

## Immobilization and mineralization of N and P by heterotrophic microbes during leaf decomposition

Beth M. Cheever<sup>1</sup>, Erika B. Kratzer<sup>2</sup>, AND Jackson R. Webster<sup>3</sup>

*Department of Biological Sciences, Virginia Polytechnic Institute and State University, 2119 Derring Hall, Blacksburg, Virginia 24061 USA*

**Abstract.** According to theory, the rate and stoichiometry of microbial mineralization depend, in part, on nutrient availability. For microbes associated with leaves in streams, nutrients are available from both the water column and the leaf. Therefore, microbial nutrient cycling may change with nutrient availability and during leaf decomposition. We explored spatial and temporal patterns of mineralization by heterotrophic microbes by placing packs of red maple leaves at sites in 5 Appalachian streams spanning a range of N and P availability. We collected packs 4 times from each site. Leaf disks from these packs were incubated in microcosms, and uptake rates and steady-state concentrations of  $\text{NH}_4^+$  and soluble reactive P (SRP) were used to calculate mineralization rates. N uptake peaked between 50 and 60 d, whereas P uptake peaked ~10 d later. Clear patterns were found for fungal biomass-specific uptake or mineralization fluxes of either nutrient over time or space, but the microbes grown in the site with the lowest nutrient availability had the highest fungal biomass-specific cycling. The ability of microbes to access nutrients from their substrate may prevent dissolved nutrient availability from being a strong driver of microbial nutrient cycling.

**Key words:** nutrient uptake, nutrient mineralization, leaf breakdown, heterotrophic microbes, nutrient availability, streams.

Understanding the flow of nutrients, such as N and P, through ecosystems is increasingly important because anthropogenic activities are altering these cycles at the ecosystem level (Vitousek et al. 1997). Organisms have certain nutrient demands or requirements based on the composition of their biomass, and these demands must be satisfied by assimilating nutrients from available resources. Therefore, nutrient flow at the organismal level is driven by the availability of the nutrient relative to the organism's demand for that nutrient (Sterner and Elser 2002). Organisms will either retain or release nutrients depending on the flexibility of their demand for nutrients and the relative availability of nutrients in their environment. Organisms retain nutrients in situations of high demand or low availability and release nutrients in situations where demand is low or nutrients are readily available (Vanni et al. 2002, Evans-White and Lamberti 2006). Nutrient demand and availability may be important drivers of nutrient cycling at the ecosystem level as well.

In ecosystems, organisms that are capable of incorporating inorganic nutrients into their biomass drive nutrient cycling by transforming nutrients from inorganic to organic forms, and making them available to higher trophic levels. The identity and diversity of these uptake organisms vary widely across ecosystems. However, all of these organisms present in a given ecosystem could be conceptualized as a single functional unit. In this case, immobilization and mineralization fluxes in the ecosystem should depend on the nutritional demand of this unit, which would be determined by the nutritional composition of its biomass (excluding recalcitrant or inactive portions) relative to nutrient availability. In this scenario, the uptake functional unit is analogous to an organism for which nutrient cycling is driven by its nutritional demand.

Redfield (1958) used this approach to explain the similarity between the nutrient composition of plankton biomass and dissolved nutrient concentrations in the open ocean. He hypothesized that dissolved nutrient concentrations in the oceans were biologically regulated through plankton nutrient cycling. The relationship between the nutritional composition of uptake organisms and their resources

<sup>1</sup> E-mail addresses: bcheever@vt.edu

<sup>2</sup> ekratzer@vt.edu

<sup>3</sup> jwebster@vt.edu

has been explored most completely in planktonic ecosystems (Hecky et al. 1993, Elser et al. 1995, 2000, Sterner et al. 2008). Much of the C in these ecosystems is fixed by photosynthetic algae that sequester limiting and nonlimiting nutrients (Sterner and Elser 2002). However, nutrient cycling in ecosystems fed by detrital C sources is driven by heterotrophic microbes. These organisms are limited in their ability to store nonlimiting nutrients (Sterner and Elser 2002) and, therefore, are much more static in nutrient composition and demand (Persson et al. 2010).

Temperate forested headwater streams are classic examples of ecosystems dominated by heterotrophic processes, particularly in autumn when a large input of C enters the streams with leaf fall. These leaves are rapidly colonized and conditioned by aquatic fungi and bacteria (Cummins 1974). Microbial nutrient demand peaks during this time of high C availability. Microbes may satisfy part of this demand by removing nutrients from the water column (Kaushik and Hynes 1971), and stream nutrient concentration often decreases during leaf fall in response to high microbial demand (Mulholland 2004).

Leaf-associated microbes should shift their nutrient demand over time. Microbial nutrient demand should be greatest during the initial stages of decomposition while microbes are actively growing and need nutrients to sustain that growth. As decomposition progresses, demand for nutrients should peak and then decline as microbes become established, grow, and eventually senesce. Nutrient immobilization and mineralization should respond to these changes in demand, resulting in a shift from net uptake of N and P to net mineralization during decomposition. This shift in function has been demonstrated during autotrophic biofilm development (Teissier et al. 2007) and has been shown in models of leaf decomposition (Webster et al. 2009).

A similar shift from net uptake to net mineralization should occur across a gradient of nutrient availability. Net uptake should occur when nutrient availability is low because microbes should retain potentially limiting nutrients. Mineralization should be greater when nutrients are more available and exceed microbial demand. Our objective was to observe patterns in nutrient uptake and mineralization by leaf-associated microbes during decomposition in sites spanning a gradient of N and P availability. We then used these observations to assess the usefulness of an organism-based conceptual model for explaining nutrient cycling in heterotrophic streams.

## Methods

### *Study sites*

The 5 study streams are in the Appalachian region of Virginia and North Carolina (USA). All are small, shaded, 1<sup>st</sup>- or 2<sup>nd</sup>-order streams with riparian vegetation dominated by deciduous hardwoods. Hugh White Creek (HW), Stonecrop Creek (SC), and Little Stony Creek (LS) are on public or private forested land, whereas sites at Little Black Creek (LB) and Smith Creek (SM) are along roadsides and are forested only on one bank. HW drains a reference watershed at Coweeta Hydrologic Laboratory.

The 5 streams span a gradient of N and P concentration (Table 1) that ranges from below detection to 896  $\mu\text{g NO}_3^-/\text{L}$  and 8.4  $\mu\text{g}$  soluble reactive P (SRP)/L. The molar ratio of total inorganic N ( $\text{DIN} = \text{NH}_4^+ + \text{NO}_3^-$  concentration) to SRP ranges from  $\sim 2.5$  to  $\sim 600$ .

### *Deployment and collection of leaf packs*

We collected red maple (*Acer rubrum*) leaves from one tree shortly after abscission and dried them at room temperature to constant mass. We placed 10 g of leaves into mesh packs (1.5-cm mesh), which we anchored to the beds of the study sites in mid-December 2008. We collected 8 packs from each site 4, 6, 8, and 10 wk after deployment by removing them from the water column and placing each pack in a separate Zip-Loc<sup>®</sup> bag of stream water. We transported packs to the laboratory on ice and stored them at 4°C until analysis ( $\leq 48$  h later). We filtered (Whatman GF/F) water samples from the sites on each collection date (except the first) and analyzed them for  $\text{NH}_4^+$  (phenate method),  $\text{NO}_3^-$  (Cd-reduction method), and SRP (ascorbic acid method) concentrations with a Lachat QuickChem flow-injection analyzer (Lachat Instruments, Loveland, Colorado; APHA 1999). We also collected 8 L of filtered stream water (Whatman GF/F) from each site on each date for use in laboratory microcosms to measure uptake and mineralization. We stored this water at 4°C.

### *Laboratory analysis*

We modified a method proposed by O'Brien and Dodds (2008) to measure nutrient cycling by heterotrophic microbes associated with leaves. We cut leaf packs open  $\leq 48$  h after each collection and placed the contents in pans of stream water to keep them moist. We used a cork borer to cut disks (2 cm diameter) from leaves but avoided stems and skeletonized areas. We reserved 30 disks from each site for determination of leaf breakdown rate and fungal biomass.

TABLE 1. Mean nutrient concentrations ( $\mu\text{g/L}$ ) at 5 study sites across 4 collection dates.  $\text{NO}_3^-$  was not measured on the first collection date. Dissolved inorganic N (DIN) =  $\text{NH}_4^+ + \text{NO}_3^-$ . Ratios are molar and were not given if soluble reactive P (SRP) was below detection limits (bd). HW = Hugh White Creek, LS = Little Stony Creek, LB = Little Black Creek, SC = Stonecrop Creek, SM = Smith Creek, Co = county, NC = North Carolina, VA = Virginia.  $n = 3$  water samples collected at each site on each collection date.

Date/variable	HW Macon Co, NC	LS Giles Co, VA	LB Roanoke Co, VA	SC Giles Co, VA	SM Montgomery Co, VA
13–14 Jan 2009					
NH <sub>4</sub> -N	2.6	19.4	12.9	bd	10.0
SRP	bd	bd	bd	bd	bd
26–27 Jan 2009					
NH <sub>4</sub> -N	8.5	4.8	25.8	7.5	5.7
NO <sub>3</sub> -N	5.2	45.0	97.8	260.0	896.0
SRP	2.7	2.9	7.3	7.4	3.3
DIN:SRP	11.4	37.6	37.3	79.8	599.0
09–10 Feb 2009					
NH <sub>4</sub> -N	10.5	4.8	6.4	5.8	6.8
NO <sub>3</sub> -N	3.7	20.0	47.3	178.7	782.3
SRP	bd	2.2	4.0	3.4	bd
DIN:SRP	–	24.6	29.8	119.6	–
23–24 Feb 2009					
NH <sub>4</sub> -N	2.8	1.5	3.2	1.9	7.5
NO <sub>3</sub> -N	bd	18.5	41.5	251.7	890.0
SRP	2.5	2.4	6.2	8.4	3.8
DIN:SRP	2.5	18.6	15.8	66.8	527.5

*Breakdown rate.*—We compared disks cut from leaves before deployment and disks cut from leaves retrieved on each collection date to calculate breakdown rate (/d) of red maple leaves at each site. We dried (45°C for  $\geq 24$  h) 3 replicates of 5 disks from each site on each collection date, weighed them, combusted them (550°C for 2 h), and reweighed them to obtain ash-free dry mass (AFDM). We calculated breakdown rate as the slope of the line describing  $\ln(\% \text{ mass remaining})$  over time in each site. We intentionally selected intact disks for this analysis, so these breakdown rates represent only mass loss caused by chemical and microbial processes. We calculated the half life (d) of red maple in each site from these rates. We used the fraction of half life (number of days in stream/half life) for comparisons across sites.

*Fungal biomass.*—We froze 3 replicates of 5 disks from each site and collection date in 5 mL of methanol. We extracted ergosterol from these samples with a liquid-phase extraction method, quantified it with high-performance liquid chromatography, and converted values to fungal biomass (Gulis and Suberkropp 2006).

*Nutrient uptake.*—We used the remaining disks from each site to measure nutrient uptake. We used 2 sets of 50-mL tubes for each site on each collection date. Both sets consisted of 27 tubes each with 35-mL of filtered stream water and 6 leaf disks collected from the same

site. We set aside 3 tubes in each set as no-spike controls and did not add nutrients to them. We filled 8 additional tubes per set with only filtered stream water to serve as container controls. Approximately 12 h after filling the tubes, we spiked them with nutrients (N or P). We added 1 mL of 1000  $\mu\text{g/L}$   $\text{NH}_4\text{-N}$  stock solution to each tube (for a total addition of 28.5  $\mu\text{g/L}$  of  $\text{NH}_4\text{-N}$ /tube) in the 1<sup>st</sup> set (except the no-spike controls) and 1 mL of 1000  $\mu\text{g/L}$   $\text{PO}_4\text{-P}$  stock solution to each tube (for a total addition of 28.5  $\mu\text{g/L}$   $\text{PO}_4\text{-P}$ ) in the 2<sup>nd</sup> set (except the no-spike controls). We incubated tubes in an environmental chamber on shaker tables (1000 rpm) at 15°C. We destructively sampled 3 tubes containing leaf disks and 1 container-control tube 0, 15, 30, 45, 75, 120, 180, and 240 min after spiking for each nutrient set. We sampled the no-spike controls after the 240-min time step. At each time step, we removed leaf disks from the tubes with hemostats and placed them in labeled Al pans. Then we capped the tubes and froze them until analysis. We measured  $\text{NH}_4^+$  or SRP concentrations in tubes as described above (APHA 1999), and we dried and combusted leaf disks to estimate AFDM as described above.

#### *Uptake and mineralization assays*

We calculated uptake rate ( $k_t$ , /min) as the slope of the line describing the  $\ln(\text{nutrient concentration/}$

AFDM of the leaf disks) over time. We calculated uptake flux ( $U$ ;  $\mu\text{g nutrient min}^{-1} \text{g}^{-1}$  AFDM) from uptake rate and the average ambient nutrient concentration in the site from which the leaves were collected ( $C_{amb}$ ;  $\mu\text{g/L}$ ) as:

$$U = k_t C_{amb} V / L$$

where  $V$  is the volume of water in each tube (L) and  $L$  is the average leaf mass in the tubes (g AFDM). After 4 h, nutrient concentration ( $\mu\text{g/L}$ ) approached a steady-state concentration ( $C'$ ;  $\mu\text{g/L}$ ), where uptake equaled mineralization. Therefore, we calculated mineralization flux ( $M$ ;  $\mu\text{g nutrient min}^{-1} \text{g}^{-1}$  AFDM) as:

$$M = k_t C' V / L$$

(O'Brien and Dodds 2008). We used the average nutrient concentration of the 3 no-spike control tubes as the steady-state concentration.

To compare across sites and time, we calculated fungal biomass-specific (FBS) uptake and mineralization. We divided uptake and mineralization fluxes by the total fungal biomass in each tube, which we estimated by scaling the estimates of fungal biomass (mg fungal biomass/g AFDM leaf) from ergosterol extractions to the AFDM of leaf disks in the tubes.

#### Statistical analysis

We compared fungal biomass and ambient nutrient concentration among sites with repeated measures analysis of variance (rm ANOVA). When ANOVAs were significant we used Tukey post hoc tests to identify those means that were different. We  $\ln(x)$ -transformed ratios to meet assumptions of normality. We used regression analysis to assess the relationships between breakdown rate for red maple and fungal biomass and to assess changes in N and P dynamics over time and space. All regressions were linear unless stated otherwise in the text. We selected nonlinear models by comparing the Akaike information criterion for small sample size (AICc) among several candidate models (SAS version 9.2; SAS Institute, Cary, North Carolina). We used SigmaPlot with SigmaStat Integration (version 10; Systat Software Inc., Chicago, Illinois) for all other statistical tests.

### Results

Ambient N and P concentrations varied across sites and over time (Table 1). Ambient  $\text{NH}_4^+$  concentration was low in all sites, whereas  $\text{NO}_3^-$  concentrations varied 1000-fold across the gradient.  $\text{NO}_3^-$  concentrations decreased slightly over time in HW, LS, and

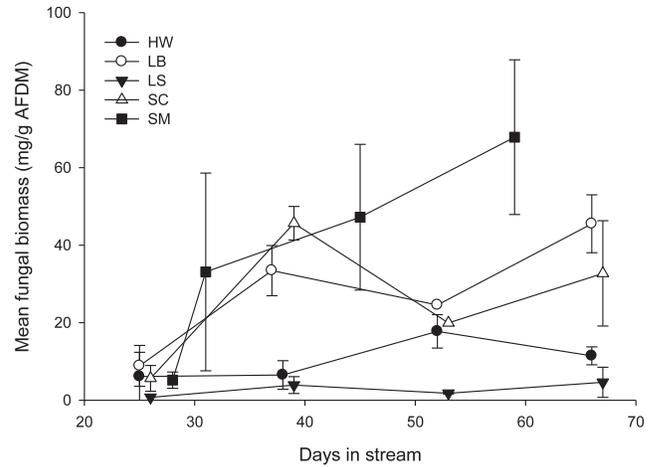


FIG. 1. Mean ( $\pm 1$  SD,  $n = 3$ ) fungal biomass (mg/g ash-free leaf dry mass [AFDM]) extracted from leaves incubated in each of the 5 sites over time. The first collections began on 13–14 January 2009, after leaves had been in sites for 4 wk. See Table 1 for site codes.

LB, but not in SC and SM, which had the highest  $\text{NO}_3^-$  concentrations (rm ANOVA,  $p < 0.001$ ). SRP was below detection in all sites on the 1<sup>st</sup> sampling date and remained low in most sites. SRP concentrations were higher in LB and SC than in the other sites in the later stages of the study (rm ANOVA,  $p < 0.001$ ). DIN:SRP also varied considerably among sites and was higher in SM than in the other sites (rm ANOVA,  $p < 0.001$ ).

Fungal biomass on red maple leaves generally increased over time in all 5 sites. Leaves accumulated more fungal biomass over 10 wk in SM than in HW and LS (rm ANOVA with Tukey post hoc test,  $p = 0.010$ ; Fig. 1). Fungal biomass increased linearly with site  $\text{NO}_3^-$  concentration and site SRP concentration but not site  $\text{NH}_4^+$  concentration (Table 2). Red maple decomposed at different rates among sites (Table 3). Red maple had the slowest breakdown rate in HW and the fastest rate in SM. Breakdown rates were  $>2\times$  faster in SM than in HW. Leaves in HW, LB, LS, and SC had completed  $\sim 40$  to  $60\%$  of their half lives by the final collection date, whereas leaves in SM had completed  $>80\%$  of their half life by that date (Table 3). Differences in breakdown rates were positively related to microbial activity. Total fungal biomass accumulated over 10 wk explained  $>70\%$  of the variation in breakdown rates, but this relationship was only marginally significant (Fig. 2).

#### Nutrient dynamics

N cycling varied spatially and temporally (Fig. 3A–H). N uptake rate in microcosms varied widely

TABLE 2. Linear regression coefficients for breakdown rates of red maple leaves and fungal biomass as functions of ambient water-column nutrient concentrations ( $\mu\text{g/L}$ ). Bolded  $p$ -values are significant at the 0.05 level. SRP = soluble reactive P.

Nutrient	Leaf breakdown rate (/d)		Fungal biomass (mg/g AFDM)	
	$r^2$	$p$	$r^2$	$p$
$\text{NH}_4\text{-N}$	0.002	0.949	0.001	0.894
$\text{NO}_3\text{-N}$	0.86	<b>0.024</b>	0.49	<b>0.004</b>
SRP	0.001	0.968	0.30	<b>0.012</b>

among sites (Fig. 3A). Peak N uptake rate of leaves collected from HW was  $>2\times$  that of leaves collected from LB and SC. Peak N uptake rate of leaves collected from LS and SM were intermediate. When all sites were analyzed together, a significant peak in N uptake rate occurred between 50 and 60 d in stream (cubic polynomial regression,  $r^2 = 0.42$ ,  $p = 0.049$ ). This relationship disappeared when N uptake rate values were standardized for stage of decomposition (Fig. 3E). Peak N uptake rate occurred after  $\sim 30\%$  of the half life in 2 low-nutrient sites (HW and LS) but occurred later in SC and LB ( $\sim 40$  and  $48\%$  of the half life, respectively).

Steady-state  $\text{NH}_4^+$  concentrations in the no-spike tubes generally were lower than ambient concentrations, although not always. Ambient and steady-state  $\text{NH}_4^+$  concentrations differed by up to  $13 \mu\text{g/L}$  (Table 4). SRP steady-state concentrations were generally greater than ambient SRP concentrations, which were often below detection (Table 4).

Temporal patterns of FBS N uptake differed among sites. FBS N uptake peaked after  $<30$  d in SM and after  $>50$  d in LS (Fig. 3B). FBS N mineralization in LS peaked after 50 d and then declined, whereas FBS N mineralization at the other sites stayed relatively constant and then declined by the last collection date (Fig. 3C). Fungal biomass was lowest in LS, and FBS N uptake and mineralization were  $\sim 3$  to  $4\times$  greater in LS than at the other sites. Standardization for stage of decomposition did not appear to influence the overall pattern of FBS N uptake or mineralization (Fig. 3F, G). Leaves were further along in decomposition in SM than in LS and HW, but FBS N uptake and mineralization remained greatest in LS and HW.

We were unable to identify a pattern in net FBS N flux (uptake – mineralization) across time, space, or stage of decomposition. Net N flux varied widely across time and space (Fig. 3D). We observed net N uptake or steady state on most collection dates across most sites, but occasionally, N mineralization flux exceeded uptake. Standardization for stage of decomposition did not influence this pattern (Fig. 3H).

P dynamics were also variable over time and space (Fig. 4A–H). P uptake rate increased linearly after 30 d in stream at HW, LB, and LS ( $r^2 = 0.57$ ,  $p = 0.005$ ; Fig. 4A). This linear relationship was not observed when P uptake rate across sites was standardized for stage of decomposition (Fig. 4E). P uptake rate was consistently faster at HW than the other sites on every collection date and was  $2\times$  that of LB by the final collection date. P uptake rate by leaves collected from LS was initially slow but increased to a rate similar to that of HW by the final collection date. We were unable to measure P uptake or to estimate steady-state concentration in several microcosm experiments (Table 4). Therefore, we did not include data from SC and SM in our analysis of P cycling. Some data from the other 3 sites also were missing because concentrations were below detection.

Temporal patterns in FBS P uptake also differed among sites. FBS P uptake increased over time in HW and LS but peaked after 50 d in LB (Fig. 4B). FBS P mineralization peaked in LB and LS after  $\sim 50$  d but continued to increase in HW (Fig. 4C). We saw a spatial trend in P fluxes similar to that of N. FBS P uptake was  $>2\times$  greater in LS than the other 2 sites by the end of the study. However, FBS P mineralization differed from FBS N mineralization in that it was

TABLE 3. Breakdown metrics for red maple leaves at each site. Fraction of half life was calculated based on the final collection date. Fraction of half life is the number of days in site (given in parentheses)/half life. See Table 1 for site codes.

Site	Leaf breakdown rate (/d)	Half life (d)	Fraction half life
HW	0.0041	169	0.39 (66)
LB	0.0062	112	0.59 (66)
LS	0.0056	124	0.54 (67)
SC	0.0052	133	0.50 (67)
SM	0.0101	69	0.86 (59)

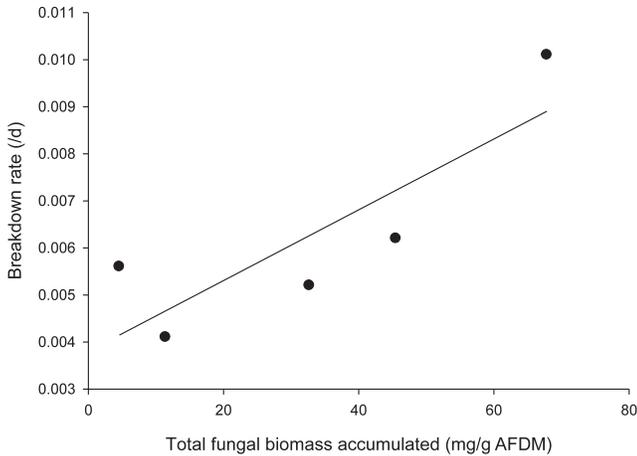


FIG. 2. Regression for breakdown rates of red maple leaves as a function of fungal biomass accumulated over 10 wk ( $r^2 = 0.71$ ,  $p = 0.072$ ).

more similar between LS and the other sites. Net P flux (uptake – mineralization) also differed from net N flux in that we observed net P mineralization at several sites (Fig. 4D). As with N dynamics, accounting for the stage in decomposition did not change the overall patterns in FBS P uptake, mineralization, or net flux (Fig. 4F–H).

Microbial nutrient cycling responded differently across the gradients in  $\text{NH}_4^+$  and  $\text{NO}_3^-$  availability (Fig. 5A–F). FBS N uptake or mineralization and  $\text{NH}_4^+$  availability did not appear to be related (Fig. 5A, B). FBS N uptake and mineralization were greatest at low  $\text{NO}_3^-$  availability (Fig. 5D, E). LS seemed to be an outlier because this site had very low fungal biomass (Fig. 1) and very high FBS N fluxes. We observed net mineralization only at low  $\text{NH}_4^+$  or  $\text{NO}_3^-$  availability (Fig. 5C, F).

Ambient SRP concentration was quite low at all sites. Thus, the SRP gradient was much more constricted than the  $\text{NO}_3^-$  gradient. However, FBS P uptake and mineralization were greatest at low SRP concentrations and decreased exponentially as SRP availability increased (Fig. 6A, B; with uptake:  $r^2 = 0.84$ ,  $p = 0.001$ ; with mineralization:  $r^2 = 0.80$ ,  $p = 0.001$ ). Net P mineralization was only observed at low SRP availability (Fig. 6C).

## Discussion

### Leaf breakdown and fungal biomass

Breakdown of leaf material is the net result of several processes including chemical leaching, physical breakage, microbial decomposition, and macroinvertebrate feeding (Webster and Benfield 1986). Direct comparisons between rates measured in our and other

studies are difficult to make for most of our sites. However, rates measured in other Coweeta reference sites are similar to our measurements in HW (0.0041/d). Red maple breakdown rates ranged from  $\sim 0.006$  to 0.018/d over several years in the stream draining reference WS 53 (Eggert and Wallace 2003) and were 0.0048/d in WS 54 (Gulis and Suberkropp 2003). In these and most other studies of leaf breakdown, rates were calculated by measuring mass lost from leaf packs over time (Benfield 2006). However, we calculated breakdown rate by measuring mass loss from leaf disks cut from intact, unskeletonized leaf surfaces in leaf packs. Thus, our breakdown rates incorporate only mass loss from chemical leaching and microbial decomposition, and the influences of most macroinvertebrate feeding and physical breakage were excluded. The breakdown rates reported here are slower than what actually occurs in these sites, but they are useful for comparing microbial processes.

Given our method for estimating breakdown rates, it is not surprising that microbial growth drove red maple breakdown rates in our study. The pattern of colonization of leaves by fungi has been described by others (Gulis and Suberkropp 2006, Gessner et al. 2007). Direct comparisons between our study and others are difficult to make, but the maximum fungal biomass we observed in HW was slightly  $< 20$  mg/g AFDM after 66 d in the site. Gulis and Suberkropp (2003) reported a maximum of  $\sim 35$  mg/g AFDM on red maple leaves in Coweeta reference WS 54 after 120 d in the stream. Fungal biomass on red maple leaves in HW might have continued to increase had we continued our collections, but it seemed to be declining by the end of our study.

Differences in fungal biomass among sites can be caused by several extrinsic factors including temperature and nutrient availability. Temperature influences microbial activity directly (Suberkropp et al. 1975, Webster and Benfield 1986, Chauvet and Suberkropp 1998, Ferreira and Chauvet 2011). We did not measure water temperature during our study, but intersite variation in water temperature probably contributed to differences in total fungal biomass. Nutrient availability can increase fungal growth (Suberkropp and Chauvet 1995, Suberkropp 1998, Grattan and Suberkropp 2001, Gulis and Suberkropp 2003, Baldy et al. 2007). Ambient  $\text{NO}_3^-$  concentration controlled fungal biomass in our study and explained  $\sim 50\%$  of the variation across sites. Fungal biomass also increased with ambient SRP concentration, but this gradient was much more constrained.

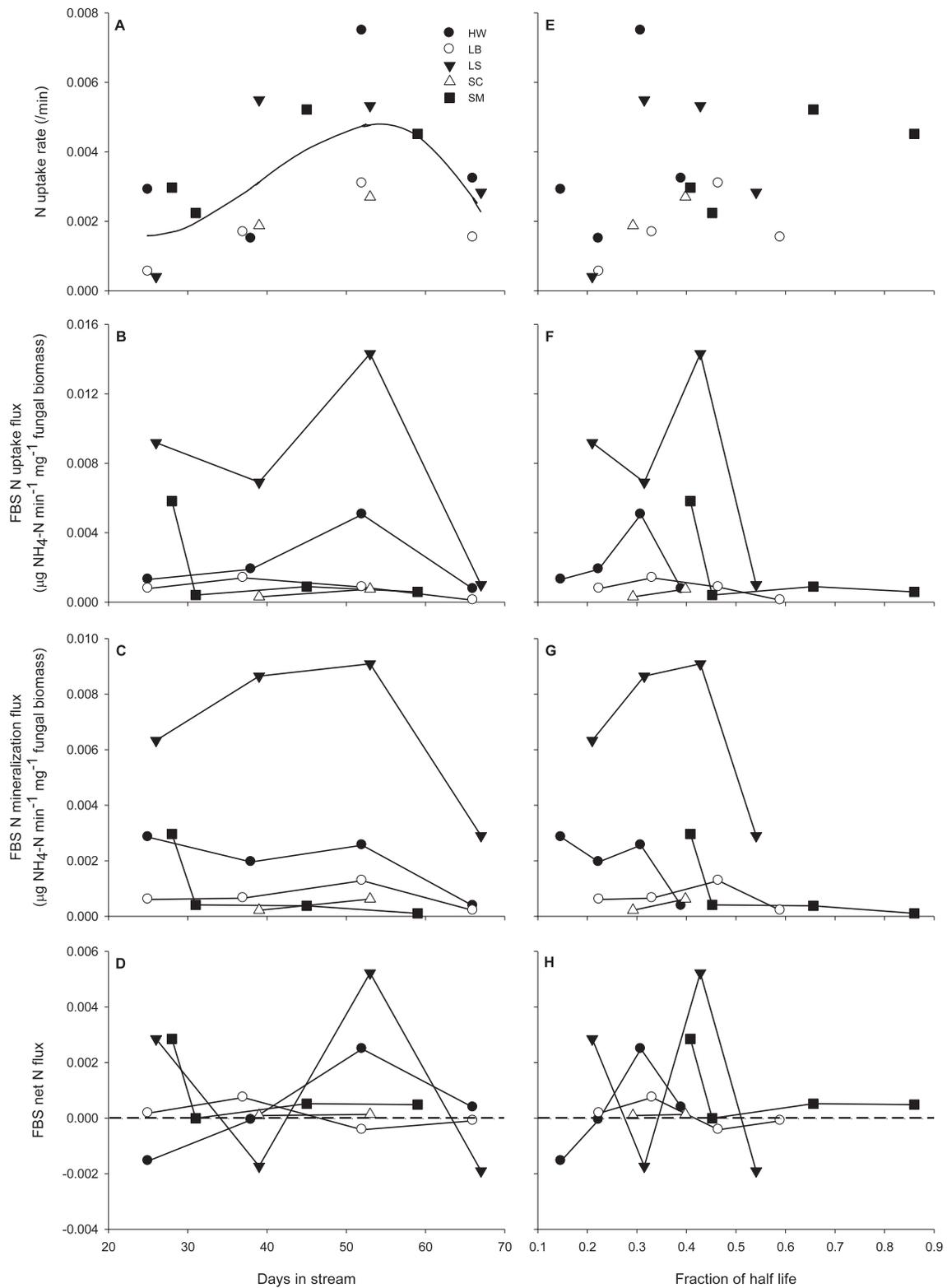


FIG. 3. N uptake rate (A, E), fungal biomass-specific (FBS) uptake flux (B, F), FBS mineralization flux (C, G), and FBS net N flux (uptake – mineralization) (D, H) of microbes from each site over time (A–D) and stage of decomposition (fraction of half life) (E–H). The curve in panel A is a cubic polynomial regression ( $r^2 = 0.42$ ,  $p = 0.049$ ,  $y = 0.0125 - 0.001x + 0.0000206x^2 + 0.000000259x^3$ ). Dashed lines in panels D and H separate net uptake (above line) from net mineralization (below line). Missing points were below detection. See Table 1 for site codes.

TABLE 4. Ambient ( $C_{amb}$ ) and steady-state ( $C'$ ) concentrations ( $\mu\text{g/L}$ ) from  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$  uptake and mineralization assays.  $C'$  was obtained from no-spike control tubes.  $C_{amb}$  was obtained from filtered water used in assays. See Table 1 for site codes.

Date/site	$\text{NH}_4\text{-N}$		$\text{PO}_4\text{-P}$	
	$C_{amb}$	$C'$	$C_{amb}$	$C'$
13–14 Jan 2009				
HW	2.6	5.7	bd	4.2
LS	19.4	13.4	bd	4.7
LB	12.9	10.0	bd	11.0
SM	10.0	5.1	–	–
26–27 Jan 2009				
HW	8.5	8.8	2.7	4.8
LS	4.8	6.0	2.9	5.1
LB	25.8	12.0	7.3	10.2
SC	7.5	5.7	–	–
SM	5.7	5.8	–	–
09–10 Feb 2009				
HW	10.5	5.3	bd	4.8
LS	4.8	3.1	2.2	5.7
LB	6.4	9.5	4.0	11.5
SC	5.8	4.8	3.4	8.3
SM	6.8	2.9	bd	3.6
23–24 Feb 2009				
HW	2.8	1.4	2.5	6.6
LS	1.5	4.4	2.4	1.2
LB	3.2	6.0	6.2	4.4
SM	7.5	1.4	3.8	3.6

### Nutrient dynamics

We observed net mineralization of both N and P on several collection dates and at several sites. Net P mineralization was particularly common and occurred more frequently than net P uptake. In contrast, net N uptake was more common than net N mineralization. Net mineralization suggests that microbial biomass is not increasing or that it is limited by other nutrients. Ambient SRP was relatively low at all sites, and microbes probably were P limited. However, the net mineralization fluxes were quite low ( $<0.002 \mu\text{g NH}_4\text{-N min}^{-1} \text{mg}^{-1}$  fungal biomass and  $<0.003 \mu\text{g PO}_4\text{-P min}^{-1} \text{mg}^{-1}$  fungal biomass), and these fluxes might be an artifact of the precision of our analytical methods.

Measurements of mineralization are scarce in stream literature. Stream ecologists have well developed and relatively straightforward methods for measuring and comparing nutrient uptake at the reach scale (Payn et al. 2005, Webster and Valett 2006, Mulholland et al. 2008). However, measuring mineralization at similar scales requires use of stable or radioactive isotopes (Newbold et al. 1983, Peterson et al. 2001, Simon et al. 2004), which is logistically impractical for most field studies. Webster et al. (2009) used a computer model that considered N and P

content of the water, microbial biomass, and leaf material to simulate microbial nutrient cycling during leaf decomposition in HW. Their simulation produced a shift for both N and P from net retention to net mineralization during decomposition. We were unable to demonstrate a relationship between net mineralization and stage of decomposition, possibly because our method was not sensitive enough to measure very low levels of net mineralization.

Microbial retention or regeneration of inorganic nutrients in headwater streams has implications for downstream nutrient processing. Nutrients exported by retentive headwaters would be largely refractory organic forms, potentially causing downstream communities to become nutrient limited. Regenerative headwaters potentially would alleviate nutrient limitation of downstream communities by exporting excess inorganic nutrients. However, in many conceptual and practical models, streams are considered as being at steady state and nutrient concentrations often are maintained over longitudinal distance (Brookshire et al. 2009). Steady state might be observed at low-resolution temporal and spatial scales because of the temporal and spatial heterogeneity of microbial activity. For instance, at any one time, leaves in streams are in various stages of

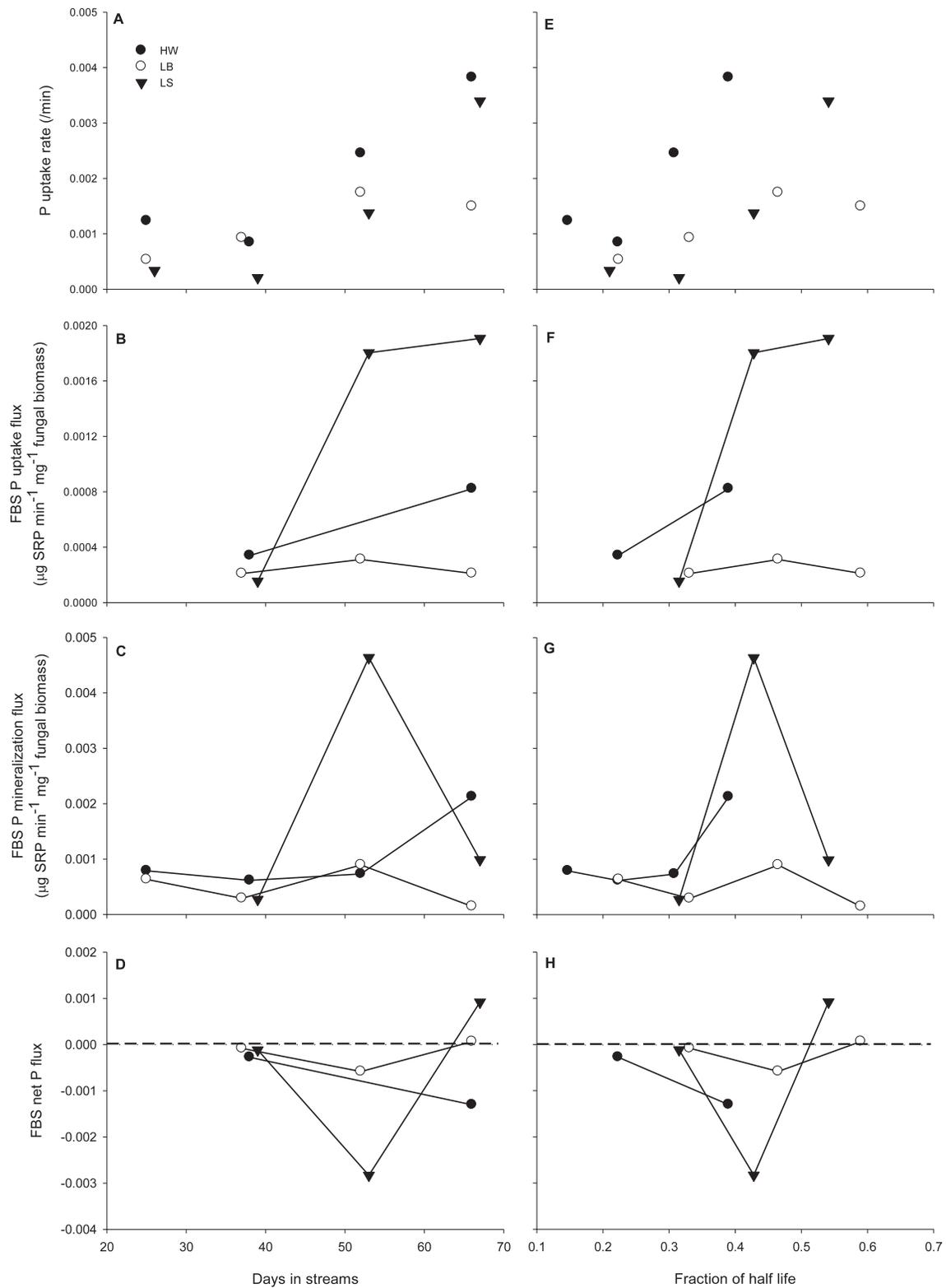


FIG. 4. P uptake rate (A, E), fungal biomass-specific (FBS) uptake flux (B, F), FBS mineralization flux (C, G), and FBS net P flux (uptake – mineralization) (D, H) of biofilms from each site over time (A–D) and stage of decomposition (fraction of half life) (E–H). Dashed lines in panels D and H separate net uptake (above line) from net mineralization (below line). Missing points were below detection. See Table 1 for site codes.

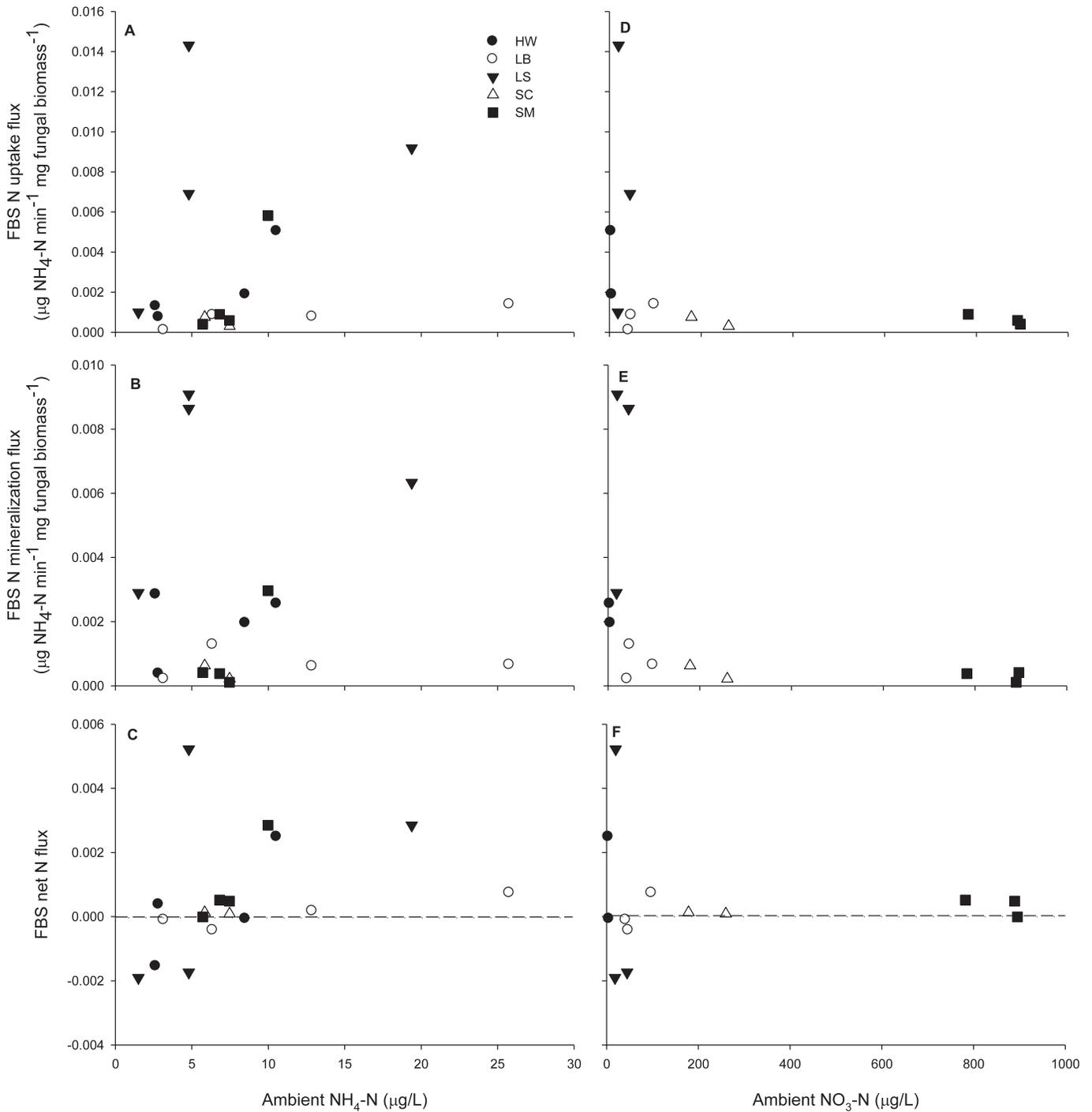


FIG. 5. Fungal biomass-specific (FBS) N uptake flux (A, D), mineralization flux (B, E), and net flux (uptake – mineralization) (C, F) along the gradient of  $\text{NH}_4^+$  (A–C) and  $\text{NO}_3^-$  (D–F) availability. Dashed lines in panels C and F separate net uptake (above line) from net mineralization (below line). See Table 1 for site codes.

decomposition (e.g., Cummins et al. 1989) and, therefore, might be supporting microbial communities with varying functions with respect to nutrient retention. This functional heterogeneity could lead to

relatively consistent nutrient concentrations over time and space.

Uptake and mineralization did not follow the pattern we predicted, but some consistent trends

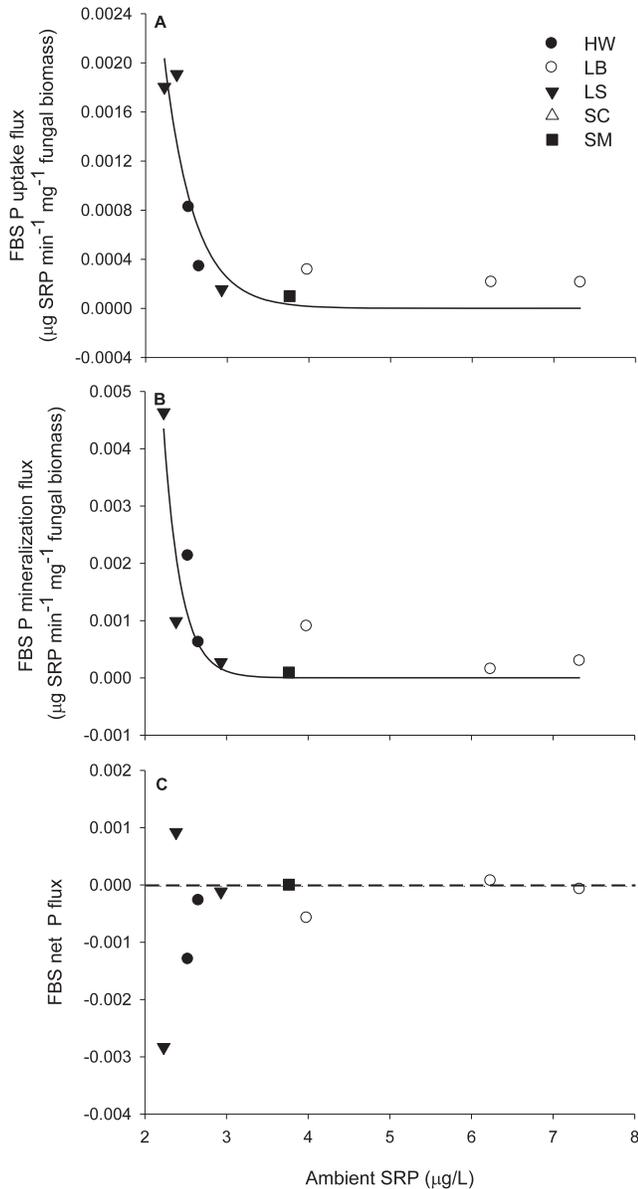


FIG. 6. Fungal biomass specific (FBS) P uptake flux (A), mineralization flux (B), and net flux (uptake – mineralization) (C) along the gradient of soluble reactive P (SRP). Solid lines in panels A and B are exponential decay regressions where FBS uptake and mineralization =  $ae^{(-b[\text{ambient SRP}])}$  (FBS uptake:  $r^2 = 0.84$ ,  $p = 0.001$ ; FBS mineralization:  $r^2 = 0.80$ ,  $p = 0.001$ ). Dashed line in panel C separates net uptake (above line) from net mineralization (below line). See Table 1 for site codes.

were present in our data. Sites with low nutrient availability had low fungal biomass and, consequently, had high FBS nutrient fluxes. For example, LS had the lowest fungal biomass but the greatest FBS uptake and mineralization of both N and P. Microbes were cycling nutrients faster per unit fungal biomass in the low- than in high-nutrient sites. We think that

differences in shredder assemblages among our sites may have contributed to the differences in fungal nutrient-cycling efficiencies. Grazing can influence both structure and function of primary producers in autotrophic ecosystems (McNaughton 1979, Gregory 1983, Lamberti and Moore 1984). Specifically, intermediate levels of grazing can enhance production by removing dead or inactive biomass. Shredding macroinvertebrates in some of our sites may have been doing something similar to heterotrophic microbes. Not all macroinvertebrates classified as shredders exhibit the shredding or chewing behavior typical of this functional feeding group. Several Plecoptera taxa, including peltoperlids, have been described as ‘microshredders’, or shredders that scrape superficial microbes and mesophilic tissue from the leaves (Wallace et al. 1970, King et al. 1988). Microshredders might enhance production by removing dead or senescent cells, which would result in a low but productive fungal biomass that would cycle nutrients at a relatively fast rate. The abundance of microshredders probably varied across sites. All of our sites have similar riparian vegetation and local conditions (with the exception of nutrient availability), but they drain catchments with different land uses. LS and HW are in forested catchments, whereas SM is in an agricultural catchment. As an order, Plecoptera are often most abundant in pristine, forested streams. We did not formally assess Plecoptera abundance, but we noticed more peltoperlids and other stonefly taxa in leaf packs collected from LS and HW than in packs collected from the other sites, an observation suggesting that microbes in LS and HW may have experienced the type of grazing that may cause high cycling rates but low biomass.

#### Evaluation of conceptual model

We did not see the patterns in nutrient cycling that we predicted from the organism-based ecosystem model. This failure to generate accurate predictions may indicate that the model is not appropriate for nutrient cycling at the ecosystem level. Nutrient cycling in the organism-based model is driven by demand and availability. For organisms, particularly animals, demand often is defined as the nutrient content of the body of the individual organism and availability as the nutrient content of its food source. We included all uptake organisms in a single functional unit analogous to a single organism and attempted to predict its nutrient cycling based on its demand and the availability of nutrients. However, we made several simplifying assumptions that might have influenced both demand and availability (Fig. 7).

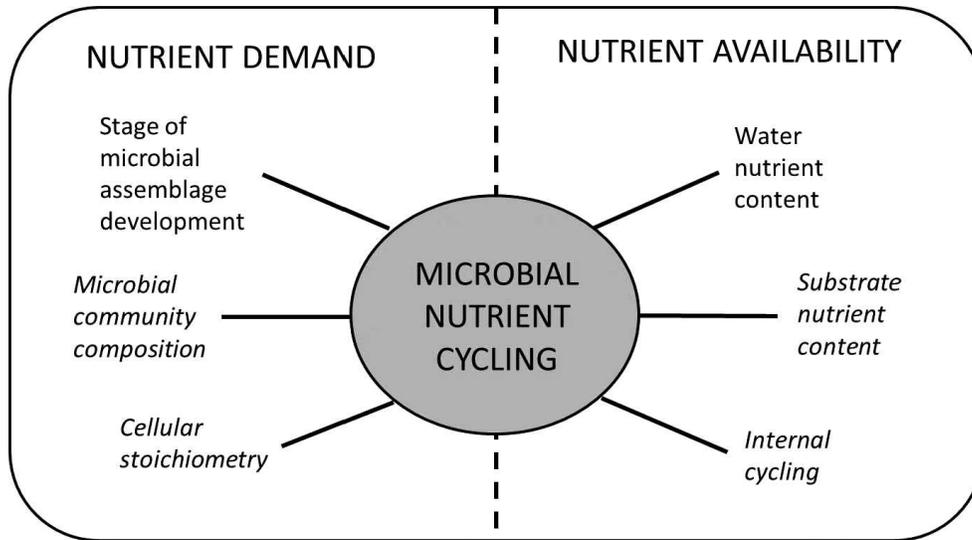


FIG. 7. Conceptual model of factors influencing nutrient cycling by leaf-associated heterotrophic microbes in streams. Microbial nutrient cycling is the product of nutrient demand and nutrient availability. At the ecosystem level, demand and availability are influenced by several factors. Italicized type indicates factors that were assumed constant or not included in our study.

First, we considered the microbe–substrate complex as a single functional unit with a single nutrient demand. However, this complex is actually a consortium of many different types of organisms including fungi, heterotrophic bacteria, and probably some autotrophic cells. These organisms, along with the extracellular enzymes and products they produce, form a matrix within and on the surface of the leaf substrate. Each of the groups of organisms in this matrix has a specific nutrient composition, growth efficiency, growth rate, and enzyme production rate, all of which contribute to a specific nutrient demand. Our model assumes that each of these groups of organisms will respond similarly to changes in nutrient availability, either over time or across a gradient. However, interactions among these organisms and their matrix may so alter conditions within the detritus that broad measures of nutrient availability are not relevant.

Second, we assumed that the nutrient content of the microbes was homeostatic, and therefore, the response in microbial nutrient demand was driven by changes in nutrient availability across space or by changes in microbial biomass over time. However, the nutrient content of microbes may be temporally or spatially variable at the individual and the assemblage level. Autotrophic microbes can store nutrients in specific compounds or in vacuoles within their cells, a process referred to as luxury uptake. This ability makes the nutrient composition of autotrophs, and potentially their nutrient demand, very responsive to nutrient

availability in the environment (Sterner and Elser 2002). Algae can colonize detritus, particularly when light levels are favorable (Rier et al. 2007, Artigas et al. 2009). We did not measure the algal content of the microbial assemblage, but it was probably fairly low, particularly in HW and LS, which were heavily shaded by *Rhododendron*. In comparison, heterotrophic microbes are considered to be stoichiometrically homeostatic. That is, the nutrient composition of heterotrophic microbes does not respond to changes in nutrient availability (Sterner and Elser 2002, Makino et al. 2003). However, results of a recent meta-analysis suggest that homeostasis should be considered as a continuum and some heterotrophic groups may be weakly plastic (Persson et al. 2010). The heterotrophic microbes associated with the leaves in our study might have exhibited some plasticity over decomposition or the nutrient gradient. The nutrient composition of the microbes may have also changed because of shifts in the composition of the microbial assemblage over time and space. Bacterial cells are much richer in N and P than fungal hyphae because of their relative lack of structural material and faster growth rate (Sterner and Elser 2002). Bacteria become more abundant in the later stages of decomposition (Suberkropp and Klug 1976), and this increase potentially could cause a shift in the stoichiometry of the microbial assemblage over time. Differences in the responses of fungi and bacteria to nutrient availability (Suberkropp et al. 2010) might cause differences in microbial stoichiometry among streams. Changes in the nutrient composition of the

microbial biomass, whether caused by individual or assemblage-level mechanisms, should be considered as drivers of microbial nutrient demand.

The final simplifying assumption of our model was that immobilization of dissolved inorganic nutrients reflects microbial demand. Several lines of evidence suggest that microbes use nutrients dissolved in the water column. The concentrations of dissolved nutrients in the water column decrease significantly during times of high microbial demand, such as peak leaf fall (Mulholland 2004, Goodale et al. 2009). Dissolved nutrient uptake is positively correlated with detrital standing stocks (e.g., Mulholland et al. 1985). Dissolved nutrient availability can stimulate microbial abundance (Meyer and Johnson 1983, Suberkropp and Chauvet 1995, Grattan and Suberkropp 2001, Baldy et al. 2007), reproduction (Suberkropp 1998, Grattan and Suberkropp 2001), and function (Meyer and Johnson 1983, Suberkropp and Chauvet 1995, Grattan and Suberkropp 2001). Studies with stable-isotope tracers have supplied direct evidence for use of dissolved nutrients by heterotrophic microbes (Tank et al. 2000, Sanzone et al. 2001). A substantial portion of microbial demand might also be satisfied by organic pools. Authors of several studies have suggested that some forms of dissolved organic N may be readily available and used quickly by stream microbes (Brookshire et al. 2005, Johnson and Tank 2009, Johnson et al. 2009).

Microbes assimilate nutrients as well as dissolved organic matter from their organic substrate. The fact that nutrient content of leaves can drive breakdown rates suggests that substrate nutrients support at least a portion of microbial nutrient demand (Ostrowsky 1997, Richardson et al. 2004, Lecerf and Chauvet 2008). Aquatic microbes produce a suite of exoenzymes that liberate nutrients from the leaves (Sinsabaugh et al. 1991). These enzymes could be used to mine nutrients as well as to acquire C (Craine et al. 2007). Knowledge of the relative importance of water-column and leaf-derived nutrients in satisfying microbial demand over the course of decomposition and at different nutrient levels would enable development of more accurate predictive models describing microbial nutrient processing in streams.

Our study showed wide variations in the rates of microbial immobilization and mineralization of N and P in 5 forested sites. We did not detect strong patterns in the fluxes of either nutrient during decomposition or across a nutrient gradient. We suggest that future investigations of microbial nutrient cycling would benefit from both methodological and conceptual improvements. First, obtaining refined measurements of microbial mineralization is difficult because immo-

bilization and mineralization occur simultaneously and the limitations of analytical methods often make detecting subtle concentration changes difficult. Stable isotopes may be useful in parsing out these simultaneous processes, despite the expense often associated with their use. Second, we suggest that the simple organism-based model is insufficient when describing nutrient cycling at the ecosystem level. This model needs to be modified to include temporal and spatial variability of nutrient demand based on microbial requirements for production and growth as well as alternative sources of nutrients available to satisfy that demand.

### Acknowledgements

This work was supported by the National Science Foundation through the Coweeta Long-Term Ecological Research Project (DEB-0823293 and DEB-02218001) and a Doctoral Dissertation Improvement Grant (DEB-478406). We thank Bobbie Niederlehner for assistance with laboratory analysis; Rob Ryland, Stephen Via, and Adam Hart for help in the field; Kathi Susano and Kenneth Godboy for access to Smith Creek; and Fred Benfield, Maury Valett, Andy Dolloff, and the Virginia Tech Stream Team and Ecosystem Research Group for their contributions to the development of this project. This manuscript was improved by comments from Bob Hall and 2 anonymous referees.

### Literature Cited

- APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). 1999. Standard methods for the examination of water and wastewater. 20<sup>th</sup> edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- ARTIGAS, J., A. M. ROMANI, A. GAUDES, I. MUÑOZ, AND S. SABATER. 2009. Organic matter availability structures microbial biomass and activity in a Mediterranean stream. *Freshwater Biology* 54:2025–2036.
- BALDY, V., V. GOBERT, F. GUEROLD, E. CHAUVET, D. LAMBRIGOT, AND J.-Y. CHARCOSSET. 2007. Leaf litter breakdown budgets in streams of various trophic status: effects of dissolved inorganic nutrients on microorganisms and invertebrates. *Freshwater Biology* 52:1322–1335.
- BENFIELD, E. F. 2006. Decomposition of leaf material. Pages 711–720 *in* F. R. Hauer and G. A. Lamberti (editors). *Methods in stream ecology*. Academic Press, New York.
- BROOKSHIRE, E. N. J., H. M. VALETT, AND S. GERBER. 2009. Maintenance of terrestrial nutrient loss signatures during in-stream transport. *Ecology* 90:293–299.
- BROOKSHIRE, E. N. J., H. M. VALETT, S. A. THOMAS, AND J. R. WEBSTER. 2005. Coupled cycling of dissolved organic

- nitrogen and carbon in a forest stream. *Ecology* 86: 2487–2496.
- CHAUVET, E., AND K. SUBERKROPP. 1998. Temperature and sporulation of aquatic hyphomycetes. *Applied and Environmental Microbiology* 64:1522–1525.
- CRAINE, J. M., C. MORROW, AND N. FIERER. 2007. Microbial nitrogen limitation increases decomposition. *Ecology* 88: 2105–2113.
- CUMMINS, K. W. 1974. Structure and function of stream ecosystems. *BioScience* 24:631–641.
- CUMMINS, K. W., M. A. WILZBACH, D. M. GATES, J. B. PERRY, AND W. B. TALIAFERRO. 1989. Shredders and riparian vegetation. *BioScience* 39:24–30.
- EGGERT, S. L., AND J. B. WALLACE. 2003. Litter breakdown and invertebrate detritivores in a resource-depleted Appalachian stream. *Archiv für Hydrobiologie* 156:315–338.
- ELSER, J. J., T. H. CHRZANOWSKI, R. W. STERNER, J. H. SCHAMPEL, AND D. K. FOSTER. 1995. Elemental ratios and the uptake and release of nutrients by phytoplankton and bacteria in 3 lakes of the Canadian shield. *Microbial Ecology* 29: 145–162.
- ELSER, J. J., R. W. STERNER, A. E. GALFORD, T. H. CHRZANOWSKI, D. L. FINDLAY, K. H. MILLS, M. J. PATERSON, M. P. STANTON, AND D. W. SCHINDLER. 2000. Pelagic C:N:P stoichiometry in a eutrophied lake: Responses to a whole-lake food-web manipulation. *Ecosystems* 3: 293–307.
- EVANS-WHITE, M. A., AND G. A. LAMBERTI. 2006. Stoichiometry of consumer-driven nutrient recycling across nutrient regimes in streams. *Ecology Letters* 9:1186–1197.
- FERREIRA, V., AND E. CHAUVET. 2011. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology* 17:551–564.
- GESSNER, M. O., V. GULIS, K. A. KUEHN, E. CHAUVET, AND K. SUBERKROPP. 2007. Fungal decomposers of plant litter in aquatic ecosystems. Pages 301–324 in I. S. Kubicek (editor). *Environmental and microbial relationships*. Springer, New York.
- GOODALE, C. L., S. A. THOMAS, G. FREDRIKSEN, E. M. ELLIOTT, K. M. FLINN, T. J. BUTLER, AND M. T. WALTER. 2009. Unusual seasonal patterns and inferred processes of nitrogen retention in forested headwaters of the upper Susquehanna River. *Biogeochemistry* 93:197–218.
- GRATTAN, R. M., AND K. SUBERKROPP. 2001. Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. *Journal of the North American Benthological Society* 20:33–43.
- GREGORY, S. V. 1983. Plant-herbivore interactions in streams. Pages 157–189 in J. R. Barnes and G. W. Minshall (editors). *Stream ecology: application and testing of general ecological theory*. Plenum Press, New York.
- GULIS, V., AND K. SUBERKROPP. 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology* 48:123–134.
- GULIS, V., AND K. SUBERKROPP. 2006. Fungi: biomass, production, and sporulation of aquatic hyphomycetes. Pages 311–326 in F. R. Hauer and G. A. Lamberti (editors). *Methods in stream ecology*. Academic Press, New York.
- HECKY, R. E., P. CAMPBELL, AND L. L. HENDZEL. 1993. The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. *Limnology and Oceanography* 38:709–724.
- JOHNSON, L. T., AND J. L. TANK. 2009. Diurnal variations in dissolved organic matter and ammonium uptake in six open-canopy streams. *Journal of the North American Benthological Society* 28:694–708.
- JOHNSON, L. T., J. L. TANK, AND C. P. ARANGO. 2009. The effect of land use on dissolved organic carbon and nitrogen uptake in streams. *Freshwater Biology* 54:2335–2350.
- KAUSHIK, N. K., AND H. B. N. HYNES. 1971. The fate of dead leaves that fall into streams. *Archiv für Hydrobiologie* 68:465–515.
- KING, J. M., J. A. DAY, P. R. HURLY, M. P. HENSHALL-HOWARD, AND B. R. DAVIES. 1988. Macroinvertebrate communities and environment in a southern African mountain stream. *Canadian Journal of Fisheries and Aquatic Sciences* 45:2168–2181.
- LAMBERTI, G. A., AND J. W. MOORE. 1984. Aquatic insects as primary consumers. Pages 164–195 in V. H. Resh and D. M. Rosenberg (editors). *The ecology of aquatic insects*. Praeger Publishers, New York.
- LECERF, A., AND E. CHAUVET. 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. *Basic and Applied Ecology* 9:598–605.
- MAKINO, W., J. B. COTNER, R. W. STERNER, AND J. J. ELSER. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. *Functional Ecology* 17:121–130.
- MCAUGHTON, S. J. 1979. Grazing as an optimization process: grass-ungulate relationships in the Serengeti. *American Naturalist* 113:691–703.
- MEYER, J. L., AND C. JOHNSON. 1983. The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. *Freshwater Biology* 13:177–183.
- MULHOLLAND, P. J. 2004. The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch watershed. *Biogeochemistry* 70:403–426.
- MULHOLLAND, P. J., A. M. HELTON, G. C. POOLE, R. O. HALL, S. K. HAMILTON, B. J. PETERSON, J. L. TANK, L. R. ASHKENAS, L. W. COOPER, C. N. DAHM, W. K. DODDS, S. E. G. FINDLAY, S. V. GREGORY, N. B. GRIMM, S. L. JOHNSON, W. H. MCDOWELL, J. L. MEYER, H. M. VALETT, J. R. WEBSTER, C. P. ARANGO, J. J. BEAULIEU, M. J. BERNOT, A. J. BURGIN, C. L. CRENSHAW, L. T. JOHNSON, B. R. NIEDERLEHNER, J. M. O'BRIEN, J. D. POTTER, R. W. SHEIBLEY, D. J. SOBOTA, AND S. M. THOMAS. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature* 452:202–206.
- MULHOLLAND, P. J., J. D. NEWBOLD, J. W. ELWOOD, L. A. FERREN, AND J. R. WEBSTER. 1985. Phosphorus spiraling in a woodland stream: seasonal variations. *Ecology* 66: 1012–1023.

- NEWBOLD, J. D., J. W. ELWOOD, R. V. O'NEILL, AND A. L. SHELDON. 1983. Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiralling. *Ecology* 64:1249–1265.
- O'BRIEN, J. M., AND W. K. DODDS. 2008. Ammonium uptake and mineralization in prairie streams: chamber incubation and short-term nutrient addition experiments. *Freshwater Biology* 53:102–112.
- OSTROFSKY, M. L. 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *Journal of the North American Benthological Society* 16:750–759.
- PAYN, R. A., J. R. WEBSTER, P. J. MULHOLLAND, H. M. VALETT, AND W. K. DODDS. 2005. Estimation of stream nutrient uptake from nutrient addition experiments. *Limnology and Oceanography: Methods* 3:174–182.
- PERSSON, J., P. FINK, A. GOTO, J. M. HOOD, J. JONAS, AND S. KATO. 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos* 119:741–751.
- PETERSON, B. J., W. M. WOLLHEIM, P. J. MULHOLLAND, J. R. WEBSTER, J. L. MEYER, J. L. TANK, E. MARTÍ, W. B. BOWDEN, H. M. VALETT, A. E. HERSHEY, W. H. MCDOWELL, W. K. DODDS, S. K. HAMILTON, S. GREGORY, AND D. D. MORRALL. 2001. Control of nitrogen export from watersheds by headwater streams. *Science* 292:86–90.
- REDFIELD, A. 1958. The biological control of chemical factors in the environment. *American Scientist* 46:205–221.
- RICHARDSON, J. S., C. R. SHAUGHNESSY, AND P. G. HARRISON. 2004. Litter breakdown and invertebrate association with three types of leaves in a temperate rainforest stream. *Archiv für Hydrobiologie* 159:309–325.
- RIER, S. T., K. A. KUEHN, AND S. N. FRANCOEUR. 2007. Algal regulation of extracellular enzyme activity in stream microbial communities associated with inert substrata and detritus. *Journal of the North American Benthological Society* 26:439–449.
- SANZONE, D. M., J. L. TANK, J. L. MEYER, P. J. MULHOLLAND, AND S. E. G. FINDLAY. 2001. Microbial incorporation of nitrogen in stream detritus. *Hydrobiologia* 464:27–35.
- SIMON, K. S., C. R. TOWNSEND, B. J. F. BIGGS, W. B. BOWDEN, AND R. D. FREW. 2004. Habitat-specific nitrogen dynamics in New Zealand streams containing native or invasive fish. *Ecosystems* 7:777–792.
- SINSABAUGH, R. L., D. REPERT, T. WEILAND, S. W. GOLLADAY, AND A. E. LINKINS. 1991. Exoenzyme accumulation in epilithic biofilms. *Hydrobiologia* 222:29–37.
- STERNER, R. W., T. ANDERSEN, J. J. ELSER, D. O. HESSEN, J. M. HOOD, E. MCCAULEY, AND J. URABE. 2008. Scale-dependent carbon : nitrogen : phosphorus seston stoichiometry in marine and freshwaters. *Limnology and Oceanography* 53:1169–1180.
- STERNER, R. W., AND J. J. ELSER. 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton, New Jersey.
- SUBERKROPP, K. 1998. Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. *Mycological Research* 102:998–1002.
- SUBERKROPP, K., AND E. CHAUVET. 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. *Ecology* 76:1433–1445.
- SUBERKROPP, K., V. GULIS, A. D. ROSEMOND, AND J. P. BENSTEAD. 2010. Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream: results of a 5-year continuous enrichment. *Limnology and Oceanography* 55:149–160.
- SUBERKROPP, K., AND M. J. KLUG. 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology* 57:707–719.
- SUBERKROPP, K., M. J. KLUG, AND K. W. CUMMINS. 1975. Community processing of leaf litter in woodland streams. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 19:1653–1658.
- TANK, J. L., J. L. MEYER, D. M. SANZONE, P. J. MULHOLLAND, J. R. WEBSTER, B. J. PETERSON, W. M. WOLLHEIM, AND N. E. LEONARD. 2000. Analysis of nitrogen cycling in a forest stream during autumn using a N-15-tracer addition. *Limnology and Oceanography* 45:1013–1029.
- TEISSIER, S., M. TORRE, F. DELMAS, AND F. GARABETIAN. 2007. Detailing biogeochemical N budgets in riverine epilithic biofilms. *Journal of the North American Benthological Society* 26:178–190.
- VANNI, M. J., A. S. FLECKER, J. M. HOOD, AND J. L. HEADWORTH. 2002. Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecology Letters* 5:285–293.
- VITOUSEK, P. M., H. A. MOONEY, J. LUBCHENCO, AND J. M. MELILLO. 1997. Human domination of earth's ecosystems. *Science* 277:494–499.
- WALLACE, J. B., W. R. WOODALL, AND F. F. SHERBERGER. 1970. Breakdown of leaves by feeding of *Peltoperla maria* nymphs (Plecoptera: Peltoperlidae). *Annals of the Entomological Society of America* 63:562–567.
- WEBSTER, J. R., AND E. F. BENFIELD. 1986. Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* 17:567–594.
- WEBSTER, J. R., J. D. NEWBOLD, S. A. THOMAS, H. M. VALETT, AND P. J. MULHOLLAND. 2009. Nutrient uptake and mineralization during leaf decay in streams – a model simulation. *International Review of Hydrobiology* 94:372–390.
- WEBSTER, J. R., AND H. M. VALETT. 2006. Solute dynamics. Pages 169–185 in F. R. Hauer and G. A. Lamberti (editors). *Methods in stream ecology*. Academic Press, New York.

Received: 11 May 2011

Accepted: 20 October 2011