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Fungi are involved in the effects of litter mixtures on consumption by shredders

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SUMMARY

1. Decomposition of litter mixtures in both terrestrial and aquatic ecosystems often shows non-additive diversity effects on decomposition rate, generally interpreted in streams as a result of the feeding activity of macroinvertebrates. The extent to which fungal assemblages on mixed litter may influence consumption by macroinvertebrates remains unknown.

2. We assessed the effect of litter mixing on all possible three-species combinations drawn from four tree species (*Alnus glutinosa*, *Betula pendula*, *Juglans regia* and *Quercus robur*) on both fungal assemblages and the rate of litter consumption by a common shredder, *Gammarus fossarum*. After a 9-week inoculation in a stream, batches of leaf discs were taken from all leaf species within litter mixture combinations. Ergosterol, an indicator of fungal biomass, and the composition of fungal assemblages, assessed from the conidia released, were determined, and incubated litter offered to *G. fossarum* in a laboratory-feeding experiment.

3. Mixing leaf litter species enhanced both the Simpson's index of the fungal assemblage and the consumption of litter by *G. fossarum*, but had no clear effect on mycelial biomass. Specifically, consumption rates of *J. regia* were consistently higher for mixed-species litter packs than for single-species litter. In contrast, the consumption rates of *B. pendula* were not affected by litter mixing, because of the occurrence of both positive and negative litter-mixing effects in different litter species combinations that counteracted each other.

4. In some litter combinations, the greater development of some fungal species (e.g. *Clavariopsis aquatica*) as shown by higher sporulation rates coincided with increased leaf consumption, which may have resulted from feeding preferences by *G. fossarum* for these fungi.

5. Where litter mixture effects on decomposition rate are mediated via shredder feeding, this could be due to indirect effects of the fungal assemblage.

Keywords: aquatic hyphomycetes, biodiversity effect, decomposition, *Gammarus fossarum*, litter mixture

Introduction

Ecosystems worldwide are currently suffering a dramatic rate of species extinctions (Butchart *et al.*, 2010). Over the past few decades, a focus in ecology has been to assess the consequences of such a biodiversity loss on ecosystem processes (Loreau, Naeem & Inchausti, 2002; Naeem, Bunker & Hector, 2009). While focussing first on the relationships between plant diversity and primary productivity (Naeem *et al.*, 1994; Tilman, Wedin & Knops, 1996), research has more recently been extended to a wide variety of ecosystems and processes (Hättenschwiler, Tiunov & Scheu, 2005; Lecerf *et al.*, 2005; Gessner *et al.*, 2010). For instance, many studies have been dedicated to the consequences of biodiversity loss at various trophic levels (e.g. litter, micro- and macrodecomposers) on leaf litter decomposition (Lecerf & Richardson, 2009; Gessner *et al.*, 2010; Kominoski *et al.*, 2010), which is a key process for carbon and nutrient cycling in forested ecosystems (including forest soils and streams) (Wallace *et al.*, 1997; Cebrian, 1999). A number of studies have shown that litter decomposition rates are predictable from traits of the

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plants and leaves concerned. These traits include those that make litter decomposition slower (e.g. lignin and tannin content, and cuticle toughness) or faster (e.g. a high content of nitrogen and phosphorus) (Melillo, Aber & Muratore, 1982; Webster & Benfield, 1986; Enriquez, Duarte & Sand-Jensen, 1993; Ostrofsky, 1997). Such traits may affect both the density (e.g. recruitment and colonisation success) and processing efficiency of decomposer organisms, mostly consisting of fungi and leaf-eating macroinvertebrates (i.e. shredders) in aquatic environments (Cummins et al., 1989; Baldy, Gessner & Chauvet, 1995; Graça, 2001; Swan & Palmer, 2006a; Kominoski et al., 2009).

Patches of litter in streams are generally composed of several leaf species, however, and recent studies have emphasised how such mixing can influence decomposition rate and nutrient flux (Srivastava et al., 2009; Gessner et al., 2010; Kominoski et al., 2010; Lecerf et al., 2011). The decomposition rate of mixtures often diverges from the simple average of the species, that is, it is non-additive (Hättenschwiler et al., 2005; Lecerf et al., 2007). Both negative and positive effects of litter mixing on decomposition rate have been reported (McArthur et al., 1994; Swan & Palmer, 2006b; Swan, Healey & Richardson, 2008), and several non-exclusive mechanisms have been proposed to explain these mixture effects in both terrestrial and aquatic environments (Hättenschwiler et al., 2005; Kominoski et al., 2010). The latter may include some transfers of nutrient or refractory compounds between leaves with different chemical properties (Hättenschwiler et al., 2005; Schimel & Hättenschwiler, 2007), or alterations of decomposer community structure (Kominoski & Pringle, 2009) and feeding activity or behaviour (Swan & Palmer, 2006b; Sanpera-Calbet, Lecerf & Chauvet, 2009). However, the relevance and relative strength of such mechanisms across ecosystems are still uncertain (Gessner et al., 2010). This lack of understanding makes the decomposition of mixtures, and thus the consequences of litter diversity loss (e.g. through species invasions, harvesting, forestry), often unpredictable from the attributes of the litter species pool.

In streams, the activity of shredders has often been suggested to be responsible for litter-mixing effects (Swan & Palmer, 2006a; Sanpera-Calbet et al., 2009). For example, the increased decomposition of fast-decomposing species within mixtures can be explained by shredders first consuming litter species with the highest concentrations of nutrients (Swan & Palmer, 2006b), whereas the preference for refractory leaves by caddis larvae for case construction could potentially lead to increased decomposition of slowly decomposing species (Kochi & Kagaya,

2005; Sanpera-Calbet et al., 2009). It has been suggested that microbial activity alone does not alter the decomposition of litter mixtures (Schädler & Brandl, 2005; Swan & Palmer, 2006a; Schindler & Gessner, 2009). The field studies assessing the relative contribution of micro- versus macrodecomposers on litter-mixing effects often rely on the use of coarse and fine mesh litter bags (Bärlocher, 2005a), assuming that the microbial contribution to decomposition corresponds to the mass loss observed in fine mesh bags, while the macroinvertebrates contribution is inferred from the difference between coarse and fine mesh bags. However, if fine mesh bags adequately assess microbial activity, the processes occurring in coarse mesh bags are not only the result of macroinvertebrate activity and microbial one, but also that of complex interactions occurring between macroinvertebrates and microorganisms such as bacteria and microalgae (Franken et al., 2005), and fungi (Lecerf et al., 2005). Consequently, the contribution of macroinvertebrate activity and these interactions cannot be disentangled using such an approach, which may result in misleading interpretations. Although the contribution of fungi is predominant for leaf litter decomposition and shredder feeding (Baldy et al., 1995; Hieber & Gessner, 2002), the structure of fungal assemblages has rarely been assessed in litter mixture experiments, and its contribution to mixture effects on decomposition (through the interactions of fungi with shredders) remains unknown.

Kominoski et al. (2009) suggested that litter mixing could influence microbial activity and diversity. For instance, the presence of recalcitrant litter could provide higher structural complexity to the leaf pack and allow a better circulation of oxygen, nutrients and conidia between leaves of different species. Leachates from different litter species could also stimulate (e.g. by leaching of nutrients; Tukey, 1970; Jensen, 1974) or inhibit (e.g. by leaching of polyphenols; Suberkropp, Godshalk & Klug, 1976) fungal growth on other leaves. Finally, fungi were shown to have some substrate preferences, with litter-associated communities being dissimilar between different litter species (Gulis, 2001). Thus, diverse leaf packs might produce a more diverse pool of conidia (released from the various leaf species), with each litter species within mixtures being more likely to be colonised by a greater diversity of fungi than in single-species litter packs.

Such alterations in both biomass and diversity of fungi associated with leaf litter are expected to influence shredder feeding (Arsuffi & Suberkropp, 1988; Graça, Maltby & Calow, 1994; Lecerf et al., 2005). Actually, fungal activity leads to increased litter quality, favouring the

activity of shredders that feed on both leaf tissues and fungal mycelium (Bärlocher & Kendrick, 1975; Arsuffi & Suberkropp, 1988; Graça, Maltby & Calow, 1993) and exhibiting preferences for different fungal species (Arsuffi & Suberkropp, 1985; Graça *et al.*, 1994). Thus, fungal diversity may alter shredder feeding through two mechanisms: (i) complementarity in resource use, leading to a better conditioning of the litter (i.e. enzymatic complementarity) and higher total fungal biomass; and (ii) complementarity as a nutritional resource, resulting in enhanced activity of shredders (Lecerf *et al.*, 2005; Duarte *et al.*, 2006).

We tested the hypothesis that litter mixture effects on decomposition result from (i) the alteration of fungal biomass and assemblage structure at the local (i.e. leaf) scale; (ii) which in turn influences the activity of shredders. In a woodland stream, we placed leaf litter from four contrasting species in fine mesh bags and subjected them to both single-species and three-species mixture treatments, thus allowing us to evaluate mixing effects in a statistically balanced design (i.e. four single-species versus four mixed treatments). Then, we compared fungal assemblages, mycelial biomass and the consumption efficiency by a common detritivore, *Gammarus fossarum* (Koch, 1836), on individual litter species across the different treatments.

Methods

Experimental design

Leaf litter was exposed in the Rémillassé, an oligotrophic second-order stream in the French Pyrenees (01°05′24″E; 42°56′36″N, 480 m a.s.l). Four tree species (alder, *Alnus glutinosa* (L.) Gaertn.; birch, *Betula pendula* Roth; walnut, *Juglans regia* L.; and oak, *Quercus robur* L.) were used. The leaves of these species differ in traits, such as the content of nutrients [e.g. nitrogen (N) and phosphorus (P)] and structural compounds (e.g. cellulose and lignin) that influence the decomposition process, it being fastest for alder and slowest for oak, with the remaining species being intermediate (Webster & Benfield, 1986; Ostrofsky, 1997; Leroy & Marks, 2006). We determined these parameters on three leaf batches for each leaf species after 24 h of leaching in tap water (Table 1).

We used four replicates each for all four single-species and all four three-species treatments, resulting in a total of 32 litter bags. Litter bags were made of fine mesh (0.3 mm), thus preventing the access of macroinvertebrates, and were introduced in the stream in late autumn (16 November 2009). Each litter bag contained a total of

Table 1 Phosphorus, nitrogen, lignin and cellulose content of leaf species (mean ± SD; $n = 3$)

	P (mg g⁻¹)	N (mg g⁻¹)	Cellulose (mg g⁻¹)	Lignin (mg g⁻¹)
	P ($mg\ g^{-1}$)	N ($mg\ g^{-1}$)	Cellulose ($mg\ g^{-1}$)	Lignin ($mg\ g^{-1}$)
Alder	0.13 ± 0.02	18.59 ± 2.5	224.6 ± 6.4	225.5 ± 4.4
Birch	0.29 ± 0.02	4.94 ± 0.5	195.3 ± 12.0	254.1 ± 15.9
Walnut	0.17 ± 0.03	10.31 ± 0.1	231.3 ± 17.7	286.1 ± 8.8
Oak	0.61 ± 0.13	9.21 ± 1.4	262.9 ± 14.6	313.4 ± 14.1

6 g (i.e. 2 g per species for mixtures) of litter dried at ambient temperature. Litter bags were removed from the stream after 9 weeks of exposure, and the leaves cleaned and separated by species. For each litter bag, three batches of ten 10-mm leaf discs were cut (i.e. one batch per species in mixtures and three batches of the same species for single-species litter bags), except for the litter mixtures containing alder, oak and walnut in which alder leaves were too far decomposed to cut discs in three of the four replicates, resulting in a total of 93 batches (i.e. 930 leaf discs).

Fungal communities

All leaf discs were placed into glass Petri dishes containing 20 mL of filtered (GF/C glass fibre filter, 1.2 μm pore size; Whatman, Clifton, NJ, U.S.A.) stream water and kept at 10°C during 48 h under constant agitation (100 rpm). The water containing released conidia was then preserved with 2% formalin (final concentration). To characterise fungal assemblages, an aliquot of these conidial suspensions was filtered on a membrane filter (SMWP, 5 μm porosity; Millipore, Bedford, MA, U.S.A.) and stained with Trypan blue (0.1% in 60% lactic acid). Trapped conidia were then counted and aquatic hyphomycete species identified under the microscope at ×200–400 (see Bärlocher, 2005b). The Simpson's dominance index was determined from conidial numbers and used as a measure of fungal assemblage structure.

Fungal biomass was assessed through determination of ergosterol by HPLC on four (of the 10 per batch) leaf discs that were stored frozen, then freeze-dried and weighed to the nearest 0.01 mg [see Gessner & Schmitt (1996) and Lecerf *et al.* (2005) for more details]. Mycelial biomass was calculated using species-specific conversion factors weighted by the proportions of conidia released by the different species in each sample. Species-specific conversion factors were available for 13 species (Table 2) from Gessner & Chauvet (1993, 1994) and Suberkropp, Gessner & Chauvet (1993), which comprised all dominant species, and accounted for 83% of total conidial production. The

Table 2 Aquatic hyphomycete species on each leaf litter species. Symbols represent the average proportion of conidia belonging to the species considered (results from single-species treatment and all mixtures combined; +++ >20%, ++ 10–20%, + 5–10% and · <5%)

	Alder	Birch	Walnut	Oak
Alatospora acuminata Ingold*	·	·	++	·
Alatospora flagellata (Gönczöl) Marvanová	·	·	·	·
Anguillospora crassa Ingold		·	·	
Anguillospora filiformis Greathead*	·	·	·	·
Anguillospora furtiva Descals	·	·	·	·
Anguillospora longissima (Saccardo and Sydow) Ingold*	·	·	+	·
Articulospora tetracladia Ingold*	+	·	++	·
Clavariopsis aquatica De Wildeman*	++	+	+	+++
Clavatospora longibrachiata (Ingold) Nilsson*	++	+++	++	++
Crucella subtilis Marvanová and Suberkropp*	·	·	+	·
Culicidospora aquatica Petersen	·	·	·	·
Flagellospora curvula Ingold*	++	+++	++	·
Geniculospora inflata (Ingold) Sv. Nilsson ex Marvanová and Sv. Nilsson	+	·	·	·
Goniopila monticola (Dyko) Marvanová and Descals		·	·	·
Heliscus lugdunensis Saccardo and Thérry	·	·	·	·
Heliscella stellata (Ingold & Cox) Marvanová*	·	·	·	·
Lemmoniera aquatica De Wildeman*	·	·	·	·
Lemmoniera terrestris Tubaki*	·	·	·	·
Stenocladiella neglecta (Marvanová and Descals) Marvanová and Descals	·	·	·	·
Tetrachaetum elegans Ingold*	++	+	+	·
Tetracladium marchalianum De Wildeman*	·	·	·	·
Tricladium chaetocladium Ingold	·	·	·	++
Tricladium splendens Ingold*		·		·
Tumularia aquatica (Ingold) Marvanová and Descals	·	·	·	·
Tumularia tuberculata (Gönczöl) Descals and Marvanová				·

*Denotes species for which specific conversion factors were available from the literature and used to calculate mycelial biomass from ergosterol content (see Methods).

average conversion factor of 5.5 mg ergosterol per g of mycelium was used for the 14 remaining species (Gessner & Chauvet, 1993). Mycelial biomass was expressed as a percentage of total detrital dry mass.

Feeding experiment

Three of the six remaining leaf discs per batch were put into $5 \times 6 \times 4$ cm containers filled with 20 mL of filtered (Whatman GF/C glass fibre filter) stream water. Each container also contained one individual of *G. fossarum* (3.56 mg ± 0.07 SE, $n = 93$). All individuals were collected in a nearby stream and acclimated 1 week in the laboratory, fed with natural litter collected in their stream of origin. They were then starved for 24 h before the beginning of the experiment.

Specimens of *G. fossarum* were kept at 10°C and allowed to feed for 90, 114, 140 and 165 h on birch, walnut, alder and oak leaves, respectively. Then leaf discs were removed, cleaned, freeze-dried and weighed to the nearest 0.01 mg. Each sample was paired with a control for the microbially mediated decomposition occurring during the feeding experiment, consisting of three additional discs under the same conditions but without detritivores, which

resulted in a total of 186 containers. The leaf mass loss because of invertebrates was determined as the difference of final leaf mass remaining between treatments where *G. fossarum* was absent and present. Then the invertebrates were oven-dried (60°C, 48 h) and weighed to the nearest 0.01 mg, and the consumption rates were calculated as the ratio between leaf consumption and invertebrate body mass and expressed in g leaf DM g^{-1} day^{-1}.

Statistical analyses

First, a non-metric multidimensional scaling was performed on fungal communities using the Bray–Curtis distance to illustrate the differences in composition between substrate species and inoculation contexts (i.e. litter diversity within litter pack).

Then the differences between treatments were tested by performing ANOVAS to assess the effect of both litter identity and litter mixing on different target variables, including the consumption rate by *G. fossarum*, the fungal biomass and the Simpson's index of dominance in fungal assemblages. Litter pack identity was included as a random factor to control the fact that these variables were not independent from each other when evaluated on

different batches from the same litter packs. As a second step, we performed the same analysis using *a priori* contrasts that allow direct comparison of litter-mixing effect on different litter species instead of overall mixing effect.

Finally, the effect of the species composition of the litter pack, rather than litter species richness, was assessed by comparing the consumption rates of the four species originating from litter packs of different species composition. Comparisons were performed using pairwise Wilcoxon nonparametric test with Bonferroni correction for multiple comparisons. Consumption rates and Simpson's dominance index in all analyses were log-transformed, while mycelial biomass was square-root transformed to meet normality and homoscedasticity assumptions (checked graphically). All statistics were performed using the R 2.6.0 software (The R Core Team, 2007).

Results

Fungal community structure

Different plant litter species supported different fungal assemblages (Fig. 1). In particular, communities on oak litter contained a large proportion of *Clavariopsis aquatica*, *Clavatospora longibrachiata* and *Tricladium chaetocladium*, the latter being in low abundance on other litter species (Table 2). Similarly, fungal assemblages associated with
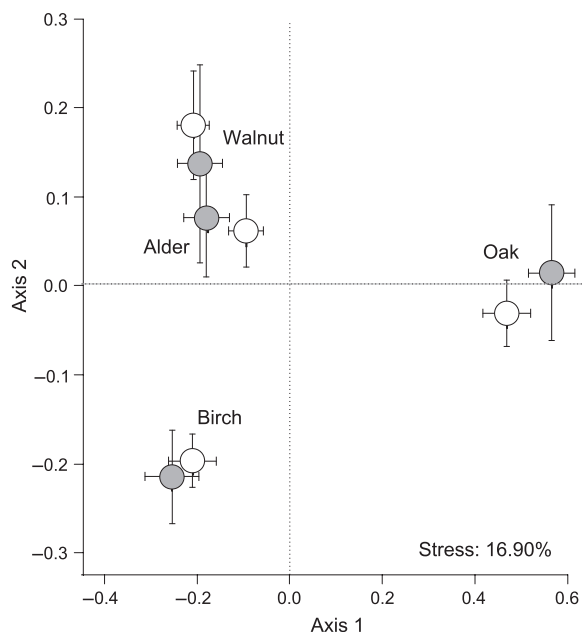
Fig. 1 Non-metric multidimensional scaling of fungal assemblages associated with litter species from single-species (white dots) and mixed (grey dots) treatments. Bars are the standard errors associated with the mean of coordinates on axes 1 and 2.

birch differed from the others because of their very high proportion of both *Flagellospora curvula* and *C. longibrachiata*, although these were present in substantial proportions on other litter species. *Alatospora acuminata* was more abundant on walnut than on the other litter species. Finally, assemblages associated with alder were intermediate, although closer to those on walnut than the two other species.

Litter mixing had little effect on fungal assemblages, especially when compared with the influence of leaf identity (Fig. 1). Nevertheless, the dissimilarity among the assemblages from different litter species tended to be higher for samples originating from mixed than single-species litter (except for those associated with walnut), corresponding to an increased abundance of species already dominant in single-species treatments. This coincided with a significant effect of litter mixing on Simpson's index of dominance (Table 3), which was higher on oak (0.26 ± 0.02 SE) and birch (0.22 ± 0.01 SE) than on alder and walnut (both: 0.14 ± 0.01 SE). Assemblages associated with samples from diverse litter packs were actually less even than those from single-species litter packs, with average Simpson's dominance indices of 0.21 (±0.01 SE) and 0.18 (±0.01 SE), respectively (Fig. 2). A net positive effect of litter mixing on fungal dominance was observed, and depended on litter pack composition (e.g. 38% more *C. aquatica* on oak when mixed with birch and walnut, and 48% more *C. longibrachiata* on birch when mixed with oak and walnut, than in other mixtures; data not shown), but was only significant on birch litter in the contrast analysis (Fig. 2).

Fungal biomass and consumption by G. fossarum

Litter species identity strongly affected the fungal biomass associated with leaves and consumption rates of *G. fossarum* (ANOVA; Table 3). The mean contribution of mycelial biomass to total detrital mass ranged from

Table 3 ANOVAs of the effect of litter species identity and litter mixing on mycelial biomass, Simpson's dominance index in fungal communities and consumption rates by *Gammarus fossarum*

|	d.f.	Mycelial biomass		Simpson's dominance		Consumption rates	
		SS	P	SS	P	SS	P
Species identity (I)	3	21471	0.014	0.10	$<1.10^{-4}$	0.66	$<1.10^{-3}$
Mixing (M)	1	3	0.966	0.01	0.048	0.03	0.244
I × M	3	1772	0.787	0.01	0.234	0.20	0.045
Residuals	25	41756		0.06		0.54	
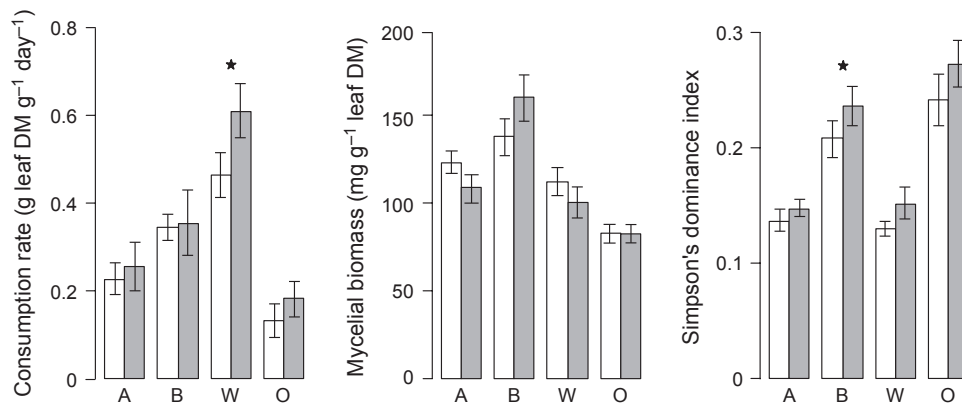
Fig. 2 Average (±SE, n = 12 per bar) consumption rate, mycelial biomass and Simpson's dominance index of leaf-associated fungal communities for each litter species within single-species (white bars) and mixtures treatments (grey bars). A, alder; B, birch; W, walnut; and O, oak. Stars indicate litter species in which mixing effect was significant (*a priori* contrasts).

82.7 mg g^{-1} (±3.5 SE) on oak to 148.7 mg g^{-1} (±8.6 SE) on birch. Mycelial biomass on alder and walnut litter was intermediate and accounted for 116.8 mg g^{-1} (±5.0 SE) and 106.4 mg g^{-1} (±6.2 SE) of total detrital mass, respectively. Fungal biomass did not differ significantly with litter pack composition for any litter species, although discrepancies between leaves of the same species originating from some litter mixtures occurred. For instance, mycelial biomass associated with walnut was low when the latter was mixed with alder and oak (76.1 mg g^{-1} ± 21.0 SE), but reached 124.3 mg g^{-1} (±13.2 SE) in litter packs containing both birch and oak (Fig. 3). Mycelial biomass on birch did not differ significantly (Table 3) when it was mixed with either alder and walnut or oak and walnut (170.5 mg g^{-1} ± 17.9 SE and 170.2 mg g^{-1} ±

31.8 SE, respectively) or in litter packs containing alder and oak (138.4 mg g^{-1} ± 16.6 SE).

Consumption of oak by *G. fossarum* was slowest (0.16 g g^{-1} day^{-1} ± 0.03 SE), and the consumption of walnut (0.57 g g^{-1} day^{-1} ± 0.05) and birch (0.35 g g^{-1} day^{-1} ± 0.04) faster than that of alder (0.25 g g^{-1} day^{-1} ± 0.04). Moreover, litter mixing and species identity had an interactive effect on consumption rate (Table 3), meaning that the mixture effect on consumption depended on the litter species considered. Specifically, mixing litter led to higher consumption rate on walnut leaves by 31%, but did not significantly influence the consumption of other litter species (Fig. 2). For birch (and, to a lesser extent, oak), the overall non-significant effect of litter mixing on consumption by *G. fossarum* (Table 3) resulted from both
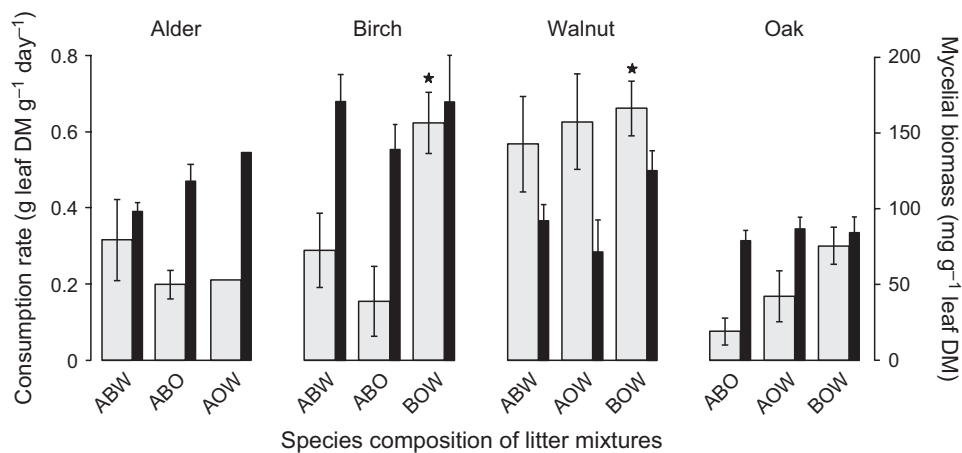
Fig. 3 Average consumption rate (grey bars) and mycelial biomass (black bars) (±SE, n = 4 per bar) for the four litter species contained in the three-species mixtures. The composition of mixtures is indicated by the first initial of the litter species' common name. Stars indicate the consumption values that are statistically different between mixture and single-species treatments for the same litter species (Wilcoxon non-parametric test).

positive and negative effects of certain litter species from mixtures (Fig. 3).

Discussion

In accordance with the literature, our results suggest that litter mixing can have both negative and positive effects on leaf litter decomposition (Swan & Palmer, 2004), fungal assemblages (Kominoski *et al.*, 2009) and shredder processing ability (Swan & Palmer, 2006a), while litter diversity *per se* has no effect (Wardle, Bonner & Nicholson, 1997; Schädler & Brandl, 2005; Lecerf *et al.*, 2007; Swan, Gluth & Horne, 2009). This is an overall result of the counteracting effects of particular species in the various mixtures (Srivastava *et al.*, 2009). This result has been consistently shown across ecosystems (e.g. terrestrial and aquatic) and may result from similar mechanisms, although their relative importance may be influenced by discrepancies in habitat conditions and in the functioning of decomposer communities in the various ecosystems (Gessner *et al.*, 2010).

Litter-mixing effects on decomposition have often been interpreted as a result of the activity of shredders. For instance, preferential feeding on labile litter (Swan & Palmer, 2006b), or indirect benefits of refractory species (e.g. providing structured habitat and shelter against predators; Hättenschwiler *et al.*, 2005; Sanpera-Calbet *et al.*, 2009), have been suggested to drive increased decomposition of labile litter within litter mixtures. Our experimental design allowed us to exclude such mechanisms and demonstrate that litter-mixing effects on decomposition can also involve microorganisms, interacting with their physical environment and shredders. Litter mixtures altered both the fungal assemblages associated with individual litter species and their palatability to *G. fossarum*, which supports the notion of an indirect fungal contribution to the effect of mixing litter on decomposition by detritivores.

The effects of litter mixing on fungal assemblages

For a given litter species, the structure of fungal assemblages from mixtures was less even (i.e. with increased fungal dominance) than that from monocultures, which partly contrasts with findings by Kominoski *et al.* (2009). Unlike Kominoski *et al.* (2009), who assessed microbial assemblages using molecular approaches, we relied on the species identity from the pool of conidia produced, which may reflect some modifications of fungal assemblages structure and/or alterations of the relative spore production in different fungal species. In our experiment, litter

mixing favoured the sporulation of the already-dominant fungal species. The fact that distinct assemblages were observed on different substrates, even in mixtures where these were close together, suggests that the structure of the fungal assemblage is more constrained by litter quality than species composition of the pool of conidia likely to colonise. To some extent, litter quality may act as an environmental filter, benefiting some species in their establishment and development (Keddy, 1992; Canhoto & Graça, 1999; Gulis, 2001; Dang, Gessner & Chauvet, 2007), potentially leading to their dominance through competitive exclusion (Hardin, 1960; Diamond, 1975; Treton, Chauvet & Charcosset, 2004). In contrast to fungal assemblage structure, no clear evidence for an effect of litter mixing on mycelial biomass associated with a given litter species was found. Again, litter identity was the main factor controlling mycelial biomass, probably reflecting the differences in the decomposition stage and nutritional value of different leaf species at the end of the experiment.

Litter-mixing effects on litter palatability

Unsurprisingly, litter identity was the most important factor influencing the consumption by *G. fossarum*. These differences may reflect litter chemistry, with the higher consumption rates on walnut and birch, two species that are relatively rich in P, suggesting that this nutrient is limiting for *G. fossarum* (Evans-White, Stelzer & Lamberti, 2005; Hladyz *et al.*, 2009). Low consumption of oak litter, although rich in P, was probably due to the high concentration of structural compounds, such as lignin, which inhibit shredder feeding (Ostrofsky, 1997). Finally, the low consumption of alder litter reflected its low N (<2%; Table 1) and high lignin (>20%; Table 1) content, relative to its usual concentration range in nature, both parameters being strongly correlated with the decomposability of alder leaves (Lecerf & Chauvet, 2008).

Overall leaf consumption by *G. fossarum* was not affected by litter mixing. However, both positive and negative effects of litter pack composition were observed, but with no clear relationship with the fungal assemblage and biomass. For instance, the consumption of oak, on which the structure of the fungal assemblage was the most responsive to litter mixture, was weakly affected by litter mixing. In contrast, the consumption of walnut was enhanced in all mixtures, while that of birch was higher in litter mixtures with walnut and oak, but lower in the others. Although these responses of walnut and birch consumption to mixture were not clearly related to any effect on fungal biomass, the highest consumption rates

were observed in samples in which mycelial biomass was also higher than average, supporting the involvement of fungi in such effects. Moreover, increased proportions of *C. aquatica* and *C. longibrachiata* on oak and birch (as found in some litter mixtures), coincided with the highest consumption rates observed on these litter species. This is consistent with the previous findings on *C. aquatica*, showing that *Gammarus* sp. exhibited high consumption rates on litter colonised by this species (Arsuffi & Suberkropp, 1989). Our result would thus suggest that *C. longibrachiata* could also be a prime resource for *G. fossarum*. Other fungal species that are known to be preferred by *Gammarus* and observed in this study include *A. acuminata*, *Heliscus lugdunensis* and *Anguillospora longissima* (Arsuffi & Suberkropp, 1989; Graça *et al.*, 1994), but the proportion of these species was low and not affected by litter mixing. Some other dominant fungal species observed in this study are not preferred by *Gammarus* (e.g. *F. curvula*) (Bärlocher & Kendrick, 1973; Arsuffi & Suberkropp, 1989; Graça *et al.*, 1994). Alterations in the fungal assemblage that result in increased proportions of such non-preferred fungal species may lead to no overall effect of litter mixing on shredder consumption, or even explain negative litter-mixing effects, if the unpalatable fungal species grow to the detriment of preferred ones, or if they include species rejected by consumers (e.g. those containing repellents). Finally, the fact that three of the four replicates of alder leaves from litter mixtures containing alder, oak and walnut were in an advanced state of decomposition suggests an increased microbial activity in this particular litter combination.

If litter mixing can influence microbial assemblages associated with a given leaf, how these alterations propagate to affect the consumption rate of shredders is hypothetical. Alterations of fungal assemblages do not necessarily lead to the changes in fungal biomass and/or leaf palatability. Conversely, the alteration of leaf palatability can occur independently of changes in the fungal assemblage, possibly implying the involvement of other mechanisms. For instance, it has been demonstrated that nutrient transfers between litter species occur in terrestrial ecosystems (Schimel & Hättenschwiler, 2007), and thus could be an important factor in determining the decomposition rate of mixtures (see Gessner *et al.*, 2010). By modifying nutrient balance in litter, such transfers are expected to alter fungal assemblages and shredder consumption rates, potentially to a different extent depending of fungal versus shredder respective needs. For instance, Güsewell & Gessner (2009) found that the $N : P$ ratios in litter influenced colonisation by fungi, the latter being N-limited at low $N : P$ ratios but becoming P

limited at higher $N : P$ ratios, leading to maximal microbial biomass at intermediate $N : P$ supply ratios. On the other hand, *Gammarus* has a low $N : P$ ratio (Evans-White *et al.*, 2005; Hladyz *et al.*, 2009) and thus may remain P-limited whichever litter it feeds on. Thus, *Gammarus* and fungi may be limited by different nutrients depending on the $N : P$ ratios of their shared resource, leading to differences in their responses to the changes in litter composition following nutrient transfer. Initial litter nutrient contents, in relation to the respective needs of decomposers, may explain why nutrient transfer (when it occurs) may have positive or no effect on fungal- and shredder-induced decomposition. However, such an interpretation remains a matter of conjecture since other compounds, such as fatty acids and vitamins, are important in determining the activity of both shredders and microbial decomposers.

In conclusion, these results suggest that further investigation of microbial decomposers and nutrient transfers are needed to understand and predict the consequences of riparian diversity loss on stream ecosystem processes. Until now, litter-mixing effects on decomposition have been suggested to be attributable to altered shredder densities and diversity (Kominoski & Pringle, 2009), preferential feeding and complementary acquisition of nutrients from different leaf species (Swan & Palmer, 2006b) and increased habitat complexity in litter packs containing refractory litter (Sanpera-Calbet *et al.*, 2009). In this experiment, litter-mixing effects on shredder feeding rates were observed without these proposed mechanisms. In contrast, our results suggest that aquatic hyphomycetes *per se* have the potential to explain mixture effects on litter decomposition by shredders. Because shredders show preferences for fungal species (Arsuffi & Suberkropp, 1985), any alteration of the structure of fungal assemblage associated with a litter species may influence shredder feeding rates, depending on the identity of the fungi (preferred or not) whose abundance is altered. Moreover, at larger spatiotemporal scales, changes in the species composition and diversity of riparian vegetation may lead to longer term alterations of aquatic hyphomycetes (Bärlocher & Graça, 2002; Laitung & Chauvet, 2005; but see Kominoski, Marczak & Richardson, 2011) and shredder assemblages (Whiles & Wallace, 1997; McKie & Malmqvist, 2009). In nature, where shredders can be diverse, concomitant alterations of fungal and shredder assemblages following the changes in litter diversity may alter resource partitioning among shredder species exhibiting different fungal preferences. This could amplify the effect of the mechanism described in this study, by which alterations in litter diversity may have ramifying the

effects on fungal assemblages and litter decomposition. Whether this mechanism is more significant than nutrient transfer among different leaf species, another mechanism possibly involving fungi (see McTiernan, Ineson & Coward, 1997; Schimel & Hättenschwiler, 2007), cannot be determined from our experiment. It remains possible, however, that the alteration in shredder feeding caused by litter mixing found in other studies (Swan & Palmer, 2006b; Sanpera-Calbet *et al.*, 2009) could be due to changes in the fungal assemblage, as observed on some litter species in the present work.

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