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# Leaf litter processing and exoenzyme production on leaves in streams of different pH

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**Abstract** We examined microbial colonization, exoenzyme activity, and processing of leaves of yellow poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), and white oak (*Quercus alba*) in three streams on the Allegheny Plateau of West Virginia, United States. Leaf packs were placed in streams that varied in their underlying bedrock geology, and therefore in their sensitivity to the high level of acidic precipitation that occurs in this region. The mean pH of the streams was 4.3 in the South Fork of Red Run (SFR), 6.2 in Wilson Hollow Run (WHR), and 7.7 in the North Fork of Hickman Slide Run (HSR). Through time, the patterns of microbial biomass and exoenzyme activity were generally similar among leaf species, but the magnitude of microbial biomass and exoenzyme activity differed among leaf species. Pectinase activity was greatest in HSR, the most alkaline stream, whereas the activity of exocellulase and xylanase was greatest in WHR and SFR, the intermediate and acidic streams. This variation in the activity of different exoenzymes was consistent with published pH optima for these exoenzymes. Variation in processing rates, both among leaf species and among streams, seems to be related to the level of microbial exoenzyme activity on the leaf detritus.

**Key words** Detrital processing · Exoenzymes · ATP · pH

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## Introduction

One recent change in regional climate patterns has been a decrease in the pH of precipitation in parts of eastern North America and northern Europe, which has resulted from increased emissions of $SO_2$ and $NO_x$ from the combustion of fossil fuels (Wisniewski and Keitz 1983; Tan 1989). In regions characterized by parent rock and soils of low carbonate content, this acidic precipitation has led to acidification of headwater streams.

Lowered streamwater pH has been shown to decrease rates of leaf litter processing (Friberg et al. 1980; Burton et al. 1985; Kimmel et al. 1985; Mulholland et al. 1987). Allochthonous leaf litter is a major source of energy for macroinvertebrates in headwater streams (Minshall 1967; Cummins et al. 1973; Anderson and Sedell 1979; Webster and Benfield 1986). Litter processing in streams is mediated by microbial activity, which increases the palatability of the leaf detritus for invertebrate shredders (Bärlocher 1981; Suberkropp et al. 1983).

Some of the effect of pH on leaf litter processing in streams has been attributed to a direct effect of pH on microbial growth and biomass (Rosset and Bärlocher 1985; Chamier 1987; Mulholland et al. 1987; Griffith 1992). Bacteria and fungi also produce several classes of exoenzymes, such as cellulases, pectinases, and hemicellulases, which degrade the structural polysaccharides that are the primary components of the leaves (Chamier 1985). Because exoenzymes often have activity optima within relatively narrow ranges of pH, Chamier and Dixon (1982) and Kok and Van der Velde (1991) suggested that differences in pH may affect the activity of these exoenzymes, and thereby may affect leaf litter processing in aquatic ecosystems.

The objective of our research was to determine whether differences in leaf litter processing rates among streams were correlated with differences in microbial biomass and exoenzyme activity on the leaf detritus. We postulated that exoenzyme activity on leaf detritus in streams of different pH would be consistent with published pH optima for those enzymes. We studied three

**Table 1** Physical characteristics and water chemistry of the study streams in or near the Fernow Experimental Forest, Tucker, County, West Virginia, USA. Means (ranges) for the period November 1992 to March 1993 are presented. SFR is South Fork of Red Run, WHR is Wilson Hollow Run, and HSR is North Fork of Hickman slide Run.

| Variable | SFR | WHR | HSR |
|---|---|---|---|
| Stream order | 2nd | 3rd | 2nd |
| Catchment area (ha) | 230.3 | 127.7 | 72.2 |
| Mean width (m) | 2.7 | 2.5 | 1.9 |
| Mean altitude (m asl) | 1198 | 738 | 852 |
| pH | 4.3 (4.1–4.5) | 6.2 (6.1–6.4) | 7.7 (7.3–8.0) |
| Alkalinity (mg/l $CaCO_3$ | 0.0 (0.0–0.0) | 1.5 (0.8–2.6) | 33.5 (31.3–39.0) |
| Conductance (μS/cm) | 28.5 (27.1–30.6) | 37.5 (26.5–49.6) | 118.5 (100.1–178.0) |
| $Ca^{2+}$ (mg/L) | 0.7 (0.6–0.8) | 1.9 (1.8–2.1) | 15.8 (14.9–17.1) |
| $SO_4^{-2}$ (mg/L) | 6.6 (5.1–8.6) | 6.8 (6.2–8.0) | 10.1 (8.9–11.6) |
| $NO_3^{-2}$ (mg/L) | 0.2 (0.1–0.3) | 0.6 (0.5–0.7) | 1.4 (1.1–2.0) |

streams of different mean pH on the Alleghany Plateau of West Virginia, United States. We measured mass loss in leaf packs, adenosine triphosphate (ATP) concentrations as an index of microbial biomass in leaf packs, and the activity of three types of microbial exoenzymes: pectinase, exocellulase, and xylanase.

## Materials and methods

### Study sites

This study was conducted in three streams in or near the Fernow Experimental Forest, Monongahela National Forest, West Virginia, United States (39°3′N, 79°40′W). These streams are tributaries of the Cheat River in the Alleghany Plateau physiographic province of the central Appalachian Mountains.

The North Fork of Hickman Slide Run (HSR) drains a 72.2-ha catchment underlain by limestones of the Greenbriar formation and shales of the Mauch Chunk formation (Table 1; Reger 1923). The riparian forest is a mature stand dominated by red oak (*Quercus borealis*), sugar maple (*Acer saccharum*), and eastern hemlock (*Tsuga canadensis*).

Wilson Hollow Run (WHR) drains a 127.7-ha catchment underlain by shales and siltstones of the Hampshire formation (Table 1; Reger 1923). The riparian forest is similar to that of HSR.

The South Fork of Red Run (SFR) drains a 230.3-ha catchment underlain by silicate sandstones of the Pottsville Formation (Table 1; Reger 1923). The riparian forest is dominated by red maple (*A. rubrum*), yellow birch (*Betula lutea*), eastern hemlock (*T. canadensis*), and rhododendron (*Rhododendron maximum*).

### Water chemistry and temperature

Water chemistry analyses were conducted in cooperation with the Timber and Watershed Laboratory of the Northeastern Forest Experiment Station at Parsons, West Virginia, United States. Grab samples for water chemistry analysis were collected on each sampling date. In addition, pH and conductivity were measured during every visit to the streams with calibrated portable meters. The methods used by personnel at the Timber and Watershed Laboratory to analyze chemical variables were described by Griffith and Perry (1991).

Water temperatures in all streams were recorded with a Ryan[1] TempMentor or Model J thermograph that was placed in a pool in each stream. Because of problems with two of the thermographs, daily mean water temperatures in January, February, and March 1993 for HSR and SFR were estimated from daily mean water temperatures in WHR. The relationship between mean daily temperatures in WHR and those in HSR and SFR was determined by least-squares regression, using data from September through December 1992.

[1] Reference to trade names or manufacturers does not imply government endorsement of commercial products.

### Leaf pack samples

Leaves of three tree species that represented a range of processing rates (Webster and Benfield 1986) were used in this study: yellow poplar (*Liriodendron tulipifera*), red maple (*A. rubrum*), and white oak (*Q. alba* L.). The leaves were collected at abscission from several trees in Morgantown, West Virginia, air-dried, weighed into 10-g leaf packs, and placed in leaf bags of 3-mm nominal mesh. Thirty-five leaf packs of each leaf species were placed in each of the three streams during November 1992. Several leaf packs of each leaf species were returned to the laboratory without placement in the streams to estimate the weight loss as a result of handling and to estimate the initial ash free dry weight (AFDW).

Five leaf packs of each species were retrieved from each stream after 2 days, 2 weeks, 4 weeks, and then at 4-week intervals until most of the leaf packs had been retrieved in March 1992. The collection from SFR in March did not occur until 2 weeks after the scheduled pick-up because of a heavy, late-season snowstorm. After collection, the leaf packs were placed in plastic bags with stream water and transported on ice. In the laboratory, the leaf packs were rinsed to dislodge any macroinvertebrates and associated particulate organic matter. Any particulate organic matter on the outside of the leaf bag or that passed through the 3-mm-mesh bag was not considered part of the remaining leaf pack.

### Microbial biomass and exoenzyme production

Subsamples for microbial analyses were taken on all sampling dates, except for the 2-day sample; 1-cm-diameter leaf discs were removed from each leaf pack with a cork borer. We conducted assays for activity of three microbial exoenzymes on the leaf packs: pectinase, xylanase, and exocellulase. We also analyzed ATP concentration as an indicator of microbial biomass.

For the pectinase assay, five leaf discs from each leaf pack were homogenized in 10 ml of 0.05 M Tris buffer solution with 0.03 M $CaCl_2$ (pH 8.0) in a Biospec biohomogenizer. The homogenate was centrifuged at 10000 rpm for 10 min, and the supernatant was used for the assay. The substrate solution was 0.5% pectin from citrus fruit (Sigma) in the Tris buffer solution. The enzyme reaction solution consisted of 1 ml each of the Tris buffer solution, the substrate solution, and the supernatant from the leaf

homogenate. The reaction solutions were incubated at 30°C for 3 h and assayed for production of unsaturated products by reaction with thiobarbituaric acid (Ayers et al. 1966). In the assay, 1 ml of the reaction solution was combined with 2.5 ml of 0.1 M HCl, 2.5 ml of 0.04 M thiobarbituaric acid, and 5 ml of distilled $H_2O$; the samples were boiled for 30 min. Absorbance was measured at 550 nm with a Perkin-Elmer Lambda 3A spectrophotometer and activity (units $g^{-1}$ AFDW leaf $h^{-1}$) was determined from the difference in absorbance between an experimental and a control assay (Arsuffi and Suberkropp 1984). One unit was defined as the amount of enzyme that produces a change in absorbance of 0.001. In the control assay, the reaction solution without the supernatant from the leaf homogenate was incubated with the experimental assays, and 1 ml of supernatant was added immediately before the assay.

In the xylanase assay, five leaf discs from each leaf pack were homogenized in 15 ml of 0.05 M acetate buffer with 0.03 M $CaCl_2$ (pH 5.0) with a Biospec biohomogenizer. The homogenate was centrifuged at 10l000 rpm for 10 min, and the supernatant was used for the assay. The substrate solution was 0.5% xylan from birchwood (Sigma) in the acetate buffer solution (Suberkropp and Klug 1980). The enzyme reaction solution consisted of 1 ml each of the acetate buffer solution, the substrate solution, and the supernatant from the leaf homogenate. The reaction solutions were incubated at 30°C for 3 h and assayed for production of reducing sugars by the Somogyi (1952) modification of the reducing sugar assay of Nelson (1944). Absorbance was measured at 660 nm, and the assay solutions were compared to standard curves prepared from known solutions of xylose. Activity (nmol xylose $g^{-1}$ AFDW $h^{-1}$) was determined from the difference in absorbance between an experimental and a control assay. In the control assay, the reaction solution without the supernatant from the leaf homogenate was incubated with the experimental assays, and 1 ml of supernatant was added immediately before the assay.

For the exocellulase assay, we used a second aliquot of the supernatant from the leaf homogenate in acetate buffer. The substrate solution was 0.5% microcrystalline cellulose (Sigmacell 20, Sigma) in the acetate buffer solution (Sinsabaugh et al. 1981). The enzyme reaction solution consisted of 1 ml each of the acetate buffer solution, the microcrystalline cellulose substrate solution, and the supernatant from the leaf homogenate. The reaction solutions were incubated at 30°C for 20 h and assayed for production of reducing sugars as in the xylanase assay, except that the standard curves were prepared from known solutions of glucose.

For ATP analysis, ten leaf discs from each leaf pack wereextracted in 4 ml of 0.5 M $H_2SO_4$ for 30 min (Suberkropp and Klug 1976). The samples were then filtered to remove particles, neutralized with 4 ml of 1 M NaOH, diluted and adjusted to pH 7.75 with 4 ml of 0.1 M Tris buffer (pH 7.75), and frozen until assayed. Known amounts of ATP were added to replicate aliquots of selected samples and assayed to estimate efficiencies. Luciferin-luciferase (Firelight, Analytical Luminescence Laboratory, San Diego, Calif.) was reconstituted with 5 ml of 0.1 M Tris buffer (pH 7.75). ATP concentrations in 100-ml aliquots were determined with a Turner TD-20e luminometer by comparing the 45-sec integration of light emission with a standard curve (Suberkropp et al. 1983).

### Leaf pack mass

Following removal of the leaf discs for the microbial analyses, we removed ten more leaf discs from each leaf pack to estimate the dry weight of the leaf discs that were used for microbial analysis. These leaf discs and the remainder of each leaf pack were dried at 65°C in a drying oven for 24 h and weighed to the nearest 1 mg. The leaf discs were then ashed at 550°C for 1 h, cooled in a desiccator, and weighed to the nearest 1 mg to determine the percent AFDW of the leaf litter. The dry weight per leaf disc was multiplied by the total number of leaf discs removed from the leaf pack for all analyses and added to the dry weight of the remaining leaf pack to calculate the remaining leaf pack dry weight after processing in the streams. This remaining leaf pack dry weight was multiplied by the percent AFDW to estimate the remaining leaf pack AFDW.

### Data analysis

The rate coefficient for leaf pack AFDW loss, $k$, for each leaf species in each stream was estimated from the regression of leaf pack AFDW against time using the exponential decay model:

$$W_t = W_i \cdot e^{-kt}$$

(Petersen and Cummins 1974), where $W_i$ was the initial leaf pack AFDW, and $W_t$ was the leaf pack AFDW at collection time, $t$. Because temperature as well as water chemistry differed among the streams, processing rates were estimated both on a $day^{-1}$ and a $degree\text{-}day^{-1}$ basis in order to separate those effects.

Analysis of covariance was used to test the null hypothesis that $k$ was not different among streams and among leaf types. A multiple regression analysis with dummy variables to distinguish among streams and among leaf types was performed using the general linear model (GLM) procedure of SAS (SAS Institute 1988).

To test the null hypotheses that microbial exoenzyme activity and ATP concentrations on the leaves were not different among streams and among leaf species, we conducted a repeated-measures analysis of variance. We also calculated Pearson correlation coefficients to test for correlations between exoenzyme activity and ATP concentrations in each of the streams. An $\alpha$ level of 0.05 was used to determine significance in all statistical analyses.

## Results

### Water chemistry and temperature

During the study, water chemistry varied little within streams but varied among streams, as was expected because of the differing bedrock geology of the streams (Table 1). Mean pH was 4.3 in SFR, 6.2 in WHR, and 7.7 in HSR. Mean alkalinity was 0.0 mg $l^{-1}$ in SFR, 1.5 mg $l^{-1}$ in WHR, and 33.5 mg $l^{-1}$ in HSR. The conductance and concentrations of $Ca^{2+}$ and $NO_3^-$ were also lowest in SFR, intermediate in WHR, and highest in HSR (Table 1). Concentrations of $SO_4^{2-}$ were similar in SFR and WHR and higher in HSR.

Daily mean water temperature was generally 1–2°C warmer in WHR and HSR than in with SFR. As a result, total cumulative degree-days during the course of the study were 233.2, 423.6, and 393.3 in SFR, WHR, and HSR, respectively.

### Leaf pack processing rates

Processing rates ($day^{-1}$) of white oak, red maple, and yellow poplar leaves differed among the streams (Fig. 1, Table 2). In general, processing rates ($day^{-1}$) were faster in the two streams with higher pH and temperature.

When processing rates ($degree\text{-}day^{-1}$) were calculated to account for the differences in temperature among streams, fewer significant differences were observed (Table 2). For yellow poplar, processing rates (degree $day^{-1}$) were fastest in SFR rather than slowest as in processing rates ($day^{-1}$).

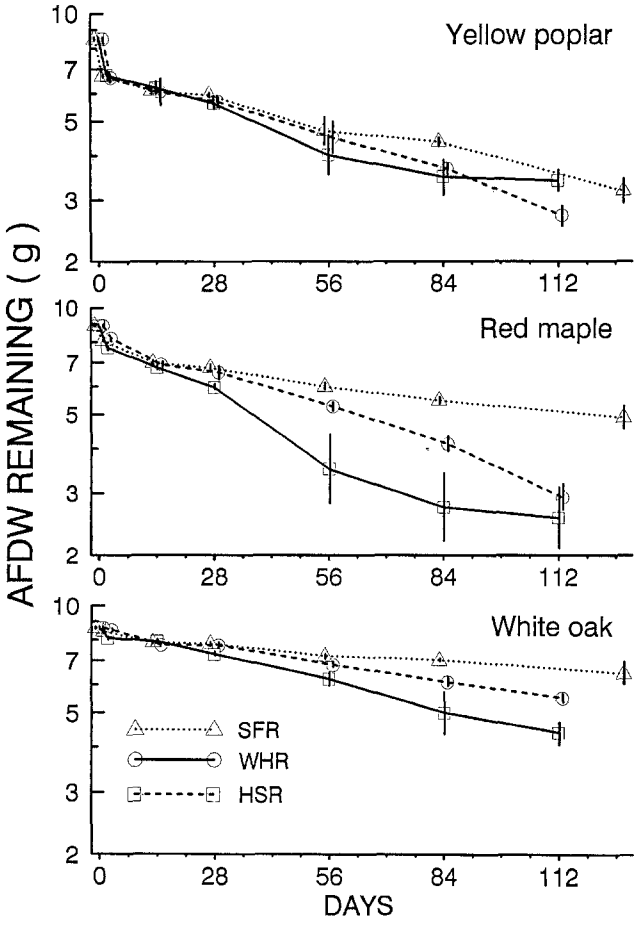


**Fig. 1** Leaf pack weight loss in the 3 study streams in Tucker County, West Virginia. The streams are South Fork of Red Run (*SFR*, pH=4.3), Wilson Hollow Run (*WHR*, pH=6.2), and North Fork of Hickman Slide Run (*HSR*, pH=7.7). Error bars represent ±1 SE (*AFDW* ash-free dry weight)

**Table 2** Processing rates per day and per degree-day mesured for yellow poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), and white oak (*Quercus alba*) leaves in leaf bags in each of the 3 study streams in or near the Fernow Experimental Forest, West Virginia, USA. Within each leaf type, processing rates followed by the same letter were not significantly different from one another ($\alpha$=0.05). The $r^2$ value is the proportion of the variation in leaf pack weight explained by the multiple regression for each leaf species

| | White oak | Red maple | Yellow poplar |
|---|---|---|---|
| $-k$ (day$^{-1}$) | | | |
| SFR | 0.0020 a | 0.0037 a | 0.0058 a |
| WHR | 0.0059 c | 0.0106 b | 0.0068 ab |
| HSR | 0.0038 b | 0.0091 b | 0.0081 b |
| $r^2$ | 0.810 | 0.753 | 0.797 |
| $-k$ (degree-day$^{-1}$) | | | |
| SFR | 0.0011 ab | 0.0022 a | 0.0033 b |
| WHR | 0.0015 b | 0.0029 a | 0.0018 a |
| HSR | 0.0011 a | 0.0026 a | 0.0023 a |
| $r^2$ | 0.835 | 0.814 | 0.863 |

**Table 3** Results of repeated measures analysis of variance comparing ATP concentration and exoenzyme activity among leaf species (LSP) and streams (STR) in or near the Fernow Experimental Forest, West Virginia, USA. STR LSP is the interaction term of stream by species.

| Variable | | STR | LSP | STR LSP |
|---|---|---|---|---|
| ATP concentration | *F* | 24.35 | 5.40 | 1.10 |
| | *P* | <0.01 | 0.01 | 0.37 |
| Pectinase activity | *F* | 160.31 | 38.12 | 9.19 |
| | *P* | <0.01 | <0.01 | <0.01 |
| Xylanase activity | *F* | 6.50 | 57.28 | 21.18 |
| | *P* | <0.01 | <0.01 | <0.01 |
| Exocellulase activity | *F* | 5.56 | 2.09 | 0.63 |
| | *P* | <0.01 | <0.14 | <0.64 |

Yellow poplar leaves seemed to degrade in a different manner from white oak and red maple leaves. White oak and red maple exhibited skeletonization, but yellow poplar did not. However, the yellow poplar leaves became very soft.

## Microbial biomass

Microbial biomass was greater on leaves in the two more alkaline streams than in the lower pH stream (Fig. 2). Repeated-measures analysis of variance indicated that ATP concentrations were significantly different among streams and among leaf species, but the interaction of the stream and leaf species effects was not significant (Table 3). In WHR and HSR, ATP concentrations in all leaf species generally increased from about 28 days to the end of the experiment at 112 days (Fig. 2). Peak ATP concentrations were greatest in red maple leaf packs, with a peak of 25 ng mg$^{-1}$ in HSR and 18 ng mg$^{-1}$ in WHR; ATP concentrations were intermediate in yellow poplar and lowest in white oak. In SFR, ATP concentrations remained low in white oak and red maple (Fig. 2), generally less than 5 ng mg$^{-1}$, but in yellow poplar, ATP concentrations increased to a peak of 10 ng mg$^{-1}$ at 84 days.

## Exoenzyme activity

Pectinase activity was greatest in the most alkaline stream and lowest in the acidic stream (Fig. 3). Pectinase activity was significantly different among streams and among leaf species, and the stream by leaf species effect was also significant (Table 3). Pectinase activity was generally greatest in HSR (pH=7.7) for all three leaf species (Fig. 3). Pectinase activity in HSR peaked at 56 days for all three leaf species. In WHR (pH=6.2), pectinase activity increased more slowly and was not as great as in HSR. Pectinase activity was low in SFR (pH=4.3) and never was greater than about 4 units mg$^{-1}$ AFDW h$^{-1}$. In comparisons of leaf species, pectinase activity was generally greatest in yellow poplar leaves, intermediate in white oak leaves, and lowest in red maple leaves.

Xylanase activity was generally greatest in the intermediate pH stream (Fig. 4). Xylanase activity exhibited

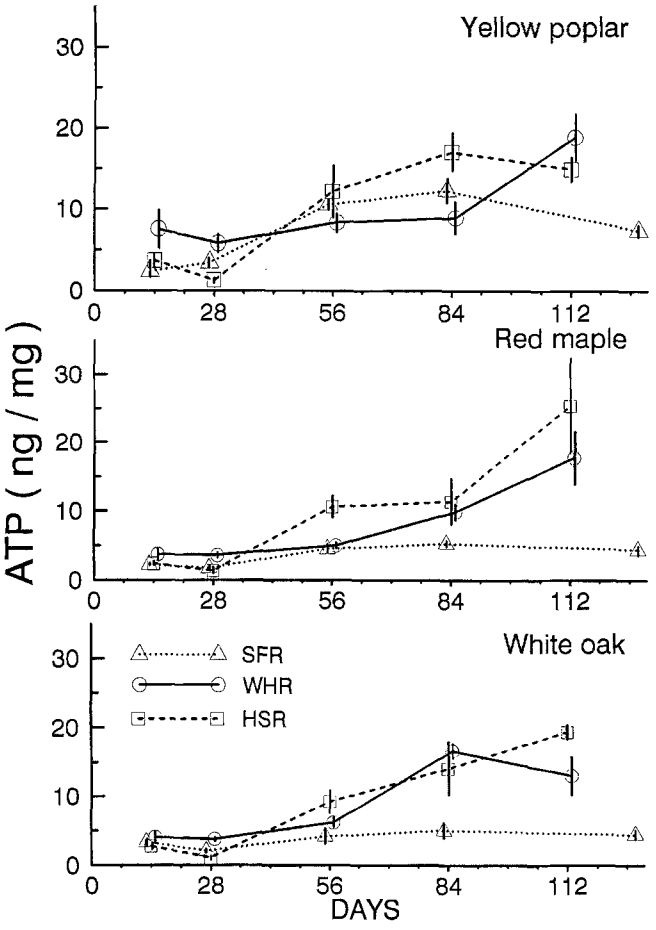


**Fig. 2** ATP concentration on leaf packs in the three study streams in Tucker County, West Virginia. The streams are South Fork of Red Run (*SFR*, pH=4.3), Wilson Hollow Run (*WHR*, pH=6.2), and North Fork of Hickman Slide Run (*HSR*, pH=7.7). *Error bars* represent ±1 SE

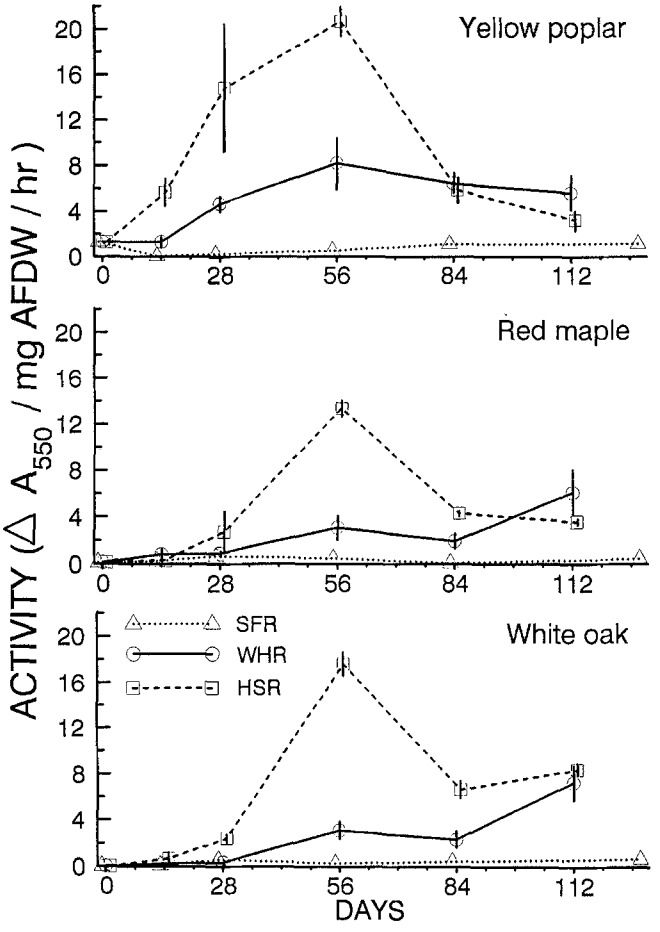


**Fig. 3** Pectinase activity on leaf packs in the three study streams in Tucker County, West Virginia. The streams are South Fork of Red Run (*SFR*, pH=4.3), Wilson Hollow Run (*WHR*, pH=6.2), and North Fork of Hickman Slide Run (*HSR*, pH=7.7). *Error bars* represent ±1 SE (*AFDW* ash-free dry weight of leaf tissue)

statistically significant stream, leaf species, and stream by leaf species effects (Table 3). On yellow poplar leaves, xylanase activity in WHR increased rapidly and remained much higher than in HSR and SFR (Fig. 4). On red maple leaves, xylanase activity in WHR did not increase as quickly and did not exceed that in HSR and SFR until after 56 days. On white oak leaves, xylanase activity was greater in HSR and SFR than in the other leaf species, and was generally more similar among all streams.

Exocellulase activity was generally greatest in the intermediate pH stream (Fig. 5). Exocellulase activity exhibited statistically significant differences only in the comparison among streams (Table 3) and was much lower than xylanase activity. The highest peak of exocellulase activity for all leaf species occurred in WHR at 56 days (Fig. 5). Exocellulase activity in SFR for all leaf species was generally intermediate between WHR and HSR, but the time of peak activity differed among leaf species. On yellow poplar and white oak in SFR, exocellulase activity increased quickly to a peak at 14–28 days, whereas on red maple in SFR, exocellulase activity increased more gradually to peak at 84 days. In HSR, exocellulase activity remained relatively low on red maple leaves and increased gradually on yellow poplar and white oak leaves to peak at 84 days.

Pearson correlation coefficients were calculated for ATP concentration and exoenzyme activity for each stream. Exocellulase activity was generally not correlated with ATP concentrations, except in the low pH stream where exoenzyme activity was often low. Exocellulase activity was not correlated with ATP concentration in any stream. Pectinase activity exhibited a low, but significant, correlation with ATP in SFR ($n$=15, $r$=0.60, $P$=0.02) and in WHR ($n$=15, $r$=0.54, $P$=0.03) but was not correlated in HSR ($n$=15, $r$=-0.14, $P$=0.63). Xylanase activity was highly correlated with ATP concentrations in SFR ($n$=15, $r$=0.84, $P$<0.01) but was not correlated in WHR ($n$=15, $r$=0.39, $P$=0.15) or in HSR ($n$=15, $r$=0.22, $P$=0.44).

## Discussion

Sinsabaugh et al. (1981) suggested two primary factors that contribute to the control of enzymatic degradation of leaf litter in streams: the initial chemical composition of

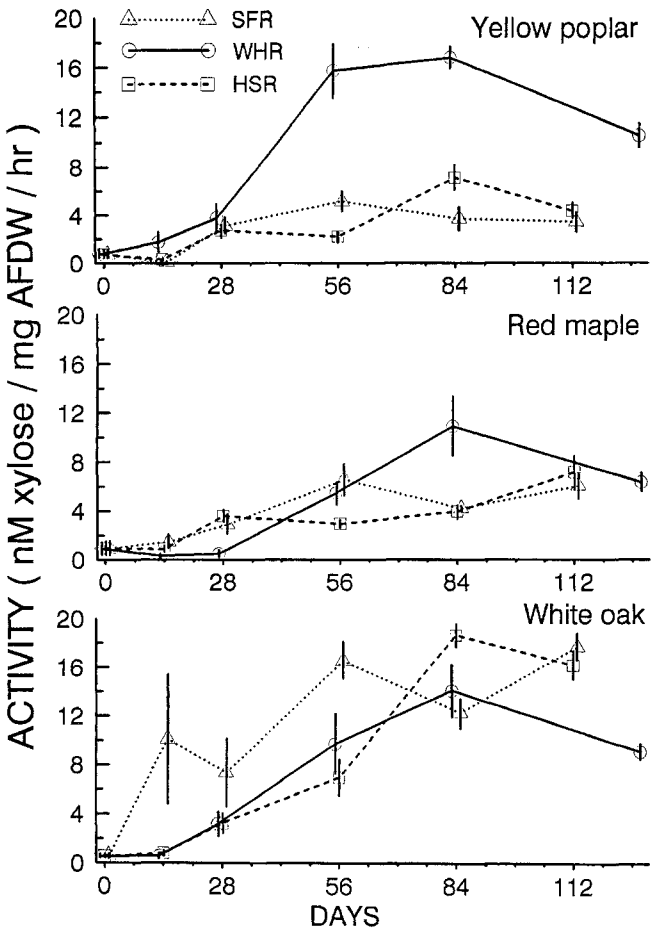


**Fig. 4** Xylanase activity on leaf packs in the three streams in Tucker County, West Virginia. The streams are South Fork of Red Run (*SFR*, pH=4.3), Wilson Hollow Run (*WHR*, pH=6.2), and North Fork of Hickman Slide Run (*HSR*, pH=7.7). *Error bars* represent ±1 SE

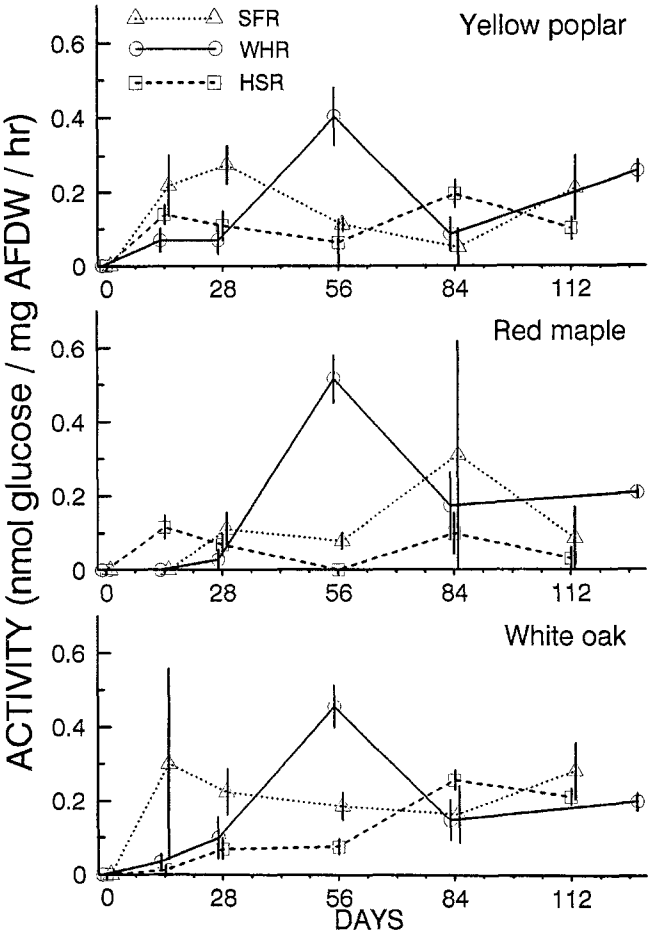


**Fig. 5** Exocellulase activity on leaf packs in the three streams in Tucker County, West Virginia. The streams are South Fork of Red Run (*SFR*, pH=4.3), Wilson Hollow Run (*WHR*, pH=6.2), and North Fork of Hickman Slide Run (*HSR*, pH=7.7). *Error bars* represent ±1 SE

the leaf detritus and environmental factors such as pH and temperature. The initial chemical composition, including the relative abundance of polysaccharides, proteins, and lignin, is thought to be a primary factor affecting the relative processing rates of different leaf species (Webster and Benfield 1986), but there is little information on the relative polysaccharide composition of deciduous leaves. The relative processing rates of white oak, red maple, and yellow poplar leaves that we measured in this study are similar to those found in the literature and to those we measured in previous studies in streams in or near the Fernow Experimental Forest (Griffith and Perry 1991; Griffith and Perry 1993). Sinsabaugh et al. (1981) found that among different leaf species, the same basic pattern occurred – increasing cellulase activity over time to a peak and then decreasing activity; the main difference among leaf species was in terms of the magnitude of cellulase activity. We found that, in general, the same pattern held among leaf species for pectinase, xylanase, and exocellulase activity.

Environmental factors such as water temperature and pH can also affect microbial exoenzyme activity and leaf processing rates in streams (Sinsabaugh et al. 1981; Chamier and Dixon 1982). Warmer water temperatures should increase enzymatic activity rates, and the faster processing rates in WHR and HSR relative to SFR were probably in part a result of warmer stream temperatures. Various microbial exoenzymes also differ in their pH activity optima. For pectinases, the optimum activity generally occurs above pH 7, whereas for exocellulases and xylanase, the optimum activity generally occurs below pH 6 (Dekker and Richards 1976; Rexova-Benkova and Markovic 1976; Sinsabaugh et al. 1981; Chamier 1987). In this study, exoenzyme activity generally reflected these pH optima. Pectinase activity was greater in HSR (pH 7.7), whereas exocellulase and xylanase activity were generally greater in WHR (pH 6.2) and in SFR (pH 4.3). Kok and Van der Velde (1991) found similiar patterns of exoenzyme activity in relation to pH on leaves of *Nymphaea alba* L. in laboratory experiments, but this has not been previously demonstrated in natural stream ecosystems. Processing rates were generally faster in WHR and in HSR than in SFR for white oak and red maple. In HSR, there was high activity by the pectinase, whereas in WHR there was significant activity by all three exoenzymes on leaves. Optimal pH levels could re-

sult in more efficient degradation of the complex polysaccharide matrix in the cell walls of the leaves (Chamier 1985; Kok and Van der Velde 1991). Yellow poplar leaves seem to be a possible exception to this trend. Processing rates (degree-day$^{-1}$) for yellow poplar were faster in SFR than in the other streams, but the leaves were not skeletonized like red maple and white oak leaves. This suggests that the importance of biological activity in processing of leaves relative to physical abrasion may differ among the leaf species.

In conclusion, leaf species and environmental factors interact to affect microbial exoenzyme activity and processing of leaf detritus in streams. Leaf species differences can affect the magnitude of exoenzyme activity, and although this may be related to the initial chemical composition of the leaves, more research is needed to ascertain the role of the leaf substrate in the induction of microbial exoenzyme activity. Various microbial exoenzymes also have pH optima, and differences in activity for pectinase, xylanase, and exocellulase among the streams were consistent with the published pH optima for these exoenzymes. While these differences were expected, this relationship has not been previously demonstrated in natural stream ecosystems. These factors can also affect microbial biomass as indicated by ATP concentrations, but the correlation between microbial biomass and exoenzyme activity was variable among streams. Differences in leaf processing rates both among leaf species and among streams seem to be related in part to the observed differences in microbial exoenzyme activity.

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