Fundamental and Applied Limnology
Archiv für Hydrobiologie
Vol. 173/1: 35–46, October 2008

Influence of shredder feeding and nutrients on fungal activity and community structure in headwater streams

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With 4 figures and 3 tables

Abstract: In stream detrital food webs, interactions occur between aquatic hyphomycetes associated with decomposing leaves and shredders consuming those leaves. However, few studies have examined how the feeding activity of shredders affects aquatic hyphomycetes. We examined the effect of shredder feeding on aquatic hyphomycete communities associated with submerged leaves in two southern Appalachian headwater streams in Coweeta Hydrologic Laboratory, North Carolina, USA. Coarse (allowing shredder access) and fine (preventing shredder access) mesh litter bags containing red maple (*Acer rubrum*) leaves were placed in the treatment stream (C54) which was enriched with nitrogen (N) and phosphorus (P), and in the reference stream (C53) and were retrieved monthly. Both shredder feeding and nutrient enrichment enhanced breakdown rates. The breakdown rates of leaves in coarse mesh bags in the reference stream ($k = 0.0275$) and fine mesh bags in the nutrient enriched stream ($k = 0.0272$) were not significantly different, suggesting that the higher fungal activity stimulated by nutrient enrichment could increase the relative contribution of fungi to leaf breakdown to the level similar to that of shredders in the reference stream. Macroinvertebrate abundance and biomass were higher in the litter bags submerged in the treatment stream. Fungal sporulation rates and biomass were higher in the treatment stream than in the reference stream, but neither fungal biomass nor sporulation rate was affected by shredder feeding in either stream. The enrichment with N and P altered fungal community composition more than shredder feeding. Species richness was higher in the nutrient enriched stream than in the reference stream, and fungal assemblages from fine and coarse mesh bag treatments within a stream were more similar to each other than the fungal assemblages from the same mesh bag treatments but from different streams.

Key words: Shredder feeding, nutrient enrichment, aquatic hyphomycetes, fungal biomass, sporulation rate, fungal community structure.

Introduction

Terrestrial plant litter forms a major part of the energy resource of forested headwater streams. The breakdown of this allochthonous organic matter, especially leaf litter, has been recognized as a vital process in the functioning of these stream ecosystems (Cummins 1988). For example, the total abundance of benthic invertebrates, especially shredders, in 'mixed substrate habitats' (heterogeneous mixture of cobbles, pebbles, sand, etc.) in the stream in which plant litter was excluded were less than 10 % of the values in a reference stream that received normal litter inputs (Wallace et al. 1997). Furthermore, total secondary production of benthic invertebrates in the treatment stream was reduced to 22 % of pretreatment values (Wallace et al. 1999).

Among the heterotrophic microorganisms colonizing submerged leaves, fungi known as aquatic hyphomycetes play a key role in litter decomposition (Bärlocher & Kendrick 1981, Suberkropp 1998). They are the predominant microorganisms associated with leaves with respect to biomass and production (Baldy et al. 1995, Weyers & Suberkropp 1996). Fungal colonization or conditioning of decaying leaves affects the

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DOI: 10.1127/1863-9135/2008/0173-0035 1863-9135/08/0173-0035 $ 3.00

nutritional value and palatability of the leaves to shredders (Suberkropp et al. 1983, Arsuffi & Suberkropp 1986, Graça et al. 1993). When a leaf with patches colonized by different fungal species was offered to limnephilid caddisflies, they discriminated among different fungal-colonized patches (Arsuffi & Suberkropp 1985). These observations suggest that fungal species composition also affects shredder feeding.

Few studies have examined the effect of macroinvertebrate feeding on the fungal community colonizing leaves (Suberkropp 1992). Shredder feeding reduced the species richness of aquatic hyphomycete communities associated with oak and larch leaves in coarse mesh litter bags, suggesting that shredders act as competitors of late-colonizing fungi by consuming leaf mass (Bärlocher 1980). Sporulation rates of fungi on leaves in coarse mesh bags that allowed shredder feeding were lower than on leaves in fine mesh bags that prevented shredder access (Bärlocher 1982). In a whole stream experiment which applied insecticide to eliminate shredders, the concentrations of fungal conidia in the water of the insecticide-treated stream were higher than in untreated streams (Suberkropp & Wallace 1992). These results suggest that leaf-eating shredders can affect the aquatic hyphomycetes associated with decomposing leaves as well.

A number of studies have indicated that the activity of fungi associated with leaves can be affected by nutrient concentrations in the water (Suberkropp 1995, Suberkropp & Chauvet 1995, Grattan & Suberkropp 2001, Gulis & Suberkropp 2003c). The addition of nutrients to streams whose ambient nutrient concentrations were low also affected fungal community structure, resulting in an increase of fungal species richness and a change in their relative abundances (Gulis & Suberkropp 2004). Robinson & Gessner (2000) suggested that the higher shredder abundance and biomass on leaves in coarse-mesh litter bags which had been enriched with nitrogen and phosphorus using fertilizer briquettes were due to higher-quality resources resulting from the nutrient addition. However, the fungal biomass and sporulation, which might have been stimulated, were not significantly different among treatments. This suggests that feeding of shredders removed elevated fungal growth and resulted in similar fungal biomass in both fertilized and unfertilized coarse mesh bags. These findings imply that nutrient enrichment and shredder feeding may change the pattern of litter breakdown and the accrual of fungal biomass through the interaction between fungi and shredding invertebrates on decomposing leaves.

The objectives of the present study were to examine how shredder feeding affects leaf breakdown and alters the pattern of fungal activities under different levels of nutrient concentrations. Leaf breakdown rates, fungal biomass, and sporulation rates were measured using coarse (allowing shredders access to leaves) and fine (preventing shredder access) mesh litter bags containing maple leaves. Litter bags were placed in two forested headwater streams whose physical and chemical characteristics were similar except their concentrations of nitrogen and phosphorus. The species richness and community structure of aquatic hyphomycetes on decomposing leaves were determined from each treatment (mesh size × nutrient level). The abundance and biomass of macroinvertebrate functional feeding groups collected from the coarse mesh bags in each stream were determined as well. We hypothesized that elevated nutrient concentrations would increase the leaf breakdown rates, fungal biomass, and sporulation rates on leaves and that shredder feeding would lower fungal biomass accrual and sporulation rate while accelerating leaf breakdown in coarse mesh bags when compared with those values in the fine mesh bags in a stream. We expected the community structure of aquatic hyphomycetes on decomposing leaves to be affected by both nutrient availability and shredder feeding.

Study sites

The study was conducted in two first-order streams at the Coweeta Hydrologic Laboratory of the United States Forest Service (Macon County, North Carolina, USA) within the Blue Ridge Mountains Physiographic Province of the southern Appalachian Mountains. The streams drain south-facing slopes of forested catchments (C) 53 and 54, which were assigned as the reference and the treatment stream respectively. Mixed deciduous species predominate in the canopy. The dense evergreen undergrowth of rhododendron (*Rhododendron maximum*) along stream corridors provides year-round shading over the streams (Swank & Crossley 1988). Since the "double canopy" of deciduous vegetation and rhododendron limits light input, the main energy source of the stream ecosystems is composed of allochthonous organic matter and associated microbial assemblages (Hall et al. 2000). The streams are small (mean discharge 1.2–1.5 l/s), circumneutral (pH 6.6–6.9), and softwater (ion concentration < 1 mg/l), having very low natural concentrations of inorganic N ($[NO_3 + NO_2]$-N: 17 µg/l, NH_4-N: 10 µg/l) and P (soluble reactive phosphorus [SRP]: 4 µg/l) (Cuffney et al. 1990, Greenwood & Rosemond 2005, Cross et al. 2006).

The reference stream (C53) and the treatment stream (C54) drain adjacent watersheds and have similar physical and chemical characteristics (Lugthart & Wallace 1992), except nutrient concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) due to the experimental nutrient enrichment of C54. Along the entire study reach of the treatment stream, a dissolved nutrient solution of NH_4NO_3, K_2HPO_4, and KH_2PO_4 was continuously added to the stream water. The nutrient solution was fed through multiple spigots in a pipe, which

was laid on the streambed and fed with stream water and a concentrated nutrient solution using a metering pump (Liquid Metronics, Inc., USA) to control the rate of nutrient addition into the pipe proportional to instantaneous discharge since July 2000. Refer to Gulis & Suberkropp (2003c) and Greenwood & Rosemond (2005) for detailed description of nutrient enrichment. Water samples for the measurement of nutrient concentrations were taken from both streams at 2-week intervals and were analyzed at the Chemical Analysis Laboratory in the Institute of Ecology, University of Georgia (Athens, Georgia, USA). During 2003–2004, the average nutrient concentrations in the treatment stream were 557 ± 357 µg/l (range: 2–1790 µg/l) for nitrate-nitrogen ($[NO_3 + NO_2]$-N), 103 ± 105 µg/l (range: below detection – 601 µg/l) for ammonium-nitrogen (NH_4-N), and 106 ± 71 µg/l (range: 6–357 µg/l) for SRP. The reference (average of C53 and upper control reach of C54) nutrient concentrations were 28 ± 39 µg/l (range: 1–219 µg/l) for nitrate-nitrogen, 11 ± 10 µg/l (range: 1–45 µg/l) for ammonium-nitrogen, and 19 ± 43 µg/l (range: 0– 23 µg/l) for SRP (A. D. Rosemond; pers. com.).

Material and methods

Leaf mass loss

Coarse and fine mesh litter-bags were deployed to measure leaf breakdown rates in the reference and the treatment stream while allowing (coarse mesh) or preventing (fine mesh) the access of macroinvertebrates to the leaf packs during November 17, 2003 – April 23, 2004. The conventional method using different mesh sizes could also affect flow and sedimentation pattern within litter-bags. Hence, the bags were placed in the marginal area of pools to minimize the effects of differences in water flow through the different mesh sizes. Both types of bags accumulated similar amount of sediments; however, the authors cannot totally eliminate these physical effects from those of shredder feeding. Red maple (*Acer rubrum*) leaves were collected after abscission along the stream banks in October 2003 and air-dried for 2 weeks. About 2 (± 0.1) g of air-dried leaves were weighed (PM600, Mettler Toledo, USA) after removing petioles. Preweighed leaves were soaked in distilled water for 2 h to impede fragmentation of dried leaves while preparing litter bags 1 d prior to being submerged in both streams. Wet leaves were placed in either coarse (15 × 35 cm, 5 mm mesh) or fine (14 × 18 cm, 1 mm mesh) mesh litter bags and labeled. A total of 20 coarse mesh and 23 fine mesh litter bags were prepared to calculate leaf mass loss rates in each stream. After bags were placed in the streams, 5 coarse and 5 fine mesh litter bags were retrieved immediately and used to determine a conversion factor to calculate ash-free dry mass (AFDM) from initial air-dry mass. Five coarse mesh and six fine mesh litter bags were grouped and anchored to the stream beds with nails at 15–20 m intervals on November 17, 2003. Subsequent samples of 3 litter bags of all four treatments were taken at 30, 59, 95, and 123 d from both streams (only 2 fine mesh bags were retrieved from C53 at 123 d). At 155 d, only 3 fine mesh litter bags from C53 could be sampled due to rapid breakdown and heavy loading of fine sediments. On each sampling date, retrieved leaves and fragments were removed from the litter bags, placed in a pan, rinsed gently with stream water to remove fine detritus, silt, and macroinvertebrates. Leaves retrieved were returned to the laboratory on ice. Leaves were dried at 60 °C for 3 d, weighed, combusted at 500 °C overnight, and re-weighed (AE163, Mettler Toledo, USA) to determine AFDM. Leaf breakdown rates were estimated based on the exponential decay model (Bärlocher 2005). Remaining material (fine detritus and silt) and organisms in the pan, which were retrieved from coarse mesh bags, were further processed as described below for macroinvertebrates.

Fungal activity

Additional coarse and fine mesh litter bags (15 and 18, respectively) for each stream, which were not preweighed, were used to determine fungal activity and fungal species composition. These litter bags were placed on both stream beds as described above. Three coarse and three fine mesh bags were retrieved after 0, 30, 59, 95, and 123 d from each stream. Leaves were rinsed with stream water, and leaf disks (15 mm diameter) were cut with a cork borer avoiding main veins. Leaf disks could not be cut when the leaves in mesh bags from both streams were almost fully skeletonized. Sporulation rate, community structure and biomass of aquatic hyphomycetes, and leaf disk AFDM were determined from subsets of leaf disks.

Ten leaf disks from each litter bag were placed in plastic containers filled with stream water and returned to the laboratory on ice. Leaf disks were transferred to autoclaved glass sporulation chambers (Suberkropp 1991), with 40 ml of filtered stream water (0.7 µm pore size, 47 mm diameter, GF/F, Whatman, USA). A total of 12 sporulation chambers (3 per treatment) were aerated continuously at the rate of 80–100 ml/min at 15 °C for 2 d to induce the production of conidia. The suspension of conidia in each sporulation chamber was drained into a 100 ml beaker and 100 µl of 0.5 % Triton-X 100 solution (ICN Chemical and Radioisotope Division, USA) was added while stirring the suspension gently to distribute conidia evenly. An aliquot of 2 ml of the suspension was filtered through a membrane filter (8 µm pore size, 25 mm diameter, Millipore, USA). Conidia retained on the filters were stained with 0.1 % trypan blue in lactic acid, and identified and counted under the microscope (Laborlux K, Leitz, Germany). Twenty to fifty fields of each membrane filter were examined at 160× magnification to calculate sporulation rates (S, conidia·mg^{-1}·d^{-1}) according to Gulis & Suberkropp (2006):

$$S = \frac{n \cdot A_e \cdot V_c}{f \cdot A_m \cdot V_a \cdot m \cdot t}$$

where n is the number of conidia counted, f is the number of fields examined, A_e is effective filtered area (mm^2), A_m is microscopic field area (mm^2), V_c is the volume of conidia suspension in a sporulation chamber (ml), V_a is the volume of aliquot filtered (ml), m is the AFDM of 10 leaf disks (mg), which was estimated from the AFDM of separate subsets of 5 leaf disks, and t is sporulation time (d). Fungal conidia were identified to species level using their characteristic morphology (Ingold 1975, Gulis et al. 2005) to determine community structure of aquatic hyphomycetes. Bray-Curtis's percent similarity indices (SI) were calculated as:

$$SI = \frac{2\sum_{k=1}^{s} \min(x_{ik}, x_{jk})}{\sum_{k=1}^{s} (x_{ik} + x_{jk})}$$

where s is the number of fungal species found in the suspension of conidia, and x_{ik} and x_{jk} are the relative abundance of species k in two samples i and j being compared, respectively (Bray & Curtis 1957, Gauch 1973).

Five leaf disks from each treatment were placed in 5 ml of methanol (HPLC grade, Fisher Scientific, USA), transported to the laboratory on ice, and stored at –20 °C in the dark until processed. Ergosterol concentration was determined to estimate fungal biomass following a liquid-to-liquid extraction method (Newell et al. 1988, modified by Suberkropp & Weyers 1996). Ergosterol was extracted and saponified by refluxing leaf disks in alcoholic KOH, the lipid fraction was partitioned into pentane which was evaporated to dryness and redissolved in methanol. The ergosterol extract was filtered (0.2 µm pore size, 13 mm diameter, Acrodisk PTFE, Gelman Laboratory, USA) and stored at –20 °C until quantified. The concentration of ergosterol in the samples was determined using high performance liquid chromatography (HPLC; LC-10AS, Shimadzu, Japan) by comparing the UV absorbance at 282 nm with that of a series of standard ergosterol concentrations (95 % HPLC grade, Fluka, Switzerland). A simple linear regression model was developed with peak areas of standard ergosterol concentrations, and the concentrations of samples were calculated. Fungal biomass associated with leaf materials, as mg mycelial dry mass (DM) per g leaf AFDM, was calculated using the conversion factor 5.5 µg ergosterol/mg mycelial DM (Gessner & Chauvet 1993). See Gulis & Suberkropp (2006) for detailed protocols.

On each sampling date, subsets of 5 leaf disks from each stream and mesh bag treatment with three replicates were used to determine leaf disk AFDM according to the protocol described above. The calculations of sporulation rate per mg leaf disk AFDM and fungal biomass per g leaf disk AFDM were based on these leaf disk mass determinations.

Macroinvertebrates

Upon retrieving the mesh bags from both stream beds, each mesh bag was put in a plastic bag and washed with stream water into a pan. After collecting leaves and leaf fragments from the coarse-mesh bags to measure leaf mass loss rates, remaining fine detritus, silt, and organisms in the pan were washed through a 250 µm sieve to remove silt and fine detritus. The material retained on the sieve was preserved in 95 % ethanol. In the laboratory, macroinvertebrates larger than 1 mm were picked and the remaining material was examined and sorted under a dissecting microscope at the magnification of 64× (Wild M38, Heerbrugg, Switzerland). All macroinvertebrates removed were preserved in 80 % ethanol, counted, and identified to the genus, except for several families of Diptera (Merritt & Cummins 1996). The body lengths of macroinvertebrates were measured to the nearest millimeter to determine the biomass of individual larvae (mg AFDM) using previously established taxon-specific body length-mass relationships (Benke et al. 1999). Macroinvertebrates were further classified into functional feeding groups (FFG) according to Merritt & Cummins (1996) and Wallace et al. (1999). Functional feeding groups designated include scrapers (SC), collector-gatherers (CG), collector-filterers (CF), shredders (SH), and predators (PR). Larval chironomids were classified as either predators (Tanypodinae) or collector-gatherers (non-Tanypodinae). Both abundance and biomass of macroinvertebrates collected were expressed as per bag or per g leaf AFDM remaining. A few individuals of chironomids were the only macroinvertebrates found in the fine-mesh bags, and other groups of macroinvertebrates included in CF, SH, and PR were not observed. Hence, macroinvertebrates from the fine-mesh bags were not used for further analysis.

Statistical analysis

Breakdown rates (k) of leaf mass were estimated using a linear regression model of ln transformed fractions of leaf AFDM remaining at each sampling date. Differences in breakdown rates were determined with analysis of covariance (ANCOVA) with time as the covariate. Fungal biomass and sporulation rates (mesh size × stream), and macroinvertebrates abundance and biomass (FFG × stream) were compared with a 2-way analysis of variance (ANOVA), or with a two-sample t-test when applicable. A Tukey's multiple-comparisons test was followed to compare the breakdown rates, fungal activities among treatments, or macroinvertebrates abundance and biomass, if the differences in ANCOVA or ANOVA test were significant ($P < 0.05$). The rapid skeletonization of red maple leaves in the coarse mesh bags in both streams as well as in the fine mesh bags in C54 allowed sampling leaf disks for the determination of fungal activities in all four treatments only at 30 d; hence the statistical analyses for fungal biomass and sporulation rates were made only at 30 d (ANOVA for all four treatments) and 59 d (t-test for fine mesh bag treatments only). Data for sporulation rates, and the abundance and biomass of macroinvertebrates were ln $(x + 1)$ transformed to comply with the assumption of normality. All statistical analyses were done with SYSTAT 10 (SPSS Inc. 2000).

Results

Leaf breakdown

The breakdown rates (k) of red maple leaves were affected by both mesh size of litter bags and nutrient concentrations in the streams (ANCOVA, $F_{3,34} = 6.77$, $P = 0.001$; Fig. 1). In both streams, the breakdown rate of maple leaves in coarse mesh bags was two to three times faster than the rate in fine mesh bags (Tukey, $P = 0.003$ for both streams; Table 1). The breakdown rate of leaves in the coarse mesh bags from the treatment stream (C54 Coarse) was about two times greater than the breakdown rate of the leaves in coarse mesh bags in the reference stream (C53 Coarse; Tukey, $P = 0.002$). The leaves in the fine mesh bags from the treatment stream (C54 Fine) exhibited a breakdown rate that was about three times faster than the leaves in fine mesh bags from the reference stream (C53 Fine; Tukey, $P = 0.011$; Table 1). There was no significant difference in the breakdown rates between C53 Coarse and C54 Fine treatments (Tukey, $P = 0.999$). Most of the leaf mass (95 %) was lost from C54 Coarse within 60 d and from C53 Coarse and C54 Fine treatments within 110 d (Table 1).

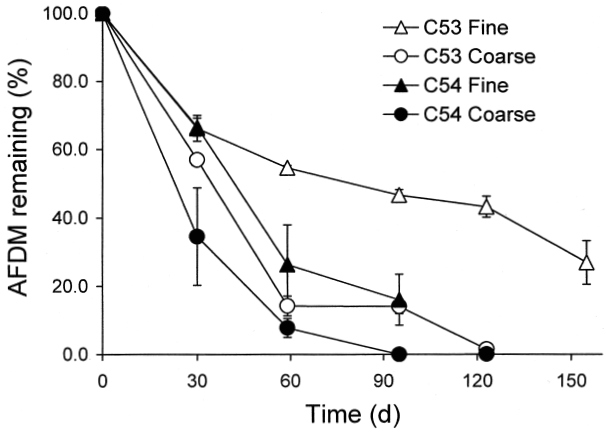

Fig. 1. Percentage of red maple leaves AFDM (mean ± 1 SE, $n = 3$; fine mesh bags from C53 at 123 d, $n = 2$) remaining at each sampling date. Leaves were retrieved from fine (triangles) and coarse (circles) mesh litter bags deployed in the reference (C53, open symbols) and the treatment (C54, closed symbols) stream during November 2003 – April 2004. Data for the fine mesh bags from C54 at 123 d were excluded as outliers.

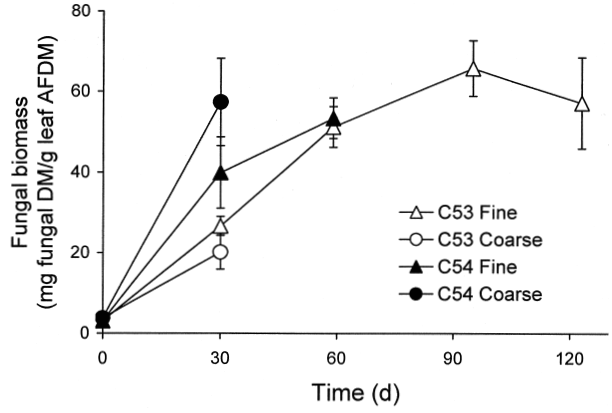

Fig. 2. Fungal biomass (mean ± 1 SE, $n = 3$) estimated from ergosterol concentrations of aquatic hyphomycetes associated with red maple leaves collected from fine (triangles) and coarse (circles) mesh litter bags submerged in the reference (C53, open symbols) and the treatment (C54, closed symbols) stream. Ergosterol associated with leaves in coarse mesh bags from both streams at 59 d was not measured due to extensive skeletonization of leaves.

Table 1. Breakdown rates (k) with 95 % confidence limits (CL) and r^2 values of the regression lines for red maple leaves in each mesh size litter bags and reference (C53) or treatment (C54) stream combination. T_{95} is the estimated number of days to 95 % mass loss.

Treatment	k (d^{-1}) ± 95 % CL	r^2	T_{95} (d)	n
C53 Fine	0.0080 ± 0.0017[a]	0.85	374	19
C53 Coarse	0.0275 ± 0.0052[b]	0.90	109	16
C54 Fine	0.0272 ± 0.0150[b]	0.57	110	14[†]
C54 Coarse	0.0496 ± 0.0164[c]	0.82	60	12[‡]

Note: Significant differences ($P < 0.05$) of breakdown rates in multiple comparisons using Tukey test were indicated by different superscript letters. All P values for the regression lines were < 0.01.
[†] Two data at 123 d were excluded as outliers.
[‡] Five data were excluded due to 0 % remaining at 95 and 123 d.

Fungal biomass

Fungal biomass was greater in the leaves submerged in the nutrient-enriched stream (C54) than the leaves in the reference stream (C53) at 30 d (ANOVA, $F_{1,8} = 11.69$, $P = 0.009$), while the differences between coarse and fine mesh bag treatments within a stream were not significant (ANOVA, $F_{1,8} = 0.55$, $P = 0.481$; Fig. 2). Fungal biomass of the C54 Coarse treatment was about three times greater than the value of the C53 Coarse treatment (Tukey, $P = 0.030$) at 30 d, but the difference between the C54 Fine and C53 Fine treatments was not significant either at 30 d (Tukey, $P = 0.605$) or at 59 d ($t_4 = 0.30$, $P = 0.776$; Fig. 2).

Sporulation rates and fungal community structure

At 30 d, sporulation rates of aquatic hyphomycetes on the leaves in the treatment stream were higher than rates on leaves from the reference stream (ANOVA, $F_{1,8} = 29.85$, $P < 0.001$), but the differences between coarse and fine mesh bags within the same stream were not significant (ANOVA, $F_{1,8} = 1.65$, $P = 0.235$; Fig. 3). Aquatic hyphomycetes associated with the leaves in the C54 Coarse treatment produced about 9 times more conidia than the leaves in the C53 Coarse treatment (Tukey, $P < 0.01$). About 4 times more conidia were produced in the C54 Fine treatment than in the C53 Fine treatment (Tukey, $P < 0.05$; Fig. 3) at 30 d. The difference in sporulation rates of C53 Fine and C54 Fine treatments at 59 d was not significant ($t_3 = 1.88$, $P = 0.147$), although the sporulation rate of C54 Fine treatment was about 2 times higher than the value of C53 Fine treatment (Fig. 3).

Eleven and fifteen species of aquatic hyphomycetes were identified from the leaf disks sampled from the reference and the treatment stream, respectively, with higher taxa richness in the fine mesh bag treatments than the coarse mesh bag treatments in both streams (Table 2). The dominant species, whose mean relative abundance was greater than 5 % in any treatment, include *Alatospora acuminata*, *Anguillospora filiformis*, *Articulospora tetracladia*, *Tetrachaetum elegans*, and *Tricladium chaetocladium*; their combined mean relative abundance was over 66 % in every treatment

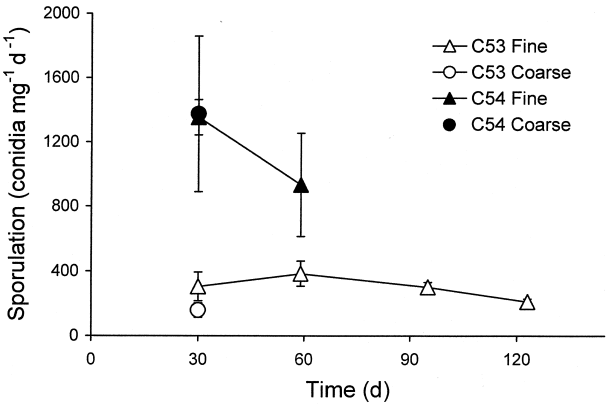

Fig. 3. Sporulation rates (mean ± 1 SE) of aquatic hyphomycetes associated with red maple leaves in fine (triangles) and coarse (circles) mesh litter bags deployed in the reference (C53, open symbols) and the treatment (C54, closed symbols) stream. Data were not taken for C53 and C54 coarse mesh bags at 59 d, and C54 fine mesh bags at 95 and 123 d due to almost completely skeletonized leaves.

(Table 2). When the relative abundance of sigmoid conidia (<60 µm) was added to that of *A. acuminata* (Gulis & Suberkropp 2004), the combined mean relative abundance of dominant fungal species exceeded 80 % in all treatments. The difference in taxa richness between the coarse and fine mesh bag treatments in the nutrient-enriched stream was greater than the difference in the reference stream (Table 2).

The addition of nutrients changed relative abundances among dominant species in the treatment stream. In the reference stream, *A. acuminata*, *A. filiformis*, *A. tetracladia*, and *T. elegans* comprised about 70 % of mean relative abundance of fungal conidia released from the aquatic hyphomycetes associated with red maple leaves. In the treatment stream, the relative abundances of *A. acuminata*, *A. filiformis*, and *A. tetracladia* were lower, whereas the values of *T. elegans* and *T. chaetocladium* were higher than in the reference stream for both coarse and fine mesh bags (Table 2). *Alatospora acuminata*, when not combined with small sigmoid conidia (<60 µm), occupied less than 5 % of total abundance through all sampling dates in both fine and coarse mesh bags in the treatment stream (Table 2). The relative abundance of *A. tetracladia* was lower in C54 Fine than in C53 Fine treatment (Table 2). In contrast, the values of *T. elegans* in both mesh bag treatments from the treatment stream were greater than the reference stream, with the greatest difference at 30 d. Nutrient enrichment also induced the earlier sporulation and codominance of *T. chaetocladium* in the treatment stream (data not presented). As a result, the most abundant fungal species was *A. acuminata*,

Table 2. Mean relative abundance (%) of conidia released from aquatic hyphomycetes on red maple leaves in coarse and fine mesh bags from the reference (C53) and the treatment (C54) stream over all sampling dates.

Fungal Species	C53 Fine	C53 Coarse	C54 Fine	C54 Coarse
Alatospora acuminata	20.4	19.8	2.4	3.8
Anguillospora filiformis	7.4	22.7	8.4	12.5
Articulospora tetracladia	25.9	16.0	19.0	14.5
Flagellospora curvula	2.3	4.2	2.0	<0.1†
Goniopila monticola			0.1†	
Heliscus lugdunensis			0.2†	
Lunulospora curvula	1.1		0.3	0.4†
Mycofalcella calcarata	0.3†		3.2	
Tetrachaetum elegans	15.5	12.7	27.8	36.6
Tricladium chaetocladium	0.3	1.8	8.6	20.0
Triscelophorus sp.			0.1†	
Unidentified tetracladiate			0.1†	
Sigmoid (<60 µm)‡§	10.4	8.9	15.8	4.0
Sigmoid (60 – 120 µm)‡	9.5	7.5	9.0	6.7
Sigmoid (>120 µm)‡	7.0	6.6	3.1	1.5
Total sample size (*n*)	12	4	8	4
Total number of taxa	11	9	15	10

† Appeared in one sample with negligible numbers.
‡ Sigmoid conidia having similar morphologies often cannot be identified without observing conidiogenesis (Gulis et al. 2005).
§ Data for *A. acuminata* and small sigmoid (<60 µm) conidia were combined for further analysis because numerous isolates of the small sigmoid conidia appeared to be *A. acuminata*. Aquatic hyphomycete species, however, having truly filiform conidia could have been present (Gulis & Suberkropp 2004).

when small sigmoid conidia (<60 µm) were included as this species, in the reference stream and was *T. elegans* in the treatment stream (Table 2).

Similarity indices (*SI*) calculated from the relative abundances of fungal species indicated that the community structure of aquatic hyphomycetes associated with red maple leaves in the reference stream (C53) differed from that in the treatment stream (C54) at 30 d (Table 3). Similarity indices between the fine and the coarse mesh bags in each stream (C53: $SI = 0.752$, C54: $SI = 0.887$) were higher than the values comparing the fungal assemblages from the reference and the treatment streams (fine: $SI = 0.595$, coarse: $SI = 0.591$).

Macroinvertebrates

A total of 24 and 32 taxa over all sampling dates, and 8 and 20 taxa of macroinvertebrates at 30 d were collected from the reference (C53) and the treatment (C54) streams, respectively (data not presented). Total invertebrate abundance associated with red maple leaves in the coarse mesh bags from the treatment stream at 30 d was about 8 times greater per bag (ANOVA, $F_{1,20} = 8.48$, $P = 0.009$) or 16 times greater per g leaf AFDM remaining (ANOVA, $F_{1,20} = 13.33$, $P = 0.002$) than the values from the reference stream (Fig. 4 A–B). At 30 d in either stream, collector-gatherers and shredders

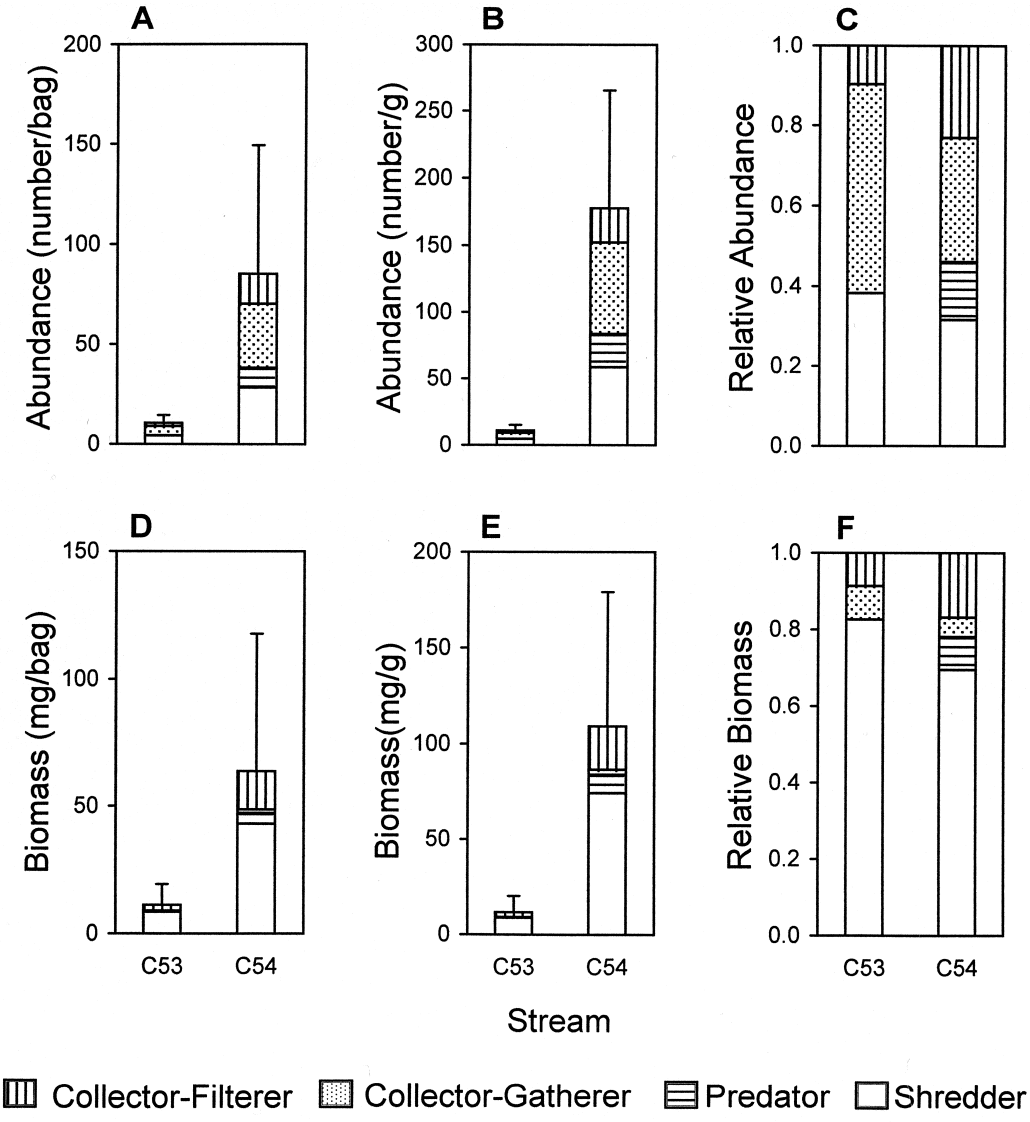

Fig. 4. The average and relative abundance (**A**–**C**) and biomass (**D**–**F**) of macroinvertebrate functional feeding groups collected from the coarse mesh bags in the reference (C53) and the treatment (C54) streams at 30 d ($n = 3$). Average abundance and biomass values are presented as either per bag (**A** and **D**) or per g leaf AFDM remaining (**B** and **E**). Error bars indicate 1 standard error of total abundance or biomass of all functional feeding groups.

accounted for more than 60 % of relative abundance (Fig. 4 C). Non-Tanypodinae chironomids (CG) and *Pycnopsyche* (Limnephilidae, SH) comprised 66 % of relative abundance in the reference stream, and 44 % in the treatment stream. In the reference stream, *Lepidostoma* (Lepidostomatidae, SH), *Soyedina* (Nemouridae, CG), *Amphinemura* (Nemouridae, CG), and *Diplectrona* (Hydropsychidae, CF), and in the treatment stream, *Tallaperla* (Peltoperlidae, SH), *Dicranota* (Tipulidae, PR), *Wormaldia* (Philopotamidae, CF), *Dolophilodes* (Philopotamidae, CF), and *Dixa* (Dixidae, CF) were co-dominant with the two predominant taxa (data not presented).

Nutrient enrichment also induced significant differences in total biomass of macroinvertebrates in the litter bags at 30 d either per bag (ANOVA, $F_{1,20} = 4.40$, $P = 0.049$) or per g leaf AFDM remaining (ANOVA, $F_{1,20} = 9.87$, $P = 0.005$). Total invertebrate biomass in the treatment stream was about 6 times (per bag) or 9 times (per g leaf AFDM remaining) greater than the biomass in the reference stream (Fig. 4 D–E). The pattern of distribution of functional feeding groups in biomass was different from the distribution in abundance. In comparison to relative abundance, shredder biomass alone accounted for about 83 % of relative biomass in the reference stream, and 70 % in the treatment stream; collector-gatherers accounted for about 9 % and 5 % relative biomass in the reference and the treatment stream, respectively (Fig. 4 F). *Pycnopsyche*, *Lepidostoma*, and *Tallaperla* were dominant taxa, with smaller contributions of *Parapsyche* (Hydropsychidae, CF) and *Soyedina* in the reference stream. In the treatment stream, *Tallaperla* and *Pycnopsyche* were two dominant taxa in the basis of relative biomass, followed by *Diplectrona* and *Wormaldia* (data not presented).

Eight taxa of predators were found in the treatment stream, which accounted for 14.5 % of relative abundance and 5.1 % of relative biomass at 30 d (Fig. 4 C, F) and through all sampling dates. In the reference stream, however, predators were not found at 30 d, but appeared at later sampling dates (data not presented). Scrapers were not found in either stream at 30 d.

Discussion

Effects of nutrients on leaf breakdown rates, fungal activity, and macroinvertebrates

The breakdown of red maple leaves was stimulated by enrichment with nitrogen and phosphorus in the treatment stream. Previous studies have indicated variable effects of enriched nutrient concentrations on litter breakdown. Elwood et al. (1981) reported that red oak leaf packs were broken down 24 % faster in a reach of a second-order woodland stream in Tennessee which was experimentally enriched with phosphorus, than in the upstream control reach (but see Peterson et al. 1993). In the same stream, however, enrichment with ammonium did not stimulate leaf breakdown (Newbold et al. 1983), suggesting P limitation of leaf processing in the stream. In a study comparing the decomposition of black locust and sweet birch leaves in two streams (undisturbed and disturbed) in North Carolina, Meyer & Johnson (1983) found 2.8× more rapid breakdown of both leaf species in the disturbed stream having higher nitrate concentrations. Leaf breakdown was stimulated in a second-order Alaskan stream which was fertilized with N and P (Benstead et al. 2005) and in several instream mesocosms in Alabama that received enrichment with either P or both N and P (Grattan & Suberkropp 2001). In contrast, Triska & Sedell (1976) reported that the decomposition rates of four leaf species were not significantly related to nitrate addition of three to four times ambient concentration, which simulated the impact of common forest management practices such as logging or nitrogen fertilization, in three (control, intermittent N input, continuous N input) streams in the Cascade Mountains, Washington (but see Ferreira et al. 2006). However, evidence that the breakdown rates of leaves are significantly correlated with nutrient concentrations (Suberkropp & Chauvet 1995, Niyogi et al. 2003, Gulis et al. 2006) suggests that leaf breakdown in streams can be affected by the availability of nutrient(s) in the water particularly if ambient concentrations are low.

The results of the present study are in agreement with previous studies examining the effect of nutrient enrichment on the breakdown of red maple and rhododendron leaves (Gulis & Suberkropp 2003c, Greenwood et al. 2007) as well as wood (Gulis et al. 2004) in Coweeta streams. In a study examining the effect of dissolved nutrients on heterotrophic biofilms of poplar veneer in C53 using nutrient-releasing substrates (agar containing N and/or P), only N + P enrichment significantly increased fungal biomass. Neither N nor P addition alone stimulated the increase in fungal biomass (Tank & Webster 1998). These studies indicate that the activity of aquatic hyphomycete communities, which play a major role in total microbial activity and decomposition (e.g., Weyers & Suberkropp 1996, Gulis & Suberkropp 2003a, c), is likely colimited by nitrogen and phosphorus in these Coweeta streams.

Fungal biomass in the reference stream accounted for 2.7 and 2.0 % of maple leaf mass remaining in fine and coarse mesh bags, respectively at 30 d. The values in the nutrient enriched stream were 4.0 % in the fine mesh bags and 5.7 % in the coarse mesh bags. Sporulation rates on the leaves in fine and coarse mesh bags were 4.4 and 8.6× greater, respectively, in the nutrient enriched stream than in the reference stream at 30 d. The increases in fungal biomass and sporulation rates induced by the elevated concentrations of nitrogen and phosphorus in the treatment stream are in accordance with the results from previous studies (Suberkropp 1995, Weyers & Suberkropp 1996, Grattan & Suberkropp 2001, Gulis & Suberkropp 2003c, Gulis et al. 2004, 2006), and the magnitude of the difference in sporulation rates between the reference and treatment stream was greater than the difference in fungal biomass. This implies that the higher nutrient concentrations had a greater effect on sporulation rates (reproduction) than on biomass (growth) (Gulis & Suberkropp 2003c). This might be a consequence of a substantial allocation of fungal secondary production to asexual reproduction by aquatic hyphomycetes. In a laboratory study which measured the biomass, respiration, and sporulation of *A. filiformis* and *Lunulospora curvula* growing on leaf disks in microcosms, total conidia production during 27–29 d of incubation accounted for 30–45 % of the net fungal production (biomass plus sporulation) by *A. filiformis* and 60–80 % of the net fungal production by *L. curvula* (Suberkropp 1991). A similar study showed that *A. tetracladia* allocated 46 % of the total fungal production to sporulation during 23 d of incubation (Gessner & Chauvet 1997). In another study, the proportion of cumulative fungal production allocated to conidia production by *A. tetracladia* increased with N and P enrichment in laboratory microcosms (Gulis & Suberkropp 2003b).

The number and biomass of macroinvertebrates were significantly greater in the leaf packs from the coarse mesh bags deployed in the treatment stream than in the reference stream at 30 d. Previous work has shown that the abundance of macroinvertebrates was positively correlated with the concentrations of DIN and SRP and was higher in enriched Portuguese streams (Gulis et al. 2006, but see Ferreira et al. 2006). In Coweeta streams, macroinvertebrate biomass in litter bags was 2 – 3 times higher with enrichment, and the pattern was similar for shredder biomass per g leaf AFDM (Greenwood et al. 2007). The abundance and biomass of shredders, in the present study, were 6.6 and 5.1× greater per bag, and 13.3 and 8.5× greater per g leaf AFDM, respectively, in C54 than in C53. Cross et al. (2006) also demonstrated that the abundance, biomass, and annual secondary production of invertebrates were significantly increased in C54 through 2 years of enrichment in the mixed substrate habitat. Robinson & Gessner (2000) inferred that the higher abundance and biomass of shredders in fertilized leaf packs were due to high quality detritus resulting from nutrient addition to stream water. Leaves submerged in C54 had higher N content (Gulis & Suberkropp 2003c) and hence lower leaf C : N ratio (Greenwood et al. 2007). The ratio of macroinvertebrate N:leaf N was increased with nutrient enrichment (Greenwood et al. 2007). These observations, with increased fungal biomass in the leaves from C54 (present study) and the predominance of fungi over bacteria in terms of biomass and production associated with decomposing leaves (Gulis & Suberkropp 2003c), indicate that the increased shredder biomass associated with leaf packs in the treatment stream was mainly mediated by aquatic hyphomycetes which enhanced the nutritional value of leaf detritus in the treatment stream. Further research including the measurements of changes in microbial production (fungi and bacteria), nutritional quality of detritus, and shredder secondary production should give more detailed understanding of the bottom-up effect of nutrient enrichment in the detrital food webs of headwater streams.

Effect of shredder feeding on fungal activity

Differences in fungal biomass and conidia production between fine and coarse mesh bags in either stream were not significant at 30 d. These results suggest that the activity of aquatic hyphomycetes on the leaves was not controlled by the feeding activity of shredders. The lack of effects by shredder feeding differs from results of other studies. In four European streams, sporulation rates on oak leaves and larch needles in coarse mesh bags were lower than in fine mesh bags, but the rates on spruce needles, which were not consumed by shredders, were similar in coarse and fine mesh bags (Bärlocher 1982). For the European streams, the difference in sporulation rate between fine and coarse mesh bags was not apparent within 2–3 months from the submersion of litter bags in the streams (Bärlocher 1982). In the present study, however, most of the leaf material in coarse mesh bags in either stream were gone by 30 d due to higher breakdown rates of the maple leaves. The more rapid breakdown rate of maple leaves in comparison with that of oak and larch may be one reason for our inability to observe a shredder feeding effect. In a previous study done in

three Coweeta streams, conidia transported in the water were higher in a stream that received insecticide to reduce the abundance and production of shredders than in two reference streams suggesting that shredders reduced sporulation of the whole stream fungal community (Suberkropp & Wallace 1992). This study also examined fungal communities growing on many leaf species with slow breakdown rates such as oak and rhododendron. However, the species composition in all three streams was similar.

Changes in fungal community structure

Nutrient enrichment resulted in shifts in dominance of fungal species in the treatment streams, compared to the reference stream. The greatest change in the community structure of aquatic hyphomycetes on leaves in C54 was the decrease in the relative abundance of *A. acuminata* and *A. tetracladia*, and the increase in the relative abundance of *T. elegans* and *T. chaetocladium*, two species with relatively large conidia (Table 2). These results are similar to previous studies (Gulis & Suberkropp 2003c, 2004). These results suggest that the increased fungal production stimulated by nutrient enrichment in the treatment stream contributed to the growth and reproduction of fungal species producing conidia with relatively large biovolume.

The addition of nutrients also induced higher fungal species richness. The fungal species richness found in each mesh size treatment from the reference or treatment stream was higher in fine mesh bags than coarse mesh bags, especially in the treatment stream (Table 2). The greater numbers of fungal species in C53 Fine and C54 Fine treatments, compared to C53 Coarse and C54 Coarse, respectively, were mainly due to the occurrence of rare species. Those species which were absent in coarse mesh bags but detected in fine mesh bags had relative abundances less than 1 %. If these rare species are excluded, the differences in the number of fungal species between C53 and C54 as well as between fine and coarse mesh bag treatment are not apparent. Bärlocher (1982) also found more fungal species in fine mesh bags than in coarse mesh bags deployed in 4 European streams, and the relative abundances of late-colonizing, rare species observed only in fine mesh bags were very small compared to those of early-colonizing, common fungi. In addition, the differences in fungal species richness were evident only at 30 d. On later sampling dates when most of leaf mass had disappeared due to feeding activity of shredders and/or decomposition by microbial activity, the numbers of fungal species in the reference and the treatment streams were similar. Bärlocher (1980, 1982) suggested the feeding activity of shredders lowers fungal species richness on leaves by removing leaf material which could be the substrate for the colonization of late-colonizing, rare fungal species, while sustaining the dominance of 4 – 5 early-colonizing common species. The result of the present study also implies that nutrient enrichment enhanced the growth of rare fungal species. Once they colonize leaves, rare fungal species require enough time to grow so that they can produce conidia without being consumed by shredders.

Several laboratory studies have demonstrated the preferential feeding of shredders for the monocultures of different fungal species and have suggested that the feeding preference of shredders could affect the relative abundance of each fungal species associated with detritus (e.g., Suberkropp et al. 1983, Arsuffi & Suberkropp 1985). However, it is uncertain whether such selective feeding actually occurs in streams (Suberkropp 1992). Any given fungal-colonized area on a decomposing leaf found in streams is the interwoven hyphal mat of several fungal species (Shearer & Lane 1983, Chamier et al. 1984), which is mainly embedded in detrital mass. Considering the high degree of overlap among dominant species across the surface of decomposing leaves (Shearer & Lane 1983), it is not likely that shredders can differentially feed on a certain species of aquatic hyphomycetes among several species within a scale of micrometers, resulting in changes in species richness of aquatic hyphomycetes. In addition, similarity indexes in the present study suggested that the change in fungal community structure associated with maple leaves was mainly driven by nutrient enrichment rather than by feeding activity of shredders. Fungal communities in fine and coarse mesh bags deployed in either the reference or treatment stream were relatively more similar to one another than to those assemblages from the other stream but in the same-sized mesh bags (Table 3). Overall, it appears that the differences in fungal community structure and species richness on maple leaves among the four treatments

Table 3. Similarity Indices based on mean relative abundances of conidia from aquatic hyphomycetes associated with leaf disks from the fine mesh and the coarse mesh litter bags in the reference (C53) and the treatment (C54) streams at 30 d.

Comparison	Similarity Index
C53 Fine – C53 Coarse	0.752
C54 Fine – C54 Coarse	0.887
C53 Fine – C54 Fine	0.595
C53 Coarse – C54 Coarse	0.591

were driven by the addition of nutrients and the loss of leaf mass, rather than the feeding preference of shredders for certain fungal species.

Conclusions

The level of nutrient concentrations in the stream water exerted greater effects on the activity and community structure of aquatic hyphomycetes associated with decomposing maple leaves than the consumption of leaves by shredders. While nutrient addition in the enriched stream increased leaf breakdown rates, fungal growth, and sporulation rates, the effect of shredder feeding on the activity of aquatic hyphomycetes was not apparent in this study. This was mainly due to rapid breakdown of leaf litter in coarse mesh bags submerged in either stream. In contrast to the breakdown rates, neither fungal biomass nor sporulation rates were affected by shredder feeding. Nutrient enrichment increased the fungal species richness and altered fungal community structure more than shredder feeding by enhancing the growth of late-colonizing fungal species. The greater abundance (mainly contributed by collector-gatherers and shredders) and biomass (mostly by shredders) found in coarse mesh bags from the nutrient enriched stream can be attributed to higher detrital food quality resulting from accelerated fungal growth in this stream.

Acknowledgements

We thank Dr. Amy Rosemond, Sue Dye, and the Analytical Chemistry Laboratory in the Institute of Ecology at the University of Georgia for providing nutrient analysis data. We also thank Drs. V. Gulis, M. Chadwick, T. L. Arsuffi, A. C. Benke, A. D. Huryn, and G. M. Ward for insightful comments on the earlier version of the manuscript. Constructive comments from two anonymous reviewers improved the manuscript. This study was supported by National Science Foundation, and the Aquatic Biology Enhancement Program at the University of Alabama.

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Submitted: 20 May 2008; accepted: 11 August 2008.