

## Effect of leaf litter exclusion on microbial enzyme activity associated with wood biofilms in streams

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**Abstract.** Leaf litter inputs have been excluded from 1 of 2 1st-order streams at Coweeta Hydrologic Laboratory, North Carolina since August 1993 to examine the bottom-up effects of resource reduction on stream ecosystems. As part of the larger project, we studied the effect of litter exclusion on the extracellular enzyme activity and fungal biomass of wood biofilms in the presence and absence of leaf litter. Replicate strips of wood veneer were incubated in both streams for 28, 58, and 86 d. Ergosterol content (to estimate fungal biomass), the activity of 5 lignocellulose-degrading enzymes, acid phosphatase activity, breakdown rates, and % organic matter were determined when the veneer strips were collected. Hydrolytic enzyme activity and wood breakdown rates in the litter-excluded stream were significantly higher than in the reference stream. Enzyme activities of all hydrolytic enzymes were highly correlated with each other. Measurement of relative activities of selected extracellular enzymes comparing carbon and nutrient acquisition suggested nutrient limitation of heterotrophic biofilms in the reference stream. Microbial activity of wood biofilms was significantly altered by the exclusion of leaf litter, and hydrolytic enzyme activity, as an indicator of carbon cycling, was higher on wood in streams without leaf litter.

**Key words:** wood biofilm, extracellular enzyme activity, fungal biomass, ergosterol, decomposition, leaf litter, nutrient, nitrogen, phosphorus, stream.

Wood has been considered an important structural component in streams (e.g., Harmon et al. 1986), but recent research has also demonstrated the importance of wood as a substrate for biofilm development (Golladay and Sinsabaugh 1991, Sinsabaugh et al. 1991, Tank and Winterbourn 1995, 1996). Wood surfaces may support higher, primarily fungal, microbial biomass than leaves (Golladay and Sinsabaugh 1991, Tank et al. 1993, Tank 1996). Few freshwater organisms consume wood directly so microbial colonization serves as a vector for wood carbon transfer to higher trophic levels in stream food webs. Wood offers physical stability allowing for extensive microbial colonization and exposure of new substrate for colonization over the course of the decomposition process (Golladay and Sinsabaugh 1991). Leaves normally arrive as a pulsed, short-term input of carbon to a stream system, whereas wood is a long-lasting source of organic matter.

Microbial colonization of wood is primarily a surface phenomenon (Aumen et al. 1983, Golladay and Sinsabaugh 1991, Sinsabaugh et al. 1992, Tank and Winterbourn 1995). Wood bio-

films consist primarily of fungi and bacteria located in a mucilaginous matrix (Lock et al. 1984, Golladay and Sinsabaugh 1991). The mucilaginous matrix can accrue extracellular enzymes, but fine organic and inorganic particulates can be trapped in the biofilm as well. Low microbial activity is often associated with the accumulation of inorganic sediments on organic surfaces in streams (Golladay and Sinsabaugh 1991, Tank et al. 1993). Inorganic sediments cover biofilms, hinder oxygen and nutrient diffusion through the biofilm, decrease microbial activity, and slow organic matter decomposition.

Microbial degradation of large particulate organic matter is mediated by enzymatic activity occurring outside the cell (e.g., Sinsabaugh et al. 1991). The enzymes involved in lignocellulose degradation, as well as nitrogen and phosphorus cycling, are most critical to degradation of organic matter (Sinsabaugh and Moorhead 1996). Because there is a strong correlation between decomposition rates of particulate organic matter and extracellular lignocellulose activity (Sinsabaugh et al. 1992, 1994b, Sinsabaugh and Linkins 1993), extracellular enzyme activity can

be used as an indicator of microbial decomposition (Jackson et al. 1995). Models derived from relationships between extracellular enzymes and decomposition predict that temperature, pH, and nutrient availability will affect microbial activity through the regulation of enzyme activity (Sinsabaugh et al. 1994b).

Previous studies have shown that activities of a few selected enzymes can be used as indices for the complex hydrolytic and oxidative reactions that drive microbial decomposition (Sinsabaugh et al. 1994b). Because wood decomposes slowly, monitoring extracellular enzymes may be more useful than weight loss as an approach to estimate microbial activity related to wood decomposition (Sinsabaugh et al. 1994a). Despite its slow decomposition, wood can support an active microbial biofilm and is thereby a potential food source for consumers.

The overall objective of this study was to determine the effect of leaf litter exclusion on the biofilm colonizing wood in a small headwater stream. Leaf litter inputs have been excluded from a 1st-order stream (Catchment 55) at Coweeta Hydrologic Lab since August 1993 in an attempt to determine how removal of the majority of the resource base will affect stream structure and function. Since leaf exclusion, woody debris in the stream has become a primary source of organic matter. Specifically, our goal was to obtain "enzymatic signatures" in time and space for wood biofilms in a leaf-excluded and reference stream by monitoring the activity of a suite of 6 extracellular enzymes involved in decomposition of wood over 3 mo. In addition to enzyme activity, fungal biomass and wood breakdown rates were used to characterize the effect of leaf litter exclusion on wood biofilm activity.

## Methods

### Study site

The study was conducted in the 1st-order streams draining Catchments 53 and 55 at Coweeta Hydrologic Laboratory, Macon County, North Carolina, USA. Coweeta is a 2270-ha experimental forest of the US Forest Service located in Nantahala National Forest in the eastern part of the southern Appalachian Mountains. The forest canopy is dominated by yellow poplar (*Liriodendron tulipifera*), white oak (*Quercus*

TABLE 1. Physical and chemical characteristics of the study streams. Values are annual means since 1985 (J. B. Wallace, University of Georgia, unpublished data).

Variable	Reference	Leaf exclusion
Temperature	12.3	12.2
Elevation (m asl) at flume	820	810
Watershed area (ha)	5.2	7.5
Average discharge (L/s)	1.06	1.72
Maximum discharge (L/s)	30.3	46.9
pH	6.8	6.7
NO <sub>3</sub> -N (µg/L)	8	10
NH <sub>4</sub> -N (µg/L)	2	3
TKN (µg/L)	30	28
SRP (µg/L)	3	1
Cl (µg/L)	0.538	0.656
K (µg/L)	0.441	0.397
Na (µg/L)	1.060	0.777
Ca (µg/L)	0.599	0.468
Mg (µg/L)	0.423	0.371
SO <sub>4</sub> (mg/L)	0.357	0.399
SiO <sub>2</sub> (mg/L)	8.015	6.812
HCO <sub>3</sub> (mg/L as CaCO <sub>3</sub> )	4.79	3.92

*alba*), red oak (*Quercus rubra*), and dogwood (*Cornus florida*). There is also a dense understory of rhododendron (*Rhododendron maxima*), which results in year-round shading and low rates of primary productivity (Wallace et al. 1997a).

The 2 streams are low-nutrient, soft-water streams having similar water chemistry, a pH between 6.7 and 6.8, similar thermal regimes, and a similar southern aspect (Table 1). Both 1st-order streams are groundwater-fed and are therefore cool in summer and relatively warm in winter. Stream-bed substrates are similar, consisting of mixed cobble-pebble with sand-gravel, and some bedrock outcrops (J. B. Wallace, University of Georgia, unpublished data).

Leaf-litter inputs have been excluded along the length of the stream (180 m) draining Catchment 55 (C55) since August 1993 by placing a 1.5-cm mesh canopy over the stream (Wallace et al. 1997b). The canopy was positioned beneath the rhododendron understory so as to maintain normal shade and yet exclude rhododendron leaves (normally 27% of total leaf litter input; J. B. Wallace, unpublished data). Small woody debris (<5 cm) and leaf litter made up most of the standing crop of benthic organic matter before leaf exclusion in C55 and in the reference

stream, C53 (60–65% and 20–24%, respectively, J. B. Wallace, unpublished data). The surface area of small woody debris represented a considerable proportion of colonizable substrate in C53 and was the only large substrate left for microbial colonization in C55 after the leaf exclusion.

#### Sample arrays

Untreated wood veneer strips (2.5 cm × 15 cm × 1 mm) of yellow poplar were used as substrates for microbial colonization in the 2 streams. Veneer strips (hereafter referred to as sticks) were attached with cable-ties to plastic mesh holders (6 sticks per holder). On 8 September 1995, mesh holders were staked to the streambed such that the long axes of the sticks were oriented parallel to stream flow. Seventeen sticks were collected from each stream after 28, 58, and 86 d and returned to the laboratory in containers of cold stream water.

#### Enzyme assays

Twelve sticks were randomly divided into 4 equal samples of 3 sticks each for each collection date in each stream. Samples were suspended in 100 mL acetate buffer (pH = 5) and homogenized with a Brinkmann polytron. The suspensions (4 samples per stream) were assayed for activity of 6 extracellular enzymes. The enzymes assayed (and their respective substrates in parentheses) were as follows: cellobiohydrolase (p-nitrophenyl (pNP)-cellobioside); B-1,4-glucosidase (pNP-B-D-glucopyranoside); B-xylosidase (pNP-B-xylopyranoside); phosphatase (pNP-phosphate); phenol oxidase (L-3,4-dihydroxyphenylalanine(L-DOPA)); and peroxidase (L-DOPA with 0.2 mL of 0.3% hydrogen peroxide solution). Final peroxidase activity was calculated as the difference between phenol oxidase activity and total peroxidase activity. All assays were incubated for 2–5 h at 20°C in pH 5 acetate buffer with 4 analytical replicates and 2 controls per sample (Sinsabaugh et al. 1994b). Five 2-mL subsamples from each suspension were placed in pre-weighed aluminum pans, dried (55°C, 24 h), weighed, ashed (550°C, 1 h), and reweighed to determine g ash free dry mass (AFDM) and % organic matter for each sample. Enzyme activity was then expressed as activity per g AFDM.

#### Relative nutrient availability

The ratios of the activities of cellulolytic enzymes compared to the activity of nutrient-acquiring enzymes were used to estimate the relative availability of nutrients to the biofilm colonizing wood in each stream (Sinsabaugh et al. 1993). These ratios were calculated for each stream as:

##### Relative P availability

$$= \frac{E_c}{E_p} = \frac{BG/P + CBH/P + X/P}{3}$$

##### Relative N availability

$$= \frac{E_c}{E_n} = \frac{BG/TO + CBH/TO + X/TO}{3}$$

where  $E_c$  = enzyme activity of the cellulolytic enzymes, B-glucosidase (BG), cellobiohydrolase (CBH), and xylosidase (X);  $E_p$  = enzyme activity of the phosphorus acquiring enzymes, phosphatase (P); and  $E_n$  = total oxidative enzyme activity represented by phenol oxidase + peroxidase activity combined (TO). Activity data for each enzyme were normalized by dividing each value by the maximum activity for that enzyme (Sinsabaugh and Moorhead 1994).

#### Fungal biomass

Fungal biomass was estimated by ergosterol content of biofilms colonizing wood using methods modified from Newell et al. (1988). Five sticks from each stream were collected on each date and subsamples of known surface area (2.5 cm × 3 cm × 1 mm) from each stick were placed in 15-mL Falcon tubes with 5 mL of methanol and refluxed in a dry block heater for 2 h at 65°C. Tubes were cooled, saponified by adding 1 mL of 4% KOH in methanol, and refluxed for another 0.5 h at 65°C. Samples were cooled, centrifuged, and the supernatants were decanted into clean 15-mL Falcon tubes. The pellets were resuspended in 2 mL of HPLC-grade methanol, centrifuged again, and the methanol used to rinse the pellets was added to the tubes containing the extracts along with 1 mL water. Each sample was extracted twice with 2 mL pentane. Pentane extracts were combined and evaporated in a fume hood. The residue was re-dissolved with 1 mL of HPLC-grade methanol and filtered through a 0.45- $\mu$ m sy-

ringe filter into clean 5-mL polypropylene tubes. Ergosterol was quantified using a reverse-phase HPLC system configured as follows: solvent = methanol; flow rate = 2 mL/min; column = Nova-Pak ODS C18 3.9 mm  $\times$  75 mm; absorbance detector = 282 nm with a range of 0.500; quantification = Waters integrator with attenuation at 256; ergosterol retention time = 1.6 min; replication = 5 per sample; standards = 5, 10, 25, and 50  $\mu$ g/mL solution of ergosterol and methanol. The general conversion factor of 6 mg ergosterol/g fungal biomass was used (Newell et al. 1988).

#### Data analyses

All data were log transformed because of non-normal distributions. Statistical analyses were carried out using log-transformed data. Two-way analysis of variance (ANOVA by date and stream) was used to compare enzyme activity and ergosterol values between streams on different sampling dates. Least squares means (LSM) were used to differentiate between means when significance was found in the ANOVA ( $p < 0.05$ ).

Breakdown rates of wood were calculated by regressing the natural log of mean % AFDM remaining of wood veneers used for enzyme analysis on exposure time in d. The negative slope of the regression line is equal to the breakdown rate ( $k$ ) of the substrate (e.g., Petersen and Cummins 1974). Analysis of covariance (ANCOVA) was used to determine whether  $k$ s differed between streams.

#### Results

Overall, wood substrates in the leaf exclusion stream had significantly higher enzyme activity than in the reference stream for 5 of 6 enzymes (Fig. 1, ANOVA, LSM,  $p = 0.0001$  for all enzymes except peroxidase). Peroxidase activity showed the reverse trend and the reference stream had significantly higher activity than the exclusion stream (ANOVA, LSM,  $p = 0.0001$ ). Enzyme activity on wood from the leaf exclusion stream steadily increased through time for all enzymes except peroxidase. Enzyme activity was always significantly higher on day 86 than on day 28 for  $\beta$ -glucosidase, cellobiohydrolase, xylosidase, phosphatase, and phenol oxidase (ANOVA, LSM,  $p < 0.05$  for all). In contrast, ac-

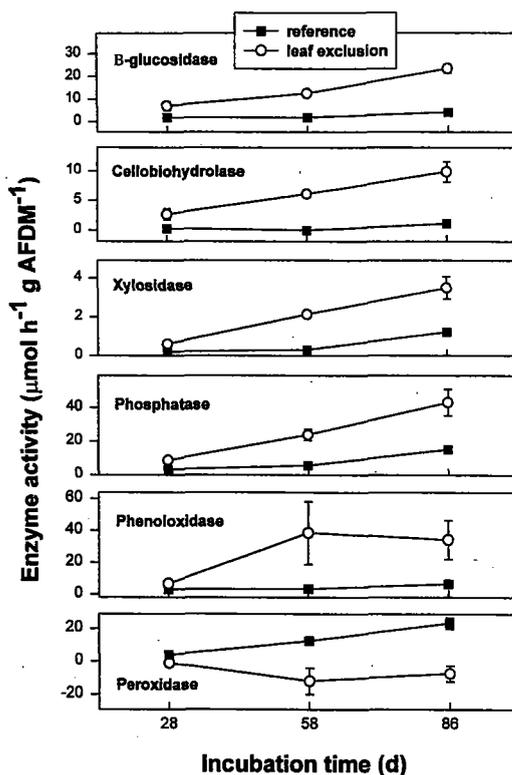


FIG. 1. Extracellular enzyme activities for 6 enzymes over time in leaf exclusion and reference streams. Each point is the mean  $\pm$  1 SE of 4 replicates. Where error bars are not evident, the SE is smaller than the symbol. AFDM = ash free dry mass.

tivity for all enzymes in the reference stream, except peroxidase, remained low and fairly constant throughout the 3-mo incubation period. Peroxidase activity in the reference stream increased through time, and activities on days 58 and 86 were significantly higher than on day 28 (ANOVA, LSM,  $p = 0.0001$ ).

Sticks lost weight in both streams over time, but weight loss in the litter exclusion stream was faster than in the reference stream (ANCOVA,  $p = 0.0115$ , Fig. 2). Calculated decomposition rates,  $k$ , were 0.0085/d in the exclusion stream and 0.0047/d for sticks in the reference stream. On days 28 and 58, total % organic matter of sticks (wood veneer + biofilm) was not significantly different between the 2 streams; organic content ranged between 65 and 80%. However by day 86, organic matter content for sticks in the leaf exclusion stream remained high, whereas % organic matter for sticks in the reference

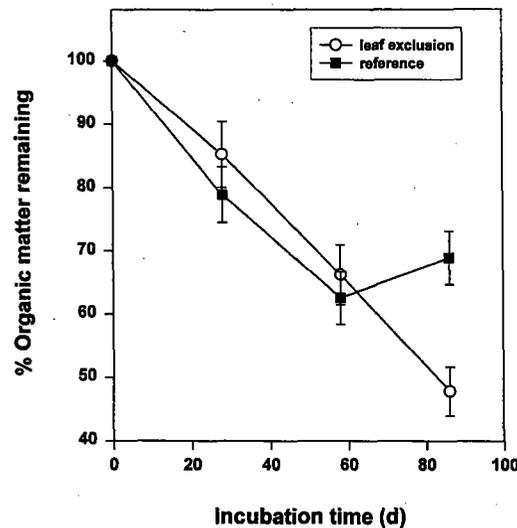


FIG. 2. Loss of wood mass expressed as % organic matter remaining of poplar veneers in leaf exclusion and reference streams. Each point is the mean  $\pm$  1 SE of 4 replicates.

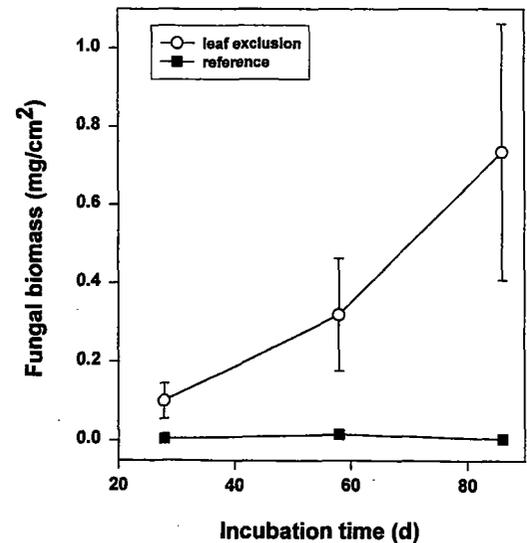


FIG. 3. Fungal biomass on wood through time in leaf exclusion and reference streams. Each point is the mean  $\pm$  1 SE of 5 replicates. Where error bars are not evident, the SE is smaller than the symbol.

stream decreased to 33%. This reduction in organic matter content coincided with a visible accumulation of sand-like sediment on sticks in the reference stream.

Fungal biomass ( $\text{mg}/\text{cm}^2$ ) on sticks in the leaf exclusion stream was significantly higher than in the reference stream on all 3 collection dates (ANOVA, LSM,  $p = 0.0001$ ) and increased over time (Fig. 3). By relating extracellular enzyme activity to trends in fungal biomass in the exclusion stream we saw that generally there was a positive relationship between fungal biomass and enzyme activity for each of the extracellular enzymes in the leaf exclusion stream, excluding peroxidase (Fig. 4), yet the relationship was not linear.

Phosphatase and total oxidative activity (phenol oxidase + peroxidase activity) expressed per unit fungal biomass can be used to estimate relative differences in energy expenditure of the microbial biofilms on nutrient acquisition (Sinsabaugh and Moorhead 1994). Phosphatase activity is representative of phosphorus acquisition, and total oxidative activity is an indicator of nitrogen acquisition (Sinsabaugh et al. 1993). Average phosphatase activity was >20 times greater in the reference stream than in the leaf exclusion stream ( $45.02$  vs.  $2.14$  expressed as activity  $\cdot \text{h}^{-1} \cdot \text{mg}$  fungal biomass $^{-1}$ ), whereas av-

erage total oxidative activity was >40 times greater in the reference stream than in the leaf exclusion stream ( $92.71$  vs.  $2.23$  expressed as average activity  $\cdot \text{h}^{-1} \cdot \text{mg}$  fungal biomass $^{-1}$ ).

Relative phosphorus availability (Ec/Ep) was 2 times higher in the leaf exclusion stream than in the reference stream (1 vs. 0.54). Relative nitrogen availability (Ec/En) was 5 times higher in the leaf exclusion stream than in the reference stream (1 vs. 0.22).

## Discussion

### *Effect of leaf exclusion on extracellular enzyme activities*

The activity of all 6 enzymes indicated that the exclusion of leaf litter from the experimental stream significantly altered the function and microbial activity of the biofilm colonizing wood. Extracellular enzyme activity over the 3-mo incubation period was significantly higher in the leaf exclusion stream for all enzymes measured except peroxidase (Fig. 1). Enzyme activities in the reference stream were always low but activities in the leaf exclusion stream were within the range of those observed in other aquatic systems (Sinsabaugh et al. 1992, 1994b, Scholz and Boon 1993, Jackson et al. 1995). B-glucosidase

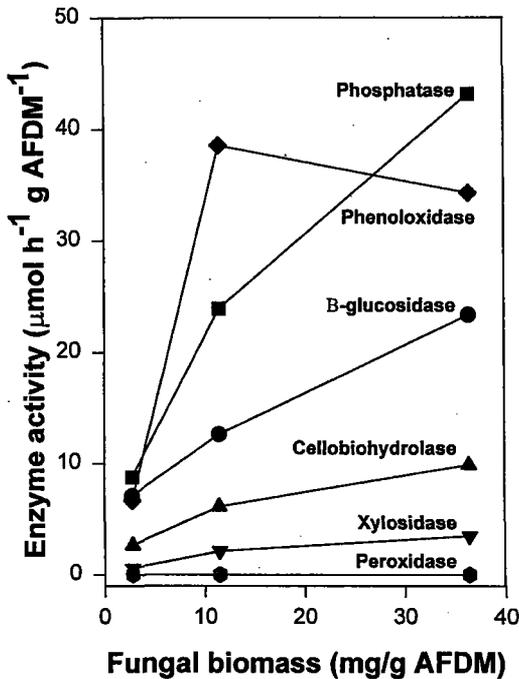


FIG. 4. Mean fungal biomass vs. mean enzyme activity for 6 extracellular enzymes in the leaf exclusion stream. Each point is a mean of 4 measurements of enzyme activity and 5 measurements of fungal biomass. AFDM = ash free dry mass.

and phenol oxidase activity in the leaf exclusion stream was at the high end of the ranges reported in other studies, indicating active decomposition of ligno-cellulose components of the sticks.

Overall, our calculated breakdown rates for wood were faster than those previously published. We attribute the quick breakdown rates in our study streams to the high surface area: volume ratios of our wood veneer strips, which enhances oxygen diffusion rates (Aumen et al. 1983). Breakdown rates were almost 2 times higher in the exclusion stream (0.0085/d) than the reference stream (0.0047/d), but both streams had breakdown rates higher than those reported by Melillo et al. (1983) for wood chips (0.0007–0.0033/d), Golladay and Webster (1988) for small woody debris in Coweeta streams (0.0004–0.0008/d), and Golladay and Sinsabaugh (1991) for birch ice cream sticks (0.0016–0.0019/d). Percent organic matter of sticks (wood substrate + biofilm) was different between the 2 streams only after 86 d when in-

organic sediment accumulated on sticks in the reference stream. Although the accumulation of inorganic sediments may have reduced biofilm activity and hence extracellular enzyme activity in the reference stream on day 86, it apparently was not a factor contributing to low activity levels in the reference stream on the 2 earlier collection dates.

In addition to faster breakdown rates, fungal biomass was always significantly higher in the exclusion stream. Fungal biomass on sticks was always very low in the reference stream, whereas fungal biomass on sticks in the leaf exclusion stream was within the range of values found in other studies (Golladay and Sinsabaugh 1991, Newell and Fell 1992, Tank 1996) and almost an order of magnitude higher than fungal biomass on leaves (Golladay and Sinsabaugh 1991). By day 86 fungal biomass was at the high end of published estimates for wood in 1st- to 4th-order streams (Golladay and Sinsabaugh 1991).

#### *Effect of nutrients on enzyme activities*

Because sticks placed in both streams for colonization were identical, differences in microbial biofilm activity must be regulated by factors other than substrate quality. We believe nutrient concentrations in streamwater were responsible for differences in biofilm enzyme activities between the exclusion and reference streams. Nitrogen has long been considered a potentially limiting nutrient in the decomposition of litter (e.g., Harmon et al. 1986, Webster and Benfield 1986, Fog 1988). Both nitrogen and phosphorus content are very low in wood and therefore nitrogen and phosphorus requirements for decomposition of woody substrates must come from the water column (Sinsabaugh et al. 1993). Fog (1988), reviewing >60 nitrogen amendment studies, found that supplying inorganic or simple organic exogenous nitrogen speeds the decomposition of labile organic matter (e.g., cellulose) while slowing degradation of refractory organic matter components (e.g., lignin) by repressing phenol oxidase activity. In this study, although phenol oxidase activity was higher in the exclusion stream than in the reference stream when expressed as activity per g organic matter (Fig. 1), phenol oxidase activity was much lower in the exclusion stream when activity was expressed per unit fungal biomass (2.23 vs. 92.71 average activity · h<sup>-1</sup> · mg fungal bio-

mass<sup>-1</sup>). These results support the contention that nitrogen was more limiting in the reference than exclusion stream.

Phosphorus has also been linked to decomposition rates of leaves in streams (Elwood et al. 1981). In addition to the nitrogen limitation described above, results from this study also indicated that extracellular activity involved in phosphorus acquisition was higher per unit fungal biomass in the reference stream than in the exclusion stream (45.02 vs. 2.14 average activity · h<sup>-1</sup> · mg fungal biomass<sup>-1</sup>, respectively). Previous studies have shown there to be an inverse relationship between phosphatase activity and environmental availability of phosphorus (Mulholland and Rosemond 1992, Sinsabaugh et al. 1993), and phosphatase activity has been used as an indicator of phosphorus limitation in aquatic systems (e.g., Wetzel 1981). Although Sinsabaugh et al. (1993) did not find phosphorus to limit wood decomposition in a lotic environment, the Coweeta streams used in this study have very low background levels of nitrogen and phosphorus (Table 1), and wood biofilms are co-limited by these nutrients (Tank 1996).

Comparative enzyme activity assays used in conjunction with nutrient concentration measurements may be an accurate indicator of nutrient limitation in streams (Sinsabaugh et al. 1993). A model was developed by Sinsabaugh and Moorhead (1996) for the Microbial Allocation of Resources among Community Indicator Enzymes (MARCIE), which connects the availability of nitrogen and phosphorus to litter decomposition rates based on the allocation of energetic resources to extracellular enzyme production by microbes (Sinsabaugh et al. 1993, 1994b, Jackson et al. 1995, Sinsabaugh and Moorhead 1996). The model predicted that mass loss rates were directly related to cellulolytic activity. Hence for this study, the model predicted that mass loss rates for wood would be higher in the exclusion stream than in the reference stream. But what was the underlying cause for higher cellulolytic activity in the exclusion stream? Microbial communities are predicted to behave as a cohesive unit and maximize production by optimizing allocation of resources among macronutrients, thereby creating trade-offs among carbon, nitrogen, and phosphorus acquisition (Sinsabaugh et al. 1993). Enzymes involved in carbon acquisition have been linked to litter quality, whereas activity of enzymes in-

involved in acquisition of nitrogen and phosphorus were related to environmental availability of those nutrients. In this study, when cellulolytic activity (Ec) was compared to nutrient acquisition (En and Ep), relative phosphorus availability was twice as high in the leaf exclusion stream and there was 5 times more nitrogen available in the leaf exclusion stream than the reference stream.

In another study conducted in the leaf exclusion and reference streams, Tank and Webster (1998) examined the effect of nitrogen and phosphorus addition on microbial respiration on wood. Nutrient-releasing substrates containing only nitrogen or phosphorus did not result in higher respiration rates, but when both N + P were added, microbial respiration increased in the reference stream to levels that were not significantly different from the exclusion stream, thereby indicating a co-limitation of nitrogen and phosphorus. In addition, long-term data from nutrient releases conducted in the study streams indicated that uptake lengths of soluble reactive phosphorus (SRP) and ammonia (NH<sub>4</sub>) were significantly shorter in the reference stream, indicating more nutrient limitation in the stream containing leaves (J. R. Webster and others, unpublished data).

Enzyme activities are a more direct estimation of the "microbial perception" of the environment than are dissolved nutrients in the water column (Sinsabaugh and Moorhead 1994). Nutrient concentrations in the exclusion and reference streams are not significantly different and have not increased measurably as a result of leaf exclusion (exclusion stream: NH<sub>4</sub>-N = 1 ug/L, NO<sub>3</sub>-N = 11 ug/L, and PO<sub>4</sub>-P = 2 ug/L; reference stream: NH<sub>4</sub>-N = 2 ug/L, NO<sub>3</sub>-N = 3 ug/L, and PO<sub>4</sub>-P = 3 ug/L; Tank 1996, J. B. Wallace, unpublished data). Yet nutrient-acquiring enzyme activity (per unit fungal biomass) was significantly higher in the reference stream. Perhaps there was no correlation between the microbial response to nutrient limitation, as represented by enzyme allocations, and water chemistry data because chemical data cannot reflect recycling dynamics at the microbe-substrate interface (Fog 1988). In addition, when leaves are absent, groundwater inputs may supply nutrients to a larger area at the substrate-water interface (Tank and Webster 1998). Water column chemistry only represents the net effect of biotic and abiotic processes in streams. Pre-

vious research has shown that the only significant correlation between nutrients and enzyme activity is between the microbial enzyme activity and the nutrient content within the organic substrate being colonized by the microbe (Sinsabaugh et al. 1993).

Results from this study indicated that the presence of leaves in forested headwater streams may mediate microbial activity on other organic substrates (e.g., wood) through the immobilization of a limited supply of nutrients from streamwater, and thereby slow the overall rate of decomposition of all types of coarse particulate organic matter. It is unknown whether proportions of different leaf species in a stream may alter rates of nutrient immobilization and therefore wood decomposition. As an alternative, nutrient limitation may strictly depend on the amount of colonizable leaf surface area as compared to wood, regardless of species. Despite these unanswered questions, results from this study indicated that nutrients may be considered a controlling factor in the cycling of carbon through stream ecosystems.

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