

# Periphyton nutrient status in a temperate stream with mixed land-uses: implications for watershed nitrogen storage

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**Abstract** We sampled periphyton communities in a highly productive stream to characterize how longitudinal changes in watershed geology and land use affect periphyton nutrient status and elemental composition. Nutrient status was evaluated from measures of periphyton nutrient composition (carbon, nitrogen, and phosphorus), stable isotope signatures ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ), and the response of periphyton to experimental enrichment with nitrogen. Biomass and nutrient content increased dramatically from the headwaters to downstream, while tissue nutrient ratios (C:P and C:N) were more consistent and did not indicate strong N- or P-limitation. Nitrogen enrichment experiments did not exhibit a consistent response upstream or downstream, and periphyton C:N:P stoichiometry showed no significant response to N-enrichment. Absolute densities of periphyton N were 5- to 90-fold greater than the overlying N concentrations in stream water (159- to 353-fold greater for P), and the  $\delta^{15}\text{N}$  signal indicates downstream enrichment from likely watershed sources

(urban and agriculture land-use). These results suggest that periphyton in Spring Creek are not N-limited and store large quantities of both N and P, which in turn can be transported downstream during high flow events.

**Keywords** Periphyton · Streams · Nutrients · Stoichiometry

## Introduction

Periphyton assemblages are often the dominant primary producers in streams (Vannote et al., 1980), and thus constitute the primary link between dissolved nutrients and higher trophic levels (Minshall et al., 1985; Mulholland et al., 2000; Finlay, 2001). Nutrient utilization by periphyton can influence longitudinal variation in both the form (dissolved, particulate, organic, inorganic) and concentrations of nutrients available to downstream communities (Newbold et al., 1982; Minshall et al., 1985). Periphyton biomass is correlated with stream nutrient concentrations (Dodds et al., 2002b), although comparatively little is known about periphyton stoichiometry relative to common environmental gradients (Sterner & Elser, 2002; Cross et al., 2005). Although few studies have examined the effects of nutrient availability on periphyton stoichiometry, those experiments have shown that periphyton communities are not strictly homeostatic (Bothwell, 1985; Kahlert, 1998;

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Stelzer & Lamberti, 2001). Despite a limited number of experimental and empirical observations, this pattern is similar to that of phytoplankton. However, C:N and C:P ratios indicating nutrient limitation are generally higher for benthic algae than phytoplankton (Kahlert, 1998).

It is important to identify the factors that regulate periphyton nutrient status because benthic communities can be a significant sink for dissolved nitrogen (Dodds et al., 2002a). Variation in periphyton stoichiometry should be directly related to the availability of nutrients for transport downstream because nutrient uptake in algae is regulated in part by internal nutrient stores (Droop, 1973). To examine this relationship in a highly productive stream system, we surveyed periphyton biomass, nutrient content (C, N, and P), stable isotope composition (C and N), and periphyton response to N-enrichment over the course of 1-year. We hypothesize that longitudinal increases in nutrient loads attenuate or eliminate nutrient limitation of periphyton growth, enrich periphyton with N and P relative to C, and increase  $\delta^{15}\text{N}$  relative to upstream reaches.

## Methods

Spring Creek is located in the ridge and valley region of the Appalachian Mountains in central Pennsylvania. The stream is a spring-fed, fourth-order tributary to the West Branch of the Susquehanna River, which ultimately flows into the Chesapeake Bay (Chang & Carlson, 2005). The Spring Creek watershed (225 km<sup>2</sup> study area) occupies the valley between two ridges, where the stream originates from springs in upland forest overlying shale and sandstone bedrock. Downstream (valley) reaches of the stream flow through agricultural, suburban, and urban areas overlying primarily karstic dolomite and high-calcium limestone bedrock. Spatial data including watersheds, land use, and geology were obtained from Pennsylvania Spatial Data Access (PASDA, [www.pasda.psu.edu](http://www.pasda.psu.edu)).

Sampling locations were selected to reflect changes in land use, stream morphology, and geology within the watershed (see Godwin & Carrick, 2008). Sites 1 and 2 were located in Galbraith Gap Run on the ridge and sites 3, 4, and 5 were located in the valley, within the main channel of Spring Creek. Site 1 was situated in mixed forest with a closed canopy at

the Rothrock State Forest boundary. Site 2 occurred in a largely forested portion of the watershed with some low-density residential areas at the base of the ridge. Sites 3 and 4 had relatively open canopies and were located in the valley where agricultural and suburban non-point sources are more predominant. Site 5 was also located in the valley, situated downstream of two fish hatcheries and a wastewater treatment facility.

## Ambient conditions

Water temperature and conductivity were measured on each sampling date using an YSI-6000 Sonde (Yellow Springs Instruments, Yellow Springs, Ohio). Nitrate ( $\text{NO}_3^-$ ) and orthophosphate ( $\text{PO}_4^{3-}$ ) concentrations were measured from the water samples using standard colorimetric methods (APHA, 1995). Stream water chemistry data for sites 3, 4, and 5 were provided by the Pennsylvania Department of Environmental Protection and the Clearwater Conservancy. Flow and nitrate data for site 1 were provided by Anthony Buda (The Pennsylvania State University, School of Forest Resources). The detection limits for orthophosphate and nitrate were 0.021 mg  $\text{PO}_4/\text{L}$  and 0.012 mg  $\text{NO}_3/\text{L}$ , respectively. Daily stream discharge data for sites 4 and 5 were provided by the United States Geological Survey. Flow data for site 3 were provided by the Clearwater Conservancy. Seasonal canopy cover was measured in the winter (January) and the spring of 2005 (May) using a LAI-2000 plant canopy analyzer (Li-Cor Environmental, Lincoln, Nebraska).

## Periphyton sampling

Samples were collected during seven sampling events, spaced at approximately 8-week intervals over 1-year (March 2004–February 2005). The seven sampling periods were categorized among four seasons (winter  $n = 2$ , spring  $n = 2$ , summer  $n = 2$ , and fall  $n = 1$ ) based upon solstice and equinox dates. Periphyton samples were collected from 2 to 3 randomly selected rocks as previously described (Godwin & Carrick, 2008). Rock scrapings were homogenized with a hand-held blender for 30 s creating a slurry that was subsampled for analyses (Biggs, 1987).

Algal biomass and nutrient content was estimated for each rock from paired subsamples. Chlorophyll

density was determined from subsamples concentrated onto glass fiber filters and frozen until analysis. Samples were extracted in a 50:50 mixture of 90% acetone and dimethylsulfoxide then the phaeophytin-corrected chlorophyll-*a* concentration in the extract was measured using a fluorometric technique (Carrick et al., 1993). Coefficients of variation among duplicate subsamples were typically <5%. Samples for determination of C and N content were filtered onto glass fiber filters and frozen, then analyzed via combustion using a Carlo-Erba CN analyzer (Horneck & Miller, 1998). Subsamples for P content were filtered onto polycarbonate membrane filters (0.2  $\mu\text{m}$  pore size) and frozen until analysis by Inductively Coupled Plasma Spectrometry (Miller 1998). Measurements of chlorophyll, C, N, and P were converted to areal densities. Ratios of C:Chl were calculated by mass, and all other ratios are molar ratios (C:N, C:P, and N:P).

A subset of periphyton samples were analyzed for their carbon and nitrogen isotopic composition. Between 9 and 11 periphyton samples were analyzed from each site on three occasions: November 2004, February, 2005, and August 2005. Aliquots (10–30 ml) of periphyton slurry were dried at 100°C in covered porcelain crucibles for at least 24 h. Isotopic analyses for nitrogen and carbon were performed using a Costech/Thermo-Finnigan Delta Plus XP, coupled elemental analyzer, continuous flow, isotope ratio mass spectrometer (EA-CF-IRMS). All analyses were performed in the Isotope Biogeochemistry Lab at The Pennsylvania State University. Powdered, decarbonated samples were weighed and sealed in tin boats for isotopic analysis.

Samples were combusted in a Costech elemental analyzer at 1020°C with a ‘zero blank’ helium atmosphere autosampler. Data are reported using delta notation relative to atmospheric  $\text{N}_2$  for nitrogen and the Vienna Pee Dee Belemnite International Standard (V-PDB) for carbon. Reference gases were calibrated relative to IAEA N3 (20.3 ‰) and N2 (4.65 ‰) isotopic standards for nitrogen and NIST polyethylene foil, graphite sucrose and NBS-19 standards for carbon. Run-to-run variations in nitrogen isotopes from instrument variability and reference gas aliquots were calibrated using a well-characterized in-house caffeine standard for carbon and nitrogen. Standard precision was often better than  $\pm 0.15\%$  for N but is reported as  $\pm 0.2\%$  to reflect

known isotopic values of IAEA N standards. Carbon isotope precision is  $\pm 0.05\%$ . Sample sizes were weighed to produce at least 2,000 mV nitrogen peak; however, analyses of standards indicate that sample peaks greater than 1,250 mV do not deviate statistically from reported standard values. The C/N ratios of a few samples required a significant helium dilution of the  $\text{CO}_2$  peaks. Without dilution of the  $\text{CO}_2$  peak, the masses required for  $\delta^{15}\text{N}$  analyses would result in  $\text{CO}_2$  peak sizes that would overload the ion source and produce unreliable results.

#### Nutrient enrichment bioassays

Periphyton N-limitation was assessed using nutrient enrichment bioassay (NEB) experiments (Fairchild et al., 1985). Nutrient-diffusing substrata were constructed from terracotta flower pot saucers (11 cm diameter, 97.4  $\text{cm}^2$  surface area) sealed with Plexiglas plates using silicone sealant. The substrata were attached Plexiglas-side down onto concrete bricks. Control substrata were filled with a solution of 2% agar in distilled water and allowed to cool before completely sealing the substrata. N-enrichment treatments were made by adding 0.5 M  $\text{NO}_3^-$  to the 2% agar solution. The substrata were assigned to bricks and positions in a randomized balanced incomplete block design to control for differences within the sampling transects and provide equal comparisons among treatments (Kuehl, 2000).

Release rate experiments were conducted to determine if the substrata released nitrogen predictably (Fairchild et al., 1985). Two substrata from each control and nitrogen treatments were placed in plastic beakers containing 1.75 l distilled-deionized  $\text{H}_2\text{O}$ . Water samples were collected from the beakers on days 1, 10, 20, and 30. The water was replaced at 48-h intervals, except on the sampling days, when the water was replaced 24 h before the sampling. Water samples were analyzed for nitrate using the automated cadmium reduction method with a minimum detect limit of 0.012 mg  $\text{NO}_3^-/\text{L}$  (APHA, 1995). Substrata made with 0.05 M  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  did not release measurable amounts of phosphorus (detection limit, 0.021 mg  $\text{PO}_4^-/\text{L}$ ) and were excluded from the analyses.

The substrata were incubated at one ridge and one valley site in Spring Creek (sites 2 and 4). These sites were selected to span the range of environmental

conditions in the stream and provide comparable light conditions. The substrata were placed across runs within the stream to minimize any differences in flow or light among the replicates. The experiments were incubated in the stream for 21–31 days, which has been shown to provide reasonable accumulation in comparison to the surrounding biomass (Godwin & Carrick, 2008). Substrata were sampled by scraping periphyton from the clay surfaces with a razor blade and a non-metallic bristle brush while rinsing with distilled-deionized water. Final chlorophyll-*a* densities were determined as described above, and expressed as accumulation rates in order to standardize effects of incubation length among the experiments. In addition, periphyton nutrient densities on natural substrata and experimental substrata were measured for the fall and winter experiments. Samples for periphyton C, N, and P were filtered and analyzed as before.

#### Data analysis

Spatiotemporal variation in periphyton nutrient content was evaluated using separate two-way multivariate analyses of variance (MANOVA) tests performed on mean densities and ratios, where site and season were considered as fixed factors. These analyses are orthogonal because the ratio of any two nutrients is assumed to be independent of the density of either nutrient. Upon revealing significant multivariate effects, one-way ANOVA tests were performed on each dependent variable to test spatial and seasonal differences. To control for the experiment-wide error rate, ANOVA *P*-values were Bonferroni corrected by multiplying by the number of dependent variables (Johnson & Wichern, 2002). One-way ANOVA tests were followed by Tukey's multiple means comparisons.

Chlorophyll accumulation rates were evaluated using an ANOVA model, where site, season, and treatment were considered as fixed factors with all interactions. Brick and replicate positions within the stream were used as random factors to account for any variance contributed by the position of the brick in the stream (Kuehl, 2000). Periphyton nutrients (C, N, and P) and nutrient ratios (C:Chl, C:N, and C:P) from the fall and winter experiments were analyzed using MANOVA tests with site, season, and treatment as fixed factors. Periphyton nutrient densities

and nutrient ratios on experimental substrata were compared with those on natural substrata using Hotelling's  $T^2$  tests assuming unequal variance-covariance matrices (Johnson & Wichern, 2002). All data were transformed prior to analyses to meet assumptions of multivariate normality and homogeneity of variances (Box & Cox, 1964). Correlations among chlorophyll and nutrient densities were computed as Pearson product-moment correlation coefficients. All statistical analyses were performed using SAS version 9 (SAS Institute, Cary, North Carolina).

## Results

### Ambient stream conditions

The physical and chemical characteristics in Spring Creek reflect a strong gradient of environmental conditions across the ridge to valley transition (Table 1). An increase in conductivity and nutrient concentrations was observed from the ridge sites to the valley sites. Concentrations of nitrate increased more than 10-fold from ridge to valley sites, while orthophosphate concentrations were low or below detection throughout the stream.

### Periphyton biomass and nutrient stoichiometry

Periphyton chlorophyll and nutrient densities exhibited considerable spatial variation following the chemical conditions in the stream (Tables 1, 2). Chlorophyll, C, N, and P densities varied several fold between ridge and valley sites (Table 2). Periphyton biomass and nutrient densities were significantly greater at sites 4 and 5 compared to densities at sites 1 and 2 (all  $P < 0.01$ ), while Site 3 was typically intermediate between the upstream (1 and 2) and downstream sites (4 and 5).

Seasonal differences were observed for C, N, and P densities (all  $P < 0.05$ ), but chlorophyll concentrations did not vary among seasons (Table 3). Summer nutrient densities increased by 2- to 3-fold over values measured in the winter and spring, and densities were the lowest in fall. However, there was no significant interaction between site and season for any variable. As expected, periphyton chlorophyll and nutrient densities were highly correlated with

**Table 1** Mean and coefficients of variation (%) for physical and chemical variables for sites in Spring Creek

Site	Flow rate (m <sup>3</sup> /s)	Canopy cover (%)	Temperature (°C)	Specific conductivity (μS/cm)	NO <sub>3</sub> -N (mg/l)	PO <sub>4</sub> -P (μg/l)
1	0.16 (82)	85.0	8.37 (50.1)	35.1 (8.4)	0.20 (44.12)	–
2	–	12.0	8.53 (62.7)	45.0 (10.3)	–	–
3	1.03 (236)	12.4	9.31 (26.6)	385.2 (13.5)	2.75 (26.5)	3.6
4	3.35 (92)	13.5	9.65 (42.5)	479.1 (16.6)	3.41 (11.9)	3.9 (16.7)
5	4.94 (85)	6.4	11.02 (35.1)	525.5 (8.1)	4.00 (13.3)	6.9 (24.8)

**Table 2** Mean and coefficient of variation (%) for periphyton chlorophyll and nutrient densities and ratios on natural substrata

Site	Chl- <i>a</i> (mg/m <sup>2</sup> )	C (g/m <sup>2</sup> )	N (g/m <sup>2</sup> )	P (g/m <sup>2</sup> )	C:Chl	C:N	C:P
1	15.6 (106.6)	2.4 (63.8)	0.9 (94.3)	0.06 (124.2)	259.8 (63.2)	11.3 (65.3)	232.2 (70.9)
2	69.2 (115.0)	8.6 (83.0)	2.9 (92.1)	0.11 (124.8)	152.7 (70.0)	10.4 (47.3)	354.4 (65.6)
3	238.6 (110.6)	28.6 (100.5)	7.2 (93.0)	0.30 (149.3)	121.5 (68.3)	10.7 (30.0)	351.2 (80.4)
4	348.5 (62.6)	50.8 (47.0)	8.3 (64.6)	0.35 (69.3)	147.0 (62.1)	17.6 (29.6)	545.6 (95.8)
5	344.7 (63.7)	46.5 (66.2)	9.9 (60.5)	0.41 (72.6)	137.3 (80.7)	12.7 (28.6)	314.9 (21.6)

C:Chl ratios are by mass; C:N and C:P ratios are molar ratios

**Table 3** MANOVA results for nutrient and chlorophyll densities on natural substrata

Test	Term	df	<i>F</i>	<i>P</i> -value
Chlorophyll and nutrient densities				
MANOVA	Site	16	2.63	0.009
	Season	12	3.39	0.003
Chl- <i>a</i>	Site	4	18.15	<0.001
	Season	3	2.75	0.329
Carbon	Site	4	18.62	<0.004
	Season	3	8.09	0.009
Nitrogen	Site	4	11.10	0.001
	Season	3	7.95	0.010
Phosphorus	Site	4	8.30	0.005
	Season	3	9.96	0.004
Chlorophyll and nutrient ratios				
MANOVA	Site	12	1.23	0.305
	Season	9	3.91	0.002
C:Chl- <i>a</i>	Site	4	1.53	NS
	Season	3	11.81	0.009
C:N	Site	4	1.18	NS
	Season	3	0.54	NS
C:P	Site	4	0.85	NS
	Season	3	2.81	0.301

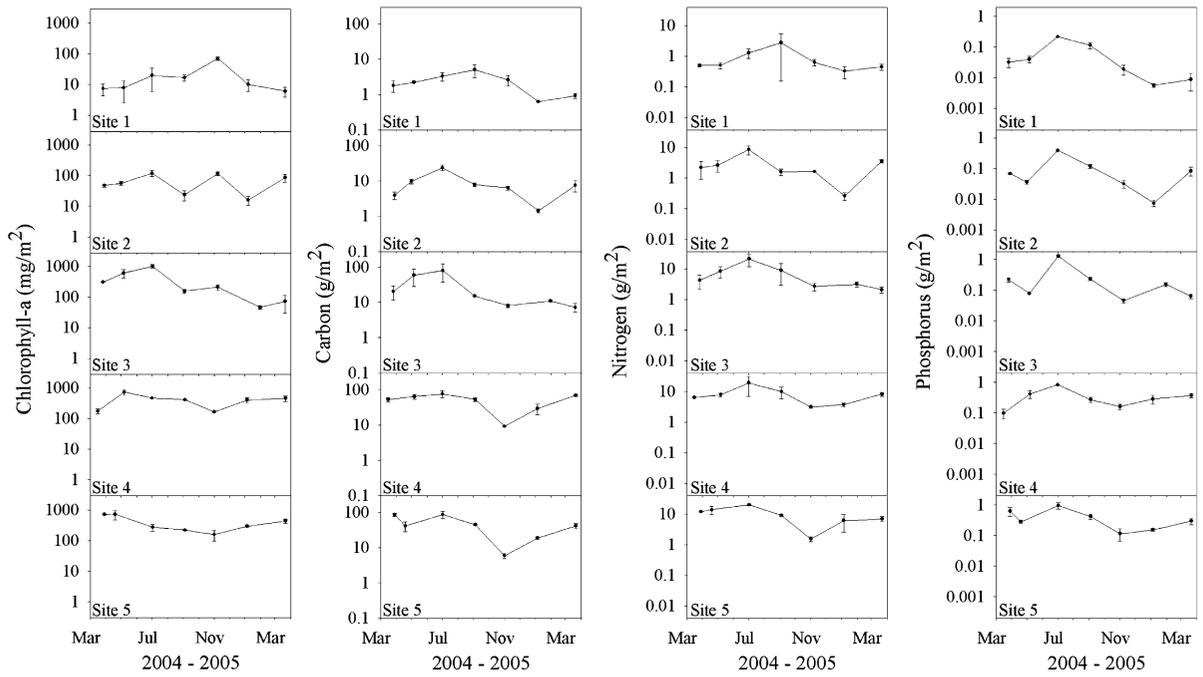
*F* values are Wilks' Lambda. Error degrees of freedom for densities = 14, ratios = 15. NS not significant (*P* > 0.50)

each other, suggesting they all reflected changes in periphyton biomass (range in  $R^2$  0.65–0.84, all  $P < 0.0001$ ).

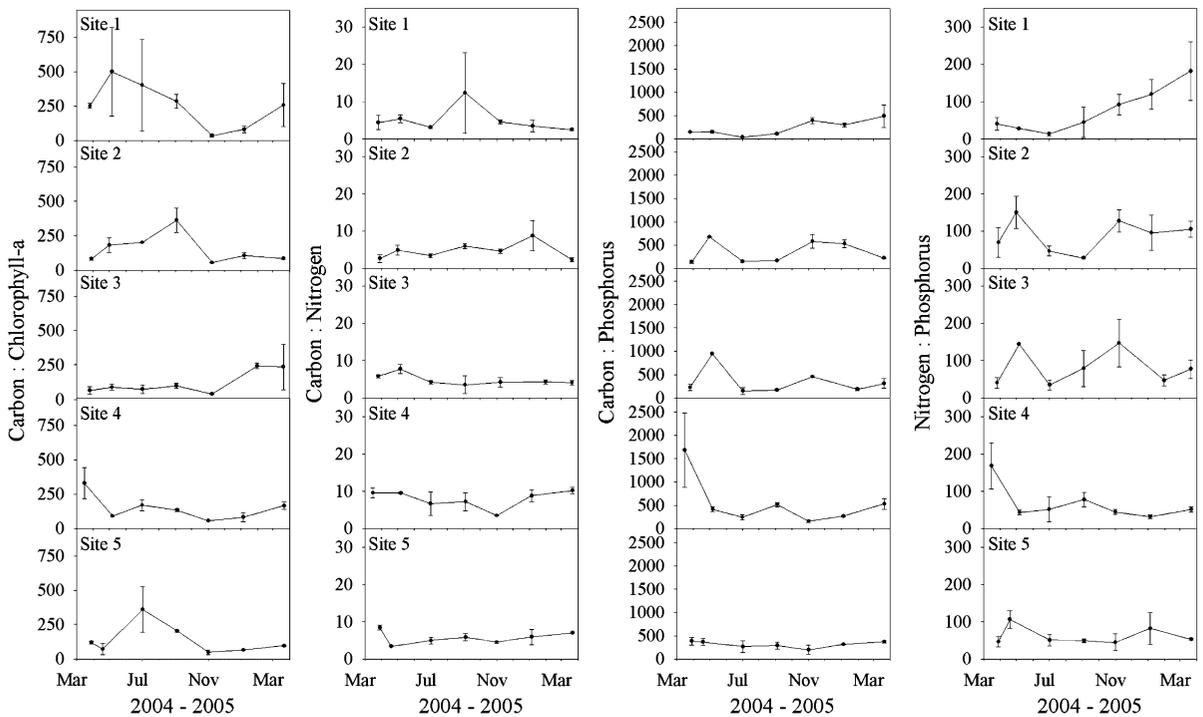
The carbon to chlorophyll ratio (C:Chl) exhibited considerable spatial variation among sites (Tables 2, 3). C:Chl ratios were nearly two-fold higher at site 1 than at the other sites, but did not show any other major differences beyond the first site. The C:Chl ratio was dramatically reduced during the fall at all sites (Fig. 2). C:N and C:P molar ratios did not exhibit significant spatial or temporal differences in Spring Creek (Fig. 2; Table 3). The only major difference among sites, although statistically insignificant, was at site 4 where both the mean C:N ratio and mean C:P ratio were at least 30% higher than those at any of the other sites. These ratios did not exhibit strong seasonal fluctuations, despite apparent seasonal changes in the densities of C, N, and P (Figs. 1, 2).

#### Periphyton response to nutrient enrichment

Nutrient diffusing substrata were used to assess whether N limits periphyton accumulation or stoichiometry (Fig. 3). There was no significant effect of treatment on chlorophyll and nutrient accumulation rates (Wilks' Lambda  $F_{4, 13} = 0.61$ ,  $P > 0.50$ ).

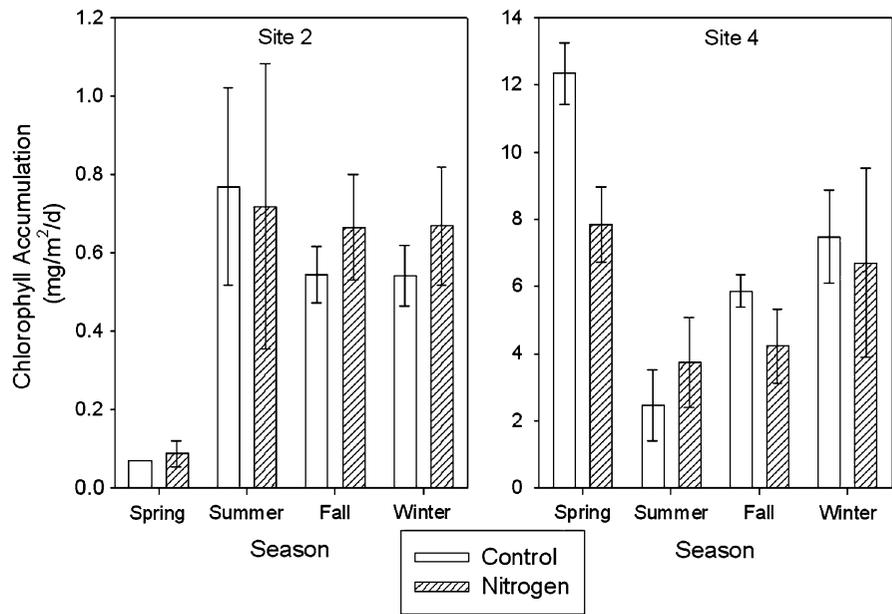


**Fig. 1** Mean periphyton chlorophyll, carbon, nitrogen, and phosphorus densities in Spring Creek ( $\pm 1$  standard error)



**Fig. 2** Mean periphyton C:Chl, C:N, C:P, and N:P ratios in Spring Creek ( $\pm 1$  standard error)

**Fig. 3** Chlorophyll accumulation rates from NEB experiments at sites 2 and 4 in Spring Creek, repeated in each season ( $\pm 1$  standard error)



Chlorophyll and nutrient densities were consistently higher for all treatments at site 4 than at site 2 ( $F_{4, 13} = 62.13$ ,  $P < 0.001$ ). Chlorophyll and nutrient accumulation rates on the nutrient-diffusing substrata also exhibited significant effects of season ( $F_{4, 13} = 3.63$ ,  $P = 0.034$ ), with the lowest rates during spring at site 2 and the highest rates occurring in spring for site 4. Chlorophyll and nutrient ratios (C:Chl, C:N, C:P) did not show a significant effect of treatment ( $F_{3, 13} = 0.48$ ,  $P > 0.50$ ). For all nutrient treatments, there were significant multivariate effects of season ( $F_{4, 13} = 4.14$ ,  $P = 0.027$ ) and site ( $F_{4, 13} = 13.68$ ,  $P < 0.001$ ) on the chlorophyll and nutrient ratios. When analyzed by individual ANOVA tests, only the C:N ratios showed significant differences, with higher ratios at site 4 during the fall ( $P < 0.001$ , site by season interaction  $P = 0.024$ ).

On average, NEB substrata at sites 2 and 4 achieved 49% and 109% of the biomass on the naturally occurring rocks at each site, respectively. The mean coefficient of variation among replicate chlorophyll measurements was similar between site 2 ( $32.4\% \pm 7.0$ , mean  $\pm 1$  standard error) and site 4 ( $28.2\% \pm 5.2$ ). The mean coefficient of variation among replicate pots was also similar between site 2 ( $33.1\% \pm 4.2$ ) and site 4 ( $37.5\% \pm 5.4$ ) and comparable to coefficients of variation from replicate natural and artificial substrata (H. Carrick, unpublished data). All of the experiments showed significant differences

between nutrient-diffusing substrata and natural substrata for at least one nutrient density or ratio (Hotelling's two-sample  $T^2$ , all  $P < 0.01$ ).

The laboratory release rate experiment revealed that both the control and N-enriched treatments released nitrate over the course of 30 days. Release of nitrate from the control substrata did not show a significant trend over the course of the experiment and ranged from 0.022 to 0.119 mg N/day ( $0.087 \pm 0.013$  mg N/day, mean  $\pm 1$  standard error). Release of nitrate from the N-enriched substrata decreased over the course of the experiment from a mean rate of 114 mg N/day ( $\pm 13$ ) on day 1 to 11 mg N/day ( $\pm 1$ ) on day 30. Release of orthophosphate from both treatments was considered to be negligible (detection for all samples,  $< 0.021$  mg  $\text{PO}_4/\text{L}$ ).

Periphyton nutrient storage and isotopic composition

To evaluate the potential impact of periphyton on stream nutrient retention, periphyton nutrient content (N and P) was compared with water column nutrient concentrations in Spring Creek (Table 4). Average values were determined among the sites for all the available data. Areal densities of N in the periphyton were 4- to 90-fold greater than  $\text{NO}_3\text{-N}$  concentrations integrated through the water column as areal densities. Periphyton P densities were 100- to 300-fold greater

**Table 4** Mean areal concentrations of N and P in stream water versus periphyton tissue along an upstream to downstream gradient in Spring Creek

Site	Depth (m)	Periphyton N (g/m <sup>2</sup> )	Water NO <sub>3</sub> -N (g/m <sup>2</sup> )	Periphyton P (mg/m <sup>2</sup> )	Water PO <sub>4</sub> -P (mg/m <sup>2</sup> )
1	0.36	0.9	0.07	60	–
2	0.37	2.68	–	95	–
3	0.22	6.38	0.60	271	0.79
4	0.53	7.74	1.81	331	2.08
5	0.24	9.02	0.96	366	1.65

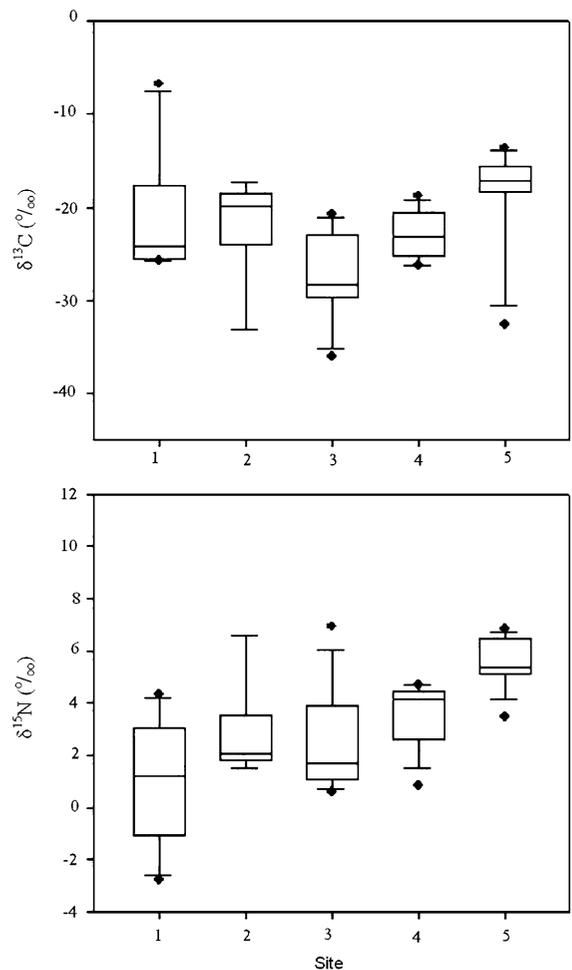
than the stream water PO<sub>4</sub>-P integrated through the water column at all the sites for which these data were available. Periphyton  $\delta^{13}\text{C}$  did not show a clear trend among the sites sampled in Spring Creek, while the  $\delta^{15}\text{N}$  content of periphyton generally increased from the headwaters to downstream (Fig. 4; Table 5).

## Discussion

### Variation in periphyton stoichiometry

The physical and chemical characteristics in Spring Creek reflect a strong gradient of environmental conditions that followed ridge-to-valley transitions common in this region (Omernik, 1987; Woods et al., 1996). Low conductivity at the ridge sites reflects poor dissolution of shale and sandstone parent material present, while high conductivity values in the valley (generally >400  $\mu\text{S}/\text{cm}$ ) reflect high dissolution of the parent limestone–dolomite material in the valley. This gradient in water conditions is similar to that observed previously from stream surveys throughout the mid-Atlantic slope (Pan et al., 1999). Strong longitudinal variation in periphyton chlorophyll and nutrient densities appears to be tied to the mixed geology of the system. However, despite similar canopies at each site, there may have been confounding effects of light availability on periphyton chlorophyll and nutrient content (Hillebrand et al., 2004).

The lack of significant seasonal patterns in periphyton nutrient ratios may be coupled with reduced temporal variation in periphyton biomass relative to most streams. Specifically, nutrients and conductivity exhibited small variation at all of the sites and the coefficients of variation for conductivity were below the 25th percentile of 98 rivers in Eastern North America (Ch  telat & Pick, 2001). Because benthic algal biomass densities at sites 3, 4, and 5



**Fig. 4**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  content in periphyton tissue along sampling sites in Spring Creek. Center lines represent the median and the upper and lower boundaries of the boxes represent the 25th and 75th percentiles, respectively. The error bars denote the 10th and 90th percentiles and the points represent the highest and lowest data points

were greater than most streams surveyed in the literature (Biggs, 2000; Dodds et al., 2002b), we might expect that periphyton in the downstream

**Table 5** Mean and coefficient of variation (%) of carbon and nitrogen isotopic compositions for periphyton sampled along an upstream to downstream gradient in Spring Creek, PA

Site	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N
1	−21.95 (21.2)	0.70 (324.5)	25.3 (21.9)
2	−21.33 (14.5)	3.09 (53.1)	20.2 (9.7)
3	−27.90 (17.5)	2.16 (88.9)	18.7 (16.5)
4	−22.50 (12.5)	4.02 (30.9)	23.9 (17.5)
5	−18.15 (18.4)	5.61 (12.4)	41.0 (29.0)

reaches of Spring Creek are not strongly limited by major nutrients (see below). Given this and the relatively stable hydrologic inputs to the stream, Godwin & Carrick (2008) hypothesized that that this stability promotes high biomass and lower temporal variation. The lack of temporal variation in biomass and high growth rates throughout the season and may be coupled to the relatively homeostatic algal C:N:P stoichiometry (Sternier & Elser, 2002). The significant declines in biomass observed in the fall were likely the result of scouring during a large flow disturbance caused by the remnants of Hurricane Ivan, which passed through central Pennsylvania just prior to our fall sampling period. This storm system produced peak discharge in excess of 60 m<sup>3</sup>/s. Following the disturbance event, periphyton biomass (Chl and C) suffers no changes in C:N:P stoichiometry, which can be taken as further evidence of balanced growth (Hillebrand & Sommer, 1999).

The C:N and C:P ratios in Spring Creek periphyton did not exhibit strong variation among sites despite very large increases in biomass (Chl) and total CNP content downstream (10-fold increase). That said, elemental ratios among stream periphyton assemblages can be quite variable (Kahlert, 1998) and may be influenced by a number of environmental conditions, such as light, temperature, and water column N:P ratios (Bothwell, 1985; Peterson et al., 1993; Stelzer & Lamberti, 2001). Cross et al. (2005) cite considerable variation in freshwater periphyton C:N (4:1–280:1) and C:P ratios (25:1–16,500:1), demonstrating that these assemblages are not strictly homeostatic (Elser et al., 2000; Sternier & Elser, 2002). The degree of homeostasis observed in Spring Creek is unusual for algae, which are generally less homeostatic than heterotrophs (Sternier & Elser, 2002; Cross et al., 2005; Frost et al., 2005a). In light of the unusually high biomass and growth rates

of these periphyton communities, these results suggest that the periphyton are likely to be N- and P-sufficient. Whether this is due to the specific nutrient composition of Spring Creek or is a property of these specific periphyton communities remains to be tested.

Periphyton at the downstream sites accumulated biomass (Chl and C) more quickly than the upstream sites, but the C:P and C:N ratios were similar among the sites. Elser et al. (1996) provide the Growth Rate Hypothesis (GRH) as an explanation for strong covariance between cellular phosphorus content and the growth rate of an organism. By the GRH, periphyton in Spring Creek may be expected to have greater amounts of ribosomal RNA due to their high relative growth rate, resulting in higher cellular concentrations of phosphorus (Elser et al., 2003). This may explain how periphyton in Spring Creek maintained relatively low C:P despite a large gradient in resource availability. It is not clear how periphyton could maintain high P content despite low P availability, but the combination of high growth rate and high cellular phosphorus is consistent with the GRH.

#### Periphyton nutrient limitation

Nutrient enrichment bioassays did not provide evidence for N-limitation of periphyton. Our results seem consistent with the overall lack of longitudinal variation in periphyton nutrient stoichiometry. Other streams also show no apparent limitation by N or P (Pringle et al., 1986). In a survey by Francoeur (2001), 40% of the experiments evaluated (101 out of 237 published bioassay experiments) involving enrichment of stream periphyton did not detect N or P limitation. The lack of a response to N-enrichment among these studies that used relatively comparable bioassay methods, suggests that stream algae are commonly N-sufficient. This conclusion is congruent with the nutrient stoichiometry from Spring Creek, which indicates that N does not likely limit periphyton growth.

Whereas nitrogen did not appear to be a limiting nutrient in Spring Creek, periphyton nutrient ratios indicated possible P limitation at all of the sites. Kahlert (1998) cites P limitation of periphytic algae at N:P > 32 or C:P > 369. By this criterion, the mean N:P ratios at all the sites indicated possible P-limitation relative to N content (see Table 2). Mean C:P ratios at sites 1, 2, 3, and 5 also did not indicate

strong P-limitation, but site 4 appeared to be marginally P-limited by this criterion (mean C:P = 546:1). Without knowing the results of experimental P enrichment, it is not possible to exclude limitation by phosphorus. In addition, the C:P ratios may be difficult to compare among studies because total periphyton nutrients were measured in Spring Creek, whereas many ratios in the literature use algal C only (Hoagland et al., 1993; Frost et al., 2005b). Our samples may have contained detrital material or heterotrophic organisms, both of which have different C:N and C:P ratios compared with algae (Cross et al., 2005). Because the contribution of algal C may only be a portion of total periphyton C (Frost et al., 2005b), correcting the C densities to reflect only algal carbon would lower the C:N and C:P ratios. The experimental and stoichiometric results indicate that periphyton in Spring Creek were not N-limited and less likely to be P-limited than indicated by C:P ratios.

Although both sites 2 and 4 showed no response to N enrichment (see below), there may be other factors limiting periphyton accumulation. Accumulation rates showed significant seasonal differences and were consistently higher at site 4 than at site 2, which was congruent with previous studies on algal accumulation in Spring Creek (Godwin & Carrick, 2008). Biomass at site 4 was exceedingly large relative to other streams (Welch et al., 1988; Dodds et al., 2002b) and appeared to be saturated with N and P. Water temperature and light availability were similar between the two sites, but conductivity was an order of magnitude greater at site 4. It is possible that accumulation rates at site 2 are limited by other dissolved substances given that the concentration of dissolved substances is low (specific conductivity < 40  $\mu\text{S}/\text{cm}$ ). In addition, periphyton biomass was reduced more severely by a known disturbance event at site 2 than at site 4 (Godwin & Carrick, 2008). The frequency and magnitude of scouring disturbances appeared to be greater at site 2, and this may explain why periphyton biomass did not approach the level of the downstream sites.

#### Role of periphyton in nutrient cycling and downstream transport

Stable isotope signatures of periphyton appear to reflect watershed influences on stream chemistry. The increase in periphyton  $\delta^{15}\text{N}$  with stream order is

likely a result of anthropogenic inputs of nitrogen to the stream. Such values are characteristic of agricultural runoff enriched with fertilizers and domestic animal waste (Kendall et al., 2001). The absence of significant spatial patterns in periphyton  $\delta^{13}\text{C}$  is likely explained by natural factors (i.e., contribution of karstic groundwater versus atmospheric inputs) as opposed to anthropogenic influences (Peterson & Fry, 1987; Fry, 2006). This is interesting given the stark contrast in stream water chemistry between ridge and valley sites (conductivity 50 and 500  $\mu\text{S}/\text{cm}$ , respectively). The periphyton C:N ratios obtained from the mass spectrometric analyses were higher than those obtained through the ICP method (Tables 2, 5). This is likely an artifact of the drying procedure used to prepare samples for isotopic analysis, as volatilization of both C and N may have occurred at higher temperatures used to dry the samples.

Our estimates of nutrient retention within periphyton in Spring Creek highlight the importance of periphyton in stream nutrient retention, similar to that observed for other stream ecosystems (Mulholland et al., 1995; Fisher et al., 1998). Scouring of periphyton is likely to contribute to downstream nutrient loading during major disturbance events. This seems evident from a scouring event measured in September 2004, when 85–99% of periphyton biomass was removed from stream rocks at all the sites. The downstream transport of materials from streams within the Susquehanna River watershed, like Spring Creek, ultimately contributes to the largest nutrient load delivered to the Chesapeake Bay. Interestingly, Paerl et al. (2001) showed that the majority of annual nutrients loaded to coastal regions of the Southeast United States can be delivered through hurricanes whose storm track passed through the area. By our conservative estimates, stream nutrient content could increase more than 10-fold for N and 100-fold for P just from the addition of periphyton into the overlying water during a high flow event.

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