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Periphyton response to longitudinal nutrient depletion in a woodland stream: evidence of upstream-downstream linkage

PATRICK J. MULHOLLAND

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6036 USA

AMY D. ROSEMOND

Department of Biology, Vanderbilt University, Nashville, Tennessee 37235 USA

Abstract. Longitudinal gradients in streamwater nutrient concentrations in Walker Branch are generated as a result of instream nutrient uptake and spatially confined groundwater inputs during the period from November to May. The response of the stream periphyton community to these longitudinal nutrient reductions was determined by measuring periphyton biomass, productivity, species composition, and phosphorus (P) cycling indices at four stations along a longitudinal transect in the stream. Phosphorus cycling indices (chlorophyll-specific phosphatase activity, phosphorus content of periphyton) exhibited significant changes along the longitudinal transect during those times of the year when streamwater soluble reactive phosphorus (SRP) concentrations also decreased along the transect. During the period from June to October, however, neither streamwater phosphorus concentrations nor phosphorus cycling characteristics exhibited longitudinal trends. Regressions between phosphatase activity and streamwater SRP concentration and between phosphorus content and streamwater SRP were highly significant for all data combined, with SRP explaining $\geq$74% of the variation in phosphatase activity and P content.

Measures of periphyton biomass (chlorophyll *a*, total biovolume), and productivity (areal carbon fixation rate, chlorophyll-specific carbon fixation rate) exhibited no consistent longitudinal patterns, even during the period of longitudinal streamwater phosphorus depletion. Regressions between productivity measures and streamwater SRP concentration for all data combined were significant, but SRP explained $\leq$56% of the variation in productivity. Periphyton biomass and productivity at all stations along the longitudinal transect appear to be maintained at low levels by high and longitudinally uniform rates of herbivory throughout the year. Algal species composition exhibited some response to longitudinal nutrient depletion. The biovolume and percentage of the blue-green alga *Chamaesiphon investiens* increased and the percentage of the chlorophyte *Stigeoclonium* sp. declined longitudinally when nutrients also declined.

Our results demonstrate an upstream-downstream biotic linkage in Walker Branch. We show that instream nutrient uptake can reduce the concentrations of nutrients in stream water and thereby influence the structure and functioning of downstream periphyton communities. However, increases in nutrient cycling in response to lower streamwater concentrations can partially compensate for nutrient depletion by upstream organisms, thereby buffering primary productivity in downstream periphyton communities from changes in nutrient supply.

Key words: stream periphyton, nutrients, phosphorus, phosphatase, primary productivity, upstream-downstream linkage, longitudinal gradients.

Nutrients are important regulators of stream ecosystem structure and function. Nutrient limitation of primary producers and organic matter decomposition is commonly observed in streams (Stockner and Shortreed 1978, Elwood et al. 1981b, Peterson et al. 1983, Grimm and Fisher 1986, Pringle 1987, Perrin et al. 1987, Bothwell 1989). Bothwell (1989) has shown that even under relatively high nutrient concentrations (exceeding the half-saturation constants for individual cells) nutrient supply can be limiting to stream periphyton communities because of diffusion-limited transport of nutrients into the periphyton matrix.

In nutrient-limited streams, algae and heterotrophic microbes can have a large effect on nutrient concentrations in stream water and on downstream nutrient flux. This is particularly true in low-order streams where water depths are generally low (wetted perimeter : water volume ratios are high). Depletion of streamwater nutrients by algal and microbial uptake have

been shown to influence the productivity and species composition of algal communities downstream. For example, in Sycamore Creek, Arizona, uptake of nitrogen in upstream reaches leads to longitudinal depletion of nitrate and dominance by blue-green algae downstream (Fisher et al. 1982). These effects illustrate strong longitudinal (upstream–downstream) linkages in stream metabolism and community composition due to nutrient transport in stream water.

Longitudinal linkages are fundamental to the way streams are viewed. In general terms, Fisher (1983) has argued that succession in stream communities can be viewed as a longitudinally distributed process dependent on upstream–downstream linkages such as nutrient retention and transport as well as immigration and emigration. An important premise of the river continuum concept (Vannote et al. 1980) is that upstream communities can influence downstream communities via effects on the quality and quantity of material in transport. While this upstream–downstream linkage has been explicitly examined as to the effects of organic matter processing and algal productivity on macroinvertebrate communities (Cummins et al. 1981, Minshall et al. 1983, Naiman et al. 1987), it has generally not been studied for other processes, such as effects of nutrient uptake and transport on periphyton.

Phosphorus is often a limiting nutrient in streams, as in other aquatic ecosystems. Several studies have indicated that the distance travelled by phosphate in stream water prior to uptake by organisms associated with the streambed (uptake length) is short in low order streams, often <100 m (Mulholland et al. 1985, 1990, Munn and Meyer 1990), and varies with season (Mulholland et al. 1985, Munn and Meyer 1990). If resupply of phosphorus to stream water via remineralization or groundwater and tributary input does not keep pace with uptake, then phosphate availability to downstream organisms will decline. In general, the strength of longitudinal linkages involving nutrients in streams should be inversely related to the relative importance of lateral inputs (e.g., groundwater inputs); however, the relative importance of longitudinal vs. lateral controls on nutrient availability in streams is not well understood (Meyer et al. 1988).

In this study we investigate the effects of longitudinal depletion of nutrient concentration in Walker Branch, a 1st-order forest stream in eastern Tennessee. Previous studies have shown the periphyton community in Walker Branch to be phosphorus limited (Elwood et al. 1981b), although more recent studies have indicated that light and nitrogen concentrations also may limit algal productivity in this stream at times (Steinman 1992; A. D. Rosemond et al. 1993), and that there are strong interactions between nutrients, light, and herbivory in the regulation of algal biomass and productivity (A. D. Rosemond, unpublished data). Other studies have indicated that groundwater inputs to Walker Branch are largely confined to certain locations, particularly four springs (Wanninkhof et al. 1990, Genereux et al. 1992), and that rates of instream N and P uptake exceed remineralization during much of the year, resulting in longitudinal depletion of phosphate and nitrate concentrations along reaches with little groundwater input (Mulholland 1992). The purpose of this study was to evaluate the response of the periphyton community to such longitudinal nutrient depletion generated as a result of instream biological processes and spatially confined groundwater inputs.

Methods

Study site

The study was conducted in the West Fork of Walker Branch, a 1st-order deciduous-forest stream on the United States Department of Energy's Oak Ridge National Environmental Research Park in eastern Tennessee. The catchment of the West Fork is underlain by dolomite of the Knox formation. Baseflow in the stream is generated primarily by several springs that are relatively constant in discharge and chemical composition. Baseflow in the stream is generally 3–10 L/s and stream water is moderately alkaline (2–3 meq/L) with pH generally 7.4–8.2. Baseflow is somewhat higher in the winter and early spring, when evapotranspiration rates are low, than in the growing season. Both stream water alkalinity and pH are inversely related to flow.

A 140-m reach about 10 m below the discharge of two perennial springs was selected for this study (Fig. 1). This reach has been previously used in several studies of phosphorus spiralling (Newbold et al. 1983, Mulholland et

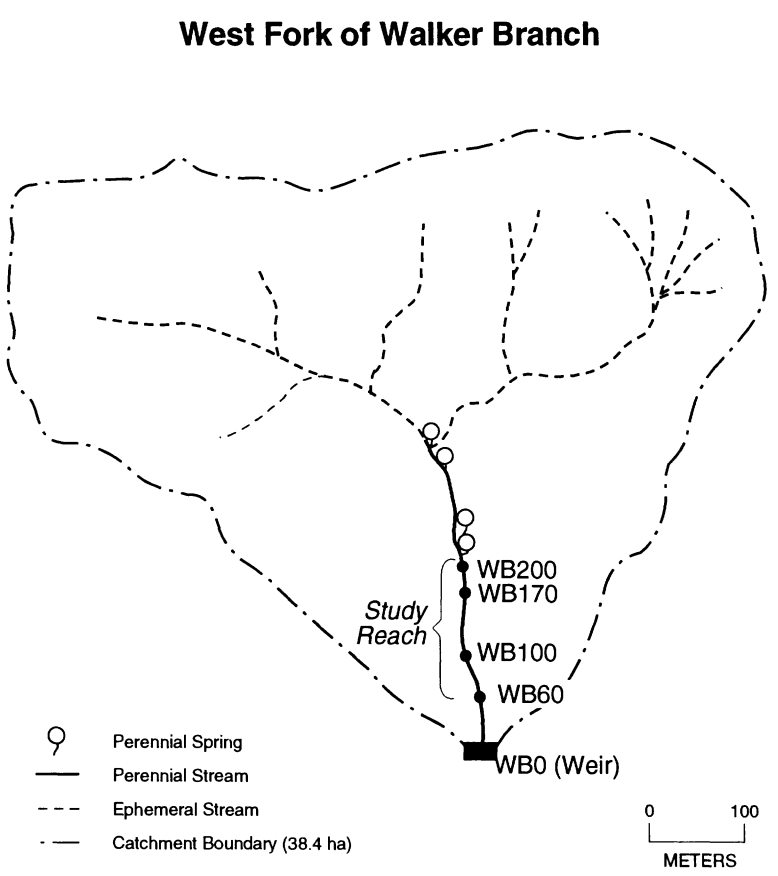

FIG. 1. Location of 140-m study reach and sampling stations.

al. 1985, 1990). The streambed consists of exposed bedrock, cobble, and gravel. The stream averages about 5–10 cm in depth and 3 m in width. During the growing season, the stream channel is heavily shaded by the deciduous forest canopy.

Four stations along the study reach were selected (WB200, WB170, WB100, and WB60). The number for each station refers to the distance in meters upstream from a V-notch weir, where stream discharge is continuously monitored. Previous studies involving injection of conservative tracers have shown that groundwater input along the study reach is relatively small (approximately 12%: Wanninkhof et al. 1990, Genereux et al. 1992). As a consequence, instream processes have been shown to exert a strong effect on streamwater nutrient concentrations (Mulholland 1992). For example, during the period from peak leaf fall in autumn (early November) until mid-late spring, high rates of instream uptake result in longitudinal gradients in NO_3 and soluble reactive phosphorus (SRP) concentrations along this reach, with highest concentrations at WB200 and lowest concentrations at WB60 (Mulholland 1992).

Analytical methods

Daily insolation at each station was estimated on several occasions during the period from October 1988 to November 1990 using Ozalid paper light meters (Friend 1961) attached to bricks placed in the stream. Three ozalid paper light meters were placed at each station, and integrated light reaching the stream surface was calculated over a 24-h period. The ozalid paper was calibrated to photosynthetically active radiation with a LiCor quantum sensor.

Density of the dominant herbivore in Walker Branch, the snail *Elimia clavaeformis*, was measured approximately bimonthly at each station

during the period from October 1988 to October 1990. At 10 randomly chosen locations at each station, a clear plexiglass grid (250 cm^2) was placed on the stream bottom, and the snails within the grid were counted.

Concentrations of SRP, NH_4, and $NO_2 + NO_3$ were measured at each station on eight dates over the period from June 1987 to December 1990. Stream water was filtered (Gelman Type A/E glass fiber filters) in the field and taken to the laboratory on ice and refrigerated until analysis. SRP was measured within 1 d of collection using the ascorbic acid method (Murphy and Riley 1962). Concentrations of NH_4 were measured within 2 d by automated phenate colorimetry and concentrations of $NO_2 + NO_3$ were measured within 3 d by automated Cu-Cd reduction followed by azo dye colorimetry (US EPA 1983).

Measurements of periphyton nutrient content, chlorophyll *a*, phosphatase activity, carbon fixation rates, and species composition were made at each station on several dates during the period from September 1988 to December 1990. Flat, unglazed ceramic tiles (5 cm × 5 cm) were placed on the streambed in riffle areas at each station at least 8 mo prior to sampling to serve as physical substrata for periphyton colonization and growth and units for sampling.

Nitrogen and phosphorus contents of periphyton were determined on three dates at each station. Two or three tiles were collected from each station and periphyton was scraped from the tile surface into a beaker and dried at 60°C for at least 48 h. Two subsamples of dried periphyton from each station (approximately 5 mg each) were weighed and analyzed for carbon and nitrogen content using a Carlo Erba Model NA1500 CNS analyzer. An additional two subsamples of dried periphyton were analyzed for total P using a modification of the Solorzano and Sharp (1980) method. These subsamples were weighed (dry mass) and ashed (500°C), and the ash was leached in 5 mL of hot 1 N HCl for 30 min to extract P. The acid leachates were diluted to 100 mL with distilled water, and phosphorus concentrations were determined using the ascorbic acid method as described above. Total P content of reference samples (orchard leaves, NBS standard reference material 1571) was 109% (SD = 3%, $n = 6$) of the certified P content (0.0021 g/g), indicating complete recovery of organic P by this method.

Phosphatase activity and chlorophyll *a* content of periphyton were determined at each station on five to seven dates, using a modification of the method of Fitzgerald and Nelson (1966) for phosphatase and the method of Palumbo et al. (1987) for chlorophyll *a*. Three to five tiles were selected from each station on each sampling date and placed in plastic jars to which 50 mL of filtered (Gelman Type A/E) stream water from the same station and 0.4 mL of a 150 mM *p*-nitrophenyl phosphate (NPP, Sigma) solution were added. All jars were then incubated for 30 min in the stream at WB60 to maintain uniform water temperature. At the end of the 30-min incubation, approximately 20 mL of the stream water from each jar was filtered (Gelman acrodisc membrane filters) and the filtrates were returned to the laboratory for colorimetric analysis. In the laboratory within 2 h of sample collection, 0.05 mL of 1 N NaOH was added to raise the pH in the filtrate to >10.5 (to achieve maximum and stable color development). Hydrolyzed nitrophenol (NP) was determined for each filtrate sample by measuring absorbance at 410 nm, and phosphatase activity was computed as moles NP produced.

To determine the chlorophyll *a* content of periphyton on each tile, tiles were removed from the jars after the field incubation, rinsed in stream water, and placed in 25 mL of dimethyl sulfoxide (DMSO) to extract chlorophyll *a*. After extraction in DMSO overnight at room temperature, the extracts were diluted 1:1 with acetone, and chlorophyll *a* was determined spectrophotometrically by measuring the absorbance at 663 nm, before and after acidification to correct for phaeopigments. Phosphatase activity was then normalized for chlorophyll *a* content.

Periphyton carbon fixation rates were determined on seven dates at each station by measuring incorporation of $NaH^{14}CO_3$ in recirculating, temperature-controlled laboratory chambers (Boston and Hill 1991). Three to five tiles were collected from each station, immersed in stream water from that station, and transported to the laboratory. Within 1 h of collection, tiles from each station were placed in glass chambers with 1 L of filtered stream water (Gelman A/E filters) from each station. Water in each chamber was recirculated by submersible pumps. The chambers were housed in a water bath maintained at a temperature within 2°C of

stream water measured in the field. Light was provided by a metal halide lamp suspended over the water bath. Light was adjusted to values typical of those measured in the field at midday at the time of sampling by varying the height of the lamp over the chambers or shading the chambers with screening. Approximately 180–360 kBq of $NaH^{14}CO_3$ (specific activity 0.74 MBq/mmol) were added to each chamber. After a 3-h incubation, tiles were removed from the chambers, gently rinsed in fresh stream water, and placed in jars containing 25 mL of DMSO for extraction of chlorophyll *a* and ^{14}C-labeled photosynthate (Palumbo et al. 1987). Subsamples of the extract were analyzed for ^{14}C by liquid scintillation spectroscopy with external standards and for chlorophyll *a* as described above. Water samples were also taken from each chamber at the beginning and end of the incubation and analyzed for ^{14}C by liquid scintillation and for total inorganic carbon (TIC) by infrared gas analysis (OI Model 700 Total Carbon Analyzer). Carbon fixation rates were calculated individually for each tile on an areal and chlorophyll-specific basis.

Periphyton species composition was determined on four dates at each station and on one date at the mouth of one of the two large springs approximately 10 m upstream from WB200. Tiles (2 to 4) were collected from each station and scraped using a toothbrush, and the resultant slurry was preserved in 2% glutaraldehyde. Identifications and counts of algal units (either single cells or colonies) were made at 400× magnification using a Palmer-Malony cell after sonication of samples. Community composition at each station on each date was based on counts of >300 algal units. Community composition is expressed in terms of biovolume by converting unit densities of different taxa to biovolume per unit tile surface area. Average dimensions of algal units were determined using an ocular micrometer, and biovolume was calculated using these dimensions in formulae for geometric shapes.

Statistical treatment

To determine whether longitudinal gradients in nutrient concentrations along the study reach resulted in similar gradients in periphyton biovolume, chlorophyll *a*, productivity, and nutrient cycling indices, linear regressions between these parameters and distance upstream from the weir were computed for each sampling date and tested for significance of slopes (*t*-tests). By applying a linear model we do not mean to imply that a linear relationship with distance best described the data (although we have no reason to believe it is not optimum). We have applied linear regression only to determine if there was a significant linear component (i.e., ascending or descending pattern) to the relationship between a given parameter and distance along the stream.

To determine relationships between periphyton characteristics and SRP concentration, linear or nonlinear (exponential) regressions were computed using mean values from each station from all dates. Multiple regression analyses of periphyton chlorophyll, nutrient content, biovolume, and community composition characteristics were performed using SRP concentration, light, and snail density as independent variables. Light was entered as average daily insolation values for each station measured within 3 wk of the measurement of periphyton characteristics. Periphyton biovolume data spanned three orders of magnitude and were log-transformed before statistical analysis. Biovolume percentages (percentage of total algal biovolume) were arcsine-square root transformed before statistical analysis. Multiple regression analyses of phosphatase activity, areal carbon fixation rate, and chlorophyll-specific carbon fixation rate were performed using SRP concentration, light, water temperature during the incubation, and snail density as independent variables.

All regression analyses were performed using the SAS REG and SAS NLIN procedures (SAS 1988). Values of $p < 0.05$ are considered significant and $p < 0.10$ are reported as marginally significant.

Results

Concentrations of SRP and $NO_2 + NO_3$ declined with distance downstream from station WB200 on all sampling dates between November and May (Fig. 2). Concentrations of NH_4 were below the detection limit (<2 μg N/L) on all sampling dates at all stations. Regressions between nutrient concentrations and distance were significant ($p < 0.05$) or marginally significant ($p < 0.10$) for the November, December,

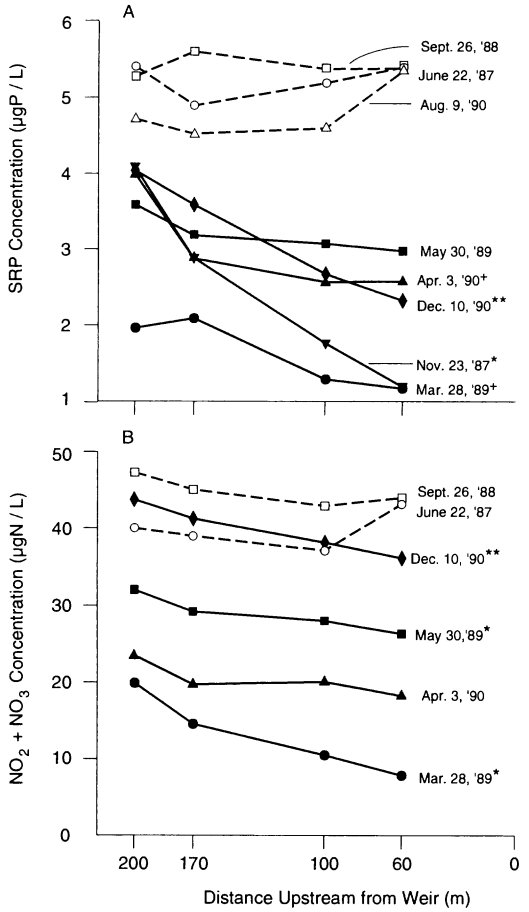

FIG. 2. Longitudinal gradients in (A) SRP concentration and (B) $NO_2 + NO_3$ concentration along the study reach ($n = 1$ at each station). Data points from the period November through May are connected by solid lines and data points from the period June through October are connected by dashed lines. Linear regressions between concentration and distance are noted for each date if slope was significant ($^+ p < 0.10$, $^* p < 0.05$, $^{**} p < 0.01$, $^{***} p < 0.001$).

and March sampling dates. Declines in SRP and $NO_2 + NO_3$ concentrations were also observed in April and May, but the regressions with distance were not significant for both nutrients. On sampling dates during the period from June to September, SRP and $NO_2 + NO_3$ concentrations showed no longitudinal trends (Fig. 2). This pattern of substantial longitudinal decline in SRP and $NO_2 + NO_3$ concentrations during the period from November to May, but little longitudinal change from June to September was also consistently observed during 2 yr of monthly sampling of stream water (Mulholland 1992). Streamwater N:P ratios ($NO_2 + NO_3$-N: SRP, atomic) varied from 12 to 35 (mean = 18.2, SD = 5.9, $n = 24$) but showed no consistent trends with distance downstream from WB200.

In addition to SRP concentration, light and herbivory may strongly influence periphyton characteristics; however, neither showed consistent longitudinal trends. Average daily insolation was highly seasonal, but regressions with distance were only marginally significant on two dates (Fig. 3). The density of snails ranged from 914/m^2 to 1961/m^2 (mean = 1434, SD = 266, $n = 32$) across all dates and stations. Of the eight dates on which longitudinal measurements were made (five of which were during the period of longitudinal nutrient decline) a significant longitudinal pattern (increase with distance downstream from WB200) was observed only on 9 August 1990.

Water temperature often exhibited a small but consistent change across the study reach, with a maximum change of 2°C measured during this study. Data from 3 yr of monthly sampling at stations WB200 and WB60 showed that water temperatures were somewhat more constant at WB200 (range: 8.2–15.5°C) than at WB60 (range:

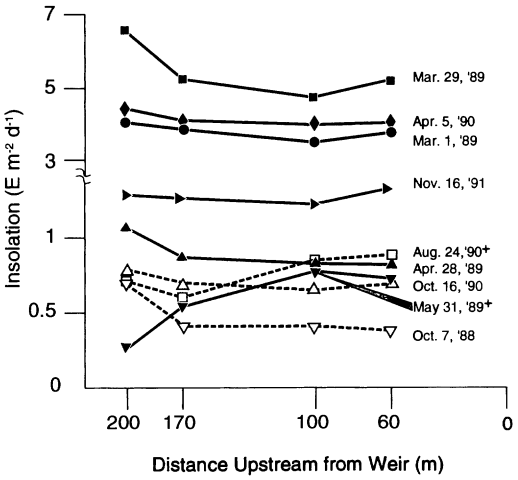

FIG. 3. Longitudinal gradients in mean values ($n = 3$) of insolation at the stream surface along the study reach. Solid and dashed lines are for the same periods described in Figure 2. Significant slopes for linear regressions between insolation and distance are noted for each date using the same convention as in Figure 2. Error bars have been omitted for clarity. Average coefficient of variation was 16.6%.

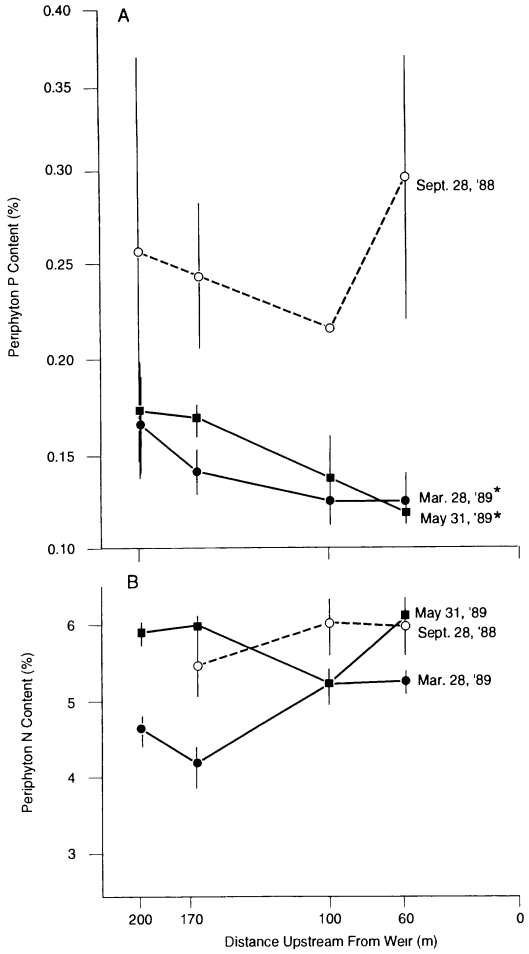

FIG. 4. Longitudinal gradients in mean values (±1 SE) of (A) phosphorus content and (B) nitrogen content of periphyton along the study reach. Solid and dashed lines are for the periods described in Figure 2. Significant slopes for linear regressions between nutrient content and distance are noted as in Figure 2.

4.9–17.3°C), owing to discharge of two relatively large springs (S3 and S3A) just above WB200 (Mulholland, unpublished data). On average, water temperature increased by 1.04°C from WB200 to WB60 during April to September, and decreased by 1.35°C over this same reach from November to March.

Periphyton P content exhibited longitudinal patterns similar to those for SRP concentration for samples collected on or near the same dates (Fig. 4A). Periphyton P content declined with distance downstream from WB200 on the March and May sampling dates, but was highly variable and showed no consistent longitudinal pattern in September. Periphyton N content exhibited no consistent longitudinal pattern at any time (Fig. 4B).

Phosphatase activity of periphyton, normalized to chlorophyll content, displayed longitudinal patterns opposite to those for SRP concentration and P content (Fig. 5). Chlorophyll-specific phosphatase increased significantly with distance downstream from WB200 on the December, March, April, and May sampling dates, but exhibited no longitudinal trend in September.

Combining data from all dates, relationships between periphyton P content and SRP concentration (Fig. 6A) and between chlorophyll-specific phosphatase activity and SRP concentration (Fig. 6B) were significant and were best described by exponential functions. SRP concentration explained 96% of the variation in periphyton P content and 74% of the variation in phosphatase activity.

Chlorophyll *a*, areal carbon fixation rate, and chlorophyll-specific carbon fixation rate were variable across the study reach and showed few consistent longitudinal patterns. Chlorophyll *a* values ranged from 0.7 to 6.1 $\mu g/cm^2$ (mean = 3.1, SD = 1.0, n = 100), areal carbon fixation rates ranged from 0.5 to 1.9 $\mu g\ cm^{-2}\ h^{-1}$ (mean

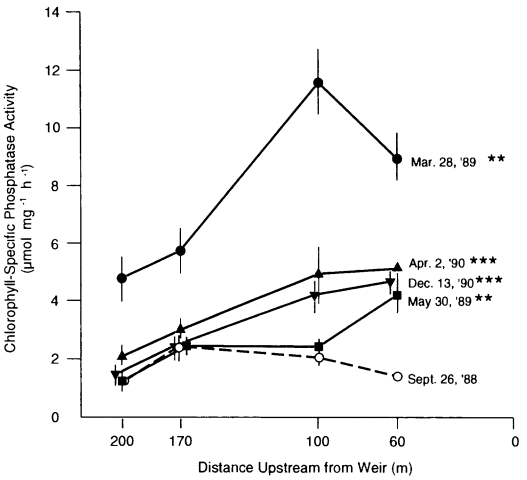

FIG. 5. Longitudinal gradients in mean values (±1 SE) of phosphatase activity along the study reach. Solid and dashed lines are for the periods described in Figure 2. Significant slopes for linear regressions between phosphatase and distance are noted as in Figure 2.

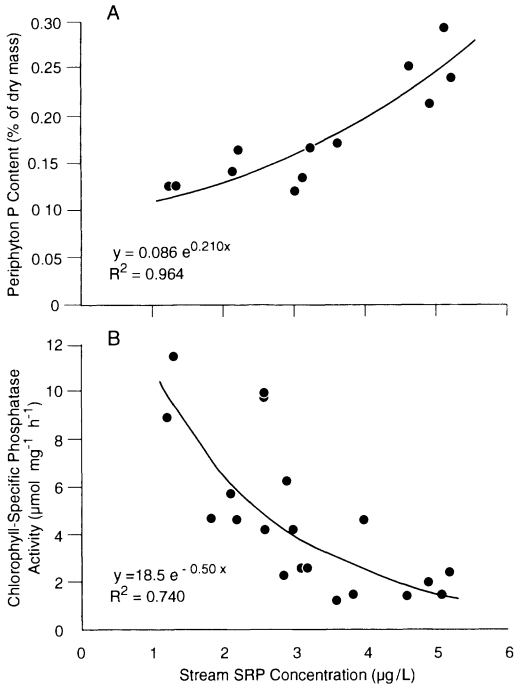

FIG. 6. Relationships between mean values of (A) periphyton phosphorus content and streamwater SRP concentration and (B) phosphatase activity and streamwater SRP concentration for all dates and stations combined. The relationships were best fit by exponential models as shown.

= 0.9, SD = 0.3, $n = 96$), and chlorophyll-specific carbon fixation rates ranged from 0.2 to 0.6 µg µg^{-1} h^{-1} (mean = 0.3, SD = 0.1, $n = 96$). Of the seven dates on which longitudinal measurements were made (four of which were during the period of longitudinal nutrient decline), only on 10 December 1989 did areal carbon fixation rate and chlorophyll-specific carbon fixation rate decline significantly ($p < 0.05$) with distance downstream from WB200. At no time were there significant declines in chlorophyll *a* with distance downstream from WB200, although on 30 May 1989 there was a marginal decline ($p < 0.10$). On one date (26 September 1988) chlorophyll *a* and areal carbon fixation rate increased significantly ($p < 0.05$) with distance downstream from WB200, and on 1 June 1989 chlorophyll-specific carbon fixation rate increased significantly downstream from WB200.

When data from all dates were combined, there were significant positive relationships between areal carbon fixation rate and SRP concentration (Fig. 7B) and chlorophyll-specific carbon fixation rate and SRP concentration (Fig. 7C). However, the relationship between chlorophyll *a* and SRP concentration was only marginally significant (Fig. 7A), with relatively little of the variation in chlorophyll *a* explained by SRP concentration ($r^2 = 0.125$). Considerably more of the variation in areal and chlorophyll-specific carbon fixation rates were explained by SRP concentration, with r^2 values of 0.556 and 0.348, respectively. Inclusion of SRP concentration, light (in situ), and snail density as independent variables in a stepwise multiple regression analysis did not significantly improve the chlorophyll prediction. Inclusion of SRP concentration, light (in situ and incubation levels), water temperature during the incubation, and snail density as independent variables in a stepwise multiple regression analysis for carbon fixation rates indicated that only SRP concentration was significant for areal carbon fixation rates and that only water temperature was significant for chlorophyll-specific carbon fixation rates ($r^2 = 0.525$, $p = 0.0001$). However, both incubation light level ($p = 0.106$) and SRP concentration ($p = 0.114$) entered the model for chlorophyll-specific carbon fixation rate at just above the $p < 0.10$ level.

Periphyton communities were dominated by two algal taxa, basal cells of a chlorophyte (*Stigeoclonium* sp.) and a small colonial blue-green alga (*Chamaesiphon investiens*) that together made up >90% of the total algal biovolume at all stations on all dates. Total algal biovolume showed few consistent patterns with distance downstream from WB200 (Fig. 8A). On 27 September 1988 total algal biovolume as well as biovolume of *Stigeoclonium* (not shown) were marginally

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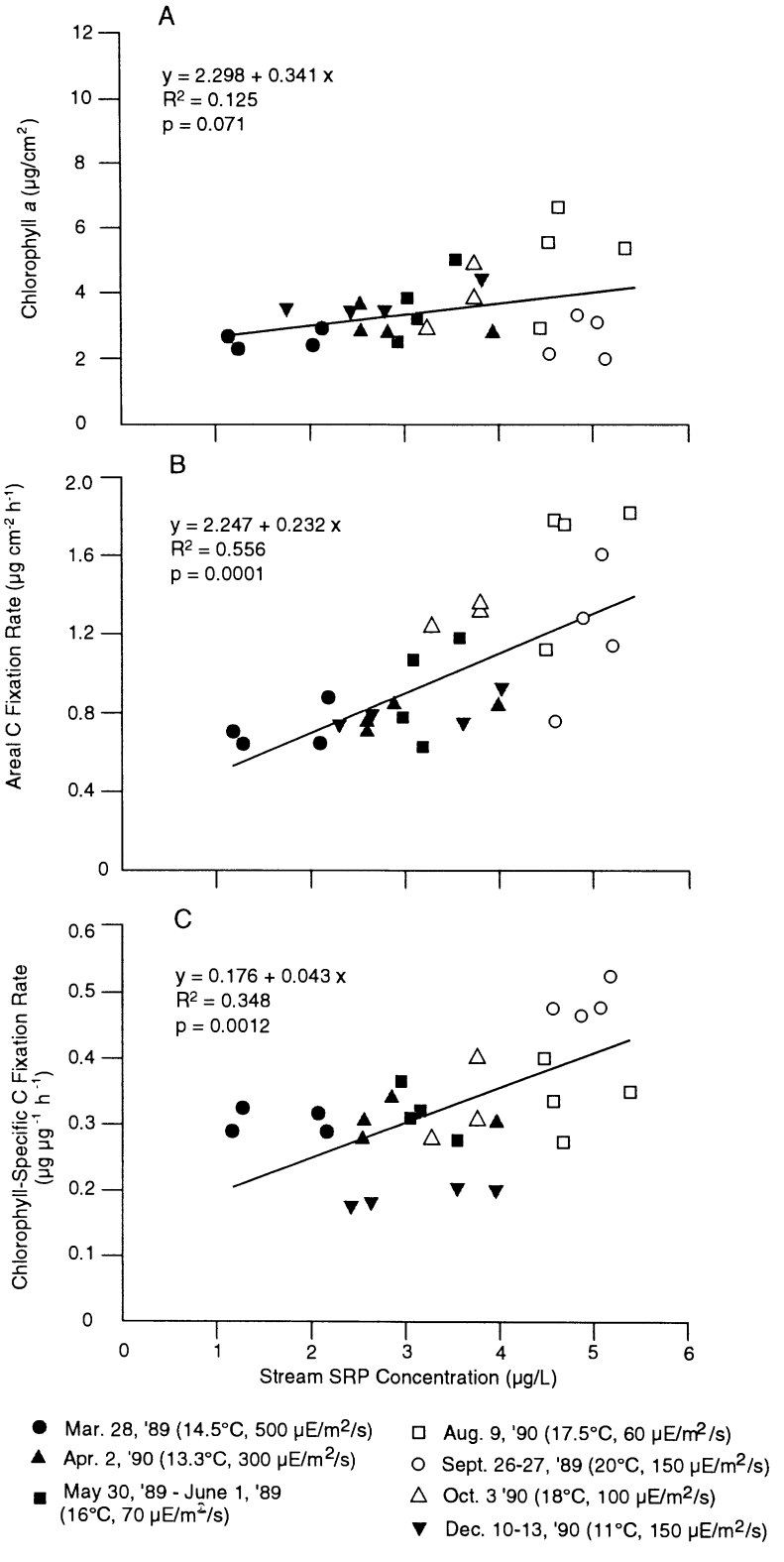
FIG. 7. Relationships between mean values of (A) chlorophyll *a*, (B) areal carbon fixation rate, and (C) chlorophyll-specific carbon fixation rate and streamwater SRP concentration for all dates and stations combined. Solid points refer to the period November to May and open points are from the period June to October. The temperature and instantaneous light conditions for each measurement are given.


A
y = 2.298 + 0.341 x
R² = 0.125
p = 0.071
Chlorophyll a (µg/cm²)
B
y = 2.247 + 0.232 x
R² = 0.556
p = 0.0001
Areal C Fixation Rate (µg cm⁻² h⁻¹)
C
y = 0.176 + 0.043 x
R² = 0.348
p = 0.0012
Chlorophyll-Specific C Fixation Rate (µg µg⁻¹ h⁻¹)
Stream SRP Concentration (µg/L)
Mar. 28, '89 (14.5°C, 500 µE/m²/s)
Apr. 2, '90 (13.3°C, 300 µE/m²/s)
May 30, '89 - June 1, '89 (16°C, 70 µE/m²/s)
Aug. 9, '90 (17.5°C, 60 µE/m²/s)
Sept. 26-27, '89 (20°C, 150 µE/m²/s)
Oct. 3 '90 (18°C, 100 µE/m²/s)
Dec. 10-13, '90 (11°C, 150 µE/m²/s)

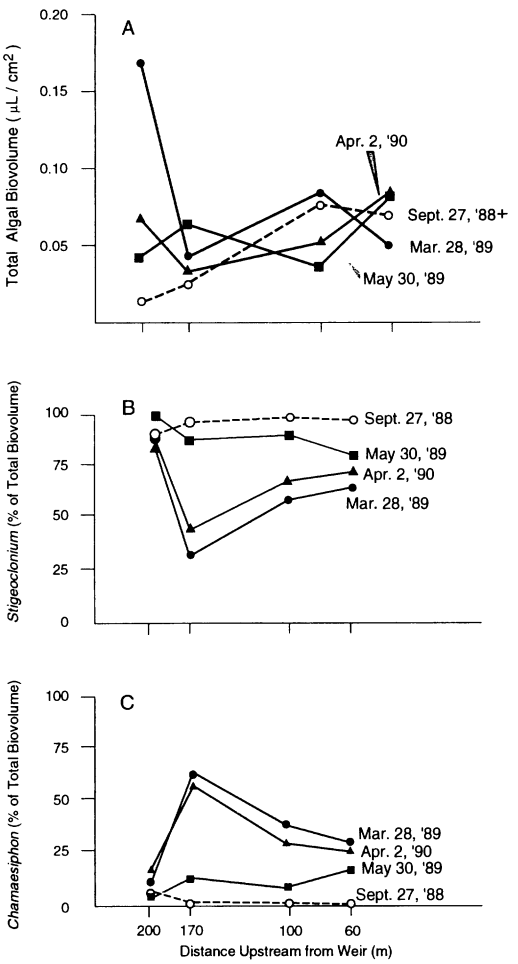


FIG. 8. Longitudinal gradients in (A) total algal biovolume, (B) percentage *Stigeoclonium*, and (C) percentage *Chamaesiphon* in the periphyton community along the study reach. Solid and dashed lines are for the periods described in Figure 2. Significant slopes for linear regressions between total biovolume or percentage of total biovolume and distance are noted as in Figure 2.

correlated with distance downstream from WB200. On 28 March 1989 total biovolume declined sharply between WB200 and stations downstream; however, regressions with distance were not significant owing to variability downstream from WB200. On this date, total algal biovolume and *Stigeoclonium* biovolume were also about 75% higher in samples collected at the mouth of the spring just upstream from WB200 than at WB200, further suggesting a sharp longitudinal decline in biovolume in the upper portion of the study reach.

Stigeoclonium was the most abundant alga on all dates. On 27 September 1988 and 30 May 1989 *Stigeoclonium* formed >80% of the community at all stations; however, its proportion in the community was somewhat lower on 28 March 1989 and 2 April 1990 when streamwater nutrient concentrations were lower (Fig. 8B). There was a general trend of decreasing importance of *Stigeoclonium* with distance downstream from WB200 on the March, April, and May sampling dates, although minimum values were observed at WB170 for the March and April samples and regressions with distance were not significant on any of the dates. In March when the *Stigeoclonium* percentage was lowest at all of the sites, the periphyton community at the mouth of the spring just upstream from WB200 (where nutrient concentrations were highest) consisted entirely of *Stigeoclonium*. Biovolume of *Chamaesiphon* was inversely correlated with *Stigeoclonium* biovolume ($r^2 = 0.713$, $p = 0.053$). Thus, as *Stigeoclonium* declined in importance downstream from WB200 on the March, April, and May sampling dates, the percentage of *Chamaesiphon* increased, although regressions between *Chamaesiphon* biovolume and distance were not significant (Fig. 8C).

Combining data for all dates and stations, there were significant negative correlations between *Chamaesiphon* biovolume and stream water SRP concentration ($r^2 = 0.555$, $p = 0.004$) and between *Chamaesiphon* percentage and SRP concentration ($r^2 = 0.573$, $p = 0.0007$), and a significant positive correlation between *Stigeoclonium* percentage and SRP concentration ($r^2 = 0.542$, $p = 0.001$). Results of stepwise multiple regression analysis with SRP concentration, light, and snail density as independent variables showed significant or marginally significant negative relationships between *Chamaesiphon* biovolume and SRP ($p = 0.004$) and snail density ($p = 0.096$), and a significant positive relationship between *Chamaesiphon* biovolume and light ($p = 0.039$), but no significant relationships with variables other than SRP concentration for *Chamaesiphon* percentage and *Stigeoclonium* percentage. Multiple regression analysis of total algal biovolume and *Stigeoclonium* biovolume showed no significant relationships with SRP concentration, light, and snail density.

Discussion

Longitudinal trends in nutrient concentrations in Walker Branch are the result of in-stream nutrient uptake and very little groundwater input along the study reach. Previous studies have documented that groundwater inputs increase streamflow by 12% or less along this reach (Mulholland et al. 1985, Genereux et al. 1992), and uptake of nutrients by microbes associated with decomposing leaves and by periphyton result in large net removal of nutrients from stream water during the period from November to May (Mulholland 1992). In some streams, spatial differences in the physicochemical sorption potential of inorganic sediments can result in longitudinal variation in streamwater SRP concentrations (Klotz 1988). However, in Walker Branch, biological processes are most important in uptake of phosphorus (Elwood et al. 1981a) and the longitudinal phosphorus gradient is due to spatially confined groundwater inputs and biological uptake (Mulholland 1992).

The response of the periphyton community to longitudinal depletion of nutrients was primarily limited to phosphorus cycling indices (phosphatase activity and P content of periphyton). Previous studies showed that periphyton growth in Walker Branch is likely limited by phosphorus rather than nitrogen (Elwood et al. 1981b, Newbold et al. 1983), although more recent work suggests that N and P may be colimiting at times (Rosemond et al. 1993). Significant longitudinal gradients in periphyton P content and the lack of such gradients in periphyton N content during periods with longitudinal gradients in streamwater SRP and $NO_2 + NO_3$ concentrations suggest that periphyton was more likely to be limited by P than by N. Lower periphyton P content under P-deficient conditions may indicate reduced internal storage of P when supplies are low or may be an indication of more rapid recycling of P from dead material within the periphyton matrix to living cells. In a study involving periphyton-dominated laboratory streams, Mulholland et al. (1991) found that reduction of streamwater nutrient concentrations resulted in reduced P content of periphyton and greater cycling of P within periphyton communities compared with streams that did not experience nutrient reduction. In a study of phosphorus cycling in two rivers with different levels of bioavailable P in water, Paul et al. (1991) also reported higher rates of P cycling by periphyton in the river with lower concentrations of P.

Phosphatase has been shown to be a reliable indicator of phosphorus deficiency in algae (Kuenzler and Perras 1965, Berman 1970, Healey and Hendzel 1979, Gage and Gorham 1985), although light conditions also may influence phosphatase levels (Klotz 1985, Wynne and Rhee 1988). Studies of Bothwell (1985, 1988, 1989) involving experimental variation of phosphorus concentrations indicate that phosphatase is a sensitive indicator of P limitation in stream periphyton communities. Our results showing a strong inverse relationship between SRP concentrations and phosphatase activity across all sites and dates indicate that periphyton consistently responds to reduced SRP concentrations, even in the range of 1–5 $\mu g/L$, by increasing production of phosphatase. We observed no significant relationship between phosphatase activity and light. Klotz (1991) reported that concentrations of phosphatase-hydrolyzable phosphorus could be an important source of phosphorus to algae, supplementing the supply of inorganic phosphorus in a small stream in New York. Taft et al. (1977) have shown the potential for phosphatase-hydrolyzable phosphorus to contribute significantly to phytoplankton phosphorus nutrition in the Chesapeake Bay. In Walker Branch, organic phosphorus concentrations are usually greater than inorganic (SRP) concentrations at the downstream station, WB60, but organic phosphorus concentrations are consistently less than inorganic concentrations at the upstream site, WB200 (Mulholland 1992). Relatively high levels of phosphatase may enhance the availability of organic forms of phosphorus to periphyton at the downstream stations, thereby partially compensating for depletion of inorganic phosphorus concentrations (SRP) with distance in Walker Branch.

Longitudinal declines in SRP concentrations had relatively little effect on periphyton biomass (chlorophyll *a*, total algal biovolume) and productivity, which are probably ultimately controlled by intense herbivory. Snail densities were high in Walker Branch (mean = $1430/m^2$) and showed little longitudinal or seasonal variation. The periphyton community at all stations was almost always dominated by basal cells of

Stigeoclonium. These prostate cells appear to be highly resistant to grazing by the dominant herbivore, the snail *Elimia clavaeformis* (Steinman 1991; Rosemond, unpublished data). In other streams nearby in which grazing snails are very abundant, *Stigeoclonium* also dominates the periphyton (Hill et al. 1992).

Although significant longitudinal declines in carbon fixation rates (areal and chlorophyll-specific) were generally not observed during periods of longitudinal decline in SRP concentration in Walker Branch, relationships between carbon fixation rate and SRP concentration were significant when data from all stations and dates were combined (Fig. 7). Multiple regression analysis showed that light level might also be important. Steinman (1992) and Rosemond (unpublished data) have shown from experimental studies that light can be an important limiting factor for periphyton growth during the summer months in Walker Branch. However, herbivory appears to be the dominant factor maintaining a periphyton community that is uniformly low in biomass and productivity at all stations, regardless of nutrient concentrations. Results of experimental nutrient enrichment studies support this finding. Nutrient enrichment of periphyton in streamside channels and in situ in Walker Branch resulted in substantial increases in periphyton biomass and productivity only in the absence of snails (Rosemond et al. 1993).

Despite the lack of a longitudinal pattern in periphyton biomass, species composition showed effects of longitudinal depletion of SRP concentration. In samples collected during March–May, when longitudinal declines in SRP concentration were evident, a small colonial blue-green alga, *Chamaesiphon investiens*, increased in biovolume and percentage of the community and *Stigeoclonium* declined in percentage at stations downstream from WB200 (Fig. 8). However, when no longitudinal gradient in SRP concentration existed (September), and at all times at the upstream station (WB200) where SRP was highest, *Stigeoclonium* formed >90% of the algal community. Further, *Stigeoclonium* formed approximately 100% of the algal community at the mouth of the spring upstream from WB200 at a time when longitudinal gradients in SRP concentration and algal composition were strong (March). This spring consistently has higher SRP concentrations than the stream, a result of weathering of the bedrock (dolomite) which contains phosphorus (Mulholland 1992). Other reports have shown that *Stigeoclonium* has a relatively high requirement for nutrients (Francke and Den Oude 1983, Rosmarin 1983). The increase in biovolume and percentage composition of *Chamaesiphon* with decline in SRP concentration and the negative correlation between *Chamaesiphon* and *Stigeoclonium* biovolumes may be the result of superior competitive ability of *Chamaesiphon* at low nutrient concentrations. Our multiple regression results suggest that light level and herbivory may also be important regulators of *Chamaesiphon* abundance.

The longitudinal increase in phosphorus cycling indices could be due at least in part to the longitudinal changes in periphyton community composition. Correlation between periphyton phosphatase activity and *Chamaesiphon* biovolume was significant ($r^2 = 0.497$, $p = 0.010$) for the dates on which we have both phosphatase and biovolume measures. The longitudinal increase in periphyton P content appears unrelated to *Chamaesiphon* biovolume ($r^2 = 0.026$, $p = 0.704$).

Other reports of longitudinal declines in streamwater nutrient concentrations resulting from instream uptake and the resultant effects on stream organisms are few. Fisher et al. (1982) have described steep longitudinal declines in streamwater nitrate concentrations resulting from periphyton uptake during regrowth in Sycamore Creek, Arizona, following a spate. Although these authors did not evaluate the effect of the longitudinal nitrate declines on biomass, productivity, or nutrient cycling processes, they did show that periphyton patches dominated by blue-green algae were more common as nitrate declined. More recent work in Sycamore Creek has shown that N-fixing taxa of periphyton become dominant with time under low nitrate conditions (Peterson and Grimm 1992). Herbivory appears to play much less of a role in controlling periphyton biomass in Sycamore Creek compared with Walker Branch, as indicated by chlorophyll *a* levels (Sycamore Creek: >100 mg/m^2 at steady state; Walker Branch: 10–60 mg/m^2). In streams where herbivory is less intense, periphyton biomass, productivity, and community composition may be even more re-

sponsive to nutrient concentration gradients than in streams highly constrained by herbivores, such as Walker Branch.

Our results here are in many ways similar to results of a nutrient reduction experiment conducted in periphyton-dominated laboratory streams (Mulholland et al. 1991). In that laboratory stream study, experimental nutrient reduction resulted in increased nutrient cycling and changes in the periphyton community composition but had little effect on periphyton biomass and productivity. The change in the periphyton community composition primarily involved increased importance of a diatom (*Epithemia adnata*) containing a N-fixing endosymbiont in the streams with reduced nutrient input, presumably because of reduced streamwater N:P ratios in these streams (Steinman et al. 1991). In our field study, N:P ratios in Walker Branch stream water showed no consistent longitudinal variation and remained above 12:1 (data not shown but see Fig. 2). The blue-green alga that increased in abundance at low SRP concentrations in Walker Branch was apparently not capable of fixing nitrogen. However, in both the field and laboratory stream studies, periphyton communities responded to reduction in nutrient supply by increasing nutrient cycling.

The results of this study show that instream processes that alter the availability of nutrients in stream water can influence species composition and nutrient cycling in downstream periphyton communities. Despite the general lack of studies on longitudinal gradients in nutrient availability and biological response in streams, such gradients should be relatively common. This may be particularly the case in small streams with relatively large nutrient demand relative to supply (low flow rates and high wetted-perimeter-to-depth ratios) and spatially confined groundwater inputs (e.g., spring-dominated streams). In Walker Branch, a 1st-order deciduous-forest stream, instream depletion of phosphorus is the result primarily of uptake by heterotrophic microbes on decomposing leaf detritus and secondarily of uptake by periphyton, and uptake exceeds remineralization during the period from November to May. Longitudinal depletion of phosphorus concentrations has little effect on periphyton biomass or productivity because these characteristics are primarily controlled by herbivory. However, longitudinal phosphorus gradients have a strong effect on phosphorus cycling indices, such as phosphatase activity and phosphorus content of periphyton, and some effect on species composition. We hypothesize that increased phosphorus cycling with distance downstream from large groundwater inputs increases the supply of phosphorus to periphyton, and thereby buffers the effect of low streamwater SRP concentrations on the productivity of downstream periphyton communities in Walker Branch.

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