9-1-2007

Algal Regulation of Extracellular Enzyme Activity in Stream Microbial Communities Associated with Inert Substrata and Detritus

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J. N. Am. Benthol. Soc., 2007, 26(3):439–449

Algal regulation of extracellular enzyme activity in stream microbial communities associated with inert substrata and detritus

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Abstract. We tested the hypothesis that algae influence the activities of extracellular enzymes involved in mineralization processes within microbial assemblages in streams. We tested the prediction that the factors that influence algal biomass and photosynthesis (i.e., diel fluctuations in photosynthetically active radiation [PAR], long-term variations in light regime, and community development stage) would have a corresponding effect on extracellular enzyme activities. We also tested the prediction that algae would influence enzyme activities on inorganic substrata and in detrital communities where they ultimately would influence plant litter decomposition rates. We allowed microbial communities to develop on inert substrata (glass-fiber filters) or on leaf litter in artificial streamside channels. For each community type, we examined the effects of long-term light manipulations, community development stage, and diel periodicity on the activities of β-glucosidase, alkaline phosphatase, leucine-aminopeptidase, and phenol oxidase. In addition, we measured the decomposition rates of the leaf litter substrata in the low- and high-light treatments. Our results support the prediction that factors that influence algal photosynthesis and biomass in the short (diel fluctuations in PAR) and long (shading, community development stage) term ultimately influence enzyme activities in microbial communities associated with both inorganic substrata and detritus. Furthermore, decomposition rates of organic detritus probably are enhanced by algal colonization and activity. Algal photosynthesis might enhance redox and pH conditions within microbial communities, and in turn, might increase the activities of oxidative and hydrolytic enzymes. As a consequence, photoautotrophic activities might stimulate heterotrophic pathways in stream ecosystems by creating conditions favorable for decomposition of both dissolved and particulate organic detritus.

Key words: streams, extracellular enzymes, light, photosynthesis, stream microbial communities, algae, fungi, bacteria, detritus, decomposition rates.

Microbial biofilms play a critical role in the C and nutrient dynamics of stream ecosystems. These communities develop on all inorganic and organic surfaces in streams and usually consist of a mixture of autotrophic and heterotrophic microorganisms such as algae, bacteria, fungi, and protozoa embedded in a polysaccharide matrix (reviewed by Lock 1993). Furthermore, in the case of fungal organisms colonizing detritus, heterotrophic assemblages also grow endogenously (i.e., hyphae) within organic substrata.

In stream ecosystems exposed to sunlight, periphytic algae often account for the bulk of the ecosystem's net primary productivity (Minshall 1978, Wetzel 2001). These photoautotrophs and associated heterotrophic microbial communities are important in the uptake and mineralization of C, N, and P in streams (Allan 1995, Mulholland 1996). In addition, these communities serve as an important food resource for consumers and form a link in the flow of C and nutrients from

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microorganisms to higher trophic levels of stream food webs (Allan 1995, Lamberti 1996).

Close spatial association and isolation from the overlying water column (Freeman and Lock 1995) probably facilitate a complex array of interactions within stream microbial assemblages (Wetzel 1993). Such interactions include bacterial use of labile organic compounds released during algal photosynthesis (e.g., Haack and McFeters 1982, Kaplan and Bott 1983), algal use of N and P released during mineralization activities by heterotrophic assemblages (Wetzel 1993) and CO_2 released during heterotrophic respiration (reviewed by Cole 1982), and heterotroph use of O_2 released during algal photosynthesis (Kuhl et al. 1996).

Mineralization of organic N and P and uptake of dissolved organic mater (DOM) within stream biofilms is facilitated by the extracellular enzymes produced by bacteria, fungi, and, in some cases, algae (Sinsabaugh et al. 1991b). A combination of hydrolytic and oxidative enzymes cleaves recalcitrant organic matter, producing molecules that can be transported across cell membranes. Enzymatic degradation of large organic molecules is a rate-limiting step, and the degradative activity of microbially derived extracellular enzymes can be considered a direct link between microbial activities and the decomposition and mineralization of organic matter (e.g., Sinsabaugh et al. 1991a, 1993, 1994).

Several environmental factors, including temperature, pH, and substrate concentration, influence the activity of extracellular enzymes (e.g., Shackle et al. 2000). For example, oxidative enzymes require the presence of O_2 to carry out substrate oxidation. Therefore, chemical conditions within the polysaccharide matrix of stream biofilms are likely to influence the activity of extracellular enzymes. The metabolic activities of microorganisms are likely to alter the internal chemical environment of biofilms. Therefore, it is probable that these microbial assemblages will directly or indirectly influence extracellular enzyme activities.

Several studies of biofilms have reported that algal photosynthesis can influence extracellular enzyme activities (Espeland et al. 2001, Francoeur and Wetzel 2003, Romani et al. 2004, Francoeur et al. 2006). Romani et al. (2004) observed that stream biofilms grown in the presence of light had higher enzyme activities than corresponding biofilms grown in the dark. Espeland et al. (2001) demonstrated that α-glucosidase, β-glucosidase, and β-xylosidase activities were stimulated by periphytic algal photosynthesis. Francoeur and Wetzel (2003) observed a diel periodicity in extracellular leucine-aminopeptidase (LAMP) activities within periphytic communities grown on artificial, inert substrata in a small subtropical wetland system. LAMP activity increased with experimental elevation of pH, leading the authors to speculate that that diel shifts in biofilm pH caused by algal photosynthesis was the probable mechanism controlling these shifts in enzyme activity (Francoeur and Wetzel 2003).

If algal photosynthesis stimulates enzyme activity in biofilms, then it is possible that photoautotrophic activities play a role in stimulating heterotrophic decomposition of DOM and particulate organic matter (POM) from both autochthonous and allochthonous sources. Previous studies have provided some evidence for stimulation of POM decomposition and DOM uptake as a result of algal photosynthesis (Neely 1994, Neely and Wetzel 1997, Romani et al. 2004). Romani et al. (2004) reported that biofilms grown in full sun were net consumers of dissolved organic C (DOC) and had higher rates of heterotrophic metabolism than corresponding biofilms grown in the dark. Earlier research by Neely (1994) demonstrated that decomposition of *Typha latifolia* in a wetland was faster when the litter was colonized by algae. If algal photosynthesis stimulates enzyme activity in biofilms, then algal biomass and photosynthetically active radiation (PAR) might ultimately play a role in regulating the decay of DOM and POM and the mineralization of organic N and P in streams.

Our study tested the hypothesis that stream algae influence the activities of representative hydrolytic and oxidative extracellular enzymes. We examined the effects of algae on the activities of β-glucosidase (GLU), which hydrolyzes β-linked polysaccharides; alkaline phosphatase (APA), which hydrolyzes organically-bound organophosphoric esters; LAMP, which hydrolyzes protein; and phenol oxidase (POA), which oxidizes polyphenolic compounds (Chróst 1991). We tested the prediction that these enzymes would respond to diel fluctuations in PAR, long-term shading, and community development stage, which would interact to influence algal biomass and photosynthetic rates. We also tested the prediction that the influence of algae on extracellular enzyme activities would not be limited only to microbial communities colonizing inorganic substrata, but also would be observed in detrital communities where aquatic fungi constituted an important component of the heterotrophic microbial community. Furthermore, we tested the prediction that increased algal biomass on detritus would lead to faster leaf-litter decomposition rates because of indirect stimulation of extracellular enzymes by algal photosynthesis.

Methods

We conducted our study between 30 June and 24 July 2004 at the University of Michigan Biological

Station's Stream Research Facility in Pellston, Michigan. This facility draws water from a 3rd-order section of the East Branch of the Maple River. To compare microbial communities associated with inorganic substrata to those associated with decomposing detritus, we grew natural assemblages on either inert substrata (glass-fiber filters [GF/Fs]) or detritus (leaf litter) in artificial stream channels (3-m-long vinyl rain gutters). Each stream channel received a continuous flow of stream water at a current velocity of 30 cm/s. We minimized colonization of stream channels by macroinvertebrates by passing the stream water through 2 nylon-mesh stockings before it entered the channel.

We placed 24 GF/Fs (47-mm diameter) or 24 quaking aspen (*Populus tremuloides* Michaux) leaves (harvested immediately after leaf fall) into each of 10 replicate stream channels. Quaking aspen is the most common tree in Northern Michigan (Schmidt et al. 1993) and dominates the riparian zones of most streams in the region (STR, personal observation). We anchored all substrata to clay tiles using silicon adhesive. We covered all stream channels with ultraviolet (UV)-opaque plastic to minimize potential influences of UV radiation and heavy precipitation on the experimental treatments.

To examine the effects of long-term differences in light regime on enzyme activities, we darkened 10 stream channels (i.e., 5 replicates of each substratum type) with 2 layers of neutral-density shade cloth to produce a low-light treatment that was ~1% ambient PAR (maximum $\approx$20 μmol m^{-2} s^{-1}, determined with Li-COR® Model LI-189 light meter (LI-COR Biosciences, Lincoln, Nebraska). We left the 10 remaining stream channels uncovered to produce a high-light treatment that was 75% ambient PAR (maximum PAR $\approx$1500 μmol m^{-2} s^{-1}). We assigned all treatments (substratum type and light level) randomly across the 20 stream channels.

We recorded stream temperature with an Onset Stow-Away® XTI temperature recorder (Onset Computer Corporation, Pocasset, Massachusetts) set to take a reading once every hour. PAR was monitored at the University of Michigan Ameriflux tower located 3 km east of the stream research facility. These data were collected with a Li-Cor LI-190SZ quantum sensor and logged every 10 min during the entire experiment. We collected water samples each week during the study period and analyzed pH, soluble reactive P, NH_4^+-N, and NO_3^--N using standard methods (APHA 1998).

To test the prediction that algal biomass accumulation on substrata through time was an important determinant of extracellular enzyme activity, we sampled substrata for enzyme activities and microbial biomass during an early stage of community development (8 d) and during a more advanced stage of community development (23 d). We chose to sample on days 8 and 23 because periphyton communities at this time of year are usually in an early stage of community development after ~8 d (thin biofilm) and begin to reach peak biomass after ~20 d of incubation in these artificial streams (STR, unpublished data). We examined diel fluctuations in extracellular enzyme activity within microbial communities during each sample date by collecting 1 GF/F or leaf from each replicate stream channel (i.e., light or dark) at intervals ranging from 3 to 4 h for 2 consecutive 24-h periods. We subsampled leaves and GF/Fs with a 7-mm-diameter cork borer for analysis of algal community composition, algal biomass, fungal biomass, bacterial biomass, and enzyme activities (GLU, APA, LAMP, POA). We estimated algal biomass as chlorophyll *a*. We stored replicate subsections of collected substrata frozen (−20°C, in darkness) until extraction. We extracted chlorophyll *a* from frozen samples in 90% acetone (APHA 1998), quantified it spectrophotometrically (665 nm), and corrected values for phaeophytin by acidification. We determined ash-free dry mass (AFDM) according to standard methods (APHA 1998). We measured algal relative abundance by direct microscopic examination (400×) of representative light-grown leaf and GF/F substrata for each sample period.

We estimated fungal biomass from concentrations of ergosterol (Gessner and Newell 2002). We stored replicate subsections of collected substrata frozen (−20°C, in darkness) until analysis. We lyophilized and weighed frozen samples, and extracted ergosterol in alcoholic KOH (0.8% KOH in high-pressure liquid chromatography [HPLC]-grade methanol, total extraction volume = 10 mL) for 30 min at 80°C in tightly capped digestion tubes. We partitioned the resultant crude extract into n-pentane and evaporated it to dryness in 15-mL glass conical vials under a stream of N gas (Kuehn and Suberkropp 1998). We redissolved ergosterol in dried samples by ultrasonication in 1 mL of methanol. We quantified ergosterol by HPLC with a LichroSpher 100 RP-18 column (0.46 × 25 cm, Merck KGaA; Merck, Darmstadt, Germany) maintained in a Shimadzu column oven (CTO-10AS; Shimadzu Scientific Instruments, Columbia, Maryland) at 40°C and connected to a Shimadzu autosampler (SIL-10AD) and Shimadzu liquid chromatograph system (Pumps LC-10AT, Controller SCL-10A, mobile phase HPLC-grade methanol, flow rate = 1.5 mL/min). We detected ergosterol at 282 nm using a Shimadzu (SPD-10A) UV/VIS detector (retention time ≈ 8 min), and identified and quantified it based on comparison with ergosterol standards (Fluka Chemical Company, Milwaukee, Wisconsin). Ergosterol concentrations were converted to fungal biomass assuming a conversion

factor of 5.5 μg ergosterol/mg fungal dry mass (Gessner and Chauvet 1993).

We measured bacterial biomass associated with substrata by epifluorescence microscopy after staining with 4′,6′-diamidino-2-pheylindole. We preserved replicate subsections of collected substrata in 3.7% (v/v) filtered (0.2 μm) buffered (sodium pyrophosphate, 0.1% w/v) formalin. We removed bacterial cells attached to substrata by ultrasonication (Fisher Model 100 dismembrator, 50 W; Thermo Fisher Scientific, Waltham, Massachusetts). After sonication, we vacuum filtered (≤20 kPa) sample aliquots through 0.2-μm pore-size Anodisc filters supported by 0.8-μm membrane filters (Fisher Scientific) and stained the filters. We enumerated bacterial cells in ≥15 fields of view (1000× magnification) with a Nikon Optiphot-2 epifluorescence microscope. We estimated bacterial C by multiplying densities by 2.0×10^{-14} g C/cell (Ducklow 2000, Findlay et al. 2002).

We determined the activities of GLU, APA, and LAMP by incubating substratum subsamples in appropriate fluorescent 4-methylumbelliferyl or amidomethylcoumarin substrate in precombusted (500°C) glass vials (600-μM final concentration) for 30 min under the appropriate light treatment (Espeland et al. 2001, Francoeur and Wetzel 2003). After incubation, we transferred subsamples of the supernatant to clean vials and placed the vials in a boiling water bath for 5 min to inactivate enzymes. After boiling, we froze the subsamples (−10°C) for storage until analysis. We thawed frozen samples and added a 100-μL aliquot from each boiled sample to 100 μL of pH 10 carbonate/bicarbonate buffer (pHydrion) in individual wells of a black 96-well plate. We measured fluorescence with a Fluoroskan Ascent® plate reader (excitation wavelength: 355 nm, emissions wavelength: 460 nm, Thermo Fisher Scientific). We converted raw fluorescence values to concentrations by running simultaneous standard curves of methlyumbelliferone sodium salt and 7-amino-4-methylcoumarin. We measured POA activity using a procedure modified from Sinsabaugh et al. (1994). We incubated subsamples of collected substrates in L-3,4-dihydroxyphenylalanine (2.5-mM final concentration) for 1 h. We read absorbance of the supernatant at 460 nm on a Spectronic Genesys 2 (Thermo Fisher Scientific). We conducted preliminary experiments before each sampling period to ensure that enzyme activity assays were conducted under saturating conditions (data not shown).

Statistical analysis

We $\log_{10}(x)$-transformed all data to normalize variances. We analyzed each substratum type independently for treatment effects because microbial dynamics on inert substrata differ from those on leaf litter. We analyzed mean microbial biomass data (chlorophyll *a*, bacterial biomass, and fungal biomass) for each replicated stream channel on each sample date with 2-way analysis of variance (ANOVA) with light level (high vs low) and sample period (day 8 vs day 23) included as factors (SYSTAT, version 10; Systat Software, Point Richmond, California). We also did correlation analyses between mean area-specific enzyme activities (averaged from the entire diel sampling period) and microbial biomass (chlorophyll *a*, bacterial biomass, and fungal biomass) individually for each substratum type. We adjusted probabilities associated with Pearson correlation coefficients with Bonferroni corrections to account for experiment-wise error rates (SYSTAT, version 10). We calculated leaf decomposition rates using the mean AFDM of subsamples collected on days 8, 18, and 23 from each treatment. We $\log_e(x)$-transformed leaf masses (as mg AFDM/cm^2) and regressed values against time. Decomposition rates (k) for leaf substrata incubated in low- and high-light treatments were compared with analysis of covariance (SYSTAT, version 10).

We analyzed diel changes in enzyme activities for each substratum type by classifying the observations into day or night categories based on PAR. To assess the degree to which changes in light level might have influenced stream temperature and, consequently, affected enzyme activities, we also ran a correlation analysis between temperature and PAR during each diel sample period. We analyzed enzyme activity data separately for each substratum type using a 3-way ANOVA with sampling period (day 8 or day 23), day/night category, and light level (low vs high) included as factors (SYSTAT, version 10).

Results

Chemical and physical measurements

Mean (±1 SE) water temperature was 17.7 ± 0.1°C for the entire study period, 16.5 ± 0.2°C during the 1st diel sample period (day 8), and 17.4 ± 0.3°C during the 2nd diel sample period (day 23). Water temperatures ranged from a mean daily minimum temperature of 14.7 ± 0.1°C to a maximum of 18.7 ± 1.6°C on day 8 and from a mean daily minimum of 14.4 ± 0.7°C to a mean daily maximum of 20.8 ± 0.6°C on day 23. Maximum PAR was 1031 μmol m^{-2} s^{-1} on day 8 and 1378 μmol m^{-2} s^{-1} on day 23 (Fig. 1). Water temperature and PAR were not significantly correlated ($p > 0.05$).

Mean (±1 SE) soluble reactive P for the entire experiment was 2.4 ± 0.4 μg/L, mean NO_3^--N was 7.1 ± 1.2 μg/L, mean NH_4^+-N was 17.7 ± 1.2 μg/L, and

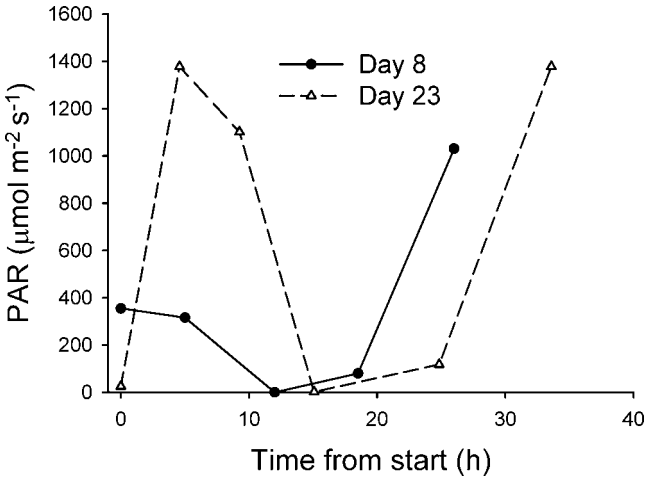

FIG. 1. Diel changes in photosynthetically active radiation (PAR) during the day 8 and day 23 sample periods.

mean pH was 7.6 ± 0.1. We did not measure DOC during our study, but Rier and Stevenson (2001) measured a concentration of 1.33 mg/L for this reach at the same time of year.

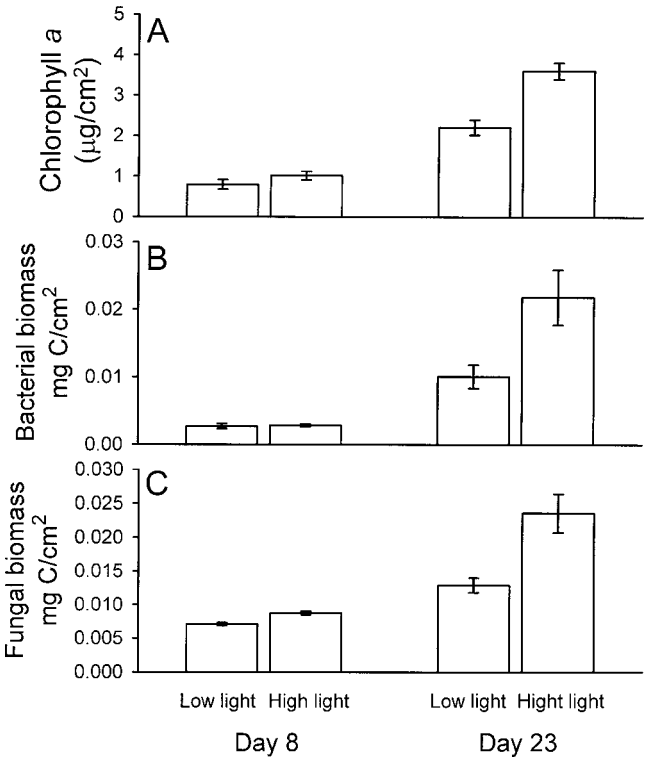

FIG. 2. Mean (±1 SE) algal biomass (as chlorophyll *a*) (A), bacterial biomass (B), and fungal biomass (C) in microbial communities grown on glass-fiber filters under low- and high-light levels on days 8 and 23.

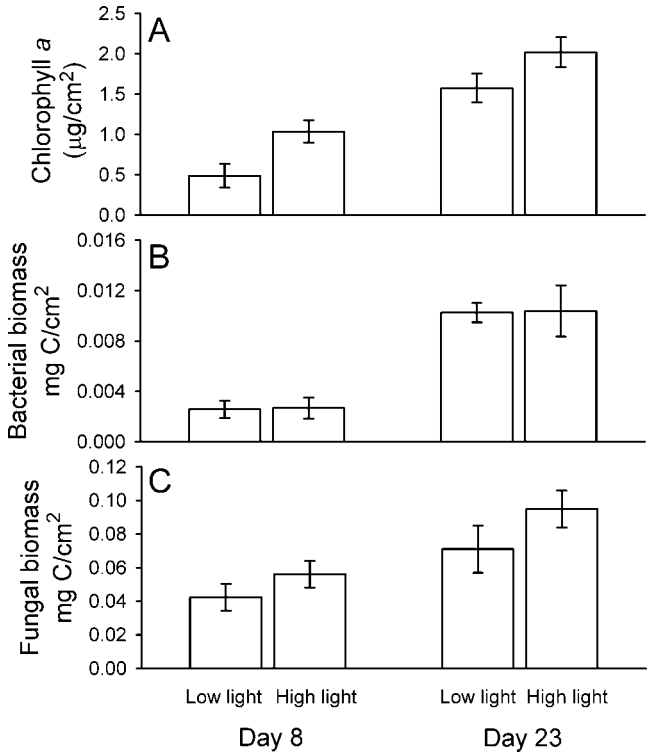

FIG. 3. Mean (±1 SE) algal biomass (as chlorophyll *a*) (A), bacterial biomass (B), and fungal biomass (C) in microbial communities grown on leaf litter under low- and high-light levels on days 8 and 23.

Microbial biomass

Diatoms dominated the algal communities on leaves and GF/Fs, and chlorophytes and cyanobacteria were present. *Cocconeis* was the most prevalent genus on leaves, and *Achnanthidium*, *Cymbella*, *Nitzschia*, *Fragellaria*, and *Gomphonema* were important components of the algal community on both substrata.

GF/Fs.—Algal biomass (chlorophyll *a*) was significantly influenced by sample period ($p = 0.002$) and light level ($p < 0.001$), but these variables did not produce a significant interaction ($p = 0.062$). Algal biomass increased between day 8 and day 23 in both light treatments, with the most substantial increase occurring in the high-light treatment (Fig. 2A). Algal biomass was greater in the high-light than in the low-light treatment during both sampling periods. Bacterial and fungal biomass closely mirrored algal biomass. The highest biomasses were observed on day 23 in the high-light treatment (Figs 2B and C). Sample period and light treatment produced significant 2-way interactions for bacterial ($p = 0.028$) and fungal biomass ($p = 0.030$).

Leaves.—Algal biomass was significantly influenced by sample period ($p < 0.001$) and light level ($p = 0.004$), but these variables did not produce a significant

TABLE 1. Probabilities associated with each factor included in the analysis of variance for alkaline phosphatase (APA), β-glucosidase (GLU), leucine-aminopeptidase (LAMP), and phenol oxidase (POA) activity. Analyses were conducted independently for communities associated with inorganic substrata (glass-fiber filters [GF/F]) and detritus (leaf litter). Bold font indicates statistical significance ($p < 0.05$).

Substratum	Variable	APA	GLU	LAMP	POA
GF/F	Light level	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Day/night	**<0.001**	0.994	0.694	0.085
	Sample period	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Light level × sample period	**0.031**	**<0.001**	**0.028**	**<0.001**
	Light level × day/night	0.446	**0.007**	0.139	**0.004**
	Day/night × sample period	0.812	0.237	0.605	**0.010**
	Light level × day/night × sample period	0.663	0.777	0.376	0.511
Leaf litter	Light level	**<0.001**	0.253	0.170	0.058
	Day/night	**<0.001**	0.090	**<0.001**	0.089
	Sample period	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Light level × sample period	0.335	0.260	0.689	0.829
	Light level × day/night	**0.037**	0.701	0.284	**<0.001**
	Day/night × sample period	0.406	0.188	0.196	0.458
	Light level × day/night × sample period	0.411	0.739	0.993	0.927

interaction effect ($p = 0.285$). Algal biomass was consistently higher in the high-light treatment than in the low-light treatment on both sample dates and nearly doubled between days 8 and 23 (Fig. 3A). Bacterial biomass was higher on day 23 than on day 8 in both light levels (Fig. 3B), producing a significant sample period effect ($p < 0.001$). Light level did not affect bacterial biomass ($p = 0.898$). Fungal biomass increased between days 8 and 23 (Fig. 3C), producing a significant sample period effect ($p = 0.007$), and was slightly higher in the high-light treatment than in the low-light treatment on both sample dates, but this effect was not statistically significant ($p = 0.100$).

Treatment effects on enzyme activities

GF/Fs.—APA activity was higher in the high-light treatment than in the corresponding low-light treatment (Table 1, Fig. 4A) and increased from day 8 to day 23. The magnitude of this increase was dependent primarily on light level, thus, the interaction between sample period and light level was significant ($p = 0.002$). APA activity was slightly higher during the day than during the night. Diel differences were more pronounced during the day 23 sample period than during the day 8 sample period, and sample period and light level produced a significant interaction effect on APA activity ($p = 0.031$). GLU activity was greater during the day 23 sample period than during the day 8 sample period, and it was greater in the high-light treatment than in the low-light treatment, particularly during the day 23 sample period (Table 1, Fig. 4B). Sample period and light level produced a significant interaction effect on GLU activity ($p < 0.001$). The most pronounced day/night effect was observed in the low-light treatment during the day 23 sample period. LAMP activity was affected by sample period and light level, with the highest activity being observed in the high-light treatment during the day 23 sample period (Table 1, Fig. 4C). Light treatment and sample period produced a significant interaction effect on LAMP activity ($p = 0.028$) LAMP activity did not appear to be affected by diel changes in PAR (day/night effect: $p = 0.694$). POA activity was greatest in the high-light treatment during the day 23 sample period (Table 1, Fig. 4D). Light level and sample period produced a significant interaction effect on POA activity ($p < 0.001$). The effects on POA activity of diel changes in PAR were complex. The greatest diel differences in POA activity were observed in the high-light treatment during the day 8 sample period. Day/night and sample period produced a significant interaction effect on POA activity ($p = 0.010$), as did day/night and light level ($p = 0.004$).

Leaves.—APA activity increased from day 8 to day 23 (Table 1, Fig. 5A), and the sample period effect was significant ($p < 0.001$). Day/night and light level produced a significant interaction effect on APA activity ($p = 0.037$). GLU also increased from day 8 to day 23 (Table 1, Fig. 5B), and the sample period effect was significant ($p < 0.001$). However, light level did not affect GLU activity ($p = 0.253$), and there was only a slight, nonsignificant indication of a day/night effect on GLU activity during the day 23 sample period ($p = 0.090$). LAMP also increased from day 8 to day 23 (Table 1, Fig. 5C), and the sample period effect was significant ($p < 0.001$). Light level did not affect LAMP

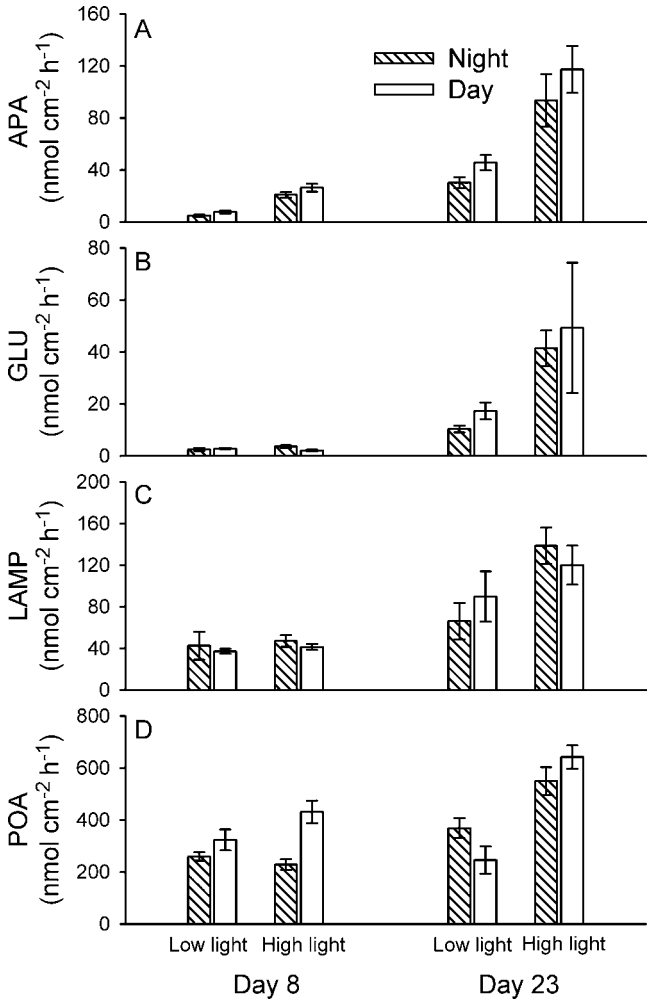

FIG. 4. Mean (±1 SE) alkaline phosphatase (APA) (A), β-glucosidase (GLU) (B), leucine-aminopeptidase activity (LAMP) (C), and phenol oxidase (POA) (D) activity measured during the day and night of the day 8 and day 23 diel sample periods in microbial communities grown on glass-fiber filters under low- and high-light levels.

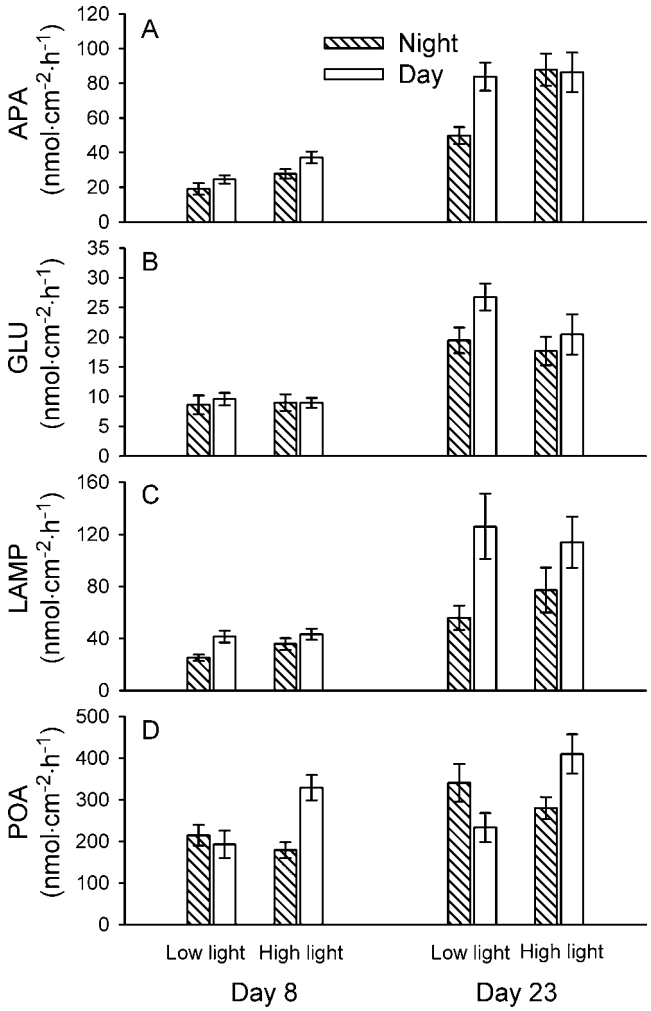

FIG. 5. Mean (±1 SE) alkaline phosphatase (APA) (A), β-glucosidase (GLU) (B), leucine-aminopeptidase activity (LAMP) (C), and phenol oxidase (POA) (D) activity measured during the day and night of the day 8 and day 23 diel sample periods in microbial communities grown on leaf litter under low- and high-light levels.

activity ($p = 0.170$), but the day/night effect was highly significant ($p < 0.001$) and indicated diel periodicity in LAMP activity. No significant interactions between variables affected LAMP activity ($p > 0.05$). Therefore, the significantly greater LAMP activity during the day was independent of other treatment effects. POA activity increased from day 8 to day 23 (Table 1, Fig. 5D), and the sample period effect was significant ($p < 0.001$). The day/night effect was highly dependent on light level, and POA activity increased during the day in the high-light treatment and decreased during the day in the low-light treatment. Day/night and light level produced a significant interaction effect on POA activity ($p < 0.001$).

Microbial biomass and enzyme activities

Mean enzyme activities usually were strongly correlated with microbial biomass (Table 2). APA was significantly ($p < 0.05$) correlated with algal, bacterial, and fungal biomass on both substrata. GLU was significantly correlated with all 3 measures of microbial biomass on GF/Fs, most notably with algal biomass. GLU was significantly correlated with fungal and bacterial biomass, but not with algal biomass ($p > 0.05$), on leaves. LAMP was strongly correlated ($p < 0.05$) with all 3 measures of microbial biomass on GF/Fs, but was significantly correlated only with bacterial and algal biomass on leaves. POA was significantly correlated ($p < 0.05$) with algal, bacterial, and fungal

TABLE 2. Pearson correlation coefficients relating the activities of each enzyme (alkaline phosphatase [APA], β-glucosidase [GLU], leucine-aminopeptidase [LAMP], phenol oxidase [POA]) to algal biomass (as chlorophyll *a*), bacterial biomass, or fungal biomass. Analyses were conducted independently for communities associated with inorganic substrata (glass-fiber filters [GF/F]) and detritus (leaf litter). Bold font indicates a significant correlation ($p < 0.05$) after Bonferroni correction for experiment-wise error rate.

Substratum	Enzyme	Algal biomass	Bacterial biomass	Fungal biomass
GF/F	APA	**0.876**	**0.782**	**0.801**
	GLU	**0.942**	**0.885**	**0.884**
	LAMP	**0.873**	**0.853**	**0.831**
	POA	**0.746**	**0.795**	**0.830**
Leaf litter	APA	**0.659**	**0.665**	**0.751**
	GLU	0.442	**0.663**	**0.606**
	LAMP	**0.814**	**0.608**	0.514
	POA	**0.796**	0.370	0.271

biomass on GF/F, but was significantly correlated only with algal biomass on leaf litter.

Leaf-litter decomposition rates

Values of *k* differed significantly between high- and low-light treatments ($p = 0.008$). Mean (±1 SE) *k* was -0.0095 ± 0.0022 in the high-light treatment, nearly 2× faster than in the low-light treatment (-0.0052 ± 0.0028).

Discussion

The evidence we present supports the overall hypothesis that stream algae influence the activities of extracellular enzymes involved in mineralization processes within stream microbial communities. Our data support the prediction that factors that influence algal photosynthesis and biomass in both the short term (i.e., diel fluctuations in PAR) and the long term (i.e., shading and stage of community development) ultimately influence enzyme activities in microbial communities associated with both inorganic substrata and detritus. We think it likely that algal stimulation of extracellular enzymes in detritus caused decomposition of leaf substrata to be faster in the high-light than in the low-light treatment.

Algae as regulators of microbial enzyme activities

Our results suggest that algal periphyton might be important regulators of microbial enzyme activities on inorganic substrata. The activities of all enzymes tested were highly correlated with algal biomass on inorganic substrata. Enzyme activities were consistently higher in the high-light than in the low-light treatment during the day 23 sample period, and the difference in algal biomass between the low- and high-light treatments was greatest on day 23. These results provide additional evidence that microbial enzyme activities are tightly coupled to algal biomass.

Phosphatases can be produced by algae, but extracellular glucosidases, peptidases, and phenol oxidases generally are produced in quantity only by heterotrophic microorganisms; thus, heterotrophic metabolism might be enhanced in the presence of algae. Our observations are consistent with other studies that have reported higher levels of heterotrophic biomass and metabolism in biofilms exposed to light (Romani and Sabater 1999, Rier and Stevenson 2002, Romani et al. 2004). Algal cells and associated exopolymeric substances provide surfaces for bacterial colonization, and these surfaces potentially increase bacterial density within a given area of substratum (Geesey et al. 1978, Sobczak 1996, Rier and Stevenson 2001). In addition, increases in heterotrophic microbial activities (i.e., enzyme production) also might be a direct response to algal release of organic compounds that require extracellular degradation before being transported across cell membranes; e.g., GLU activity increases in the presence of extracellular cellobiose (e.g., Romani and Sabater 2001).

The most convincing evidence in our study and in others for this direct stimulation of enzyme activity is the tendency for extracellular enzyme activities to fluctuate on a diel cycle (Francoeur and Wetzel 2003) or to be affected by short-term light/dark incubations (Espeland et al. 2001, Francoeur and Wetzel 2003, Francoeur et al. 2006). In our study, short-term variations in PAR stimulated the activities of APA, GLU, and POA. Similar to other studies (e.g., Francoeur and Wetzel 2003, Francoeur et al. 2006), this stimulation was variable and often depended on light level and sample period (stage of community development). These short-term fluctuations might have been driven by fluctuations in pH in the case of hydrolytic enzymes (Francoeur and Wetzel 2003) or by oxidative conditions, in the case of POA.

Possible role of algae in decomposition of organic matter

Microbial decomposition of detritus usually is thought to be primarily a heterotrophic process, but our results suggest that algal accumulation and subsequent activities (i.e., photosynthesis) on leaf litter also could play an important role by stimulating enzyme activities and, ultimately, the overall decomposition process. The activities of all enzymes tested, except GLU, were highly correlated with algal biomass on leaves. Furthermore, POA was significantly correlated with algal biomass, but not fungal or bacterial biomass, even though heterotrophic microorganisms are the ultimate source of these enzymes. POA was significantly related to the day/night cycle in substrata exposed to high light during both sample periods. This interaction may indicate that a sufficient algal biomass was necessary to stimulate a corresponding shift in POA activity in response to changes in photosynthetic rate. POAs are important in the degradation of phenolic compounds, such as lignins and tannins (e.g., Sinsabaugh et al. 1994), which are important structural components of leaf litter (Swain 1979). Thus, algal colonization might possibly contribute to faster rates of leaf-litter decomposition in streams where irradiance levels are sufficient to enable algal accumulation on detritus. This explanation could be one reason for the faster leaf decomposition rates observed in high light in our study.

POAs are oxidative enzymes; thus, a possible mechanism for the stimulation of POA by algal photosynthesis is the release of O_2 during photosynthesis. Neely (1994) reported that wetland plant litter (*Typha*) decomposed more rapidly when colonized by algae than when algae were not present. Neeley (1994) speculated that enhanced leaf-litter decay might have resulted from increased heterotrophic microbial activities caused by greater O_2 availability from algal photosynthesis. Freeman et al. (2004) observed that POA activity in peatlands can be O_2 limited and that POA rates can be substantially increased when O_2 is provided. O_2 concentrations generally are high in streams. However, O_2 demand in biofilms also can be high because of diffusion limitations (Freeman and Lock 1995), and therefore, O_2 availability within the biofilm is influenced strongly by algal photosynthesis (Carlton and Wetzel 1987).

Leaf breakdown in streams is often N and P limited (e.g., Robinson and Gessner 2000). Therefore, photosynthetic activity of algal inhabitants on leaf litter also might enhance inorganic nutrient availability by stimulating greater mineralization activities by microbial autotrophs or heterotrophs. The water feeding the artificial streams in our study had low concentrations of inorganic N and P. Therefore, indirect algal stimulation of organic P- and N-mineralizing enzymes might possibly have contributed to faster leaf-litter decomposition rates in the high-light treatment. Further evidence for this mechanism was provided by the strong significant correlations between algal biomass and the activities of enzymes involved in mineralization of P and N (i.e., APA and LAMP) on leaf litter. In addition, APA was higher on leaves incubated in high-light than on leaves incubated in low-light treatments in both early (day 8) and later (day 23) stages of community development, and both APA and LAMP displayed at least some diel periodicity in response to changing levels of PAR.

The amount of light reaching the streambed is an important determinant of ecosystem function. Riparian canopy regulates the amount of PAR that is available for algal photosynthesis, and PAR ultimately determines the relative importance of photoautotrophic and heterotrophic pathways within the stream system. The results of our study suggest that, when light regimes are favorable, photoautotrophs might enhance heterotrophic pathways in streams directly by creating favorable conditions for the decomposition of both dissolved and particulate organic detritus within biofilms and the leaf matrix.

Acknowledgements

The University of Michigan Biological Station, Bloomsburg University, and Eastern Michigan University provided facilities and support for this research. PAR data were provided by Chris Vogel and the University of Michigan Ameriflux tower. Streamwater nutrient data were provided by Mike Grant at the University of Michigan Biological Station. We thank Bob Vande Kopple, Jackie Pilette, Audrey Johnson, and Cathy Haffner for their support of this research. This research was supported by a Research Opportunity Award Supplement to National Science Foundation grant DEB-0315686.

Literature Cited

ALLAN, J. D. 1995. Stream ecology: structure and function of running waters. Chapman and Hall, London, UK.

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). 1998. Standard methods for the examination of water and wastewater. 20th edition. American Public Health Association, American Waterworks Association, and Water Environment Federation, Washington, DC.

CARLTON, R. G., AND R. G. WETZEL. 1987. Distributions and fates of oxygen in periphyton communities. Canadian Journal of Botany 65:1031–1037.

CHRÓST, R. J. 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. Pages 29–59 *in*

R. J. Chróst (editor). Microbial enzymes in aquatic environments. Springer Verlag, New York.

COLE, J. J. 1982. Interactions between bacteria and algae in aquatic ecosystems. Annual Review of Ecology and Systematics 13:291–314.

DUCKLOW, H. 2000. Bacterial production and biomass in the oceans. Pages 85–120 *in* D. L. Kirchman (editor). Microbial ecology of the oceans. Wiley–Liss, New York.

ESPELAND, E. M., S. N. FRANCOEUR, AND R. G. WETZEL. 2001. Influence of algal photosynthesis on biofilm bacterial production and associated glucosidase and xylosidase activities. Microbial Ecology 42:524–530.

FINDLAY, S., J. L. TANK, S. DYE, H. M. VALETT, P. J. MULHOLLAND, W. H. MCDOWELL, S. L. JOHNSON, S. K. HAMILTON, J. EDMONDS, AND W. K. DODDS. 2002. A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microbial Ecology 43:55–66.

FRANCOEUR, S. N., M. SCHAECHER, R. K. NEELY, AND K. A. KUEHN. 2006. Periphytic photosynthetic stimulation of extracellular enzyme activity in aquatic microbial communities associated with decaying *Typha* litter. Microbial Ecology 52:662–669.

FRANCOEUR, S. N., AND R. G. WETZEL. 2003. Regulation of periphytic leucine-aminopeptidase activity. Aquatic Microbial Ecology 31:249–258.

FREEMAN, C., AND M. A. LOCK. 1995. The biofilm polysaccharide matrix: a buffer against changing organic substrate supply. Limnology and Oceanography 40:273–278.

FREEMAN, C., N. J. OSTLE, N. FENNER, AND H. KANG. 2004. A regulatory role for phenol oxidase during decomposition in peatlands. Soil Biology and Biochemistry 36:1663–1667.

GEESEY, G. G., R. MUTCH, AND J. W. COSERTON. 1978. Sessile bacteria: an important component of the microbial population in small mountain streams. Limnology and Oceanography 23:1214–1223.

GESSNER, M. O., AND E. CHAUVET. 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Applied and Environmental Microbiology 59:502–507.

GESSNER, M. O., AND S. Y. NEWELL. 2002. Biomass, growth rate, and production of filamentous fungi in plant litter. Pages 390–408 *in* C. J. Hurst, R. L. Crawford, G. R. Knudsen, M. J. McInerney, and L. D. Stetzenbach (editors). Manual of environmental microbiology. American Society for Microbiology Press, Washington, DC.

HAACK, T. K., AND G. A. MCFETERS. 1982. Nutritional relationships among microorganisms in an epilithic biofilm community. Microbial Ecology 8:115–126.

KAPLAN, L. A., AND T. L. BOTT. 1983. Microbial heterotrophic utilization of dissolved organic matter in a piedmont stream. Freshwater Biology 13:363–377.

KUEHN, K. A., AND K. SUBERKROPP. 1998. Diel fluctuations in rates of CO_2 evolution from standing dead leaf litter of the emergent macrophyte *Juncus effusus*. Aquatic Microbial Ecology 14:171–182.

KUHL, M., R. N. GLUD, H. PLOUG, AND N. B. RAMSING. 1996. Microenvironmental control of photosynthesis and photosynthesis-coupled respiration in an epilithic cyanobacterial biofilm. Journal of Phycology 32:799–812.

LAMBERTI, G. A. 1996. Role of periphyton in benthic food webs. Pages 533–572 *in* R. J. Stevenson, M. Bothwell, and R. Lowe (editors). Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, California.

LOCK, M. A. 1993. Attached microbial communities in rivers. Pages 113–138 *in* T. E. Ford (editor). Aquatic microbiology: an ecological approach. Blackwell Scientific Publications, Boston, Massachusetts.

MINSHALL, G. W. 1978. Autotrophy in stream ecosystems. BioScience 28:767–771.

MULHOLLAND, P. J. 1996. Role in nutrient cycling in streams. Pages 609–640 *in* R. J. Stevenson, M. Bothwell, and R. Lowe (editors). Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, California.

NEELY, R. K. 1994. Evidence for positive interactions between epiphytic algae and heterotrophic decomposers during the decomposition of *Typha latifolia*. Archiv für Hydrobiologie 129:443–457.

NEELY, R. K., AND R. G. WETZEL. 1997. Autumnal production by bacteria and autotrophs attached to *Typha latifolia* L. detritus. Journal of Freshwater Ecology 12:253–267.

RIER, S. T., AND R. J. STEVENSON. 2001. Relation of environmental factors to density of epilithic lotic bacteria in 2 ecoregions. Journal of the North American Benthological Society 20:588–600.

RIER, S. T., AND R. J. STEVENSON. 2002. The effects of labile dissolved organic carbon, inorganic nutrients and light on algal-bacterial relationships in stream periphyton. Hydrobiologia 489:179–184.

ROBINSON, C. T., AND M. O. GESSNER. 2000. Nutrient addition accelerates leaf breakdown in an alpine springbrook. Oecologia (Berlin) 122:258–263.

ROMANI, A. M., H. GUASCH, I. MUNOZ, J. RUANA, E. VILALTA, T. SCHWARTZ, F. EMTIAZI, AND S. SABATER. 2004. Biofilm structure and function and possible implications for riverine DOC dynamics. Microbial Ecology 47:316–328.

ROMANI, A. M., AND S. SABATER. 1999. Effect of primary producers on the heterotrophic metabolism of a stream biofilm. Freshwater Biology 41:729–736.

ROMANI, A. M., AND S. SABATER. 2001. Structure and activity of rock and sand biofilms in a Mediterranean stream. Ecology 82:3232–3245.

SCHMIDT, T. L., J. S. SPENCER, AND R. BERTSCH. 1993. Michigan's forests 1993: an analysis. Resource Bulletin NC-179. Forest Service, US Department of Agriculture, North Central Experiment Station, St. Paul, Minnesota. (Available from: Forest Service, US Department of Agriculture, North Central Experiment Station, 1992 Folwell Avenue, St. Paul, Minnesota 55108 USA.)

SHACKLE, V. J., C. FREEMAN, AND B. REYNOLDS. 2000. Carbon supply and the regulation of enzyme activity in constructed wetlands. Soil Biology and Biochemistry 32:1935–1940.

SINSABAUGH, R. L., R. K. ANTIBUS, AND A. E. LINKINS. 1991a. An enzymatic approach to the analysis of microbial activity during plant litter decomposition. Agriculture Ecosystems and Environment 34:43–54.

SINSABAUGH, R. L., R. K. ANTIBUS, A. E. LINKINS, C. A. MCCLAUGHERTY, L. RAYBURN, D. REPERT, AND T. WEILAND. 1993. Wood decomposition—nitrogen and phosphorus

dynamics in relation to extracellular enzyme activity. Ecology 74:1586–1593.

SINSABAUGH, R. L., M. P. OSGOOD, AND S. FINDLAY. 1994. Enzymatic models for estimating decomposition rates of particulate detritus. Journal of the North American Benthological Society 13:160–169.

SINSABAUGH, R. L., D. REPERT, T. WEILAND, S. W. GOLLADAY, AND A. E. LINKINS. 1991b. Exoenzyme accumulation in epilithic biofilms. Hydrobiologia 222:29–37.

SOBCZAK, W. 1996. Epilithic bacteria responses to variations in algal biomass and labile dissolved organic carbon during biofilm colonization. Journal of the North American Benthological Society 15:143–154.

SWAIN, T. 1979. Tannins and lignins. Pages 657–682 *in* G. A. Rosenthal and D. H. Janzen (editors). Herbivores: their interactions with secondary plant metabolites. Academic Press, New York.

WETZEL, R. G. 1993. Microcommunities and microgradients: linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. Netherlands Journal of Aquatic Ecology 27:3–9.

WETZEL, R. G. 2001. Limnology: lake and river ecosystems. 3rd edition. Academic Press, San Diego, California.

Received: 21 November 2006
Accepted: 11 April 2007