doi:10.1111/j.1365-2427.2010.02483.x

Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams

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SUMMARY

1. Leaf litter constitutes the major source of organic matter and energy in woodland stream ecosystems. A substantial part of leaf litter entering running waters may be buried in the streambed as a consequence of flooding and sediment movement. While decomposition of leaf litter in surface waters is relatively well understood, its fate when incorporated into river sediments, as well as the involvement of invertebrate and fungal decomposers in such conditions, remain poorly documented.

2. We tested experimentally the hypotheses that the small interstices of the sediment restrict the access of the largest shredders to buried organic matter without compromising that of aquatic hyphomycetes and that fungal decomposers in the hyporheic zone, at least partly, compensate for the role of invertebrate detritivores in the benthic zone.

3. Alder leaves were introduced in a stream either buried in the sediment (*hyporheic*), buried after 2 weeks of exposure at the sediment surface (*benthic-hyporheic*), or exposed at the sediment surface for the entire experiment (*benthic*). Leaf decomposition was markedly faster on the streambed surface than in the two other treatments (2.1- and 2.8-fold faster than in the benthic-hyporheic and hyporheic treatments, respectively).

4. Fungal assemblages were generally less diverse in the hyporheic habitat with a few species tending to be relatively favoured by such conditions. Both fungal biomass and sporulation rates were reduced in the hyporheic treatment, with the leaves subject to the benthic-hyporheic treatment exhibiting an intermediate pattern. The initial 2-week stage in the benthic habitat shaped the fungal assemblages, even for leaves later subjected to the hyporheic conditions.

5. The abundance and biomass of shredders drastically decreased with burial, except for *Leuctra* spp., which increased and was by far the most common leaf-associated taxon in the hyporheic zone. *Leuctra* spp. was one of the rare shredder taxa displaying morphological characteristics that increased performance within the limited space of sediment interstices.

6. The carbon budgets indicated that the relative contributions of the two main decomposers, shredders and fungi, varied considerably depending on the location within the streambed. While the shredder biomass represented almost 50% of the initial carbon transformed after 80 days in the benthic treatment, its contribution was <0.3% in the hyporheic one and 2.0% in the combined benthic-hyporheic treatment. In contrast,

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mycelial and conidial production in the permanently hyporheic environment accounted for 12% of leaf mass loss, i.e. 2–3 times more than in the two other conditions. These results suggest that the role of fungi is particularly important in the hyporheic zone.
7. Our findings indicate that burial within the substratum reduces the litter breakdown rate by limiting the access of both invertebrate and fungal decomposers to leaves. As a consequence, the hyporheic zone may be an important region of organic matter storage in woodland streams and serve as a fungal inoculum reservoir contributing to further dispersal. Through the temporary retention of litter by burial, the hyporheic zone must play a significant role in the carbon metabolism and overall functioning of headwater stream ecosystems.

Keywords: aquatic hyphomycetes, litter breakdown, organic matter, river sediment, shredders

Introduction

Leaf litter constitutes the major source of organic matter and energy in woodland stream ecosystems (Cummins *et al.*, 1989). A substantial part of leaf litter entering running waters may be buried into the streambed during storms (Herbst, 1980) or as a consequence of flooding and sediment movement (Metzler & Smock, 1990; Naegeli *et al.*, 1995). Thus, the hyporheic zone of streams is potentially a major site of organic matter storage. Comparisons between benthic and hyporheic zones have shown that the latter may account for 25–82% of the total stored organic matter (Cummins *et al.*, 1983; Metzler & Smock, 1990; Smock, 1990; Jones, 1997; Jones *et al.*, 1997). During the last four decades, studies on the decomposition of leaf litter have mostly been limited to benthic habitats, i.e. with processes occurring above the streambed surfaces (Kaushik & Hynes, 1971; Webster & Benfield, 1986; Suberkropp, 1998). Although a few authors have attempted to determine the fate of leaf litter when incorporated into stream sediments, its ecological significance for this compartment of headwater streams remains poorly documented (but see Herbst, 1980; Rounick & Winterbourn, 1983; Metzler & Smock, 1990; Smith & Lake, 1993; Naamane, Chergui & Pattee, 1999). From these studies, however, burial has been generally shown to reduce the rate of leaf litter decomposition (Reice, 1974; Herbst, 1980; Rounick & Winterbourn, 1983; Metzler & Smock, 1990; Naamane *et al.*, 1999), with a few other studies reporting ambiguous or less well-defined patterns (Mayack, Thorp & Cothran, 1989; Smith & Lake, 1993). In contrast, Nichols & Keeney (1973) observed an enhancement of leaf litter decomposition rate by the presence of sediment in the lentic environment.

Aquatic hyphomycetes, or Ingoldian fungi, are a phylogenetically heterogenous group of fungi that are involved in leaf litter colonisation and decomposition in streams, i.e. environments where aerobic conditions generally prevail (Bärlocher, 1992; Storey, Fulthorpe & Williams, 1999; Gulis, Kuehn & Suberkropp, 2009). Despite their key importance in the functioning of stream ecosystems, aquatic fungi have historically received much less attention than macroinvertebrates, algae, fish or even bacteria (Gulis & Suberkropp, 2006). These fungi play an essential role in the decomposition of leaf litter in aquatic environments (Bärlocher, 1992; Gessner & Chauvet, 1994; Gessner, Chauvet & Dobson, 1999) by producing extracellular enzymes able to degrade the structural constituents of vascular plants such as cellulose, hemicelluloses, pectin and, to a lesser degree, lignin, and in transforming leaf material into a more suitable food source for invertebrates in streams (Bärlocher, 1992). Therefore, aquatic hyphomycetes are important mediators in the energy and nutrient transfer to higher trophic levels (Gulis *et al.*, 2009), and more generally in the ecological functioning of headwater streams. Fungal abundance, distribution and population dynamics are strongly linked to the seasonal timing and extent of terrestrial plant matter inputs into the surface zone of running waters (Suberkropp, 1997). Hyporheic fungi might thus play a fundamental role in processing allochthonous organic matter, and the incorporation into the trophic webs like fungi do in surface waters

(Storey *et al*., 1999). Surprisingly, and despite the considerable amount of plant matter buried in sediment, little is known about the role of microorganisms – and aquatic hyphomycetes in particular – on the decomposition of leaf litter in the hyporheic zone of headwater streams. From the very few studies available, the conclusions concerning the role of aquatic hyphomycetes remain unclear.

Essafi *et al*. (1994) and Metzler & Smock (1990) suggested that because of constraints prevailing in the hyporheic zone, fungal conditioning was more limited than in surface waters and thereby led to a decrease in the attractiveness of leaves to invertebrates. In more extensive studies, Rounick & Winterbourn (1983) recorded a lower protein content and microbial activity in buried litter, while Herbst (1980) found no consistent difference in the protein content of leaves incubated above and below the surface of sediment, and Naamane *et al*. (1999) did not find aquatic hyphomycete spores on poplar leaves buried in the sediments of a Moroccan stream. Finally, Smith & Lake (1993) observed microbial colonisation, particularly by fungal hyphae, on leaf litter incubated either above or below the surface of sediment. Bärlocher *et al*. (2006, 2008) found that the occurrence of aquatic hyphomycetes in streambed sediment was closely linked to that of deciduous leaves and suggested that aquatic hyphomycetes readily disperse within the hyporheic zone. Clearly, additional data are needed to fully understand the abundance, the role, the activities and the dynamics of aquatic fungi colonising leaves in the hyporheic zone of headwater streams.

The aim of this study was to determine how the location of leaf litter within the streambed, i.e. at the surface or buried, may affect the leaf-associated decomposer communities and therefore leaf litter decomposition. Hyporheic sediment may act as a physical barrier, allowing only shredders with thin and elongated bodies to penetrate the substratum layers. Similarly, the specific conditions prevailing in the hyporheic zone are likely to considerably influence the presence, dynamics and activity of aquatic hyphomycetes. As a result of such constraints, our first hypothesis was that the density and biomass of both detritivorous macroinvertebrates and aquatic hyphomycetes would be lower in the hyporheic zone than in the benthic habitat. We expected leaf litter burial to lead to an impoverished community and lowered activity of aquatic hyphomycetes, not only because of the physical barrier but also because of the decreased oxygen concentration in the hyporheic water. Our second hypothesis was that leaf litter decomposition depends on initial microbial colonisation. Specifically, we predicted that leaves exposed for a short period at the sediment surface before burial, because of higher microbial inoculation, would be decomposed more rapidly than those buried from the beginning, but at a rate lower than leaves decomposing entirely at the sediment surface. We tested these hypotheses by conducting an experimental study in a second-order stream located in southern France, where leaf-associated microbial and macroinvertebrate communities, as well as decomposition rates, were compared across controlled treatments differing by their location within the streambed (buried in the sediment, buried after 2 weeks of exposure at the sediment surface or exposed at the sediment surface throughout the experiment).

Methods

Study site

The experimental site was located in the Alzeau, a second-order stream of the Montagne Noire in South-Western France (02°13′23′′E, 43°25′51′′N; elevation 743 m a.s.l). The surrounding forest consisted of mixed broadleaf species including alder (*Alnus glutinosa* (L.) Gaertn.), oak (*Quercus petraea* (Mattus.) Liebl.), hazel (*Corylus avellana* L.) and aspen (*Populus tremula* L.). The area was subject to a mountain climate with oceanic influences marked by high rainfall in the autumn and winter (1300 mm year^{-1}). The mean monthly temperatures ranged from −0.7 to 21.4 °C. Forestry was the only anthropogenic disturbance, although limited within the study area. The study was conducted in a pool-riffle-pool sequence, extending over *c*. 100 m. At baseflow, the stream was 3.5–4.7 m wide and 0.25–0.35 m deep in riffles. The mean water temperature during the leaf decomposition study was 7.7 °C in both the benthic and hyporheic zones, and the average discharge was *c*. 500 L s^{-1}. Vertical hydraulic gradients (VHGs) through the streambed were calculated as follows: VHG (m m^{-1}) = $\Delta h/\Delta l$, where $\Delta h = h_{\text{stream}} - h_{\text{piezometer}}$ and Δl is the distance between the streambed and the top

of the plexiglas minipiezometer screen (Baxter, Hauer & Woessner, 2003). Vertical hydraulic gradients were measured to identify downwelling (positive VHG) and upwelling (negative VHG) areas in the hyporheic zone, and the sediment characteristics (particle size and hydraulic conductivity) were studied in four representative riffles of the study site. The substratum was unconsolidated and mostly made of coarse sediments as determined from fractionation of the grain size of sediment cores (20 cm length × 30 cm diameter) sampled from downwelling zones in the middle of four representative riffles of the study site: >20 mm: 8.6%; 20–10 mm: 25.9%; 10–5 mm: 23.4%; 5–2 mm: 19.3%; 2–1 mm: 9.5%; 1–0.5 mm: 9.3%; 0.5–0.25 mm: 3.9%. The average flow rate through the sediment of the study site was relatively fast (22 cm h^{-1}). The stream water temperature was monitored every 2 h with calibrated data loggers (Smart-Button; ACR System Inc., Surrey, Canada). Water chemistry was determined at the six dates when leaf bags were introduced into or retrieved from the stream. pH, conductivity, and dissolved oxygen concentration were measured in the field using portable instruments (pH-meter 320i and Oxi 330i; WTW, Weilheim, Germany; Conductimeter Dist5; HANNA, Woonsocket, RI, U.S.A.). Water samples were filtered in the field with 0.7- μm glass fibre filters (Glass fibre GF/F, Whatman, Clifton, NJ, U.S.A.), stored in pre-rinsed polyethylene bottles and placed in an icebox until they were returned to the laboratory. Concentrations of P-PO_4^{3+}, measured as soluble reactive phosphorous (SRP), N-NO_3^-, N-NO_2^- and N-NH_4^+, and alkalinity were measured using standard colorimetric methods by flow injection analysis with an Alpkem Flow Solution IV system (OI Analytical, College Station, TX, U.S.A.) and potentiometric titration, respectively.

The stream water was slightly acidic (pH 6.0–6.6) with low conductivity (24–34 μS cm^{-1}) and low buffering capacity (1–5 mg $CaCO_3$ L^{-1}). Concentration of SRP (0.7–2.9 μg L^{-1}) was low, while concentration of N-NO_3^- (0.802–0.980 mg L^{-1}) was relatively high. The stream was always well oxygenated (88–111% of the saturation). Interstitial water was pumped from plexiglas minipiezometers using a hand-held vacuum pump. Physical and chemical conditions in the hyporheic zone (i.e. at 15 cm below the sediment surface) were comparable to those at the surface level, except for dissolved oxygen (45–86%) and SRP (8.3–12.4 μg L^{-1}) that were usually lower and higher, respectively, in the hyporheic zone.

Leaf decomposition

We selected alder as the test decomposing leaf species because it was by far the most common deciduous riparian tree species in the study area and provided a large part of leaf litter input to the stream. Alder leaves were collected from trees at abscission using nets in the autumn of 2007. Leaf bags consisted of 3.00 g (mean air-dry mass ± 0.03 g) of leaves enclosed in plastic net bags (15 × 15 cm, 5 mm mesh) to simulate natural accumulations of leaf detritus in the stream. Before incorporated into bags, the leaves were moistened with distilled water from a vaporizer to prevent breakage during handling and transport.

A total of 60 leaf bags were introduced in the stream at the head of riffles (downwelling zones). Leaf bags were subjected to three treatments: benthic, hyporheic (i.e. buried in the sediment) or benthic-hyporheic (i.e., buried in the sediment after 17 days of benthic exposure). Benthic leaf bags were placed at the sediment surface and anchored to an iron bar driven into the streambed. Hyporheic leaf bags were positioned approximately 15–20 cm below the sediment surface using a small shovel, with a coloured plastic wire attached to facilitate localisation and retrieval. At the time of the burial of the benthic-hyporheic leaf bags, these were disassembled and all macroinvertebrates associated with leaves were meticulously removed. Four replicate bags per treatment were randomly retrieved after 17, 31, 45, 59 and 80 days.

In parallel, artificial leaves were used to test whether invertebrates utilised leaves as refuge rather than food. Artificial leaves were made of polyethylene sheets with the shape and thickness similar to that of real leaves. Before use, these 'leaves' were pre-soaked for 2 weeks with several changes of distilled water to extract any soluble artificial chemicals (Boulton & Foster, 1998). A total of 24 bags with artificial leaves were introduced at the head of riffles as previously, with two treatments (i.e. benthic artificial and hyporheic artificial) applied to four replicate leaf bags and three sampling times (17, 45 and 80 days).

During leaf bag sampling, a Surber net (500 μm mesh size) was used to minimise invertebrate loss because of passive or active drift. Leaf bags were stored individually in plastic zip-lock bags and

transported to the laboratory in an icebox. Leaves were washed individually to remove sediments, exogenous organic matter and macroinvertebrates, which were collected in a 500 -μm screen sieve and then preserved in 70% ethanol until processing. Two sets of five 12 -mm-diameter discs and another set of 10 were cut from leaves, avoiding the central vein. One set of five leaf discs was frozen at −18 °C until processing for ergosterol extraction, the second one was used to determine microbial respiration and the last set of 10 to characterise fungal sporulation. The remaining leaf litter was dried at 65 °C to constant mass and weighed to the nearest 0.01 g. The leaf material was ground using a micro hammer mill (Culatti, Zurich, Switzerland) with a 0.5 -mm mesh. Portions of leaf material of about 250 mg were ashed at 550 °C for 4 h and weighed to determine the organic matter content. The leaf mass remaining in bags was expressed as the ratio of the ash-free dry mass (AFDM) between the final and initial leaf litter. Four unexposed batches of leaf litter were used to determine the leaf initial oven-dried mass by unit of air-dried mass and the initial AFDM according to the procedures described earlier. Ground aliquots of leaf material were used to determine the nitrogen content with a CHN analyzer (NA 2100 Protein; CE Instruments, Milan, Italy).

Microbial respiration on leaves

Respiration rates associated with decomposing leaf material were inferred from measurements of oxygen consumption from five leaf discs. Once cut, leaf discs were quickly placed in a measuring chamber containing 3 mL of membrane-filtered (0.45 μm pore size, 47 mm diameter; Millipore Corporation, Bedford, MA, U.S.A.) stream water and incubated in a water bath at stream temperature. Oxygen concentrations were monitored with oxygen electrodes connected to a data acquisition module (SI 130 Microcathode and 928 6-Channel Oxygen System; Strathkelvin Instruments, North Lanarkshire, U.K.) and a computer serving as a control and storage unit. Oxygen concentration was recorded every second for l.5 h. Determinations were carried out simultaneously with six electrodes, i.e. in five chambers containing samples plus one control chamber containing stream water but no leaf material. Respiration rates (mg O_2 g^{-1} leaf AFDM h^{-1}) were calculated as the slope of regression lines during periods of linear decreases in oxygen concentrations and were corrected for oxygen loss in the control chamber.

Spore production and fungal diversity

Once cut, the 10 fresh leaf discs were quickly placed in glass Petri dishes filled with 20 mL filtered (Glass fibre GF/F, Whatman) stream water. The production of fungal asexual spores was stimulated by gentle shaking (0.05 *g*, 25.4-mm orbital path) at 10 °C for 48 h. Thereafter, spore suspensions were transferred into 50- mL polyethylene centrifuge tubes, the Petri dish and discs were rinsed with distilled water to collect spores no longer in suspension, and the volume was adjusted to 35 mL with 2 mL of 37% formalin and distilled water. The discs were then lyophilised and weighed to the nearest 0.1 mg. To the spore suspensions, 0.5 mL of Triton X-100 (0.5% solution) was added and they were stirred gently to ensure uniform distribution of spores. Five millilitres of aliquots was then filtered through a membrane filter (5 μm pore size, 25 mm diameter ; Millipore Corporation), and the spores on the filter were stained with 0.1% Trypan blue in 60% lactic acid (Iqbal & Webster, 1973). Spores were counted and identified under the microscope (×200). Spore production was calculated as the number of spores released per gram leaf AFDM per day. The Shannon and Weaver index of diversity (H′) and the Shannon and Weaver index of evenness ($E_{H'}$) were computed for fungal communities associated with each of the three treatments.

Fungal biomass

Mycelial biomass in leaves was assessed through the content of ergosterol (Gessner & Chauvet, 1993). Leaf material was lyophilised and weighed to the nearest 0.1 mg, and then lipids were extracted with alkaline methanol heated at 80 °C for 30 min. Extracts were purified using solid-phase extraction cartridges (Oasis HLB, 60 mg, 3 cc; Waters, Milford, MA, U.S.A.) and ergosterol quantified by high-performance liquid chromatography (procedure slightly modified from Gessner, 2005). The extraction efficiency (85–92%) was determined for each series from controls to which known amounts of ergosterol were added and was applied to calculate the ergosterol content in leaf litter.

Macroinvertebrates

Macroinvertebrates retained over a 500 -μm mesh sieve were counted and identified to the lowest practicable level. Identification was to the genus/species level whenever possible, except for Oligochaeta and Diptera (family and subfamily or tribe, respectively), and individuals were classified as shredders, grazers and others (Tachet *et al.*, 2000). Macroinvertebrates assigned to the shredder group were measured to the nearest 0.5 mm, and individual body mass was calculated using body length–dry mass relationships from the literature (Meyer, 1989; Burgherr & Meyer, 1997).

Carbon budget

Mycelial biomass was estimated from the leaf content of ergosterol using 182 as conversion factor (Gessner & Chauvet, 1993; Charcosset & Chauvet, 2001). The biomass of released conidia was calculated from individual conidial mass for species listed in Bärlocher & Schweizer (1983) and Chauvet & Suberkropp (1998), or the average value of 200 pg for the other species (Gessner, 1997). The daily consumption rate of leaf litter by shredders was assumed to be 10% of the animals' body mass (Hieber & Gessner, 2002). Cumulative production of CO_2, conidial biomass and shredder biomass was calculated by interpolating production rates (CO_2, conidia) and consumption rates (shredders) between sampling dates and summing over the decomposition period. A factor of 0.5 was used to convert leaf AFDM, shredder biomass, mycelial biomass and conidial biomass into carbon (Baldy *et al.*, 2007). Partial decomposition budgets were built based on either initial leaf carbon or leaf carbon loss.

Statistics

Leaf decomposition rates (k) for each treatment were calculated using a nonlinear regression of the proportion of leaf mass remaining versus time, following an exponential decay model, $M_t = M_o \cdot e^{-kt}$, where M_o is the initial AFDM, M_t is the AFDM remaining at time t and t is the time in days (Petersen & Cummins, 1974). Leaf decomposition rates of the three treatments were compared using generalised linear models based on the log-link function and by a likelihood type I test (Lecerf & Chauvet, 2008). We used a two-way factorial ANOVA to assess differences in microbial and macroinvertebrate parameters (leaf-litter-associated respiration, spore production, and fungal biomass and abundance) and nitrogen content with treatment (i.e. benthic, hyporheic, or benthic-hyporheic) and exposure time as the main effects. When significant differences were detected between treatments, Tukey HSD tests were then carried out for *post hoc* pairwise comparisons. The ergosterol content and abundance of shredders were log-transformed to meet the assumptions of ANOVA. In cases of persistent heterogeneity of variances, a nonparametric test was applied. The Kruskal–Wallis test was used to investigate for differences in total shredder biomass across the three treatments. Mann–Whitney U tests (with Bonferroni corrected P values) were then carried out for pairwise comparisons. Differences in total shredder abundance and biomass associated with artificial leaves were tested using two-way ANOVA with treatments and exposure time as the main effects and Mann–Whitney U test, respectively. STATISTICA 6.0 (StatSoft Inc., 2001) was used for all statistical analyses. Differences were considered significant when $P < 0.05$. The similarity between aquatic hyphomycete communities at each sampling date under the various treatments was measured with the Steinhaus index (Legendre & Legendre, 1998) using R software version 2.6.0 (R Development Core Team, 2007). The differences among the indices were estimated by Monte-Carlo procedure. For this purpose, these indices were compared to 9999 pseudo-values of the Steinhaus index obtained from comparisons of virtual aquatic hyphomycete samples randomly generated (using a normal distribution for each species, with its observed mean and standard deviation in the original data set).

Results

Litter decomposition and nitrogen dynamics

The leaf decomposition rate was 2.8 and 2.1 fold higher at the sediment surface than for samples buried in the sediment and those buried after 17 days of exposure at the sediment surface, respectively (Fig. 1a and Table 1). Consequently, the decomposition rates differed significantly among these three conditions (GLM test, $\chi^2 = 12.5$, $P = 0.002$). The loss in

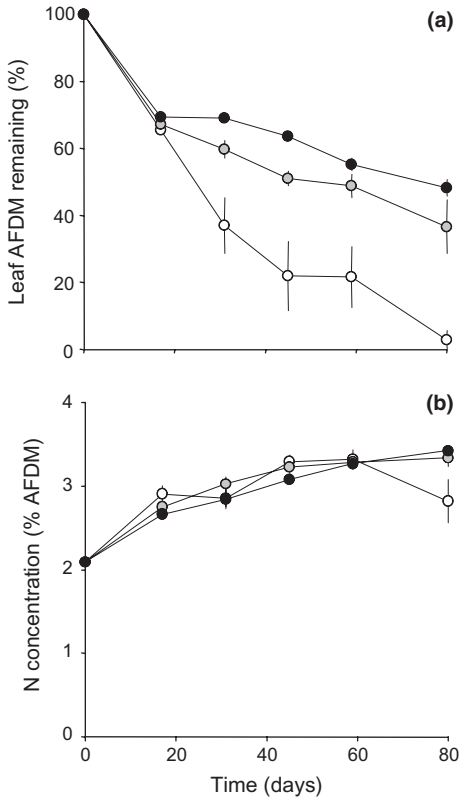

Fig. 1 Remaining ash-free dry mass (a) and nitrogen concentration (b) of alder leaves in the three treatments, hyporheic: buried in the sediment (●), benthic-hyporheic: buried after 2 weeks of exposure at the sediment surface (◉) and benthic: exposure at the sediment surface throughout the experiment (○). Vertical bars indicate ±1 SE.

Table 1 Decomposition rates of alder leaves in the three treatments: benthic (exposure at the sediment surface throughout the experiment), benthic-hyporheic (buried after 2 weeks of exposure at the sediment surface) and hyporheic (buried in the sediment). The 95% confidence limits and the regression coefficient are indicated

Treatment	k (day^{-1})	95% CL	R^2
Hyporheic	0.0103	0.0090–0.0114	0.83
Benthic-hyporheic	0.0138	0.0120–0.0156	0.83
Benthic	0.0292	0.0240–0.0345	0.88

leaf mass after 17 days did not differ between treatments: not only were the benthic (67.5 ± 1.9%, mean ± SE of leaf AFDM remaining) and benthic-hyporheic (69.1 ± 3.0%) samples identical during this initial period but also the hyporheic one was the same (69.8 ± 0.5%; Kruskal–Wallis test; $n = 12$; $P = 0.584$). Clearly, divergences between treatments occurred between d17 and d31, with the differences remaining constant thereafter (Fig. 1a). After 80 days, only 3% of initial leaf AFDM remained for the samples at the sediment surface, which contrasted with the 49% remaining for leaves exposed in the hyporheic habitat. The combined benthic-hyporheic treatment gave an intermediate value (37%). Extrapolation from the decomposition rates indicated that an incubation period of 255 and 341 days would be required for the benthic-hyporheic and hyporheic treatments, respectively, to reach the same decomposition stage as the benthic treatment after 80 days.

Total nitrogen concentrations during the course of leaf decomposition ranged from 2.09 to 3.47%, which corresponded to the initial litter and the last sampling date in the hyporheic treatment, respectively (Fig. 1b). Nitrogen concentrations for all treatments showed constant increases, which, however, seemed to level off at the end of the decomposition. The concentrations were very similar across the treatments (ANOVA, $F_{2,40} = 1.26$, $P = 0.295$).

Leaf-litter-associated microbial respiration

At day 0, microbial consumption of O_2 was 0.063 mg g^{-1} AFDM h^{-1}. Subsequently, respiration rates increased from 0.084 (hyporheic, d17) to 0.462 mg g^{-1} AFDM h^{-1} (benthic, d31; Fig. 2a), reached a maximum and then generally decreased. At any date, oxygen consumption from leaf material in the benthic habitat exceeded that from the hyporheic habitat with the combined benthic-hyporheic treatment showing intermediate values (ANOVA, $F_{2,34} = 9.123$, $P < 10^{-3}$ and HSD test). Microbial O_2 consumption was 1.4 and 1.6 fold higher in the treatment at the sediment surface for the whole experiment compared with those samples buried after 2 weeks of exposure at the sediment surface and those buried in sediment throughout experiment, respectively.

Spore production and fungal diversity

The pattern of leaf-associated sporulation rate of aquatic hyphomycetes was comparable across the treatments, however, with very different dynamics (Fig. 2b). In the former, the sporulation rate showed a sharp increase to a peak of 1.9 conidia μg^{-1} AFDM day^{-1} at d31 followed by a rapid and pronounced

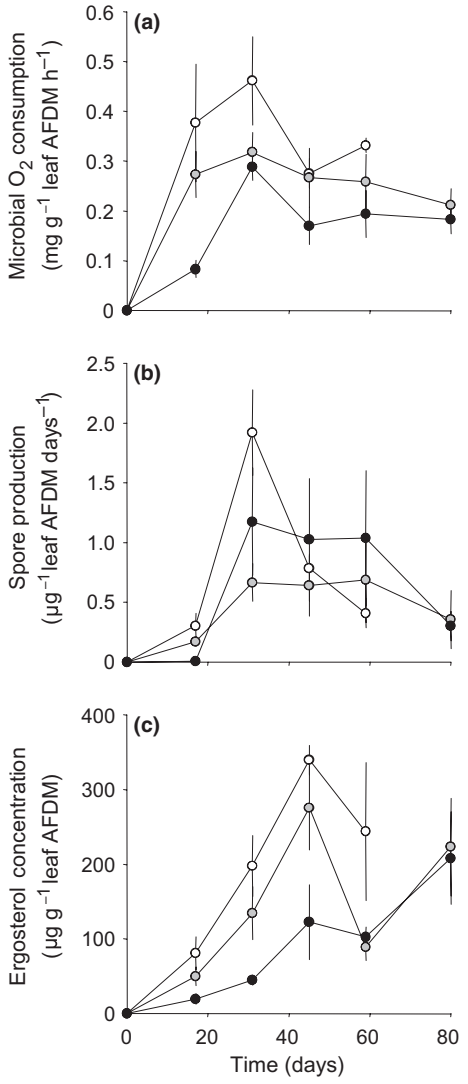

Fig. 2 Microbial respiration (a), sporulation rate of leaf-associated aquatic hyphomycetes (b), and ergosterol concentration in alder leaves (c) in the three treatments, hyporheic: buried in the sediment (●), benthic-hyporheic: buried after 2 weeks of exposure at the sediment surface (◍) and benthic: exposure at the sediment surface throughout the experiment (○). Vertical bars indicate ±1 SE.

decline, whereas the high rates also reached at d31 in the latter treatments were maintained up to the fourth sampling date before declining. The maximum rates were, however, lower in the hyporheic and benthic-hyporheic treatments with values of 1.2 and 0.7 conidia μg^{-1} AFDM day^{-1}, respectively. As a consequence, sporulation rates in the hyporheic habitat were intermediate between those of the two other treatments, which differed from patterns of both microbial respiration and fungal biomass. Owing to the large variations between dates and in spite of the differences in maximum values, sporulation rates were found not to differ significantly across treatments (ANOVA, $F_{2,34} = 1.048$, $P = 0.631$). Finally, because of the sustained sporulation rates over a longer period in the hyporheic treatment, the cumulative conidial production in relation to the initial leaf mass was much higher than in the other two treatments (data not shown, but see *Carbon budgets*).

A total of 23 sporulating species of aquatic hyphomycetes were identified from decomposing leaves with a similar number, i.e. 19 or 20, found in the three exposure treatments (Table 2). However, species richness at any time was generally much lower, i.e. by about 50%, in the permanently hyporheic treatment than in the two other treatments (data not shown). Only very slight differences in species abundance were observed between benthic and benthic-hyporheic treatments (Table 2). Similarly, the species composition of fungal assemblages on the leaves was much more comparable between benthic and benthic-hyporheic treatments than between any of these treatments and the permanently hyporheic one. At the first sampling date (i.e. before burial of leaf material in the benthic-hyporheic treatment), aquatic hyphomycete communities were very similar between benthic and benthic-hyporheic treatments (Steinhaus similarity index = 0.839, $P = 0.003$). In contrast, these two treatments differed markedly from the hyporheic one (Steinhaus similarity index amounting to 0.219 and 0.270 with P values of 0.962 and 0.930, respectively). The similarity between benthic and benthic-hyporheic treatments slowly decreased thereafter, but remained significant until the third sampling date, and was still high at the fourth sampling date although no longer significant (Steinhaus similarity index = 0.70, $P = 0.101$). At this date, the similarity of the hyporheic treatment with the benthic-hyporheic and benthic ones was only 0.48 and 0.37, respectively. The Shannon–Weaver diversity index observed for benthic and benthic-hyporheic treatments was much higher than that for the hyporheic one denoting lower evenness in the latter community (Table 2). The relative abundance of *Flagellospora curvula* conidia was higher in the benthic treatments, where it almost reached half of the overall conidial production (42–46%). Similarly, the abundance of *Tricladium chaetocladium* was one order of magnitude higher in the benthic and benthic-hyporheic treatments than in the

Table 2 Relative abundance (%) of the leaf-associated aquatic hyphomycete species and diversity indices for the three exposure treatments, determined from the cumulative conidial production over the first four sampling dates (four replicate leaf bags combined)

Species	Hyporheic	Benthic-hyporheic	Benthic
Alatospora acuminata Ingold	0.35	0.27	0.37
Alatospora flagellata (Gönczöl) Marvanová	0.09	0.60	0.37
Anguillospora filiformis Greathead	3.78	5.03	4.30
Anguillospora longissima (Sacc. & Syd.) Ingold	0.44	3.16	3.18
Articulospora tetracladia Ingold	33.21	10.35	8.08
Casaresia sphagnorum Gonz. Fragoso	1.62	0.03	0.03
Clavariopsis aquatica De Wild.	0.33	0.54	1.79
Clavatospora longibrachiata (Ingold) Marvanová & Nilsson	1.22	2.50	0.43
Crucella subtilis Marvanová	0.28	0.06	0.30
Culicidospora aquatica Petersen	0.05	1.78	1.69
Flagellospora curvula Ingold	29.79	42.09	46.34
Heliscella stellata (Ingold & Cox) Marvanová & Nilsson	0.46	0.12	0.03
Heliscus lugdunensis Sacc. & Thérry	20.47	5.91	0.65
Lemonniera aquatica De Wild.	1.71	2.93	4.12
Lemonniera cornuta Ranzoni	–	0.04	–
Lemonniera terrestris Tubaki	0.12	0.13	0.23
Mycofalcella calcarata Marvanová, Om-Kalth. & Webster	–	–	0.03
Taeniospora gracilis Marvanová	–	0.03	0.03
Tetrachaetum elegans Ingold	4.12	8.55	9.97
Tricladium chaetocladium Ingold	1.73	15.85	17.99
Tricladium splendens Ingold	–	0.03	0.03
Varicosporium elodeae Kegel	0.18	–	–
Unknown tetraradiate	0.06	–	–
Total number of species	19	20	20
Shannon–Weaver Diversity Index	0.91	1.54	1.53
Shannon–Weaver Evenness Index	0.52	0.67	0.64

hyporheic one (16–18% versus <2%, respectively). The same pattern, although less marked, applied to *Tetrachaetum elegans*. In contrast, *Articulospora tetracladia* was dominant (33%) in the hyporheic treatment, and had a lower abundance (8–10%) in the other two. The greatest difference was found in *Heliscus lugdunensis*, which contributed up to 20% of the overall conidial production in the hyporheic treatment compared to <1% in the benthic one, and an intermediate 6% for the combined treatment.

Fungal biomass

Freshly collected leaves of alder in this study contained minute amounts of ergosterol, indicating that fungal colonisation was negligible at the beginning of the experiment (Fig. 2c). The ergosterol content in the leaf material in the three treatments was significantly different (ANOVA, $F_{2,34} = 19.43$, $P < 10^{-4}$): the benthic treatment had constantly higher values than the combined treatment, which itself was higher than the hyporheic one at the first three sampling dates. However, only the two extreme treatments differed significantly (benthic versus hyporheic, HSD test, $P < 10^{-4}$). The ergosterol content at the sediment surface reached a maximum of 340 μg g^{-1} (i.e. corresponding to a mycelial biomass of 6.2% leaf detrital mass) after 45 days, whereas maxima of 276 and 208 μg g^{-1} (5 and 3.8%) were attained in benthic-hyporheic and hyporheic treatments, respectively. The treatments did not differ, however, with regard to these maximum ergosterol values (ANOVA, $F_{7,40} = 1.387$, $P = 0.238$ and HSD test). The maximum ergosterol content for the hyporheic treatment was delayed by 35 days in comparison with the benthic and benthic-hyporheic treatments, even though the latter also showed a strong increase at the final sampling date (Fig. 2c).

Shredders

The shredder abundance differed significantly between treatments (four last sampling dates; ANOVA, $F_{2,33} = 12.37$, $P < 10^{-4}$) and the HSD test indicated that

the differences were significant for all comparisons except between the two treatments subject to burial. The highest abundance was associated with benthic leaves and the lowest with hyporheic leaves (Fig. 3a, Table 3). A considerable reduction in the abundance on the benthic-hyporheic leaves was observed following burial (d17), with the densities thereafter resembling those on the hyporheic leaves (Fig. 3a). Apart from this, shredder numbers tended to increase gradually over time, except at the last sampling date (d80) for both benthic and benthic-hyporheic treatments, which showed large increases to 200 and 50 individuals g^{-1} leaf AFDM, respectively.

The same although more contrasted patterns were observed with the total shredder biomass (Fig. 3b, Table 3). The benthic-hyporheic leaves showed similarly intermediate values between benthic and hyporheic ones, being lower and higher by factors of 5 and 10, respectively (Table 3). The peak in shredder biomass observed on benthic leaves at d31 was only found in two replicate bags and was mostly attributable to movements of individuals of *Sericostoma* and *Potamophylax*, i.e. the largest dominant shredder taxa in the stream (Table 3). In contrast, *Leuctra* spp. was a strongly dominating taxa associated with hyporheic leaves, in terms of both biomass and numbers. The structure of the shredder community for the benthic-hyporheic treatment was intermediate between the two others, with the three latter taxa together representing 90% of the total shredder biomass.

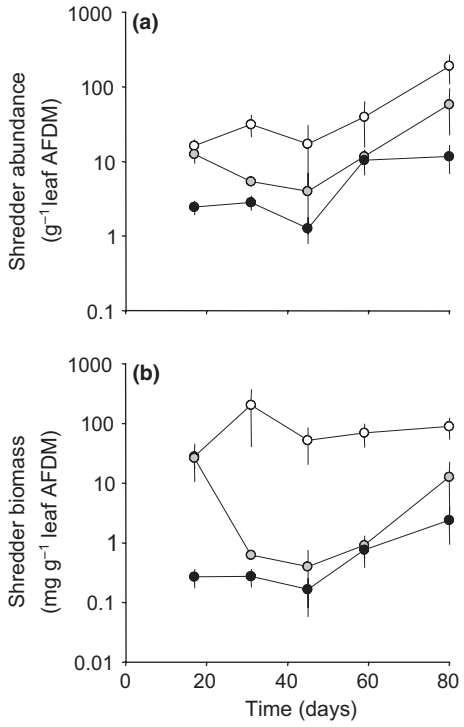

Fig. 3 Total shredder abundance (a) and biomass (b) associated with alder leaves in the three treatments, hyporheic: buried in the sediment (●), benthic-hyporheic: buried after 2 weeks of exposure at the sediment surface (◍) and benthic: exposure at the sediment surface throughout the experiment (○). Note the logarithmic scale. Vertical bars indicate ±1 SE.

The synthetic leaves exhibited the same differences between benthic and hyporheic treatments as natural leaves, although the absolute values were lower by *c*. one order of magnitude (maximum numbers of 13 and 11 individuals, and 29 and 1 mg per leaf bag, respectively). The same patterns were also observed in the community structure.

Carbon budget

The relative contribution of carbon products of leaf decomposition differed substantially among treatments, especially benthic (Fig. 4a) versus the other two (Fig. 4b,c). While shredder biomass represented almost 50% of the initial carbon transformed after 80 days in the benthic treatment, its contribution was <0.3% in the hyporheic one and 2.0% in the combined benthic-hyporheic treatment. In contrast, the cumulative release of microbial C-CO_2 was comparable, with values ranging from 14.3% (hyporheic) and 18.6% (benthic-hyporheic). The maximum mycelial biomass ranged from 2.0 and 2.8% for the hyporheic and combined treatments, respectively. The cumulative conidial production was twice as high in the hyporheic (4.1%) as in the benthic and combined treatments (1.9 and 2.2%, respectively). Overall, the four carbon products represented a total of 21–36% of initial leaf carbon transformed, depending on the treatment (Fig. 4). Table 4 shows that these four carbon products accounted for a total of up to 40% of leaf carbon loss at the time of their maximum contribution (i.e. around the end of the decomposition period). The same, although generally more pronounced, patterns as for the budgets of initial leaf carbon were observed. Microbial CO_2 accounted for as much as 28–30% in the two hyporheic treatments, while the contribution of the shredders consumption was almost as high as that of microbial respiration in the benthic treatment. Mycelial and conidial production in the permanently hyporheic environment accounted for 12% of leaf

Table 3 Relative occurrence (%) and biomass (%) of shredder genera associated with alder leaves in the three treatments, hyporheic: buried in the sediment, benthic-hyporheic: buried after 2 weeks of exposure at the sediment surface and benthic: exposure at the sediment surface throughout the experiment (all sampling dates and replicates combined)

	Abundance			Biomass		
	Hyporheic	Benthic-hyporheic	Benthic	Hyporheic	Benthic-hyporheic	Benthic
Average per bag per date	8.8	18.5	21.7	1.1	12.0	59.5
Plecoptera						
Leuctra	91.4	84.1	32.6	83.9	25.5	3.9
Amphinemura	2.9	5.9	29.8	1.9	1.5	3.0
Nemoura	1.1	3.0	2.3	0.2	0.7	0.2
Protonemura	1.7	1.9	16.6	1.4	1.0	1.1
Trichoptera						
Potamophylax	–	0.3	2.8	–	18.8	25.2
Pseudopsilopteryx	–	0.3	–	–	7.0	-
Limnephilinae	–	0.8	0.9	–	0.2	1.4
Micrasema	0.6	–	–	0.1	–	–
Sericostoma	2.3	3.5	14.1	12.5	45.1	60.0
Others						
Tipula	–	–	0.2	–	–	4.8
Gammarus	–	0.3	0.7	–	0.2	0.4

mass loss, i.e. 2–3 fold more than in the two other conditions.

Discussion

Benthic versus hyporheic litter breakdown rates

Our results indicate that the decomposition of alder leaves was strongly affected by burial in the stream sediment, with rates being 2.8 and 2.1 higher in the benthic habitat than when buried either for the whole period or after 2 weeks of initial exposure at the sediment surface, respectively. The few studies that have compared leaf litter decomposition rates in benthic and hyporheic habitats have provided contradictory results, although most of them have also shown a reduced decomposition in the hyporheic zone. In line with this study, Metzler & Smock (1990) reported very low decomposition rates from leaves of black gum, red maple and water ash buried in the substratum of a first-order Coastal Plain stream with a sand bottom. Likewise, leaves of silver maple and cottonwood were found to decompose 2.7–5.1 faster at the sediment surface than when buried in a headwater stream (Herbst, 1980).

In contrast, Smith & Lake (1993) did not observe significant differences in decomposition rates for *Eucalyptus viminalis* leaves in an Australian stream, and Mayack *et al.* (1989) in South Carolina reported a reduction in decomposition rates of sweet gum in winter but not in spring where decomposition was, on the contrary, increased by burial. Such discrepancies from the literature are probably linked to the substratum and hydrological conditions, together with the biological characteristics of the stream biota, like in the latter study where the activity of tipulid shredders was responsible for the increased decomposition in the spring. Overall, the results support the conclusion that leaf decomposition is generally depressed in the hyporheic zone, which leads to speculation about the causes and mechanisms of such an effect.

Physical constraints

An examination of Fig. 1 reveals lower coefficients of variation in loss of leaf mass, indicating that the decomposition process was more stable over time within rather than above the sediment, and this also holds for leaves buried after a short exposure at the sediment surface. We suspect that the reduced mechanical abrasion in the 'protected' hyporheic habitat is at least partly responsible for the slower decomposition of buried leaves and also explains the reduced variability of leaf mass loss. This effect of current velocity on the breakdown rate of leaf litter has been reported in many situations (e.g. Canton & Martinson, 1990; Chergui & Pattee, 1990; Heard *et al.*,

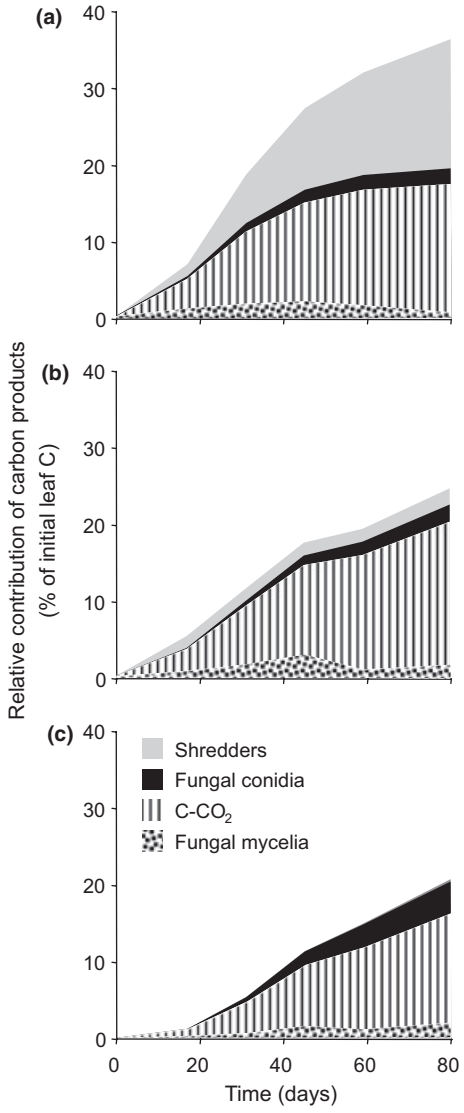

Fig. 4 Changes in the relative contributions of shredders consumption and microbial products to leaf decomposition during decomposition for the three treatments: (a) benthic, (b) benthic-hyporheic and (c) hyporheic. Values are expressed as percentage of initial leaf carbon.

Table 4 Relative contribution (%) of the leaf decomposition carbon products to carbon mass loss, derived from microbial activity and shredders consumption for the three treatments after 59 or 80 days.

	Hyporheic	Benthic-hyporheic	Benthic
Time (days)	80	80	59
Fungal mycelia	3.8	2.4	1.9
Fungal conidia	8.0	3.5	2.2
Microbial CO_2	27.7	29.5	19.2
Shredders	0.5	3.3	17.1

1999), with the study by Ferreira *et al.* (2006) being an exception.

Burial within the substratum can also reduce the access of decomposers to leaves with the small interstices of the gravelly sediment acting as a constraint, especially for the largest shredders. Within a few centimetres, the sediment of the hyporheic zone constitutes a physical barrier, allowing only the taxa with appropriate morphological characteristics (i.e., smaller, narrower, more flexible) to penetrate to the deeper layers (Omesová, Horsák & Helešic 2008). Indeed, shredders were particularly rare in both numbers and biomass in the hyporheic zone, although they were abundant on leaf detritus at the surface, and the only taxa with the appropriate morphology to penetrate the substratum in this study were also the less efficient decomposers (e.g. *Leuctra* spp.).

Oxygen limitation and biotic responses

Not only physical parameters but also the related chemical characteristics such as dissolved oxygen concentration may explain the observed patterns. Indeed, Strommer & Smock (1989) observed a significant correlation between dissolved oxygen concentration and invertebrate abundance, and Strayer *et al.* (1997) found a significant relationship between levels of dissolved oxygen, sediment grain size, organic matter concentration and the density of hyporheic animals. Shredders probably were limited in the subsurface sediments by low oxygen concentrations, unsuitable substratum and a lower palatability of buried versus surface leaves.

Like the shredder biomass, leaf-associated fungal biomass was strongly affected by burial in our study. Crenshaw, Valett & Tank (2002) found a significantly reduced fungal biomass on woody debris decomposing in the hyporheic zone compared to the benthic zone of a headwater mountain stream. The lower dissolved oxygen concentrations in the hyporheic zone probably explained this reduction in fungal biomass. Medeiros, Pascoal & Graça (2009) recently reported a decrease in fungal biomass associated with decomposing alder leaves from 99 to <30 mg g^{-1} leaf AFDM together with a comparable reduction in fungal sporulation rate, at exposures of 94 and 76% of O_2 saturation, respectively. Dissolved oxygen is a limiting factor for biological colonisation and activity in the interstitial habitats, with its depletion leading to

qualitative and quantitative changes in macroinvertebrate and microbial assemblages (Ward *et al*., 1998).

The composition and structure of fungal assemblages were also strongly affected by burial in sediment, which suggests a better adaptation of some aquatic hyphomycete species to the physical and chemical conditions prevailing in the hyporheic zone. *Heliscus lugdunensis* and *A. tetracladia*, for example, seem to be relatively favoured in such conditions, in contrast to *F. curvula* and *T. chaetocladium*. Whether these species respond differently to depletion of oxygen concentrations is currently unknown. However, the initial stage of fungal inoculation was clearly of major importance depending on whether it occurs in the stream flow, i.e. with abundant and highly diverse spores, or in the quantitatively and qualitatively impoverished hyporheic habitat. This led to diverging communities, potentially differing in enzymatic capacities and performance in litter breakdown.

Relative contribution of benthic and hyporheic decomposers

Decomposition rates of leaf litter buried in sediment whether or not initially exposed for 2 weeks at the sediment surface were similar and markedly low compared with the benthic habitat. This observation shows that microbial decomposers did not fully compensate for the lack of shredders, and underlines the fundamental role of macroinvertebrates as mediators of leaf litter processing. The carbon budgets drawn from our study, however, indicated that the relative contributions of shredders and fungi varied considerably with the location within the streambed. While this variation was obviously related to the unconstrained abundance of shredders in the benthic zone, trophic interactions in both habitats were also modified. Shredders are known to act both as competitors and predators of fungi, because of their preferential feeding on leaf patches colonised by fungi, thus reducing microbial activity (Bärlocher, 1980; Suberkropp, 1992, 1998). The lack of shredders in the hyporheic zone virtually suppressed the competition between the two decomposers and allowed fungi to grow and decompose the leaf litter to a higher extent than in the benthic habitat. Over the whole decomposition period, a much higher fungal contribution than shown in Fig. 4 and Table 4 is even expected in the hyporheic habitat. This is discernible when extrapolating data beyond the final sampling date, i.e. at a time where the 50% leaf matter remaining can sustain further fungal growth in contrast to the benthic habitat where the decomposition budget is nearly completed. From Fig. 4 it is thus expected that fungal decomposers in the hyporheic zone compensate, if not fully, at least partly for the action of invertebrate detritivores in the benthic zone. At late decomposition stages, i.e. in spring and summer at a period of high biological activity and limitation of resource on the streambed, the buried leaf litter may also become essential in supporting a portion of the invertebrate community (Mayack *et al*., 1989). In such conditions, we hypothesise the leaves buried after exposure at the sediment surface to be preferentially consumed by shredders in comparison with constantly buried leaves, because of their higher fungal colonisation and palatability. This highlights the fundamental role of the history of leaf litter, i.e. the sequence of events responsible for multiple microbial assemblage scenarios, which in turn can alter leaf litter processing.

Links between benthic and hyporheic food webs

In natural environments, the community structure is in part driven by requirements for the resource, which is distributed heterogeneously in space and time (Bulling *et al*., 2008). However, Strayer *et al*. (1997) suggest that the influence of organic matter depends on, and is sometimes outweighed by, other mechanisms and processes determining the number and composition of co-occurring species in a local environment. For instance, the variability of pore sizes in the three dimensions of the hyporheic zone results in complex fluid distribution patterns (Zilliox, 1994). These spatial complexity and heterogeneity are reflected in the dispersion patterns of invertebrates. Our results lead us to conclude that at this site the food sources, whether leaf litter or fungal biomass, are not as determinant for controlling hyporheic invertebrate distribution as other site-specific factors such as the characteristics of sediment and water physico-chemical parameters. Clearly, the functioning of surface and hyporheic subsystems is interdependent, being linked through hydrological exchange as a transport vector for materials and nutrients (Jones, Fisher & Grimm, 1995). In hyporheic zones with abiotic characteristics of the sediment matrix less

limiting to invertebrates and biotic interactions (e.g. large pore), a greater involvement of macroinvertebrates in leaf litter processing is expected as suggested by the findings of Brunke & Fischer (1999) and Navel *et al.* (2010). Physically, the higher interstitial water flow rates at such sites may allow greater access for invertebrates to migrate to deeper layers. Owing to a higher channel water infiltration and a shorter retention time of hyporheic water, chemical conditions may be less constraining as well.

Extrapolation from decomposition rates indicated a required incubation period of 255 and 341 days for the benthic-hyporheic and hyporheic treatments, respectively, to reach the same decomposition stage as in the benthic treatment after 80 days (i.e. 3% of mass remaining). This suggests that the hyporheic zone of woodland streams is an important compartment for organic matter storage with the release of decomposition products being extended for up to 1 year for fast-decomposing leaf species. Depending on the structure and porosity of the sedimentary matrix, together with the amount, quality and seasonality of litter input, this temporary reservoir may differently affect the overall stream metabolism. Compositional changes in species assemblages, as well as their relative abundance and biomass resulting from limiting dispersal conditions, are likely to affect leaf litter decomposition. In addition to providing organic sources for the stream food web, plant matter decomposing in the hyporheic zone sustains a substantial fungal biomass that can serve as a complementary food source and as a source of fungal inoculum. Fungal conidial production in the hyporheic zone was surprisingly high, but whether this is linked to changes in the metabolic strategy of fungi in adverse conditions is questionable. Nevertheless, it remains likely that the high release of spores by fungal species partly differing from those dominating the benthic habitat constitutes a major trait of hyporheic decomposition of leaf litter, with potential relevance for the overall stream functioning.

Acknowledgments

We are grateful to Verónica Ferreira and Michael Danger for their help in laboratory analyses and discussions, and Sylvain Lamothe and Daniel Dalger for their expert technical assistance. The comments by two anonymous reviewers on an earlier version of the manuscript were most appreciated. This study was funded through the InBioProcess project of the ANR Biodiversity programme (ANR-06-BDIV-007).

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