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Leaf diversity influences in-stream litter decomposition through effects on shredders

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SUMMARY

1. The functioning of many aquatic ecosystems is controlled by surrounding terrestrial ecosystems. In a view of growing interest in linking biodiversity to ecosystem-level processes, we examined whether and how leaf diversity influences litter decomposition and consumers in streams.

2. We tested experimentally the hypothesis that the effects of leaf diversity on decomposition are determined by the responses of leaf consumers to resource–habitat heterogeneity. Leaves from three common riparian trees, beech (*Fagus sylvatica*), hazel (*Corylus avellana*) and ash (*Fraxinus excelsior*), were exposed alone and in all possible mixtures of two and three species in a stream. We analysed individual leaf species for decomposition rate, microbial respiration and mycelial biomass, and we determined the species composition, abundance and biomass of shredders in leaf bags.

3. We found that the decomposition of the fastest decomposing leaves (hazel and ash) was substantially stimulated (up to twofold higher than single species leaf packs) in mixtures containing beech leaves, which are refractory. In contrast, the decomposition of beech leaves was not affected by leaf mixing. Such species-specific behaviour of leaves in species mixtures has been overlooked in previous studies that examined the overall decomposition of litter mixtures.

4. The effects of leaf diversity on decomposition varied with the abundance and biomass of shredders but not with microbial parameters. Beech leaves alone were less attractive to shredders than leaf packs made of hazel, ash or any mixture of species. Moreover, the presence of beech leaves in mixtures led to higher shredder abundance and biomass than we had expected from data from single species exposed alone. Lastly, we found that early instars of the caddisfly *Potamophylax* (the dominant shredder in terms of biomass) almost exclusively used the toughest material (i.e. beech leaves) to construct their cases.

5. Leaf pack heterogeneity may have altered shredder-mediated decomposition. Shredders colonising diverse leaf packs benefited from the stable substratum provided by beech leaves, whereas ash and hazel leaves were primarily used as food. Thus, our findings provide strong evidence for an intimate linkage between the diversity of riparian vegetation and aquatic communities.

Keywords: biodiversity, leaf mixing, litter breakdown, shredders, terrestrial–aquatic linkage

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Introduction

Leaf litter supplied by riparian vegetation is an essential resource to the food webs of forest streams (Cummins *et al.*, 1989; Wallace *et al.*, 1997). There is compelling evidence that the amount of litter input is a limiting factor for in-stream secondary production (Richardson, 1991; Dobson & Hildrew, 1992; Wallace *et al.*, 1997). A complementary issue is the consequence for streams of qualitative variations in litter-fall, such as those resulting from differences in forest structure and composition (Cummins *et al.*, 1989; Griffith & Perry, 1991; Pozo *et al.*, 1998; Benfield *et al.*, 2001; Lecerf *et al.*, 2005, 2007a; Ferreira *et al.*, 2006a).

According to Cummins *et al.* (1989), diverse riparian vegetation that produces leaf litter of different degradability would promote efficiency of resource use by leaf-shredding detritivores (hereafter shredders). Degradability refers to variation in the rate of leaf decomposition associated with various physical (e.g. toughness) and chemical (e.g. contents in nutrients and soluble and insoluble organic compounds) leaf traits (Gessner & Chauvet, 1994; Lecerf & Chauvet, 2008b; Hladyz *et al.*, 2009). Labile leaf species are rapidly exploited by shredders, whereas microbial decomposers largely account for initial processing of slow decomposing leaf species and improve leaf palatability (Cummins *et al.*, 1989).

Consistently, leaf decomposition rate has been found to be higher in streams lined by species-rich forest than in adjacent streams lined by species-poor forest (Pozo *et al.*, 1998; Lecerf *et al.*, 2005; Ferreira *et al.*, 2006a). This may be due to the effect of resource-habitat heterogeneity on the structure and activity of consumer assemblages in streams running through species-rich forests (Lecerf *et al.*, 2005, 2007a; Kominoski *et al.*, 2007; Swan, Healey & Richardson, 2008). It is possible, however, that the amount of labile litter (i.e. fast decomposing species) among that retained in streams is more important than leaf diversity *per se* in controlling the effect of forest diversity on litter dynamics (Griffith & Perry, 1991; Benfield *et al.*, 2001).

How the diversity and composition of riparian vegetation really influences stream ecosystems can be properly addressed through *in situ* experimental manipulation of leaf species number and identity. With the notable exception of an earlier study conducted at the reach scale (Sweeney & Vannote,

1986), such experiments have been carried out at the patch scale using leaf bags exposed in streams (e.g. Swan & Palmer, 2004; Kominoski *et al.*, 2007; Lecerf *et al.*, 2007b). Mixing leaves has been reported to have non-additive effects on leaf decomposition, such as those measured by the deviation between observed decomposition rate and an expected value calculated from single species alone (Wardle, Bonner & Nicholson, 1997; Swan & Palmer, 2004; Lecerf *et al.*, 2007b).

Non-additive effects have been hypothesised to be due: (i) to the presence/absence of leaf species having particular characteristics (Wardle *et al.*, 1997) or (ii) to emergent effects of leaf pack heterogeneity (Epps *et al.*, 2007). Hypothesis 1 assumes that a single leaf species with atypical traits invariably has the same directional effect on the decomposition of other component species. This may be due to the translocation of stimulating (nutrients, vitamins, labile carbon) or inhibitory (polyphenols) compounds from leaf species with high concentrations to those poor in such elements (McArthur *et al.*, 1994). Alternatively, tough leaves may decelerate the decomposition of fragile leaves affected by intense physical abrasion (i.e. an armouring effect: Swan *et al.*, 2008). Hypothesis 2 assumes that differences in physical and chemical traits among individual leaf species create a heterogeneous micro-environment and food resources that enhance coexistence and complementarity of leaf consumers, ultimately resulting in higher consumption rates of all the leaf component species (Leff & McArthur, 1989; Bastian, Pearson & Boyero, 2008).

Elucidating the relative importance of key leaf species and leaf pack heterogeneity in mediating the effects of leaf diversity on decomposition can be achieved by examining: (i) the decomposition rate of individual leaf species in mixtures (Ostrowsky, 2007) and (ii) leaf consumer assemblages. It is worth noting that the conclusions of most *in situ* studies rely on the decomposition rate of leaf mixtures as a whole. This approach can mask the species-specific behaviour of leaves in diverse mixtures (Hättenschwiler, Tiunov & Scheu, 2005). The few studies that have isolated leaf species effects were limited either to a single mixture of leaves (Leff & McArthur, 1989; McArthur *et al.*, 1994; Moretti, Gonçalves & Callisto, 2007) or a single leaf species isolated from several mixtures (Swan *et al.*, 2008).

In this study, we examined whether and how mixtures of leaves, which reflect the riparian

vegetation diversity, influence decomposition and stream consumers. From the results of a previous field survey (Lecerf *et al.*, 2005), we predicted decomposition of a refractory leaf species (beech) to be faster when mixed with more labile leaves. We also expected this increase to be proportional to the degree of heterogeneity of leaf packs. To conduct our experiment we selected a stream with a very high diversity of shredders and leaf litter.

Methods

Study area

The experiment was conducted in the Ruisseau de Peyreblanque, a second-order forested stream situated in the Montagne Noire, SW France (43°25'52"N, 2°13'12"E, altitude 750 m). Riparian vegetation consisted of mixed deciduous tree species dominated by beech (*Fagus sylvatica* L.). The streambed was composed of heterogeneous sediments dominated by sand and gravel along with large boulders. In this region, there is a variety of shredders, such as amphipods, stoneflies and caddisflies (Dobson, 1994; Lecerf *et al.*, 2005). The slightly acidic water (pH: 5.5–6.4; alkalinity: 2.0–8.0 mg CaCO₃ L⁻¹) in the study stream has probably prevented amphipods (*Gammarus*) from dominating the shredder assemblage. Microbial activity is limited by the low P concentration (1.4–2.4 µg SRP L⁻¹). In contrast, biological N demand was probably saturated by the high nitrate concentrations (>300 µg NO₃-N L⁻¹; Lecerf & Chauvet, 2008a; Ferreira, Gulis & Graça, 2006b). Water temperature ranged from 2.5 to 7.0 °C during the course of the experiment.

Leaf bags

We selected three co-occurring tree species known to produce leaf litter of contrasting quality. Ash (*Fraxinus excelsior* L.) and beech leaves are among the fastest and slowest decomposing species within this region, respectively, which is consistent with their N content (Lecerf *et al.*, 2007b; Table 1). Hazel (*Corylus avellana* L.) was chosen for its intermediate degradability and toughness as indicated by acid detergent fibre content, i.e. fibre containing cellulose and lignin (Table 1). These three leaf species were exposed in the stream alone and in all possible species combinations.

Table 1 Total leaf dry mass (mean and SE) enclosed in mesh bags for the seven experimental assemblages of leaf species. Initial leaf chemistry (N, C, ADF) and percent dry mass of the component leaf species in mixtures are also given

Treatments	Initial DM (g)	N (% DM)	C (% DM)	ADF (% DM)
Single species				
Beech	6.0 (0.1)	1.2 (0.1)	47.3 (0.7)	67.9 (0.1)
Hazel	4.5 (0.1)	1.7 (0.1)	45.9 (0.9)	47.8 (0.2)
Ash	6.3 (0.1)	1.8 (0.1)	43.2 (0.2)	34.5 (0.8)
		Beech (% DM)	Hazel (% DM)	Ash (% DM)
Mixtures				
Hazel + ash	5.4 (0.1)		42	58
Beech + hazel	5.5 (0.1)	59	41	
Beech + ash	6.4 (0.1)	51		49
Beech + hazel + ash	5.8 (0.1)	38	26	36

N, nitrogen; C, carbon; ADF, acid detergent fibre content of each species in percentage of leaf dry mass (mean and SE).

Freshly abscised leaves were collected from the ground. These leaves were returned to the laboratory shortly after collection. Leaves were not air-dried before leaf bag construction in order to preserve their physical and chemical integrity (Gessner, 1991). Due to differences in the moisture content of the leaf species, we standardised leaf batch size by leaf area. The total size of each batch of leaves was 1116.5 ± 6.5 cm² (mean ± SE), which was divided evenly among component species in mixtures. Leaf area was estimated by weighing the fresh leaves and using the mass-to-surface area ratios determined for this study. On a dry mass basis, the beech–ash mixture contained equal amounts of both, whereas other mixtures contained a lower proportion of hazel leaves than of beech or ash (Table 1).

The leaf packs were enclosed in 10-mm mesh bags constructed of rigid plastic net and closed in a tetrahedral shape (Jonsson, Malmqvist & Hoffsten, 2001). They were stored overnight in plastic bags at 4 °C and then transported to the field. Iron bars driven into the sediment were used to secure the bags in the stream. The seven species combinations were replicated four times using four shallow riffles as random blocks. Leaf bags were harvested after 10, 40

and 81 days of exposure in the stream to achieve roughly 25%, 50% and 75% of leaf mass loss in the three-species mixture. The samples were stored individually in zip-lock bags and transported to the laboratory in a coolbox. Four extra batches of each leaf species were used to determine the initial leaf dry mass by unit of leaf fresh mass.

Leaves were carefully washed with tap water to remove fine debris and sediment. The invertebrates retained in a 0.5-mm sieve were preserved in 70% alcohol until processing. Remaining leaves in mixtures were sorted by species, and each leaf species was analysed individually for leaf dry mass and microbial parameters. For the determination of leaf-associated microbial respiration rate and mycelial biomass, two sets of five 10-mm leaf discs were cut from three to five leaves of each species (avoiding central veins). Due to rapid decomposition of hazel and ash leaves, there was insufficient leaf material remaining to cut whole discs of these species from several samples collected at the second and third sampling occasions. The remaining leaf material was oven-dried at 105 °C for 48 h and weighed by species to the nearest 0.01 g.

Invertebrates

Shredders were identified to genus and counted (Tachet *et al.*, 2000). Other taxa with different feeding habits and diets were disregarded. After measurement of animal length to the nearest 0.5 mm, individual body mass was calculated using body length–dry mass relationships for European fauna (Smock, 1980; Meyer, 1989; Burgherr & Meyer, 1997).

Microbial parameters

Microbial respiration was measured as the rate of oxygen consumption by microbial communities in decomposing leaves incubated at 10 °C. Five leaf discs of each species were placed in 3 mL of filtered (Whatman International, Florham Park, NJ, U.S.A.; 0.45 µm pore size) stream water in respiration chambers (Strathkelvin Instruments, Motherwell, U.K.). Oxygen concentration was recorded every second over a 1-h period. Oxygen consumption rate ($\mu\text{g O}_2 \text{ g}^{-1} \text{ leaf DM s}^{-1}$) was estimated from the slope of the regression line of oxygen concentration versus time and related to leaf disc mass. This value was corrected for oxygen loss in a control chamber without leaf discs.

Mycelial biomass in leaves was assessed through the content of ergosterol (Gessner & Chauvet, 1993). The method required a chemical extraction of ergosterol in each set of leaf discs, purification of the extracts using a solid-phase extraction cartridge and ergosterol quantification by high-performance liquid chromatography (procedure slightly modified from Gessner, 2005). Mycelial biomass was calculated by applying a general conversion factor of 182 to the ergosterol mass. Results were expressed as fungal mass per leaf dry mass.

Statistics

An exponential daily decay rate (k) was calculated for each species decomposing alone or in mixtures, using a nonlinear regression of the proportion of leaf mass remaining versus time. Better R^2 for all regressions were achieved with a floating intercept, which did not significantly differ from the theoretical value of 1. Decomposition rates of a leaf species were compared among species combinations using generalised linear models based on the log-link function (Lecerf & Chauvet, 2008a). When k -values differed among species combinations according to a likelihood type III test, we conducted a likelihood type I test and sequential introduction of categorical variables to perform planned contrasts.

We used a two-way factorial ANOVA to assess differences in shredder biomass per bag across leaf species combinations and exposure time. We then conducted a stepwise multiple regression to assess the relationship between shredder biomass and leaf mass remaining. A forward selection procedure was applied to identify the most important predictor among inter-correlated variables.

Observed shredder biomass in leaf mixtures was compared to the expected value from the component species exposed alone. This was calculated as the mean of total shredder biomass (by leaf mass) found in single species bags from the same riffle, weighted by the leaf mass remaining of each component species in mixtures (e.g. Lecerf *et al.*, 2007b). Leaf mixing effects on the total number of shredders were assessed in the same way.

We compared shredder assemblages between species combinations using abundance data pooled across sampling dates and riffles. An individual-based rarefaction algorithm was used to correct generic richness for differences in the total number

of invertebrates (Gotelli & Entsminger, 2008). We used the Mann–Whitney test to assess differences between treatment groups. Besides differences in diversity, we assessed differences in both structure and composition of shredder assemblages using the Bray–Curtis similarity index.

We assessed treatment effects on the body mass of the dominant shredder. We first calculated the median body mass of individuals for each leaf bag. The median was preferred over the mean due to a skewed distribution of body mass (Sokal & Rohlf, 1981). ANOVA was then used to compare median body mass per leaf bag between treatments. Since the median was determined on samples of different size, each median was weighted by the ratio of the number of individuals per leaf bag to the grand sum of individuals.

A two-way factorial MANOVA was used to assess variation in microbial parameters on a given leaf species, between species combinations and across time.

‘Riffle’ was included as a random factor in the generalised linear models, ANOVAS and MANOVAS

(see Table 2). Total shredder biomass and median body mass of *Potamophylax* were log-transformed to meet the assumptions of ANOVA. Leaf mixing effects on total shredder biomass were indicated when the deviation between observed and expected values was significantly different from zero (one sample *t*-test). STATISTICA 6.0 (StatSoft Inc., 2001) was used for all statistical analyses.

Results

Leaf decomposition

Leaf decomposition rate varied by a factor of up to five among species exposed alone, representing a broad gradient of leaf degradability (Fig. 1). Ash leaves had been decomposed almost entirely before the last sampling date, so the rate for this species was calculated from the first two sampling dates only. The decomposition rate of beech leaves did not differ across species combination (Table 2: *cf.* interaction time-by-combination). In contrast, the decomposition rates of hazel ($P = 0.0043$) and ash ($P = 0.0003$) leaves were not consistent across species combinations (Table 2). Leaves of these two species decomposed up to twice as slowly when alone or mixed together as when mixed with beech leaves (Fig. 1). The presence/absence of beech leaves in leaf bags was thus a significant predictor of the decomposition rates of hazel ($\chi^2 = 9.4$, $P = 0.0021$) and ash ($\chi^2 = 17.8$, $P < 0.0001$). The residual variation of these two generalised linear models was not significantly related to species composition (likelihood type I test: $\chi^2 < 3.7$, $P > 0.156$), indicating that the presence/absence of beech leaves in leaf bags was a key determinant of mixing effects on decomposition.

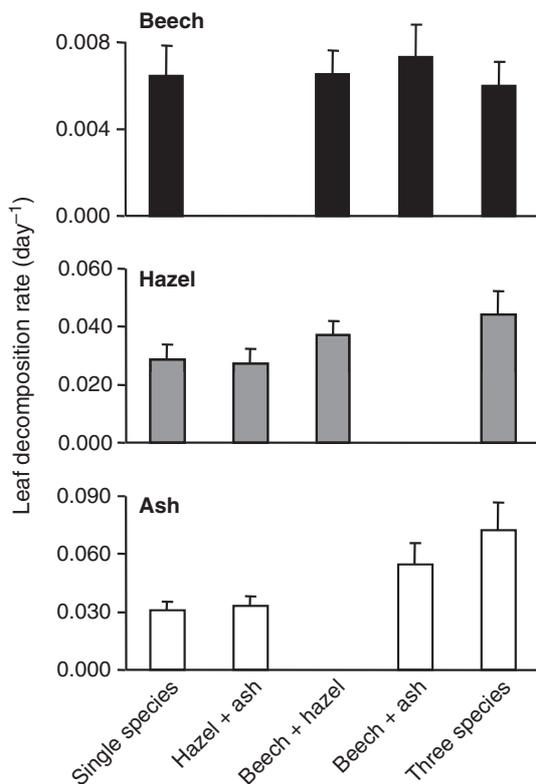


Fig. 1 Decomposition rates of beech, hazel and ash leaves exposed in single- and mixed-species bags. Note that the scales are different on the *y*-axes for each species.

Shredders

Although beech leaves persisted longer in the stream, shredders were half as abundant in beech-only leaf bags as in other species combinations (Table 3). On the first two sampling dates, total shredder biomass in leaf bags was found to be up to seven times lower in beech leaves exposed alone than in other monospecific or species mixture combinations (Fig. 2a). Such a difference was not observed on the later sampling date (Fig. 2a). Indeed, the effect of leaf species combination on total shredder biomass depended on

Table 2 Summary of statistical analyses

Analysis		Time (<i>T</i>)		Species combination (<i>S</i>)		<i>S</i> × <i>T</i>		Riffle	
		Statistic _{d.f.}	<i>P</i> -value	Statistic _{d.f.}	<i>P</i> -value	Statistic _{d.f.}	<i>P</i> -value	Statistic _{d.f.}	<i>P</i> -value
GLZ	Mass remaining of beech leaves	$\chi^2_1 = 68.7$	<0.0001	$\chi^2_3 = 1.7$	0.63	$\chi^2_3 = 0.7$	0.87	$\chi^2_3 = 2.7$	0.44
GLZ	Mass remaining of hazel leaves	$\chi^2_1 = 113.8$	<0.0001	$\chi^2_3 = 0.5$	0.91	$\chi^2_3 = 13.1$	0.0043	$\chi^2_3 = 0.8$	0.85
GLZ	Mass remaining of ash leaves	$\chi^2_1 = 105.0$	<0.0001	$\chi^2_3 = 7.1$	0.0687	$\chi^2_3 = 19.2$	0.0003	$\chi^2_3 = 2.8$	0.42
ANOVA	Total shredder biomass (log)	$F_{2,58} = 7.5$	0.0013	$F_{6,58} = 1.6$	0.16	$F_{12,58} = 2.5$	0.0114	$F_{3,58} = 2.2$	0.0947
ANOVA	Median value of <i>Potamophylax</i> body mass by leaf bags (log)	$F_{2,53} = 16.0$	<0.0001	$F_{6,53} = 4.2$	0.0015	$F_{12,53} = 2.2$	0.0246	$F_{3,53} = 1.5$	0.21
MANOVA	Microbial parameters measured on beech leaves	$F_{4,64} = 15.3$	<0.0001	$F_{6,64} = 1.2$	0.30	$F_{12,64} = 1.3$	0.24	$F_{6,64} = 0.7$	0.63
MANOVA	Microbial parameters measured on hazel leaves	$F_{2,16} = 227.7$	<0.0001	$F_{6,32} = 1.8$	0.12	$F_{6,32} = 4.4$	0.0024	$F_{6,32} = 3.2$	0.0129
MANOVA	Microbial parameters measured on ash leaves	$F_{2,16} = 55.5$	<0.0001	$F_{6,28} = 0.6$	0.71	$F_{2,14} = 1.3$	0.30	$F_{6,28} = 1.1$	0.39

Bold values denote significance at $P < 0.05$.

GLZ, generalised linear model.

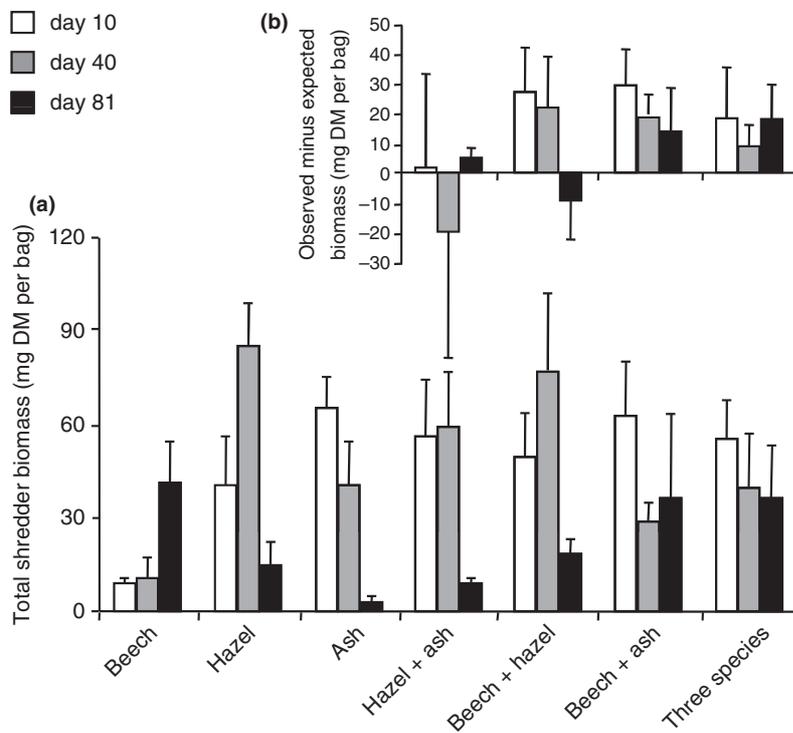


Fig. 2 Total shredder biomass in leaf bags after 10, 40 and 81 days of exposure. (a) Mean (+SE) by species combination. (b) Differences (+SE) between observed and expected shredder biomass in mixed-species bags (see Methods for the calculation of expected values). Deviations from zero indicate non-additive effects of leaf mixing on shredder biomass.

time (interaction time-by-combination, $P = 0.0114$), and species combination *per se* had no significant effect (Table 2). Total shredder biomass significantly varied with time of exposure (Table 2). The amount of high quality leaves (sum of hazel and ash leaf mass) was the only significant variable in the stepwise multiple regression between shredder biomass and leaf mass remaining (F-to-enter = 21.1, $P < 0.0001$). Total leaf quantity and the mass of each individual species were less powerful predictors of shredder biomass in bags (F-to-enter < 10) and did not account for the residual variation ($P > 0.50$).

The total count of shredders from species mixtures with beech leaves was 25–30% higher than expected values calculated from single leaf species bags. In contrast, such a non-additive effect of leaf mixing on shredder abundance accounted only marginally (1%) for the total observed number of shredders in hazel–ash bags. Consistently, shredder biomass in leaf mixtures often deviated from values expected from biomasses associated with the component species exposed alone in the same riffle (Fig. 2b). The deviation between observed and expected values was significantly different from zero overall ($t_{47} = 2.0$, $P = 0.0057$). However, it is clear that the hazel–ash mixture did not behave like the three mixtures with beech leaves (Fig. 2b). In the former, observed shredder biomass was not statistically different than expected ($t_{11} = -0.27$, $P = 0.79$), whereas the latter

(i.e. mixtures including beech leaves) had higher shredder biomass than expected ($t_{35} = 2.9$, $P = 0.0021$).

The shredder assemblage differed among species combinations in terms of diversity and structure. Estimated richness was higher in leaf bags with beech leaves than without (Mann–Whitney test: $P = 0.0339$; Table 3). Shredder assemblages did not strikingly differ among leaf species combinations (Bray–Curtis similarity index; $n = 7$ treatments; grand mean = 82%, SE = 1.9%). Twelve genera of shredders were identified (Table 3). Plecoptera dominated the assemblage numerically, with *Amphinemura* and *Nemoura* together representing more than half of the individuals counted. Non-plecopteran shredders consisted of *Potamophylax*, *Gammarus* and five rarer taxa. *Potamophylax* was found in 74 of the 84 leaf bags, accounting for an average of 72% of the total biomass of shredders. Biomass of other taxa was $< 5\%$ of the total biomass.

Body size structure of the *Potamophylax* population varied in time and between leaf species combinations (Table 2). Median body mass per leaf bag increased from 10 to 40 days in all leaf species combinations (Fig. 3a). It fell below these values from 40 to 81 days in leaf bags without beech and the combination beech + hazel (Fig. 3a). Leaf bags with beech alone, in combination with ash leaves, and the three-species mixtures were colonised by bigger individuals than other leaf bags (Table 2: cf. species combination,

Table 3 Relative occurrences (%) of shredder genera in single- and mixed-species leaf bags (all sampling dates and replicate bags combined)

Genus	Beech	Hazel	Ash	Hazel + ash	Beech + hazel	Beech + ash	All 3 species
Plecoptera							
<i>Amphinemura</i>	38.4	46.3	36.5	42.4	42.1	45.6	34.9
<i>Capnioneura</i>	12.4	8.7	7.5	10.3	7.6	7.7	9.6
<i>Leuctra</i>	9.5	7.2	5.6	5.6	10.4	8.5	7.4
<i>Nemoura</i>	27.2	19.4	31.1	22.2	23.6	22.6	27.7
<i>Protonemura</i>	2.9	2.1	6.8	3.8	2.4	2.7	4.4
Trichoptera							
<i>Notidobia</i>		0.1		0.1	0.1		0.1
<i>Odontocerum</i>						0.2	
<i>Potamophylax</i>	4.3	14.2	11.5	14.1	8.6	10.3	14.1
<i>Sericostoma</i>	0.2		0.1		0.5	0.2	0.1
Other genera							
<i>Gammarus</i>	5.0	1.7	0.9	1.6	4.3	2.2	1.6
<i>Elodes</i>		0.1					0.1
<i>Tipula</i>	0.2	0.1			0.3	0.1	
Abundance	581	1261	1022	1160	997	926	936
Estimated richness	8.7	8.1	7.5	7.4	9.3	9.1	8.6

Richness was estimated for 500 individuals.

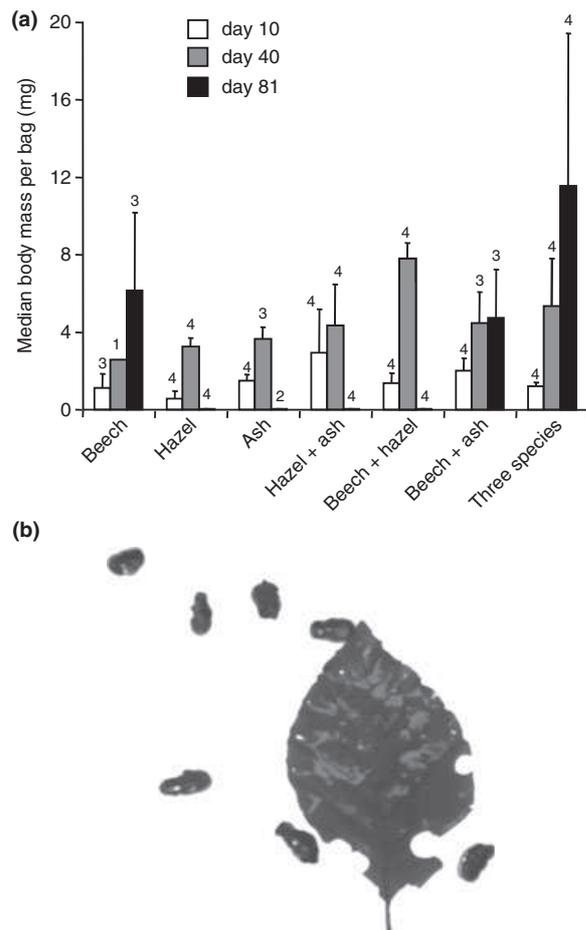


Fig. 3 (a) Median body mass of the cased caddisfly *Potamophylax* (mean + SE) determined after 10, 40 and 81 days of exposure (number of leaf bags colonised by this taxon displayed above bars). (b) Picture of cases of early instars of *Potamophylax* individuals and a beech leaf from which circular leaf discs were cut out by the larvae for case construction.

$P = 0.0015$), a fact that became even more obvious at the last sampling date (Table 2: cf. interaction time-by-combination: $P = 0.0246$; Fig. 3a).

Microbial parameters

Microbial parameters varied across leaf species and in time, whereas mixing leaf species had at most a weak and inconsistent effect. Microbial respiration rate on beech leaves was on average half that on hazel and ash (Fig. 4a). Mycelial biomass also differed among leaf species, with beech and ash having higher biomass than hazel (Fig. 4b). The time of leaf exposure was also an important determinant of the variability of microbial parameters across samples of a given leaf

species (MANOVAS; $P < 0.0001$; Table 2). By contrast, microbial parameters did not significantly vary according to species combination *per se* (Table 2). In addition, the time-by-combination interaction did not account for variation in microbial parameters associated with beech and ash leaves (Table 2). There were only subtle differences for hazel leaves at certain time (interaction time-by-combination: $P = 0.0024$; Table 2). Notably, mycelial biomass on the second sampling date was 1.4 times higher in hazel leaves decomposing in mixtures than alone (Fig. 4b).

Discussion

Our results indicate that leaf diversity can considerably alter the decomposition of particular species. Diversity of the riparian vegetation can therefore be an important factor controlling leaf decomposition in streams, since the diversity of leaf litter inputs to streams is primarily controlled by the nature of the riparian vegetation (Cummins *et al.*, 1989; Griffith & Perry, 1991; Lecerf *et al.*, 2005, 2007a). In the same region as the present study, leaf decomposition rate exhibited up to a 2.7-fold difference between streams lined by species-poor and species-rich riparian forest (Lecerf *et al.*, 2005). We found that leaf mixing consistently produced up to a twofold increase in the decomposition rate of single leaf species. Swan & Palmer (2004) reported antagonistic leaf diversity effects but, more importantly, there were large deviations between observed and expected decomposition rates of their leaf mixtures. Such fairly large diversity effects should be considered in perspective, however, as they are small in comparison to intra- and inter-specific variability in leaf degradability, which can range over orders of magnitude (Webster & Benfield, 1986; Lecerf & Chauvet, 2008b; Hladysz *et al.*, 2009).

In this study, the most refractory leaf species (beech) enhanced the decomposition of the fastest decomposing leaf species (hazel and ash). Our finding challenges our prior hypothesis, which was based on the general assumption that the highest quality leaves enhance the decomposition of the lowest quality leaves (Gartner & Cardon, 2004). The occurrence of labile leaves, similar to these in the present study, resulted in accelerated decomposition of refractory leaves in previous research (Lecerf *et al.*, 2005). Our result is consistent with preferential feeding by shredders, however, a mechanism reported from a

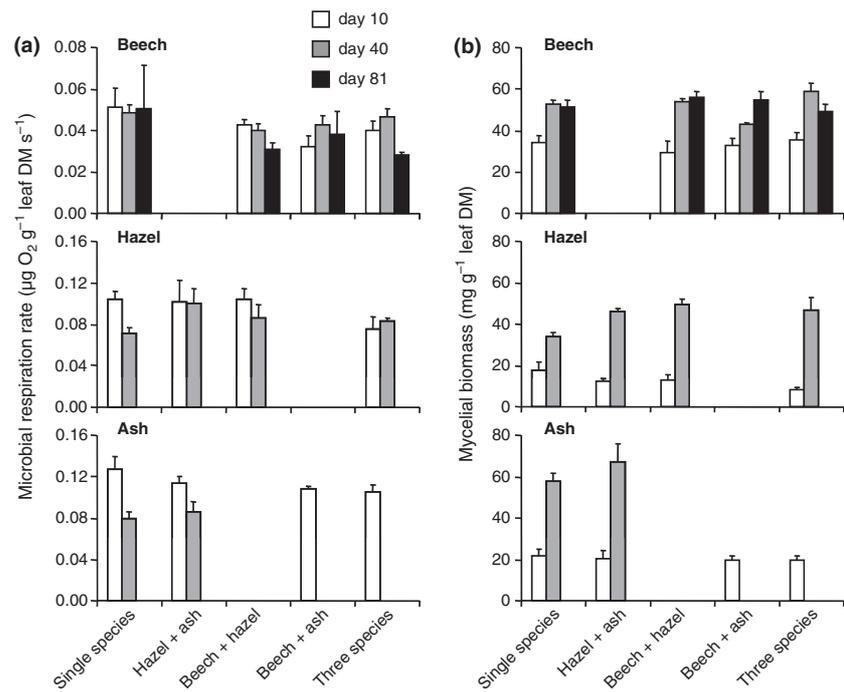


Fig. 4 Microbial respiration rate (a) and mycelial biomass (b) of beech, hazel and ash leaves exposed in single- and mixed-species bags and determined after 10, 40 and 81 days of exposure (mean + SE). Missing data for hazel and ash on the second and third sampling dates are due to insufficient leaf material remaining to determine microbial parameters.

microcosm study (Swan & Palmer, 2006b). The rationale is that, when a choice of leaf quality is offered to shredders, they consume preferentially the highest quality leaves available. If so, this mechanism would have been accompanied by a sharp decrease in the decomposition rate of beech leaves as a result of shredders aggregating on the highest quality leaves. Rather, beech leaves were decomposed at a fairly similar rate across all species combinations, suggesting that additional mechanisms mediated leaf mixing effects on decomposition observed in this study.

Beech leaves significantly influenced leaf mixing effects on decomposition, making it a key species (*sensu* Wardle *et al.*, 1997). Physical traits rather than nutritional value *per se* may have been the determinant of this effect. It is obvious that shredders avoid eating beech leaves, at least in the initial stages, i.e. when leaves have not yet been conditioned by microbial decomposers (Dobson, 1994; Dangles & Chauvet, 2003). Shredders can take advantage of the high structural stability of beech leaves, however, which may enhance invertebrate diversity (Table 3; Dobson, 1994). The importance of refractory leaves in streams is also highlighted by the fact that early instars of the cased caddisfly *Potamophylax* used beech leaves almost exclusively for case construction (Fig. 3b; also noted by Otto & Svensson, 1980). This behaviour did not directly alter decomposition of

beech in leaf mixtures (Fig. 1), probably because of a limited demand for case materials by *Potamophylax* in our bags. The selection of the strongest and most durable materials available for case construction may reduce energy cost of case maintenance/renewal (Kochi & Kagaya, 2005). In addition, cases made of beech leaves are not attractive to other shredders, which reduces the risk of case material being eaten.

The presence of beech leaves in species mixtures also implied that co-occurring leaf species (hazel and/or ash) were less abundant relative to single species bags, whereas shredder biomass was broadly similar in these bags. The ratio of shredders-to-preferred resource (hazel and ash leaves) was thus increased by the presence of beech leaves, which probably accounted for the accelerated decomposition rate of hazel and ash leaves in such mixtures. Accordingly, functional evenness of leaf mixtures (i.e. the balance between refractory and palatable leaves) may be as important as the number of functional leaf types (refractory versus palatable) or leaf species richness in mediating leaf diversity effects. Disentangling the effects of the different facets of plant diversity on leaf decomposition should help to elucidate how changes in the riparian vegetation, for instance by the invasion of non-native plants or by forest management, may alter in-stream litter dynamics (Lecerf *et al.*, 2005, 2007a).

Leaf mixing influenced shredders more than it did microbial consumers. It is thus unlikely that microbial consumers directly mediated leaf mixing effects on decomposition. Consistently, Swan & Palmer (2006a,b) did not find any clear evidence from their microcosm experiments that microbial decomposition leads to non-additive effects. The small and inconsistent effect of leaf mixing on mycelial biomass and microbial respiration in our study may indicate that micro-environment conditions on the surface of leaves were not altered in mixtures or, if so, that resident communities were relatively insensitive to these alterations. This is apparently opposite to predictions that the performance of microbial communities on low-quality leaves should have been enhanced by the release of soluble nutritional compounds from high quality leaves in mixtures (Gartner & Cardon, 2004). However, such a transfer of soluble compounds among leaf species is more likely to occur in soils or standing waters than in running waters, where leaf leachates are constantly washed out.

To conclude, our study lends support to the prediction that riparian vegetation diversity is important for stream ecosystem processes (Cummins *et al.*, 1989). Consistent with our data and previous studies (Lecerf *et al.*, 2005; Swan & Palmer, 2006a,b), shredders may be instrumental in mediating plant diversity effects on litter decomposition. We propose that mixing leaf species of contrasting quality can alter shredder populations and assemblages through improvements to resource quality and microhabitat. It is, however, intriguing that diverse litter types did not affect the use of refractory leaf species (beech) by consumers in this study. Further investigations should elucidate the mechanisms involved in the decomposition of low-quality leaf species alone and in species mixtures in a longer-term study than the one reported here.

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