

Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream

V. GULIS and K. SUBERKROPP

Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, U.S.A.

SUMMARY

1. Decomposition of red maple (*Acer rubrum*) and rhododendron (*Rhododendron maximum*) leaves and activity of associated microorganisms were compared in two reaches of a headwater stream in Coweeta Hydrologic Laboratory, NC, U.S.A. The downstream reach was enriched with ammonium, nitrate, and phosphate whereas the upstream reach was not altered.
2. Decomposition rate, microbial respiration, fungal and bacterial biomass, and the sporulation rate of aquatic hyphomycetes associated with decomposing leaf material were significantly higher for both leaf types in the nutrient-enriched reach. Species richness and community structure of aquatic hyphomycetes also exhibited considerable changes with an increase in the number of fungal codominants in the nutrient-enriched reach.
3. Fungal biomass was one to two orders of magnitude greater than bacterial biomass in both reaches. Changes in microbial respiration rate corresponded to those in fungal biomass and sporulation, suggesting a primary role of fungi in leaf decomposition.
4. Nutrient enrichment increased microbial activity, the proportion of leaf carbon channelled through the microbial compartment and the decomposition rate of leaf litter.

Keywords: aquatic hyphomycetes, bacteria, conidial production, fungal biomass, respiration

Introduction

Leaf litter is an important energy source for food webs in woodland streams (Minshall, 1967; Fisher & Likens, 1973). Aquatic heterotrophic microorganisms (fungi and bacteria) are crucial for the mineralization of leaf litter and also render it more palatable for leaf-shredding invertebrates (Kaushik & Hynes, 1971; Bärlocher, 1985; Suberkropp, 1992). Consequently, along with driving decomposition processes, fungi, especially aquatic hyphomycetes, and bacteria are important intermediaries in energy flow in lotic ecosystems (Suberkropp & Klug, 1976; Bärlocher &

Kendrick, 1981; Findlay & Arsuffi, 1989; Bärlocher, 1992).

Eutrophication caused by anthropogenic disturbances can affect stream biota and ecosystem functioning directly, through increased nutrient concentrations, as well as indirectly through oxygen depletion. Of major concern in streams are non-point sources of inorganic nitrogen and phosphorus resulting from agriculture runoff, urban activity and atmospheric deposition (Carpenter *et al.*, 1998). Although a number of studies have examined the effects of nutrient enrichment on primary producers (Pringle, 1987; Rosemond, Mulholland & Elwood, 1993) and unshaded, autotrophic streams (Peterson *et al.*, 1993), less is known about how nutrient enrichment alters the metabolism of woodland streams that are primarily heterotrophic. Several studies have indicated that leaf litter decomposition and fungal activity in lotic

Correspondence: Vladislav Gulis, Departamento de Zoologia, Universidade de Coimbra, 3004-517 Coimbra, Portugal.

E-mail: vgulis@ci.uc.pt

ecosystems can be affected by the concentration of nutrients (e.g. nitrogen and phosphorus) in the water (Elwood *et al.*, 1981; Meyer & Johnson, 1983; Suberkropp & Chauvet, 1995; Sridhar & Bärlocher, 2000; Grattan & Suberkropp, 2001; Rosemond *et al.*, 2002). It is also known that microorganisms associated with submerged leaf litter can obtain nitrogen (Kaushik & Hynes, 1971; Suberkropp, 1995) and phosphorus (Mulholland *et al.*, 1984) from water. High nutrient concentrations potentially stimulate the activity of heterotrophic microorganisms associated with submerged leaf litter and, hence, influence decomposition rates and the availability of detrital resources to invertebrates.

The present study is part of a larger project examining the response of a small woodland stream to continuous enrichment with nitrogen and phosphorus. The specific objective of the present study was to examine the effect of increased nutrient concentrations on the decomposition of red maple (*Acer rubrum* L.) and rhododendron (*Rhododendron maximum* L.) leaves placed in an unaltered and a nutrient enriched reach of a headwater stream. We also determined the rate of microbial respiration, fungal and bacterial biomass, and sporulation rate and community composition of aquatic hyphomycetes associated with these leaves, to link changes in microbial performance with decomposition processes.

Methods

Study site, experimental set up and decomposition

The study was conducted in two reaches of a headwater stream that drains catchment 54 in Coweeta Hydrologic Laboratory, NC, U.S.A. (Swank & Waide, 1988; Cuffney, Wallace & Lugthart, 1990). This is a small (mean discharge, *ca.* 1.5 L s⁻¹) softwater stream on a south-facing slope that drains mixed deciduous forest in the southern Appalachian Mountains at an altitude of *ca.* 850 m a.s.l. Rhododendron forms a dense understory, which results in shading of the stream throughout the year. The downstream reach was enriched with ammonium, nitrate and phosphate with a pump that added a concentrated solution of ammonium nitrate and potassium phosphate into a pipe laid along the streambed and fed with streamwater. The rate at which nutrients were added to the water in the pipe was controlled by an

Isco data logger situated at the downstream weir so that the amount of nutrient solution added was proportional to instantaneous discharge. The pipe had multiple openings along the downstream reach where nutrient-enriched water dripped into the stream. The reach immediately upstream from the pump was not altered and served as a control. Because of the close proximity of the two reaches, they did not obviously differ prior to nutrient addition. Water samples were taken every 2 weeks at six sites along the stream, filtered through membrane filters (0.45 µm pore-size), transported to the laboratory on ice and frozen. Samples were subsequently thawed and analysed for ammonium-N (automated phenate colorimetry), nitrate plus nitrite-N (cadmium reduction followed by automated colorimetry) and orthophosphate-P (automated ascorbic acid) (United States Environmental Protection Agency, 1983) with a Technicon autoanalyser by the Stable Isotope/Soil Biology Laboratory in the Institute of Ecology, University of Georgia. Water temperature was monitored with Optic StowAway temperature probes (Onset Computer Corp., Pocasset, MA, U.S.A.) that recorded temperature every 30 min.

Autumn-shed red maple and rhododendron leaves were picked up soon after abscission in October, 2000, along stream banks, brought back to the laboratory and air dried for 2 weeks. Portions of about 2 g of air-dry material (rhododendron) and 1 g (red maple) were preweighed for placement into litterbags. One day before they were placed in the stream, leaves were wetted in deionised water for 2 h and enclosed in fibreglass mesh bags (1 mm mesh size to reduce invertebrate feeding). On 1 November 2000, leaf bags were transported to the stream in a cooler. They were tied to lines and secured to the stream bottom with nails. Three groups of bags were placed in the upstream and three in the downstream reach. On each sampling date, six bags of each leaf type (three for dry mass estimation and three for punching out leaf disks) were recovered from each reach. To determine dry mass of the leaves remaining, leaf material was removed from bags, rinsed with stream water and then returned to the laboratory. Samples were dried at 100 °C for at least 2 days, weighed, combusted at 500 °C overnight, weighed again and their ash free dry mass (AFDM) calculated. Six bags of both leaf types recovered at the beginning of the experiment were used to determine the conversion

factor that allowed initial AFDM to be calculated from air-dry mass. On each collection date, leaf material from other sets of bags was sampled at the stream by punching out disks (11.8 mm diameter). Subsets of disks were used to determine fungal and bacterial biomass, microbial respiration, sporulation rate of aquatic hyphomycetes and AFDM. Leaf material that remained after punching out leaf disks was dried, ground in a Wiley mill, and used to determine carbon and nitrogen content with a C/H/N analyser (Carlo Erba Strumentazione, Milan).

Respiration

Microbial respiration was measured as the oxygen uptake of decomposing leaf material at stream water temperatures. On each collection date, 10 leaf disks from each bag were placed in 26 mL stream water in respiration chambers placed in the stream and oxygen concentration was recorded every 5–7 min with YSI 5100 Dissolved Oxygen Meters (Yellow Springs, OH, U.S.A.) for 30 min in darkness. Additional chambers containing only stream water served as a control. Oxygen consumption was determined from the slope of the regression of oxygen concentration on time minus the control slope (determined from stream water alone) and expressed per leaf AFDM per hour. The proportion of total carbon loss of the leaf litter due to respiration was calculated by summing up oxygen uptake over the entire experiment (after linear interpolation for missing data), applying a respiratory quotient of 1, and assuming 50% carbon content of leaf AFDM.

Fungi

Fungal biomass associated with decomposing leaf litter was monitored by measuring ergosterol concentration. Five leaf disks from each of three replicate bags per leaf type and treatment were placed in 5 mL methanol in scintillation vials and transported back to the laboratory. Ergosterol was extracted (Newell, Arsuffi & Fallon, 1988; as modified by Suberkropp & Weyers, 1996) and determined by comparing absorbance at 282 nm after separation by HPLC (Shimadzu) with a standard concentration of ergosterol (Fluka). To convert ergosterol concentration to fungal biomass, we assumed an ergosterol concentration of $5.5 \mu\text{g mg}^{-1}$ of mycelial dry mass (Gessner & Chauvet, 1993).

Sporulation rates and community structure of aquatic hyphomycetes were determined by inducing sporulation from 10 leaf disks from each bag in laboratory microcosms simulating stream conditions (Suberkropp, 1991). Chambers were filled with 40 mL of filtered stream water (Whatman GF/F) from the upstream or downstream reach and aerated with 80–100 mL air min^{-1} at 15 °C for 24–30 h. Chambers were drained into beakers, 0.1 mL of a 0.5% Triton X-100 was added and suspensions were stirred to ensure a uniform distribution of conidia. Two to four millilitres of suspension was filtered through membrane filters (5 μm pore size, Millipore) and stained with trypan blue in lactic acid (0.1%). Two filters were prepared for each replicate and the conidia in a total of 50 fields were identified and counted (Leitz Laborlux, 160 $\times$).

The concentration of conidia and the community structure of aquatic hyphomycetes in stream water from both reaches were also determined on each collection date. Stream water was filtered (300–500 mL) through membrane filters (5 μm pore size, Millipore) (Iqbal & Webster, 1973) and conidia were stained with trypan blue in lactic acid (0.1%). Filters were taken to the laboratory where conidia were identified and counted (50–75 fields, Leitz Laborlux, 160 $\times$).

Bacteria

Bacteria were counted using epifluorescence microscopy following staining with DAPI (Velji & Albright, 1993). Five leaf disks from each bag were preserved in 5 mL of 2% buffered formalin solution made in saline (8.5 g NaCl L^{-1}) and stored until analysis. One millilitre of tetrasodium pyrophosphate solution (NaPPi – 60 mM, Tween 80 – 60 mg L^{-1} , formalin – 1%) was added to aid in bacterial dispersion, tubes were then vortexed and incubated for 20 min on a shaker at 150 rpm. To dislodge bacteria from leaf surfaces, samples were sonicated for 20 min in a Branson 1200 sonication bath and vortexed. This preparation was then diluted appropriately, 1.5 mL was transferred into 2 mL polypropylene centrifuge tubes and 15 μL of DAPI (final concentration 5 $\mu\text{g mL}^{-1}$) were added. Tubes were incubated for 10 min in the dark and bacteria suspensions were loaded on to a 12-port Millipore filter apparatus. Samples were filtered through black polycarbonate membrane filters

(0.22 µm pore size, Poretics) supported by cellulose nitrate backing filter (5 µm pore size, Millipore) and rinsed with 2 mL of 2% buffered formalin. All solutions used in sample preparation were filter sterilised (0.22 µm pore size membrane filters). Filters mounted in Cargille type FF non-fluorescent immersion oil were scanned at 1000× with a Leitz Diaplan microscope equipped for epifluorescence observation. At least 10 grids per filter (about 20–30 cells per grid) were counted. Photographs (Kodak Elite Chrome 400 slide film) were taken for biovolume estimates. They were scanned and four bacteria classes were sized using Scion Image software (Scion Corp., Frederick, MD, U.S.A.).

Bacterial biovolume was converted to bacterial C using the following empirical formula (Simon & Azam, 1989), to take into account differences in the carbon-to-volume ratios of different bacterial classes: $C = 89 \cdot V^{0.59}$, where the conversion factor has units of fg C µm⁻³. For our bacterial classes with biovolume ranging from 0.15 to 0.59 µm³ it yielded values from 195 to 110 fg C µm⁻³. To calculate bacterial C associated with leaf samples, total bacterial counts and the contribution of each class, biovolume data and specific conversion factors were used for each sampling date. We found that this conventional technique, i.e. sonication of leaf litter to dislodge bacteria (Velji & Albright, 1993; Weyers & Suberkropp, 1996; Komínková *et al.*, 2000), underestimated bacterial populations by a factor of 10 in comparison with counts made from leaf material that was homogenised with a tissue grinder (unpublished data). Consequently, our bacterial counts have been multiplied by this conversion factor.

Statistical analysis

Decomposition rate, k , was estimated by linear regression of ln transformed data (exponential model). Differences in k were determined with analysis of covariance (ANCOVA) followed by Tukey's test to compare slopes among treatments. Effects of enrichment and leaf type on total nitrogen, ergosterol, the sporulation rate of aquatic hyphomycetes, the number of conidia in transport, bacterial biomass and microbial respiration were tested by repeated measures ANOVA (Zar, 1984). Data derived from conidial and bacterial counts were $\ln(x + 1)$ transformed. Statistical analyses were done with SYSTAT (version 10).

Results

In the downstream reach during the study period, the mean (range) total inorganic N (nitrate plus ammonium) concentration was 199 (19–691) µg L⁻¹ and soluble reactive phosphorus (SRP) was 34 (5–129) µg L⁻¹, compared with 11 (5–25) and 4 (0–9) µg L⁻¹, respectively, in the upstream reach. Mean daily water temperatures were very similar in both reaches (data not shown) and ranged from 14 to 15 °C at the beginning (November) and the end of study (June) to 4 °C in January.

Decomposition was significantly faster (ANCOVA, $P < 0.001$) for both leaf types in the nutrient-enriched reach than in the control reach (Fig. 1a, Table 1). Decomposition was faster for maple than for rhododendron leaves in the control reach (ANCOVA, $P < 0.001$), but not in the enriched reach ($P = 0.13$).

The initial nitrogen content of maple and rhododendron leaves was low (0.54 and 0.36% of AFDM, respectively). The nitrogen content of leaves increased sharply after submergence in the enriched reach (Fig. 1b), whereas in the control reach the increase was delayed and the maximum values attained were lower. The differences between the two reaches and leaf types were highly significant (ANOVA, $P \leq 0.005$).

Respiration rates associated with leaves from the nutrient-enriched reach were higher than values from the control reach for both leaf types, and respiration rates associated with maple leaves were higher than those associated with rhododendron leaves in both reaches (ANOVA, $P < 0.001$ for all four comparisons, Fig. 2).

The differences in fungal biomass associated with leaves in the control and treatment reaches were significant for both maple and rhododendron leaf litter (ANOVA, $P < 0.001$ and $P = 0.003$, respectively, Fig. 3a). Fungal biomass associated with maple leaves in the nutrient-enriched reach increased markedly faster than in the control reach, with values of 57 mg of fungal carbon g leaf AFDM⁻¹ after 13 days (17 times higher than initial values) but only 13 mg g⁻¹ in the control reach. Fungal biomass associated with maple leaves generally exceeded that of rhododendron within a given reach (Fig. 3a, ANOVA, $P < 0.001$ in the control reach, $P = 0.006$ treatment reach).

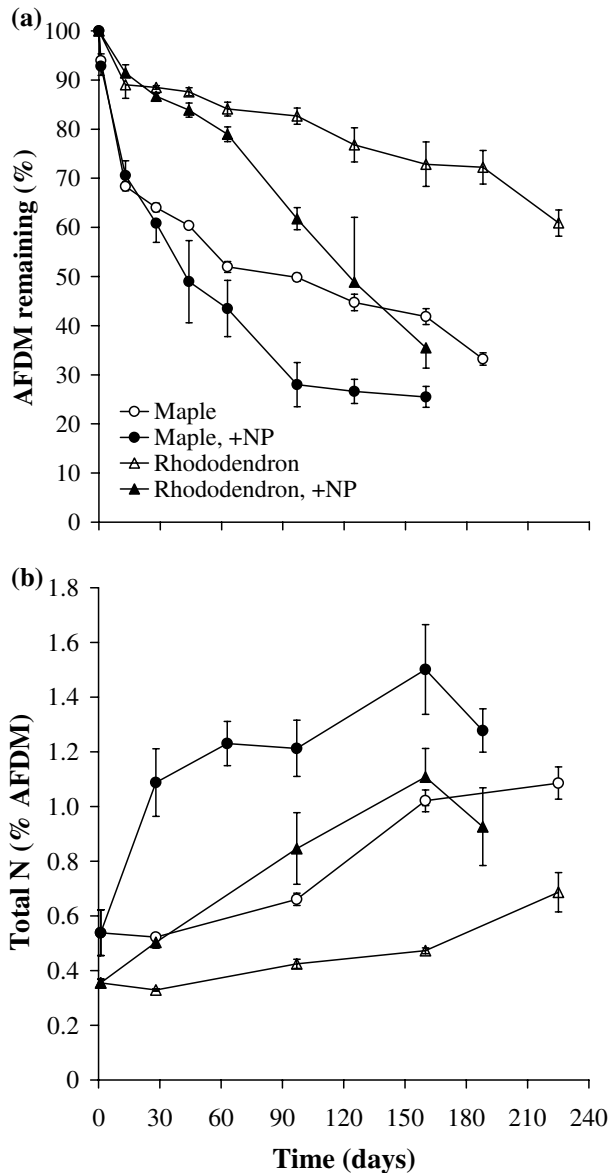


Fig. 1 Mass loss (a) and total nitrogen concentration (b) of maple and rhododendron leaf material in the control and nutrient-enriched (+NP) reaches of the experimental stream. Symbols indicate mean $\pm$ 1 SE. Study started on 1 November 2000.

Table 1 Decomposition rates (k , $\pm 95\%$ CL) of maple and rhododendron leaves in the control and nutrient-enriched (+NP) reaches of stream 54

Leaf species and site	k (day ⁻¹) $\pm 95\%$ CL	r^2
Maple	0.0048 $\pm$ 0.0007	0.87 a
Maple, +NP	0.0089 $\pm$ 0.0017	0.83 b
Rhododendron	0.0018 $\pm$ 0.0003	0.83 c
Rhododendron, +NP	0.0065 $\pm$ 0.0016	0.78 a,b

Different letters indicate significant differences ($P < 0.001$) among slopes using Tukey's test.

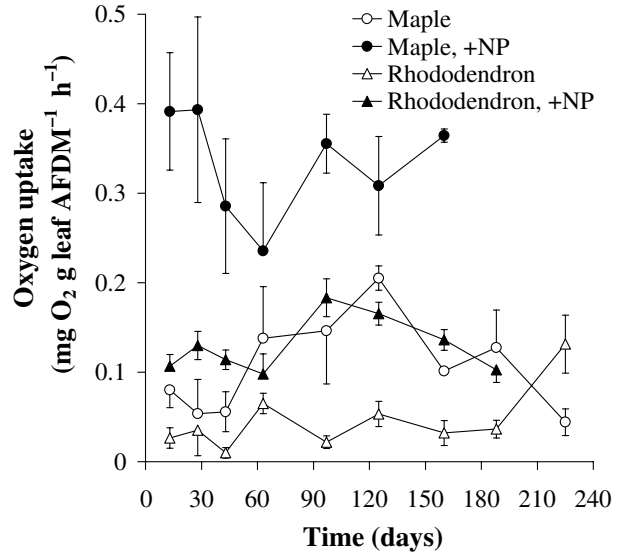


Fig. 2 Microbial respiration (mean oxygen uptake $\pm$ 1 SE) from rhododendron and maple leaf litter in the control and nutrient-enriched (+NP) reaches.

Sporulation rate of aquatic hyphomycetes associated with leaf litter (Fig. 3b) was significantly higher for both maple and rhododendron in the nutrient-enriched reach than in the control reach (ANOVA, $P < 0.001$ for both comparisons). Sporulation reached a peak of 4.1 and 5.1 conidia $\mu\text{g leaf AFDM}^{-1} \text{ day}^{-1}$ for maple and rhododendron, respectively, in the treatment reach against 0.2 and 0.15 conidia $\mu\text{g leaf AFDM}^{-1} \text{ day}^{-1}$ in the control reach. With respect to the differences between leaf species, sporulation rate of fungi from maple leaves was higher than from rhododendron in the control reach (ANOVA, $P < 0.001$). In the enriched reach sporulation rate was high from both leaf types but still statistically different (ANOVA, $P = 0.013$) because of distinctive temporal patterns of sporulation (Fig. 3b). The concentration of aquatic hyphomycete conidia in water in the nutrient-enriched reach was also significantly higher than in the control reach (ANOVA, $P < 0.001$, Fig. 4).

A total of 35 species of aquatic hyphomycetes were identified from the nutrient-enriched reach and 32 from the control reach (Table 2). The species richness of aquatic hyphomycetes in transport was generally higher from the enriched reach, except on two dates at the very beginning and the end of study (Fig. 5a). The number of fungal species associated with leaves recovered from the nutrient-enriched reach on each

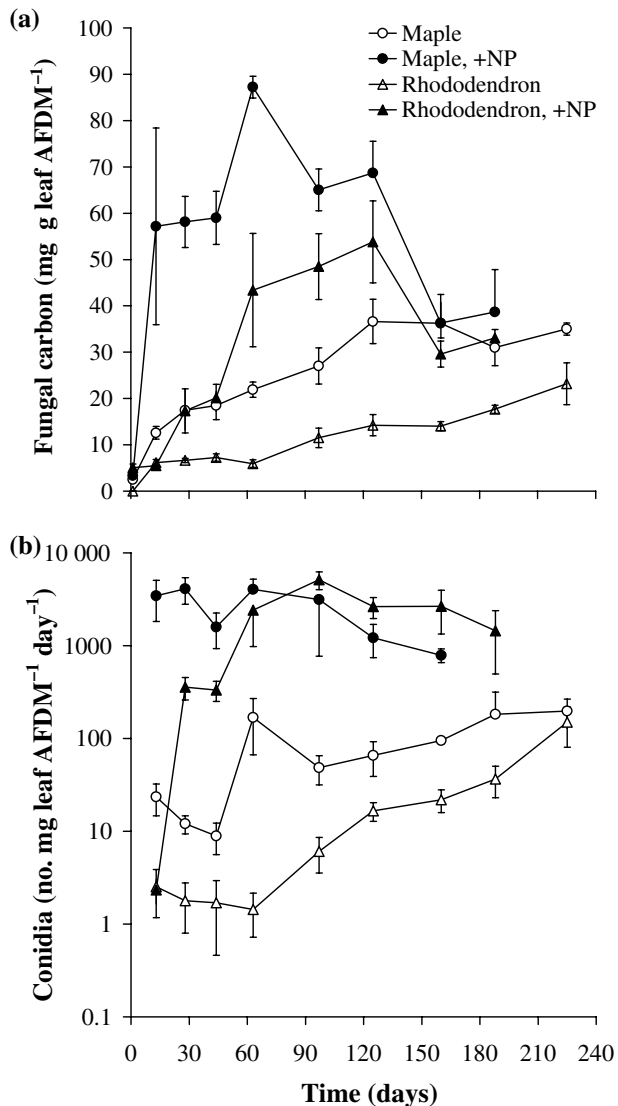


Fig. 3 Fungal biomass (a) and sporulation rate of aquatic hyphomycetes (b) associated with maple and rhododendron leaf litter in the control and nutrient-enriched (+NP) reaches. Symbols indicate mean $\pm$ 1 SE.

date was nearly twice that from the control reach (Fig. 5b). No clear differences were observed between leaf types. Some differences in relative abundances of dominant species between reaches were evident (Fig. 6, Table 2). The relative abundance of *Tetrachaelum elegans* conidia in transport was higher in the nutrient-enriched reach than in the control reach, whereas that of *Articulospora tetracledia* was lower (Figs 6a, b). The most striking difference between aquatic hyphomycete assemblages from leaves is the appearance and codominance of *Dimorphospora folii-*

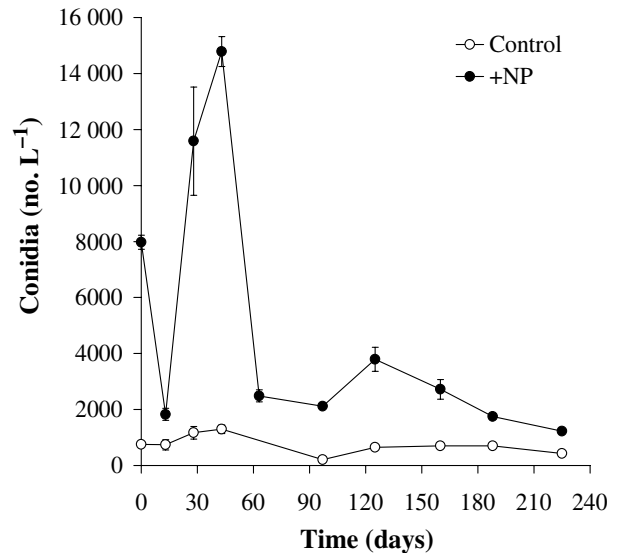


Fig. 4 Concentration of conidia (mean $\pm$ 1 SE) of aquatic hyphomycetes in the control and nutrient-enriched (+NP) reaches.

cola in the nutrient-enriched reach (Fig. 6c–f). *Anguillospora filiformis* was also more abundant in the nutrient-enriched reach in comparison with the control reach. Generally, fungal communities from the control reach were dominated by only two species (*Alatospora acuminata* and *Articulospora tetracledia*) whereas four to six species were codominant on substrata from the nutrient-enriched reach.

Bacterial numbers associated with maple and rhododendron leaves in the nutrient-enriched reach were significantly higher than in the control reach (ANOVA, $P = 0.001$ for both comparisons; Fig. 7a). The same trend was apparent if we consider bacterial carbon per unit of leaf AFDM (ANOVA, $P < 0.01$ and $P < 0.02$; Fig. 7b). Differences between leaf species were also significant in both reaches (ANOVA, $P < 0.01$, bacterial C). During the study, we observed shifts in the contribution of different bacteria classes to total biomass for both stream reaches and leaf types. The relative importance of bacteria with higher biovolume, and hence biomass, increased over time (data not shown).

Discussion

Decomposition of both leaf species was stimulated by enrichment with N and P as has been demonstrated previously in laboratory microcosms (Kaushik & Hynes, 1971; Howarth & Fisher, 1976). In whole

Table 2 Aquatic hyphomycete conidia from water (W) and associated with rhododendron (R) and maple (M) leaves from control and nutrient-enriched (+NP) reaches of stream 54 (mean of percent contributions over all sampling dates)

Species	W	W, +NP	M	M, +NP	R	R, +NP
<i>Alatospora acuminata</i> Ingold aggreg.	9.5	5.9	9.0	4.6	3.1	0.7
<i>Anguillospora filiformis</i> Greathead	17.9	16.0	1.6	19.7	11.2	28.4
<i>Anguillospora</i> cf. <i>furtiva</i> J. Webster & Descals		0.1				
<i>Anguillospora</i> cf. <i>rosea</i> J. Webster & Descals		0.1				
<i>Articulospora tetracladia</i> Ingold	20.5	11.3	32.9	5.3	11.2	1.8
<i>Casaresia sphagnorum</i> Gonz. Frag.	2.1	0.8	3.7		0.4	
<i>Clavariopsis aquatica</i> De Wild.	0.1	0.2				
<i>Clavatospora longibrachiata</i> (Ingold) Marvanová & Sv.Nilsson	0.4	0.1				
<i>Culicidospora aquatica</i> R.H. Petersen	0.2	+				+
<i>Dendrospora erecta</i> Ingold	0.6	0.2				
<i>Dimorphospora foliicola</i> Tubaki	?	?		21.9	2.8	19.2
<i>Dwayaangam</i> cf. <i>dichotoma</i> Nawawi	0.1					
<i>Goniopila monticola</i> (Dyko) Marvanová & Descals	+	0.3		+		
<i>Flagellospora curvula</i> Ingold	0.3	0.5	2.9	0.2		0.1
<i>Fontanospora alternibrachiata</i> Dyko	0.2					
<i>Fontanospora eccentrica</i> (R.H. Petersen) Dyko		+				
<i>Heliscella stellata</i> (Ingold & V.J. Cox) Marvanová	1.3	0.6	0.5	0.9		0.1
<i>Heliscina antennata</i> Marvanová	0.4	0.1		+		
<i>Heliscina campanulata</i> Marvanová	0.6	0.7		1.1		
<i>Heliscus lugdunensis</i> Sacc. & Thérre	1.4	1.0		0.1	17.2	6.4
<i>Lateriramulosa uni-inflata</i> Matsush.	0.1	0.8		1.0		+
<i>Lemonniera pseudofloscula</i> Dyko	3.9	0.4	0.7	+	9.8	
<i>Lunulospora curvula</i> Ingold		1.4	1.7	0.9	0.3	1.1
<i>Monotosporella microaquatica</i> (Tubaki) Sv.Nilsson		0.1		2.2		0.2
<i>Mycofalcella calcarata</i> Marvanová, Om-Kalth. & J.Webster	0.3	0.7				+
<i>Taeniospora gracilis</i> var. <i>enecta</i> Marvanová & Stalpers	7.0	5.6		+		
<i>Tetrachaetum elegans</i> Ingold	2.2	11.0	2.7	16.9	3.2	5.5
<i>Tricladium biappendiculatum</i> (G.R.W. Arnold) Marvanová & Descals		+				
<i>Tricladium chaetocladium</i> Ingold	0.9	4.3		7.6	2.8	10.9
<i>Trinacrium</i> sp.		0.2				
<i>Tripospermum</i> sp.	0.1				3.7	
<i>Triscelophorus konajensis</i> K.R. Sridhar & Kaver.	3.1	2.0	0.6	+	2.1	0.1
<i>Triscelophorus monosporus</i> Ingold	2.8	2.6	1.2	+	1.0	+
Sigmoid (<60 µm)*	17.5	26.2	35.3	17.2	31.0	25.4
Sigmoid (60–120 µm)	2.9	3.0	1.9			
Sigmoid (>120 µm)	1.9	1.2	1.9			
Unidentified no. 1	0.2					
Unidentified no. 2 (pentaradial)		0.1				
Unidentified tetradia	1.2	1.3	3.5	+		
Sum	100.0	100.0	100.0	100.0	100.0	100.0
Total no. of species	31	35	15	21	14	17

*Aquatic hyphomycetes with sigmoid conidia often cannot be identified on the basis of detached spores alone.

+, Species present; ?, could not be identified on filters from water.

stream studies, however, nutrient enrichment has had variable effects on decomposition rates. Triska & Sedell (1976) found no significant effect of nitrogen

addition even though N : P ratios in the water suggested N limitation. Enrichment of a small hard-water stream with P stimulated leaf decomposition

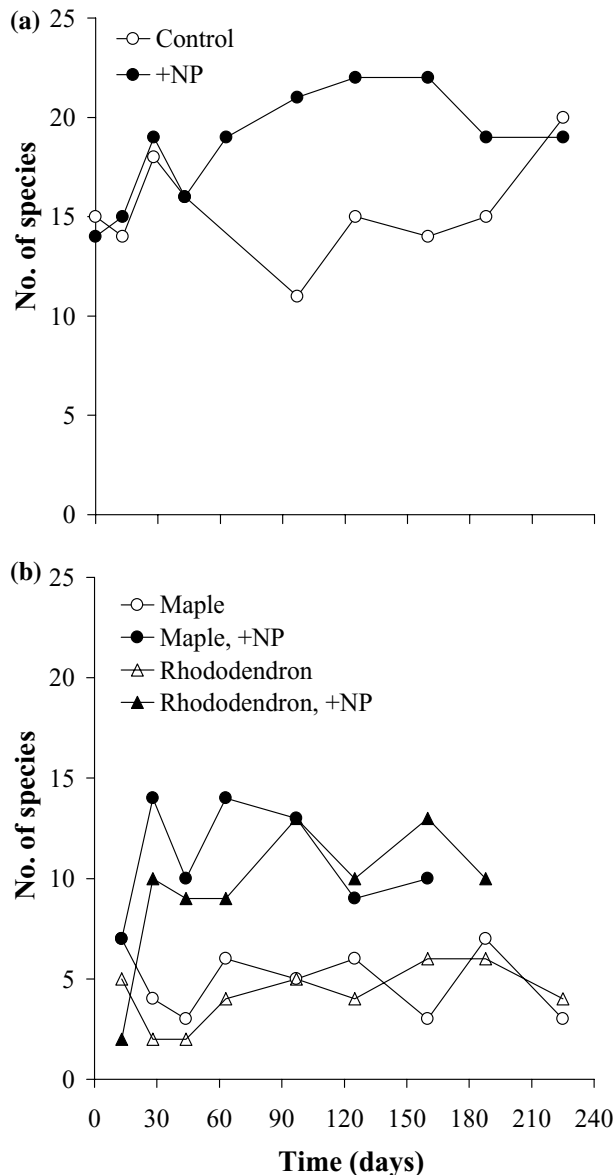


Fig. 5 Species richness of aquatic hyphomycetes (as conidia) in the control and nutrient-enriched (+NP) reaches. (a) Conidia in transport (stream water). (b) Conidia released from leaf material after incubation in the laboratory.

(Elwood *et al.*, 1981) whereas enrichment of the same stream with N did not (Newbold *et al.*, 1983), suggesting the process was P limited in this stream. Studies using slow-release fertilizer pellets (containing both N and P) placed within litter bags have also produced variable results. In a stream with low phosphorus concentration, decomposition rates increased with the addition of fertilizer pellets (Robinson & Gessner, 2000). Using similar methods, Royer

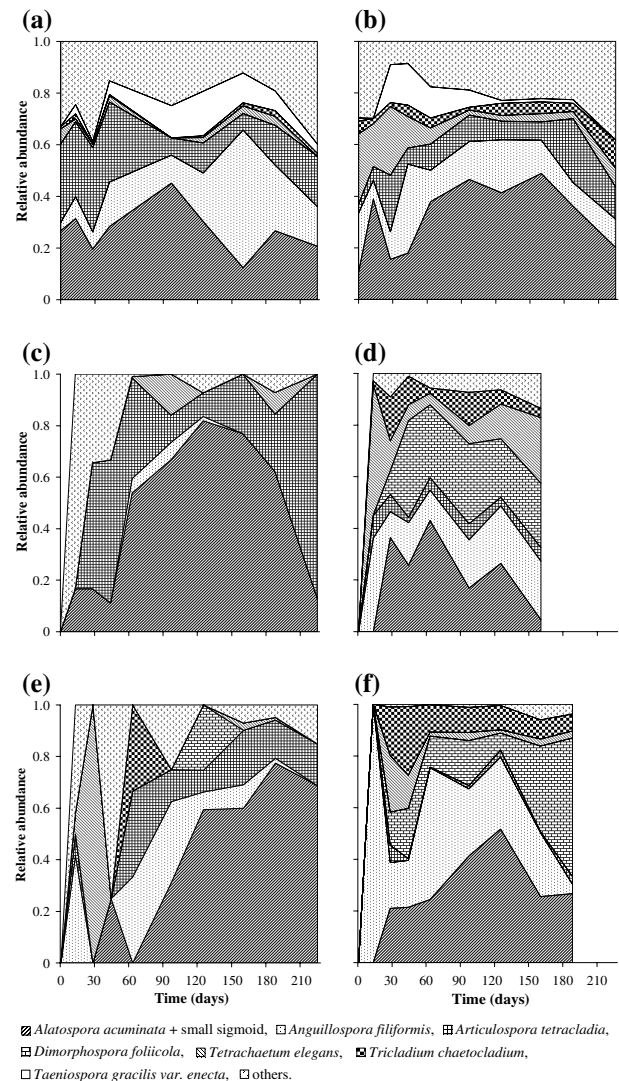


Fig. 6 Relative abundance of dominant aquatic hyphomycetes (as conidia in transport in water and from leaf material) in the control and nutrient-enriched (+NP) reaches. (a) Water. (b) Water, +NP. (c) Maple. (d) Maple, +NP. (e) Rhododendron. (f) Rhododendron, +NP. A dominant species has mean per cent contribution of more than 10% over all sampling dates from at least one leaf substrate- or water-treatment combination.

& Minshall (2001) found that the addition of fertilizer pellets did not stimulate decomposition. Nutrient concentrations in the stream they studied were moderate and therefore possibly not limiting to the microorganisms involved in decomposition (Royer & Minshall, 2001). Enrichment of flow-through channels in three streams indicated that decomposition was colimited by N and P, or limited by P alone, depending on the nutrient concentrations of the

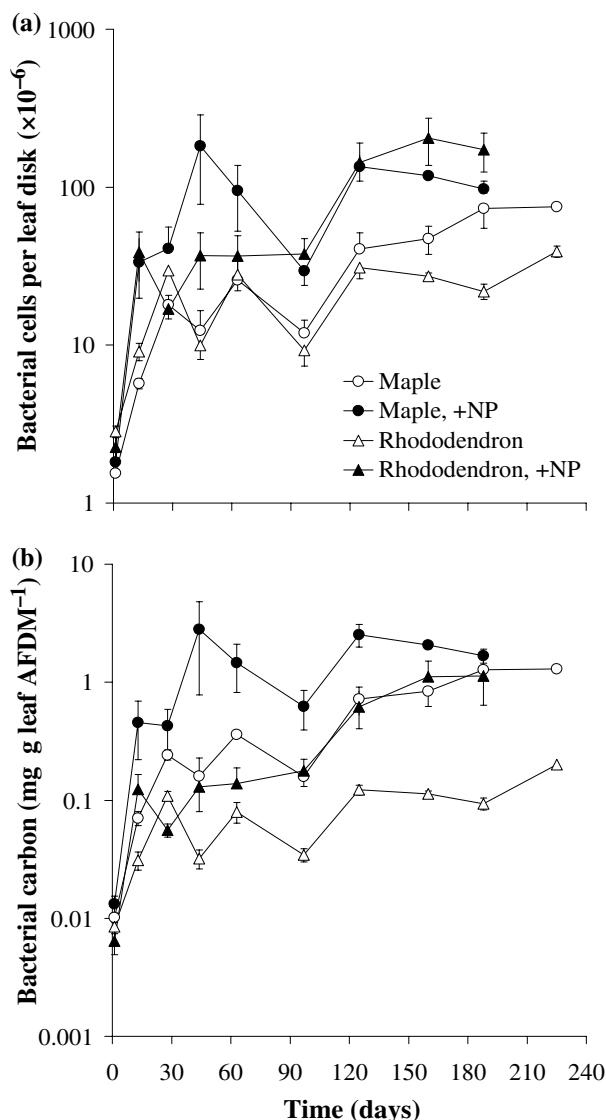


Fig. 7 Bacterial numbers (a) and biomass (b) associated with maple and rhododendron leaf litter in the control and nutrient-enriched (+NP) reaches. Symbols indicate mean ± 1 SE.

streams studied (Grattan & Suberkropp, 2001). In the present study, the experimental stream was supplemented with both N and P as the concentrations of both these nutrients were very low. In addition, previous research indicated that fungal biomass associated with decomposing wood veneers in two headwater streams at Coweeta similar to our study stream was stimulated only when N and P were both supplied (Tank & Webster, 1998).

Both leaf types used in our study had relatively low initial nitrogen concentrations (Fig. 1b) but were reported as having different decomposition rates

(Webster & Benfield, 1986). In the control reach, maple leaf litter decomposed faster and maintained higher microbial biomass and activity than rhododendron. Similar differences were apparent in the nutrient-enriched reach but they were less pronounced and decomposition rate did not differ between leaf types. This suggests that microorganisms colonising rhododendron leaf litter are more nutrient limited than those on maple, the former having an initially lower nitrogen content and lower surface area to volume ratio than maple. The slower decomposition of rhododendron leaves could also be because of a poorer quality of carbon available than in maple. The nitrogen content of leaf litter increased faster and reached higher values in the downstream reach than in the control (Fig. 1b). At least in part, this can be attributed to higher microbial activity and accrual of biomass associated with leaf material following nutrient enrichment.

Changes in microbial respiration generally corresponded to patterns of change in fungal biomass and sporulation, suggesting a major role of fungi in total microbial activity and hence decomposition. Respiration was somewhat influenced by lower water temperatures in winter, however, as it did not follow the pattern known from laboratory experiments at constant temperature (Suberkropp, 1991, unpublished data). This was especially pronounced for the downstream (+NP) reach with higher microbial biomass (Fig. 3a). On average, respiration accounted for 31 and 33% of carbon loss in maple and rhododendron leaves in the control reach, respectively, and for 56 and 43% in the nutrient-enriched reach. Elwood *et al.* (1981) estimated that microbial respiration accounted for between 33 (control reach) and 39% (high-level P enriched reach) of the mass loss of red oak (*Quercus rubra* L.) leaves. However, Baldy & Gessner (1997) found that microbial respiration accounted for only 17% of alder [*Alnus glutinosa* (L.) Gaertn.] leaf mass loss in a stream with a moderately high nutrient concentrations.

Fungal biomass associated with leaf litter in the nutrient-enriched site reached 17.4% of total detrital mass for maple and 10.8% for rhododendron compared with only 7.3 and 4.6% in the control reach, respectively. Our results in this respect are similar to those of others who have found a positive response of fungi to enrichment both in laboratory experiments (Sridhar & Bärlocher, 2000) and in streams

(Grattan & Suberkropp, 2001). Robinson & Gessner (2000) found no differences in fungal biomass associated with leaves enriched with fertiliser pellets in coarse mesh litter bags, however, possibly as a result of extensive invertebrate feeding that may have been stimulated by nutrients and masked fungal production. The rate of accumulation and maximum values of fungal biomass for leaves decomposing in our fine-mesh litter bags were considerably higher than found previously for both leaf types in coarse-mesh litter bags in another Coweeta stream with low nutrient concentrations (Paul & Meyer, 1996). Paul & Meyer (1996) reported that the initial (and maximum) fungal biomass associated with maple leaves was 1.5% of the total detrital mass and then dropped to about 0.5% over 100 days. Fungal biomass of rhododendron leaves was 0.4% initially and then declined below detection limits over the next 100 days. These differences suggest that invertebrate feeding may alter the pattern of fungal biomass accumulation during leaf decomposition.

High nutrient concentrations in the enriched reach had a greater effect on the rate of fungal reproduction than on fungal biomass (Fig. 3). This was reflected by the higher concentration of conidia in transport at the downstream end of the nutrient-enriched reach than in the control reach (Fig. 4). Although the concentration of conidia in water was much higher in the nutrient-enriched reach than in the control reach, fluctuations were similar suggesting common factors such as temperature and discharge also affected fungal reproduction. Sporulation rates of aquatic hyphomycetes from leaves in the enriched reach were about an order of magnitude greater than from leaves in the control reach. Similar strong positive effects on conidial production have been found in laboratory microcosms supplemented with nitrate and phosphate, either alone or in combination (Sridhar & Bärlocher, 1997, 2000; Suberkropp, 1998), and in field experiments (Grattan & Suberkropp, 2001). Consequently, reproduction in aquatic hyphomycetes appears to be more sensitive to nutrients than is growth.

The effect of N and P on species richness and composition of aquatic hyphomycetes was appreciable, although less pronounced than for fungal biomass or reproduction. Despite a similar number of species identified from each reach over the entire

study, species richness was higher in the nutrient-enriched reach than in the control reach on each sampling date (Fig. 5). We also noticed shifts in the relative contribution of dominant species (Fig. 6). These findings are contrary to previous observations (Sridhar & Bärlocher, 2000) where no obvious shifts in species composition were observed in response to nutrient addition.

In contrast to fungi, the maximum bacterial contribution to total detrital mass was very low: 0.58 and 0.23% (maple and rhododendron, respectively) in the nutrient-enriched reach or 0.27 and 0.04% in the control reach. This was 17–325 times lower than fungal biomass and is close to values obtained from another softwater stream (32–185 times lower; Weyers & Suberkropp, 1996). Bacteria may exhibit higher turnover rates than fungi but it has been found that fungal production associated with decomposing leaf litter in lotic ecosystems is similar to (Baldy & Gessner, 1997) or as much as 100 times higher than bacterial production, depending on the stage of decomposition (Suberkropp & Weyers, 1996; Weyers & Suberkropp, 1996). In both reaches, fungal contribution to total microbial (fungal + bacterial) biomass ranged from 94.5 to 99.7% throughout the study. Consequently, increased nutrient concentrations did not significantly shift the fungal–bacterial equilibrium. Similar values (95–99%) have been obtained from studies in lotic ecosystems, ranging from a first order woodland stream to a large river, with natural nitrogen concentrations even greater than we were able to achieve experimentally (0.6, 0.7 and 1.8 mg nitrate-N L⁻¹; Baldy, Gessner & Chauvet, 1995; Baldy & Gessner, 1997; Hieber & Gessner, 2002). Thus, it appears that fungi are the primary agents of leaf decomposition in streams over a relatively wide range of nutrient concentrations.

Overall, this whole-stream nutrient addition stimulated microbial activity and increased the proportion of leaf carbon channelled through microbial compartment (both CO₂ evolution and production). These results suggest that one of the major effects of eutrophication on naturally oligotrophic heterotrophic streams would be to increase the decomposition rate of leaf litter. This should reduce the amount of leaf detritus in the stream through much of the year and may interfere with the seasonal feeding activities of leaf-shredding invertebrates.

Acknowledgments

This work was supported in part by the North Atlantic Treaty Organization under NSF-NATO Postdoctoral Fellowship in Science and Engineering awarded to VG and by an NSF grant, DEB 9806610. We thank H. Weyers for excellent assistance in the field and A. Rosemond and two anonymous reviewers for critical comments on the manuscript.

References

- Baldy V. & Gessner M.O. (1997) Towards a budget of leaf litter decomposition in a first-order woodland stream. *Comptes Rendus de l'Academie des Sciences – Series III. – Sciences de la Vie*, **320**, 747–758.
- Baldy V., Gessner M.O. & Chauvet E. (1995) Bacteria, fungi and the breakdown of leaf litter in a large river. *Oikos*, **74**, 93–102.
- Bärlocher F. (1985) The role of fungi in the nutrition of stream invertebrates. *Botanical Journal of the Linnean Society*, **91**, 83–94.
- Bärlocher F. (1992) *The Ecology of Aquatic Hyphomycetes*. Springer-Verlag, Berlin.
- Bärlocher F. & Kendrick B. (1981) Role of aquatic hyphomycetes in the trophic structure of streams. In: *The Fungal Community: Its Organization and Role in the Ecosystem* (Eds D.T. Wicklow & G.C. Carroll), pp. 743–760. Marcel Dekker, New York.
- Carpenter S.R., Caraco N.F., Correll D.L., Howarth R.W., Sharpley A.N. & Smith V.H. (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, **8**, 559–568.
- Cuffney T.F., Wallace J.B. & Lugthart G.J. (1990) Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. *Freshwater Biology*, **23**, 281–299.
- Elwood J.W., Newbold J.D., Trimble A.F. & Stark R.W. (1981) The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. *Ecology*, **62**, 146–158.
- Findlay S.E. & Arsuffi T.L. (1989) Microbial growth and detritus transformations during decomposition of leaf litter in a stream. *Freshwater Biology*, **21**, 261–269.
- Fisher S.G. & Likens G.E. (1973) Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecological Monographs*, **43**, 421–439.
- Gessner M.O. & Chauvet E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology*, **59**, 502–507.
- Grattan R.M. II & Suberkropp K. (2001) Effects of nutrient enrichment on yellow poplar leaf decomposition and fungal activity in streams. *Journal of the North American Benthological Society*, **20**, 33–43.
- Hieber M. & Gessner M.O. (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026–1038.
- Howarth R.W. & Fisher S.G. (1976) Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. *Freshwater Biology*, **6**, 221–228.
- Iqbal S.H. & Webster J. (1973) Aquatic hyphomycete spora of the River Exe and its tributaries. *Transactions of the British Mycological Society*, **61**, 331–346.
- Kaushik N.K. & Hynes H.B.N. (1971) The fate of dead leaves that fall into streams. *Archiv für Hydrobiologie*, **68**, 465–515.
- Komínková D., Kuehn K.A., Büsing N., Steiner D. & Gessner M.O. (2000) Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. *Aquatic Microbial Ecology*, **22**, 271–282.
- Meyer J.L. & Johnson C. (1983) The influence of elevated nitrate concentration on rate of leaf decomposition in a stream. *Freshwater Biology*, **13**, 177–183.
- Minshall G.W. (1967) Role of allochthonous detritus in the trophic structure of a woodland springbrook community. *Ecology*, **48**, 139–149.
- Mulholland P.J., Elwood J.W., Newbold J.D., Webster J.R., Ferren L.A. & Perkins R.E. (1984) Phosphorus uptake by decomposing leaf detritus: effect of microbial biomass and activity. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **22**, 1899–1905.
- Newbold J.D., Elwood J.W., Schulze M.S., Stark R.W. & Barmeier J.C. (1983) Continuous ammonium enrichment of a woodland stream: uptake kinetics, leaf decomposition and nitrification. *Freshwater Biology*, **13**, 193–204.
- Newell S.Y., Arsuffi T.L. & Fallon R.D. (1988) Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. *Applied and Environmental Microbiology*, **54**, 1876–1879.
- Paul M.J. & Meyer J.L. (1996) Fungal biomass of 3 leaf litter species during decay in an Appalachian stream. *Journal of the North American Benthological Society*, **15**, 421–432.
- Peterson B.J., Deegan L., Helfrich J. et al. (1993) Biological responses of a tundra river to fertilization. *Ecology*, **74**, 653–672.
- Pringle C.M. (1987) Effects of water and substratum nutrient supplies on lotic periphyton growth: an

- integrated bioassay. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**, 619–629.
- Robinson C.T. & Gessner M.O. (2000) Nutrient addition accelerates leaf breakdown in an alpine springbrook. *Oecologia*, **122**, 258–263.
- Rosemond A.D., Mulholland P.J. & Elwood J.W. (1993) Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. *Ecology*, **74**, 1264–1280.
- Rosemond A.D., Pringle C.M., Ramírez A., Paul M.J. & Meyer J.L. (2002) Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. *Limnology and Oceanography*, **47**, 278–289.
- Royer T.V. & Minshall G.W. (2001) Effects of nutrient enrichment and leaf quality on the breakdown of leaves in a hardwater stream. *Freshwater Biology*, **46**, 603–610.
- Simon M. & Azam F. (1989) Protein content and protein synthesis rate of planktonic marine bacteria. *Marine Ecology Progress Series*, **51**, 201–213.
- Sridhar K.R. & Bärlocher F. (1997) Water chemistry and sporulation by aquatic hyphomycetes. *Mycological Research*, **101**, 591–596.
- Sridhar K.R. & Bärlocher F. (2000) Initial colonization, nutrient supply, and fungal activity on leaves decaying in streams. *Applied and Environmental Microbiology*, **66**, 1114–1119.
- Suberkropp K. (1991) Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. *Mycological Research*, **95**, 843–850.
- Suberkropp K. (1992) Interactions with invertebrates. In: *The Ecology of Aquatic Hyphomycetes* (Ed. F. Bärlocher), pp. 118–134. Springer-Verlag, Berlin.
- Suberkropp K. (1995) The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams. *Canadian Journal of Botany*, **73** (Suppl. 1), S1361–S1369.
- Suberkropp K. (1998) Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. *Mycological Research*, **102**, 998–1002.
- Suberkropp K. & Chauvet E. (1995) Regulation of leaf breakdown by fungi in streams: influences of water chemistry. *Ecology*, **76**, 1433–1445.
- Suberkropp K. & Klug M.J. (1976) Fungi and bacteria associated with leaves during processing in a woodland stream. *Ecology*, **57**, 707–719.
- Suberkropp K. & Weyers H. (1996) Application of fungal and bacterial production methodologies to decomposing leaves in streams. *Applied and Environmental Microbiology*, **62**, 1610–1615.
- Swank W.T. & Waide J.B. (1988) Characterization of baseline precipitation and stream chemistry and nutrient budgets for control watersheds. In: *Forest Hydrology and Ecology at Coweeta* (Eds W.T. Swank & D.A. Crossley Jr), pp. 57–79. Springer-Verlag, New York.
- Tank J.L. & Webster J.R. (1998) Interaction of substrate and nutrient availability on wood biofilm processes in streams. *Ecology*, **79**, 2168–2179.
- Triska F.J. & Sedell J.R. (1976) Decomposition of four species of leaf litter in response to nitrate manipulation. *Ecology*, **57**, 783–792.
- United States Environmental Protection Agency (USEPA) (1983) *Methods of Chemical Analysis of Water and Wastes*. Environmental Monitoring and Support Laboratory, Cincinnati, OH, USA. Report number EPA-600/4-79-020.
- Velji M.I. & Albright L.J. (1993) Improved sample preparation for enumeration of aggregated aquatic substrate bacteria. In: *Handbook of Methods in Aquatic Microbial Ecology* (Eds P.F. Kemp, B.F. Sherr, E.B. Sherr & J.J. Cole), pp. 139–142. Lewis Publishers, Boca Raton, FL, USA.
- Webster J.R. & Benfield E.F. (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **17**, 567–594.
- Weyers H.S. & Suberkropp K. (1996) Fungal and bacterial production during the breakdown of yellow poplar leaves in two streams. *Journal of the North American Benthological Society*, **15**, 408–420.
- Zar J.H. (1984) *Biostatistical Analysis*, 2nd edn. Prentice Hall, Englewood Cliffs, NJ, USA.

(Manuscript accepted 30 July 2002)