Applied and Environmental Microbiology, Feb. 2008, p. 1094–1101 Vol. 74, No. 4
0099-2240/08/$08.00+0 doi:10.1128/AEM.01903-07

Comparison of Fungal Activities on Wood and Leaf Litter in Unaltered and Nutrient-Enriched Headwater Streams[▽]

Vladislav Gulis,[1]* Keller Suberkropp,[1] and Amy D. Rosemond[2]

Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487,[1] and Odum School of Ecology, University of Georgia, Athens, Georgia 30602[2]

Received 18 August 2007/Accepted 6 December 2007

Fungi are the dominant organisms decomposing leaf litter in streams and mediating energy transfer to other trophic levels. However, less is known about their role in decomposing submerged wood. This study provides the first estimates of fungal production on wood and compares the importance of fungi in the decomposition of submerged wood versus that of leaves at the ecosystem scale. We determined fungal biomass (ergosterol) and activity associated with randomly collected small wood (<40 mm diameter) and leaves in two southern Appalachian streams (reference and nutrient enriched) over an annual cycle. Fungal production (from rates of radiolabeled acetate incorporation into ergosterol) and microbial respiration on wood (per gram of detrital C) were about an order of magnitude lower than those on leaves. Microbial activity (per gram of C) was significantly higher in the nutrient-enriched stream. Despite a standing crop of wood two to three times higher than that of leaves in both streams, fungal production on an areal basis was lower on wood than on leaves (4.3 and 15.8 g C m^{-2} $year^{-1}$ in the reference stream; 5.5 and 33.1 g C m^{-2} $year^{-1}$ in the enriched stream). However, since the annual input of wood was five times lower than that of leaves, the proportion of organic matter input directly assimilated by fungi was comparable for these substrates (15.4 [wood] and 11.3% [leaves] in the reference stream; 20.0 [wood] and 20.2% [leaves] in the enriched stream). Despite a significantly lower fungal activity on wood than on leaves (per gram of detrital C), fungi can be equally important in processing both leaves and wood in streams.

Terrestrial detritus in the form of leaves and wood is an important carbon and energy source for microorganisms and food webs in freshwaters. Detritus-associated microorganisms also control important processes in aquatic ecosystems, including the uptake and sequestration of dissolved inorganic nutrients (33, 37). Fungi, rather than bacteria, dominate the microbial biomass on coarse particulate organic matter (CPOM), such as leaves and wood in streams, and fungal production on submerged leaves is also greater than that of bacteria (10, 14, 23, 28, 56). A recent study suggests that fungal ribotype richness levels (as determined by denaturing gradient gel electrophoresis) may be higher than those of bacteria and actinomycetes on submerged leaves (10). Thus, fungi are likely the most important biological driver of processes such as CPOM carbon dynamics and detritus-associated nutrient uptake in headwater streams.

Both allochthonous leaf litter and submerged wood in streams harbor diverse communities of fungi, mainly ascomycetes and their anamorphs (hyphomycetes) (see, e.g., references 4, 39, and 51) though chytrids, zygomycetes, and basidiomycetes have also been detected by using molecular techniques (35). Communities can be species rich. For example, more than 200 species from wood in a single river have been reported (51), whereas the diversity of aquatic hyphomycetes that colonize mostly leaves is considerably lower (e.g., see reference 5). Aquatic fungi also show some substrate preferences (13, 22), so leaves and wood harbor different communities that may result in different biomass and activity. While several studies have quantified biomass and production of fungi associated with submerged leaves (see reference 23), very few studies have quantified fungal biomass on wood. These studies have primarily used artificial substrates, such as wood veneers (see, e.g., references 24 and 50) or introduced branches (11, 42), while only one study has quantified fungal biomass on naturally occurring wood in streams (14). Estimates of fungal production from wood in freshwater systems are lacking, but would likely differ from those previously determined from leaves because of differences in chemical composition of wood versus leaves (higher C/N ratio and higher lignin concentration) and different surface area-to-volume ratios and residence times in streams. Interestingly, even though it is generally acknowledged that fungi are the major decomposers of wood in terrestrial ecosystems as well, fungal production on wood (based on rates of incorporation of radiolabeled acetate into ergosterol) has also never been estimated in these systems.

Fungi associated with submerged leaves and wood may respond differently to increases in limiting nutrients. Stelzer et al. (44) and Gulis et al. (24) found that microbial response to nutrient enrichment depended on the nutrient content of detritus, with wood veneers exhibiting a greater response to nutrient addition than did leaves, whereas Ferreira et al. (13) did not find such an effect. Such substrate-specific differences may affect whole-ecosystem functioning, as the relative availability of different types of detritus (due to a variation in leaf and wood input and standing crop) and other factors that affect microbial activity (e.g., temperature and nutrients) are altered due to land use change.

* Corresponding author. Present address: Department of Biology, Coastal Carolina University, P.O. Box 261954, Conway, SC 29528-6054. Phone: (843) 349-2576. Fax: (843) 349-2201. E-mail: vgulis@coastal.edu.

▽ Published ahead of print on 14 December 2007.

The importance of microbially driven processes on an ecosystem scale is a function of temporally and substrate-specific microbial activity (31) and substrate availability. Our study quantified and compared fungal biomass, production, assimilation and microbial respiration on leaves and wood both at the substrate scale and at the ecosystem scale on an annual basis. The objectives of this study were to (i) obtain the first estimates of fungal production on submerged wood, (ii) compare fungal parameters associated with submerged wood and leaf litter and fungal importance in decomposition of these substrates at the ecosystem scale, and (iii) determine the effects of whole-stream nutrient enrichment on microbial activity associated with submerged wood and leaf litter.

MATERIALS AND METHODS

Study sites and sampling. The study was conducted from July 2004 to July 2005 in two headwater streams at the Coweeta Long Term Ecological Research site, Macon County, NC, at an altitude of ca. 850 m above sea level. This area of the southern Appalachian Mountains is covered by mixed hardwoods with a dense understory of *Rhododendron maximum* L. in stream valleys. These hardwoods and understory result in year-round shading, and consequently, stream ecosystems at Coweeta are primarily heterotrophic, i.e., they rely on allochthonous organic matter, nutrients, and energy (27). The experimental streams situated about 200 m apart are similar with respect to physicochemical characteristics. They are small (average discharge about 1 liter s^{-1}), circumneutral, softwater streams and have low ambient nutrient concentrations (see reference 20 for additional stream information). One of the streams was designated as a reference (catchment 53), while the other (catchment 54) received continuous nutrient enrichment initiated in July 2000 (i.e., 4 years before our study started) and continued throughout our 1-year study. The nutrient-enriched stream received a solution of ammonium nitrate and potassium phosphate. A metering pump situated upstream of a 145-m experimental reach was connected to a flow meter and delivered nutrient solution (proportionally to instantaneous discharge) to a pipe with multiple sprinklers fed with stream water and laid along the stream (see references 20 and 24 for details). Dissolved nutrient concentrations in the treatment stream during the study period were six to eight times higher than those in the reference stream: NH_4-N, 93 ± 73 versus 17 ± 14 μg $liter^{-1}$; NO_3-N, 385 ± 184 versus 40 ± 38 μg $liter^{-1}$; and soluble reactive phosphorus, 82 ± 40 versus 12 ± 20 μg $liter^{-1}$ (values are means ± standard deviations) (26 or 27 samples; P values of <0.001 for all comparisons by the paired t test). See references 20 and 24 for details of water sampling and analysis. Water temperature was continuously monitored with Optic StowAway temperature probes (Onset Computer Corp., Pocasset, MA), and temperatures were similar between the two streams, ranging annually from 5.3 to 17.8°C (mean 12.2 and 12.5°C in the reference and nutrient-enriched streams, respectively).

The standing crop of small wood (<40 mm diameter) in both streams was determined by a line intersect technique (52) on June 1, 2005. In contrast to leaves, wood input is not seasonal, decomposition is slow, and downstream export is small in highly retentive Coweeta streams, and hence, the standing crop is relatively constant seasonally and between years (see, e.g., references 53 and 54). Nineteen transects (lengths equal to wetted stream widths) were set up in each stream, and the diameters of all intersected fully submerged or wetted wood pieces were measured. The volume of wood per m^2 of stream bottom for three size classes (<11, 11 to 25, and 25 to 40 mm in diameter) was calculated according to the method in reference 52, and ultimately ash-free dry mass (AFDM) per m^2 was determined based on a specific gravity estimate of wood from a Coweeta stream (53). We also sampled wood quarterly for microbial analyses. From each of five transects in both streams, we collected four to six pieces of wood in each of three size classes. The pieces were placed in plastic bags with stream water and brought back to the laboratory on ice. Mean diameter estimates of size classes were 7.1 ± 0.1 (n = 200), 15.0 ± 0.2 (n = 147), and 34.1 ± 0.6 mm (n = 64) (values are means ± standard errors [SE]). Two disks (or segments for the largest size class) 1 to 2 mm thick were cut from each branch, pooled within the respective size class/transect/stream, and used for microbial analyses (see below).

Leaf litter to determine the standing crop was collected monthly from 10 transects randomly selected on each sampling date within 106-m- and 130-m-long reaches in the reference and treatment streams, respectively. Leaf material was collected from 0.15-m-wide transects spanning the wetted stream width. Leaves were weighed, subsampled if needed, taken to the laboratory, oven-dried (100°C), weighed, combusted (500°C overnight), and reweighed to determine AFDM per m^2. At streamside, two sets of 5 leaf disks and a set of 10 disks were cut from leaves collected from each of five transects in each stream and used for microbial analyses (see below).

Fungal biomass, growth rate, production, and microbial respiration. Fungal biomass was estimated from ergosterol content of plant litter, while fungal growth rates were estimated from rates of [^{14}C]acetate incorporation into ergosterol (34, 49) as described previously (26). Briefly, sets of leaf or wood disks were incubated at stream temperature in glass tubes filled with 3.95 ml filtered stream water (HA, 0.45 μm; Millipore) with aeration. Sodium [1-^{14}C]acetate (MP Biomedicals, Irvine, CA) was added (final acetate concentration, 0.4 mM; activity, 1 MBq per sample) and tubes were incubated for 3 (leaf disks) or 4 h (wood). Formalin-killed controls were run in parallel to correct for background radioactivity. Samples were filtered (934AH glass fiber; Whatman), rinsed, and either preserved with 5 ml methanol (leaf disks) or frozen (wood) in scintillation vials. A corresponding set of leaf disks was dried at 100°C, weighed, ashed at 500°C overnight, and reweighed to estimate AFDM of the extracted set. Frozen wood samples were later freeze-dried and weighed before ergosterol extraction, while leaf samples were directly extracted with alcoholic KOH. Lipids were partitioned into pentane, evaporated to dryness, reconstituted in methanol, and filtered [poly(tetrafluoroethylene), 0.2 μm; Fisher Scientific]. Ergosterol was quantified with a high-performance liquid chromatograph (Shimadzu, Columbia, MD) equipped with a Whatman Partisphere C18 column and an UV detector set at 282 nm, and results were compared with external ergosterol standards (Acros, Morris Plains, NJ). Ergosterol fractions were collected with a fraction collector (Advantec) and mixed with Ecolume scintillation cocktail (ICN Biomedicals, Cleveland, OH). Radioactivity in the ergosterol fraction was measured in a scintillation counter (Beckman, Fullerton, CA) that corrected for quenching. Fungal biomass of plant litter was calculated using the conversion factor of 5.5 mg ergosterol g^{-1} fungal dry mass (15). Instantaneous fungal growth rates were calculated assuming an exponential growth model as described previously (16). Empirical conversion factors of 19.3 μg fungal biomass $nmol^{-1}$ acetate incorporated for leaf litter (49) and 27.0 μg $nmol^{-1}$ for wood sticks (V. Gulis, unpublished data) were used. Daily fungal production was calculated based on fungal growth rate and biomass estimates.

Microbial respiration was measured as oxygen uptake of plant litter with YSI 5100 dissolved oxygen meters (Yellow Springs, OH) at stream water temperatures (25). On each collection date, sets of 10 leaf disks or wood samples were placed in 26 ml stream water in respiration chambers and oxygen concentrations were recorded periodically for 30 min in the dark. Oxygen consumption was determined from the slope of regression of oxygen concentration versus time minus the control slope determined from stream water alone in the control chambers. The AFDM levels of samples were determined as described above to calculate the respiration rate per unit of plant litter.

Calculations and statistical analyses. Since leaves had much higher associated fungal activity than did wood and because of different sampling schedules, we compared microbial parameters from these substrates separately. Wood-associated microbial data were analyzed by analysis of covariance (ANCOVA), with wood diameter as a covariate and stream (two levels) and temperature (three levels, same temperatures in both streams for each date) as categorical variables. For leaves, microbial data were analyzed by analysis of variance (ANOVA), with stream and temperature (six levels, same temperatures in both streams for each date) as categorical variables. Even though we took advantage of using wood diameter as a continuous variable in our ANCOVA model, we had to use size classes to calculate fungal parameters on an areal basis since the line intersect technique used to estimate wood standing crop does not allow calculation of the volume of each piece of wood.

Daily fungal production on an areal basis was calculated as the product of fungal production and plant litter standing crop. Consequently, variances for these products were calculated as described previously (19, 32). Annual fungal production and microbial respiration (per gram of plant litter and per m^2 of stream bottom) were estimated by interpolating between sampling dates and summing up daily production or respiration estimates over the year. Microbial parameters for the three wood size classes were estimated separately and then summed up. Since fungal activity and respiration rate could be affected by temperature (see Results), we adjusted each daily estimate depending on actual water temperature using the Q_{10} coefficient of 2.62 (mean from references 2 and 49). We used bootstrapping to estimate annual fungal production and microbial respiration means and 95% confidence limits (CL). Each raw data set was resampled with replacement, and the data were recombined to produce 1,000 bootstrap sets, from which the means and 95% CL were calculated (32). To test for differences between annual production and respiration estimates between

two litter types or two streams, we used a bootstrap test of significance (30), testing whether the mean difference between estimates is greater than zero. To convert our data to carbon units, 50% C content of fungal biomass and plant AFDM and a respiratory quotient of 0.95 were assumed. Fungal assimilation was calculated based on production data using a growth efficiency estimate of 33% (45).

To estimate the importance of microorganisms in the carbon budget of the stream ecosystem, we also calculated the percentage of annual CPOM input assimilated by fungi and lost through microbial respiration. Annual CPOM input data (A. D. Rosemond, unpublished data) were derived from biweekly estimates of leaf litter and small wood input in direct fall and lateral traps positioned along both streams. Mean annual input data (July 2004 to July 2005) were used for leaf litter, while average annual input data from 6 years of observations (2000 to 2005) were used for small wood since wood input was found to be highly variable. Means and 95% CL were calculated by bootstrapping. Statistical analyses were carried out with SPSS 14.0, and bootstrapping was performed with custom VBA macro in Microsoft Excel.

RESULTS

Microbial parameters per unit of detrital carbon. Fungal biomass per unit of detrital carbon on wood was about two to three times lower than that associated with leaves (Fig. 1A and B), whereas the fungal growth rate, production, and microbial respiration were about an order of magnitude lower on wood than on leaves (Fig. 1C to H). All fungal parameters and microbial respiration associated with both wood and leaves were higher in the nutrient-enriched stream than in the reference stream (the P value was ≤ 0.004 by ANCOVA or ANOVA), except for fungal biomass on leaves, which was comparable in both streams ($P = 0.077$) (see Table 1 for detailed results). As expected, water temperature had no significant effect on fungal biomass associated with wood ($P = 0.572$), but it positively affected fungal growth rate and production ($P \leq 0.005$) (Table 1). The fungal growth rate and respiration from leaf litter were also positively affected by temperature ($P \leq 0.016$). However, a higher fungal biomass was observed at lower stream water temperatures ($P < 0.001$). Although the latter relationship between fungal biomass and temperature is counterintuitive, it can be explained by the pronounced seasonality of leaf litter input and standing crop (Fig. 2G). As a result, fungal production from leaf litter that depends on both biomass and growth rate was not affected by temperature ($P = 0.116$).

We found a significant correlation between fungal production associated with both wood and leaves and microbial respiration ($r = 0.64$ and $P < 0.001$ for wood, and $r = 0.79$ and $P < 0.001$ for leaves), suggesting that the considerable proportion of microbial respiration may be fungal (see Discussion).

All fungal parameters and microbial respiration were higher on wood sticks of smaller diameters ($P \leq 0.005$, ANCOVA) (Table 1). The relationship between wood diameter and fungal production measured in August 2004 and June 2005 (same water temperatures) is shown in Fig. 3. Both linear and negative exponential models gave a significant fit. The latter model has a slightly better fit for both streams and is more realistic since fungal production on larger sticks should be low but not equal to zero.

Microbial parameters on an areal basis. Fungal parameters per m^2 of stream bottom for both wood and leaves were estimated based on plant litter standing crops and production estimates per gram of detrital C discussed above. Fungal biomass on wood on an areal basis was very stable over the

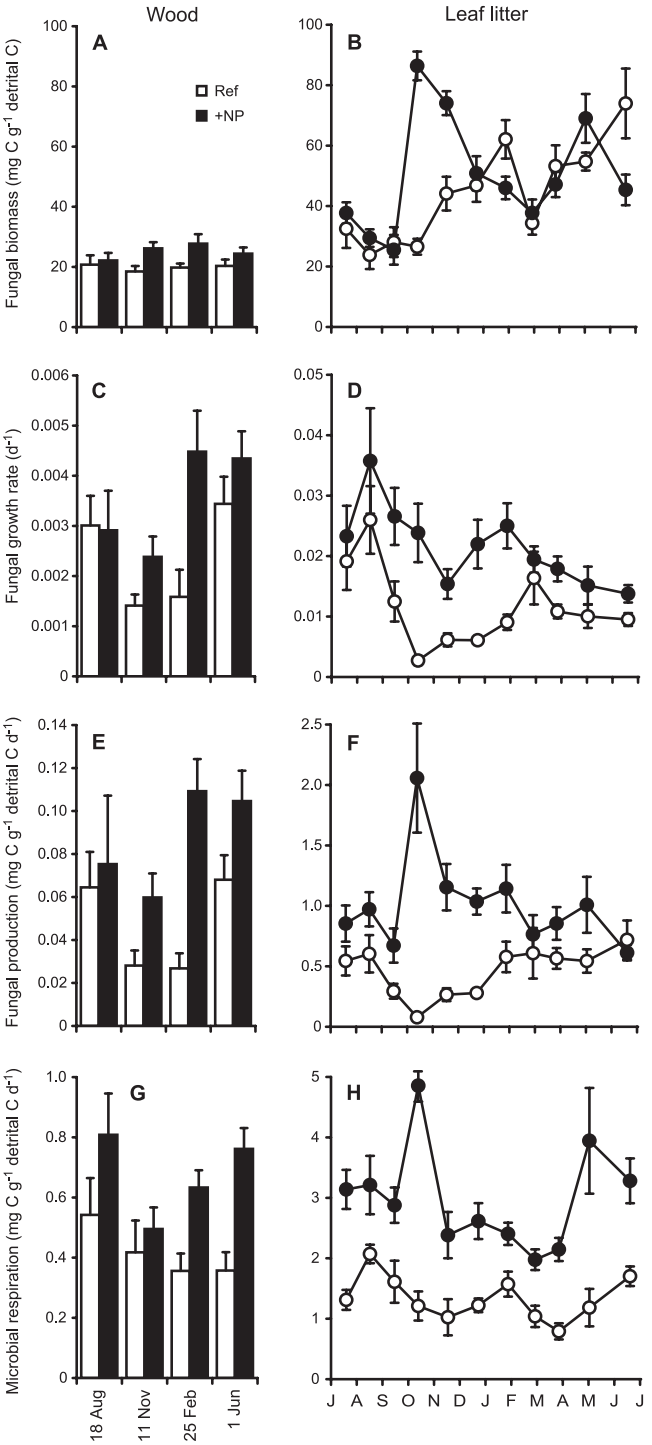

FIG. 1. Fungal biomass (A and B), instantaneous fungal growth rate (C and D), daily fungal production (E and F), and microbial respiration (G and H) associated with decomposing small wood and leaf litter in the reference and the nutrient-enriched streams during the 1-year study period. Data are per unit of detrital carbon. Error bars indicate 1 SE ($n = 15$ for wood and $n = 5$ for leaves). d^{-1}, per day; Ref, reference stream; +NP, nutrient-enriched stream.

TABLE 1. Results of ANCOVA or ANOVA on the effects of stream, temperature, and wood diameter on fungal parameters and microbial respiration associated with submerged wood and leaves[a]

Type of detritus and variable	Fungal biomass			Fungal growth rate			Fungal production			Microbial respiration		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Wood												
Stream	1	12.47	<0.001	1	8.84	0.004	1	21.39	<0.001	1	20.76	<0.001
Temperature	2	0.56	0.572	2	5.20	0.007	2	5.52	0.005	2	2.54	0.083
Wood diam	1	21.43	<0.001	1	8.37	0.005	1	23.66	<0.001	1	14.60	<0.001
Error	113			113			113			113		
Leaves												
Stream	1	3.20	0.077	1	31.18	<0.001	1	42.67	<0.001	1	120.03	<0.001
Temperature	5	4.67	<0.001	5	2.95	0.016	5	1.81	0.116	5	5.95	<0.001
Error	102			102			102			99		

[a] Results shown for wood are from ANCOVA, and those for leaves are from ANOVA. All microbial parameters except fungal growth rate are expressed per gram of detrital carbon. Wood diameter is a continuous variable.

sampling dates (Fig. 2A), whereas fungal biomass on leaves (Fig. 2B) showed clear seasonal peaks in both streams, resulting from dramatic changes in leaf litter standing crop following leaf litter input in autumn (Fig. 2G). Fungal production (Fig. 2C) and microbial respiration (Fig. 2E) on wood on an areal basis did not show marked seasonality, even though production and respiration per gram of wood C (Fig. 1E and F) were affected by temperature and were slightly different between sampling dates. Fungal production (Fig. 2D) and microbial respiration (Fig. 2E) on leaves per m^2 of stream bottom showed highly seasonal patterns similar to that of biomass.

Annual estimates of microbial activity at the ecosystem scale and importance in decomposition of wood and leaves. Fungal assimilation and microbial respiration proceeded significantly faster in the nutrient-enriched stream than in the reference stream for both substrates (bootstrap test of significance, *P* values of <0.001) (Fig. 4A and B). Fungal assimilation of leaves was about an order of magnitude higher than that of wood. Absolute fungal assimilation values (>1 g C g^{-1} detrital C $year^{-1}$) indicated that leaves would disappear in the nutrient-enriched stream in less than a year due to fungal activity alone.

The small wood standing crop was almost twice as high in the reference stream as in the nutrient-enriched stream ($P = 0.038$, *t* test) (Fig. 4C). This led to comparable estimates of annual fungal production, assimilation, and microbial respiration on wood on an areal basis in both streams ($P > 0.05$, bootstrap test of significance) (Fig. 4D and E), despite the twofold higher production and respiration per gram of wood C in the nutrient-enriched stream (Fig. 1E and G and 4A and B). Annual fungal production and microbial respiration on leaves were significantly higher in the nutrient-enriched stream than in the reference stream ($P \leq 0.001$) (Fig. 4D and E).

Annual small wood input was about five times lower than leaf litter input for both streams, while no differences in either leaf or wood input were found between streams (*P* values of >0.05 by the *t* test) (Fig. 4F). The proportion of annual wood input assimilated by fungi was similar between streams ($P = 0.19$, bootstrap test of significance) (Fig. 4G), while fungi assimilated a higher proportion of annual leaf input in the nutrient-enriched stream ($P = 0.001$). No difference was found between streams in the proportion of annual wood or leaf input respired by microorganisms ($P > 0.077$) (Fig. 4H).

DISCUSSION

Virtually nothing is known about the activity and the relative importance of fungi in the decomposition of submerged wood. Since our study was the first to estimate fungal production associated with naturally occurring submerged wood in streams, direct comparisons with other studies cannot be made. However, estimates of fungal biomass associated with naturally occurring wood (14) or corticated sticks introduced in streams for decomposition experiments (11, 42) are available. Fungal biomass on randomly collected small wood in our study was on average 2 to 2.5% of the detrital mass, while the value was 0.9% for nine North American streams (14). The relatively short duration of wood decomposition experiments in two earlier studies (11, 42) and the relatively slow growth of fungi on wood precluded the development of sizeable fungal biomass, while in our study, sticks were of unknown and probably considerable age. Unfortunately, several studies where attempts to estimate fungal biomass on wood have been made either used wood veneers or presented data per unit of wood surface area (7, 17, 41), and thus, their results are not comparable with our estimates per unit of wood mass/carbon. Clearly, fungal hyphae penetrate several millimeters deep inside wood tissues (V. Gulis, unpublished observation). Therefore, the use of the term "biofilm" to refer to the fungal colonization of wood is inappropriate. However, the penetration of fungi even deeper into wood tissue could be limited by oxygen availability (1). Consequently, we found a significant decline in fungal production as the wood diameter increased (Fig. 3). Fungal activity on wood pieces larger than those used in our study (i.e., >40 mm diameter) should be relatively low. Our preliminary trials with deep cores taken from logs larger than 20 cm in diameter showed fungal biomass and production to be close to or below the detection limits.

Fungal biomass on wood per m^2 of stream bottom in this study (ca. 5 g C m^{-2}) was comparable to estimates from a somewhat similar headwater woodland stream in Tennessee (ca. 2 g C m^{-2} [14]), but it was much higher than that in other

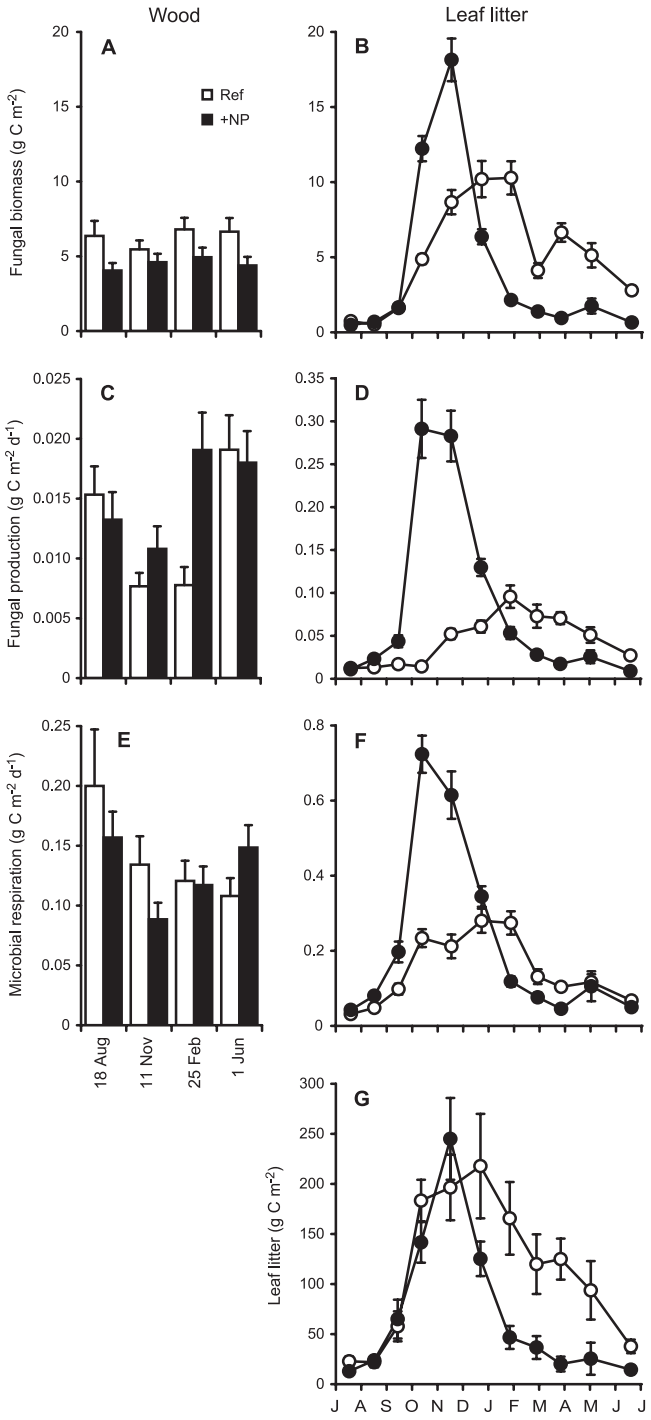

FIG. 2. Seasonal changes in fungal biomass (A and B), production (C and D), and microbial respiration (E and F) per unit of stream bottom area for small wood and leaf litter in the reference and the nutrient-enriched stream. Highly seasonal dynamic of leaf litter standing crop is given in panel G, to aid in interpreting leaf-associated fungal and respiration data. Small wood standing crop is given on Fig. 4C. Error bars indicate 1 SE. d^{-1}, per day; Ref, reference stream; +NP, nutrient-enriched stream.

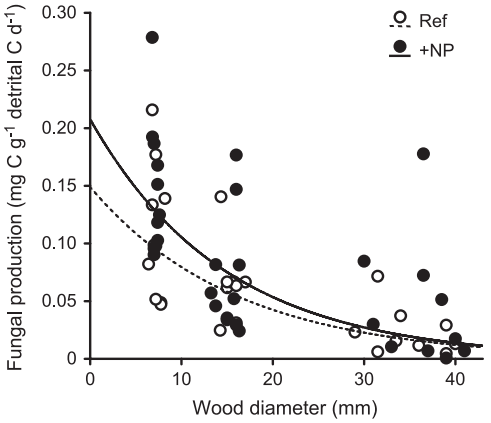

FIG. 3. The relationship between wood diameter and wood-associated fungal production. Since fungal production depends on temperature, the raw production data from August 2004 and June 2005 only (stream and incubation temperatures of 15°C) were used for this graph. By the linear regression model (not shown), $R^2 = 0.42$ and $P < 0.001$ for the reference stream and $R^2 = 0.33$ and $P = 0.001$ for the nutrient-enriched stream. By the negative exponential model, $y = 0.149e^{-0.063x}$, $R^2 = 0.60$, and $P < 0.001$ for the reference stream and $y = 0.207e^{-0.068x}$, $R^2 = 0.43$, and $P < 0.001$ for the nutrient-enriched stream. d^{-1}, per day; Ref, reference stream; +NP, nutrient-enriched stream.

North American streams (below 0.2 g C m^{-2} [14]). This result could be explained by a standing crop of wood in our highly retentive streams about an order of magnitude higher than that in the mostly larger streams included in the study by Findlay et al. (14) and by different sampling approaches, i.e., the scraping of fungal "biofilm" in their study that underestimates total fungal biomass.

Fungal production and microbial respiration per gram of detrital C were 3 to 13 times lower on wood than on leaves in this study. Annual fungal production-to-biomass ratios on wood were only 0.63 and 1.22 in the reference and the nutrient-enriched streams, respectively, while on leaves they reached 3.7 and 7.9. Fungal production-to-biomass ratios on leaves, however, were also relatively low compared to the values reported for other streams (8 to 21; see references 6, 32, and 47). Even though fungal activity in our streams was not exceptionally high, it translated into direct assimilation of a considerable proportion of annual CPOM inputs in these streams.

Fungal parameters and microbial respiration on wood on an areal basis in this study did not fluctuate considerably throughout the year, even though we found a significant effect of temperature on fungal activity. The high seasonality of fungal activity on leaves in this (determined by litter input rather than temperature) (Fig. 2) and other studies (6, 32) resulted in fungal parameters and microbial respiration on an areal basis on wood being higher than those on leaves in summer months but much lower during other seasons due to high leaf litter standing crop. This suggests that wood may be an important resource to invertebrate consumers in summer when leaf litter is scarce and may also serve as a source of some fungal inoculum for colonization of newly fallen leaves in autumn (38).

Annual fungal production from wood on an areal basis ranged from 4.3 to 5.5 g C m^{-2} $year^{-1}$, which is considerably lower than that from leaves in this study (15.8 to 33.1 g C m^{-2} $year^{-1}$) and other recent estimates of fungal production on leaves in small streams (8 to 23 g C m^{-2} $year^{-1}$) (6, 32, 47). Our estimates of annual fungal production on submerged wood are about an order of magnitude higher than available

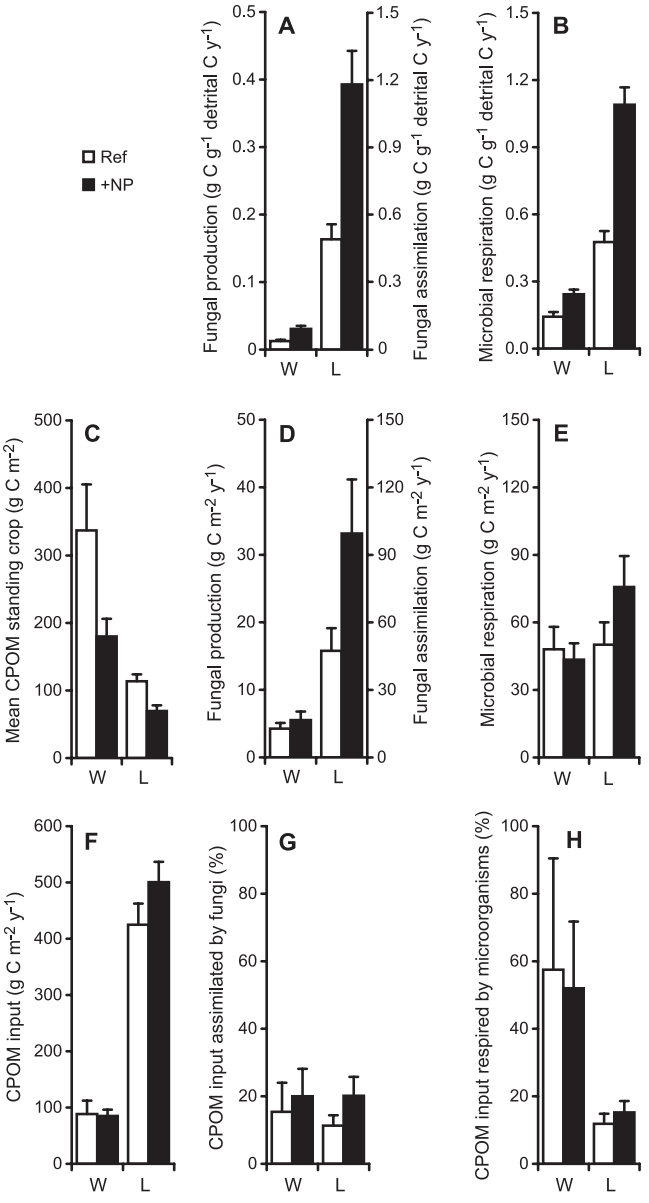

FIG. 4. Annual ecosystem-level estimates of microbial activity associated with and microbial importance in the decomposition of small wood and leaf litter in the reference and the nutrient-enriched streams for July 2004 to July 2005. (A) Annual fungal production and assimilation (fungal growth efficiency of 0.33 was used to calculate assimilation) (45). (B) Annual microbial respiration (respiratory quotient of 0.95 was assumed). (C) Mean small wood and leaf litter standing crop. (D) Annual fungal production and assimilation per stream bottom area. (E) Annual microbial respiration on an areal basis. (F) Annual small wood and leaf litter input. (G and H) Estimates of CPOM input assimilated by fungi and lost through microbial respiration. Means and upper 95% CL are shown since values were estimated by bootstrapping, except in panels C and F, where SE are shown. Note that panels A and B answer the question of how fast submerged detrital carbon is used by microorganisms, while panels G and H show what proportion of annual riparian subsidies is utilized by microorganisms. L, leaves; W, wood; Ref, reference stream; +NP, nutrient-enriched stream.

estimates of bacterial production on wood from aquatic ecosystems. Annual bacterial production on snags in a large blackwater river was estimated at ca. 0.7 g C m^{-2} $year^{-1}$ (12), while bacterial production on wood in a subtropical wetland was 0.007 to 1.2 g C m^{-2} $year^{-1}$, depending on season and habitat, with a grand mean of 0.12 g C m^{-2} $year^{-1}$ (43).

Our estimates of annual wood breakdown rates based on fungal assimilation alone (0.02 to 0.14 $year^{-1}$ depending on wood size class and stream) are of the same order of magnitude as annual breakdown rates of hardwood sticks of similar diameter (0.5 to 3 cm) in stream decomposition experiments calculated from mass loss data (0.06 to 0.28 $year^{-1}$ (11, 18, 40, 55), underscoring fungal importance in submerged wood decomposition.

Microbial respiration on both wood and leaves significantly correlated with and followed a seasonal pattern similar to that of fungal production in our study, suggesting that a considerable proportion of microbial respiration was fungal and that fungi may play a greater role than bacteria in the decomposition of not only leaves (e.g., 23, 36, 56) but also wood in streams. We feel, however, that the importance of bacteria may be greater on wood than on leaves. If we compare our estimates of fungal importance based on fungal production data and based on microbial respiration data (Fig. 4D versus E or G versus H), it is apparent that fungal respiration (assumed to be equal to 67% of fungal assimilation) accounts for almost all microbial respiration on leaves, while on wood, other organisms, probably bacteria, protists, and meiofauna, contributed more than 50% to total microbial respiration. An alternative explanation is that fungal growth efficiency on such a recalcitrant substrate as wood was lower than the assumed 33%, i.e., we underestimated fungal respiration since fungi might have respired more and invested less in biomass production on wood than on leaves.

Total fungal production on CPOM (wood plus leaves) was 20.1 and 38.7 g C m^{-2} $year^{-1}$ in the reference and the nutrient-enriched streams, respectively, which is higher than bacterial production on leaves (8 to 14 g C m^{-2} $year^{-1}$) (K. Suberkropp, V. Gulis, and A. D. Rosemond, unpublished data) and invertebrate secondary production on a habitat-weighted basis (rockface and mixed substrates) in these streams (4.0 and 8.4 g C m^{-2} $year^{-1}$, based on averages from the first 2 years of nutrient enrichment, 2000 to 2002 [9; W. F. Cross, unpublished data]).

Although it was not our primary goal, we also followed the effects of nutrient enrichment on microbial activity associated with decomposing wood and leaf litter. Our 3-year studies of the same streams (1999 to 2001) that employed before-after control-impact design or upstream-downstream comparisons showed a large effect of nutrient enrichment on the decomposition of introduced leaves and wood veneers and associated microbial parameters (21, 24, 25). The stimulation of fungal activity on leaves and wood continued through 2004-2005 when the present study was conducted. We found generally higher fungal biomass, production, and microbial respiration in the nutrient-enriched stream than in the reference stream per gram of detritus. However, microbial parameters per m^2 of stream bottom were not always higher in the nutrient-enriched stream. This was caused by wood and leaf litter standing crops twofold lower in the nutrient-enriched than in the reference stream, presumably a result of the disappearance of organic matter in the treatment stream due to faster decomposition rates brought about by elevated microbial activity during 5 years of enrichment (21, 24). Increased fungal biomass buildup

and nitrogen immobilization in plant litter following nutrient addition (24, 25) resulted in an enhanced food quality for consumers and higher invertebrate biomass and production of some invertebrate groups in this stream (8, 9, 21). At the same time, the availability of detrital resources decreased, which should have an opposite effect on some consumers over longer periods (8).

How important are fungi at the ecosystem scale? Based on our production data, we estimated that for both wood and leaves and in both streams fungi directly assimilated from 11 to 20% of the annual litter input (Fig. 4G), which is comparable to previous estimates for leaves in several streams (5 to 40%) (6, 32, 47). Independent estimates based on microbial respiration measurements were comparable for leaf litter, but were even higher for wood (Fig. 4H). These estimates of fungal importance in litter decomposition do not include fungus-mediated losses of plant carbon as dissolved organic carbon and fine particulate organic carbon, which can be comparable to losses of plant C due to respiration (3). A considerable portion of allochthonous litter input may be also lost from a stream reach due to downstream transport and other breakdown factors, such as abiotic leaching, mechanical fragmentation, and invertebrate feeding. The last two processes are facilitated by fungi through leaf litter softening and maceration due to fungal enzymatic activities (29, 48), rendering leaf litter a more palatable and nutritious food source for invertebrates through fungal biomass accrual, nutrient (N and P) immobilization, and enzymatic breakdown of refractory plant polymers (46). Consequently, our estimates of fungal participation and importance in wood and leaf litter decomposition in streams are clearly underestimates since we did not address all indirect effects and pathways of plant carbon losses due to fungal activity.

At the ecosystem scale, the proportion of wood or leaf input directly assimilated by fungi in this study was significant (at 11 to 20%), and it may increase with higher water nutrient concentrations and, possibly, higher temperatures. Thus, predictions of global changes, such as increased nutrient mobilization and rising temperatures, may result in an increased role of fungi in the decomposition of detritus in aquatic ecosystems.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Science Foundation (DEB 0318063).

We thank Nathan Taylor, Sue Dye, John Davis and Cindy Tant for excellent field assistance. Sue Eggert provided useful advice on estimating wood standing crop. We also thank Michael Chadwick for advice and help with bootstrapping. We also appreciate the assistance and contributions of Holly Weyers, Jon Benstead, and Roger Hilten to the long-term data sets on organic matter inputs.

REFERENCES

1. **Aumen, N. G., P. J. Bottomley, G. M. Ward, and S. V. Gregory.** 1983. Microbial decomposition of wood in streams: distribution of microflora and factors affecting [C-14]lignocellulose mineralization. Appl. Environ. Microbiol. **46:**1409–1416.
2. **Bååth, E.** 2001. Estimation of fungal growth rates in soil using ^{14}C-acetate incorporation into ergosterol. Soil Biol. Biochem. **33:**2011–2018.
3. **Baldy, V., V. Gobert, F. Guerold, E. Chauvet, D. Lambrigot, and J. Y. Charcosset.** 2007. Leaf litter breakdown budgets in streams of various trophic status: effects of dissolved inorganic nutrients on microorganisms and invertebrates. Freshw. Biol. **52:**1322–1335.
4. **Bärlocher, F.** 1992. The ecology of aquatic hyphomycetes. Springer Verlag, Berlin, Germany.
5. **Bärlocher, F.** 2000. Water-borne conidia of aquatic hyphomycetes: seasonal and yearly patterns in Catamaran Brook, New Brunswick, Canada. Can. J. Bot. **78:**157–167.
6. **Carter, M. D., and K. Suberkropp.** 2004. Respiration and annual fungal production associated with decomposing leaf litter in two streams. Freshw. Biol. **49:**1112–1122.
7. **Collier, K. J., B. J. Smith, and N. J. Halliday.** 2004. Colonization and use of pine wood versus native wood in New Zealand plantation forest streams: implications for riparian management. Aquat. Conserv. Mar. Freshw. Ecosyst. **14:**179–199.
8. **Cross, W. F., B. R. Johnson, J. B. Wallace, and A. D. Rosemond.** 2005. Contrasting response of stream detritivores to long-term nutrient enrichment. Limnol. Oceanogr. **50:**1730–1739.
9. **Cross, W. F., J. B. Wallace, A. D. Rosemond, and S. L. Eggert.** 2006. Whole-system nutrient enrichment increases secondary production in a detritus-based ecosystem. Ecology **87:**1556–1565.
10. **Das, M., T. V. Royer, and L. G. Leff.** 2007. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. Appl. Environ. Microbiol. **73:**756–767.
11. **Díez, J., A. Elosegi, E. Chauvet, and J. Pozo.** 2002. Breakdown of wood in the Agüera stream. Freshw. Biol. **47:**2205–2215.
12. **Edwards, K. T., J. L. Meyer, and S. E. G. Findlay.** 1990. The relative contribution of benthic and suspended bacteria to system biomass, production and metabolism in a low-gradient blackwater river. J. N. Am. Benthol. Soc. **9:**216–228.
13. **Ferreira, V., V. Gulis, and M. A. S. Graça.** 2006. Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecologia **149:**718–729.
14. **Findlay, S., J. Tank, S. Dye, H. M. Valett, P. J. Mulholland, W. H. McDowell, S. L. Johnson, S. K. Hamilton, J. Edmonds, and W. B. Bowd.** 2002. A cross-system comparison of bacterial and fungal biomass in detritus pools of headwater streams. Microb. Ecol. **43:**55–66.
15. **Gessner, M. O., and E. Chauvet.** 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl. Environ. Microbiol. **59:**502–507.
16. **Gessner, M. O., and E. Chauvet.** 1997. Growth and production of aquatic hyphomycetes in decomposing leaf litter. Limnol. Oceanogr. **42:**496–595.
17. **Golladay, S. W., and R. L. Sinsabaugh.** 1991. Biofilm development on leaf and wood surfaces in a boreal river. Freshw. Biol. **25:**437–450.
18. **Golladay, S. W., and J. R. Webster.** 1988. Effects of clear-cut logging on wood breakdown in Appalachian mountain streams. Am. Midl. Nat. **119:** 143–155.
19. **Goodman, L. A.** 1960. On the exact variance of products. J. Am. Stat. Assoc. **55:**708–713.
20. **Greenwood, J. L., and A. D. Rosemond.** 2005. Periphyton response to long-term nutrient enrichment in a shaded headwater stream. Can. J. Fish. Aquat. Sci. **62:**2033–2045.
21. **Greenwood, J. L., A. D. Rosemond, J. B. Wallace, W. F. Cross, and H. S. Weyers.** 2007. Nutrients stimulate leaf breakdown rates and detritivore biomass: bottom-up effects via heterotrophic pathways. Oecologia **151:**637–649.
22. **Gulis, V.** 2001. Are there any substrate preferences in aquatic hyphomycetes? Mycol. Res. **105:**1088–1093.
23. **Gulis, V., K. A. Kuehn, and K. Suberkropp.** 2006. The role of fungi in carbon and nitrogen cycles in freshwater ecosystems, p. 404–435. *In* G. M. Gadd (ed.), Fungi in biogeochemical cycles. Cambridge University Press, Cambridge, United Kingdom.
24. **Gulis, V., A. D. Rosemond, K. Suberkropp, H. S. Weyers, and J. P. Benstead.** 2004. Effects of nutrient enrichment on the decomposition of wood and associated microbial activity in streams. Freshw. Biol. **49:**1437–1447.
25. **Gulis, V., and K. Suberkropp.** 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw. Biol. **48:**123–134.
26. **Gulis, V., and K. Suberkropp.** 2006. Fungi: biomass, production and sporulation of aquatic hyphomycetes, p. 311–325. *In* F. R. Hauer and G. A. Lamberti (ed.), Methods in stream ecology. Academic Press, San Diego, CA.
27. **Hall, R. O., J. B. Wallace, and S. L. Eggert.** 2000. Organic matter flow in stream food webs with reduced detrital resource base. Ecology **81:**3445–3463.
28. **Hieber, M., and M. O. Gessner.** 2002. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology **83:**1026–1038.
29. **Jenkins, C. C., and K. Suberkropp.** 1995. The influence of water chemistry on the enzymatic degradation of leaves in streams. Freshw. Biol. **33:**245–253.
30. **Manly, B. F. J.** 1991. Randomization and Monte Carlo methods in biology. Chapman & Hall, London, United Kingdom.
31. **McClain, M. E., E. W. Boyer, C. L. Dent., S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, E. Mayorga, W. H. McDowell, and G. Pinay.** 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems **6:**301–312.
32. **Methvin, B. R., and K. Suberkropp.** 2003. Annual production of leaf-decaying fungi in 2 streams. J. N. Am. Benthol. Soc. **22:**554–564.
33. **Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J.**

Peterson, J. R. Webster, and J. L. Meyer. 2000. Nitrogen cycling in a forest stream determined by a N-15 tracer addition. Ecol. Monogr. **70:**471–493.
34. **Newell, S. Y., and R. D. Fallon.** 1991. Toward a method for measuring instantaneous fungal growth rates in field samples. Ecology **72:**1547–1559.
35. **Nikolcheva, L. G., and F. Bärlocher.** 2004. Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. Mycol. Prog. **3:**41–49.
36. **Pascoal, C., and F. Cássio.** 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Appl. Environ. Microbiol. **70:**5266–5273.
37. **Sanzone, D. M., J. L. Tank, J. L. Meyer, P. J. Mulholland, and S. E. G. Findlay.** 2001. Microbial incorporation of nitrogen in stream detritus. Hydrobiologia **464:**27–35.
38. **Shearer, C. A.** 1992. The role of woody debris, p. 77–99. *In* F. Bärlocher (ed.), The ecology of aquatic hyphomycetes. Springer Verlag, Berlin, Germany.
39. **Shearer, C. A.** 1993. The freshwater Ascomycetes. Nova Hedwigia **56:**1–33.
40. **Shearer, C. A., and S. B. von Bodman.** 1983. Patterns of occurrence of ascomycetes associated with decomposing twigs in a midwestern stream. Mycologia **75:**518–530.
41. **Sinsabaugh, R. L., S. W. Golladay, and A. E. Linkins.** 1991. Comparison of epilithic and epixylic biofilm development in a boreal river. Freshw. Biol. **25:**179–187.
42. **Spänhoff, B., and M. O. Gessner.** 2004. Slow initial decomposition and fungal colonization of pine branches in a nutrient-rich lowland stream. Can. J. Fish. Aquat. Sci. **61:**2007–2013.
43. **Stanley, E. H., M. D. Johnson, and A. K. Ward.** 2003. Evaluating the influence of macrophytes on algal and bacterial production in multiple habitats of a freshwater wetland. Limnol. Oceanogr. **48:**1101–1111.
44. **Stelzer, R. S., J. Heffernan, and G. E. Likens.** 2003. The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. Freshw. Biol. **48:**1925–1937.
45. **Suberkropp, K.** 1991. Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. Mycol. Res. **95:**843–850.
46. **Suberkropp, K.** 1992. Interactions with invertebrates, p. 118–134. *In* F. Bärlocher (ed.), The ecology of aquatic hyphomycetes. Springer Verlag, Berlin, Germany.
47. **Suberkropp, K.** 1997. Annual production of leaf-decaying fungi in a woodland stream. Freshw. Biol. **38:**169–178.
48. **Suberkropp, K., and M. J. Klug.** 1980. The maceration of deciduous leaf litter by aquatic hyphomycetes. Can. J. Bot. **58:**1025–1031.
49. **Suberkropp, K., and H. Weyers.** 1996. Application of fungal and bacterial production methodologies to decomposing leaves in streams. Appl. Environ. Microbiol. **62:**1610–1615.
50. **Tank, J. L., and J. R. Webster.** 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. Ecology **79:**2168–2179.
51. **Tsui, C. K. M., K. D. Hyde, and I. J. Hodgkiss.** 2001. Longitudinal and temporal distribution of freshwater ascomycetes and dematiaceous hyphomycetes on submerged wood in the Lam Tsuen River, Hong Kong. J. N. Am. Benthol. Soc. **20:**533–549.
52. **Wallace, J. B., and A. C. Benke.** 1984. Quantification of wood habitat in subtropical Coastal Plain streams. Can. J. Fish. Aquat. Sci. **41:**1643–1652.
53. **Wallace, J. B., J. R. Webster, S. L. Eggert, and J. L. Meyer.** 2000. Small wood dynamics in a headwater stream. Verh. Int. Ver. Limnol. **27:**1361–1365.
54. **Wallace, J. B., J. R. Webster, S. L. Eggert, J. L. Meyer, and E. R. Siler.** 2001. Large woody debris in a headwater stream: long-term legacies of forest disturbance. Int. Rev. Hydrobiol. **86:**501–513.
55. **Webster, J. R., E. F. Benfield, T. P. Ehrman, M. A. Schaeffer, J. L. Tank, J. J. Hutchens, and D. J. D'Angelo.** 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. Freshw. Biol. **41:**687–705.
56. **Weyers, H. S., and K. Suberkropp.** 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in two streams. J. N. Am. Benthol. Soc. **15:**408–420.