fluorometer (July 1999 – May 2001) and a Turner TD-700 fluorometer (July 2001 – July 2002) (Turner Biosystems Inc., Sunnyvale, California). Photosynthetically active radiation (PAR) was measured starting July 1999 at the reference stream and November 1999 at the treatment stream at each sampling site during the study period with a LI-COR LI-250 model hand-held light meter (LI-COR, Linclon, Nebraska).

Growth rates

We sought to determine a measure of algal productive capacity between the two streams that was not influenced by potential losses owing to grazers and that also examined potential effects of irradiance on nutrient response. Algal productivity in these streams was too low to measure with an oxygen change method and measuring ^{14}C uptake was logistically problematic. Thus, we used algal cellular growth rates as a proxy for productive capacity by assessing accrual rates of algal cells on glass slides before and after canopy closure. This method also allowed us to measure algal accrual in the absence of grazers, which were excluded from experimental chambers. In-stream channels were deployed for 4 weeks during four separate trials in "March" (21 March – 4 April), "April" (18 April – 1 May), "June" (21 May – 5 June), and "July" (26 June – 10 July) 2002. In-stream channels were constructed using 30-cm lengths of vinyl rain gutter. To exclude macrograzers, we glued two layers of 200-μm Nitex mesh with silicon sealant to both ends of the gutter. No grazers were detected in the gutters during the experiment. Five gutters each were placed in the treatment and reference streams. Microscope slides were used as the colonization substratum. Three slides were glued horizontally into each channel with silicon sealant. One slide was collected after 2 weeks (2 weeks of accrual time) and another slide at 4 weeks (total of 4 weeks of accrual time) from each of the five gutters in each stream. Slides were scraped with a razorblade and fixed in 1 mL of 2.5% formalin. Cell densities were enumerated in a Palmer–Maloney cell as for species composition, below. New cell accrual between weeks 2 and 4 was calculated as the increase in number of cells per day, which was compared between each stream for each date. This time frame was adequate for distinguishing growth period (2- to 4-week accrual) from confounding factors of the colonization period (0- to 2-week accrual) and avoided the potential for longer-term biomass accrual obscuring differences in growth rates.

Species composition and biovolume

Beginning in September 1999, one additional tile was collected with each set of biomass tiles for algal taxonomic analysis. Tiles were brushed with a toothbrush, rinsed, and

Table 2. Average pretreatment (July 1999 – July 2000) and treatment (July 2000 – July 2002) nutrient levels ($\mu g \cdot L^{-1}$) for the reference and treatment streams.

Site	$(NO_2^-+NO_3^-)$-N	NH_4^+-N	SRP
Pre-enrichment			
Reference			
Mean (1 SD)	15.4 (6.6)	9.4 (14.1)	7.6 (8.0)
Range (*n*)	9–26 (5)	BD–30 (5)	BD–20 (5)
Treatment			
Mean (1 SD)	18.8 (11.5)	9.9 (8.6)	8.8 (8.1)
Range (*n*)	4–40 (12)	BD–25 (12)	BD–22 (12)
Post-enrichment			
Reference			
Mean (1 SD)	16.9 (29.8)	10.4 (16.9)	3.7 (4.7)
Range (*n*)	BD–151 (33)	BD–76 (33)	BD–17 (33)
Treatment			
Mean (1 SD)	308.9 (377.8)	105.5 (119.7)	51.2 (55.6)
Range (*n*)	11–1711 (44)	6–566 (44)	BD–268 (44)

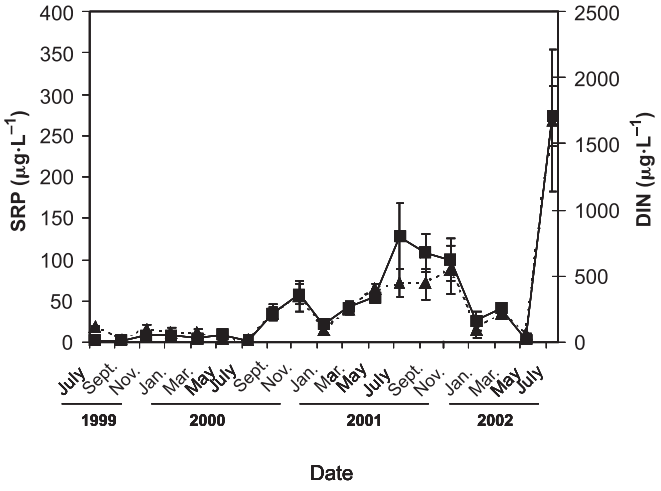
Note: SRP, soluble reactive phosphorus; BD, below detection.

the entire slurry fixed in a total of 20 mL of 2.5% formalin solution. Community composition and cell density were determined by counting at least 500 cells in a Palmer–Maloney nanoplankton counting chamber at 400× magnification. In cases where cell densities were too low to find 500 cells, 10 transects of the Palmer cell were examined. Diatom species were identified after cleaning with 30% H_2O_2 and $K_2Cr_2O_7$ to clear cell contents and permanently mounting in Naphrax high-resolution mounting medium. Species composition was assessed by identifying 300 complete frustules at 1000× magnification. In cases where cell densities were too low to find 300 frustules, 10 transects of the diatom slide were examined and all complete frustules found were identified. To calculate algal cell biovolume, we applied average dimensions of up to 10 cells in each taxon to standard geometric shapes that best represented the shape of each taxon (Hillebrand et al. 1999). Biovolumes from the Academy of Natural Sciences database from the Phycology Section of the Patrick Center for Environmental Research biovolume database (http://diatom.acnatsci.org/nawqa/2001biovol.asp) were used to estimate biovolumes for rarely encountered taxa.

Data analysis

Randomized intervention analysis (RIA) (Carpenter et al. 1989) was used to assess differences between the reference and treatments streams in AFDM, chlorophyll *a*, total biovolume, and relative biovolume of algal species with >5% average biovolume from tiles. RIA compares differences between unreplicated reference and treatment systems before and after an intervention (in this case, nutrient addition). A significant difference with RIA would result from an increase or decrease in the difference between reference and treatment streams. Appropriateness of parametric versus nonparametric tests for comparisons of seasonal levels of PAR and algal biomass and also growth rate data was determined by testing the data for normality by fitting to a normal distribution model. Owing to lack of fit with the normal distribution, Kruskall–Wallis with a Bonferroni correction for multiple tests (Ott and Longnecker 2001) and Tukey's multiple comparison tests were used to compare PAR and biomass data. Algal growth rates fit a normal distribution and differences between streams and individual trials were assessed with a two-way ANOVA and a Tukey test. Tests for normal distribution, Kruskall–Wallis, Tukey's, and the two-way ANOVA were performed with JMP (release 5.0.1a) (SAS Institute Inc., Cary, North Carolina).

Fig. 1. Stream water nutrient concentrations in the treatment stream ± 1 SE as soluble reactive phosphorus (SRP) (triangles) and dissolved inorganic nitrogen (DIN) (squares). Data points represent means from data collected during the previous 2 months.



Results

Nutrient enrichment

On average, the enrichment increased dissolved inorganic nitrogen approximately 13 times and SRP approximately five times to a long-term mean of ~400 µg NO_3^--N + NH_4^+-N·L^{-1} and 45 µg SRP·L^{-1} in the treatment stream (Fig. 1; Table 2). These nutrient concentrations are within the range of those found regionally in streams (Scott et al. 2003). Phosphorus and nitrogen varied during the enrichment period (Fig. 1) with decreased nutrient concentrations during early winter

Fig. 2. Algal biomass ± 1 SE as (*a*) chlorophyll *a* and (*b*) ash-free dry mass (AFDM) from the reference stream (open circles and broken line) and treatment stream (solid circles and solid line) (randomized intervention analysis (RIA), $p < 0.05$ for both chlorophyll *a* and AFDM).

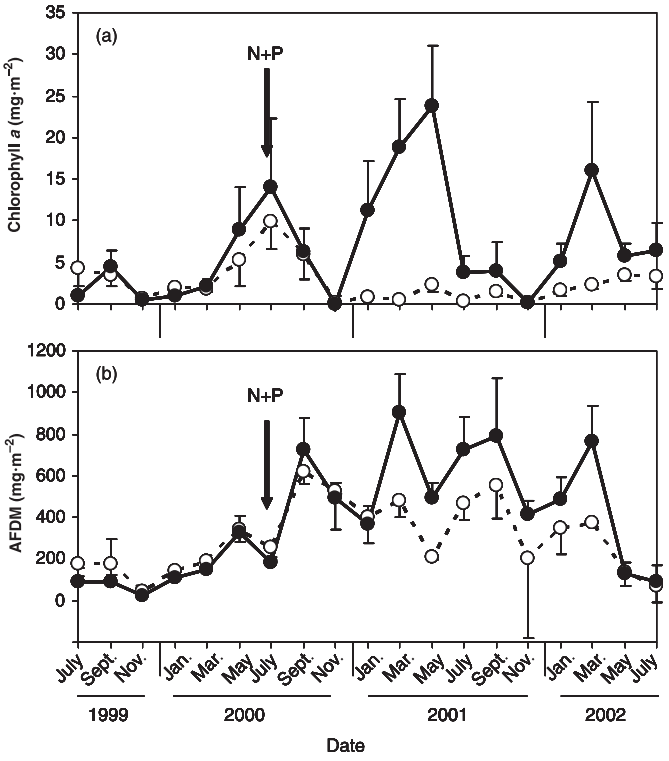
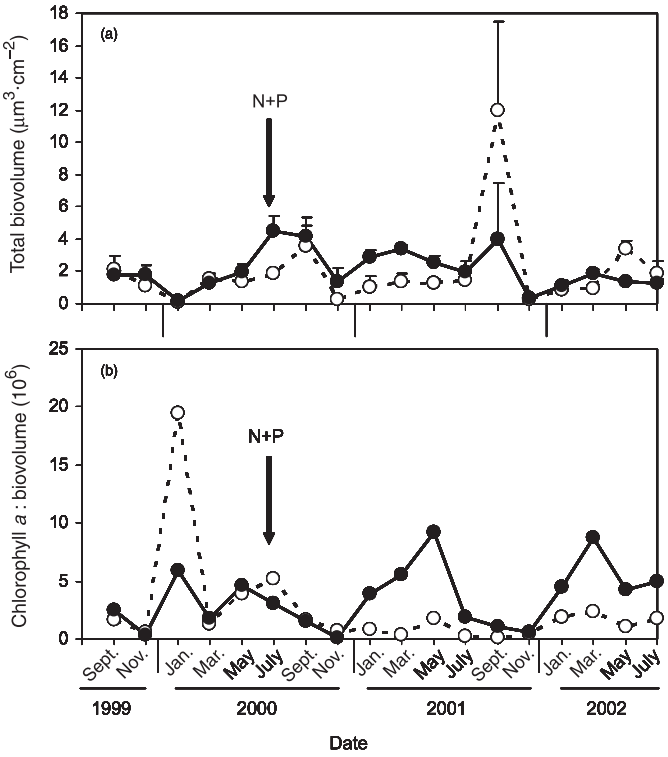


Fig. 3. Algal biomass ± 1 SE as (*a*) total biovolume and (*b*) chlorophyll *a* per biovolume from the reference stream (open circles and broken line) and treatment stream (solid circles and solid line). Randomized intervention analysis (RIA) showed that differences between streams before and after enrichment were not significantly different ($p > 0.05$).

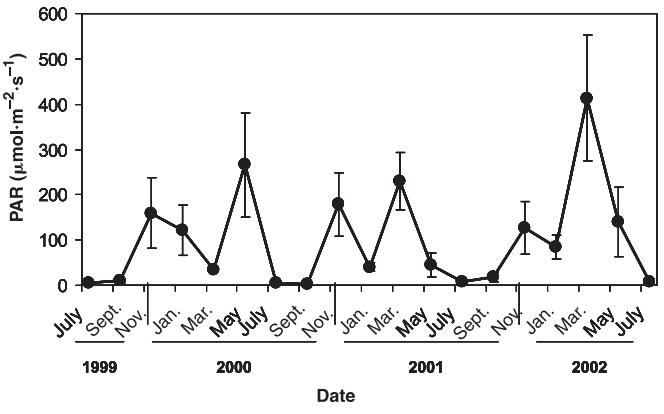


2001 and winter–spring 2002 that increased again in the summer and early fall of both years. Nutrient concentrations in the reference stream remained consistent across pretreatment and treatment periods with total inorganic nitrogen <30 μg·L^{-1} and SRP < 5 μg·L^{-1}. The mean nitrogen to phosphorus ratio of stream water collected from the treatment stream over the enrichment period was 19.7. This ratio contrasts with the 11.1 ratio that was added in our stock solution, indicating greater in-stream uptake of phosphorus relative to nitrogen.

Biomass response

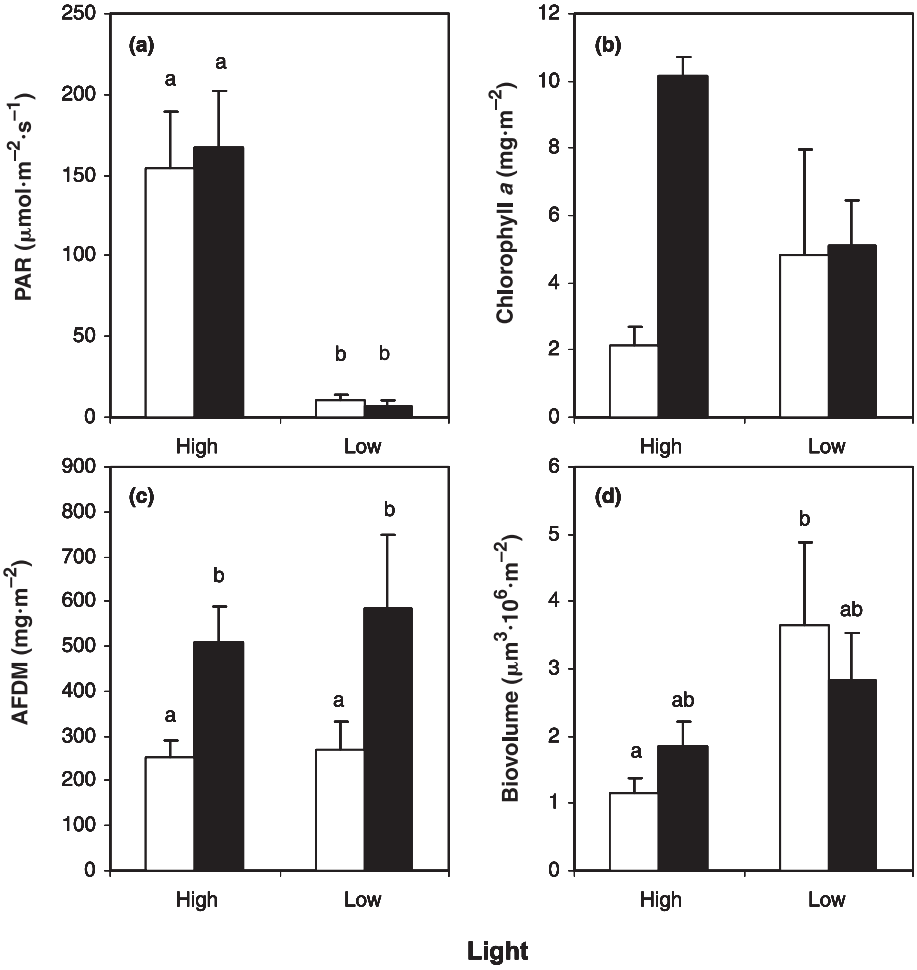
Periphyton biomass, as both AFDM (Fig. 2*b*) and chlorophyll *a* (Fig. 2*a*), was significantly greater in the treatment stream relative to the reference stream after nutrient enrichment (RIA, $p < 0.05$). Chlorophyll *a* was typically 2–9 mg·m^{-2} prior to enrichment and increased to 23 mg·m^{-2} in the treatment stream in year 1 (in May) of enrichment but only up to 17 mg·m^{-2} in year 2 (in March). Pretreatment values for ADFM were low and similar in the two streams (100–300 mg·m^{-2}), and although AFDM in both streams was higher in the period after July 2000, AFDM was roughly 50% greater in the treatment versus reference stream beginning in March 2001 (up to 900 mg·m^{-2}). Chlorophyll *a* showed a greater increase during the spring of each year compared with summer and fall. Increased AFDM in the treatment stream showed no such seasonal effect. There was very little difference in either AFDM or chlorophyll *a* between treatment and reference streams on our last two sampling dates in summer 2002.

Fig. 4. Photosynthetically active radiation (PAR) ± 1 SE averaged from the treatment stream ($n = 5$) and reference stream ($n = 5$).



Algal biomass as total biovolume (Fig. 3*a*) was not significantly different between streams after nutrient enrichment (RIA, $p > 0.05$). Biovolume exhibited a slight seasonal pattern where biovolume was lowest, <2 × 10^6 μm^3·cm^{-2}, during November or January and reached a maximum of ~4 × 10^6 μm^3·cm^{-2} in mid- to late summer. An extremely high value for biovolume in the reference stream after enrichment

Fig. 5. Average (*a*) photosynthetically active radiation (PAR), (*b*) chlorophyll *a*, (*c*) ash-free dry mass (AFDM), and (*d*) total biovolume ± 1 SE in the reference stream (open bars) and treatment stream (solid bars) during seasonal periods of high and low light availability. Different letters above the bars indicate Kruskal–Wallis at $p < 0.0125$ and Tukey's multiple comparison at $p < 0.05$.



was associated with one replicate having unusually high algal accrual. The ratio of chlorophyll *a* to biovolume (Fig. 3*b*) contained a very high value in the reference stream prior to enrichment owing to a very low biovolume value (0.1×10^6). This data point was considered spurious and was removed before RIA. Thus, there was also no significant difference between streams in the ratio of chlorophyll *a* to biovolume (RIA, $p > 0.05$) (Fig. 3*b*). However, there was a trend of higher values during the spring months in the treatment stream, as was seen for chlorophyll *a*.

Relationships between periphyton biomass and irradiance

Instantaneous PAR levels showed seasonal trends, with lowest levels in July and September and seasonal peaks in November and in May the first year and in March the next 2 years (Fig. 4) (light data were lost for July 2000 and January 2001 in the treatment stream because of instrument malfunction). To determine whether variation in periphyton response to nutrients was due to season, we compared average light levels during periods of higher (January, March, May, and November) and lower (July and September) light and then compared algal biomass between treatment and reference streams during these time periods. Regressions run between biomass measurements and light levels with and without enrichment were nonsignificant ($p > 0.05$) with little variation explained by the model ($r^2 < 0.05$), most likely because of the variability inherent in instantaneous light readings. Thus, we decided to use longer-term averages to assess the relationship between seasonal light and chlorophyll *a* levels. Data from the treatment stream during pretreatment were included with values for the reference stream. Instantaneous PAR levels averaged ~150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during November–May and 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during July–September (Fig. 5*a*). Light levels were significantly (Bonferroni-corrected significance value = 0.0125) higher during November–May in both streams compared with July–September (Kruskal–Wallis, $\chi^2 = 19.16$, df = 3, $p < 0.0005$; Tukey's multiple comparison, $p < 0.05$). Chlorophyll *a* levels were not significantly different between streams but peaked overall in the treatment stream during November–May (Kruskal–Wallis, $\chi^2 = 7.85$, df = 3, $p = 0.05$) (Fig. 5*b*). AFDM levels were significantly higher in the treatment versus the reference stream during both periods (Kruskal–Wallis, $\chi^2 = 11.22$, df = 3, $p < 0.01$; Tukey's multiple comparison, $p < 0.05$) (Fig. 5*c*). Biovolume was significantly higher only in the reference

Table 3. *F* values from two-way ANOVA of log algal cell accrual rate.

Treatment	df	Accrual rate
Nutrient	1	5.58* (enriched stream > reference)
Month	3	6.73** (March (=April) > June and July (=April))
Nutrient × month	3	ns

Note: Multiple comparison tests were conducted for significant ($p < 0.05$) effects of month with Tukey's test ($p < 0.05$). *, $p < 0.05$; **, $p < 0.005$; ns, not significant.

stream between high-light and low-light times of the year (Kruskal–Wallis, $\chi^2 = 13.71$, df = 3, $p < 0.005$; Tukey's multiple comparison, $p < 0.05$) (Fig. 5*d*). This difference was likely due to the unusually high biovolume measured in the reference stream in September 2001.

Growth rates

Nutrients had a significant positive effect on accrual rates of algal cells (Table 3; Fig. 6). Accrual rates were also significantly higher in March compared with June and July (Table 3), and the range in accrual rates in the treatment stream decreased by an order of magnitude from April to June and again by an order of magnitude from June to July (Fig. 6). However, differences between streams did not vary seasonally (nutrient by stream interaction was not significant) (Table 3).

Species composition

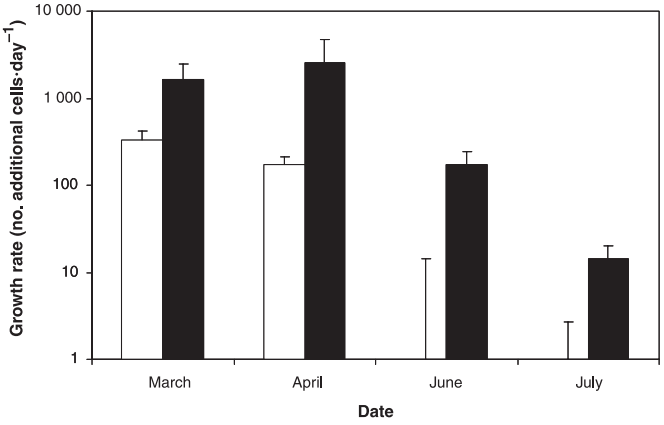
Diatom taxa were dominant (>98% of algal biovolume on average) in both the reference and treatment streams throughout the experiment (Table 4). Of the 35 taxa identified, most were rare, with five taxa making up nearly 95% of the biovolume in both streams (Table 5). None of these taxa showed significant differences in biovolume between the reference and treatment streams (RIA $p > 0.05$) (Fig. 7). By far, the dominant taxa in the streams were *Euntoia pectinalis* var. *minor* and *Meridion constrictum*, which exhibited somewhat predictable seasonal trends. Biovolume of *E. pectinalis* var. *minor* was low in the spring months and peaked in the late summer and fall (Fig. 7*a*), whereas biovolume of *M. constrictum* was greatest in spring and declined through the summer months (Fig. 7*b*). Although important contributors to total biovolume, *Eunotia pectinalis* var. *recta* (Fig. 7*c*) and *Gomphonema parvulum* (Fig. 7*d*) showed no strong seasonal patterns or treatment effects, except for an increase in *G. parvulum* in spring 2002. *Navicula tantula* showed a potentially delayed response to enrichment, increasing in relative biovolume from about 10% to nearly 50% in the treatment stream during the last two sampling dates (Fig. 7*e*).

Discussion

Effects of nutrient addition on stream periphyton

Our study showed how different aspects of the periphyton responded differently to nutrient enrichment, which may be useful in predicting chronic nutrient enrichment effects in similar headwater streams. Overall, we observed increased

Fig. 6. Increase in cell density per day ± 1 SE (logarithmic scale) between week 2 and week 4 of the algal growth experiment during March, April, June, and July trials for the reference stream (open bars) and treatment stream (solid bars).



algal productivity that resulted in small increases in periphyton biomass and no detectable change in algal assemblage composition. Algal cellular growth rates were stimulated by nutrient addition, but the magnitude of the response was small under seasonally low light levels (<100 cells·cm^{-2}·day^{-1}), suggesting constraints resulting from light availability. Periphyton biomass increased by some measures (chlorophyll *a* and AFDM) but not others (algal biovolume) and was small compared with standing crops of other food resources (e.g., leaf detritus) in the study streams (e.g., 0.5 g AFDM periphyton·m^{-2} compared with ~300–500 g AFDM leaves·m^{-2}; K. Suberkropp, Biology Department, University of Alabama, Tuscaloosa, AL 35487, USA, unpublished data). Notably, even the highest biomass values that we observed were low compared with benthic stream periphyton biomass measurements worldwide (Feminella and Hawkins 1995, Biggs 1996; Dodds et al. 1998). Effects of nutrient enrichment on biomass as chlorophyll *a* and cellular growth rates peaked during months with relatively higher irradiance; only biomass measured as AFDM, which includes heterotrophic components of periphyton, was consistently positively affected by nutrient enrichment. Short-term experimental studies have shown that a transient response to nutrient enrichment can be quite high in the absence of grazers and under high-light conditions (e.g., Rosemond 1993; Hillebrand 2002), whereas such responses are small when top-down consumption or other limiting factors also constrain algal biomass (e.g., Lowe et al. 1986; Peterson et al. 1993; Rosemond et al. 1993). Results from our long-term study in which periphyton biomass and taxonomic response was subdued or apparently controlled by other factors are consistent with such studies.

The size of effect that we observed on variables that responded to enrichment can be put into context of other responses of periphyton to environmental variation. For example, Feminella and Hawkins (1995) provided data from a wide range of stream systems examining grazing impacts on periphyton. For experiments that ran >4 weeks, chlorophyll a was ~40 mg·m^{-2} under grazed conditions and ~100 mg·m^{-2} under ungrazed conditions. In terms of defining nutrient-driven trophic state, Dodds et al. (1998) sug-

Table 4. Average cell biovolume ($\mu m^3 \cdot cm^{-2}$) and standard deviations (SDs) of all cells and algal divisions for reference and treatment streams during the pretreatment year and both years of nutrient enrichment.

Site	Total	Diatoms	Chrysophyta	Chlorophyta	Cyanobacteria
Pre-enrichment					
Reference					
Mean	1.35×10^6	1.3×10^6	21 259	1 004	116
SD (n = 30)	0.72×10^6	0.72×10^6	44 721	2 460	285
Treatment					
Mean	1.90×10^6	1.89×10^6	13 632	0	0
SD (n = 30)	1.44×10^6	1.45×10^6	21 201	1	1
Post-enrichment year 1					
Reference					
Mean	1.49×10^6	1.45×10^6	28 547	423	252
SD (n = 29)	1.10×10^6	1.12×10^6	39 317	1 037	617
Treatment					
Mean	2.71×10^6	2.70×10^6	427	4 721	15
SD (n = 29)	1.00×10^6	1.00×10^6	744	11 565	37
Post-enrichment year 2					
Reference					
Mean	3.22×10^6	3.18×10^6	33 631	1 865	550
SD (n = 29)	4.42×10^6	4.43×10^6	46 535	2 515	953
Treatment					
Mean	1.65×10^6	1.65×10^6	403	4 714	23
SD (n = 30)	1.23×10^6	1.23×10^6	987	11 546	57

gested mean chlorophyll *a* of 20 $mg \cdot m^{-2}$ and maximum chlorophyll *a* of 60 $mg \cdot m^{-2}$ as the boundary between oligotrophic and mesotrophic systems. Our average chlorophyll during high-light months in the nutrient-enriched stream was ~10 $mg \cdot m^{-2}$, and there were only three monthly average measurements for which chlorophyll *a* was >15 $mg \cdot m^{-2}$. Biggs (1996) found a median of 1.7 mg chlorophyll $a \cdot m^{-2}$ for benthic periphyton in unenriched streams compared with a median of 21 mg chlorophyll $a \cdot m^{-2}$ for enriched streams. Our observed values in the treatment stream approached the median for Biggs' (1996) enriched values at certain times but were not sustained at that level. Our AFDM values were extremely low, even in the nutrient-enriched stream, compared with other studies. Feminella and Hawkins (1995) reported values of 5000 – 40 000 mg AFDM $\cdot m^{-2}$ from various studies and Biggs (1996) reported medians of 1500 mg AFDM$\cdot m^{-2}$ (unenriched) and 4800 mg AFDM$\cdot m^{-2}$ (enriched). Our values, even in the nutrient-enriched stream, were <1000 mg AFDM$\cdot m^{-2}$ (~250–550 in unenriched versus enriched stream on average). The lack of a strong biomass response in this study may have been due to control by other limiting factors that determine the chronically low standing crop of periphyton in these streams.

The duration of our experiment was likely long enough for potential changes in periphyton biomass and algal assemblages to occur. The other long-term whole-stream enrichment that we are aware of, conducted in the Kuparak River, represents 16 years of enrichment during the growing season (for ~45 days each year, total = 720 days) (Slavik et al. 2004). Because of the more moderate climate in our study area compared with the Alaskan tundra, our continuous enrichment represents 730 days for potential periphyton response. These conditions represent several to hundreds of generation times for algal populations, allowing for a maximum potential bottom-up response. Lengthening the time of our study would have likely only affected the potential for a nutrient response of periphyton to be constrained by consumption. Specifically, a longer time frame would allow greater potential for increased production of consumers to control periphyton response; larval life span of dominant potential herbivores in these streams ranges from 120 to 365 days (W. Cross, El Verde Field Station, HC-05 Box 8974, Rio Grande, Puerto Rico 00745, unpublished data).

Irradiance was likely an important factor in the expressed response of periphyton to nutrient enrichment. Seasonal variation in chlorophyll *a*, the amount of chlorophyll *a* per cell, and measures of algal cell growth pre- and post-canopy closure suggests that irradiance influenced all of these variables. The variation in nutrient concentrations in the enriched stream over the course of the enrichment did not likely have overriding effects on the seasonal responses of the above variables, as the peaks in nutrient levels measured in the stream did not coincide with peaks in chlorophyll measurements or growth rates. Seasonal variation in chlorophyll *a* appeared to be at least partly due to a trend of increased chlorophyll *a* produced per cellular unit rather than an increase in biovolume or cell densities. In previous studies, increased chlorophyll *a* per cell in microalgae occurred in response to increased nutrients (Rosen and Lowe 1984; Geider et al. 1993; Rosemond 1993). Interestingly, the nutrient-driven response of higher chlorophyll *a* per cell under high-light conditions that we observed is opposite to the predicted response (in the absence of enrichment) in which taxa with greater relative chlorophyll *a* per cell or a physiological shift to greater chlorophyll *a* per cell is observed in response to low-light environments (e.g., Rosemond 1993; Felip and Catalan 2000). Contributing to this pattern was that total biovolume was temporally vari-

Table 5. Algal taxa encountered from periphyton samples on tiles.

	Reference	Treatment
Bacillariophyta		
Achnanthes deflexa	—	R
Achnanthes stewartii	R	R
Achnanthes subrostrata var. *appalachiana*	U	U
Achnanthidium minutissimum	R	R
Cymbella tumida	—	R
Diatoma hiemale var. *mesodon*	R	R
Encyonema minutum	R	R
Eunotia curvata	R	R
Eunotia exigua var. *exigua*	R	R
Eunotia pectinalis* var. *minor	**A**	**A**
Eunotia pectinalis* var. *recta	**C**	**C**
Fragilaria vaucheriae	R	R
Frustulia rhomboidies	R	R
Gomphonema acuminatum var. *pusillum*	R	R
Gomphonema gracile	R	R
Gomphonema parvulum	**C**	**C**
Melosira varians	—	R
Meridion constrictum	**A**	**A**
Navicula angusta	R	R
Navicula placenta	U	R
Navicula tantula	**U**	**C**
Nitzschia palea	R	R
Nizschia dissipata	R	R
Pinnularia mesogongyla	R	R
Pinnularia subcapitata var. *paucistriata*	—	R
Planothidium lanceolatum	R	R
Surirella angusta	R	R
Synedra minuscula	R	R
Synedra rumpens var. meneghiniana	R	R
Synedra ulna	R	R
Chrysophyta		
Unidentified stomatocysts	U	R
Chlorophyta		
Mougeotia sp.	R	R
Cyanobacteria		
Chamaesiphon sp.	R	R
Unidentified spheres	R	R
Unidentified filaments	R	R

Note: Categories based on average relative biovolume across all samples (% of relative biovolume): A, abundant (>20%); C, common (5–20%); U, uncommon (1–5%); R, rare (<1%). A long dash (—) means that the species was not detected in that stream. Combined species in bold accounted for 95% of the biovolume and were analyzed for responses to nutrient enrichment.

able, was not affected by enrichment, and showed a trend to be higher in low- versus high-light months, whereas chlorophyll *a* exhibited a response to nutrients in only high-light months. This suggests that physiological changes and the manufacturing of the nitrogen-containing chlorophyll molecule were limited by nutrient availability but were not consistently accompanied by increased algal biomass.

Stoichiometric changes in epilithon were also observed in which both the carbon to nitrogen and carbon to phosphorus ratios (8.7 to 4.6 and 1741 to 845, respectively) of epilithic scrapings in the treatment stream were greatly reduced compared with the reference stream (Cross et al. 2003). In the absence of substantial changes in periphyton assemblage composition, changes in stoichiometry may signal other physiological changes in periphyton in response to enrichment. However, since these nutrient concentrations were measured from the entire epilithic community, it cannot be determined if the source of change was from autotrophic, heterotrophic, or both components of the periphyton.

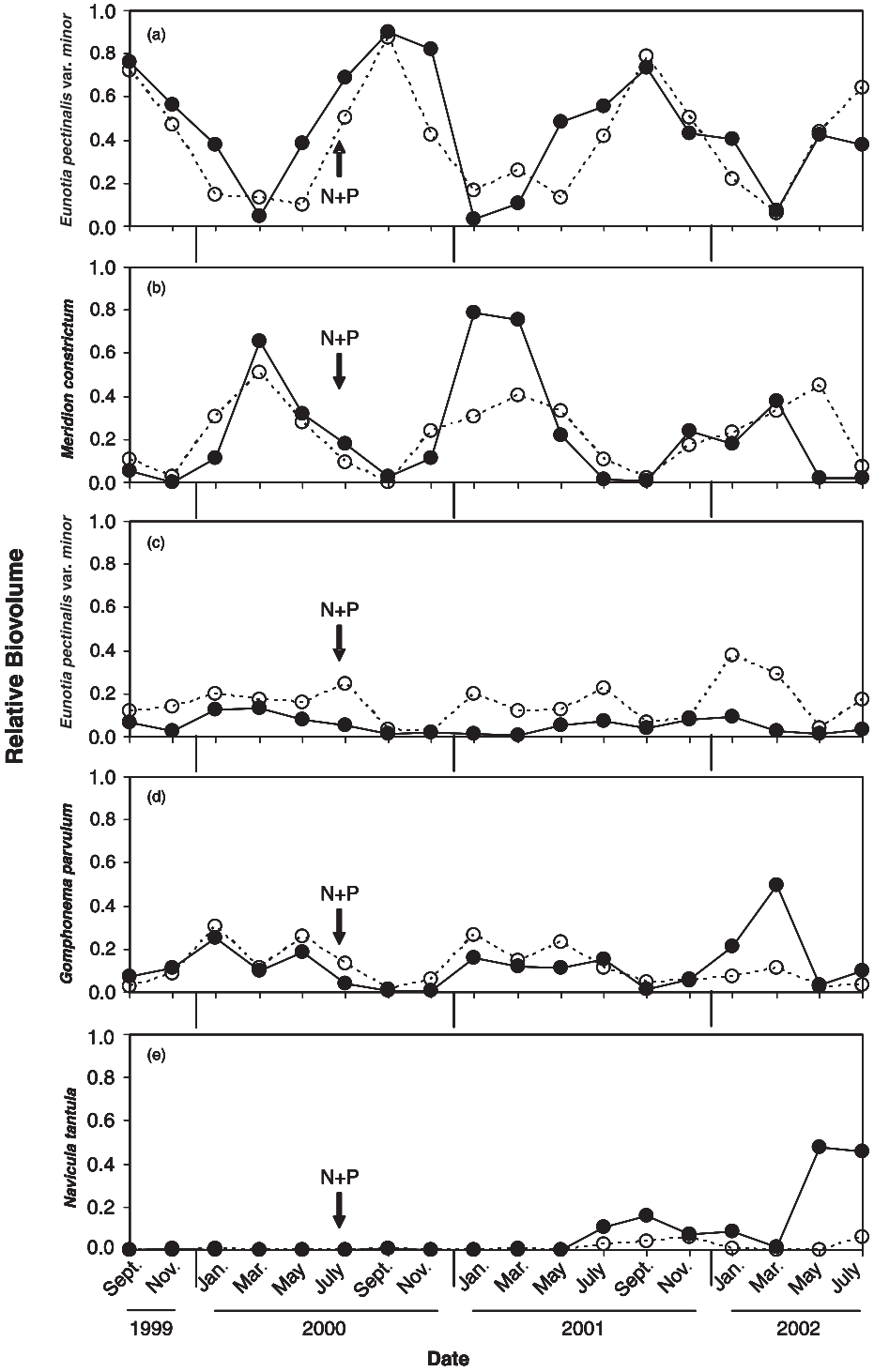
Our data indicate that heterotrophic compared with autotrophic components of periphyton biomass responded more consistently to nutrient enrichment. AFDM, which is a measure of periphyton biomass that likely includes heterotrophic components (e.g., fungi, bacteria, and trapped organic particles), increased in response to enrichment, but quantification of the strictly algal response, algal biovolume, did not. Heterotrophic microorganisms associated with leaves (Gulis and Suberkropp 2003) and wood (Gulis et al. 2004) increased dramatically in the treatment stream during this study, indicating that positive effects of nutrients on heterotrophs living on other substrates (e.g., tiles) would be likely. However, autotrophs may also have contributed to variation in AFDM via the production of extracellular organic material by algae, which could potentially increase in response to enrichment (Hoagland et al. 1993).

How much of a lack of algal response could be attributed to herbivore consumption versus light availability?

The seasonal pattern in algal growth rates and chlorophyll *a* was most likely the result of light availability in these headwater streams. The maximum response observed in chlorophyll *a* and growth rates, the only definitive responses to enrichment, occurred when light levels reaching the stream were high. Also, data from whole-stream metabolism measurements (P.J. Mulholland, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA, unpublished data) showed the highest rate of gross primary production (GPP) in both treatment and reference streams during April compared with other seasonal measurements, although overall, GPP was always very low (typically <3 mg $O_2 \cdot m^{-2} \cdot day^{-1}$ with one value of ~9 mg $O_2 \cdot m^{-2} \cdot day^{-1}$). Other studies have shown that benthic algal productivity can be primarily limited by light (Hill et al. 1995, 2001; Hill and Knight 1988) and that the importance of light, relative to other limiting factors, can change seasonally (Rosemond et al. 2000). Rates of photosynthesis generally reach a maximum at light levels between 100 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in forested streams (Hill et al. 1995). Instantaneous irradiance levels during spring and fall often reached or exceeded 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in both streams, indicating that light limitation was most likely less important during this time than during the summer when irradiance levels were <20 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Overall biomass of periphyton remained low in both streams despite increased productivity of algal assemblages as cellular growth rates in the treatment stream. Our measurements of cellular growth rates, which were greatest in magnitude prior to canopy closure (March and April), were

Fig. 7. Relative biovolume of the five most common species: (*a*) *Eunotia pectinalis* var. *minor*, (*b*) *Meridion constrictum*, (*c*) *Eunotia pectinalis* var. *recta*, (*d*) *Gomphonema parvulum*, and (*e*) *Navicula tantula* from the reference stream (open circles) and treatment stream (closed circles). Randomized intervention analysis (RIA) showed that differences between streams before and after enrichment were not significantly different.



made in the absence of potential macroinvertebrate grazers. It is possible that in situ consumption by invertebrate grazers precluded such increased productivity from resulting in higher algal biomass in the study stream. Specifically, despite observed nutrient-driven increases in algal growth rates that were fairly large in magnitude, rates of primary productivity in these streams are so low that invertebrate grazers can likely consume any accumulated biomass. As a potential indication of herbivores tracking and controlling algal resources in these streams, we examined whether invertebrate scraper biomass was similarly higher in higher light months, as we had observed with chlorophyll *a*. Over the time period

of enrichment, mean monthly scraper biomass from rockface substrata was as follows: for June–October (n = 10 per stream), 23.1 mg·m^{-2} (3.4 SE) in the reference stream and 26.2 mg·m^{-2} (8.6 SE) in the treatment stream and for May–November (n = 14 per stream), 41.4 mg·m^{-2} (8.7 SE) in the reference stream and 91.2 mg·m^{-2} (29.6 SE) in the treatment stream (overall means based on n = 3 means from rockface substrate taken monthly; methods in Cross 2004). Scraper biomass was highest in the treatment stream during the high-light period but not significantly so and overall differences in scraper biomass between streams were not significant (Cross 2004). Carbon flow to scrapers was also on the order of GPP in these streams, suggesting that they could keep up with any excess primary production. Carbon production estimates translate to 0.2 g AFDM·m^{-2}·year^{-1} (based on GPP of 3 mg O_2·m^{-2}·day^{-1}), whereas carbon flow to scrapers was on the order of 0.3 and 0.5 g AFDM·m^{-2}·year^{-1} (reference versus treatment, year 1) and 0.2 and 0.4 g AFDM·m^{-2}·year^{-1} (reference versus treatment, year 2) (Cross 2004). These values suggest that overall primary production and flow of energy to scrapers was low, but an increased value in the treatment stream suggests some increased carbon going to scrapers under elevated nutrient conditions. Further, the increase in scrapers provides support for the possibility that autotrophic components of periphyton were under dual control by nutrients and light levels, since potential grazer response to nutrient addition was only during the spring months.

Limited assemblage composition response

There was remarkable constancy in the composition of the benthic algal assemblages in both study streams despite long-term changes in the availability of nutrients in the treatment stream. There were actually more pronounced seasonal effects in algal assemblages compared with nutrient effects, suggesting that light availability was driving the species distribution in this study. *Eunotia pectinalis* var. *minor* and *M. constrictum* seemed to trade off dominance in the community, with *E. pectinalis* var. *minor* making up most of the biovolume when light availability was seasonally low and *M. constrictum* dominating during periods of high light. *Eunotia pectinalis* var. *minor* also exhibited preference for low light levels in another deciduous forest stream study (Rosemond 1993), and *Meridion circulare*, a taxon related to *M. constrictum*, was shown to be stimulated by higher light levels (Rosemond et al. 2000), consistent with seasonal patterns observed in this study. However, *Meridion* has been shown to prefer colder temperatures (Patrick 1971; Lowe 1974), which may explain its peak during the colder months of the year. Toward the end of the study period, two taxa, *G. parvulum* and *N. tantula*, were potentially responding to enrichment, indicating that possibly the algal assemblage was beginning to change toward the end of the experiment. The increase in *G. parvulum* occurred during the high-light months and in another study at Coweeta was more common in a clearcut stream (Lowe et al. 1986), suggesting a preference for higher light levels. *Navicula tantula* has been described as preferring slightly enriched environments (Bahls 1993; Kentucky Division of Water 2002), which may explain its increase during the last two sampling dates.

The species pool found on tiles in these headwater streams was largely limited to diatoms, with little presence of chlorophytes or cyanobacteria. Whatever factors restrict taxonomic distribution in our samples may have ultimately constrained any response that we observed of benthic algae to nutrient enrichment. Chlorophytes might be expected more during seasonal periods of high light, as was observed in a nearby stream at Coweeta that had been logged (Lowe et al. 1986). Although no seasonal response of chlorophytes was seen, biovolume was slightly higher in the treatment stream with enrichment, indicating a potential response to nutrients. However, chlorophytes were too rare to submit to statistical analysis. Cyanobacteria have been known to outcompete diatoms in low-light situations (Stevenson et al. 1985) and could be expected to thrive during nutrient enrichment, but cyanobacteria were rarely encountered in our tile samples.

An analysis of algal communities from different substrata (moss, liverwort, and bedrock) from approximately the last two sampling dates of this study indicates that communities from tiles were largely representative of algal communities on natural substrates (Greenwood 2004). Bryophytes were important primary producers in these streams but showed no biomass response to nutrient enrichment (Greenwood 2004). About 80% of the taxa found on bedrock were also found on tiles (the other 20% dominated by cyanobacterial filaments), but dominant taxa from tiles only comprised 5%–65% of the biovolume on bedrock. In addition, the differences between assemblages on moss, liverworts, or bedrock in treatment versus reference streams were tested and no consistent changes from enrichment were found (Greenwood 2004). These results suggest that regardless of substrata, response of the algal community to nutrient enrichment was minimal.

The species pool of algae in these streams was mostly limited by factors other than nutrients. Other studies have found very little change in algal species composition in response to nutrients under conditions of intense grazing (assemblage composition was driven by grazer resistance more than by response to nutrients (e.g., Hill et al. 1992; Rosemond et al. 1993). However, the dominant taxa in our study streams would not necessarily be characterized as a flora indicative of a heavily grazed system (e.g., some taxa are stalked and produce upright cells in contrast with dominance by prostrate adnate taxa that are grazer resistant; Steinman 1996). Physical or chemical characteristics (overall low irradiance levels, high turbulence, and softwater chemistry) of these streams may also have overriding controls on potential assemblage composition. Regardless of which other controlling factors were most important, subtle physiological changes such as increased chlorophyll *a* or nutrient content did not translate into enough differential activity among different algal species to result in any shifts in species composition.

Two years of continuous moderate nutrient enrichment resulted in neither shifts in algal assemblage composition nor sustained large effects on algal biomass. This limited response can probably be attributed to the low initial standing crop of periphyton, other factors controlling biomass and productivity, and to a limited taxonomic pool of potential responders to nutrient addition. In contrast with nutrient effects on primary producers, analyses of heterotrophic

microbial response in these study streams has revealed dramatic effects of nutrient addition on bacteria and fungi associated with leaves and wood (Gulis and Suberkropp 2003; Gulis et al. 2004; K. Suberkropp, Biology Department, University of Alabama, Tuscaloosa, AL 35487, USA, unpublished data). Although we have evidence that shading and potentially invertebrate consumption contributed to the limited response of benthic algae, other factors that ultimately limited the algal species pool are unknown but are likely associated with the physiochemistry of undisturbed shaded headwater streams. Our data show that in such streams, if otherwise undisturbed, periphyton will respond very little to moderate levels of long-term nutrient enrichment.

Acknowledgements

This work was funded in part by the National Science Foundation (DEB-9806610) (A.D. Rosemond, J.B. Wallace, K. Suberkropp, and P.J. Mulholland). JLG acknowledges additional support from the University of Georgia Graduate School via a Dissertation Completion Award. We thank Holly Weyers, Ted Siler, Sue Eggert, Megan Hagler, Paula Marcinek, and Mike Busbee for field and laboratory assistance. Suggestions from Judy Meyer, Bruce Wallace, Sue Eggert, Wyatt Cross, and two anonymous reviewers improved earlier versions of this manuscript.

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