Bacteria, fungi and the breakdown of leaf litter in a large river — **Source link**

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We examined the dynamics of leaf mass loss and microbial biomass associated with decomposing leaf litter in a seventh order river. This was in an attempt to test the hypothesis that fungal contribution to the breakdown process is less important in major rivers than was previously found for headwater streams. Bacterial biomass was estimated from direct cell counts coupled with determinations of bacterial biovolumes. Fungal biomass was estimated on the basis of ergosterol measurements, species-specific conversion factors, and the relative abundance of the dominant fungal species. Sporulation rates of aquatic hyphomycetes were determined by counting conidia released from leaf litter during brief laboratory incubations. Compared to low-order streams, the breakdown of willow, poplar and plane leaves was slow with exponential decay coefficients k ranging from 0.0045 to 0.0091 d^{-1}. Numbers of bacteria first increased exponentially on all leaf species but reached a plateau of almost 10^8 cells per mg AFDM after 4–8 weeks of leaf submergence. This corresponds to a peak bacterial biomass of 0.3–0.5% of detrital carbon. Fungal biomass attained peaks of 5–10% of detrital carbon after 4–8 weeks and greatly exceeded bacterial biomass at any instance. On average, fungi accounted for 96% of the total microbial (fungal plus bacterial) biomass in leaf litter. Dynamics of sporulation rates of aquatic hyphomycetes were characterized by early peaks of 1.2–1.4 conidia µg^{-1} AFDM d^{-1}, followed by sharp declines to about 0.2 µg^{-1} d^{-1}. Peaks occurred before the corresponding peaks in fungal biomass. Rough organic matter budgets suggest that fungi assimilated a minimum of 16–23% of the initial leaf carbon, and accounted for 42–65% of the overall carbon loss from leaves during periods of highest fungal activity. Taken together, these findings indicate that fungi play an eminently important role in the biological transformation of leaf litter even in major rivers. Bacterial contribution is likely to be small in spite of increases in biomass at advanced stages of breakdown. With regard to leaf decomposition, large fluvial systems would thus appear to behave like their headwater counterparts, suggesting that the present results can be generalized for lotic ecosystems.

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Streams and rivers that flow through forested catchments receive substantial amounts of leaf litter from the riparian vegetation, and the decomposition of this allochthonous material has been recognized as a vital process in the functioning of running waters (Webster and Benfield 1986, Boulton and Boon 1991, Maltby 1992a). Emphasis in past research on leaf decomposition in streams has been primarily laid on the determination of breakdown rates as a function of leaf species (Petersen and Cummins 1974, Hill et al. 1992, Gessner and Chauvet 1994), the chemical transformations that accompany leaf breakdown (Suberkropp et al. 1976, Rosset et al. 1982, Chauvet 1987, Gessner 1991) and the pattern of macroinvertebrate colonization on decomposing leaf material (Anderson and Sedell 1979, Cuffney et al. 1990, Chauvet et al. 1993).
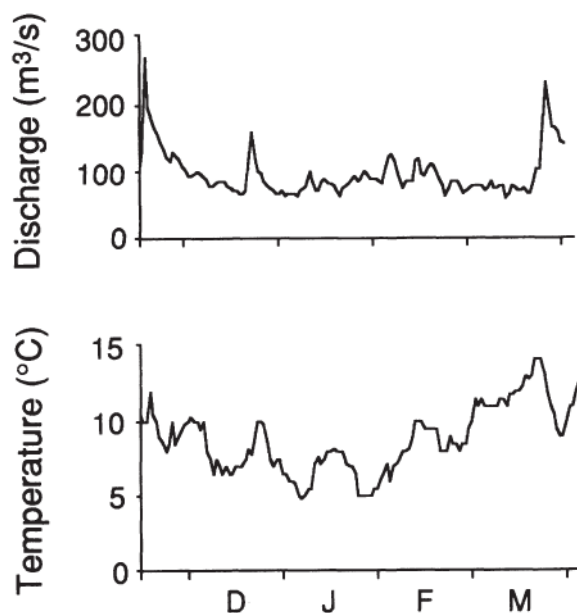
Fig. 1. Daily means of discharge and water temperature of the Garonne River during the field experiment from November 1991 to April 1992.

Most of these studies were carried out in small headwaters, because the contribution of allochthonous organic matter to stream metabolism tends to diminish with increasing channel width (Conners and Naiman 1984). However, even in large rivers, inputs of organic matter derived from the terrestrial environment may be significant. Chauvet and Jean-Louis (1988) estimated, for example, that the seventh order Garonne River receives about 10% of the coarse particulate organic matter (CPOM) typically entering fully canopied low order streams. Additional material may be imported from upstream reaches and the floodplain (Conners and Naiman 1984, Cuffney 1988). Thus, the decomposition of CPOM and particularly leaf litter would appear to constitute a central process not only in low order streams but also in major rivers (cf. Thorp and Delong 1994). Before the fundamental alterations of channel morphology and riparian vegetation by man, the importance of the process in whole system functioning would have been even greater (Naiman et al. 1988).

Leaf breakdown in aquatic ecosystems is mainly a biological process involving three types of organisms: large particle detritivores commonly referred to as shredders, fungi and bacteria. Given the considerable attention devoted to invertebrates and their role in the leaf breakdown process, surprisingly little work has been accomplished on the microbial assemblages associated with decomposing leaves (e.g., Kaushik and Hynes 1971, Suberkropp and Klug 1976, Chamier 1987) although the critical role of microorganisms is clearly established (Suberkropp 1992a, Maltby 1992b). Recent estimates indicate in fact that peak fungal biomass in leaf litter is normally greater than 5% of detrital mass and may even clearly exceed 10% of detrital mass (Suberkropp 1992b, Gessner and Chauvet 1994).

Those studies that have taken microorganisms into account, were generally restricted to either the fungal (Bärlocher 1992a, Chergui and Pattee 1993) or the bacterial (Cuffney et al. 1990, Leff and McArthur 1990) component, or measured bulk parameters of microbial biomass and activity such as ATP content and respiration (Petersen and Cummins 1974, Mulholland et al. 1984, Cuffney et al. 1990, Hill et al. 1992). A separate quantitative consideration of the two groups of microorganisms has rarely been attempted (but see Padgett 1976, Findlay and Arsuffi 1989). However, owing to the fundamentally different life forms of fungi (generally filamentous) and bacteria (apart from Actinomycetes typically single-celled), these two groups probably exert distinct impacts on decomposing plant material. This idea ties in with the observation that fungi appear to dominate microbial assemblages in leaves initially as long as the tissue is more or less intact, while bacteria tend to increase when leaves become partially broken down (Suberkropp and Klug 1976). If so, a clear distinction between fungal and bacterial activity would be paramount to understanding the leaf decomposition process in running waters.

In this paper, we report an investigation into the breakdown of three leaf species in a large river and the dynamics of both the prokaryotic (bacteria) and eukaryotic (fungi) component of the microbial assemblage associated with these leaves. Our specific intention was (1) to test whether leaf-associated fungal biomass would be as important in the seventh order Garonne River as was previously found in two low order streams (Suberkropp et al. 1993, Gessner and Chauvet 1994), (2) to determine the relative importance of bacterial and fungal biomass in this river, and (3) to test the general hypothesis that microbial assemblages on decomposing leaf litter in running waters are initially dominated by fungi and, with progressing leaf breakdown, gradually shift to assemblages in which bacteria assume greater importance (Suberkropp and Klug 1976).

Materials and methods

Study site

The study was conducted in the Garonne River, a seventh order hardwater river draining large portions of southwestern France. We chose a site downstream from the confluence with the Ariege River some 7 km upstream from the city of Toulouse. At this site, the river channel is 200 m wide. Dominant tree species of the fragmented floodplain forest are willow (mainly *Salix alba* L.) and poplar (*Populus nigra* L. and various hybrids), but generally only willows grow immediately adjacent to the river channel. River discharge was recorded continuously at a

gauging station near the experimental site. The long-term annual mean is 192 m^3/s. During the study period, it ranged from 61 to 270 m^3/s (Fig. 1). Water temperature was measured once daily and varied between 5°C and 14°C. Discharge and temperature data were kindly communicated by local authorities. Additional physical and chemical characteristics of the Garonne River were determined according to standard procedures and are summarized in Table 1.

Experimental set up and field procedures

Freshly fallen leaves of willow, poplar and London plane (*Platanus hybrida* Brot.) were collected at the study site on 14 November 1991. London plane is a species uncommon to riparian forests of western Europe but was included in this study because it is frequently planted along water courses in France and elsewhere in Europe, while being more recalcitrant to decomposition than the two other natural species. The collected leaves were stored overnight, then weighed into portions of 2–7 g fresh mass (depending on leaf species), and placed in mesh bags (15 cm sides) made from rigid plastic netting with a mesh size of 2 mm (Chauvet 1987). Four replicate bags were set aside to determine initial leaf dry mass, fungal and bacterial biomass. The remaining leaf bags were hot-sealed and secured in the river by means of metallic rings slipped over steel rods, which were anchored in the river bottom. Water depth at base flow was 30–50 cm at the place where leaf packs were exposed. After 2, 4, 8, 12, 16 and 20 weeks of immersion, four bags of each leaf species were retrieved and returned to the laboratory in a bucket containing river water.

Leaf mass loss

In the laboratory, the leaves were removed from the litter bags and individually rinsed with deionized water so as to remove the greater part of extraneous sediments and adhering invertebrates. Subsamples of the leaf material were taken for the determination of microbial biomass and fungal community structure. The remaining leaves were dried at 105°C for at least 24 h, cooled in a desiccator, weighed to the nearest 0.1 g, and ground to pass a

1-mm-mesh screen. Subsamples of about 250 mg were ashed for 3 h in a muffle furnace at 550°C to determine ash free dry mass (AFDM). Carbon content was determined by a conductimetric analysis of CO$_2$ released after combustion of 250-mg subsamples (Carmhograph 8 analyser, Wösthoff oHG, Germany).

Fungal community structure and sporulation rate

Three willow leaves, one poplar leaf or a quarter of a plane leaf were arbitrarily taken from each litter bag, placed in Erlenmeyer flasks containing 100 ml of filtered river water (Whatman GF/D, approximate pore size 2.7 μm), and incubated on a rotary shaker for 3 d at 10°C so as to induce sporulation of aquatic hyphomycetes. At the end of incubation, 250 μl of a 0.5% Triton X-100 (Prolabo, France) solution were added to ensure a uniform distribution of conidia, and 2 ml of the supension was passed through a Whatman membrane filter (pore size 5 μm). The conidia trapped on the filter were stained with 0.01% trypan blue in lactic acid, and identified and counted under a Zeiss Axioplan microscope at a magnification of 200. Two filters per sample were prepared and at least 100 conidia per filter were counted. Conidial numbers were converted to conidial carbon based on published values of biovolumes of the identified species (Bärlocher and Schweizer 1983) and assuming an average carbon density of 250 fg C/μm^3 (Findlay and Arsuffi 1989, Newell 1992).

A particle-plating method was used to identify leaf-colonizing fungi other than aquatic hyphomycetes. Six leaf discs (4 mm diameter) were cut from leaf material of each replicate litter bag using a cork borer. Discs were placed on 0.1% malt extract agar (Merck) and incubated at 10°C. Colonies growing out of the discs' edges were transferred to fresh plates and selected strains of each colony type identified to genus or species. Isolated strains showing macroscopically identical characteristics were assumed to belong to the same taxon.

Fungal biomass

Fungal biomass was estimated as ergosterol content. Analyses were performed with six willow leaves, two poplar leaves or one half of a plane leaf from each litter bag. Ergosterol was extracted from lyophilized leaf samples by 30 min refluxing in alcoholic base and quantified by measuring UV-absorbance after purification by HPLC (Gessner et al. 1991). The chromatographic system consisted of a Kontron pump, autoinjector and a variable wavelength detector set at 282 nm. The column was a 25 cm × 4.6 mm RP18 Lichrospher (5 μm particle size). Column temperature was maintained at 28°C in a water bath. The system was run isocratically with HPLC-grade methanol at a flow rate of 1.5 ml/min. Ergosterol eluted

Table 2. Exponential breakdown coefficients (mean ± asymptotic standard error) of leaf litter decomposing in the Garonne River as determined with non-linear regression analysis (N = 21–23).

Leaf species	Breakdown coefficient (d^{-1})	Breakdown coefficient (degree-d^{-1})
Salix	0.0091±0.0013	0.00112±0.00007
Populus	0.0070±0.0006	0.00086±0.00004
Platanus	0.0045±0.0004	0.00054±0.00002

after 9 min; peak identity was checked on the basis of retention times of commercial ergosterol purchased from Fluka (> 98% purity). Ergosterol contents were converted to fungal biomass using species-specific factors established for the eight dominant fungal species sporulating on the leaves, coupled with estimates of their relative abundance in the fungal community (Gessner and Chauvet 1993). Fungal carbon content was determined for the three dominant species of aquatic hyphomycetes identified in the present study, thus permitting us to express fungal biomass on a carbon rather than on a dry mass basis. To obtain mycelia for these analyses, fungal strains were isolated from single spores, and grown in shake culture on a glucose mineral-salt solution (Gessner and Chauvet 1993). They were harvested during late growth and analyzed in the same way as leaf material.

Bacterial numbers and biomass

At each sampling date, 15 discs (7 mm diameter) were cut from 2–4 leaves of each litter bag with a cork borer, preserved in 2% filter-sterilised formalin, and stored until analysis. Five ml of filtered autoclaved water was added before homogenizing the leaf discs for 3 min with an Ultra Turrax blender. Samples were diluted with 20 ml of water and vigorously vortexed. After allowing the homogenate to settle for 1 min (Suberkropp and Klug 1976), a 0.1-ml subsample was taken about 5 mm below the surface and completed to a final volume of 10 ml. DAPI (4',6-diamidino-2-phenylindole) was added to a final concentration of 5 mg/l before the suspension was passed through a black polycarbonate filter (Nuclepore, pore size 0.22 µm). A cellulose nitrate filter (Whatman or Millipore, pore size 0.45 µm) was placed under the black filter in order to spread the vacuum evenly, ensuring an even distribution of the particles over the entire filter surface (Fry 1988). After filtration, the filter was rinsed with 10 ml of filtered autoclaved water in order to remove excess staining solution (Fry 1988). Three filters were prepared for each replicate leaf pack and the bacteria retained on the filter were counted at 1000× by using epifluorescence microscopy with 10 or 20 microscopic fields being viewed per filter.

Photographs were taken from filter portions from one of the four replicate leaf packs per species per sampling date. Photographic slides (Ektachrome ISO 400/27°)

were projected on a screen, and normally 150–200 bacteria were sized and assigned to one of 34 classes defined on the basis of bacterial size and shape. The distribution of bacteria among classes was assumed to be representative of the bacterial assemblage on a given leaf species at a given date. To account for size-dependent differences in the dry mass density of bacterial cells, we converted bacterial biovolume (V in µm^3) to carbon (C in fg) according to the empirically determined relationship $C = 89.6 \cdot V^{0.59}$ (Simon and Azam 1989). This equation yields carbon densities ranging from 130 to 446 fg/µm^3 for the usually encountered bacterial size spectrum of 0.02 to 0.40 µm^3 per cell. Occasionally, we found large cells measuring up to 1.1 µm^3, corresponding to carbon densities of 95 fg/µm^3. To calculate the total amount of bacterial carbon associated with leaves, the information on carbon densities was used in combination with total cell counts and the size-frequency structure of bacterial assemblages.

Statistical analysis

Using non-linear regression analysis, mass loss data were adjusted to the simple exponential model $m_t = m_0 \cdot e^{-kt}$, where m_t is the leaf mass remaining at time t, m_0 is the initial leaf mass, and k is the exponential breakdown coefficient. One-way and repeated-measures analysis of variance were used to test for differences among leaf species in terms of bacterial numbers, ergosterol concentrations, and sporulation rates of aquatic hyphomycetes. Differences were considered significant when P < 0.05. All calculations were made with the statistical package SYSTAT (Wilkinson 1990).
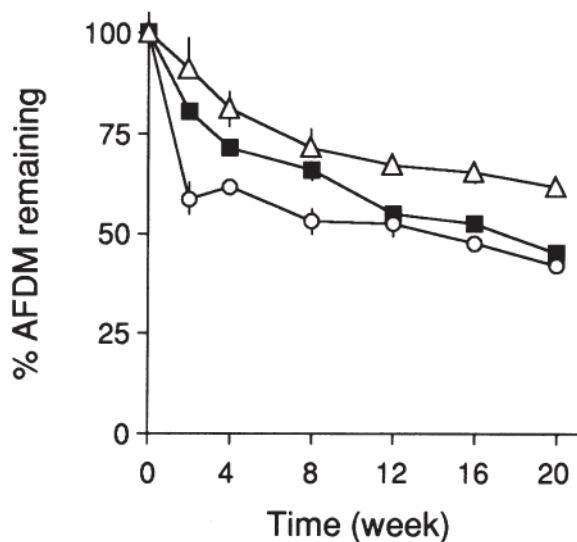

Fig. 2. Loss in ash-free dry mass (AFDM) from leaves of willow (O), poplar (■) and London plane (△) decomposing in the Garonne River. Vertical bars indicate ± 1 SE.
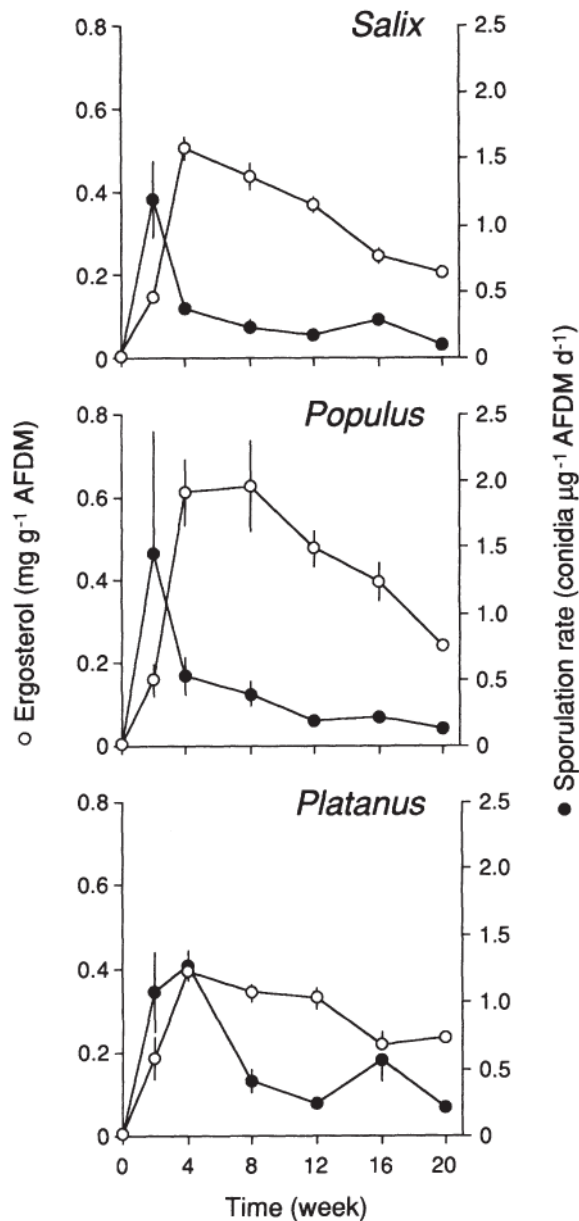
Fig. 3. Dynamics of ergosterol concentrations (in leaves) and sporulation rates of aquatic hyphomycetes associated with leaf litter decomposing in the Garonne River. Vertical bars indicate ± 1 SE.

their initial AFDM (Fig. 2). After 20 weeks, 60% of the initial AFDM remained in leaf packs with London plane while less than half of the initial leaf mass remained in poplar and willow at that time.

Sporulation rates of aquatic hyphomycetes on leaf litter reached peaks of 1.2 (willow), 1.4 (poplar) and 1.3 (London plane) conidia per µg AFDM per day after 2 or 4 weeks of leaf submersion in the river (Fig. 3). Subsequently, sporulation rates declined to 0.09–0.57 conidia μg^{-1} AFDM d^{-1}. Differences in sporulation rates between leaf species were not significant. At the two-week sam-

Fig. 4. Dynamics of bacterial numbers associated with leaf litter decomposing in the Garonne River. Vertical bars indicate ± 1 SE.

Results

Exponential breakdown coefficients ranged from 0.0044 to 0.0091 per day (Table 2) and appeared to be different among leaf species, as judged from the visual comparison of confidence limits around means of AFDM remaining at each sampling date. However, much of the difference between willow and poplar was due to the first two weeks of breakdown when willow leaves lost nearly 40% of
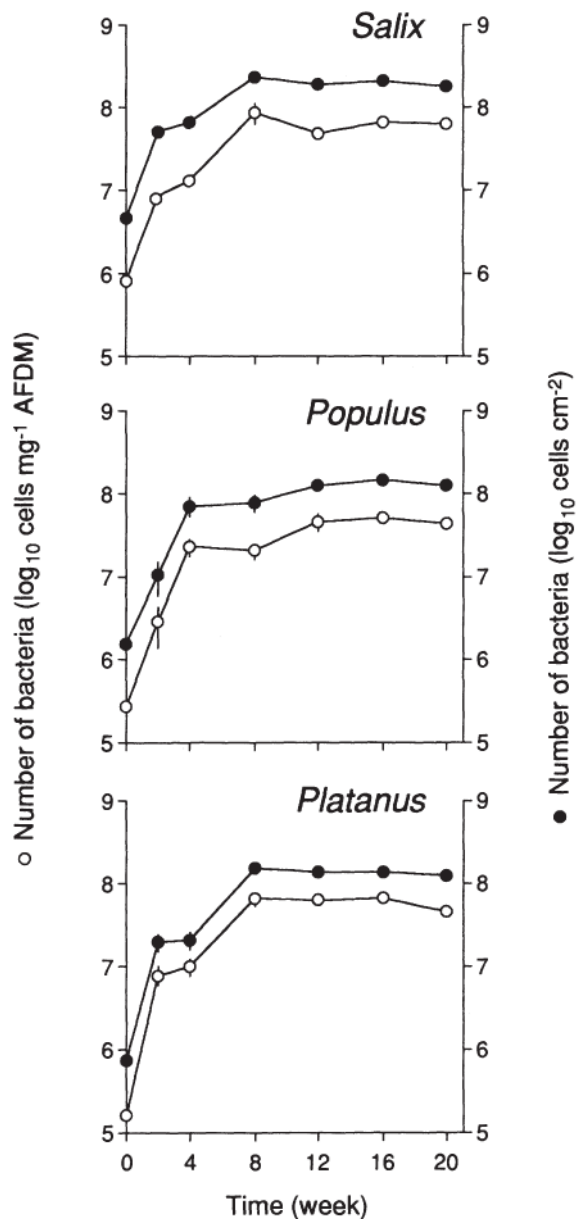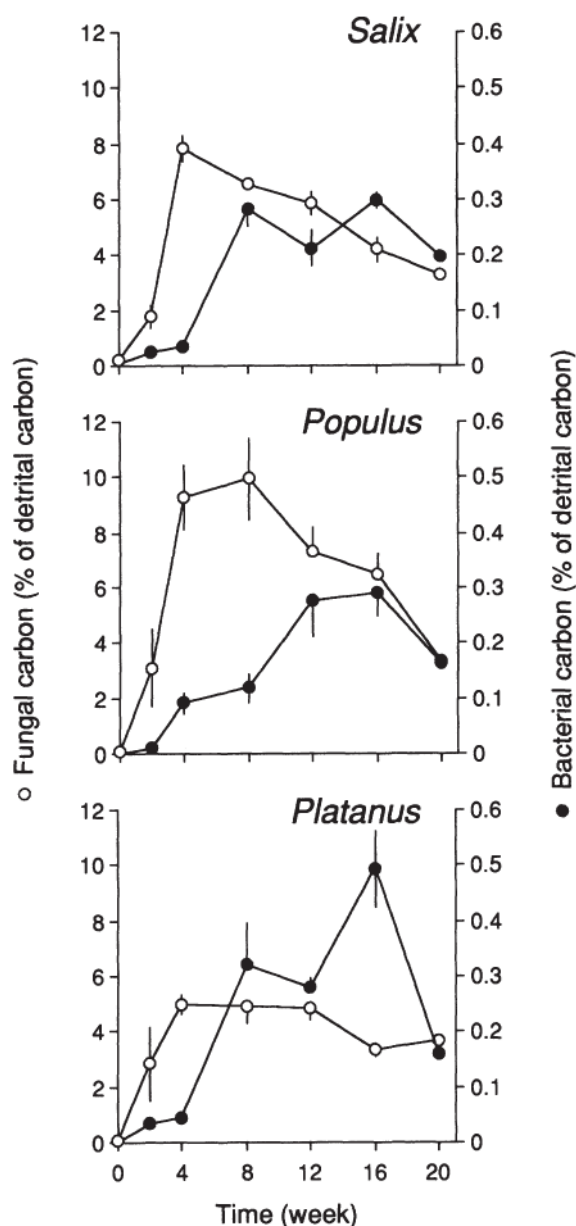
Fig. 5. Dynamics of fungal and bacterial biomass associated with leaf litter decomposing in the Garonne River. Vertical bars indicate ± 1 SE.

pling date, *Flagellospora curvula* Ingold made up 54–75% of the total number of conidia released from leaf surfaces. Subsequently, *Tetracladium marchalianum* de Wildeman and *Clavariopsis aquatica* de Wildeman co-dominated the aquatic hyphomycete assemblages. These three species together accounted for 75–87% of the conidia produced on leaf surfaces.

Frequent isolates of fungi other than aquatic hyphomycetes included *Epicoccum purpurascens* Ehrenb. ex Schlecht., *Cladosporium cladosporioides* (Fres.) de Vries, species of the genera *Fusarium*, *Pythium*, and *Saprolegnia*, and sterile mycelia. Quantitative comparisons of these fungi with aquatic hyphomycetes were not possible owing to the differences in methods used to detect the different fungal groups.

Concentrations of ergosterol in leaf litter were minute (<15 µg/g AFDM) before the start of the experiment but increased rapidly within the first 4 weeks after submersion of leaves in the river (Fig. 3). Peak concentrations of 0.50, 0.39 and 0.63 mg/g AFDM, respectively, were reached after 4 (willow and London plane) or 8 weeks (poplar). In willow and poplar, ergosterol peaks occurred later than the corresponding peaks in sporulation rate. Subsequent decreases in ergosterol concentration were more striking in willow and poplar than in London plane, but declines were consistently less pronounced than those in sporulation rate. Differences in ergosterol concentrations between leaf species were significant both when peak values were compared and when the whole data sets were considered using repeated-measures ANOVA (P < 0.001).

Bacterial numbers increased exponentially during the first weeks of the study and remained constantly high for the remainder of the experiment (Fig. 4). Peaks of 8.7, 6.6 and $5.2 \cdot 10^7$ bacterial cells per mg AFDM, corresponding to 2.4, 1.5 and $1.3 \cdot 10^8$ bacterial cells per cm^2, were attained 8 (willow) or 16 (London plane and poplar) weeks after immersion of leaves in the river. On an AFDM basis, peak values were not significantly different between leaf species irrespective of whether statistical tests were performed using log-transformed or untransformed data. Likewise, in spite of significant differences between leaf species at several sampling dates, the significant interaction term between sampling date and leaf species in a repeated-measures ANOVA indicated that, on the whole, bacterial colonization of the three leaf species was similar. When data were expressed on an area basis, however, results of ANOVA indicated that bacterial numbers were significantly higher on willow leaves than on the other two species.

Estimates of bacterial and fungal biomass associated with decomposing leaf litter in the Garonne River are presented in Fig. 5. Fungal biomass, expressed in terms of carbon, greatly exceeded bacterial biomass in each of the three leaf species and on each sampling occasion, with differences being more pronounced in willow and poplar than in London plane. As a consequence of both increases in bacterial mass and decreases in fungal mass, differences between standing stocks of bacterial and fungal carbon tended to diminish with progressing leaf breakdown in all leaf species. Fungal mass nevertheless continued to exceed bacterial mass even after 20 weeks of leaf breakdown. In addition to the accumulation of mycelial biomass in leaves, a substantial fraction of carbon was transformed to conidia of aquatic hyphomycetes over the course of the experiment, with cumulative conidial biomass ranging from 10 (willow) to 30 (London plane) mg C per g detrital C (Table 3).

Table 3. Maximum microbial biomass and production rates at times of highest activity (indicated as footnotes). Total microbial biomass was calculated at each sampling date as the sum of bacterial and mycelial biomass. Bacterial and mycelial production rates were calculated as the change in standing stocks during the indicated periods divided by the elapsed time in d; leaf C refers to the amount of carbon present at the start of the considered period, which had been corrected for microbial carbon. Conidial production rates were deduced from conidial biovolumes and numbers at times of peak sporulation rates. Cumulative conidial production was calculated by interpolating conidial production rates between sampling dates and summing up over the entire study period of 20 weeks. All values are means ± 1 SE, if available.

Parameter	Salix	Populus	Platanus
Bacterial biomass (mg C g^{-1} detrital C)	1.7±0.2[c]	1.6±0.3[d]	3.2±0.3[d]
Mycelial biomass (mg C g^{-1} detrital C)	49±3[b]	67±7[b]	42±1[b]
Ratio of mycelial biomass to total microbial biomass (%)	94–100	95–100	88–99
Bacterial production rate (mg C g^{-1} leaf C d^{-1})	0.053[g]	0.041[f]	0.075[g]
Mycelial production rate (mg C g^{-1} leaf C d^{-1})	2.8[f]	3.4[f]	1.8[e]
Conidial production rate (mg C g^{-1} detrital C d^{-1})	0.34±0.05[a]	0.47±0.18[a]	0.62±0.05[b]
Cumulative conidial production (mg C g^{-1} initial leaf C)	10	19	30

[a] 2 weeks, [b] 4 weeks, [c] 8 weeks, [d] 16 weeks, [e] 0–2 weeks, [f] 2–4 weeks, [g] 4–8 weeks.

Discussion

Significance of fungal biomass in a large river

In large rivers, the breakdown of leaf litter tends to be slower than in small headwater streams (Webster and Benfield 1986, Chauvet et al. 1993 and references therein). The rather low breakdown rates in the present study (Fig. 2, Table 2) compared to literature values for low order streams lend further support to this contention. According to current theory, the phenomenon is due to the structure of macroinvertebrate communities which is thought to shift from one dominated by shredders (large particle detritivores) and collectors (fine particle feeders) in upstream reaches to one mainly composed of collectors in downstream reaches of river systems (Cummins 1988). In a previous investigation (Chauvet et al. 1993), however, we found that shredders were clearly not responsible for the large differences in breakdown rates observed between the seventh-order Garonne River (slow breakdown, shredders abundant) and the Touyre, a third-order tributary in the upper drainage basin (rapid breakdown, shredders rare). Furthermore, we found evidence that in small streams such as the Touyre, rates of leaf breakdown are primarily controlled by microfungi, par-

ticularly members of the so-called aquatic hyphomycetes (Gessner and Chauvet 1994). These fungi are thought to have their preferred habitat in small, clean, and turbulent headwater streams (Webster and Descals 1981, Bärlocher 1992b, Maltby 1992b, Suberkropp 1992a), leading us to hypothesize that the reduction of leaf breakdown in high order rivers results primarily from a diminution in fungal activity (Chauvet et al. 1993).

The results of the present study show that this hypothesis is untenable. Fungal carbon attained 5 to 10% of the total detrital carbon in leaf litter decomposing in the Garonne River (Fig. 5). These values are similar to those measured in leaf litter decomposing in the headwater (Gessner and Schwoerbel 1991, Suberkropp et al. 1993, Gessner and Chauvet 1994, see also Golladay and Sinsabaugh 1991) and are in the upper range of values determined for other low order streams using different methodology (Findlay and Arsuffi 1989, Suberkropp 1992b). As a result, rates of mycelial production inferred from differences in fungal standing stocks (Table 3), were likewise within the range previously encountered in the headwater stream (Gessner and Chauvet 1994). Sporulation rates on leaves in the Garonne River were in the lower range of values observed in the headwater (Gessner and Chauvet 1994) but they were not strikingly low

Table 4. Fungal net production and potential contribution to initial leaf breakdown in the Garonne River. All values refer to times when the sums of mycelial net production and cumulative conidial production were maximal. This occurred after the first 4 weeks of immersion in willow, after 8 weeks in poplar and after 12 weeks in London plane. Total fungal production is the sum of net mycelial production (equivalent to the accumulated standing stock at the respective sampling date) and conidial production, which was calculated by interpolating conidial production rates between sampling dates and summing up over the considered periods. The percentage of initial leaf carbon assimilated by fungi was calculated by dividing total fungal net production by an average growth efficiency of 0.35 which had been determined for aquatic hyphomycetes growing in leaf litter (Suberkropp 1991). The contribution of fungal assimilation to overall loss of leaf carbon was estimated by dividing fungal carbon assimilation by the loss in leaf carbon.

Parameter	Salix	Populus	Platanus
Loss in leaf carbon (%)	37.5	34.9	27.6
Total fungal net production (mg C g^{-1} initial leaf carbon)	55	80	61
Percentage of initial leaf carbon assimilated by fungi (%)	15.7	22.9	17.4
Contribution of fungal carbon assimilation to overall loss of leaf carbon (%)	41.9	65.5	63.1

Table 5. Initial concentrations of lignin, nitrogen and phosphorus in leaf litter (% of dry mass, mean±SE).

Species	Lignin	Nitrogen	Phosphorus	Reference
Salix	20.0±0.3	1.64±0.02	0.073±0.001	Chauvet (1987)
Populus	23.2±0.3	0.93±0.01	0.053±0.001	Chauvet (1987)
Platanus	30.9±0.5	0.52±0.02	0.017±0.001	Gessner and Chauvet (1994)

compared to other studies in small streams (Suberkropp and Chauvet 1995). Differences between streams become somewhat larger when data are expressed in terms of conidial mass rather than numbers (Table 3; Gessner and Chauvet 1994). The implication is that in spite of a high mycelial production in the Garonne River, overall fungal productivity might have been reduced with the proportion of resources allocated to hyphal growth being increased at the expense of conidial production. On the small current data basis, however, this argument remains tentative.

In order to get an idea of fungal contribution to the overall loss in leaf mass, we calculated partial organic matter budgets for the initial periods of breakdown until fungal biomass reached peaks (Table 4). These estimates of microbially mediated carbon flow are based on measurements of biomass and cumulative conidial production and an assumed net production efficiency of 0.35 (average calculated from Table 3 in Suberkropp 1991). Accordingly, fungi transformed between 16% (willow, 4 weeks) and 23% (poplar, 8 weeks) of the initial leaf mass (as carbon) to either fungal biomass (mycelial or conidial carbon) or to CO_2 within the first 4 to 12 weeks of leaf submergence in the Garonne; this is equivalent to 42 to 65% of the total leaf carbon lost during the considered periods (Table 4). Thus, fungi would appear to assume a critical role in the biological transformation of leaf litter even in large rivers such as the Garonne. If so, in this respect, the downstream reaches of river networks would behave exactly like their headwater counterparts.

Litter breakdown is regulated by exogenous variables such as temperature and nutrient availability (Suberkropp and Chauvet 1995), and by endogenous factors such as litter chemistry. Among the endogenous factors that have been proposed (see Gallardo and Merino 1993 for an overview), the initial lignin content of leaves appears to have the highest predictive power and most universal applicability in both forest ecosystems (e.g., Taylor et al. 1991) and low order streams (Gessner and Chauvet 1994). The present results from the Garonne River suggest that this principle holds also for large rivers, since the ranking of leaf species according to breakdown rate (Table 2) was predictable from the initial lignin content of leaves (Table 5). Note, however, that in contrast to our previous study (Gessner and Chauvet 1994) lignin and nutrient contents of leaves were intercorrelated (Table 5) so that we cannot rule out the possibility that the nutrient content of leaves affected breakdown rates as well. Gessner and Chauvet (1994) showed that the control of leaf breakdown rate through lignin can be mediated by

the activity of microfungi. This finding also is broadly consistent with the present results in that the two leaf species with lower lignin contents, willow and poplar, accumulated significantly greater amounts of fungal mass than the high-lignin leaves of London plane (Fig. 5).

Relative importance of fungi and bacteria

Throughout the study of nearly 5 months, fungal biomass in willow, poplar and plane leaves exceeded bacterial mass by an order of magnitude (Fig. 5). This finding is consistent with reports by Findlay and Arsuffi (1989), which, to our knowledge, is the only other study comparing bacterial and fungal biomass associated with decomposing leaves in a freshwater ecosystem. The combined use of three techniques in Findlay and Arsuffi's (1989) study, viz. direct counts of bacteria, hyphal length measurements for filamentous fungi, and ATP determinations for total microbial biomass, suggests that mycelial mass accounted in general for 87–96% of the total microbial biomass associated with leaves. Similar results were obtained for standing-dead *Spartina* leaves in a salt marsh (Newell 1993). In the present study, the corresponding mean percentage was as high as 96% (Table 3) although our bacterial counts were in the upper range of values available from the literature (e.g., Suberkropp and Klug 1976, Findlay and Arsuffi 1989, Cuffney et al. 1990, Leff and McArthur 1990, Sridhar and Bärlocher 1993), demonstrating that fungal mass greatly outweighed bacterial mass at any instant of time in the Garonne River. Probably not all leaf-attached bacteria were dislodged or visualized with the extraction technique we used, and DAPI staining may have resulted in an underestimation of bacterial numbers and cell size compared to staining with acridine orange (Suzuki et al. 1993). However, these potential sources of error alone cannot account for the large difference between bacterial and fungal biomass observed in the present study (Fig. 5). An important consequence of the high proportion of fungal to total microbial biomass in leaves is that increases in leaf palatability resulting from microbial colonization, a process known as conditioning (Bärlocher 1985, Suberkropp 1992b), would be chiefly caused by eukaryotic as opposed to prokaryotic microorganisms. A corollary of this conclusion is that fungi rather than bacteria would govern the microbially mediated energy flow from leaf litter to higher trophic levels such as the leaf-shredding detritivores.

Estimates of microbial biomass, whether bacterial or fungal, reflect a particular state in time and hence are not fully adequate to describe a dynamic process such as microbial growth. How would bacteria and fungi, therefore, compare on a production basis? Because of their small size, prokaryotes are generally believed to exhibit higher growth rates than eukaryotes including fungi. Supposing, therefore, that losses of bacterial cells from decomposing leaf litter were high, bacterial production could be underestimated when inferred from measurements of standing stocks, and this would lead to an inappropriate assessment of bacterial importance for the breakdown process. Findlay and Arsuffi (1989) provide some support for this idea. On the basis of measurements of [³H]thymidine incorporation into DNA, these authors calculated bacterial doubling times of 0.5–3 d for decomposing leaf litter in a stream, which are similar to values available for other detrital systems (Moran and Hodson 1989, Newell 1993). Recently, measurements of fungal growth rates also have become possible owing to the development of a novel tracer technique (Newell and Fallon 1991). For decomposing leaf material, fungal doubling times determined with this method range from 7 to 139 d (Gessner and Chauvet unpubl., Newell and Fallon 1991, Suberkropp 1995), which is considerably longer than the time taken by the bacterial populations in Findlay and Arsuffi's study (1989). However, by measuring bacterial and fungal growth rates simultaneously, Weyers and Suberkropp (pers. comm.) demonstrated that the contribution of bacteria to total microbial production on leaf litter in a stream was as small as their contribution to microbial biomass, i.e. <5%. Therefore, it seems unlikely that the bacteria associated with decomposing leaf litter in the Garonne River assumed much greater importance than is suggested by our estimates of bacterial biomass.

Do bacteria replace fungi in later stages of breakdown?

Current concepts on microbial dynamics during leaf breakdown in running waters view fungi as the initial colonizers of fresh litter. Bacteria are not thought to assume notable importance before the leaf material has become partially broken down (Suberkropp and Klug 1976). Our results on the dynamics of microbial biomass support these ideas to some extent. Bacterial numbers increased exponentially during the first 4 or 8 weeks of breakdown (Fig. 4), but because numbers were low initially, this steep increase had minor consequences for the accumulation of bacterial biomass at that time (Fig. 5). Therefore, in spite of potentially high growth rates, bacteria were probably of minor importance for the breakdown process during the first few weeks after leaf submergence. Fungi, in contrast, clearly exhibited the most rapid accumulation of biomass and most dense sporulation initially (Fig. 5). This phenomenon was especially pronounced in the present study and confirms previous find-

ings (Findlay and Arsuffi 1989, Suberkropp 1991, Gessner and Chauvet 1994) indicating that stream microfungi are most active in the early stages of leaf breakdown.

Bacterial biomass exhibited the sharpest increase when fungal biomass started to decline (Fig. 5). Interestingly, this decrease in fungal mass was retarded in poplar leaves and thus coincided with a delay in the increase of bacterial mass in this leaf species. These opposite dynamics of bacteria and fungi tend to support the idea that, with progressing breakdown, the microbial assemblages associated with leaves in streams shift towards the bacterial component. We emphasize, however, that sporulation of aquatic hyphomycetes continued beyond the "turning point" (Fig. 3) and mycelial mass remained considerably higher than bacterial mass throughout (Fig. 5) with mycelial production probably also continuing at this time (Suberkropp 1995, Gessner and Chauvet unpubl.). This suggests that at advanced stages of leaf breakdown bacteria complement rather than replace the fungi, a point that applies especially when considering the leaf litter system in streams from a foodweb perspective.

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