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Effects of N : P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition

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Abstract

The effects of nutrient ratios on algal community structure and algal growth have been examined extensively in lakes and marine environments, but rarely in streams. We manipulated stream water N : P ratio (65 : 1, 17 : 1, 4 : 1) and total nutrient concentration (low and high) in a factorial experiment using once-through streamside flumes and measured responses in abundance, community structure, and elemental composition of periphyton communities. Early in the experiment, periphyton chlorophyll *a* and total algal biovolume were higher for treatments where N was added (high total nutrient concentration) but were not affected by N : P ratio. This response is contrary to our prediction that P would limit periphyton growth based on the high N : P ratio in the source water and unamended periphyton mats. The relative abundance of nine of eleven common algal taxa was affected by N : P ratio, total nutrient concentration, or both. Overall, algal community structure was more sensitive than bulk measures of periphyton abundance to changes in N : P ratio and total nutrient concentration. Periphyton %N and %P increased with the N and P concentration of stream water, and periphyton N : P tracked stream water N : P ratio. Responses in periphyton chemical composition to nutrients could affect the food quality of periphyton for consumers.

The relative amounts of essential nutrients required for growth and reproduction typically differ among plant species. This knowledge led researchers to develop the concept of optimal nutrient ratios, at which the supplies of nutrients are balanced to the growth requirements of the plant (Tilman 1977; Rhee and Gotham 1980; Tilman 1982). Resource-ratio theory, first developed for phytoplankton (Tilman 1977, 1982, 1985), predicts that changes in the environmental ratios of two essential nutrients will cause changes in plant community structure due to exploitative competition among taxa with different optimal nutrient ratios. Two species with different optimal nutrient ratios are able to coexist only if each species is a better competitor for the nutrient most limiting to the other species. It is unclear whether benthic algae compete exploitatively for nutrients, although evidence is rapidly accumulating that periphyton in lakes (e.g., Fairchild et al. 1985) and streams can be nutrient limited (Bothwell 1989; Pringle 1990; Harvey et al. 1998). Benthic algae in flowing waters may have less ability to affect nutrient availability than phytoplankton in lakes because lotic algae are generally exposed to a continuous supply of nutrients (but *see* Mulholland et al. 1995). Exploitative competition for nutrients is probably most intense in dense algal mats, where algae probably affect nutrient availability through nutrient uptake and where diffusibility is reduced (Burkholder et al. 1990).

Because resource-ratio theory assumes that plants compete exploitatively for nutrients, care should be exercised when applying resource-ratio theory to benthic algal communities. Reasons other than exploitative competition for nutrients, however, suggest that benthic algae should respond to variation in environmental nutrient ratios and concentration. For example, at high environmental N : P ratios, a benthic algal species with a high affinity for P (sp. A) may be able to grow faster, and monopolize space better, than a species that is less efficient at P uptake (sp. B). When the external N : P ratio is low, a greater maximum growth rate may allow sp. B to become dominant. Thus, shifts in the relative abundance of benthic algal taxa could occur along a N : P ratio gradient, without exploitative use of nutrients. The effects of resource ratios on the community structure of benthic algae have been examined in the laboratory (Sommer 1996), in lakes (Fairchild et al. 1985), and to a limited extent in streams (Stockner and Shortreed 1978). Investigations of the effects of resource ratios on phytoplankton, in culture (Tilman 1977, 1981; Fujimoto et al. 1997), in lakes (Schindler 1977; Tilman 1977; Smith 1983; Interlandi et al. 1999), and in marine environments (Sommer 1988) have been more common.

Ecologists often use dissolved and cellular nutrient ratios to predict which nutrient will limit overall algal production (e.g., Hillebrand and Sommer 1997, 1999), as measures of nutrient deficiency (e.g., Bothwell 1985), and sometimes as a tool for nutrient abatement efforts (*see* Smith 1998). Most predictions of nutrient limitation use as a benchmark the Redfield ratio of 106C : 16N : 1P, a composite optimal nutrient ratio empirically determined for oceanic seston (Redfield

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Table 1. Chemical and physical variables measured in the East Branch of the Maple River at the inflow to the stream research facility during the experiment.

Parameter*	Unit	Mean ± SD†
NO_3-N + NO_2-N	μg liter^{-1}	13.8 ± 6.3
NH_4-N	μg liter^{-1}	31.4 ± 10.1
DIN	μg liter^{-1}	45.2 ± 12.2
SRP	μg liter^{-1}	1.3 ± 0.7
TP	μg liter^{-1}	9.1 ± 6.2
N : P	—	84.3 ± 28.9‡
SiO_2	mg liter^{-1}	6.4 ± 1.3
Cl^-	mg liter^{-1}	2.7 ± 1.3
pH	—	8.0 ± 0.0
Water temperature	°C	13.9 ± 1.5

* DIN = dissolved inorganic nitrogen, SRP = soluble reactive phosphorus, TP = total phosphorus, N : P = DIN : SRP ratio in moles.
† n = 5 for all parameters except water temperature, where n = 18.
‡ The high N : P ratio treatment (*see Table 2*) is within the range of N : P ratios measured in ambient inflow water; because SRP is near the limit of detection in the EBMR, small changes in the SRP of ambient water have large effects on ambient N : P ratio.

1958). Deviation from the Redfield ratio has been used as an indication of which nutrient is limiting, especially when nutrient concentrations are low to moderate. For example if N : P $\gg$ 16 : 1, P is assumed to be limiting. Nutrient ratios have value in determining potential nutrient limitation, but nutrient concentrations must also be considered to determine actual nutrient limitation (Bothwell 1985).

In addition to influencing algal community composition and production, nutrients can also affect algal elemental composition, as illustrated with nutrient kinetics theory (Borchardt 1996). For example, Michaelis-Menten kinetics predicts that the nutrient content of algae will be an increasing saturating function of the concentration of a limiting nutrient. Workers have shown that the nutrient content of phytoplankton (e.g., Rhee 1978) and periphyton (e.g., Bothwell 1985; Mulholland and Rosemond 1992) increases with nutrient availability.

In this study, we investigated how N : P ratio and total nutrient concentration affect the community structure, biomass, and elemental composition of stream periphyton. We manipulated N : P ratio and total inorganic N and P concentration in artificial streams and measured the response of periphyton communities. Our study is the first replicated experiment that has explicitly examined how the community structure of stream algae responds to nutrient ratios. Our objectives were to determine (1) whether algal community structure is affected by N : P ratio and total nutrient concentration, (2) whether the response of periphyton biomass to N : P ratio and N and P concentration is consistent with predictions based on the Redfield ratio, and (3) whether periphyton elemental composition responds to external nutrients as predicted from nutrient kinetics theory.

Methods

Stream research facility—The experiment was conducted in 30 once-through flumes (2.8 m long × 10 cm wide × 7 cm high vinyl gutters) at the Stream Research Facility (SRF), University of Michigan Biological Station (UMBS), Douglas Lake, Michigan. Flumes were fed by water pumped from the East Branch of the Maple River (EBMR) using a Monarch pump with 2.54-cm holes in the impeller. EBMR is a third-order, low-nutrient stream that flows through second-growth forest (Table 1). EBMR drains Douglas Lake but also receives substantial groundwater input that contributes to relatively stable surface water nutrient concentrations, water temperature, and discharge (Robert Vande Kopple, UMBS, unpubl. data). Thus, EBMR water is ideally suited for nutrient manipulation experiments.

Table 2. Treatments, measured nutrient concentrations and ratios in flumes (Mean ± SD, n = 4). Ambient (unamended streamwater) treatment is indicated with bold.

Treatment		DIN*	SRP†	Molar
N : P	TNC‡	(μg liter^{-1})	(μg liter^{-1})	N : P ratio
High	**Low**	**43.4 ± 29.8**	**1.4 ± 0.6**	**62.9 ± 16.7**
High	High	152.9 ± 34.4	5.1 ± 1.5	68.9 ± 16.1
Medium	Low	60.1 ± 42.3	7.1 ± 0.7	18.3 ± 11.9
Medium	High	135.4 ± 17.5	18.0 ± 2.3	16.8 ± 2.7
Low	Low	51.7 ± 37.2	27.9 ± 3.4	4.0 ± 2.6
Low	High	136.2 ± 32.4	68.9 ± 6.0	4.4 ± 1.1

* DIN = dissolved inorganic nitrogen.
† SRP = soluble reactive phosphorus.
‡ TNC = total nutrient concentration.

Experimental design and nutrient addition—Three different N : P ratios (low, medium, and high) were crossed with two levels of total nutrient concentration (TNC) (low and high) for a total of six treatments, each replicated five times (Table 2). Low and high TNC treatments had low and high N concentrations, respectively; however, the range in P concentrations overlapped considerably between TNC treatments in order to provide appropriate N : P ratio levels. Therefore, the TNC treatment levels mainly reflect N rather than P concentrations in stream water. Treatments were randomly assigned to the 30 flumes. N : P ratios were determined as DIN (dissolved inorganic nitrogen) divided by soluble reactive phosphorus (SRP) in molar quantities. The high N : P, low N treatment was unamended EBMR water. The other N : P ratio treatments were achieved by adding P to EBMR water, thus lowering the N : P ratio. Nitrogen, in addition to P, was added to achieve the high TNC treatments. Nutrient concentrations were chosen to cover a range at which algal mats might be nutrient limited at the low end and nutrient saturated at the high end (Bothwell 1989) and were representative of Midwestern U.S. streams (Johnson et al. 1997*a*). Nutrients were added continuously as solutions of $NaNO_3$ and $NaH_2PO_4 \cdot H_2O$ directly into the inflow water stream using Manostat multichannel peristaltic pumps (pump rate ~ 0.8 ml min^{-1}). A tile dam 40 cm from the head of each flume helped to ensure complete mixing of water as verified by dye releases. Because of the stability of background nutrients in the EBMR and the precise flow capabilities of the peristaltic pumps, nutrient ratios and concentrations in the flumes were fairly consistent throughout the experiment. However, DIN in the EBMR decreased by ~15

μg L^{-1} over the course of our experiment. We began adding nitrogen as $NaNO_3$ to low N flumes on day 22 to bring N closer to the 45 μg DIN L^{-1} target concentration for these flumes.

Nylon mesh (nominal mesh size 0.2 mm) was placed over the inflow nozzles of each flume to reduce invertebrate colonization. Flumes were shaded during the experiment with fiberglass window screening to reduce light to 15% of incident levels, which were typically 1,500 μmol m^{-2} s^{-1} during full sun at the SRF. This reduced light level ($\sim$225 μM) was representative of light conditions in the moderately shaded EBMR. Water temperature in the flumes was measured regularly between 900 and 1100 h with a handheld thermometer and ranged from 12 to 17°C. Water discharge was measured in all flumes daily and was adjusted as necessary with valves at the head of each channel. Mean discharge (n = 29 measurements from each flume) in the flumes ranged between 0.251 and 0.256 L s^{-1} among treatments. Water velocity within the flumes was maintained at 35–40 cm s^{-1}.

Seventy clay tiles (49 mm long × 35 mm wide × 10 mm high), previously leached in distilled water and then autoclaved, were placed in each flume for periphyton colonization. The experiment began on the same day as nutrient addition. Periphyton was sampled for chlorophyll *a* (Chl *a*), algal taxonomic composition, and elemental composition on days 9, 17, and 28. The 28-d interval encompassed a full cycle of periphyton colonization, growth, and sloughing. On each sampling day, three pairs of tiles were chosen at random from each replicate flume (one pair from each nominal third of flume). Two tiles were placed on ice and stored at −20°C for subsequent Chl *a* analysis (on day 28 four tiles were sampled for Chl *a* due to increased patchiness of periphyton as a result of sloughing episodes). Two tiles for elemental composition were gently agitated to remove loose fine particulate organic matter (FPOM), rinsed gently with distilled water, placed on ice, and then frozen. Periphyton for algal taxonomic composition was scraped from surfaces of two additional tiles (total area of 13.5 cm^2) with a razor blade and preserved in Lugols solution. Water samples for analysis of SRP, NH_4-N, and NO_3-N + NO_2-N were taken at the outflow of each flume every 5 to 7 d to confirm nutrient treatments. Samples were placed on ice, vacuum filtered the same day through 25-mm Whatman GF/F filters, and stored at −20°C until nutrient analysis was performed.

Sample processing and analysis—Stream water SRP was measured using the ascorbic acid method (American Public Health Association 1992). To increase sensitivity we modified the method for use with a 10-cm cuvette and measured absorbance on a Milton Roy Spectronic 501 spectrophotometer. NO_3-N + NO_2-N was measured on a Lachat QuickChem 8000 autoanalyzer using cadmium reduction. NH_4-N was measured colorimetrically on a Perkin-Elmer Lambda 6 spectrophotometer after Solórzano (1969).

Periphyton for Chl *a* analysis was scraped from the tiles with a razor blade into a beaker and then homogenized using a tissue grinder. Two 1-ml aliquots were extracted in a final concentration of 90% buffered acetone overnight at 4°C. Absorbance was read on a Spectronic Genesys 2 spectrophotometer and Chl *a* corrected for pheophytin was calculated after APHA (1992). Residual Chl *a* on scraped tiles was measured by whole extraction of tiles in 90% buffered acetone and added to Chl *a* obtained from scraping. On day 9, Chl *a* was measured solely by whole-tile extraction because of low periphyton abundance.

Samples for periphyton elemental composition were lyophilized with a Virtis FreezMobile 12 at −45°C under a 100 millitorr vacuum, homogenized with a glass rod and scissors, and stored in a desiccator in the dark until chemical analysis. Dry masses of subsamples for elemental analysis were measured to ±1 μg on a Perkin-Elmer AD-4 Autobalance. Carbon and N (%) were measured on a Perkin-Elmer 2400 Series CHN analyzer with acetanilide as an internal standard. Periphyton total carbon was calculated by multiplying periphyton dry mass by %C from the CHN analysis. Periphyton for total P analysis was combusted at 500°C for 1 hr, followed by addition of 1N HCl and incubation at 80°C for 30 min (method after Mulholland and Rosemond 1992). After dilution, aliquots were analyzed for SRP using the ascorbic acid method (APHA 1992). Apple leaves (U.S. National Institute of Standards and Technology) were used as an external standard for all periphyton elemental analyses. Percentage recovery averaged 93% for P and 101% for N.

Periphyton samples for algal taxonomic identifications were homogenized and diluted as necessary. Temporary wet mounts were made by placing 80 μl of the mixture on a microscope slide and sealing the edges of a coverslip with nail polish. Algae were identified and enumerated at 1,000 times magnification using oil immersion with an Olympus BH2 microscope under bright field illumination. All cells containing protoplasm within a microscope field were counted in a series of 40–100 fields (generally 400 to 600 cells per slide). For common taxa the dimensions of 8–12 cells (fewer cells measured on rare taxa) per treatment per date were measured with an ocular micrometer and were used to calculate biovolume with BIOVOL v. 2.1 software (created by D. Kirschtel, University of Vermont). Taxa were identified to species in most cases. Because responses of individual species to nutrients generally were consistent with the overall response of the genus, taxa were pooled at the generic level for analysis.

Statistical analysis—Effects of N:P and total nutrient concentration on periphyton abundance, periphyton elemental composition, and relative abundances for individual algal taxa were analyzed using a repeated-measures two-way analysis of variance (ANOVA). Results of univariate analyses were confirmed with multivariate repeated-measures ANOVA when compound symmetry was in doubt (Crowder and Hand 1990). In some cases, one-way ANOVAs were performed for separate dates followed by Tukey multiple comparison tests (Tukey HSD) to make specific contrasts. To protect against experiment-wise error rate, α values were adjusted using the standard Bonferroni correction based on the number of ANOVAs conducted within a related class of variables (e.g., six for periphyton elemental composition resulting in $\alpha = 0.05/6 = 0.008$).

To relate the responses of algal communities to nutrients, we performed principal components analysis (PCA) on cor-

relation matrices of algal relative abundance for each sampling date. Correlation matrices were used in the PCA to standardize the data.

The characteristic ''horseshoe effect'' often observed in plots of principal components scores was minimized because the relative abundance of individual taxa was typically linearly related to N : P ratio.

Periphyton abundance and elemental composition data were log-transformed as needed to stabilize variance and to improve normality. All statistical analyses of algal taxa were restricted to those groups whose relative abundance by biovolume was greater than 1%. Algal taxonomic composition percentages were arcsin square-root transformed for ANOVAs. Untransformed data are presented in all figures. Statistical analyses were performed using SYSTAT® 7.0 software.

Results

Periphyton abundance—Periphyton Chl *a* abundance increased by about twofold at high TNC compared with low TNC but was not significantly affected by nutrient ratio (Fig. 1A, Table 3). The effects of total nutrient concentration were most pronounced early and midway in the experiment (Fig. 1A, time × concentration effect $P = 0.0061$). A tendency for Chl *a* to be higher at high N : P ratio on day 17 and 28 resulted in a ratio × time interaction ($P = 0.0005$). The response in algal biovolume was broadly similar to that of Chl *a*. Overall, algal biovolume was higher at high N, but this effect was most pronounced early in the experiment (Fig. 1B, Table 3). By day 17 and 28 algal biovolume responded to high TNC only at high N : P ratio ($P < 0.05$, Tukey HSD). Algal biovolume peaked on day 17. Visible sloughing of the periphyton mats had begun to occur by day 28, which likely contributed to the decline in biovolume late in the experiment. Like Chl *a*, periphyton total carbon increased steadily during the experiment (Fig. 1C). Both nutrient ratio and concentration had significant but small effects on total carbon (Table 3).

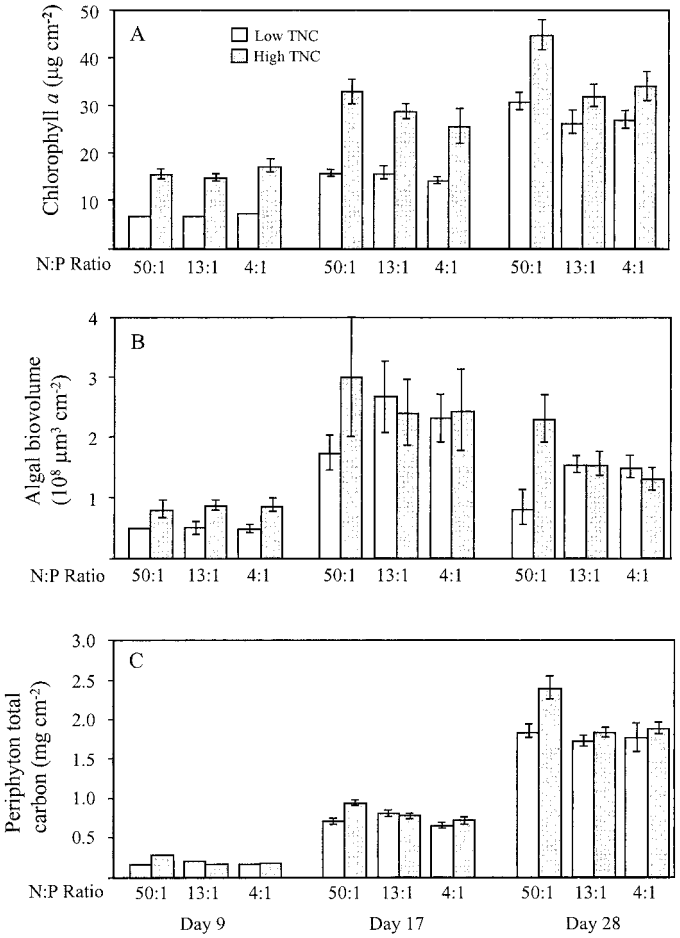

Fig. 1. Measures of periphyton abundance at high, medium, and low target N : P ratio and low and high total nutrient concentration (TNC) on days 9, 17, and 28. Bars are means ± SE; $n = 5$. (A) Chl *a* of periphyton. (B) Algal biovolume. (C) Periphyton total carbon.

Algal community structure—Diatoms were the dominant algal group, comprising 93% of the total algal biovolume on average. Algal taxa were grouped according to their response to nutrient treatments. Several diatom taxa responded primarily to nutrient ratio. *Achnanthidium* (almost all *A. minutissima*, a small, monoraphid diatom) was primarily affected by nutrient ratio and was most abundant at high N : P ratio on all three sampling dates (Fig. 2A, Table 4). On days 17 and 28 *Achnanthidium* also appeared to be positively affected by nutrient concentration at lower N : P ratios. *Achnanthidium* reached 20% relative abundance of the community by biovolume early in the study, but declined later. *Amphipleura* (*A. pellucida*) also responded primarily to nutrient ratio, generally peaking at high to medium N : P (Fig. 2B, Table 4). The response to ratio depended on nutrient concentration. At low total nutrient concentration relative abundance was highest at medium N : P, whereas at high TNC relative abundance was highest at high N : P. *Fragilaria* (e.g., *F. construens* and *F. capucina*) also responded to nutrient ratio, exhibiting highest abundances at low to medium N : P (Fig. 2C, Table 4). *Fragilaria* increased in abundance throughout the experiment, reaching 18% relative abundance on day 28.

Two algal groups responded primarily to nutrient concentration and were not significantly affected by nutrient ratio. *Gomphonema* (mostly *G. truncatum, G. acuminatum,* and *G. parvulum*) was more abundant at high TNC, especially later in the experiment (Fig. 2D, Table 4, $P = 0.0036$ for time × concentration). Green algae (e.g., *Closterium* spp. and *Mougeotia* spp.) were more abundant at low TNC, especially later in the study (Fig. 2E, Table 4) when chlorophytes were 5–20% of total algal abundance.

Several taxa responded strongly to both nutrient ratio and total concentration. *Cymbella* (mostly *C. affinis*) was consistently more abundant at low TNC (Fig. 2F, Table 4). A strong ratio effect was also evident on day 28 (Fig. 2F, Table 4) when the relative abundance of *Cymbella* reached 50% in the ambient treatment of high N : P and low TNC. *Cyclotella* diatoms also were affected by TNC but, unlike *Cymbella,* were more abundant at high TNC (Fig. 2G, Table 4). Early in the experiment, *Cyclotella* was most abundant at low and medium N : P ratios. *Navicula* (e.g., *N. cryptocephala*) was

Table 3. Repeated measures two-way analysis of variance tables for descriptors of periphyton abundance and elemental composition. Total carbon and biovolume data were log transformed. Significant *P* values after standard Bonferroni correction are shown in bold.

Parameter	Source of variation (between groups)	*P*	Source of variation (within groups)	*P*
Chl *a*	Ratio (*R*)	0.0235	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	**0.0005**
	R × *C*	0.2474	Time × *C*	**0.0061**
			Time × *R* × *C*	0.2771
Biovolume	Ratio (*R*)	0.3619	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	0.9583
	R × *C*	**0.0041**	Time × *C*	0.0543
			Time × *R* × *C*	**0.0019**
Total C	Ratio (*R*)	**0.0027**	Time	<**0.0001**
	Concentration (*C*)	**0.0011**	Time × *R*	0.3423
	R × *C*	**0.0010**	Time × *C*	0.7934
			Time × *R* × *C*	0.1348
Percent N	Ratio (*R*)	0.4029	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	0.0568
	R × *C*	0.7089	Time × *C*	0.0590
			Time × *R* × *C*	0.0168
Percent P	Ratio (*R*)	<**0.0001**	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	<**0.0001**
	R × *C*	**0.0080**	Time × *C*	0.0104
			Time × *R* × *C*	0.2844
N : P	Ratio (*R*)	<**0.0001**	Time	0.2543
	Concentration (*C*)	<**0.0001**	Time × *R*	<**0.0001**
	R × *C*	**0.0006**	Time × *C*	0.0299
			Time × *R* × *C*	0.0100
Percent C	Ratio (*R*)	0.2603	Time	**0.0003**
	Concentration (*C*)	0.1577	Time × *R*	0.4768
	R × *C*	0.1804	Time × *C*	0.4742
			Time × *R* × *C*	0.5385
C : N	Ratio (*R*)	0.0101	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	0.0111
	R × *C*	0.9173	Time × *C*	0.8146
			Time × *R* × *C*	**0.0013**
C : P	Ratio (*R*)	<**0.0001**	Time	**0.0062**
	Concentration (*C*)	<**0.0001**	Time × *R*	**0.00012**
	R × *C*	<**0.0001**	Time × *C*	0.1359
			Time × *R* × *C*	0.0250

most abundant at high TNC and low to medium N : P ratios, especially later in the experiment (Fig. 2H, Table 4). *Nitzschia* spp. were rare in the ambient treatment but relatively common in all treatments receiving added nutrients (Fig. 2I, Table 4). Two groups, *Synedra* (mostly *S. ulna*) and blue-green algae (Cyanophyta) did not respond significantly to either nutrient concentration or ratio (Fig. 2J,K, Table 4). *S. ulna* was most abundant early in the experiment, whereas blue-green algae increased in relative abundance late in succession, but both were unaffected by added nutrients.

The first two principal component axes combined explained from 40 to 50% of the total variance in the relative abundance of common algal taxa. *Navicula, Cyclotella,* and *Nitzschia* loaded positively, whereas *Cymbella, Achnanthidium,* and *Amphipleura* loaded negatively on PC1 for all three sampling dates. Loadings of taxa onto PC2 were more variable. A plot of PC1 and PC2 scores shows that the six nutrient treatment combinations resulted in generally distinct algal communities (Fig. 3). Algal communities tended to align along a high to low N : P ratio gradient along PC1, particularly at low TNC. High TNC communities were typically segregated from those of low TNC. The PCA suggests that algal communities responded to N : P ratio and TNC.

Periphyton elemental composition—Periphyton elemental composition did not change substantially with time over the experiment (e.g., periphyton %N and %P decreased by 12 and 17% from day 9 to day 28), so we focused on patterns midway through the experiment (day 17), although the statistics presented here apply to the entire study. Periphyton percent N was higher at high total nutrient concentration but was not affected by N : P ratio (Fig. 4A, Table 3). Periphyton

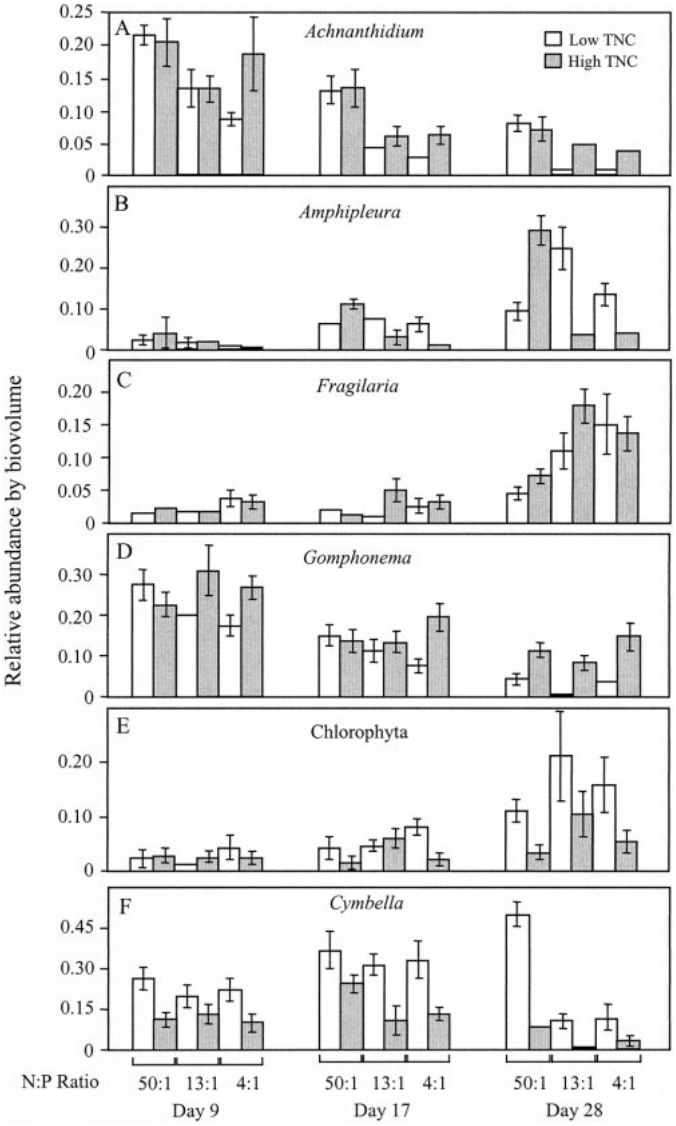

Fig. 2. Mean (± SE; $n = 5$) relative abundance by biovolume of common algal taxa (>1% relative abundance) at high, medium, and low N : P ratio and low and high total nutrient concentration (TNC) on days 9, 17, and 28.

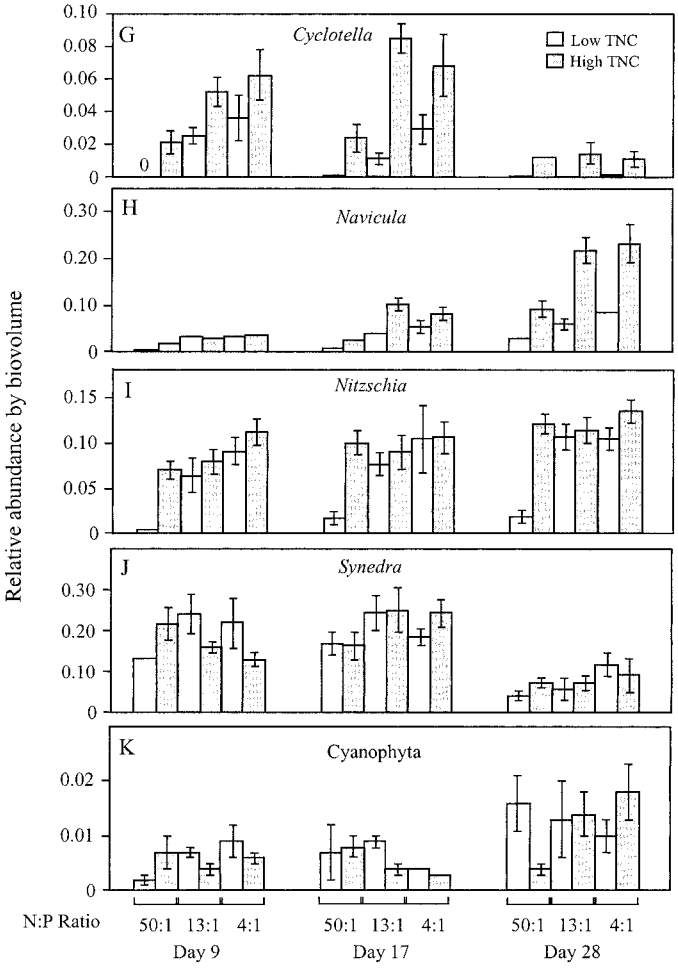

Fig. 2 Continued.

P was affected by both nutrient ratio and TNC (Fig. 4B, Table 3), although the effect of TNC was most pronounced at high N : P ratio (ratio × concentration $P = 0.008$). The effect of N : P ratio on %P generally decreased with time (Table 3). Periphyton N : P closely tracked stream water N : P, particularly at medium and low N : P ratio (Fig. 4C). This pattern was driven primarily by variation in periphyton %P. Periphyton percent carbon was not affected by either nutrient ratio or concentration (Fig. 4D, Table 3). Periphyton C : N and C : P mirrored the responses of periphyton %N and %P, respectively, to nutrients (Fig. 4E,F, Table 3).

Discussion

Periphyton nutrient limitation—Based on the high ambient N : P ratio (relative to Redfield proportions) in the water of the East Branch of the Maple River and in periphyton from the ambient treatment, and the low DIN and SRP concentrations in the EMBR, we expected periphyton biomass to be strongly P limited. Contrary to our prediction, two lines of evidence suggest that periphyton accrual was stimulated by N and not P concentration early in the study. First, periphyton Chl *a* and biovolume were high in all high TNC (high N concentration) treatments and low in all low TNC (low N concentration) treatments on day 9. Second, increasing stream water P concentrations within a level of TNC (decreasing N : P ratio) did not lead to increases in periphyton biomass. Thus, early in the study nutrient ratios did not indicate which nutrient would limit periphyton accrual. A recent study in New Zealand streams also found N : P ratios in stream water and periphyton to be of limited use in predicting nutrient limitation (Francoeur et al. 1999). On days 17 and 28 of our study there was some indication that algae may have responded to P concentration, as biovolume was lowest at the lowest P concentration (ambient treatment). Based on DIN concentration alone, it was not surprising that N limitation occurred. Other investigators have found periphyton growth to be N limited when ambient levels were 50–90 μg NO_3-N L^{-1} (Grimm and Fisher 1986; Lohman et al. 1991), higher than the ambient DIN in our study. In our

Table 4. Repeated measures two-way analysis of variance tables for algal relative abundance by biovolume. Data were arc sin transformed. Significant *P* values after standard Bonferroni correction are shown in bold.

Taxa	Source of variation (between groups)	*P*	Source of variation (within groups)	*P*
Achnanthidium	Ratio (*R*)	<**0.0001**	Time	<**0.0001**
	Concentration (*C*)	0.0073	Time × *R*	0.7949
	R × *C*	0.0271	Time × *C*	0.6335
			Time × *R* × *C*	0.2241
Amphipleura	Ratio (*R*)	<**0.0001**	Time	<**0.0001**
	Concentration (*C*)	0.0082	Time × *R*	0.6932
	R × *C*	<**0.0001**	Time × *C*	0.3872
			Time × *R* × *C*	**0.0013**
Fragilaria	Ratio (*R*)	**0.0003**	Time	<**0.0001**
	Concentration (*C*)	0.2052	Time × *R*	0.0346
	R × *C*	0.0670	Time × *C*	0.6115
			Time × *R* × *C*	0.4680
Gomphonema	Ratio (*R*)	0.1799	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	0.0602
	R × *C*	0.0106	Time × *C*	**0.0036**
			Time × *R* × *C*	0.3437
Chlorophyta	Ratio (*R*)	0.0834	Time	<**0.0001**
	Concentration (*C*)	**0.0036**	Time × *R*	0.4545
	R × *C*	0.2103	Time × *C*	0.0378
			Time × *R* × *C*	0.9066
Cymbella	Ratio (*R*)	**0.0003**	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	**0.0001**
	R × *C*	0.4521	Time × *C*	0.0367
			Time × *R* × *C*	0.0101
Cyclotella	Ratio (*R*)	**0.0001**	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	<**0.0001**
	R × *C*	0.4825	Time × *C*	0.0466
			Time × *R* × *C*	0.4051
Navicula	Ratio (*R*)	<**0.0001**	Time	<**0.0001**
	Concentration (*C*)	<**0.0001**	Time × *R*	0.2427
	R × *C*	0.5272	Time × *C*	<**0.0001**
			Time × *R* × *C*	0.2472
Nitzschia	Ratio (*R*)	<**0.0001**	Time	**0.0021**
	Concentration (*C*)	<**0.0001**	Time × *R*	0.6097
	R × *C*	<**0.0001**	Time × *C*	0.8627
			Time × *R* × *C*	0.9673
Synedra	Ratio (*R*)	0.1499	Time	<**0.0001**
	Concentration (*C*)	0.7851	Time × *R*	0.3140
	R × *C*	0.1988	Time × *C*	0.5115
			Time × *R* × *C*	0.2041
Cyanophyta	Ratio (*R*)	0.4613	Time	**0.0001**
	Concentration (*C*)	0.6674	Time × *R*	0.3085
	R × *C*	0.6761	Time × *C*	0.8003
			Time × *R* × *C*	**0.0027**

study stream water N : P ratio did not affect the overall abundance of periphyton. Similarly, Bothwell (1985) found that lotic periphyton productivity did not respond to N : P ratio but was affected by nutrient concentration.

Nutrient ratios only suggest potential nutrient limitation—nutrient concentration must also be considered (Bothwell 1985). Although nutrient concentrations in ambient stream water were fairly low in our study, P concentration may have been sufficient to saturate growth rates for many algal taxa and, thus, make N : P ratio irrelevant to overall periphyton abundance. More likely, the taxa-specific responses to N : P ratio (i.e., some taxa were more abundant at high N : P ratio, whereas others did better at low N : P ratio) may have simply ''canceled out'' at the community level.

The weak response in total carbon to nutrients may have been related to the production of extracellular polymeric sub-

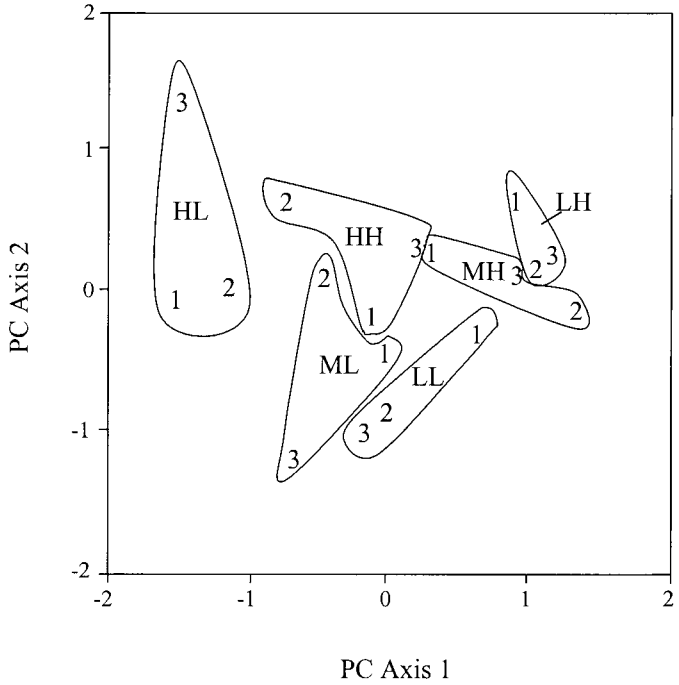

Fig. 3. Principal components analysis (PCA) of common algal taxa. Labels (1–3) are mean PCA scores for each of three sampling dates (days 9, 17, and 28) for six treatments: HL = high N : P ratio, low total nutrient concentration (TNC); ML = medium N : P ratio, low TNC; LL = low N : P ratio, low TNC; HH = high N : P ratio, high TNC; MH = medium N : P ratio, high TNC; LH = low N : P ratio, high TNC. Groups were drawn by hand.

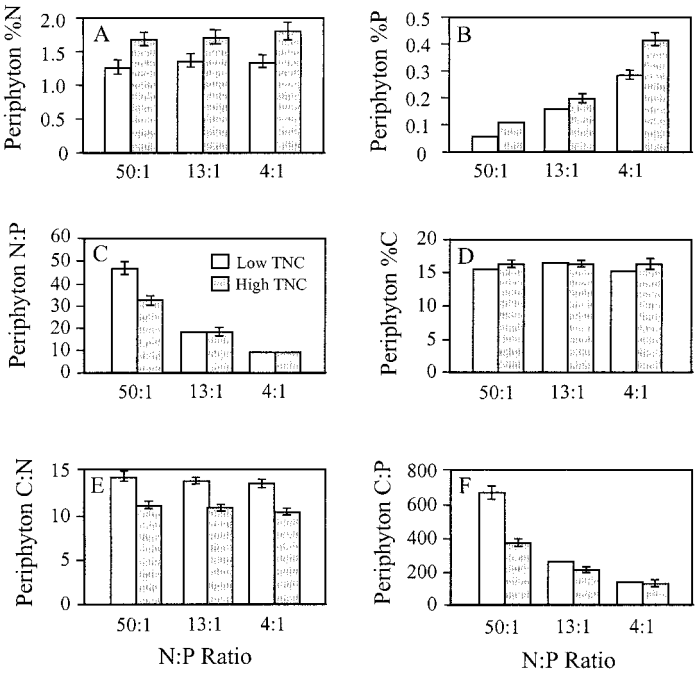

Fig. 4. Measures of periphyton elemental composition at high, medium, and low N : P ratio and low and high total nutrient concentration (TNC) on day 17 (other dates showed similar patterns). Bars are means ± SE; $n = 5$. (A) Periphyton %N of dry mass. (B) Periphyton %P. (C) Periphyton N : P as moles. (D) Periphyton %C. (E) Periphyton C : N. (F) Periphyton C : P.

stances (Hoagland et al. 1993) by periphyton mats in the low TNC treatments. These mats had a distinct gelatinous texture, suggesting high polysaccharide content. *Cymbella affinis* was abundant in mats grown under low TNC, and this diatom is known to produce copious polysaccharides (Rex Lowe, pers. comm.). Although total Chl *a* and algal biovolume were lower at the low TNC treatments, higher carbon-rich polysaccharide production by these mats may have obfuscated a total carbon response. The increase in total carbon through day 28 for all treatments does not mirror the decline in algal biovolume on day 28. This discrepancy suggests that nonalgal carbon (e.g., fine particulate organic matter and bacteria) continued to accumulate after the algal component had declined. The discrepancy between the response of Chl *a* (positive) and algal biovolume (neutral) on day 17 to TNC may be related to an increase in the cellular quota of Chl *a*. Rhee (1978) observed a twofold increase in Chl *a* content per cell of *Scenedesmus* after nitrate addition. Peterson and Grimm (1992) observed a pattern similar to ours in a desert stream where periphyton Chl *a*, but not algal biovolume, increased after NO_3-N enrichment.

Algal community structure—In our study, the relative abundance of 9 of the 11 most common algal taxa was affected by N : P ratio, total nutrient concentration, or both. Three taxa responded primarily to N : P ratio, two taxa responded mainly to TNC, and four taxa responded to both. PCA analysis also revealed that algae, when considered as communities, were affected by both TNC and N : P ratio. The mechanism by which algal taxa responded to N : P ratio and TNC is unclear. The response to nutrient ratio is predicted from resource-ratio theory. However, it is not known whether algal taxa that responded to nutrient ratio did so through exploitative competition for nutrients, which is assumed by theory (Tilman 1982). Nutrient availability is lower at the base of periphyton mats than at the surface (Stevenson and Glover 1993). For exploitative competition for nutrients to occur, however, nutrient uptake rates must approach supply rates, which has yet to be documented for periphyton mats in flowing waters. Mechanisms other than exploitative competition could have been responsible for responses of algal taxa to nutrients. Fast growth rates and abilities to monopolize space and other resources are traits that can also lead to dominance in plant communities (Grime 1979). *Cymbella affinis*, for example, attained a high biomass at low TNC, particularly late in the experiment at high N : P ratio, where it constituted about 50% of the total algal biovolume. *Cymbella* produced polysaccharides that resulted in a low-density gelatinous mat that appeared to inhibit colonization and growth by other algal taxa. Although *C. affinis'* apparent low overall requirements for nutrients likely contributed to its prominence at low TNC, its ability to monopolize space probably played a role in its high relative abundance. Regardless of the mechanisms responsible for the observed patterns of algal community structure, the response to nutrient ratios suggests that benthic algal taxa in running waters, like planktonic algae, have optimum N : P ratios. Our results sug-

gest, for example, that *Achnanthidium minutissima, Amphipleura pellucida,* and *Cymbella affinis* all have high to medium optimal N : P ratios.

The response of algal taxa to total nutrient concentration was not predicted by resource-ratio models (Tilman 1982, 1985). According to these models, changes in TNC at a fixed resource ratio should not alter algal species composition because increasing TNC does not change regions of resource space at which specific pairs of species are competitively dominant (Tilman 1985). Variation in TNC at fixed nutrient ratios is not important in these models because algae are assumed to decrease nutrient concentrations to levels defined by their optimal nutrient ratios, regardless of initial total concentration. For benthic algae in open systems such as streams, however, total nutrient concentration may be important. In streams, algae may be less able to affect nutrient supply through uptake. In addition, diffusion of solutes through periphyton mats is limited, in part, by the concentration of solutes in stream water. In our experiment, algal taxa that were affected by TNC may have primarily responded to N concentration, as was suggested for total periphyton abundance. However several of the taxa that responded to TNC also responded to N : P ratio, suggesting that N and P concentrations were both important to these taxa. For example, *Navicula* had its highest relative abundance at high TNC and medium to low N : P ratios, where both nitrogen and phosphorus concentrations were high. Other investigators have similarly found that naviculoid diatoms flourish at high N and P concentrations (Fairchild et al. 1985; Pringle 1990).

In our study, changes in algal community composition mostly involved shifts in the relative abundance of diatom taxa. Dramatic shifts among higher taxonomic orders (e.g., from diatoms to green algae), a possible consequence of eutrophication in streams (Biggs 1996), were notably absent. Low N : P ratios did not lead to dominance by blue-green algae, a common response observed in lakes (Schindler 1977; Smith 1983) and streams (Peterson and Grimm 1992; Mulholland et al. 1995). Shading the flumes to ambient levels of $\sim$250 μmol m^{-2} s^{-1} in our study might have limited the ability of green algae to respond to nutrients (Graham et al. 1982). High SiO_2 concentration (6 mg L^{-1}) in the EBMR may have favored diatoms, as Sommer (1996) observed in a study of marine periphyton. Diatoms were the dominant group in some other fertilization studies of periphyton (Pringle 1990; Lohman et al. 1991; Hillebrand and Sommer 1997) whereas shifts to green and blue-green algae have been observed elsewhere (Sommer 1996).

Tests of resource-ratio theory for algae fall into three categories: competition experiments using laboratory cultures with selected algal taxa (e.g., Tilman 1977, 1981; Fujimoto et al. 1997), experimental enrichments of natural periphyton communities in the laboratory (e.g., Sommer 1996) or in lentic habitats (e.g., Fairchild et al. 1985), and natural surveys of mixed algal communities along nutrient gradients (e.g., Smith 1983; Sommer 1988). Simple competition experiments between pairs of algal taxa with different optimal nutrient ratios (e.g., Tilman 1981; Fujimoto et al. 1997) have produced the ''cleanest'' results and argue persuasively for the importance of nutrient ratios in affecting the distribution and abundance of algae. When the effects of nutrient ratios and nutrient concentration have been examined in species-rich natural algal communities, responses of algae have been more variable. For example, in enrichment experiments in a lake (Fairchild et al. 1985), the abundances of several common algal taxa were related to the external N : P ratio, although some less common algal taxa did not respond to nutrients. In a descriptive study in a temperate lake, Sommer (1993) found few examples of unimodal responses of phytoplankton taxa to nutrient ratios that changed seasonally. However, 14 of 16 species showed relationships between relative biomass and at least one nutrient ratio by rank correlation. Stockner and Shortreed (1978) manipulated N and P in flumes in British Columbia in an early test of the effects of resource ratios on stream algae. In their study, the relative abundance of algal groups changed considerably among different N : P supply ratios, but because treatments were unreplicated, there was no statistical power to determine if N : P ratio truly caused the community shift. Mulholland et al. (1995) showed that benthic algal community structure changed with longitudinal position in experimental stream channels. Concomitant changes in N : P ratio along the lengths of the channels suggest that N : P ratio may have played a role in the algal community dynamics. The general finding that algae in mixed natural communities do not respond as consistently to nutrient ratios and TNC as do taxa in laboratory experiments suggests that factors in addition to nutrients (e.g., light, grazers, hydraulic disturbance) affect the abundance and distribution of algae in nature (Lamberti 1996).

Responses of algal community structure to N : P ratio and total nutrient concentration in our study were more dramatic than those of Chl *a,* algal biovolume, and total carbon, particularly midway and late in the experiment. The differential response between overall periphyton abundance and individual diatom taxa to N : P ratio was particularly striking. On day 17, for example, diatom relative abundance was substantially different among N : P ratio treatments but differences in total algal biovolume were less pronounced. Taken together, this suggests that by midway and late in the experiment, algal taxa were generally substitutable in regard to their biomass contribution to the periphyton mats. Redundancy of function (sensu Walker 1992) among these taxa cannot be assumed, however, because we have little knowledge of the relevant metabolic parameters of specific taxa (e.g., maximum growth rates). In a desert stream, total algal biovolume was not affected by nutrient amendments, whereas functional groups of algae (those capable of N_2 fixation and nonfixers) were affected differentially (Peterson and Grimm 1992). Our results also suggest that benthic algal taxonomic composition may be a more sensitive indicator of nutrient enrichment than bulk measures of periphyton such as AFDM or total carbon.

Periphyton elemental composition—The increase in N content in periphyton exposed to elevated DIN concentrations is consistent with the observed pattern of N limitation of periphyton accrual. The increase in periphyton P content with SRP concentration does not necessarily mean that periphyton incorporated more P into organic compounds at

high levels of SRP. Our measures of periphyton P may have overestimated organic P because the digestion procedure we used hydrolyzes inorganic mineralogical P such as apatite in addition to organic P. The range of periphyton %N (1.2–2.1) and %P (0.06–0.47) across our treatments is similar to values reported by other investigators (e.g., Mulholland et al. 1991). Positive relationships between dissolved nutrients and periphyton nutrients have been found in streams (Mulholland and Rosemond 1992; Stelzer, unpubl. data), marshes (Grimshaw et al. 1993), and seas (e.g., Hillebrand and Sommer 1997). Our study demonstrated a strong relationship between lotic periphyton N : P and the N : P of dissolved nutrients. Changes in periphyton nutrient content in response to the availability of dissolved nutrients may be important to consumers because algal food quality has been shown to be related to its chemical composition (e.g., Sterner 1993).

Periphyton successional dynamics—As periphyton mats become thicker during succession, resource availability in the mat (especially in the lower layers) is expected to change, even if resource supply external to the mat remains constant. Studies have documented changes in nutrient turnover rates (Mulholland et al. 1994) and light availability (Jørgensen and Des Marias 1988) within periphyton mats as biomass (thickness) increases. In a laboratory study, Johnson et al. (1997*b*) found that light availability at the base of periphyton mats decreased approximately tenfold from early to late in succession (43 d span). Light availability likely decreased over time within the periphyton mats in the present study, which may partly explain the increase in algal Chl *a* to biovolume ratio late in the experiment. As periphyton mats became thicker, the concentration of Chl *a* per algal cell may increase to compensate for reduced light availability (Rhee and Gotham 1981). The decline in biovolume on day 28 was associated with a sharp increase in the percentage of dead cells (empty diatom frustules), perhaps due to resource limitation or natural cycles of cell death. An increase in the percentage of dead diatom cells late in periphyton mat development has been observed in another system (Johnson et al. 1997*b*).

Periphyton mat thickness likely affected nutrient availability to algae, especially those in lower layers, because of limitation of the rate of nutrient diffusion from bulk water into the mat (Bothwell 1989; Burkholder et al. 1990; Stevenson and Glover 1993). Pringle (1990) found that algal taxa in the upper layers of periphyton appeared to interfere with inorganic nutrient procurement by understory sessile taxa. Thus, nutrients may become limiting within periphyton mats even when nutrient supply in the water column is constant. However, nutrient recycling has been shown to increase within periphyton mats as biomass increases (e.g., Mulholland et al. 1994) and may partially compensate for diffusional constraints. In our study, periphyton N decreased by 12% and P decreased by 17% on average from day 9 to day 28. The decline in percent nutrients through time may be due to preferential remineralization of N and P relative to C in the mats. These ''bulk'' measurements of periphyton nutrients may not reflect fine-scale gradients and patchiness of nutrients that likely were present in the periphyton mats. Overall, mat development appeared to add a dynamic component to the nutrient regimes that affected specific algal taxa.

The relative abundances of all 11 common algal taxa changed significantly during the course of our experiment, and several taxa exhibited strong linear changes with time. Some of the successional patterns can be explained in terms of how the growth form of various taxa influenced their response to changes in resource availability during succession. The decrease in *Achnanthidium* during succession may have been related to its adnate growth form and lack of motility, traits that probably make it vulnerable to light limitation as periphyton mats thicken. *Navicula* and *Fragilaria* increased in relative abundance during succession. Many of the *Navicula* species found in our study are motile, which would provide an advantage under vertical gradients in light and nutrients (Pringle 1990). *Fragilaria* grew as long ribbon-like chains loosely associated with the periphyton mats, particularly late in the study. This growth form is probably well suited for light and nutrient procurement in thick periphyton mats. Johnson et al. (1997*b*) also observed proliferations of araphid, chain-forming diatoms such as *Fragilaria* late in periphyton succession. The decrease in *Gomphonema* during our study was somewhat surprising, as its cells grow attached to stalks, which would presumably impart an advantage by allowing cells to extend into the upper parts of periphyton mats, giving them greater access to resources. In thick periphyton mats, motility may be a more flexible strategy than a stalked growth form for resource acquisition.

Conclusions

We showed that the overall abundance of lotic periphyton responded positively to increased TNC, but not to N : P ratio. In our experiment, periphyton biomass was primarily limited by DIN and not SRP concentration, despite high N : P ratio in the ambient stream water and periphyton. This suggests that predicting nutrient limitation from stream water or periphyton nutrient ratios alone may have limitations. Algal community structure responded strongly to both N : P ratio and TNC, although the mechanisms are unclear. The response to N : P ratio is consistent with resource-ratio theory, but further study is needed to determine if benthic algae, like their pelagic counterparts, compete exploitatively for nutrients. Research is also needed to determine how bulk nutrient availability in stream water interacts with periphyton mat morphology (e.g., thickness) and physical factors (e.g., flow) to affect nutrient availability to algae and other microbes within periphyton mats. Our results showed that N : P ratio affected the community structure of the periphyton mats in this study, but had little effect on bulk biomass measures. This result suggests that periphyton community structure may be more sensitive to perturbations of stream water nutrients than composite measures of periphyton abundance.

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