RESEARCH PAPER

The effect of periphyton stoichiometry and light on biological phosphorus immobilization and release in streams

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Abstract Periphyton stoichiometry can vary substantially as a result of differences in stream nutrient availability. A decrease in the periphyton carbon to phosphorus (C:P) ratio should decrease the demand for new P to be immobilized from stream water, but no studies to our knowledge have explored the relationship between periphyton stoichiometry and net P immobilization and release by periphyton. We sought to model biological P immobilization and release (flux) in streams by measuring periphyton stoichiometry and light availability. We measured P flux to and from intact periphyton on stream cobbles (20–100 mm diameter) in 1 L microcosms incubated with streamwater under variable light conditions. Net P immobilization occurred in 75% of microcosms, net P release occurred in only 5% of microcosms, and 20% of microcosms had neither net immobilization nor net release. When normalized to stream conditions, net P immobilization was highest when light availability was high (<60% canopy attenuation) and the periphyton C:P ratio was also high. In contrast, net P release occurred only when light availability was low (>60% canopy attenuation) and the periphyton C:P ratio was also low.

A multiple regression model that included both periphyton stoichiometry and light availability from the growing season only, and the interaction term of these two variables, explained 99% of the variation in daily periphyton P flux observed in the study. These results indicate that in order to predict periphyton P immobilization, periphyton stoichiometry and light availability should be considered together. Furthermore, the results indicate that net P immobilization occurs even in very P-rich periphyton, which can act as a P sink when light availability is high.

Keywords Phosphorus spiraling · Transient storage · Element ratios

Introduction

The effect of nutrient enrichment on stream ecosystems (stream eutrophication) is an understudied component of aquatic ecology, particularly in comparison to lakes (Dodds 2007). Streams and rivers not only act as conduits that transport nutrients, but also transform nutrients during their downstream transport from the continents to the ocean. Elemental ratios in periphyton can reflect the availability of nutrients in streams (Stelzer and Lamberti 2001, O'Brien and Wehr 2010). Several recent works have also focused on how stream nutrient enrichment and light availability can interact to influence periphyton chemical composition and stoichiometry (Fanta et al. 2010, Hill et al. 2011). What remains unclear is how differences in periphyton stoichiometry may subsequently alter a stream's capacity for biological phosphorus (P) immobilization from streamwater under variable light conditions, and how efficiently P immobilization or release (spiraling) occurs due to variation in both light climate and periphyton stoichiometry (Mulholland et al. 1985).

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Abiotic P immobilization and release in streams has been shown to be negatively correlated with the particle size of the stream substrate, where greater abiotic P immobilization occurs with soil-sized particles and less with larger, rocky substrate (Lottig and Stanley 2007). However, biological P immobilization and release is positively correlated with particle size, presumably because larger substrates provide a more stable location for microflora growth (Lottig and Stanley 2007). Indeed, periphyton growing on substrate greater than ∼ 20 mm diameter can have a major influence on stream and river P flux (Gainswin et al. 2006). Therefore, P spiraling in streams dominated by cobble and bedrock bottoms should be controlled largely by biological processes, but to our knowledge few data are available to test this hypothesis (Withers and Jarvie 2008).

Periphyton in headwater streams may have a tremendous capacity for nutrient storage in the landscape (Godwin et al. 2009). Environmental factors such as light and temperature can influence biological metabolism and nutrient immobilization and release in streams. Sterner et al. (1997) suggested that an increase in the ratio of light to water column soluble reactive phosphorus (SRP) would result in an increased sestonic carbon to phosphorus ratio (C:P ratio). Fanta et al. (2010) tested this "light-nutrient hypothesis" for stream periphyton and found that P availability affected periphyton C:P ratios more strongly than light availability in both experimental and natural streams. This pattern was attributed to the nonalgal portions of periphyton, which in terms of P immobilization did not respond directly to light. These results were consistent with another study which reported that water column P levels alone, rather than in combination with light, have a stronger effect on periphyton C:P ratios in lakes (Frost and Elser 2002).

Stream periphyton are dynamic in their capacity to obtain nutrients through the enzymatic hydrolysis of dissolved organic nutrients in streamwater (Scott et al. 2009), and retain nutrients within the periphyton matrix through efficient recycling (Wetzel 1993). However, elevated stream nutrient concentrations can decouple autotrophic and heterotrophic processes in periphyton due to decreased demand for recycled nutrients (Scott et al. 2008). We hypothesized that high light availability and high periphyton C:P ratios would result in net P immobilization from streamwater, and that low light and low periphyton C:P ratios would result in net P release to streamwater. Further, we hypothesized that the magnitude of P immobilization and release rates would be affected by the interaction between periphyton stoichiometry and light availability. To test these hypotheses, we conducted a series of microcosm experiments using intact periphyton collected from Ozark streams in the nutrient surplus area in Northwest Arkansas (Slaton et al. 2004). These streams were similar in their nutrient concentrations but differed drastically in their surrounding land use and riparian cover characteristics.

Methods

Stream characterization and periphyton sampling

Five streams (second or third order) in the Illinois River Watershed of Northwest Arkansas's Ozark Plateau were selected for the study. The Ozark Plateau is located in Northern Arkansas and Southern Missouri, USA. Ozark streams frequently experience high flow due to a moderate to high gradient. They are typically constantly flowing streams that contain cobble and gravel substrate. The dominant landcover in the region is mixed forest and cattle pasture. The Illinois River has experienced nutrient enrichment corresponding to human activity in its watershed, but recent data indicate that water quality conditions are improving due to watershed management (Scott et al. 2011). The streams chosen for this study represented a variety of land-use characteristics in each watershed, were similar in size and geomorphology, and were similar in bottom substrate (Table 1). We used the 2006 National Land Cover Database to estimate the relative contributions of various land uses to the drainage area of each stream.

Water quality samples were collected seasonally (October 2008, January–February 2009, April–May 2009, and July–August 2009) from each stream and were analyzed for SRP, total P, and total N using standard methods (APHA 2005). Samples for stream SRP analysis were filtered through a 0.45 μm membrane filter and analyzed colorimetrically using segmented-flow automated analysis on a SkalarSan autoanalyzer. Total P concentrations were determined similarly on unfiltered samples following persulfate digestion (APHA 2005). Total N concentrations were determined as nitrite following persulfate digestion and cadmium reduction (APHA 2005).

Periphyton stoichiometry was also quantified seasonally in each stream from 2008 to 2009 (October 2008, January–February 2009, April–May 2009, and July–August 2009). Photosynthetically active radiation (PAR) was measured at several locations in a ∼ 50 m stream reach and also under direct sun to determine percent light attenuation by the stream canopy. Ten similar sized stones (20–100 mm diameter) were collected from each stream during each season. Preliminary data indicated that periphyton biomass and stoichiometry did not differ among stones of different sizes within individual streams. This gave us confidence that choosing stones within a relatively narrow size range (which would fit inside the microcosms; see below for details) would still potentially result in differences in periphyton biomass and stoichiometry among streams, rather than being controlled by the size of stones alone.

Periphyton were scraped from the stones using a stiff-bristled brush and rinsed with stream water to form a periphyton slurry. A composite slurry was created from the

Table 1 Stream drainage characteristics, streambed geomorphology, stream light climate, and average stream nutrient concentrations during the study period

Stream	Land use			Flow rate $(m^3 \, s^{-1})^a$	% Substrate <20 mm	Light reaching stream (%)				Mean SRP $(mg \, L^{-1})$ $n = 4$ (SE)	Mean TP $(mg \, L^{-1})$ $n = 2$ (SE)	Mean TN $(mg \, L^{-1})$ $(n = 1)$
	% Pasture	% Forest	% Urban			Oct. 2008	Feb. 2009	May 2009	Aug. 2009			
Chamber's Springs	28	71	1	0.08	51	20	82	70	69	0.04 (0.003)	0.04 (0.003)	1.89
L. Wildcat Creek	61	20	19	0.16	51	93	89	93	94	0.03 (0.011)	0.03 (0.007)	5.33
Moore's Creek	33	59	8	0.20	35	54	82	11	6	0.07 (0.007)	0.12 (0.024)	3.03
Mud Creek tributary	7	25	68	0.02	53	48	55	49	44	0.02 (0.009)	0.03 (0.012)	1.03
Wildcat Creek	70	26	4	0.19	56	32	57	23	20	0.04 (0.005)	0.04 (0.005)	4.68

PAR ranged from 43 to 2870 $\mu E \, m^{-2} \, s^{-1}$ in stream reaches throughout the study

[a] Rogers et al. (2011), Aug 2008, during base flow

combined biomass of the ten stones collected from each stream on each date. The composite slurry total volume was recorded and the slurry was divided into two sub-samples. Five milliliters of slurry were filtered onto a pre-muffled (500°C for 4 h) and pre-weighed Whatman GFF filter for ash-free dry mass (AFDM) analysis (APHA 2005). The remaining slurry was poured into a 70 mL weigh boat and dried at 60–75°C. Dried slurries were ground into a fine powder and stored frozen in a scintillation vial. The dried slurry powder was analyzed for C and N content using a Thermo Flash 2000 elemental analyzer (Thermo Scientific, Flash 2000). Phosphorus content was determined by autoclaving samples in a mixture of peroxodisulfate, boric acid, and sodium hydroxide, then analyzed via spectrophotometry (Færøvig and Hessen 2003).

Possible seasonal differences across sites and site differences across seasons were tested for periphyton AFDM (g cm^{-2}), dry mass (g cm^{-2}), %C, %N, %P, total C (μg cm^{-2}), total N (μg cm^{-2}), total P (μg cm^{-2}), C:P ratios, C:N ratios, and N:P ratios using a one-way analysis of variance (ANOVA) in SAS 9.1. The Ryan–Einot–Gabriel–Welsch post hoc test was performed where we found significant ($P = 0.05$) differences.

P flux experiments

Periphyton samples for P flux experiments were collected seasonally on the same dates as the periphyton stoichiometry samples. Periphyton intact on stones (20–100 mm in diameter) were collected from riffle habitat in each stream and returned to the laboratory. Forty liters of stream water were also collected. Fifteen 1 L microcosms were filled with stream water and placed in a temperature-controlled water bath at ambient stream temperature at the time of sampling. Stones were placed in each microcosm with the periphyton side up and incubated under an artificial light gradient (i.e., $\sim$400, $\sim$140, $\sim$60, $\sim$20, and 0 $\mu E \, m^{-2} \, s^{-1}$). Three replicate microcosms were incubated at each light level for each stream.

Microcosms were spiked with inorganic P (as K_2HPO_4) at T_0 to a final concentration of $\sim$0.5 mg P L^{-1} in order to saturate microcosms with P (Borchardt 1996). Controls were created by adding streamwater to flasks (500 mL) without stones under high light and dark conditions. Controls were spiked to the same concentrations as microcosms with stones, and the sampling and analysis protocol was the same. A 10 mL sample was collected from each microcosm at 0, 3, 5, 7, 9, 13, 17, and 24 h after the P addition. Water samples from treatment and control microcosms were stored in acid-washed, triple-rinsed plastic scintillation vials at 4°C and analyzed for SRP within one week, as described previously. A periphyton slurry was harvested for each stone as described previously, and analyzed for ash-free dry mass (APHA 2005). Phosphorus concentrations in each microcosm were converted to mass by multiplying the measured concentration by the volume of water in each microcosm at the time of sampling.

The mean P mass from the three replicate microcosms for each light level was plotted against average incubation time for each microcosm in a treatment. A linear best fit line was constructed using Sigma Plot version 11.0, where the slope of the line represented hourly P flux (μg P h^{-1}). Slopes of controls were estimated in the same manner and subtracted from each microcosm slope to create a corrected slope value. A negative slope indicated net P immobilization by periphyton, whereas a positive slope indicated net P release by periphyton. A 99% confidence interval was used to determine if the corrected slope supported the null

hypothesis that P flux was zero. In cases where the P concentration changes were not statistically significant at $P \leq 0.01$, the P flux rate for that microcosm was set to 0 μg P h^{-1}. Corrected P flux was then normalized to mean periphyton AFDM (μg P g AFDM^{-1} h^{-1}), or mean rock surface area (μg P cm^{-2} h^{-1}) for each light level.

Daily P flux rates in study streams were estimated by integrating light-specific P flux rates over the appropriate duration. To determine this duration, light intensity data collected at 30 min intervals were obtained from the weather station at University of Arkansas Agricultural Experiment Station, Fayetteville, for the entire study period. Light intensity from the continuous data were averaged for a ten-day period bracketing each sample date (five days before and after light sampling) to estimate each season's potential irradiance. This potential irradiance was adjusted to light attenuation data obtained during sampling to estimate an average daily light intensity for each stream in each season. The experimental light conditions were matched with the appropriate light climate data to determine the duration of a 24 h period over which each set of P flux rates were appropriate. The duration (h) was multiplied by the biomass-specific or areal-specific P flux rates from appropriate experimental light levels to determine the fractional contribution of each light condition to daily P flux. These fractional rates were summed to estimate daily biomass-specific P flux (μg P g AFDM^{-1} day^{-1}) or daily areal-specific P flux (μg P cm^{-2} day^{-1}) for each stream in each season. Nutrient limitation status was inferred from stoichiometric ratios according to the following: C:P >180 and N:P >22, P limitation; C:N >10 and N:P <13, N limitation (Hillebrand and Kahlert 2001). A multiple regression model using both periphyton C:P ratios and average light intensity data, as well as the interaction of these terms, was also constructed using SAS 9.1 to predict periphyton daily P flux rates.

Results

Periphyton biomass and stoichiometry

Land-use and canopy cover varied substantially among streams but water column nutrient concentrations were similar among most streams (Table 1). Average total P in Moore's Creek was 3–4 times higher than total P in the other streams. Periphyton AFDM ranged from 0.1 to 4.7 mg AFDM cm^{-2} with an average of 1.7 mg AFDM cm^{-2} across all streams and dates. Although some variability did exist among streams and seasons, there was no significant difference in AFDM over seasons ($F_{3,15} = 1.31$, $P = 0.31$) or among streams ($F_{4,14} = 0.26$, $P = 0.90$).

Mean periphyton elemental composition varied among streams regardless of season (Fig. 1), but there were no
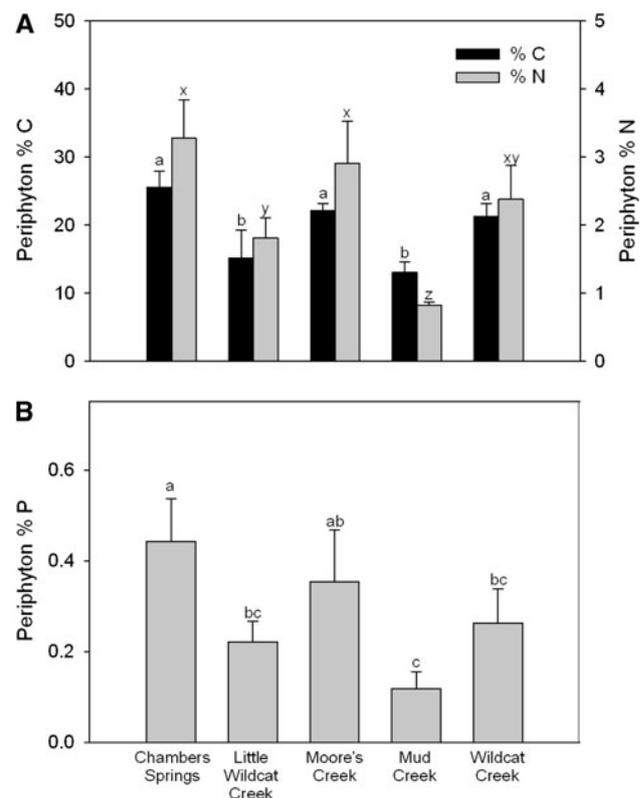

Fig. 1 Mean periphyton stoichiometry for each stream across all seasons. **a** Mean % carbon and nitrogen in periphyton biomass. **b** Mean % phosphorus in periphyton biomass. Streams with *different letters* are statistically significantly different at $P = 0.05$

seasonal differences in mean periphyton elemental composition within streams. Periphyton %C, %N, and %P were correlated across all streams and seasons (Fig. 2). Periphyton C:N and C:P ratios were always greater than the Redfield ratio of 106C:16N:1P (Fig. 2a, b). Periphyton N:P ratios were greater than 16:1 (by moles) in almost all samples (Fig. 2c). Periphyton biomass expressed on a per area basis (mg C cm^{-2}) was not statistically different among streams, but areal periphyton P (mg P cm^{-2}) varied by 4× with the greatest amount of periphyton P in Chamber's Springs and the least in Mud Creek (Fig. 3). Periphyton C:P ratios were not strongly correlated with water column SRP or total P concentrations, but these samples were not usually collected at the same times.

Periphyton C:P ratios ranged from 114 (Moore's Creek in May) to 579 (Mud Creek in July), with an average of 211. There was no difference in C:P ratios over seasons ($F_{3,16} = 2.75$, $P = 0.08$) or among streams ($F_{4,15} = 2.12$, $P = 0.13$). Periphyton C:N ratios ranged from 7.61 (Moore's Creek in July) to 21.01 (Mud Creek in July). There was no difference in C:N ratios over seasons ($F_{3,16} = 0.23$, $P = 0.87$), but there was a difference in C:N ratios among streams ($F_{4,15} = 23.36$; $P \leq 0.0001$). The Mud Creek tributary had a significantly greater C:N ratio than all other
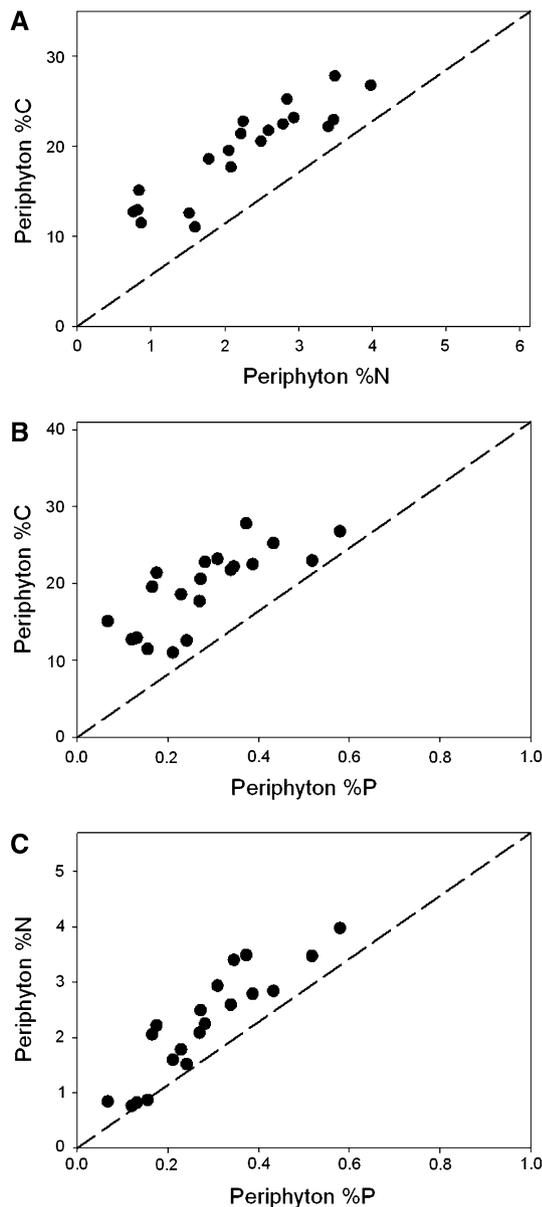
Fig. 2 Relationships between periphyton carbon, nitrogen, and phosphorus across all streams and seasons. *Dashed line* in all panels represents the Redfield ratio, and is used here as an indicator of approximate nutrient balance in periphyton
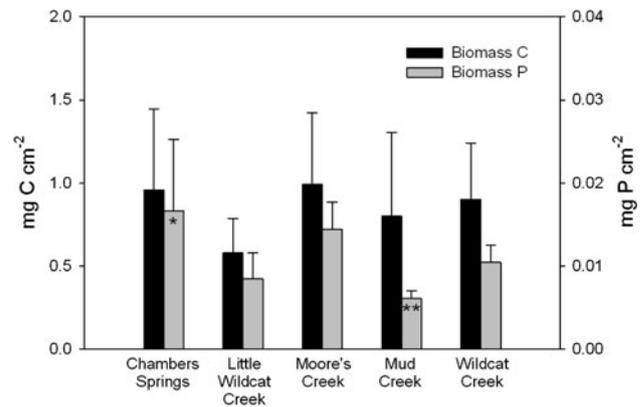

Fig. 3 Areal coverage of periphyton biomass (as carbon per square centimeter) and biomass-bound P for each stream across all seasons. *Asterisks* indicate statistical differences at $P = 0.05$ (*one asterisk*) and $P = 0.01$ (*two asterisks*)

immobilization rate of 18.3 μg P h^{-1} across all streams and seasons (Table 2). Net P immobilization occurred more frequently and at a greater magnitude during the growing season (May and July) than in the nongrowing season (October and January). Statistically significant net immobilization occurred across all streams and incubation light levels in summer, except for samples from Moore's Creek and Mud Creek that were incubated in the dark. Net P release occurred less frequently, but at a similar magnitude, and was observed only during the nongrowing season. In general, hourly P flux rates for each microcosm followed a positive relationship with light during fall, spring, and summer, but hourly P flux was actually lower in microcosms with light intensity exceeding 20 μE m^{-2} s^{-1} in winter. The greatest release and least immobilization always occurred under no light or low light conditions, and the least release and greatest immobilization occurring at high light availability (Table 2).

When periphyton stoichiometry was used to classify periphyton into N, P, or no nutrient limitation categories, statistically significant ($P < 0.01$) P immobilization occurred in more than 80% of samples that were considered P-limited (Table 3). Periphyton P limitation was most frequent during the growing season, occurring in 74% of samples. However, statistically significant P flux also occurred in 24% of samples from the nongrowing season. Periphyton N limitation was more common during the nongrowing season, but growing season N limitation did occur at Chamber's Springs in July. Similarly, N + P co-limitation primarily occurred in the nongrowing season, with one exception: Mud Creek tributary in May. The frequency of statistically significant hourly P flux values varied under both N and N + P co-limited conditions (Table 3).

Daily periphyton P flux values calculated for each stream were weakly correlated with seasonal light availability and periphyton stoichiometry (Fig. 4). However,

streams. Periphyton N:P ratios ranged from 12.33 (Mud Creek tributary in January) to 28.03 (Wildcat Creek in July). There was no statistically significant difference in N:P ratios among streams ($F_{4,15} = 0.44$, $P = 0.78$), but there was a difference in N:P ratios across seasons ($F_{3,16} = 12.41$, $P = 0.0002$). Streams in summer (July) had greater N:P ratios when compared to the other seasons.

Periphyton phosphorus flux

Hourly P flux rates from individual microcosms ranged from a net release rate of −18.8 μg P h^{-1} to a net

Table 2 Phosphorus immobilization (positive) or release (negative) for individual microcosms

Stream	Light level (μE m⁻² s⁻¹)	Phosphorus flux (μg P h⁻¹)			
		October 2008 (n = 3)	January 2009 (n = 3)	May 2009 (n = 3)	July 2009 (n = 3)
Chamber's Springs	400	12.0	**3.6****	2.8	**12.2****
	140	**14.3****	**3.0****	**7.6****	**9.5****
	60	12.9	**5.8****	**5.0****	**10.1****
	20	8.3	**5.7****	**6.5****	**8.7****
	0	4.1	**1.2***	**2.5****	**3.4****
Little Wildcat Creek	400	4.5	3.8	**10.3****	**10.1****
	140	5.1	**6.0***	**10.8****	**12.1****
	60	12.4	6.3	**4.2***	**10.6****
	20	6.2	14.1	**6.2***	**4.1****
	0	6.9	1.2	**4.8***	**4.6****
Moore's Creek	400	−24.1	1.6	**−4.3***	**4.5****
	140	**−19.5***	3.4	−2.6	**7.9****
	60	**−18.8****	3.6	0.2	**5.3****
	20	−9.6	**4.2****	**3.6***	**3.2****
	0	**−27.9***	2.6	**2.1***	4.8
Wildcat Creek	400	15.0	**3.8***	**18.3****	**5.0****
	140	12.5	**0.4****	**13.6****	**10.0****
	60	**6.3***	**2.6****	**17.0****	**8.9****
	20	**4.0****	8.6	**16.5****	**9.5****
	0	4.7	−2.4	8.7	**6.1****
Mud Creek tributary	400	**9.8***	**−0.2***	**7.6****	**8.5****
	140	**10.1****	**2.1***	**8.0****	**9.2****
	60	**14.3****	**3.4****	**9.1****	**8.1****
	20	**5.8***	**−3.4***	**9.1****	**6.9****
	0	6.4	0.0	**5.7****	3.2

Rates in bold are statistically different from zero at α = 0.05 (*one asterisk*) or α = 0.01 (*two asterisks*)

a multiple regression model that included both growing season periphyton stoichiometry and growing season light availability, and the interaction term of these two variables, was able to explain 99% of the variation in daily periphyton P flux observed in the study (Fig. 5). The best fit model that derived the strongest fit to the data was

$$P_f = -2.01445 + 0.01070 \times L_{GS} + 0.01350 \times R_{C:P} - 0.00004 \times (L_{GS} \times R_{C:P}),$$

where P_f is the daily P flux value, L_{GS} is the growing season stream-specific daytime light availability (μE m⁻² s⁻¹), and $R_{C:P}$ is the growing season stream-specific periphyton C:P ratio. Fit statistics for the multiple regression model are provided in Fig. 5.

The details of the interaction between periphyton stoichiometry, light availability, and periphyton P flux can be found from the information in Figs. 4 and 5. For instance, the periphyton C:P ratios in Moore's Creek, Chamber's Springs, and Little Wildcat Creek were virtually identical in May, but daily P immobilization rates across these three streams spanned the entire range observed in the study (Fig. 4b). Daily P flux was strongly related to light availability in May,

with the highest rates occurring in Little Wildcat Creek and the lowest occurring in Moore's Creek (Fig. 4a). The combination of both high light availability and low C:P ratios in Little Wildcat Creek in May resulted in the greatest daily P immobilization rates observed in the study. Daily P flux rates were generally lower in July, but were again related to light availability. The three-dimensional model shown in Fig. 5 also shows that daily P immobilization was lowest when both light availability and periphyton C:P were also low, but increased with increases in both light and periphyton C:P. Interestingly, light appeared to have no effect on daily P flux rates when periphyton C:P was always high. However, daily P immobilization increased with decreasing C:P when light availability was high.

Discussion

Periphyton P flux, stoichiometry, and light climate

The results of this study demonstrate that periphyton stoichiometry, light availability, and the interaction between

Table 3 Periphyton stoichiometry and the % of microcosms that exhibited significant net phosphorus immobilization or release ($P < 0.01$)

Stream	Date	C:P	C:N	N:P	% Significant immobilization values[a]
Chamber's Springs	October	150	10	15	20
	January	150	9	16	80
	May	119	8	15	80
	July	192[b]	9	21	100
Little Wildcat Creek	October	169	10	17	0
	January	134	10	14	0
	May	135	8	17	40
	July	306[b]	11[c]	28[b]	100
Moore's Creek	October	209[b]	12[c]	18	0
	January	195[b]	10	20	20
	May	114	8	15	0
	July	165	8	22	80
Mud Creek tributary	October	273[b]	19[c]	14	60
	January	191[b]	15[c]	12[c]	20
	May	254[b]	18[c]	14	100
	July	579[b]	21[c]	28[b]	80
Wildcat Creek	October	166	10	17	0
	January	209[b]	12[c]	17	40
	May	193[b]	9	21	80
	July	315[b]	11[c]	28[b]	80

Nutrient limitation status inferred from stoichiometric ratios according to the following: C:P >180 and N:P >22, P limitation; C:N >10 and N:P <13, N limitation (Hillebrand and Kahlert 2001)

[a] Net P immobilization at a significance of 0.01

[b] P limitation

[c] N limitation

these two variables, can predict periphyton P immobilization and release in streams during the growing season. These findings support our hypothesis that the magnitude of P immobilization and release rates would be driven by periphyton stoichiometry and light availability. However, during the nongrowing season, we were unable to predict P immobilization and release based upon light availability and periphyton stoichiometry. Estimates of daily P flux determined from this study did not support our hypothesis that low light and low C:P would result in net P release. We did observe net P release in individual experimental microcosms, but these rates were rarely statistically significant (Table 2), which resulted in net zero daily P flux when the experimental data were scaled up to in-stream conditions.

We expected to find that diurnal variation in light availability would result in net P immobilization during the day and net P release during night. Furthermore, we expected to find that streams with low periphyton C:P would act as net P sources (i.e., net P release), particularly when incubated in the dark. We also expected that periphyton from many of our streams would have low C:P ratios due to their location in the nutrient surplus area of Arkansas (Slaton et al. 2004).

Indeed, water column total P concentrations measured in this study approached or exceeded the recommended total P water quality criteria for Illinois River Watershed (0.037 mg/L), and periphyton in these streams also exhibited low C:P ratios. Although there was some variation in periphyton %P (Fig. 1) and areal periphyton P

(Fig. 3), there were no statistical differences in periphyton C:P ratios among streams. This may have been an artifact of our reasonably small sample size ($n = 5$ streams).

The range of net P immobilization rates observed in this study (-2.26 to 8.14 µg P cm^{-2} day^{-1}) was similar to or lower in magnitude than rates observed in an in-stream nutrient retention study conducted in Spain (4.98–35.15 µg P cm^{-2} day^{-1}; Marti and Sabater 1996). Although daily periphyton P flux varied substantially among streams in this study, it was not strongly related to variability in periphyton stoichiometry alone (Fig. 4b). However, when common stoichiometric thresholds indicative of N or P limitation (C:P >180 and N:P >22 indicating P limitation; C:N ratios >10 and N:P ratios <13 indicating N limitation; Hillebrand and Kahlert 2001) were used to assign nutrient limitation status to each stream, P-limited periphyton exhibited the greatest percentage (80% or greater) of statistically significant P immobilization measurements in each season (Table 3). This indicates that net P immobilization occured more often when periphyton were P-limited, regardless of light availability. The frequency of periphyton P limitation and net P immobilization was similar across most seasons, but most frequent during summer.

When daily periphyton P flux data were restricted to those generated for the growing season (May and July), periphyton P flux was best predicted by a multiple regression model that included light availability, periphyton stoichiometry, and the
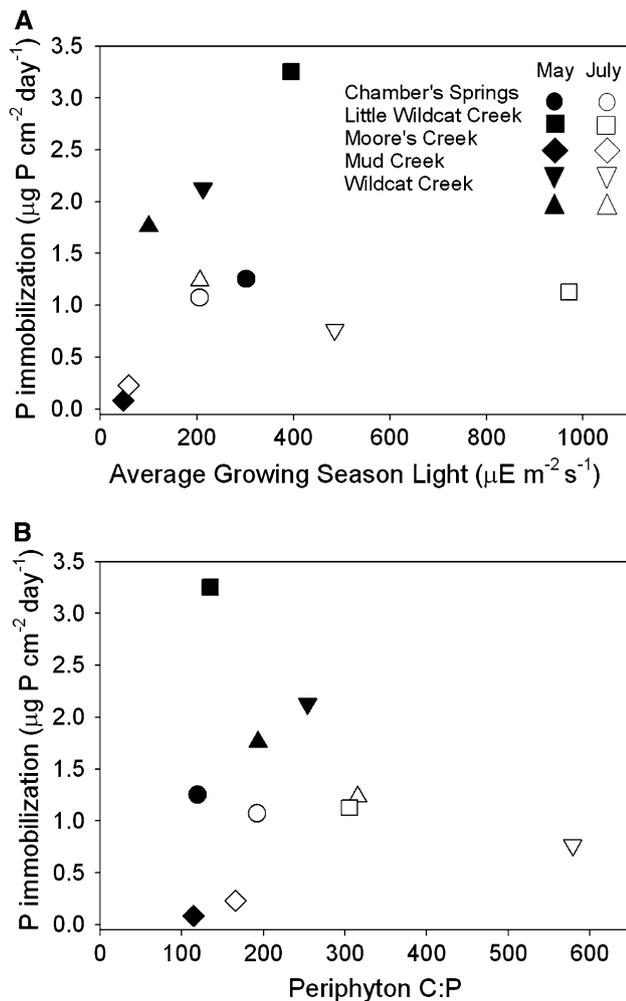
A

B

Fig. 4 Daily periphyton phosphorus immobilization as a function of **a** average light conditions, and **b** periphyton C:P during the growing season from May to July 2009. *Filled symbols* represent samples collected in May, *open symbols* represent samples collected in July. Individual sites are represented by the following *symbols*: *circles* Chamber's Springs, *triangles* Wildcat Creek, *squares* Little Wildcat Creek, *diamonds* Moore's Creek, *inverted triangles* Mud Creek tributary
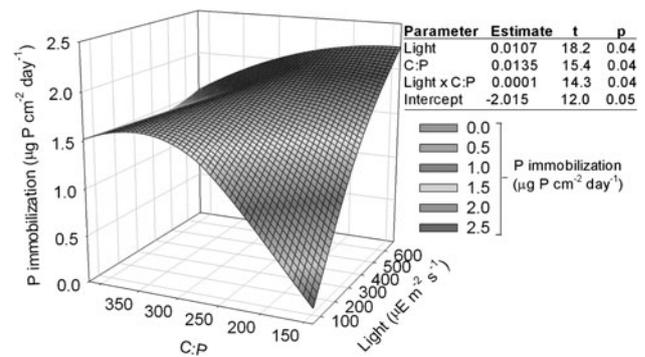

Fig. 5 Three-dimensional model of periphyton phosphorus immobilization predicted from the interaction between periphyton stoichiometry and average stream light conditions. Multiple regression model statistics for daily P immobilization are also shown

interaction of these terms (Fig. 5). In evaluating this model, we found that periphyton C:P ratios in Little Wildcat Creek in May were very similar to periphyton C:P ratios in Moore's Creek in May, but daily P immobilization at these two sites in May represented the largest and smallest values of daily periphyton P flux observed in the study (Fig. 4b). Interestingly, daily mean light values in Little Wildcat Creek during May exceeded 500 μE m^{-2} s^{-1}, but daily mean light values in Moore's Creek in May never exceeded 100 μE m^{-2} s^{-1} (Table 1).

Variability in P immobilization in all streams during the growing season was related to light availability, but the relationship between light and P immobilization was steepest in May and less steep in July (Fig. 4a). The decreases in periphyton P immobilization from May to July observed in Little Wildcat Creek, Mud Creek, and Wildcat Creek corresponded with increases in periphyton C:P ratios over this same period. Therefore, it seems that P-rich periphyton immobilize more streamwater P than P-poor periphyton when light availability is high. This suggests that periphyton P flux in streams is related to both light availability and periphyton stoichiometry. Interestingly, the interaction of these two variables in our model was negative, suggesting that high light availability and low P availability influence periphyton P flux antagonistically (Townsend et al. 2008). This finding is in contrast with the light-nutrient hypothesis, which would suggest that the greatest P immobilization rates should occur under high-light and low-nutrient (high periphyton C:P) conditions (Sterner et al. 1997; Fanta et al. 2010).

Mulholland et al. (1985) found the greatest P immobilization rates in the Walker Branch during the spring, which were attributed to high light levels prior to the development of the leaf canopy. The results of this study indicate that P immobilization from streamwater should be highest and occur most frequently during the spring and summer in Ozark streams. Therefore, this study suggests that P immobilization would be greatest in the growing season and least in the nongrowing season, with only one exception. The greatest daily P immobilization value at Chamber's Springs was observed in October. However, unlike the other streams in this study, the periphyton stoichiometry in Chamber's Springs was never indicative of periphyton P limitation. Therefore, greater P immobilization does not necessarily occur due to more intense periphyton P limitation when nutrient limitation is inferred from elemental ratios.

Overall, we found that periphyton were generally a sink for streamwater P when light availability was high. We also found that periphyton were sometimes a source of P

when light availability was low and periphyton C:P ratios were also low. These results agree with other studies highlighting the importance of light availability in nutrient processing in streams (Rosemond et al. 1993). Only one time did we observe P release in microcosms incubated under high light, and this corresponded with our lowest observed periphyton C:P ratio, indicating that under extreme conditions P leaking can occur even with high light intensity. However, the same periphyton assemblage under less light showed net P immobilization, which underscores the degree of in-stream variation that can occur between individual periphyton assemblages. More importantly, our results indicate that moderately P-rich periphyton more often act as a P sink, particularly when light availability is high. This suggests that net biological P release due to diurnal variations in light climate does not have a large effect on P loss from streams on a daily timescale. Rather, P export through the physical removal of periphyton is probably much more important to reach-scale P retention in streams.

Seasonal and spatial variability of periphyton stoichiometry and P immobilization

When considered across all streams in this study, periphyton elemental composition did not vary in a consistent manner over seasons, except for trends in periphyton N:P ratios. Periphyton N:P ratios increased in all streams during summer, which has been shown in other studies (Hillebrand and Kahlert 2001; Kahlert et al. 2002; Liess and Hillebrand 2005). This pattern is probably related to increases in biological demand drawing down inorganic N availability during summer (Marti and Sabater 1996). Although this study had no replicate stoichiometric data and only a small number of streams were studied, the lack of substantial variability in periphyton C:P ratios among streams or seasons may indicate that all streams were generally saturated in P relative to biological demand.

The variability in nutrient concentrations and periphyton nutrient content across streams with such variable land use and riparian cover was expected to be greater (Table 1). For example, Little Wildcat Creek was initially expected to be more nutrient-rich than other streams due to the relatively dense pasture cover in its watershed and the proximity of pastures to the stream at the sampling location (Carpenter et al. 1998). We expected landscape-scale controls of in-stream nutrient concentrations to create a more distinct pattern of periphyton stoichiometry across streams (Stelzer and Lamberti 2001; O'Brien and Wehr 2010). Only Mud Creek had statistically lower periphyton P than the other streams in the study, yet it recently served as a receiving stream for treated wastewater, which should lead to higher periphyton P. The Mud Creek tributary's

watershed is highly urbanized, which can result in frequent flow disturbances that cause shear stress and scouring (Walsh et al. 2005). As a result, periphyton biomass may slough off substrate due to frequent exposure to scouring flows, ultimately exporting large quantities of biologically immobilized P. This supports the notion that the physical removal of P-rich periphyton, rather than the leaking of P from stable periphyton communities, may be a dominant mechanism for P export in streams.

The results of our study suggest that periphyton can be a P sink, even in P-rich streams, when sufficient light is available to support algal growth. Periphyton stoichiometry, light availability, and the interactive effects of these two variables can control P immobilization and release by periphyton in nutrient-rich streams. This pattern may be particularly pronounced during the growing season. Even though periphyton P content was relatively high in all study streams, net P immobilization by periphyton occurred in over 75% of the sampling events, and net P release by periphyton occurred in only 5% of sampling events in the experimental microcosms. The pattern of net P release was dampened further when flux rates were scaled up to daily estimates. Daily periphyton P immobilization was greatest when light availability was high and periphyton C:P ratios were low, suggesting that P-rich periphyton exhibit greater P demand when energy supplies are sufficient.

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