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IMPORTANCE OF STREAM MICROFUNGI IN CONTROLLING BREAKDOWN RATES OF LEAF LITTER[1]

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Abstract. Breakdown of seven leaf species covering a broad range of litter qualities (lignin: 7–31% of leaf dry mass; tannin: 0.0–6.7%; nitrogen: 0.5–2.6%; phosphorus: 0.017–0.094%) and dynamics of fungal biomass and reproductive activity were studied in a softwater mountain stream. Litter breakdown proceeded at exponential rates k ranging from 0.0042 d^{-1} (evergreen oak) to 0.0515 d^{-1} (ash). Fungal colonization of litter was generally rapid, with the fungus-specific indicator molecule ergosterol increasing from initially negligible concentrations to 375–859 $\mu g/g$ of detrital mass. Using species-specific factors relating ergosterol concentrations to mycelial dry mass, maximum fungal biomass associated with litter was estimated as 61–155 mg/g of total system mass. Minimum estimates of net mycelial production during active growth varied between 0.3 and 3.8 $mg \cdot g^{-1} \cdot d^{-1}$, and maximum sporulation rates of aquatic hyphomycetes ranged from 760 to 7500 conidia $\cdot mg^{-1} \cdot d^{-1}$. Initially, reproductive activity was largely synchronized with increases in ergosterol concentrations, but it declined dramatically after peak sporulation rates were reached, whereas ergosterol concentrations levelled off or decreased at considerably slower rates. Periods of highest fungal productivity were thus limited to an initial breakdown stage of ≈2–8 wk. Strong correlations were found between the exponential breakdown coefficient and each of three parameters reflecting fungal activity in leaf litter, that is, maximum ergosterol concentration ($P = 0.002$, $r = 0.96$), net mycelial production ($P = 0.02$, $r = 0.92$), and sporulation rate ($P < 0.001$, $r = 0.99$). The initial lignin content of leaves was also significantly correlated with the rate constant k ($P = 0.02$, $r = -0.83$), suggesting that lignin was the primary factor determining litter quality and thus breakdown rate. The correlation was even stronger when data were logarithmically transformed ($P < 0.01$, $r = -0.95$). Tannin concentration was significantly correlated with k only when two high-lignin species were excluded from the analysis ($P = 0.19$, $r = -0.56$ compared with $P = 0.05$, $r = -0.88$), while initial concentrations of phosphorus ($P = 0.17$, $r = 0.58$) and particularly nitrogen ($P = 0.82$, $r = 0.06$) were poor predictors of litter decomposability. These results suggest that the initial lignin content of leaves controlled litter breakdown rate through a kinetic limitation of carbon sources for saprotrophic microfungi. The decomposer activity of these organisms, in turn, would then have governed breakdown rates. In doing this, fungi produced substantial amounts of both mycelial and conidial biomass that was potentially available to higher trophic levels of the food web.

Key words: aquatic hyphomycetes; decomposition; ergosterol; fungal biomass; fungal reproductive activity; leaf litter; lignin; nitrogen; phosphorus; substrate quality; stream; tannin.

INTRODUCTION

An essential prerequisite for the analysis of ecosystem structure and functioning is information on the biomass and productivity of the organisms driving the basic processes in the system. Much like forest floors, many running water systems receive (Webster et al. 1990) and retain (e.g., Jones and Smock 1991, Snaddon et al. 1992) large amounts of leaf litter derived from riparian vegetation; the breakdown of this material therefore constitutes a key process in the metabolism of such streams (Cummins 1988). Despite considerable research efforts into leaf breakdown over the last two decades (see reviews by Webster and Benfield 1986, Boulton and Boon 1991) and the realization of the critical importance of aquatic microfungi in this process (Kaushik and Hynes 1971, Suberkropp 1992*a*), information on the quantity of fungal mass developing in decomposing leaves is notably scarce. Bärlocher and Kendrick (1981) and Suberkropp (1992*b*), in their reviews, used published accounts of ATP and hyphal length measurements (for a discussion of the limits of these approaches see Frankland et al. 1990 and Newell 1992) in conjunction with conversion factors adopted from different systems to calculate litter-associated fungal mass. These estimates range from 0.004 to 12% of detrital mass. It is not surprising, therefore, that conclusions about the importance of microfungi in the trophic structure of streams are currently debated (Suberkropp 1992*b*). While in terrestrial systems the decomposition of leaf litter has been studied in more detail than in streams, current knowledge of the fungal

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TABLE 1. Physical-chemical characteristics of the Touyre during field experiments from November 1989 to May 1990. TDP = total dissolved phosphorus.

Parameter	Mean	Range
Catchment area (km^2)	19.4	...
Discharge (m^3/s)*	0.57	0.14–2.54
Water temperature (°C)	7.1	3.3–12.0
pH	7.5	7.2–7.9
Conductivity ($\mu S/cm$, 25°C)	52	35–83
Alkalinity (mmol/L)	0.38	0.25–0.66
Ca^{2+} (mg/L)	7.1	4.5–13.1
Mg^{2+} (mg/L)	0.49	0.35–0.55
NO_3-N (mg/L)	0.21	0.12–0.28
PO_4-P ($\mu g/L$)	...	<2–3
TDP ($\mu g/L$)	...	<2–5

* Generally lower than 0.3 m^3/s at the location where leaf packs were exposed.

mass developing during decay is similarly incomplete. Recent advances in methodology, however, in particular the development of the ergosterol assay, now facilitate the determination of litter-associated fungal mass with greater accuracy (Newell 1992, 1994).

Leaves of different plant species are well known to lose mass at different rates (Swift et al. 1979, Webster and Benfield 1986). While the absolute breakdown rate of a given leaf species varies with environmental conditions, the sequence of breakdown of a set of species is predictable (Petersen and Cummins 1974, Chauvet et al. 1993). Consequently, for woodland soils, attempts have been made to establish relationships between breakdown rates of various types of litter and the chemical nature of these materials. The general conclusion of these studies is that concentrations of nutrients, lignin, or a combination of these constituents are critical in determining litter decomposability (e.g., Melillo et al. 1984, Taylor et al. 1989, 1991, Geng et al. 1993, but see Schaefer et al. 1985). Additionally, phenolic compounds, particularly tannins, have been suggested as major determinants of breakdown rate (Nicolai 1988, Stout 1989), and physical leaf properties may also be important (Gallardo and Merino 1993).

In streams, notably few studies have compared the breakdown of more than three types of leaves directly (e.g., Petersen and Cummins 1974, Hill et al. 1992). None of these investigations attempted to relate leaf litter quality and decomposition rate in a formal manner (but see Melillo et al. [1983] for a study using wood chips). Above all, we are unaware of any study attempting to pinpoint the missing organismic link between the chemical nature of leaf litter and the rate at which it is broken down, that is, to identify the biological mechanisms by which leaf quality affects the rate of litter transformation. The basic rationale that breakdown rate is primarily controlled by microbial activity also underlies, however, the enzymic approach to litter decomposition developed by Sinsabaugh et al. (1992, Sinsabaugh and Linkins 1993) and others.

Taking advantage of the recently developed ergosterol assay (Newell 1992) and the availability of specific conversion factors for our system (Gessner and Chauvet 1993), the aims of the present study were thus (1) to provide comprehensive data on the biomass of aquatic microfungi developing on decomposing leaf litter in freshwater, (2) to document the range of variation in litter-associated fungal mass among a variety of leaf species, and (3) to relate leaf breakdown rates to both chemical leaf properties and parameters reflecting fungal activity in decomposing litter. The hypothesis implicit in the third point is that aquatic microfungi control rates of leaf litter breakdown in streams with their activity in turn being controlled by substrate quality.

MATERIALS AND METHODS

Study site

Litter breakdown and fungal dynamics on decomposing litter were studied in the Touyre, a third-order stream in the French Pyrenees (42°52′ N, 1°45′ E). This stream drains a valley underlain with crystalline bedrock and covered by a mixed beech–coniferous forest (*Fagus sylvatica* L. and *Abies alba* Mill.) on the northeast-facing slope and oak (*Quercus pubescens* Willd.) on the southwest-facing slope. About 40% of the drainage basin is above the tree line. The riparian vegetation is primarily composed of ash (*Fraxinus excelsior* L.), alder [*Alnus glutinosa* (L.) Gaertn.], and beech (*Fagus sylvatica* L.) with an understory of hazel (*Corylus avellana* L.). Leaf fall begins in mid-October and is completed by the end of November. A study reach, some 100 m in length, was chosen at 890 m elevation downstream from a pool from where water is partially diverted to a hydroelectric power plant at high discharge. Stream width was 5 m in that reach, average water depth at base flow 15–20 cm, and the channel gradient was 5%. Bed sediments were mainly cobble and boulder. Physical and chemical characteristics of the stream are summarized in Table 1; additional information can be found in Gessner et al. (1993).

Leaf material

On 3 November 1989, leaf litter of five riparian tree species, i.e., alder (*Alnus glutinosa*), ash (*Fraxinus excelsior*), beech (*Fagus sylvatica*), wild cherry (*Prunus avium* L.), and hazel (*Corylus avellana*), were collected at the study site. Leaves of sycamore (*Platanus hybrida* Brot.) and evergreen oak (*Quercus ilex* L.) were obtained at a site in the Black Mountains some 80 km north. Sycamore and evergreen oak are not natural components of the riparian vegetation in the catchment of the Touyre but were included in the study in order to cover a wide range of litter qualities. Apart from oak leaves, which were directly taken from trees, litter consisted of naturally shed leaves or leaflets (the rachis of ash leaves was discarded) picked from the ground just after abscission. Some leaves of alder and hazel

were picked off trees or shrubs but care was taken to include only material that had a well-developed parting tissue, leading to ease of detachment from twigs.

Four chemical parameters were chosen to describe the initial quality of litter. Total nitrogen content was measured in ground subsamples of leaves (50–70 mg) using an automated phenate procedure operating on a Technicon Autoanalyzer II, after mineralization with 50% H_2O_2 and concentrated H_2SO_4 in a Hach-Digesdahl apparatus. For phosphorus analyses, ground subsamples (100 mg) were placed in acid-cleaned Teflon screw-cap vials, mineralized for 10 h with concentrated HNO_3, and quantified using the phosphomolybdate assay. Lignin was determined gravimetrically following the procedure of Goering and Van Soest (1970). After refluxing of leaf powder in acid detergent, cellulose was hydrolyzed with 72% sulphuric acid and lignin determined as the ignition loss of the material remaining. Protein-precipitating potential (tannin content) was estimated with the radial diffusion assay after the extraction of 100 mg ground leaf material with 1 mL of 70% acetone (Hagerman 1988); 36 μL of an extract was applied to a well punched in an agarose gel (1% strength) containing 0.01% bovine serum albumin (BSA). The precipitation of protein by tannins results in the formation of plaques whose surface is proportional to the amount of tannin applied. A standard curve was prepared with commercial tannic acid (Merck).

Field manipulation and sample processing

In total, 214 monospecific leaf packs were constructed from the collected litter, weighed fresh in the field, enclosed in nylon mesh bags (9-mm mesh openings) surrounding a rigid wire frame, and attached to house bricks (Gessner 1991). On 3 November 1989, within 7 h after the collection of leaves, 144 packs were randomly distributed in the study reach. The remaining 70 packs, 10 of each leaf species, were returned immediately to the laboratory and served as standards to relate litter fresh mass to dry mass (Table 2). After 2 wk, 4 wk, and at subsequent 4-wk intervals, three replicate packs per leaf species were retrieved from the stream, placed individually in plastic containers half filled with stream water, and returned to the laboratory in an ice box. On arrival, individual leaves were cleaned of debris and macroinvertebrates. As long as sufficient leaf material was remaining, three leaves were removed from each pack for the analysis of fungal community structure and the determination of conidial production. The remaining material was frozen at −18°C, later freeze dried, weighed, and ground to pass a 1-mm mesh screen. Subsamples of 250 mg were ashed for 3 h at 550°C to determine ash-free dry mass (AFDM).

Conidial production

Six discs were cut from each leaf using a cork borer (7 mm diameter) and incubated for 3 d at 10°C in 10 mL filtered stream water. Discs were then fixed in lactophenol, mounted on slides, stained with 0.01% trypan blue in lactic acid, and examined microscopically to determine fungal community structure and the total number of conidia produced per unit leaf surface. Relative abundance of fungal species was estimated in eight classes: 0 = 0%, 1 = 0–1%, 2 = 1–5%, 3 = 5–20%, 4 = 20–40%, 5 = 40–60%, 6 = 60–80%, and 7 = 80–100%. One-half of a leaf disc was scanned at a magnification of 200 and the aquatic hyphomycete species encountered assigned to the classes according to the relative frequency of their conidia. Class means were then used to calculate the average proportion of each species in a given fungal assemblage. Total numbers of conidia produced per unit leaf surface were estimated from counts in five microscopic fields per leaf disc (1.23 mm^2 each), i.e., in 90 fields per leaf pack. Additional leaf discs were cut, dried, and weighed to establish relationships between disc surface and dry mass, allowing the expression of conidial production on a leaf dry mass basis. Conidial numbers were converted to dry mass using published biovolumes of conidia (Bärlocher and Schweizer 1983) and assuming an average dry mass density of 500 $fg/\mu m^3$ (cf. Findlay and Arsuffi 1989).

TABLE 2. Initial fresh mass (range) and corresponding dry mass (mean ± 1 SD) of standard leaf packs. N = 10 packs.

Leaf species	Fresh mass (g)	Dry mass (g)
Ash	17.0–17.7	3.96 ± 0.23
Cherry	13.4–14.5	3.65 ± 0.22
Alder	9.5–10.7	3.75 ± 0.05
Hazel	13.2–13.9	3.58 ± 0.16
Sycamore	9.8–11.2	6.06 ± 0.47
Beech	10.3–11.6	3.44 ± 0.07
Oak	9.8–10.1	5.74 ± 0.09

Mycelial biomass and production

Ergosterol, a membrane component largely restricted to eumycotic fungi (Newell 1992), has recently proven a useful index of fungal biomass both in a marine detrital system (Newell et al. 1989) and in two decomposition studies in streams (Gessner and Schwoerbel 1991, Golladay and Sinsabaugh 1991) and was therefore chosen in the present study for this purpose. The compound was extracted from 250-mg subsamples of ground leaf material by homogenization in methanol and quantified by measuring absorbance at 280 nm after separation from other lipids by high-performance liquid chromatography (HPLC; Gessner et al. 1991). Ergosterol concentrations were converted to fungal biomass using species-specific factors established for the dominant fungal species of the examined litter in conjunction with estimates of their relative abundance in the fungal community (Gessner and Chauvet 1993). According to the proportion of conidia produced, the species for which specific conversion factors were available accounted on average for 93% of the total

fungal biomass associated with leaves. Fungal biomass is expressed as a concentration of both detrital mass, i.e., leaf mass plus fungal mass, and leaf mass, i.e., detrital mass minus estimated fungal mass.

The daily net production of mycelium was calculated according to the equation

$$\frac{m_f}{m_l} \cdot \frac{1}{t},$$

where m_f is the fungal dry mass per leaf pack in milligrams, m_l is the initial leaf dry mass in grams, and t is the elapsed time in days. The underlying assumption is that losses in fungal mass were negligible over the considered period. Results therefore reflect minimum estimates. True mycelial production cannot be estimated on the basis of the available data because of the multitude of events that affect losses of foliar and fungal mass to varying degrees (e.g., selective grazing on mycelium by shredders, leaching, fungal assimilation of leaf material).

Statistics

Nonlinear regression analysis was used to adjust mass loss data to the model

$$m_t = m_0 e^{-kt},$$

where m_t is the mass remaining at time t, m_0 the initial mass, and k the breakdown coefficient (Wieder and Lang 1982). Analysis of covariance (ANCOVA) was used to test for differences between breakdown rates provided that linear regressions of logarithmically transformed data produced parameter estimates similar to those obtained with nonlinear procedures. Apart from cherry, this was the case when the first 4 mo of breakdown were considered. Thereafter, rates of mass loss increased in beech, hazel, sycamore, and oak, an effect that was presumably caused by an enhanced mechanical fragmentation of litter due to increased shear stress during a period of high discharge. As a result, the initial dry mass of several species was grossly overestimated by linear regressions on log-transformed data and estimates of breakdown coefficients differed from those obtained with nonlinear procedures. As we were primarily interested in the biological processing of leaf litter and considered comparisons of k values more meaningful when these were calculated for identical time periods, we decided to perform ANCOVA with the data encompassing the first 3 mo of breakdown only. Note that the ranking of leaf species according to breakdown coefficients was independent of whether the whole or the limited data set were used.

Analysis of variance (ANOVA) was chosen to test for differences among leaf species in regard to initial concentrations of lignin, tannin, nitrogen, and phosphorus, peak ergosterol concentration, mycelial production, and reproductive activity. Bonferroni's test was used for a posteriori pairwise comparisons when overall differences were significant (Day and Quinn 1989, Wilkinson 1990). Correlation analyses were performed to depict relationships between breakdown coefficients, parameters reflecting fungal activity (peak ergosterol concentration, mycelial production, and reproductive activity), and parameters describing initial litter quality (concentrations of lignin, tannin, nitrogen, and phosphorus). Correlation analyses were based on mean values. All statistical calculations were made using the SYSTAT computer package (Wilkinson 1990). Differences were considered significant when $P < 0.05$.

Results

Fungal biomass

Freshly collected leaves of the seven species examined in the present study contained either no ergosterol or minute amounts (beech and hazel), indicating that fungal colonization was negligible at the beginning of the experiment. During breakdown in the stream, ergosterol concentrations increased rapidly with peak values being reached within a few weeks in six of the seven species (Fig. 1). The subsequent decrease was rapid in some leaf species such as ash and hazel but slower in others such as beech and sycamore. Oak leaves differed somewhat from the general pattern in that ergosterol was undetectable even after a 2-wk exposure in the stream, and in that subsequent increases occurred slowly so that peak concentrations were not reached until 5 mo later. Leaf species differed in regard to both the maximum concentrations of ergosterol attained ($P < 0.001$) and the timing of reaching these peak values with ash and oak representing the two extremes (Fig. 1).

The 12 most abundant species of aquatic hyphomycetes producing conidia on leaf surfaces are listed in Table 3. Based on ergosterol-to-biomass conversion factors established for these species (Table 3) and their relative abundance in the fungal communities on leaves, peak fungal biomass was estimated to range from 61.2 to 154.5 mg/g detrital dry mass (Table 4). This is equivalent to 6.5–18.4% of leaf dry mass.

Fungal production

The phase of mycelial net growth on leaf litter was generally confined to the first weeks of leaf breakdown (Fig. 1) but was extended over a 16-wk period in the case of oak. Assuming that losses of mycelium were negligible during this period, daily increases in fungal mass were estimated to vary between 0.3 and 3.8 mg/g leaf mass depending on leaf species (Table 5). These values reflect minimum estimates of daily net production. Differences among leaf species were significant ($P < 0.001$) with half of the pairwise comparisons following ANOVA also yielding significant differences.

For any given leaf species, the dynamics of reproductive activity ran relatively parallel with changes in ergosterol concentrations (Fig. 1). However, peaks usually occurred before the corresponding ergosterol peaks and the subsequent decrease was more pronounced

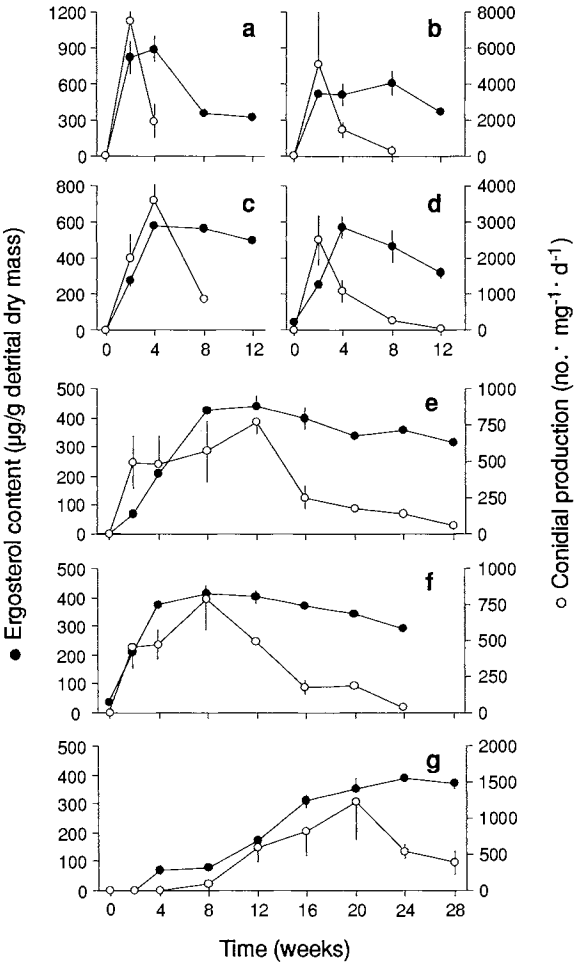

FIG. 1. Dynamics of ergosterol concentrations and sporulation rates of aquatic microfungi associated with decomposing leaf litter of ash (a), cherry (b), alder (c), hazel (d), sycamore (e), beech (f), and oak (g). Vertical bars denote ± 1 SD; for clarity, error bars of the sporulation rates on ash and cherry at day 14 are not fully represented (see Table 5).

TABLE 4. Peak biomass (mean ± 1 SD) of aquatic microfungi associated with decomposing leaf litter. Leaf mass is referred to as the difference between total detrital mass and estimated fungal mass.

Leaf species	Fungal biomass (mg/g detrital dry mass)	Fungal biomass (mg/g leaf dry mass)
Ash	154.5 ± 35.5	184.2 ± 50.7
Cherry	116.7 ± 29.3	133.0 ± 37.0
Alder	90.4 ± 9.9	99.4 ± 12.0
Hazel	108.3 ± 15.8	121.7 ± 19.9
Sycamore	76.8 ± 7.3	83.2 ± 8.6
Beech	62.9 ± 2.0	67.1 ± 2.3
Oak	61.2 ± 1.1	65.2 ± 1.2

than the decrease in ergosterol. Among species, differences in reproductive activity were significant and more pronounced than differences in ergosterol concentrations (Table 5), but pairwise comparisons following ANOVA produced no significant differences between leaf species due to the great variability among replicate packs. In terms of dry mass, conidial production ranged from 0.6 to 5.2 mg/g detrital dry mass per day (Table 5).

Leaf breakdown rates

Rates of leaf breakdown varied considerably among species (Fig. 2) with exponential breakdown coefficients ranging from 0.0042 d^{-1} to 0.0515 d^{-1} (Table 6). Calculation of k values on an ash-free dry mass basis gave nearly identical results. Analysis of covariance revealed significant differences between breakdown rates of most species.

Due to the accumulation of microbial biomass on decomposing organic matter, determination of litter mass loss by subtracting the mass remaining at a given time from that initially present results in an underestimation of real breakdown rates. However, these underestimates were not significant and relatively small

TABLE 3. Ergosterol contents of dominant aquatic hyphomycetes used to calculate mycelial biomass and production on decomposing litter, and approximate dry mass of a single conidium.

Fungal species	Ergosterol content (mg/g dry mass)*	Conidial dry mass (pg)†
Alatospora acuminata Ingold	5.9	135
Anguillospora longissima (Sacc. & Syd.) Ingold	4.0	1197
Articulospora tetracladia Ingold	4.6	1413
Clavariopsis aquatica de Wildeman	8.0	738
Clavatospora longibrachiata (Ingold) S. Nilsson ex Marvanová & S. Nilsson	8.3	153
Crucella subtilis Marvanová	3.3	135
Flagellospora curvula Ingold	10.2	207
Heliscella stellata (Ingold & Cox) Marvanová	6.0	153
Lemonniera aquatica de Wildeman	5.8	1188
Lemonniera centrosphaera Marvanová	5.5	585
Lemonniera terrestris Tubaki	5.3	963
Tetrachaetum elegans Ingold	2.9	3150

* Except for *H. stellata*, data are from Gessner and Chauvet (1993); a general conversion factor for aquatic hyphomycetes was applied to *L. centrosphaera*.

† Calculated from Bärlocher and Schweizer (1983); the value used for *C. subtilis* corresponds to the one determined for *A. acuminata*.

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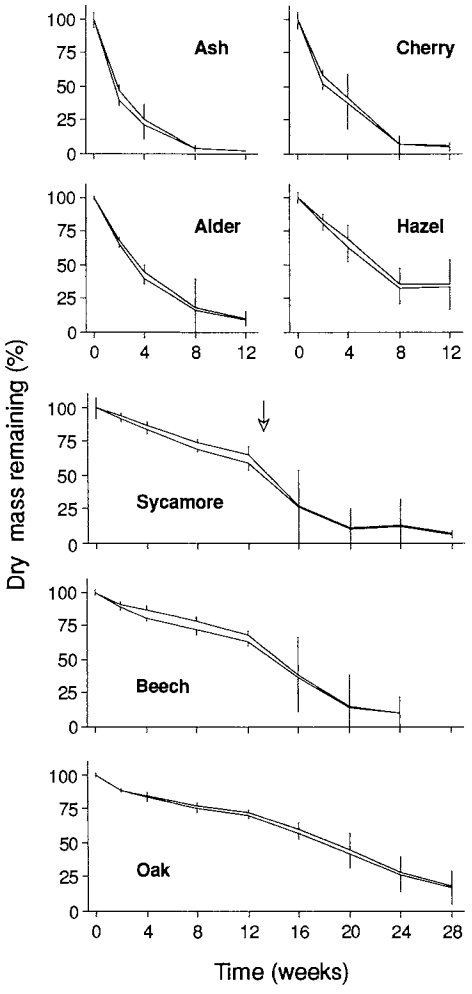

FIG. 2. Loss in dry mass of leaf packs decomposing in the Touyre. The upper curve in each panel represents the loss in detrital mass and the lower one the loss in leaf mass, i.e., detrital mass minus estimated fungal mass. Vertical bars denote ± 1 SD and the arrow indicates the occurrence of a major spate.

Relationships among breakdown rate, fungal activity, and litter quality

The highly significant correlations between leaf breakdown coefficients and fungal growth parameters (Table 9) provide a second line of evidence indicating that the rate of litter breakdown in the Touyre was primarily controlled by saprotrophic microfungi. This fungal control may be exerted by direct decomposer activity, which leads to fragmentation, leaching, and assimilation of leaf material (Suberkropp 1992*a*), and/or via leaf conditioning, which promotes feeding on leaves by shredders (Suberkropp 1992*b*). Therefore, whatever the main mechanism, the key to understanding leaf breakdown processes in streams like the Touyre would appear to lie primarily in the assessment of the relative importance of factors that affect fungal activity in litter. Possible factors include the quality, quantity, and accessibility of carbon sources, the availability of nutrients within the substratum or in the environment, the impact of fungistatic leaf components, and the effectiveness of physical barriers of the leaf tissue.

Lignin content of leaf litter was originally proposed as an indicator for its degradability in temperate woodlands and subsequently this parameter was found useful to predict breakdown rates of both foliar and woody litter in various terrestrial ecosystems (see references in Melillo et al. 1984, Taylor et al. 1991, and Gallardo and Merino 1993). The results of the present study indicate that lignin is also the primary constituent determinant of leaf breakdown rate in streams (Table 10). Lignin is particularly recalcitrant to enzymic degradation; therefore, the higher the proportion of this constituent is in a given leaf species, the lower is the relative amount of more readily available carbon compounds. In addition, the intimate association of lignin with cellulose fibers results in masking of a large fraction of carbohydrate, which otherwise would be accessible to the leaf-associated microorganisms. Lignin content in leaf litter may hence be viewed as an inverse index for the availability of carbon to decom-

TABLE 8. Correlations between parameters of initial leaf quality. $N = 7$.

Parameter	F value	P	r
Lignin vs. tannin	0.05	0.84	0.10
Lignin vs. nitrogen	1.26	0.31	−0.45
Lignin vs. phosphorus	13.07	0.02	−0.85
Tannin vs. nitrogen	2.19	0.20	0.55
Tannin vs. phosphorus	0.04	0.85	0.09
Nitrogen vs. phosphorus	7.14	0.04	0.77

TABLE 7. Initial concentrations of lignin, tannin, nitrogen, and phosphorus (mean ± 1 SD) in leaves of seven broad-leaved tree species. Means with identical superscript letters are not significantly different.

Leaf species	Lignin (% dry mass)	Tannin (% dry mass)	Nitrogen (% dry mass)	Phosphorus (% dry mass)
Ash	6.8 ± 0.3[a]	0.0 ± 0.0	1.10 ± 0.14[a]	0.073 ± 0.005[a]
Cherry	8.4 ± 1.0[a]	3.1 ± 0.3[a]	0.52 ± 0.09[b]	0.048 ± 0.003[b]
Alder	8.0 ± 0.7[a]	5.2 ± 0.2	2.55 ± 0.14	0.094 ± 0.010
Hazel	13.3 ± 0.9	3.6 ± 0.1	1.05 ± 0.05[a]	0.072 ± 0.008[a]
Sycamore	30.9 ± 0.8	2.7 ± 0.1[a]	0.52 ± 0.04[b]	0.017 ± 0.002[c]
Beech	25.5 ± 0.8	2.9 ± 0.1[a]	0.68 ± 0.01[b]	0.032 ± 0.001[b,c]
Oak	18.5 ± 0.9	6.7 ± 0.2	1.56 ± 0.04	0.047 ± 0.003[b]

TABLE 9. Correlation between the exponential breakdown coefficient k and parameters characterizing the activity of leaf-associated microfungi. $N = 7$.

Parameter	F value	P	r
Peak ergosterol concentration	39.3	0.002	0.94
Mycelial production	13.2	0.02	0.85
Peak sporulation rate	280.6	$\ll 0.001$	0.99

posers. If so, the leaf-associated fungi in the present study would appear to have experienced a more or less severe carbon limitation, and this kinetically controlled shortage of suitable carbon sources to saprotrophic fungi would in turn have governed breakdown rates.

While lignin explained much of the difference in breakdown rates among the tested leaf species, results of correlation analysis suggest that tannins affected litter breakdown as well (Table 10). In particular, the large amounts present in oak leaves presumably delayed the breakdown of this species, while the absence of protein-precipitating compounds may have contributed to the remarkably rapid mass loss of ash leaves.

Nutrient effects on leaf breakdown

Nitrogen clearly did not account for differences in breakdown rate among leaf species (Table 10). This finding is contrary to the widely cited tenet that concentration of this nutrient is critical in determining breakdown rates of detrital particles (Mann 1988) including leaf litter (Taylor et al. 1989), although its failure to predict litter breakdown rates has also been reported elsewhere (Melillo et al. 1984, Schaefer et al. 1985). In four Canadian streams, Melillo et al. (1983) did find significant relationships between breakdown rate and the initial nitrogen concentration of wood chips, but that material had only 1/10 the nitrogen concentration of the leaves used in our study. N:P ratios of both leaf litter (24–72 on a molar basis) and stream water (> 100) indicate that if nutrient limitation of leaf breakdown occurred in our stream, P rather than N would have been the critical element. Like nitrogen, however, the initial phosphorus concentration was correlated neither with fungal activity (data not shown) nor with breakdown rate (Table 10), suggesting that in the present study phosphorus availability also was of subordinate importance as a rate-regulating factor. With low-nutrient materials such as wood, again, one may expect nutrient effects on heterotrophic microbial activity and litter breakdown to be stronger (Sinsabaugh et al. 1993).

TABLE 10. Correlations between the exponential breakdown coefficient k and concentrations of constituents characterizing initial leaf quality. $N = 7$.

Parameter	F value	P	r
Lignin	11.4	0.02	−0.83
Lignin*	43.6	0.001	−0.95
Tannin	2.3	0.19	−0.56
Tannin†	10.6	0.05	−0.88
Nitrogen	0.06	0.82	0.06
Nitrogen‡	0.04	0.86	−0.10
Phosphorus	2.53	0.17	0.58

* After double-logarithmic transformation.

† Beech and sycamore with very high lignin concentrations excluded ($N = 5$).

‡ Alder with a very high nitrogen concentration excluded ($N = 6$).

As an alternative or in addition to substrate control, external control of leaf litter breakdown may be effective. Elwood et al. (1981), for example, reported a stimulation of leaf breakdown following continuous amendment of phosphate to a small stream, and K. Suberkropp and E. Chauvet (*unpublished manuscript*) found strong relationships between nitrate concentrations of various streams with rates of leaf breakdown in these systems. Most other studies attempting to demonstrate similar effects in streams have, however, failed to do so (e.g., Newbold et al. 1983, Peterson et al. 1993). Effects of nutrients on litter breakdown are more difficult to assess than effects on autotrophic processes since fungi and bacteria, apart from critically relying on organic carbon supplies, can make use of nutrient resources located both within the litter material and in the surrounding medium (Sinsabaugh et al. 1993). Both nutrient pools are apparently exploited as implied by the net immobilization of N and P by the decomposing leaf litter in the present study (data not shown), close similarities in the temporal pattern of ^{32}P uptake and changes in respiration rate (Mulholland et al. 1984), and phosphatase production by stream fungi when growing in leaves, even irrespective of ambient phosphate concentrations (Suberkropp and Jones 1991). The conflicting results as regards potential nutrient control of the leaf breakdown process hence might be reconciled if the complexity of the regulating mechanisms is fully appreciated. In many situations the external supply of nutrients may simply be sufficient to satisfy microbial needs (e.g., Newbold et al. 1983). This argument appears to be particularly relevant to streams in which the constant flow of water across the substratum replenishes nutrient resources continuously. As a result, threshold concentrations for external nutrient limitation would probably be rather low in these systems, a prediction that matches the situation in those studies demonstrating an influence of N or P on leaf breakdown (Elwood et al. 1981, Meyer and Johnson 1983; K. Suberkropp and E. Chauvet, *unpublished manuscript*). The strongest effect, for instance in enrichment experiments, would be predicted

for leaf species with a low lignin content (equivalent to ready carbon availability) and a low concentration of the critical nutrient, which may be either N or P depending on the stream. If so, it would even seem possible that fungi growing on a rapidly decomposing leaf species like ash or cherry are limited by N or P in the same stream where fungi degrading a recalcitrant species like beech or sycamore are not. Results by Triska and Sedell (1976), who compared the breakdown of four leaf species in response to nitrate manipulation, partly support this prediction, although differences between treatments and controls were not significant in that study.

Dynamics of fungal mass and reproductive activity

According to current ideas of microbial succession during leaf decomposition in running waters, fungi dominate the leaf-associated microbial community in the early stages of breakdown but are gradually replaced by bacteria as particles become smaller and more refractory. This concept was primarily derived from experiments with antibiotics (Kaushik and Hynes 1971) and from measurements of the reproductive activity of aquatic hyphomycetes coupled with counts of bacteria attached to leaf surfaces (Suberkropp and Klug 1976, Bärlocher 1982, Chamier 1987, Findlay and Arsuffi 1989). Additional support comes from direct measurements of bacterial growth rates, which tend to increase with progressing leaf decay (e.g., Mulholland et al. 1987 in their circumneutral stream, Findlay and Arsuffi 1989).

Our results on reproductive activity and mycelial biomass dynamics (Fig. 1) are partly consistent with these expectations. In contrast to many other fungi, aquatic hyphomycetes sporulate abundantly during exponential mycelial growth (Suberkropp 1991). Single-species microcosm studies have shown, moreover, that temporal changes in sporulation roughly parallel changes in respiration, although the drop in respiration rate is less sharp than that in conidial production (Suberkropp 1991). Therefore, the dramatic decline in sporulation rate (Fig. 1) may be indicative (but not necessarily so) of a general decrease in fungal vitality, resulting in reduced ectoenzyme activity (Golladay and Sinsabaugh 1991) and hence fungal contribution to breakdown. However, in contrast to conidial production concentrations of ergosterol decreased much less rapidly or even levelled off (Fig. 1), indicating that living fungal mass persisted during advanced stages of leaf breakdown. This finding is in agreement with observations from other river systems where fungal mass associated with decomposing leaves consistently exceeded bacterial mass (Findlay and Arsuffi 1989, V. Baldy et al., *unpublished manuscript*), which supports the idea that, on a biomass basis, fungi dominate the microbial assemblages associated with leaves throughout the breakdown process.

In the present study, aquatic hyphomycetes continued to sporulate after having attained peak rates (Fig. 1), indicating that fungi were metabolically active at this stage. A part of this conidial production may have resulted from a reallocation of hyphal mass (Suberkropp 1991), while another part would probably have been derived from newly acquired resources becoming available through litter degradation. Furthermore, in nature, fungal respiration and implicitly degradative activity may decline less markedly than was observed in the single-species laboratory studies conducted by Suberkropp (1991; compare his concomitant stream studies), because natural multispecies assemblages have an additional degree of flexibility in that community structure may change with progressing breakdown. Such successional patterns of aquatic hyphomycetes on decomposing leaves have been described for our study stream (Gessner et al. 1993); associated with these may be functional shifts related to enzymic performance (Tanaka 1993) and resource allocation to maintenance metabolism and biomass production of vegetative and reproductive structures.

Thus, while it is debatable whether shifts from eukaryotically to prokaryotically mediated leaf litter breakdown are a typical feature in streams, circumstantial evidence suggests that it may be less conspicuous than originally envisaged. Direct comparisons of the temporal dynamics of bacterial (e.g., Mulholland et al. 1987, Findlay and Arsuffi 1989) and fungal instantaneous growth rates (Newell and Fallon 1991) have potential to clarify this issue. Even if it turned out, however, that bacterial importance outweighs that of fungi in advanced stages of breakdown, fungi may still promote the process indirectly by increasing leaf palatability to detritivores.

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