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# Effects of burial on leaf litter quality, microbial conditioning and palatability to three shredder taxa

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## SUMMARY

1. Heterotrophic microorganisms are crucial for mineralising leaf litter and rendering it more palatable to leaf-shredding invertebrates. A substantial part of leaf litter entering running waters may be buried in the streambed and thus be exposed to the constraining conditions prevailing in the hyporheic zone. The fate of this buried organic matter and particularly the role of microbial conditioning in this habitat remain largely unexplored.

2. The aim of this study was to determine how the location of leaf litter within the streambed (i.e. at the surface or buried), as well as the leaf litter burial history, may affect the leaf-associated aquatic hyphomycete communities and therefore leaf consumption by invertebrate detritivores. We tested the hypotheses that (i) burial of leaf litter would result in lower decomposition rates associated with changes in microbial assemblages compared with leaf litter at the surface and (ii) altered microbial conditioning of buried leaf litter would lead to decreased quality and palatability to their consumers, translating into lower growth rates of detritivores.

3. These hypotheses were tested experimentally in a second-order stream where leaf-associated microbial communities, as well as leaf litter decomposition rates, elemental composition and toughness, were compared across controlled treatments differing by their location within the streambed. We examined the effects of the diverse conditioning treatments on decaying leaf palatability to consumers through feeding trials on three shredder taxa including a freshwater amphipod, of which we also determined the growth rate.

4. Microbial leaf litter decomposition, fungal biomass and sporulation rates were reduced when leaf litter was buried in the hyporheic zone. While the total species richness of fungal assemblages was similar among treatments, the composition of fungal assemblages was affected by leaf litter burial in sediment.

5. Leaf litter burial markedly affected the food quality (especially P content) of leaf material, probably due to the changes in microbial conditioning. Leaf litter palatability to shredders was highest for leaves exposed at the sediment surface and tended to be negatively related to leaf litter toughness and C/P ratio. In addition, burial of leaf litter led to lower amphipod growth rates, which were positively correlated with leaf litter P content.

6. These results emphasise the importance of leaf colonisation by aquatic fungi in the hyporheic zone of headwater streams, where fungal conditioning of leaf litter appears particularly critical for nutrient and energy transfer to higher trophic levels.

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*Keywords:* aquatic fungi, hyporheic zone, leaf litter stoichiometry, organic matter breakdown, shredder growth rate

## Introduction

Low-order forested streams, where light limitation restricts primary production, rely upon the input of plant organic matter from the riparian zone to fuel in-stream processes (Vannote *et al.*, 1980). Therefore, their metabolism is primarily heterotrophic with carbon and energy flow from coarse particulate organic matter (CPOM) to the stream food web being mediated by microorganisms (Bärlocher & Kendrick, 1976; Gessner & Chauvet, 1994; Suberkropp, 1998; Graça, 2001; Hieber & Gessner, 2002). A large quantity of this organic matter input is stored in the hyporheic zone of streams after its burial during storms (Herbst, 1980) or flooding (Metzler & Smock, 1990; Naegeli *et al.*, 1995). Despite the hyporheic zones of streams having long been recognised as sustaining many biotic and abiotic processes (Orghidan, 1959; Williams & Hynes, 1974; Gibert *et al.*, 1990; White, 1993), the specific significance of such processes in the whole stream functioning has long been neglected. Most studies on the dynamics of organic matter in streams have been limited to benthic habitats, with processes occurring above the streambed surfaces (Kaushik & Hynes, 1971; Webster & Benfield, 1986). As underlined by Boulton (2000), these lotic ecosystems were at that time only considered as conducting water, solutes and OM from the catchment downstream, without considering other flow paths above or below the ground where constant exchanges of water and materials occur. The few authors who have attempted to determine the fate of leaf litter once incorporated into stream sediments have generally reported a reduction in rates of leaf litter decomposition (Reice, 1974; Herbst, 1980; Rounick & Winterbourn, 1983; Metzler & Smock, 1990; Naamane, Chergui & Pattee, 1999; Cornut *et al.*, 2010), even though ambiguous or less well-defined patterns have also been observed (Mayack, Thorp & Cothran, 1989; Smith & Lake, 1993). Overall, the ecological significance of leaf litter decomposition in the hyporheic zone for headwater stream metabolism remains poorly documented.

Cornut *et al.* (2010) have recently examined how the location of leaf litter within the streambed, either at the surface or buried, may affect the leaf-associated decomposer communities and leaf litter degradation. Their findings indicate that burial within the substratum reduces the litter breakdown rate by limiting the access

of invertebrate detritivores to leaves, suggesting a potential major role of fungi like aquatic hyphomycetes. Fungal colonisation results in increased leaf litter nutritional value and palatability to shredding invertebrates through fungal biomass and associated changes in leaf tissue because of their enzymatic activity (Rounick & Winterbourn, 1983; Graça, 1993). Shredders seem to prefer feeding on leaves with certain characteristics, such as high nitrogen (N) content, low levels of structural or secondary leaf compounds and a high degree of fungal colonisation (Schlief & Mutz, 2006). The enhancement of leaf detritus food quality by fungi has been shown to affect the life-history traits of shredders such as growth, survivorship and reproduction (Chung & Suberkropp, 2009). Herbst (1980) first reported that burial within the sediment depressed the consumption rates of leaves by shredders compared with those incubated at the streambed surface. This response was suggested to be related to the higher crude protein content of surface-incubated leaves. Other hypotheses have also been proposed, including differences in leaf toughness or the intensity of microbial colonisation. Because of the scarcity of literature on the subject, the role of aquatic hyphomycetes in the conditioning of leaves within the hyporheic zone and its consequences in terms of palatability to shredder and leaf decomposition rate remain largely unknown.

Improvement in the elemental composition of resources through fungal activity is recognised as a main driver of nutrient transfer efficiency in food webs, affecting in turn a wide range of ecological processes (Cebrian, 1999; Sterner & Elser, 2002). Phosphorus (P) and nitrogen (N) are often limiting elements for the growth and activity of microbial decomposers in many ecosystems (Elser *et al.*, 2003) and particularly in streams (Rosemond *et al.*, 2002; Cross *et al.*, 2003). Leaf litter nutrient content has been shown as positively altering shredder consumption rates in headwater streams (Irons, Oswood & Bryant, 1988). Yet, the relationship between leaf litter nutrient contents and growth rates of shredding invertebrate in these ecosystems has been far less examined.

The aim of the present study was to determine how the location of leaf litter within the streambed (i.e. at the surface or buried), as well as the leaf litter burial history, may affect the leaf-associated aquatic hyphomycete communities and leaf consumption by invertebrate detritivores. Burial of leaf litter should result in lower

decomposition rates associated with changes in microbial assemblages compared with leaf litter at the surface. Consequently, altered microbial conditioning of buried leaf litter should lead to decreased quality and palatability to their consumers, translating into lower growth rates of detritivores. We tested experimentally these hypotheses in a second-order stream, where the leaf-associated fungal community, and the leaf decomposition rate, elemental composition, and consumption by three common shredder taxa were compared across four treatments regarding the effects of leaf burial and history of exposition to the benthic and hyporheic zones. Consequences of these treatments on invertebrate growth rate were determined for one amphipod species.

## Methods

### Study sites

The field site was located in the Alzeau, a second-order stream (3.5–4.7 m wide, 0.30 m deep, mean discharge *c.* 0.5 m<sup>3</sup> s<sup>-1</sup>) in the Montagne Noire (south-western France; 02°13'23"E, 43°25'51"N; elevation 743 m a.s.l). The area is surrounded by a mixed broadleaf forest dominated by alder, *Alnus glutinosa* (L.) Gaertn, and oak, *Quercus petraea* (Mattus.) Liebl., and is subject to a mountainous climate. The study was conducted from January to May 2009 in a pool–riffle–pool sequence, extending over *c.* 100 m, similar to those described by Cornut *et al.* (2010). Four representative riffles were selected, each riffle being considered as a replicate site for leaf exposition. To identify downwelling and upwelling areas in the hyporheic zone, vertical hydraulic gradients (VHG) were measured from the equation  $VHG (m m^{-1}) = \Delta h / \Delta l$ , where  $\Delta h = h_{stream} - h_{piezometer}$  and  $\Delta l$  is the distance between the streambed and the top of a Plexiglas minipiezometer screen (Baxter, Hauer & Woessner, 2003). The sediment was unconsolidated and mostly made of coarse particles (35% of particles being superior to 5 mm in diameter). The average hydraulic conductivity through the sediment of the four riffles reached 22 cm h<sup>-1</sup>. Stream water temperatures were recorded every 2 h throughout the experiment using calibrated data loggers (SmartButton, ACR Systems Inc., BC, Canada). Water chemistry was determined on seven dates, at the beginning of the experiment and then on every sampling date. Water sampling was carried out on the four replicate sites, both at the surface of the sediment and within the hyporheic zone at 15 cm below the sediment surface. In the hyporheic zone, water was sampled using a hand-held vacuum pump immersed in a Plexiglas minipiezometer.

Conductivity, pH and dissolved oxygen concentrations were measured in the field using portable instruments (pH-meter 320i and Oxi 330i, WTW, Weilheim, Germany; Conductimeter Dist, HANNA, Woonsocket, RI, U.S.A.). Water samples were filtered in the field using GF/F filters (Glass fibre filters, Whatman, Clifton, NJ, U.S.A.; nominal cut-off 0.7 µm) and stored at 4 °C in pre-rinsed polyethylene bottles until analyses in the laboratory. Concentrations of PO<sub>4</sub>-P, measured as soluble reactive phosphorous (SRP), and NO<sub>3</sub>-N were determined using standard colorimetric methods (AFNOR, 1990).

### Leaf litter conditioning and mass loss

Oak was selected as a slow decomposing leaf species and with a particularly low palatability in the absence of microbial colonisation. Oak leaves were collected at abscission using a suspended net in the riparian zone of the Alzeau river in autumn 2008. About 2.5 g (±0.02 g) of air-dried leaves was enclosed in fine (0.5-mm) nylon mesh bags, 10 × 10 cm sized. The fine mesh excluded most invertebrates without interfering with microbial colonisation (Boulton & Boon, 1991).

A total of 144 litter bags were introduced into the stream in mid-January 2009, following four conditions that simulate different scenarios after leaf litter introduction into a stream: (i) strictly benthic (B), that is, leaf litter exposed at the surface of the sediment throughout the experiment; (ii) benthic–hyporheic (BH), that is, leaf litter exposed at the surface of the sediment for 19 days allowing inoculation by and initial development of benthic microorganisms, then buried into the sediment until the end of the experiment; (iii) hyporheic–benthic (HB), that is, leaf litter directly buried into the sediment, then exposed at the surface of the sediment after 19 days; and (iv) strictly hyporheic (H), that is, leaf litter buried in the sediment throughout the experiment. Hyporheic leaf bags were buried at 15–20 cm depth into the sediment using a small shovel. In the selected stream, some shredder taxa (mainly *Leuctra* spp. and to a lesser extent *Sericostoma*) were found to occur at this depth and thus be able to access buried leaf litter and be directly affected by differences in leaf litter palatability (Cornut *et al.*, 2010). The experiment lasted 117 days, and one litter bag of each treatment was randomly sampled from each of the four replicate riffles at six dates (after 7, 19, 35, 56, 82 and 117 days) to follow leaf conditioning and decomposition. In addition, after 35, 56 and 82 days, one supplementary litter bag was randomly sampled for each treatment from each of the four riffles to determine the leaf palatability to three shredder taxa. Each sampled litter bag was stored

individually in plastic zip-lock bag and brought back to the laboratory at 4 °C. Then, leaves were quickly washed individually to remove small sediments until processing for diverse analyses and consumption measurements. For each sample, a set of 12 leaf discs of 12 mm diameter was cut from which five discs were stored at -18 °C until ergosterol determinations and seven used to quantify the spore production by aquatic hyphomycetes. The remaining leaf material was stored at -18 °C until being freeze-dried and weighted. It was then ground using a bead-grinder (Culatti, Zurich, Switzerland), and aliquots of 300–400 mg were combusted at 450 °C for 5 h to estimate leaf ash-free dry mass (AFDM). Leaf litter decomposition rate was determined from ratios of remaining to initial leaf AFDM.

#### *Microbial colonisation of leaves and litter quality*

At each sampling date, several parameters were followed to estimate the leaf litter microbial colonisation and quality for consumers. To estimate the fungal diversity in leaves, seven freshly cut leaf discs were quickly placed in glass Petri dishes filled with 20 mL of pre-filtered (GF/F, Whatman, nominal cut-off: 0.7 µm) stream water. Dishes were gently agitated in the dark on an orbital shaker (60 rpm, 25.4-mm orbital path) at 10 °C for 48 h to stimulate the production of fungal asexual spores. Thereafter, spore suspensions were transferred into 50-mL polyethylene tubes, the Petri dish and leaf 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 leaf discs were then freeze-dried and weighed to the nearest 0.1 mg. Triton X-100 (0.5 mL of 0.5% solution) was added to the spore suspensions, which were stirred gently to ensure the uniform distribution of spores. Aliquots of 5 mL were then filtered through a membrane filter (SMWP, 25 mm diameter, pore size 5 µm; Millipore, Bedford, MA, U.S.A.), and the spores on the filter were stained with 0.1% Trypan blue in 60% lactic acid. Spores were counted and identified under the microscope (×200), according to Gulis, Marvanová & Descals (2004). Spore production was calculated as the number of spores released per g leaf AFDM per d. The Shannon diversity index ( $H'$ ) and the Shannon evenness index ( $E_{H'}$ ) were computed from the cumulative spore production after 19, 35, 56 and 82 days of exposure for fungal communities associated with each of the four treatments.

Fungal biomass in leaf litter was quantified using ergosterol content as an estimator of mycelial biomass (Gessner & Chauvet, 1993). The five frozen leaf discs were

freeze-dried, weighed to the nearest 0.1 mg and heated at 80 °C for 30 min with alkaline methanol to extract the lipids. Extracts were purified using solid-phase extraction cartridges (Oasis HLB, 60 mg, 3 cc; Waters, Milford, MA, U.S.A.), then quantified by high-performance liquid chromatography. Ergosterol content was then converted to mycelium biomass ( $\text{mg g}^{-1}$  AFDM of litter), assuming that 5.5 mg of ergosterol represents 1 g of fungal dry matter (Gessner & Chauvet, 1993).

To describe oak litter quality to shredding invertebrates, two parameters were measured: leaf litter toughness and elemental composition. Leaf toughness is an important parameter for leaf litter consumption by invertebrates (Arsuffi & Suberkropp, 1984), particularly in low palatability species such as oak, and was estimated by measuring the force needed to penetrate leaf samples using a penetrometer similar to that described in the study by Graça, Bärlocher & Gessner (2005). The punch (diameter: 1.55 mm) was placed in the centre of an area bounded by the leaf veins. Water was loaded on a piston until the punch broke through the leaf. The mass (mg) of water necessary to break through the leaf was converted into penetration pressure (kPa) and then used as a measure of leaf toughness. For each treatment replicate, leaf toughness corresponded to the mean of 15 measurements on at least five distinct leaves. Finally, leaf litter elemental ratios were chosen as good estimators of detrital organic matter decomposability (Enriquez, Duarte & Sand-Jensen, 1993) and essential parameters affecting consumer life-history traits (Sterner & Elser, 2002). To estimate the leaf litter elemental quality for consumers, C, N and P contents were measured at each sampling date. Analyses were carried out on previously ground material. The percentages of C and N contained in leaf litter were determined using a CHN elemental analyzer (NA 1500 Series 2; Fisons, Manchester, U.K.). Total P content was determined after mineralisation by alkaline oxidation with sodium persulphate (Danger *et al.*, 2008). All elemental ratios were expressed as molar ratios.

#### *Invertebrate feeding rates*

To investigate the effect of leaf litter burial in the hyporheic zone of streams on its palatability to invertebrates, we chose three distinct representative shredder taxa of Western European headwater streams. The crustacean *Gammarus fossarum* Koch is widespread and common in headwater streams, often playing a major role in leaf litter breakdown (Felten *et al.*, 2008). While present at the surface of the sediment, this species was

never found in the hyporheic zone of the studied stream (Cornut *et al.*, 2010). *Sericostoma personatum* Kirby & Spence is the most common trichopteran present in the studied stream, and small individuals are able to access buried leaf litter (Cornut *et al.*, 2010). Finally, the plecopterans *Leuctra* spp. are among the most efficient taxa at penetrating the hyporheic zone thanks to their body morphology, and have access to buried organic matter (Cornut *et al.*, 2010).

Leaf litter palatability to selected shredder taxa was determined through short-term non-choice feeding assays (Elger & Lemoine, 2005) after 35, 56 and 82 days of leaf litter conditioning in the stream. Individuals of the three invertebrate taxa were acclimatised in the laboratory for at least 7 days at 10 °C before the experiments and supplied *ad libitum* with plant detritus from the stream. The experiments were carried out in plastic containers (125 mL), filled with 60 mL of filtered stream water (GF/C filters, Whatman, nominal cut-off 1.2 µm), at 10 °C. For *G. fossarum* and *S. personatum*, tests were performed individually, whereas for *Leuctra* spp., relatively small species compared with the two others, five individuals were used for each trial to limit the duration of the test and consequently respect the conditions necessary for non-choice palatability measurements (Elger & Lemoine, 2005). For each taxon, individuals were sorted out by size class (dry mass ± SD: 6.8 ± 0.7 mg for *G. fossarum*, 0.59 ± 0.07 mg for *Leuctra* spp., and 13.4 ± 2.3 mg for *S. personatum*). Three pairs of 10-mm leaf discs were cut from the same leaf, that is, one pair for each taxon. All leaf discs were weighted to the nearest 0.01 mg after being quickly dried on a clean absorbent paper. For each invertebrate taxon, one leaf disc was available as food on the bottom of the container and the second one (control) was enclosed in a small fine-mesh (250-µm) bag attached to the container's edge. The length of palatability test was adjusted to reach *c.* 50% of mass loss in at least one treatment, that is, 11 h for *S. personatum*, from 45 to 48 h for *Leuctra* spp. and from 68 to 72 h for *G. fossarum*. Then, unconsumed and control leaf discs as well as invertebrates were dried at 65 °C to constant mass and weighed to the nearest 0.01 mg. Dry mass (DM) of the leaf disc available as food for consumers was estimated from its initial fresh mass multiplied by the ratio of DM to fresh mass of the control leaf disc. Relative consumption rates were calculated as the DM consumed divided by the DM of consumers and by the time of the consumption experiment. Relative consumption rates were expressed as mg mg<sup>-1</sup> consumer per day. Consumption rates were measured independently for each

invertebrate taxon four times per treatment per replicate riffle (i.e. 16 measurements per treatment per date) and then averaged by riffle to avoid pseudoreplication (four independent data per treatment per date).

#### *Individual consumer growth rates*

To test for the effects of leaf litter conditioning treatments on consumer life-history traits, we carried out individual growth measurements on males of *G. fossarum* fed with leaf litter subject to these treatments. This crustacean species was preferred to the two other taxa, owing to its robustness for relatively long-term experiments. As for palatability measurements, *G. fossarum* were collected in the field and acclimatised in the laboratory for at least 7 days at 10 °C, then sorted by size and put into clear water without food 48 h before starting the experiments. We verified that organisms did not differ significantly between treatments and dates at the beginning of the experiment (15.6 ± 1.8 mg wet mass). To test for the effect of the duration of leaf litter conditioning on the different treatments, the experiment was carried out at two sampling dates, after 35 and 82 days of conditioning. At these sampling dates, 12-mm leaf discs were cut. To limit any alteration in leaf litter quality during the 3-week experiment, leaf discs were immediately frozen at -18 °C before their distribution to consumers. Males of *G. fossarum* were put individually in 125-mL containers filled with 60 mL of filtered stream water. Organisms were fed *ad libitum* with two leaf discs. Every second day, leaf discs were recovered and replaced by new leaf discs and water was changed and replaced by filtered aerated stream water. Experiments were carried out at 10 °C. All organisms were weighed at the beginning and at the end of the experiment to the nearest 0.01 mg after being quickly drained on clean absorbent paper. As for palatability tests, each measurement was conducted on sixteen samples, that is, four per riffle, which were averaged to obtain a mean value for each treatment and replicate riffle. One individual died on the first day of the experiment with 35-days litter and was immediately replaced. *G. fossarum* growth was calculated as the percentage of the difference between final and initial mass against initial mass.

#### *Statistical analyses*

Leaf litter decomposition rates (*k*) were calculated using the exponential decay model as follows:  $M_t = M_0 \cdot e^{-kt}$ , where  $M_0$  represents the initial AFDM,  $M_t$  the remain-

ing AFDM at time  $t$  and  $t$  the time in  $d$  spent since the beginning of the experiment. Analysis of covariance (ANCOVA) made on logarithmically transformed AFDM data was used to test for the effects of burial treatment on leaf litter decomposition. Differences between physicochemical conditions in hyporheic and benthic zones of the stream were tested using a two-way ANOVA, with time and location as categorical predictors. For all measured parameters (leaf litter toughness, elemental quality, fungal biomass, aquatic hyphomycete sporulation rates, leaf litter consumption rate and *G. fossarum* growth rate), the effects of burial treatment, time and their potential interactions were examined using two-way ANOVAs. Data were log-transformed to improve the homoscedasticity when necessary. Because of systematic differences in physicochemical conditions in one of the four replicate riffles, and to correct for this potential bias, this parameter was introduced in all analyses as a block effect. In fact, this parameter was only shown as significantly affecting the P content of buried leaf litter and was thus removed from the other analyses. For all analyses, multiple comparisons were made using Tukey's HSD test. STATISTICA 6.0 (StatSoft Inc, 2001) was used for all statistical analyses cited above. 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). For all analyses, a significance threshold of  $P = 0.05$  was chosen.

## Results

### *Physicochemical conditions in benthic and hyporheic zones*

All physicochemical parameters measured throughout the experiment differed significantly between the hyporheic zone and the benthic zone of the river (Table 1). Oxygen was significantly lower in the hyporheic than in the benthic zone, showing a reduction by *c.* 40%. Conductivity and P content of interstitial water were systematically higher than those of surface water. Temperature was on average 0.4 °C higher in the hyporheic compartment. This result can be related to the fact that one of the four riffles showed higher temperature of interstitial water during the first 80 days of the experiment and less variation in temperature throughout the experiment than other riffles. This riffle was characterised by a positive VHG, indicating an upwelling-type functioning (discharge from the interstitial zone into surface water), whereas the three other

riffles, which showed negative VHG throughout the experiment, were downwelling zones.

### *Leaf litter decomposition and conditioning*

There was a marked variation in the decomposition of oak leaves as a function of burial treatment (Table 2). In all treatments, leaves lost up to 10% of their mass during the first 7 days of the experiment. Then, decomposition followed a classical exponential pattern (data not shown), which differed among burial treatments. Leaf litter decomposition was significantly faster when exposed in the benthic zone of the stream, intermediate in the HB treatment and the lowest in the H and BH treatments (Table 2).

Mycelial development of aquatic fungi, as measured by ergosterol content, was significantly affected by burial treatment ( $F_{15,72} = 1.87$ ,  $P = 0.041$ ). Fungal biomass increased quickly to a first plateau during the first 20 days (Fig. 1), this plateau being independent of burial treatment. After 60 days, fungal biomass reached a second plateau, this one being significantly higher (by more than 30%) in B than in the three other treatments.

The pattern of spore production of leaf-associated aquatic hyphomycetes was quite similar between treatments, increasing throughout the experiment (Fig. 2). However, the sporulation rate in treatment B showed a sharp increase to reach a peak of 195 spores  $\text{mg}^{-1}$  AFDM  $\text{d}^{-1}$  at day 82, whereas maximal values of sporulation rate were lower in the BH, H and HB treatments. As a consequence, sporulation rates in the B treatment were higher than those of the three other treatments ( $F_{3,56} = 5.73$ ,  $P = 0.002$ ).

A total of 30 sporulating species of aquatic hyphomycete were identified from decomposing leaves with a quite similar number (19–24) found in the four exposure treatments (Table 3). Considering the heterogeneity of fungal assemblages, no single species was shown as significantly differing between treatments. However, similarity analyses carried out on communities showed that aquatic hyphomycete communities were relatively similar between B and BH treatments (Steinhaus similarity index = 0.654) at day 19, the time of burial or removal of leaf material from sediment. In contrast, these two treatments differed markedly from those subject to an initial exposition to the hyporheic zone. Steinhaus similarity index at this date ranged from 0.022 to 0.080 between the four treatments. The similarity between B and HB treatments increased throughout the experiment to reach its maximum with a Steinhaus similarity index of 0.868 at day 82. At this date, the similarity of the H treatment with the HB and B ones had

**Table 1** Physical and chemical characteristics of the benthic and hyporheic zones of the stream. Values are means  $\pm$  SE of data collected during the experiment (from January to May 2009)

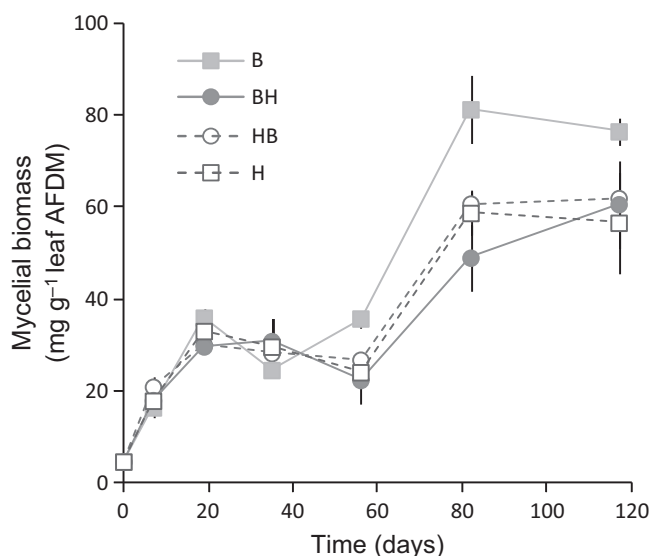
Variable	Benthic	Hyporheic	<i>P</i>
O <sub>2</sub> (% saturation)	104.9 $\pm$ 0.2 (101–107)	61.4 $\pm$ 17.7 (39–95)	<10 <sup>-4</sup>
pH	6.2 $\pm$ 0.1 (5.6–6.6)	5.9 $\pm$ 0.1 (5.0–6.6)	<10 <sup>-4</sup>
Conductivity ( $\mu$ S cm <sup>-1</sup> )	27.4 $\pm$ 0.9 (14–35)	35.3 $\pm$ 2.5 (22–46)	<10 <sup>-4</sup>
Temperature ( $^{\circ}$ C)	6.2 $\pm$ 0.2 (4.0–10.4)	6.6 $\pm$ 0.5 (4.1–10.4)	0.011
NO <sub>3</sub> -N (mg L <sup>-1</sup> )	0.91 $\pm$ 0.02 (0.71–1.09)	0.83 $\pm$ 0.09 (0.52–1.01)	0.005
SRP ( $\mu$ g L <sup>-1</sup> )	1.55 $\pm$ 0.23 (0.45–5.07)	9.61 $\pm$ 1.89 (3.98–19.62)	<10 <sup>-4</sup>

Numbers in parentheses represent minimal and maximal values recorded. *P*-values indicate the significance of differences between average determinations in the two stream compartments.

**Table 2** Daily exponential decay rates (*k*) of oak leaves as a function of exposure treatment: B, leaf litter exposed at the surface of the sediment throughout the experiment; BH, leaf litter exposed for 3 weeks at the surface of the sediment before being buried in the hyporheic zone; HB, leaf litter exposed for 3 weeks in the hyporheic zone before being exposed at the surface of the sediment; H, leaf litter exposed in the hyporheic zone throughout the experiment

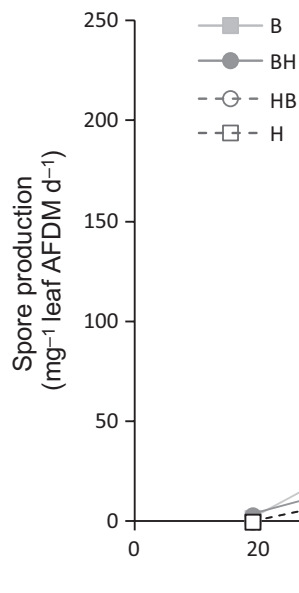
Treatment	<i>k</i> (d <sup>-1</sup> )	<i>r</i> <sup>2</sup>	<i>T</i> <sub>50</sub> (d)
Benthic (B)	0.0036 <sup>a</sup>	0.95	191
Benthic–Hyporheic (BH)	0.0029 <sup>b</sup>	0.88	240
Hyporheic–Benthic (HB)	0.0031 <sup>ab</sup>	0.91	221
Hyporheic (H)	0.0029 <sup>b</sup>	0.87	242

Different letters represent significantly different treatments. Regression coefficients and time to reach 50% of leaf mass loss are also indicated.



**Fig. 1** Changes in mycelial biomass (derived from ergosterol contents) in oak litter as a function of exposure treatment in the benthic and hyporheic zones of the stream. Legends of treatments are the same as in Table 2. Vertical bars correspond to  $\pm 1$  SE.

also increased, but was only 0.438 and 0.452, respectively. The Shannon diversity indices observed for H and HB were higher than those for the two other treatments, whereas



**Fig. 2** Sporulation rates of leaf-associated aquatic hyphomycetes as a function of exposure treatment in the hyporheic zone of the stream. Legends of treatments are the same as in Table 2. Vertical bars correspond to  $\pm 1$  SE.

evenness values across the four treatments were quite similar (Table 3).

#### Leaf litter quality to consumers

Oak leaf litter toughness (Fig. 3) decreased continuously during the experiment ( $F_{3,48} = 44.4$ ,  $P < 10^{-4}$ ), but was on average 20% lower in the benthic-exposed leaves than in the three other treatments ( $F_{3,48} = 7.6$ ,  $P < 10^{-3}$ ).

Both C/P and C/N ratios (Fig. 4) of leaf litter exposed in the stream decreased significantly throughout the experiment ( $F_{5,69} = 19.4$ ,  $P < 10^{-4}$  and  $F_{5,72} = 9.76$ ,  $P < 10^{-4}$ , respectively). In contrast, leaf litter N/P ratio was constant during the experiment (time:  $F_{5,72} = 0.82$ ,  $P = 0.535$ ; interaction time  $\times$  treatment:  $F_{15,72} = 0.15$ ,  $P = 0.999$ ). Leaf litter C/N and N/P ratios were not significantly affected by the exposure treatment ( $F_{3,72} = 0.52$ ,  $P = 0.663$  and  $F_{3,72} = 0.42$ ,

**Table 3** Relative abundance (%) of the leaf-associated aquatic hyphomycete species and diversity indices determined from the cumulative spore production after 19, 35, 56 and 82 days of exposure for the four exposure treatments (four replicate leaf bags combined)

Species	B	Benthic–hyporheic	Hyporheic–benthic	H
<i>Alatospora acuminata</i> Ingold	2.13	1.02	1.06	2.33
<i>Anguillospora filiformis</i> Greathead	0.18	0.83	0.03	0.10
<i>Anguillospora furtiva</i> Descals & Marvanová	0.72	–	0.04	–
<i>Anguillospora longissima</i> (Sacc. & Syd.) Ingold	1.17	0.99	0.18	–
<i>Articulospora tetracladia</i> Ingold	0.18	1.52	5.14	1.29
<i>Casaresia sphagnorum</i> Gonz. Fragoso	0.13	–	–	–
<i>Clavariopsis aquatica</i> De Wild.	1.59	1.66	0.22	3.50
<i>Clavatospora longibrachiata</i> (Ingold) Marvanová & Nilsson	2.20	0.46	7.89	12.39
<i>Culicidospora aquatica</i> Petersen	1.45	1.33	1.86	0.75
<i>Dendrospora</i> sp.	–	–	0.10	–
<i>Flagellospora curvula</i> Ingold	21.33	26.10	7.93	22.92
<i>Fontanospora alternibrachiata</i> Dyko	0.05	0.03	6.35	–
<i>Geniculospora inflata</i> (Ingold) Sv. Nilsson ex Marvanová & Sv. Nilsson	0.15	–	–	–
<i>Heliscella stellata</i> (Ingold & Cox) Marvanová & Nilsson	0.03	–	0.23	5.45
<i>Heliscus lugdunensis</i> Sacc. & Théry	2.70	6.43	9.29	1.57
<i>Lemonniera aquatica</i> De Wild.	3.72	3.32	0.20	0.16
<i>Lemonniera cornuta</i> Ranzoni	–	–	3.40	1.21
<i>Lemonniera terrestris</i> Tubaki	–	0.03	–	0.01
<i>Mycocentrospora</i> sp.1 cf. <i>angulata</i> Petersen	44.14	26.27	35.20	21.24
<i>Mycocentrospora</i> sp.2	–	–	–	0.02
<i>Mycofalcella calcarata</i> Marvanová, Om-Kalth. & Webster	0.16	–	0.33	–
<i>Taeniospora gracilis</i> Marvanová	13.17	19.73	7.85	9.54
<i>Tetrachaetum elegans</i> Ingold	0.13	0.04	0.04	–
<i>Tricladium chaetocladium</i> Ingold	3.99	10.05	1.30	2.33
<i>Tricladium splendens</i> Ingold	–	–	–	0.05
<i>Tripospermum camelopardus</i> Ingold, Dann & P.J. McDougall	–	0.05	0.05	0.61
<i>Tripospermum myrti</i> (Lind) S. Hughes	0.66	0.08	0.15	11.52
<i>Tumularia aquatica</i> (Ingold) Descals & Marvanová	–	–	–	0.57
Sigmoid (<60 µm)	–	0.05	3.33	–
Sigmoid (60–120 µm)	–	–	7.86	2.42
Total number of species	21	19	24	21
Shannon Diversity Index	1.01	0.86	1.19	1.16
Shannon Evenness Index	0.60	0.60	0.66	0.59

Legends of treatments are the same as in Table 2.

$P = 0.737$ , respectively), whereas leaf litter C/P ratio was significantly lower in B than in the three other treatments ( $F_{3,69} = 2.76$ ,  $P = 0.048$ ). C/N and C/P ratios were negatively correlated with the mycelial content of the leaf ( $r^2 = 0.34$ ,  $n = 96$ ,  $P < 10^{-4}$  and  $r^2 = 0.49$ ,  $n = 96$ ,  $P < 10^{-4}$ , respectively). In addition, leaf litter toughness was also negatively correlated with the mycelial content of the leaf ( $r^2 = 0.54$ ,  $n = 80$ ,  $P < 10^{-4}$ ).

#### Feeding rate of invertebrates

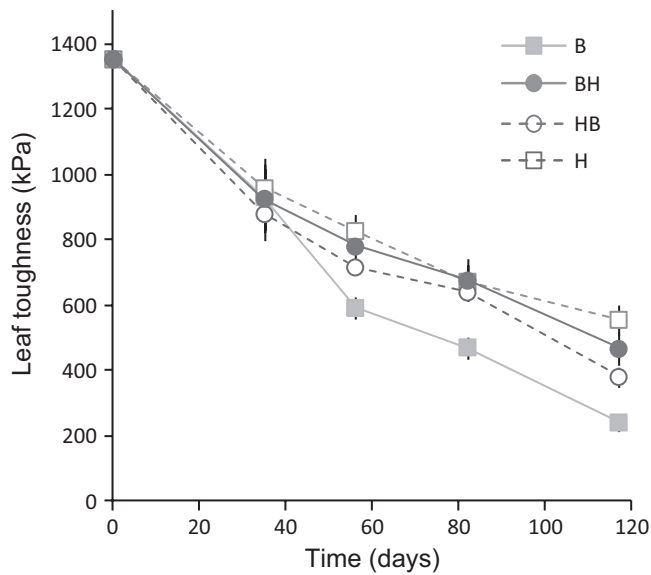
Leaf litter palatability, as measured by short-term consumption rates of a leaf disc, differed as a function of the duration of the exposure and the type of consumer tested (Fig. 5).

Leaf litter consumption rate by *G. fossarum* significantly increased with the length of conditioning in the stream

( $F_{2,36} = 17.7$ ,  $P < 10^{-4}$ ). Consumption rates of leaf litter by *S. personatum* were slightly higher after 56 days of exposure in the stream than for the two other conditioning durations tested ( $F_{2,36} = 5.06$ ,  $P = 0.011$ ). In contrast, consumption rates of leaf litter by the plecopteran *Leuctra* were unaffected by the duration of leaf litter conditioning ( $F_{2,36} = 0.78$ ,  $P = 0.465$ ).

For the three invertebrates tested, leaf litter exposed in the benthic zone of the stream was on average consumed more than leaf litter exposed in the hyporheic zone throughout the experiment ( $F_{3,36} = 3.69$ ,  $P = 0.021$ ;  $F_{3,36} = 3.20$ ,  $P = 0.035$ ; and  $F_{3,36} = 3.44$ ,  $P = 0.027$  for *G. fossarum*, *S. personatum* and *Leuctra*, respectively).

Leaf litter palatability to *S. personatum* was significantly related to leaf litter C/P ratio ( $r^2 = 0.14$ ,  $n = 48$ ,  $P = 0.009$ ). Leaf litter palatability to *Leuctra* was slightly negatively related to leaf litter toughness ( $r^2 = 0.09$ ,  $n = 48$ ,  $P = 0.041$ ).



**Fig. 3** Leaf litter toughness as a function of exposure treatment. Legends of treatments are the same as in Table 2. Vertical bars correspond to  $\pm 1$  SE.

and C/P ratio ( $r^2 = 0.09$ ,  $n = 48$ ,  $P = 0.037$ ). Finally, leaf litter palatability to *G. fossarum* was more strongly, negatively related to leaf litter toughness ( $r^2 = 0.31$ ,  $n = 48$ ,  $P < 10^{-4}$ ) and C/P ratio ( $r^2 = 0.13$ ,  $n = 48$ ,  $P = 0.011$ ) and positively related to the mycelial content ( $r^2 = 0.31$ ,  $n = 48$ ,  $P < 10^{-4}$ ).

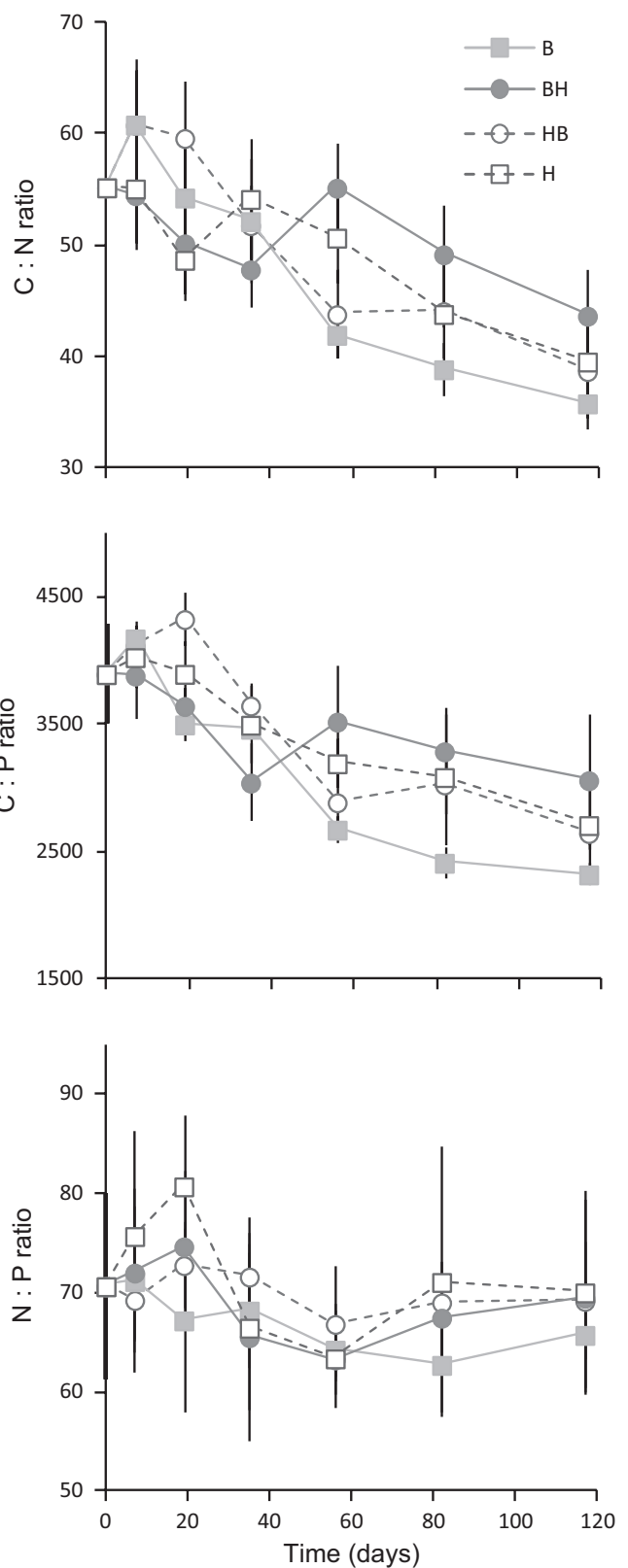
#### Effect of leaf litter burial on consumer growth rates

Individual growth rates of *G. fossarum* showed a significant effect of leaf litter exposure treatment ( $F_{1,27} = 3.53$ ,  $P = 0.028$ , Fig. 6). Growth rates of *G. fossarum* did not differ significantly when fed with leaf litter conditioned for 35 and 82 days in the stream ( $F_{1,24} = 2.37$ ,  $P = 0.135$ ). However, there were significantly higher growth rates when fed with leaves from B than from the H treatment. *G. fossarum* growth rate was neither correlated with leaf litter toughness nor correlated with palatability. However, it was negatively related to leaf litter C/P ratio ( $r^2 = 0.13$ ,  $n = 32$ ,  $P = 0.046$ ).

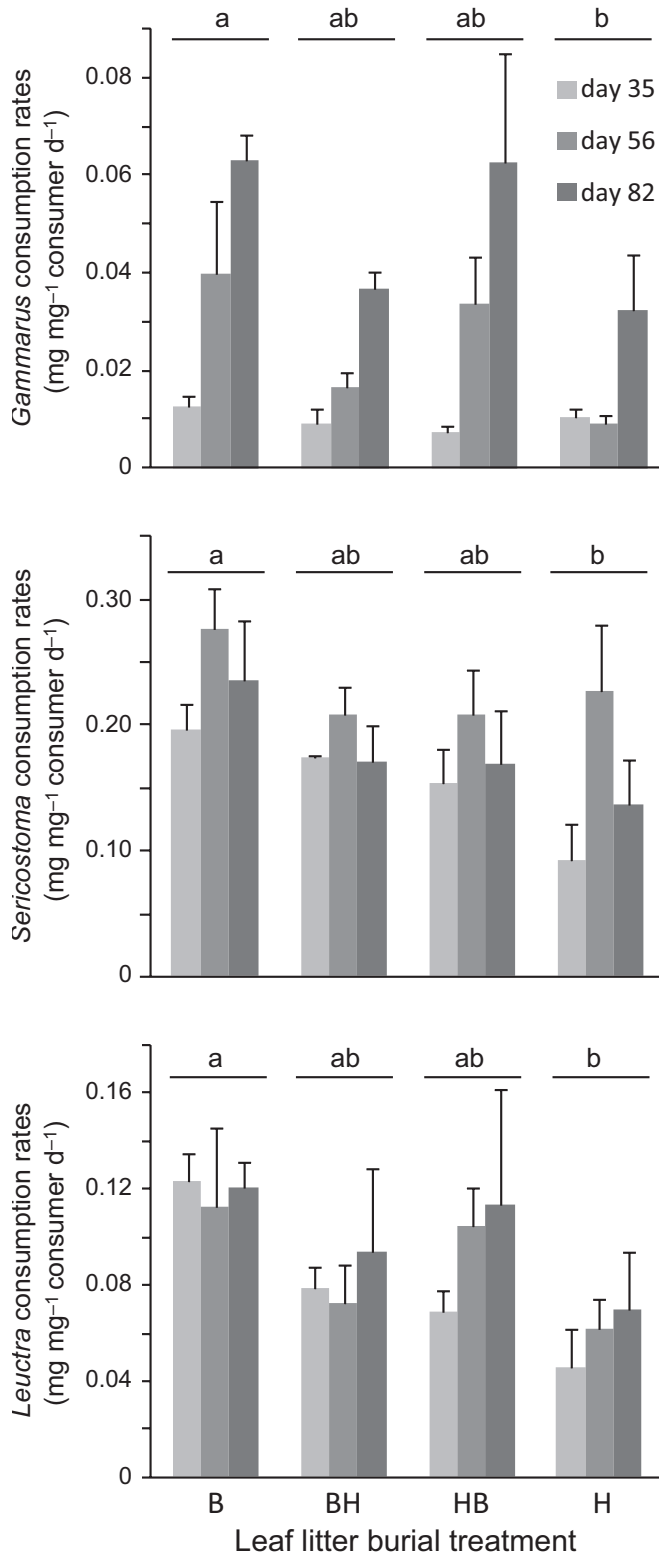
## Discussion

### Leaf litter decomposition and fungal communities

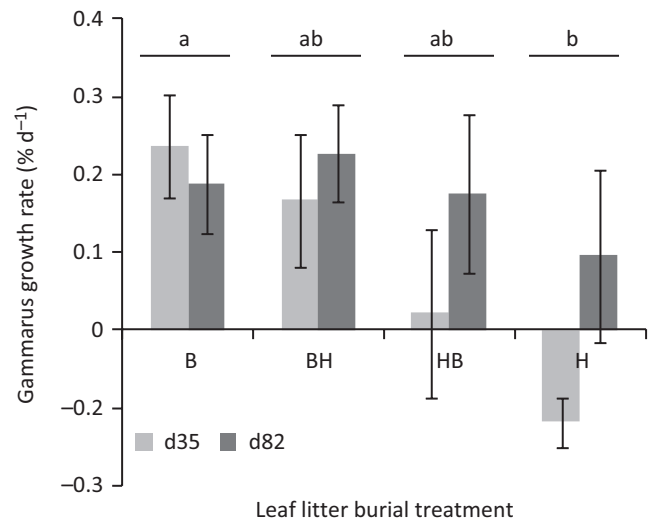
Our results indicate that leaf litter decomposition by microorganisms is significantly reduced in the hyporheic zone compared with the benthic zone of headwater streams. This result is in contradiction with the only other study comparing litter decomposition in fine-mesh bags between hyporheic and benthic zones of streams



**Fig. 4** Leaf litter elemental ratios as a function of exposure treatment. Elemental ratios are molar ratios. Legends of treatments are the same as in Table 2. Vertical bars correspond to  $\pm 1$  SE.



**Fig. 5** Leaf litter palatability to three taxa of benthic invertebrate consumers: *Gammarus fossarum* (Crustacea), *Sericostoma personatum* (Trichoptera) and *Leuctra* spp. (Plecoptera), as a function of leaf litter exposure treatment. Leaf litter palatability measurements were taken on leaf litter exposed for 35, 56 and 82 days in the different treatment conditions. Legends of treatments are the same as in Table 2. Vertical bars correspond to  $\pm 1$  SE.



**Fig. 6** Effects of leaf litter burial on *Gammarus fossarum* growth rates during 3-week experiments. Values are expressed as percentages of initial mass. Growth rates were determined for animals fed with 35- and 82-day-old leaf material. Legends of treatments are the same as in Table 2. Vertical bars correspond to  $\pm 1$  SE.

(Rounick & Winterbourn, 1983), where decomposition patterns were found not to differ. Interestingly, we found no differences in mycelial biomass or in decomposition rates between leaves that were subject to a burial stage whatever its duration or timing (i.e. H, BH or HB), with both variables being *c.* 20% lower than in the benthic zone at the end of the exposition period. The spore production, being two to three times lower than in the permanently benthic condition, was more impacted. Consequently, burial of leaf litter in the hyporheic zone of the stream, whatever its duration, significantly altered the microbial colonisation and delayed the decomposition of detrital organic matter.

In line with the results of Cornut *et al.* (2010) for alder leaves, the structure of fungal assemblages associated with oak leaves was affected by burial in sediment. While benthic and benthic-hyporheic communities were initially quite similar, they strongly diverged after several weeks of exposure in the stream, in contrast with benthic and hyporheic-benthic fungal communities that converged. This, together with the patterns related above, suggests that (i) the fungal inoculum is more abundant or efficient when originating from the benthic environment than from the hyporheic and (ii) the latter environment is less favourable to fungal development. In addition, aquatic hyphomycete communities developing in hyporheic and benthic zones differed, probably reflecting better adaptation of individual species to the conditions prevailing in either environment. Whether some species were sensitive to the reduction in oxygen concentration (Medeiros,

Pascoal & Graça, 2009), the higher P availability in the hyporheic zone or other parameters remains unknown. Overall the positive effect of inorganic P in water was, nevertheless, small as judged by the C/P ratios in hyporheic litter, which was higher than in benthic litter.

The qualitative and quantitative differences in fungal assemblages and development were more pronounced on alder leaves (Cornut *et al.*, 2010) than on oak leaves (this study), with both studies being carried out in the same stream with a gap of 1 year. These differences observed between two contrasting types of leaf litter probably result from differences in substratum recalcitrance, which in the case of oak led to a delayed and limited extent of the development of fungal communities (Bengtsson, 1983; Gessner & Chauvet, 1994; Chauvet *et al.*, 1997; Gulis, 2001).

#### *Leaf litter consumption by invertebrates*

In this study, we showed that leaf litter palatability to invertebrate consumers was higher when leaf litter was incubated in the benthic zone than in the hyporheic zone of the stream. These results are in accordance with those reported by Herbst (1980) of reduced palatability of ash and poplar leaves to invertebrate consumers. In our study, leaf litter palatability greatly differed between the three invertebrate consumer taxa tested, with consumption rates increasing with time only for *G. fossarum*. In addition, while palatability was related to leaf litter toughness for two of the three invertebrates, the relationship was far stronger for *G. fossarum*. These results may be related to differences in feeding behaviour and mouth parts anatomy of these invertebrate taxa. Leaf toughness can influence food selection by building a physical barrier for invertebrate feeding, harder leaves being more difficult to tear than soft ones (Pennings *et al.*, 1998; Motomori, Mitsunashi & Nakano, 2001). This is especially relevant for *G. fossarum*, which eats the whole leaf matter, consisting of both fungi and leaf matrix, and is limited by its feeding apparatus when leaf remains too hard (Graça, Maltby & Calow, 1993). This species appears to be a particularly interesting biological model because of its dependence on microbial conditioning of leaf detritus. In contrast, the small plecopteran *Leuctra* only feeds by scraping the surface of the leaf discs. Because toughness measurements integrate the whole leaf thickness, this parameter is certainly not pertinent for this type of consumer. The independence of the consumption by the trichopteran *S. personatum* from leaf toughness is probably related to its ability to eat poorly conditioned and hard materials.

Only a small part of the observed patterns of leaf litter palatability to consumers can be explained from the

measured biological and physicochemical parameters. Several studies have showed that, more than total fungal biomass in leaf litter, the fungal species present can shape the response of invertebrate consumers (e.g. Butler & Suberkropp, 1986). Given that shredding invertebrates have feeding preferences for litter colonised by certain fungal species (Bärlocher & Kendrick, 1976; Arsuffi & Suberkropp, 1984, 1985, 1989; Butler & Suberkropp, 1986), differences can be anticipated in consumption and growth rate between invertebrates fed on litter colonised by different assemblages (Arsuffi & Suberkropp, 1984, 1985). In our study, fungal assemblage but not total species richness was altered by leaf exposure in the hyporheic zone of the stream. Recently, Chung & Suberkropp (2009) demonstrated that ecological efficiencies (i.e. larval survivorship, relative consumption rate, gross growth efficiency and instantaneous growth rate) of limnephilid larvae were more affected by fungal species identity than diversity. Similarly, we can hypothesise burial to indirectly affect leaf litter palatability through the selection of fungal species that are more or less palatable. Consumption rates of leaf litter by the three selected invertebrate taxa of our study could thus, at least partly, be driven by alterations in the fungal community. Experimental approaches using litter inoculated with controlled fungal assemblages are, however, required to confirm this hypothesis.

Overall, our results clearly show that burial of leaf litter can strongly decrease its consumption by invertebrate consumers and thus lower nutrients and energy transfer to higher trophic levels. However, the timing and sequences of leaf litter burial, for example a short colonisation period occurring in the benthic zone before burial, could alleviate these effects. In addition, the impact of leaf litter burial on its consumption by invertebrates certainly depends on the shredder identity and its ability to access buried organic matter. Cornut *et al.* (2010) showed that *Leuctra* spp. and small individuals of *S. personatum* but not *G. fossarum* were able to colonise leaf litter buried in the hyporheic zone of this stream. Reduced litter palatability should thus directly impact leaf litter breakdown by species able to access buried leaf litter, while consequences of the reduction in leaf litter palatability to shredders unable to penetrate sediment should only depend on sediment movements that make this litter accessible.

#### *Consumer growth rates*

Exposure in the hyporheic zone not only negatively impacted leaf litter palatability, but also impacted growth rates of *G. fossarum*. Maximal growth rates were observed

when organisms were fed with leaf litter exposed at the surface of sediment throughout the experiment. *G. fossarum* growth rate was negatively correlated with leaf litter C/P ratio but was independent of leaf litter toughness and palatability. These results are in accordance with the 'Growth Rate Hypothesis', the resource content in P being often limiting consumer growth (Elser *et al.*, 2003). To our knowledge, it is the first time that such a relationship has been shown for detritivorous invertebrates. Nevertheless, the relationship is weak, and several other factors (e.g. secondary compounds) may interact with P content to influence the invertebrate growth. The role of resource P content in regulating key life-history traits of headwater stream detritivores, food web structure and related functional processes remains to be explored in further studies.

In conclusion, our study shows that the exposure of leaf litter in the hyporheic zone of a headwater stream, even for a short period, alters the microbial development and leaf litter conditioning. These modifications can in turn negatively impact leaf litter palatability to consumers as well as a major detritivorous consumer life-history trait, i.e. growth rate. The results highlight the key role of aquatic hyphomycetes in the conditioning of leaf litter in the hyporheic zone, which is at least as influential as in surface habitats. The constraining conditions of the hyporheic zone and the disturbances related to the burial history have markedly impacted fungal development not only by delaying the accrual of mycelial biomass on leaf litter, but also by modifying the structure and composition of the species assemblages. Even if the mechanisms underlying the feeding preferences of invertebrate consumers remain unclear, fungal species identity may be crucial in controlling leaf consumption by shredders. The low decomposition rates observed, as well as the delay in quality improvement compared with benthic-exposed leaves, also imply that the hyporheic zone of woodland streams acts as an important compartment for organic matter storage and food provision to detritivorous organisms after sediment movements, particularly when new detrital inputs are scarce. This temporary reservoir of resources may affect the overall stream metabolism and should thus be taken into account in further studies considering the functioning, the conservation or the restoration of headwater stream ecosystems.

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