

Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms

Steven T. Rier^{1,2,*} & R. Jan Stevenson¹

¹*Department of Zoology, Michigan State University, Michigan, 48824, USA*

²*Department of Biological & Allied Health Sciences, Bloomsburg University, Hartline Science Center, 400 East 2nd Street, Bloomsburg, PA 17815, USA*

(*Author for correspondence: E-mail: srier@bloomu.edu)

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Abstract

In this study we manipulated both nitrogen and phosphorus concentrations in stream mesocosms to develop quantitative relationships between periphytic algal growth rates and peak biomass with inorganic N and P concentrations. Stream water from Harts Run, a 2nd order stream in a pristine catchment, was constantly added to 36 stream-side stream mesocosms in low volumes and then recirculated to reduce nutrient concentrations. Clay tiles were colonized with periphyton in the mesocosms. Nutrients were added to create P and N concentrations ranging from less than Harts Run concentrations to 128 $\mu\text{g SRP l}^{-1}$ and 1024 $\mu\text{g NO}_3\text{-N l}^{-1}$. Algae and water were sampled every 3 days during colonization until periphyton communities reached peak biomass and then sloughed. Nutrient depletion was substantial in the mesocosms. Algae accumulated in all streams, even streams in which no nutrients were added. Nutrient limitation of algal growth and peak biomass accrual was observed in both low P and low N conditions. The Monod model best explained relationships between P and N concentrations and algal growth and peak biomass. Algal growth was 90% of maximum rates or higher in nutrient concentrations 16 $\mu\text{g SRP l}^{-1}$ and 86 $\mu\text{g DIN l}^{-1}$. These saturating concentrations for growth rates were 3–5 times lower than concentrations needed to produce maximum biomass. Modified Monod models using both DIN and SRP were developed to explain algal growth rates and peak biomass, which respectively explained 44 and 70% of the variance in algal response.

Introduction

Nuisance algal blooms resulting from N and P contamination have become a major problem in many streams (USEPA, 1998; Dodds & Welch, 2000). Protection and remediation of these resources is therefore contingent on a better understanding of the quantitative relationships between algae and inorganic nutrients. However, most studies of nutrient effects on periphytic algae in streams have only assessed the presence or absence of nutrient limitation through enriching streams or stream habitats with nutrient

diffusing substrates (e.g., Pringle & Bowers, 1984; Hepinstall & Fuller, 1994; Pan & Lowe, 1994; Francoeur et al., 1999), once-through artificial stream channels (e.g., Mundie et al., 1991; Hill et al., 1992), or whole-stream enrichments (e.g., Petersen et al., 1993; Slavik et al., 2004). Nutrient limitation has also been investigated by applying regression approaches to field survey data (Lohman et al., 1992; Dodds et al., 1997, 2002; Biggs, 2000), and these empirical methods confirm the experimental evidence that N and P are both important determinants of stream community structure and function.

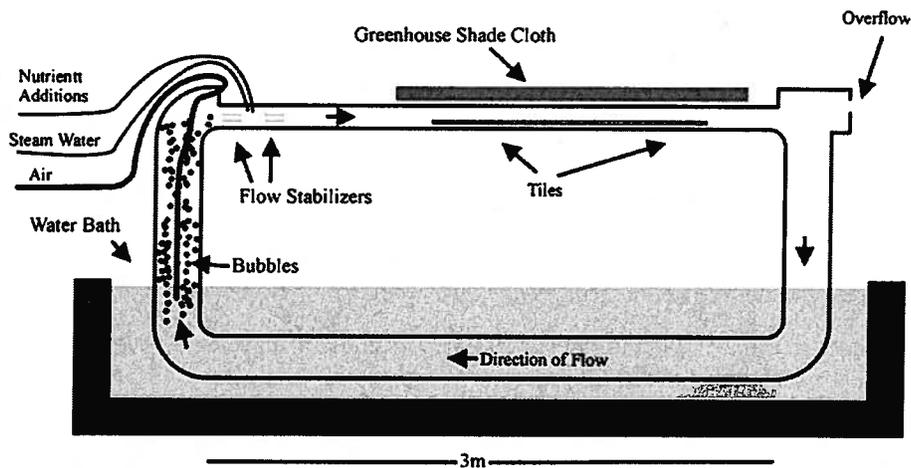


Figure 1. Diagram of the partially re-circulating stream system used in the experiment.

stream water. This method was shown to reduce nutrient concentrations in source water (Mulholland et al., 1991).

Stock solutions with different concentrations of NaNO_3 and KH_2PO_4 were delivered from 1 l containers to each channel using peristaltic pumps (Manostat™ model STD) set at a drip rate of 0.3 ml min^{-1} . We added N and P throughout the experiment at a rate that would elevate ambient nutrient concentrations by the amounts shown in Table 1, not taking into account uptake within the channels. Nutrient treatments were assigned randomly throughout the 36 channels. We used a total of three replicate channels for each treatment and used three channels as controls, which received no nutrient additions.

Twenty unglazed ceramic tiles (29.2 cm^2) were placed at the downstream end of each channel where turbulence was lowest. Two layers of greenhouse shade cloth, supported by 1 cm^2 wire mesh screen were placed over the tiles to simulate light regimes in nearby streams. The shades

allowed 12% of incident photosynthetically active radiation (LiCor™ Model Li 189 light meter) to reach the tiles.

A large species pool of potential algal colonists was collected from streams varying in nutrient conditions by adding 500 ml of an algal inoculum to each stream mesocosm one day prior to the start of experiment. This inoculum was produced by scraping rocks from three streams differing in the level of human impact in the watershed: Harts Run, the low nutrient stream where the experiment was run; Wilson Creek with moderate agriculture in the watershed and moderate concentrations of N and P (R. J. Stevenson unpublished data); and the Middle Fork of Beargrass Creek, located within the city of Louisville, Kentucky. Beargrass Creek has an urban watershed and periodically has high nutrient concentrations (R. J. Stevenson, unpublished data). Scrapings from the three streams were combined in a single container and homogenized before aliquots were added to each experimental stream. In using this approach, we

Table 1. Nutrient addition treatments utilized in the study

	Nitrogen concentration(s) ($\mu\text{g l}^{-1}$)	Phosphorus concentration(s) ($\mu\text{g l}^{-1}$)
Control channels	0	0
Nitrogen treatments	0, 16, 64, 256, 512, 1024	50
Phosphorus treatments	500	0, 2, 8, 32, 128

All values are projected concentrations above ambient stream water for each treatment not taking into account uptake within each channel.

where K_μ and K_{PB} were the half saturation constants for the particular nutrient manipulation and μ_{\max} or PB_{\max} were the maximum growth rate or maximum PB respectively. With respect to the Monod (1950) model, we used a "working" definition of saturating nutrient concentrations as those when μ and PB attained 90% of their respective maxima.

Relations between μ and PB and S were only determined when the other nutrient was assumed to be above saturating conditions; i.e. the relation between μ and PB and N was determined with results from treatments with PLOAD > 49 $\mu\text{g PO}_4\text{-P l}^{-1}$ and algal response to P was determined with NLOAD > 499 $\mu\text{g NO}_3\text{-N l}^{-1}$. Coefficients of determination (r^2) (Sokal & Rohlf, 1995) for the models were used to compare the goodness of fit of the different models as proportion of error in μ or PB explained by the model.

Predictive models of μ and PB that simultaneously considered both N and P were assessed using DIN and SRP concentrations, TP and TN concentrations, and PLOAD and NLOAD. Linear, log-linear, and modified Monod models were used. Multiple regression and nonlinear regression were used to calculate the model parameters and determine goodness of fit with coefficients of determination (Systat[®] 10). The linear equation took the form

$$\mu \text{ or PB} = \alpha + \beta_N S_N + \beta_P S_P$$

where the subscripts N and P denote nutrient concentrations and the rate of increase in μ or PB per unit of each specific nutrient. The log-linear equation took the form

$$\mu \text{ or PB} = \alpha + \log_e \beta_N S_N + \log_e \beta_P S_P$$

The modified Monod equation took the forms:

$$\begin{aligned} \mu &= \mu_{\max} (S_N / (S_N + K_{\mu-N})) * (S_P / (S_P + K_{\mu-P})) \\ PB &= PB_{\max} (S_N / (S_N + K_{PB-N})) * (S_P / (S_P + K_{PB-P})) \end{aligned}$$

where the subscripts N and P denote nutrient concentrations and half saturation constants specific to those nutrients. Molar ratios of DIN:SRP, TN:TP, and NLOAD:PLOAD were also used to develop predictive models of μ and PB in different nutrient conditions using simple linear regression.

Results

Temperature and water chemistry

Water temperatures in the re-circulating channels averaged approximately 20 °C. Stream water NO_3^- ranged from 17.5 to 38.0 $\mu\text{g NO}_3^- \text{-N l}^{-1}$ and SRP ranged from 1.5 to 7.3 $\mu\text{g l}^{-1}$. Both nutrients generally decreased throughout the experiment due to a lack of rain with the exception of day 14, which immediately followed a moderate rain (Table 2). During the period we defined as the early growth stage (days 1, 3, and 6), stream water $\text{NO}_3^- \text{-N}$ averaged 30.1 \pm 5.6 $\mu\text{g l}^{-1}$ (± 1 SE) and SRP averaged 4.8 \pm 1.4 $\mu\text{g l}^{-1}$ (± 1 SE). In the period we defined as the peak biomass stage (days 6, 8, and 10), stream $\text{NO}_3^- \text{-N}$ concentrations averaged 19.1 \pm 0.5 $\mu\text{g l}^{-1}$ (± 1 SE) and SRP averaged 2.6 \pm 0.3 $\mu\text{g l}^{-1}$ (± 1 SE). Ammonium and silica concentrations fluctuated throughout the experiment ranging from 6.8 to 51.0 $\mu\text{g NH}_4^+ \text{-N l}^{-1}$ and 10.53–11.99 mg Si l^{-1} .

Nutrient depletion was evident in recirculating channels for all nutrients and was related to stage of colonization and nutrient concentration (Figs. 2 and 3). In the control channels NO_3^- and NH_4^+ uptake were apparent from day 3 to the end of the experiment (Table 2). Starting on day 3, $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ concentrations were significantly lower ($p < 0.05$) in control channels than in ambient stream water (Harts Run). Nitrate concentrations in recirculating streams decreased during this time from ambient levels to a range varying between 3.7 and 8.5 $\mu\text{g NO}_3^- \text{-N l}^{-1}$. SRP uptake was not apparent in the control channels, which may be due to ambient levels that were at or near detection limits. No difference in silica concentrations was observed between control channels and ambient stream water (Table 2).

Algal response

Diatoms dominated algal communities in all treatments throughout the experiment. Although relative abundances differed between treatments and despite a mixture to potential colonists from three different streams with varying levels of nutrient enrichment, only 14 diatom species dominated algal species composition in both low and high N and P treatments (Table 3) (K. Manoylova

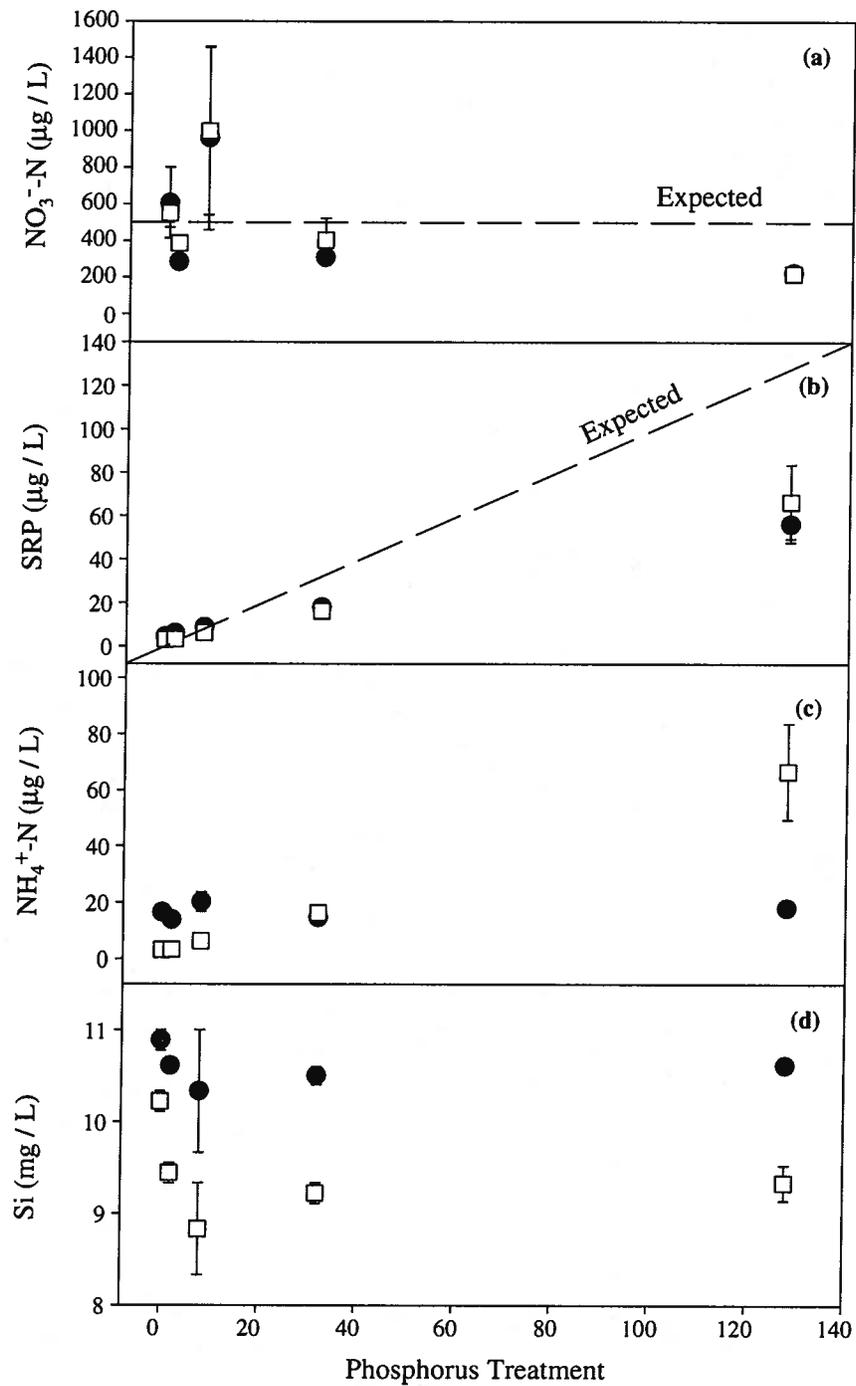


Figure 2. Nutrient concentrations measured in each treatment across the phosphorus gradient in both the early growth stage (darkened circle) and peak biomass stages (open square) of community development. Panel a = $\text{NO}_3^- \text{-N}$, panel b = SRP, panel c = $\text{NH}_4^+ \text{-N}$, panel d = Si.

and $100 \mu\text{g N l}^{-1}$, but then continued to increase linearly along the remainder of the DIN gradient. However, a log-linear model of PB as a function of DIN only explained 2% more variation than the Monod model (Table 4), even though the log-linear model does not saturate at high N and should fit the suspected pattern.

PB across the P gradient increased sharply between 2 and $20 \mu\text{g SRP l}^{-1}$ and then did not change with higher SRP concentration. K_{μ} was

$4.20 \pm 1.78 \mu\text{g P l}^{-1}$ (± 1 SE) and PB_{max} was $8.65 \pm 0.90 \mu\text{g chlorophyll } a \text{ cm}^{-2}$ (± 1 SE) when fit to the Monod equation (Fig. 6). Saturation of peak biomass occurred at $38 \mu\text{g SRP l}^{-1}$. This model explained 53% of the variance.

The Monod model usually explained as much or more variation in μ and PB as a function of nutrient concentrations as the log-linear model, and it always explained nutrient effects better than the linear model (Tables 4 and 5). For models

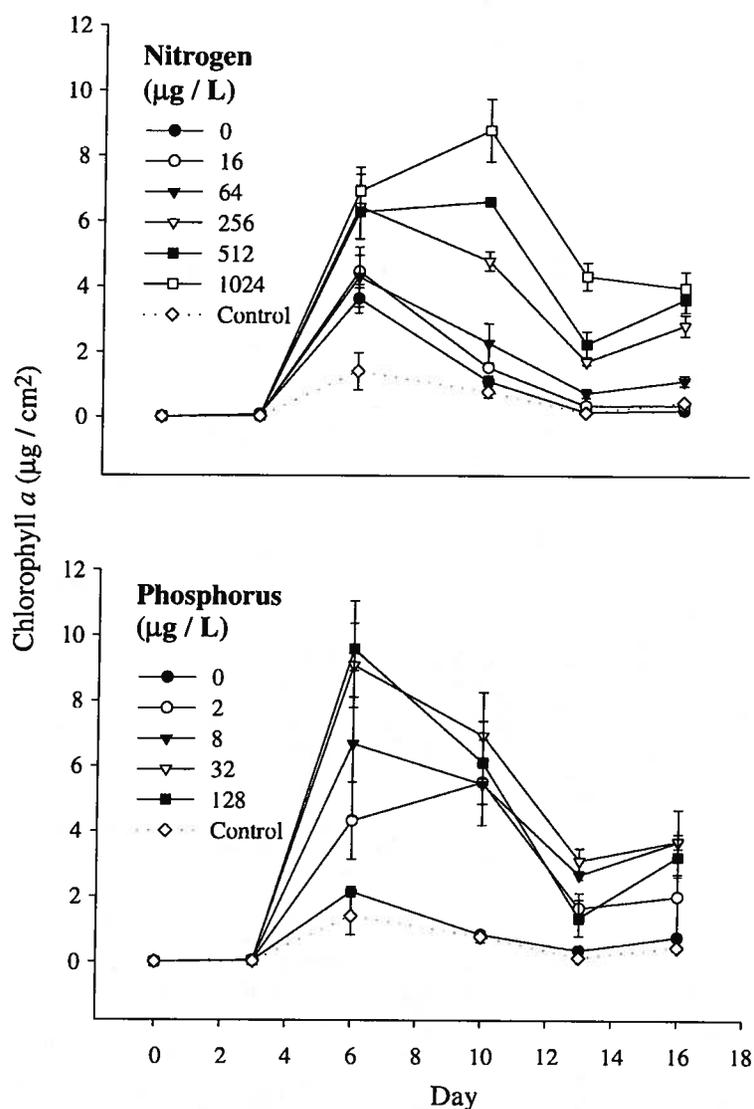


Figure 4. Algal biomass (Chlorophyll *a*) throughout the experiment in different nitrogen and phosphorus treatments.

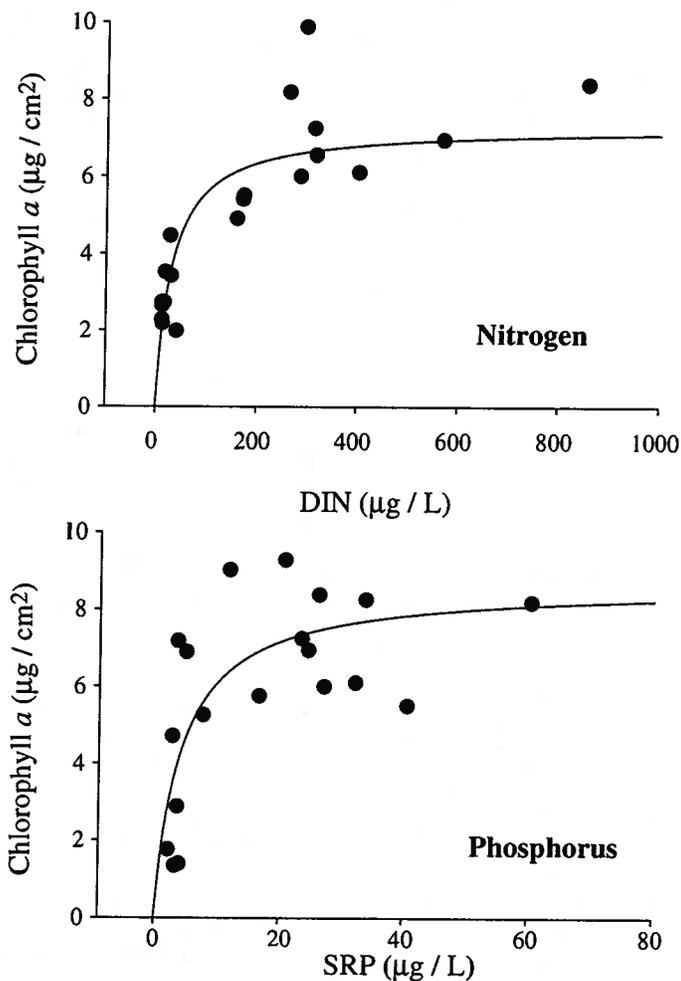


Figure 6. Peak algal biomass (chlorophyll *a*) as a function of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP). Curve represents the best fit of the data to the Monod (1950) equation.

Soluble nutrient concentrations (SRP and DIN) explained as much or more variation in μ with the Monod model as TP or TN, but the relationship between soluble nutrients and PB was somewhat less than for TP and TN (Table 4). The log-linear model explained more variation than the Monod model for μ and PB as a function of loading rates (PLOAD and NLOAD). Using log-linear models, PLOAD and NLOAD explained more variation in μ than measured nutrient concentrations, but only PLOAD explained more variation in PB. NLOAD explained less variation

in PB than measured N concentrations (DIN, NO_3 , and TN).

Mat N and P concentrations explained little variation in peak biomass. Mat N varied from 153 to 569 mg N g^{-1} AFDM and mat P varied from 12 to 107 mg P g^{-1} AFDM. Peak biomass was better related to mat P than mat N with ($r^2=0.365$ and 0.106 for log-linear models using mat P and mat N, respectively, Table 4).

Monod models with nutrient concentrations were better able to explain μ and PB than models between molar ratios of nutrients (DIN:SRP,

Table 5. Coefficients of determination (r^2) for linear, log-linear, Monod, or modified Monod models (if two nutrient variables are involved) of algal growth rate (μ), and peak biomass (PB) as a function of concentrations of soluble reactive phosphorus (SRP), total phosphorus (TP), PO_4^{3-} -P or NO_3^- -N loading concentrations in experimental channels (NLOAD & PLOAD), dissolved inorganic nitrogen concentration (DIN), NO_3^- -N, NH_4^+ -N, total nitrogen (TN)

Algal attribute	Nutrient	r^2		
		Linear	Log-linear	Monod
μ	DIN & SRP	0.207	0.362	0.436
μ	TN & TP	0.213	0.306	0.377
μ	NLOAD & PLOAD	0.397	0.464	0.488
μ	TN:TP	N.S.		
μ	DIN:SRP	N.S.		
μ	NLOAD:PLOAD	N.S.		
PB	DIN & SRP	0.404	0.663	0.701
PB	TN & TP	0.477	0.659	0.744
PB	NLOAD & PLOAD	0.603	0.637	0.783
PB	TN:TP	N.S.		
PB	DIN:SRP	N.S.		
PB	NLOAD:PLOAD	N.S.		

N.S. denotes those relationships where one or more of the parameters estimated were not significant in the regression ($p < 0.05$).

ences in the chemical and physical conditions of our channels and in Bothwell's experiment could account for our higher saturating P levels. Current velocities in our systems were 50% slower than in Bothwell's experiment (1988). Since higher current velocities can enhance nutrient uptake (e.g., Whitford & Schumacher, 1964; Horner & Welch, 1981; Horner et al., 1983; Horner et al., 1990), high current velocities in Bothwell's experiments may have allowed for a greater flux of nutrients between the algal cells and the overlying water, thereby reducing the nutrient concentrations required to saturate algal growth. Bothwell reported DIN concentrations that ranged from 60 to 80 $\mu\text{g N l}^{-1}$ during the winter and spring, but only 20 $\mu\text{g N l}^{-1}$ during the summer. NH_4^+ -N was undetectable throughout his study. Since we show saturation for stream algae above 86 $\mu\text{g N l}^{-1}$, N limitation could have constrained growth responses at high P concentrations in Bothwell's study. Although, we attempted to estimate cellular growth in the absence of density effects by using the change in biomass between days 3 and 6, a 100-fold increase in biomass between these two dates may have resulted in density effects in the later stages of this period. Stevenson (1990) has shown that algal density can reduce growth rates. In addition, higher nutrient concentrations are

required to saturate accrual at high biomass than low biomass (Bothwell, 1988; this study).

In general, algal growth in this experiment was extraordinarily high with respect to other values reported in the literature for stream periphyton (Bothwell, 1985, 1988, 1989; Biggs, 1990; Stevenson et al., 1991; Humphrey & Stevenson, 1992) and similar to only a few phytoplankton observations (e.g., Hecky & Fee, 1981). A number of factors could have contributed to these high growth rates. Average temperature during this experiment was 20 °C, which has been shown to be within an optimal range for algal growth (Rhee & Gotham, 1981). Current velocity over the tiles was 25 cm s^{-1} , which may have been high enough to overcome some diffusion limitation (Whitford, 1960) without creating excess drag (see review by Stevenson, 1996). Saturation with respect to the other nutrient in question in all treatments except the controls, likely contributed to the high growth rates by preventing a shift to limitation by the other nutrient. Initially seeding the experimental channels with a diverse species pool representing a wide range of nutrient conditions may have increased chances that species were present in each treatment that were best adapted to grow and compete under the given nutrient conditions. Using chlorophyll *a* in the algal growth rate

which probably better reflect the nutrient availability that enables development of peak biomass in streams than nutrient concentrations measured during periods of peak biomass. Even in our recirculating streams, the commonly higher correlations between loading concentrations (PLOAD and NLOAD) and growth rates and peak biomass, vs. measured nutrient concentrations with algal responses, indicated that nutrient availability was difficult to characterize with soluble inorganic nutrient concentrations.

The lack of relation between N and P concentrations in periphyton mats may be due to variability in measures of mat nutrients or complex relations between cell nutrient concentrations and growth rates. Mat nutrient concentrations have been suggested as potential indicators of nutrient status in streams and have been related to water column nutrient concentrations (Humphrey & Stevenson, 1992; Biggs, 1995). N:P ratios in mats may indicate relative limitation by N or P availability (Shanz & Juon, 1983; Borchardt, 1996). Complex relations between mat nutrient concentrations and algal growth or peak biomass are probably related to accrual of excess nutrients (i.e., high N:AFDM or P:AFDM ratios), when cells are limited by other nutrients or other resources (Stevenson & Stoermer, 1982). At peak biomass, either N or P concentrations may be limiting further accrual of periphyton. Therefore, mat N or P may provide some information about trophic status of streams, but concentrations of water-column nutrients provided better predictors of peak biomass under the controlled settings of this study.

Many challenges remain in solving the relations between algae and nutrients. Differing responses of species to different temperature, light, and current environments surely affect our prediction of periphyton growth as a function of nutrients. Not only do different diatoms respond to nutrients differently, but also filamentous algae add complexity by responding to nutrients independently and producing additional substratum for epiphytic algal colonization. Even in our study, where nutrients in water from a stream draining a relatively pristine forested catchment were decreased in recirculating streams, we were not able to stop diatom growth with low nutrients. Can benthic diatoms accumulate under any natural nutrient

conditions where grazers do not constrain accrual?

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