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Relevance of large litter bag burial for the study of leaf breakdown in the hyporheic zone

Pierre Marmonier · Christophe Piscart · Pierre Emmanuel Sarriquet · Didier Azam · Eric Chauvet

Abstract Particulate organic matter is the major source of energy for most low-order streams, but a large part of this litter is buried within bed sediment during floods and thus become poorly available for benthic food webs. The fate of this buried litter is little studied. In most cases, measures of breakdown rates consist of burying a known mass of litter within the stream sediment and following its breakdown over time. We tested this method using large litter bags (15 × 15 cm) and two field experiments. First, we used litter large bags filled with *Alnus glutinosa* leaves (buried at 20 cm depth with a shovel) in six stations within different land-use contexts and with different sediment grain sizes. Breakdown rates were surprisingly high (0.0011–0.0188 day^{-1}) and neither correlate with most of the physico-chemical characteristics measured in the interstitial habitats nor with the land-use around the stream. In contrast, the rates were negatively correlated with a decrease in oxygen concentrations between surface and buried bags and positively correlated with both the percentage of coarse particles (20–40 mm) in the sediment and benthic macro-invertebrate richness. These results suggest that the vertical exchanges with surface water in the hyporheic zone play a crucial role in litter breakdown. Second, an experimental modification of local sediment (removing fine particles with a shovel to increase vertical exchanges) highlighted the influence of grain size on water and oxygen exchanges, but had no effect on hyporheic breakdown rates. Burying large litter bags within sediments may thus not be a relevant method, especially in clogged conditions, due to changes induced through the burial process in the vertical connectivity between surface and interstitial habitats that modify organic matter processing.

Keywords Rivers · Groundwater · Organic matter cycling · Connectivity · Land-use

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Introduction

The input of dead leaves from terrestrial riparian forests is the major source of organic matter in many low-order forested streams (Kaushik & Hynes, 1971; Cummins, 1974; Vannote et al., 1980; Polis et al., 1997; Boulton & Foster, 1998), and it may represent up to 95% of the total carbon input for such streams (Fisher & Likens, 1973; Hall et al., 2000). Anthropogenic disturbances of aquatic ecosystems can severely affect the rate of litter processing (Sponseller & Benfield, 2001; Gessner & Chauvet, 2002; Piscart et al., 2009). Such disturbances are related to decreases in litter quantity and quality (Benfield et al., 1991; Lecerf & Chauvet, 2008a), changes in nutrient availability that modify microbial activities (Lecerf et al., 2006; Piscart et al., 2009), modifications in the density, and composition of microbial and invertebrate assemblages (Hagen et al., 2006; Lecerf & Chauvet, 2008a, b; Piscart et al., 2009), or physical disturbances of the bed sediment.

Among physical perturbations, the deposition of suspended sediments that leads to leaf litter burial can reduce breakdown rates (Crenshaw & Valett, 2002). The movement of sediments in rivers typically occurs during natural floods (Schumm, 1977; Schalchili, 1992; Vanek, 1997; Brunke, 1999), but is also linked to anthropogenic activities such as changes in the land-use, destruction of the riparian zone, or sediment extraction from the river channel (Bramley & Roth, 2002; Roy et al., 2003; Dodds & Whiles, 2004; Downes et al., 2006). Litter burial is frequent in natural and disturbed rivers, but almost nothing is known about organic matter processing within sediments (e.g., breakdown rates and efficiency of the degradation). The few studies that have addressed this question generally highlighted a large decrease in biological activities leading to reduced breakdown rates within sediments compared to surface habitats (Rulik et al., 2001; Crenshaw & Valett, 2002; Tillman et al., 2003; Boulton & Foster, 1998). Most of these studies used experimental burying of leaves or wood. Nothing is known, however, about the effect of this burial method on local characteristics and resulting processes. However, the vertical connectivity between surface water and interstitial habitats is likely to be altered by this practice, and is known to be crucial for biological activities (Dahm et al., 1991; Boulton, 1993; Hendricks, 1993; Findlay, 1995; Boulton et al., 1998).

This study aimed to test the suitability of the benthic litter bag method to assess litter breakdown within sediments through two successive experiments. In the first experiment, we tested the efficiency of large buried litter bags in contrasted river contexts. We buried litter bags at 20 cm depths within the sediment in six streams with different land-uses in their watershed (from forested to intensive agriculture) and with different grain sizes (from sand to pebble substrata). We attempted to relate breakdown rates to water characteristics and invertebrate assemblages in both surface and interstitial habitats to evaluate the relevance of burying litter bags for the determination of hyporheic breakdown rates. If the litter bag method was appropriate, we predicted significant correlations between decomposition and both interstitial characteristics and land-use. In a second experiment, we studied the effects of burying through a modification of grain size characteristics of the river sediments surrounding the large litter bags. We buried litter bags in six riffles at two stations (surrounded by forest or agriculture), three riffles were slightly modified when litter bags were carefully buried (reference riffles), while fine particles were removed from the bottom sediment in the three others (modified riffles). If the method used was relevant, we predicted a significant decrease between surface and interstitial breakdown rates in control riffles, while this difference was expected to be less in modified riffles.

Materials and methods

Study sites

In the first experiment (Exp 1), four sites (FF, GF, IC, and IF; Fig. 1) were chosen in the Long Term Ecological Research site of Pleine-Fougeres (LTER of “Continental Brittany”, Western France). This area consisted of a patchy landscape with forests, pastures, crop cultures, and farming zones, exhibiting a wide gradient of agricultural pressures (Burel et al., 2003). Two other sites (FC and GC; Fig. 1) were chosen 50 km to the west, in the forest of Broceliande (FC) and in a dairy farm region (GC). This resulted in two sites located in a forested area (noted F: FF and FC), two in grassland area devoted to cattle farming (noted G: GF and GC), and two in intensive

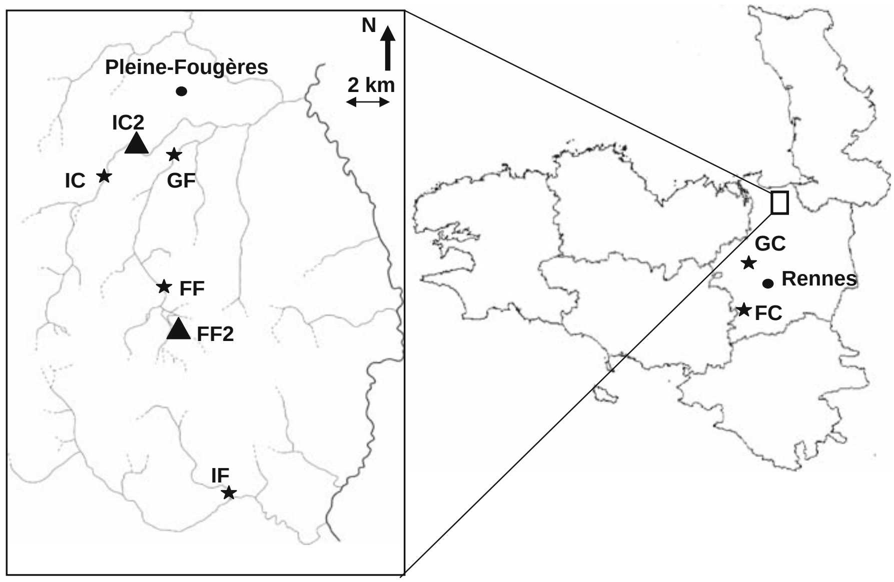

Fig. 1 Location of the stations of the first (*stars*) and second (*triangles*) experiments in Brittany (W. France)

agriculture area (noted I: IF and IC, Table 1). At each site, a riffle was chosen that was long enough to bury the 12 litter bags needed for the determination of breakdown rates (four exposure times × three replicate bags).

In the second experiment (Exp 2), two sites were chosen with similar sediment grain size (dominated by sand and gravel) in the LTER area, one in a forested area (FF2) and one surrounded by intensive agriculture (IC2; Fig. 1). At each site, six riffles were selected: three were not modified, except when litter bags were buried within sediments (noted "control"). In the three other sites (noted "modified"), a 15 cm layer of bottom sediment was removed with a shovel in a 1-m^2 plot, washed in a basket with clear water to remove fine particles (silt and sand) and placed back in the river. During the manipulation, invertebrates were removed together with fine particles. We chose this very destructive method to insure a massive hydrological connection between surface and interstitial water. Three litter bags were then buried at 15 cm depth in each riffle to evaluate leaf litter breakdown rates using three durations of exposure.

Land-cover patterns were established by coupling geographical data from 1:25,000 maps and field observations. The database was processed using a Geographical Information System (ArcView 8.2; http://www.esri.com) to assess the surface area (in percent) of each land-cover type within a 300 m diameter circle around each site (Table 1).

Water and sediment characteristics

For both experiments, three water samples were collected at each site directly for the surface water, three others at a 10 cm depth in the sediment for the interstitial water, and three samples were taken from inside three litter bags, after 1 month for the first experiment ($n = 54$) and at each sampling date for the second experiment ($n = 108$). Surface water was collected directly from the flowing water. Interstitial water was sampled with a syringe and three screened mini-piezometers (1 m long, 1.7 cm diameter, and 3 cm screen length) pushed to a 10 cm depth into sediment using an internal metallic rod (Boulton, 1993; Dahm & Valett, 1996; Lefebvre et al., 2004). Water from the litter bags was sampled using a thin plastic tube inserted between leaves before bags were buried and long enough to reach the surface of the stream to be sampled with a syringe. The three bags equipped with these tubes were removed at the last sampling date.

Water temperature, electrical conductivity (LF92, WTW™, Weilheim, Germany), pH, and dissolved oxygen content (HQ20, HACH™, Dusseldorf, Germany) were determined in the field. In the laboratory, filtered water samples (GF/C, 1.2 µm pore size, Whatman™, Maidstone, UK) were analyzed by colorimetric methods: molybdate–antimony for Soluble Reactive Phosphorus (P-SRP; Murphy & Riley, 1962), indophenol blue for ammonium (N-NH_4^+; Rossum &

Table 1 Percentage of land-use type (buffer of 300 m around the station) and surface water chemical characteristics of the six stations studied (mean ± SD)

	Built	Water	Forest	Agri.	Grass	W.land	Cond.	pH	$N\text{-}NO_3$	$P\text{-}PO_4$	20–40 mm (%)	2–20 mm (%)	0.2–2 mm (%)	<0.2 mm (%)
FC	0	3	93	0	4	0	94.1 ± 2.0	5.3 ± 0.5	0.7 ± 0.3	10 ± 10	21.2	61.0	16.4	1.4
FF	1.6	2	96.4	0	0	0	145.2 ± 2.6	6.7 ± 0.4	0.6 ± 0.04	46 ± 7	2.6	29.2	50.4	17.8
GC	7.5	2	6	30.4	51.8	2.4	335.3 ± 12.2	7.2 ± 0.1	5.3 ± 1.1	103 ± 3	42.4	53.3	4.0	0.4
GF	3.4	0	13	30.3	48.4	5	284.3 ± 38.6	7.4 ± 0.2	3.7 ± 0.5	103 ± 4	0	35.8	55.4	8.8
IC	10.1	0	6.8	48.3	34.7	0	295.6 ± 13.4	7.0 ± 0.2	4.3 ± 0.5	54 ± 1	71.9	1.0	12.9	0.3
IF	6.7	0.4	18.1	52.2	21.5	1.1	225.8 ± 21.2	6.9 ± 0.5	2.6 ± 0.6	82 ± 16	0	28.1	59.8	2.2

Built villages and roads, *Water* ponds and rivers, *Forest* forested area, *Agri.* cultivated fields, *Grass* grasslands, *W.land* waste lands not used for agriculture production, *Cond.* electrical conductivity (in µS cm^{-1}), *$N\text{-}NO_3$* nitrate (in mg N l^{-1}), *$P\text{-}PO_4$* phosphate (in µg P l^{-1}). Grain size fractions (in %)

Villaruz, 1963), diazotation for nitrite ($N\text{-}NO_2^-$; Barnes & Kollard, 1951), and nitrate after a reduction to nitrite by activated cadmium ($N\text{-}NO_3^-$; APHA, 1976).

Sediment grain sizes were measured from 500 g dried samples (48 h, 105°C) and the results expressed as percentages of fine grain size (<200 µm), two classes of medium size (between 0.2 and 2 mm and between 2 and 20 mm), and coarse (20–40 mm) particles. All particles larger than 40 mm were excluded due to the reduced size of the samples (Beschta & Jackson, 1979). The three sites with coarse sediment (noted C) had bottom layers dominated by coarse gravel (IC; Table 1) or fine gravel (FC and GC). The three sites with fine sediment (noted F: FF, GF, and IF) were dominated by coarse sand (Table 1). Site FF had the highest content of fine particles. For each landscape, we thus studied both a fine and a coarse sediment station. In order to evaluate the vertical connectivity between the stream and the hyporheic zone, we calculated the decrease (expressed as a %) in dissolved oxygen concentrations between the surface water and (i) the interstitial water and (ii) the water pumped from inside the litter bags.

Leaf litter decomposition and invertebrate sampling

The litter bag method (Chauvet, 1987; Boulton & Boon, 1991) was used to assess breakdown rates. Freshly fallen leaves of *Alnus glutinosa* were collected in December from adjacent forests. A total of 1 ± 0.05 g (Exp 1) and 4 ± 0.05 g (Exp 2) of air-dried leaves were enclosed in mesh bags (15 × 15 cm size, 6-mm mesh). The coarse mesh allowed large shredders such as Gammaridae and Limnephilidae to enter the bags and feed on leaves (Piscart et al., 2009). Twelve bags (Exp 1) and nine bags (Exp 2) per station were buried within bottom sediments from the beginning of January to the end of February: three bags were retrieved after 7, 14, 28, and 53 days (Exp 1) or 7, 28, and 42 days of immersion (Exp 2). In order to reduce the disturbance of the river bed and the modification of the sediment structure, we carefully introduced a fine spade obliquely into the sediment and slowly pushed the litter bags below the spade. In the second experiment, 18 additional litter bags (i.e., three replicates for each date at each site) were exposed at the surface of sediment and removed at the same dates as for buried litter bags. In order to reduce invertebrate loss, a Surber net (0.5 mm mesh size) was placed downstream during litter bag removal. Upon retrieval, leaves were washed individually to remove sand, exogenous organic matter, and invertebrates. The remaining leaf material was dried at 105°C for 24 h and weighed to the nearest mg. For each experiment, three control litter bags were subjected to a short immersion in the streams and drying at 105°C for 24 h to estimate the initial dry mass of leaf litter and the handling loss. The exponential leaf litter breakdown rate (k) was calculated using the relationship

$$M_t = M_i * e^{-k*t},$$

where M_t is the leaf dry mass remaining at time t and M_i the leaf dry mass at the initial time (Petersen & Cummins, 1974).

The invertebrates that colonized buried leaves in both experiments were preserved in 70°C ethanol. The assemblages present in the benthic layer were characterized from three samples taken in each stream using a Surber net sampler (0.375 m^2; Exp 1) or from the six litter bags sampled after 42 days of incubation in the benthic layer (Exp 2). Invertebrates were identified to the genus or species levels when possible, except for Diptera (family) and Nematoda, Oligochaeta, and Copepoda (not identified further). Shredders were counted separately (using the biological traits given in Tachet et al., 2000).

Statistical analyses

In the first experiment, Spearman's rank correlations were used to test the relationship between land-cover and nutrient load. In both Exp 1 and Exp 2, differences in physico-chemical characteristics between stations were tested using one-way repeated measures ANOVAs and pair-wise comparisons were investigated through a post-hoc analysis based on Tukey's HSD test. ANCOVAs on log-transformed % remaining mass data were performed to compare breakdown rates among sites in Exp 1 and locations in Exp 2 (litter at the surface or at 15 cm depth in control and modified riffles). Tukey's HSD tests were used for pair-wise comparisons.

In each experiment, we computed biocenotic indices (i.e., total taxonomic richness, total abundance, abundance of shredders, and abundance of *G. pulex*). Differences in the biocenotic indices between sites (Exp 1) and locations of litter bags (Exp 2) were tested using Kruskal–Wallis nonparametric ANOVAs. In this case, changes between two pair-wise comparisons were investigated through a post-hoc analysis based on multiple comparison tests (Siegel & Castellan, 1988) using procedures from Statistica 7.1 (Statsoft, 2005).

Results

Experiment 1: litter breakdown rates and stream characteristics

The two forested sites (FC and FF) were located in areas where forest represents more than 90% of the land surface (Table 1). Here, the water had low electrical conductivity (<150 $\mu S\ cm^{-1}$), acid pH, and low nutrient contents (<1 mg $N\text{-}NO_3\ l^{-1}$ for and <50 $\mu g P\text{-}SRP\ l^{-1}$). The two sites located in the dairy farm region (GC and GF) were surrounded by 50% grassland (Table 1), with water characterized by high electrical conductivity, basic pH, and high nutrient concentrations (e.g., $P\text{-}SRP > 100\ \mu g\ l^{-1}$ at both sites). Finally, the two sites located in the intensive agriculture area (IC and IF) were surrounded by 50% cultivated fields (mainly maize and wheat), with water characterized by high electrical conductivity, neutral pH, and intermediate nutrient concentrations (Table 1). The mean $N\text{-}NO_3$ concentrations not only correlate with the percentage of cultivated fields but also correlate with the percentage of grassland devoted to intensive cattle farming ($r^2 = 0.905$, $P < 0.01$).

Oxygen concentrations decreased significantly between the surface (ranged from 10.4 to 11.3 mg l^{-1}) and interstitial habitats at all stations (ranged from 2.7 to 9.5 mg l^{-1}; $F_{1,4} > 75.7$; $P < 0.001$). SRP concentrations significantly increased with depth at four stations (i.e., IC, FF, GC, and FC; $F_{1,4} > 7.7$; $P < 0.05$) but remained similar at IF and GF ($P = 0.283$ and $P = 0.643$, respectively). Changes in the N concentrations with depth were less homogeneous: $N\text{-}NO_3$ concentrations remained similar at four stations (i.e., FF, FC, IC, and GC; $P > 0.05$), but significantly decreased with depth at the two others (i.e., IF and GF; $P < 0.017$), while N-NH_4 concentrations significantly increased within stream sediment at two stations (i.e., FF and IC; $P < 0.048$), but remained similar at the four others (i.e., IF, GF, GC, and FC; $P > 0.05$).

Water pumped from within litter bags had oxygen and N-NO_3 concentrations ($P > 0.147$) similar to that of water pumped from the interstitial habitats. In contrast, N-NH_4 and SRP concentrations were generally lower in the litter bags than in the interstitial samples. N-NH_4, for example, was slightly lower in samples pumped from within the litter bags than in the sediment ($F_{1,24} = 7.03$; $P = 0.014$) and the SRP concentrations in water pumped from within the litter bag were also lower at five out of the six stations, but this difference was not significant when the stations were considered altogether ($P > 0.05$).

Breakdown rates varied significantly among the six sites (ANCOVA: $F_{5,65} = 4.74$, $P = 0.001$; Fig. 2). They were higher in stations with coarse sediment (0.0188 ± 0.0127 day^{-1} in IC, 0.0011 ± 0.0077 day^{-1} in FC, and 0.0103 ± 0.0063 day^{-1} in GC) than in the sites with fine sediment (0.0073 ± 0.0023 day^{-1} in FF, 0.0074 ± 0.0030 day^{-1} in GF, and 0.0033 ± 0.0040 day^{-1} in IF). These differences resulted in a significant positive correlation between the breakdown rates and the coarse fraction of the bottom sediment (>20 mm; Fig. 3a), and a negative correlation with the estimate of the vertical connectivity between the stream and the litter bags (the percentage of oxygen decrease; Fig. 3b). Breakdown rates were not only significantly correlated with any chemical characteristics of the water pumped from the interstitial habitats of streams ($P > 0.05$) but also they were significantly correlated with the chemical characteristics of both surface water and water pumped from the litter bags. The values of k were negatively correlated with N-NH_4 concentrations in surface water ($r^2 = 0.76$, $P = 0.012$) and within the litter bags ($r^2 = 0.67$, $P = 0.022$), and positively correlated with N-NO_3 in the surface water ($r^2 = 0.81$, $P = 0.007$) and within the litter bags ($r^2 = 0.65$, $P = 0.026$). Concentrations of N-NO_3 in the surface water and within the litter bags were closely related ($r^2 = 0.79$, $P = 0.009$) suggesting again a high vertical connectivity between the stream and the litter bags.

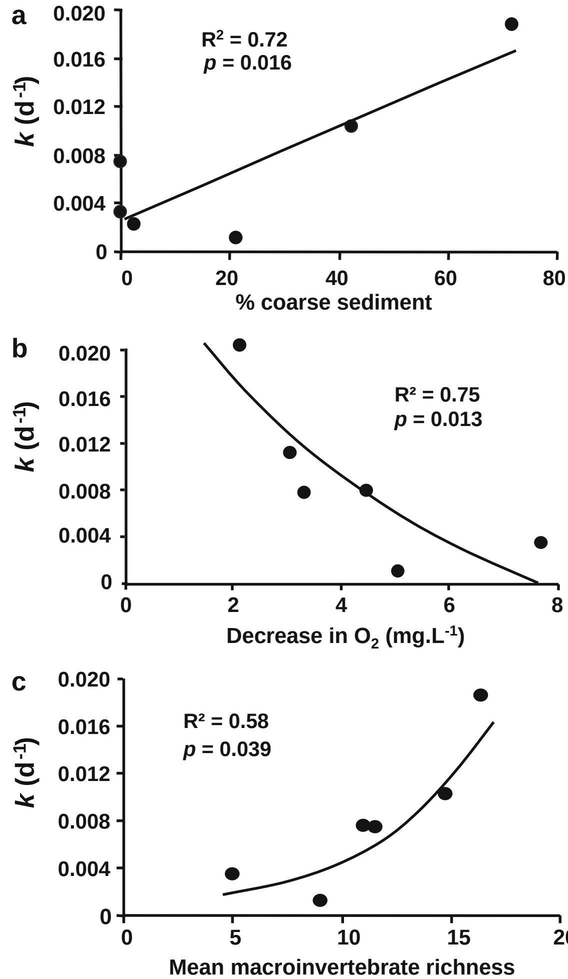

Fig. 3 Relationships between litter breakdown rates (k in day^{-1}) and the percentage of fine sediment (**a**), the decrease in oxygen concentrations (mg l^{-1}) between surface and interstitial waters (**b**), and the mean taxonomic richness measured in the benthic layer (**c**). R^2 are the coefficients of determination of the linear (**a**) or exponential (**b**, **c**) regressions

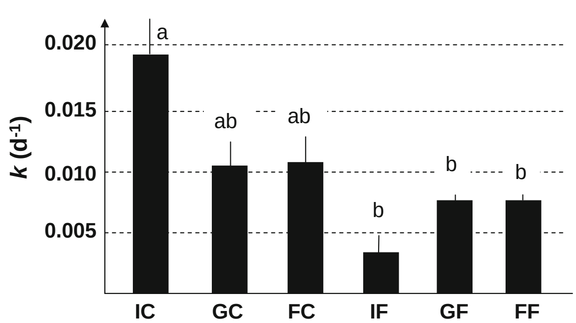

Fig. 2 Mean litter breakdown rates (±SE, $n = 3$) measured within the sediment of the six studied stations ranked according to fine sediment contents (see Table 2)

Table 2 Mean (± SD) taxa richness (S tot) and abundance (Q tot) of invertebrates, and taxa richness (S shred.) and abundance (Q shred.) of shredders sampled in the litter bags (after 28 days of incubation) and in the benthic layer

	Within the litter bags		In the benthic layer			
	S tot	Q tot	S tot	Q tot	S shred	Q shred
FC	6.7 ± 3.1	21.7 ± 2.5	9.1 ± 1.0	50.7 ± 5.5	3 ± 1	11 ± 11.3
FF	2.3 ± 1.5	22.7 ± 17.2	11.5 ± 3.5	260.7 ± 441.1	5 ± 2.8	5.3 ± 5.5
GC	5.7 ± 3.8	16.0 ± 6.0	13.3 ± 5.9	253.3 ± 389.6	6 ± 2	54.7 ± 80.0
GF	5 ± 3.6	114.7 ± 151.9	9.7 ± 0.6	26 ± 12.1	4 ± 1	6.3 ± 4.9
IC	3.0 ± 2.0	5.7 ± 0.6	14.7 ± 4.9	67.7 ± 31.8	9.3 ± 3.1	50 ± 28.7
IF	2.3 ± 2.5	5.7 ± 8.1	12.7 ± 1.5	67.7 ± 39.8	7 ± 1	34.7 ± 33.2
P-values	n.s.	0.026	n.s.	n.s.	n.s.	n.s.
r^2	0.11	0.008	0.53	0.006	0.58	0.49
With *k*	n.s.	n.s.	($P < 0.05$)	n.s.	($P < 0.05$)	n.s.

P-values and *letters* refer to Kruskal–Wallis ANOVAs and result of post-hoc pair-wise comparisons of mean ranks. *Last line*: linear correlations between litter breakdown rates (*k*) and characteristics of the assemblages. Note that sampling methods for litter bags and the benthic layer are different and values cannot be directly compared. n.s. = not significant at $P = 0.05$

The invertebrates in the litter bags showed that significant differences in abundance ($P = 0.026$), although very low (5–114 individuals) and with a low taxonomic richness (2.3–6.7 taxa; Table 2). In the benthic layer, abundance (26–260 individuals) and taxonomic richness (9.1–14.7 taxa; Table 2) were also low due to winter conditions. No significant correlation was found between invertebrate abundance or taxa richness within litter bags and litter breakdown rates (Table 2). However, breakdown rates were significantly correlated with both total invertebrate and shredder taxa richness in the benthic assemblages (Table 2; Fig. 3c).

Experiment 2: modification of the connectivity between surface and interstitial habitats

During the incubation period, slight changes were observed for pH, electrical conductivity, $N\text{-}NO_3$, and $N\text{-}NH_4$ (data not shown), but a complex pattern was observed for dissolved oxygen concentrations. At FF2, dissolved oxygen concentrations in the interstitial habitat (−10 cm deep within the sediment; Fig. 4A) were significantly lower in control riffles and, to a lesser extent, in modified riffles, than in the surface water at all dates ($P = 0.002$ and 0.019, respectively). In modified riffles, oxygen concentrations progressively decreased during the incubation period, from values similar to those in surface water (date 1) to values close to those in control riffles (date 3). In litter bags (Fig. 4B), oxygen concentrations were similar in surface and in both types of riffles at the first date ($P > 0.05$) and then decreased, first in the control riffles (date 2) and then in the modified riffles (date 3).

At IC2, removing fine particles from the bottom sediment of the modified riffles had a slight effect on oxygen concentrations at 10 cm depth (Fig. 4C) those were similar in both types of riffles and significantly lower than in the surface water at all dates ($P < 0.001$). In the litter bags (Fig. 4D), the oxygen concentrations were also significantly lower than in the surface water ($P < 0.02$), but a decreasing pattern with incubation duration was observed in both types of riffles.

Breakdown rates measured at the surface were 2–5 times higher at site FF2 which was surrounded by forest compared to site IC2 which was surrounded by intensive agriculture (Table 3). Breakdown rates in litter bags buried within sediments at site FF2 were lower, although not significantly, than rates measured in the benthic layer (Table 3). Despite a slight increase in leaf litter breakdown rates in modified riffles, the removal of fine particles did not induce a significant increase in these rates.

Invertebrate assemblages sampled in benthic litter bags had similar low densities (around 20 individuals per bag) and low taxonomic richness (around 7 taxa) at

Fig. 4 Mean oxygen concentrations (±SE, $n = 3$) expressed as the percentage of the saturation, measured in surface water (*white*), within sediment (**A** and **C**) and within litter bags (**B** and **D**) of modified (*hatched*) and control (*black*) riffles, in the two studied stations (FF2: **A** and **B**; IC2: **C** and **D**) and at the three sampling dates

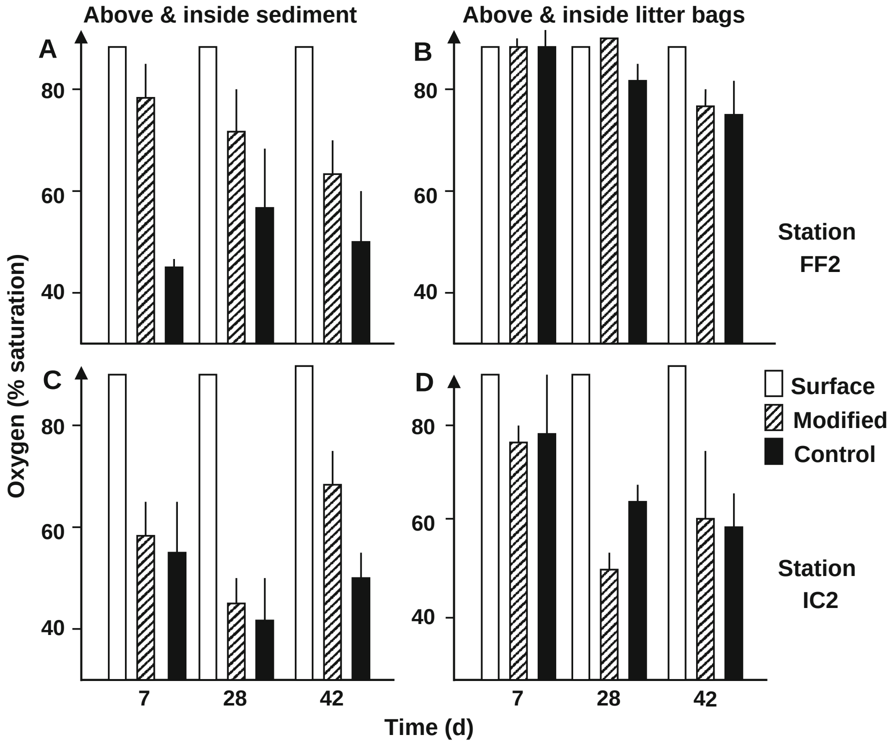

Table 3 Litter breakdown rates (±SD, in day^{-1}) in mesh bags located in the benthic layer or buried in the sediment at the control and at the modified riffles (experiment 2)

	FF2	IC2
Benthic layer	0.0541 (±0.0251)	0.0112 (±0.0079)
Buried in control riffles	0.0273 (±0.0345)	0.0136 (±0.0150)
Buried in modified riffles	0.0358 (±0.0350)	0.0157 (±0.0144)
ANCOVA test	$F_{2.44} = 1.09$; $P = 0.34$	$F_{2.44} = 1.59$; $P = 0.21$

both sites (Table 4). Litter bags buried in the control riffles were even less populated and diversified (without any shredders or *G. pulex* at IC2), while those buried in the modified riffles had an intermediate abundance of total invertebrates ($P < 0.05$ at IC2), taxonomic richness ($P < 0.05$, both stations), and abundance of shredders ($P < 0.05$ at IC2), and the highest abundance of *G. pulex* ($P = 0.05$ in FF2, Table 4). In all cases, no significant correlations were found between leaf litter breakdown rates and mean abundance, mean species richness (for both total assemblages and shredders) or abundance of *G. pulex*.

Discussion

During these two methodological experiments, we tested the suitability of large litter bags (15 × 15 cm) used in the benthic habitat to measure breakdown rates of buried leaf litter within stream sediments. Four observations resulted from this methodology as follows.

(1) There were very high breakdown rates within stream sediments, especially in the first experiment. The k values measured at 20 cm depth within sediment (i.e., from 0.003 to 0.019 day^{-1}) were surprisingly close to the values reported for the benthic zone, by considering either intra-specific variation within alder (0.0129–0.104 day^{-1}; Lecerf & Chauvet, 2008a) or habitat variation among streams (0.0087–0.0410 day^{-1}; Tiegs et al., 2009). Furthermore, in our second experiment, we did not observe any significant decrease in k in the hyporheic zone compared with the benthic layer. However, low values in the hyporheic zone (0.002 and 0.005 day^{-1}) compared with the surface (0.02 day^{-1}) have been reported for alder leaves (Rulik et al., 2001). Similarly, Crenshaw & Valett (2002) observed lower biological activities on wood buried in the hyporheic

Table 4 Mean (±SD) mean taxonomic richness (S) and total abundance (Q total, individuals per bag) and of invertebrate assemblages, mean abundance of shredders (Q shredders), and mean abundance of *G. pulex* (Q Gp) sampled within the litter bags in the benthic layer, in control and modified riffles

Station	Location	S	Q total	Q shredders	Q Gp
FF2	Benthic layer	7.2^{a} (±1.5)	19.0 (±6.7)	6.0 (±6.8)	4.8^{a} (±6.4)
	Buried in control Riffle	3.0^{b} (±1.0)	6.7 (±5.7)	1.7 (±1.1)	0.3^{b} (±0.5)
	Buried in modified Riffle	6.7^{a} (±2.5)	27.7 (±19.0)	18.7 (±15.0)	18.3^{a} (±14.5)
	P-values	$P = 0.048$	ns	ns	$P = 0.05$
IC2	Benthic layer	7.8^{a} (±2.9)	20.5^{a} (±11.3)	3.3^{a} (±2.0)	0.7 (±1.2)
	Buried in control Riffle	2.0^{b} (±1.0)	4.0^{b} (±3.6)	0^{b}	0
	Buried in modified Riffle	$4.7^{a,b}$ (±1.5)	$12.0^{a,b}$ (±8.0)	2.3^{a} (±0.6)	1.0 (±1.0)
	P-values	$P = 0.019$	$P = 0.049$	$P = 0.020$	ns

P-values refer to Kruskal–Wallis ANOVAs between characteristics of invertebrate assemblages sampled in the benthic and buried litter bags, with *letters* referring to post-hoc comparisons of mean ranks of all pairs of groups

zone than on wood located in the benthic layer. The low breakdown rates measured by these authors may be explained by the low availability of dissolved oxygen that supports heterotrophic activities (Chauvet, 1988; Claret et al., 1998; Dahm et al., 1998; Lefebvre et al., 2005) and by changes in invertebrate assemblage composition.

(2) Another surprising result was the linkage between k and sediment characteristics. Leaf litter breakdown rates were indeed higher in streams with coarse bottom sediments compared with sandy sediments and they were significantly correlated with the percentage of gravel (the 20–40 mm fraction). This relationship may be explained by the higher hydraulic conductivity of coarse material compared with sand (Dahm & Valett, 1996) and the resulting higher exchange rate between the surface and the interstitial water in downwelling zones (negative hydraulic heads). In a different, but converging way, Tillman et al. (2003) observed a large decrease in leaf litter breakdown rates in some areas of the hyporheic zone fed by upwellings of groundwater (3-fold less compared with the surface habitat) where the exchanges with surface water were very low compared with downwelling zones. The connection with surface water seems thus crucial for leaf litter breakdown within stream sediment.

(3) The importance of vertical connectivity between surface water and interstitial habitats for litter breakdown rates was also supported by the negative correlation between k and the oxygen depletion between surface water and the litter bags. When the vertical connectivity decreased, the depletion of oxygen increased and the breakdown rate at 20 cm depth decreased. Similarly, Crenshaw & Valett (2002) observed decreases in the biomass of fungi on buried wood together with oxygen concentrations from the surface to the hyporheic zone (just above the main channel) and to the parafluvial zone (in lateral gravel bars). Such a response pattern is consistent with the high sensitivity to water chemistry reported for decomposing wood (Diez et al., 2002). In the second experiment, we observed a very slight decrease in oxygen concentration between the surface water and the water pumped from within the litter bags, especially during the first week of incubation. This suggests a relatively high turnover of the water within bags and thus a high vertical connectivity with surface water, which can stimulate biological activities and enhance breakdown during the first part of the incubation period. Later, the depletion in oxygen increased with the duration of the incubation, suggesting a progressive clogging of the interstices around the bags (Findlay, 1995; Brunke, 1999; Lefebvre et al., 2004) and/or an increase in microbial respiration within and around the bags (Chauvet, 1988; Hendricks, 1993). Overall, the removal of fine particles from the bottom sediments had no or very little effect on breakdown rates measured by large litter bags, which were similar in both types of riffles and in the benthic layer. The burying process of large litter bags finally had the same effect as the direct removal of fine particles.

(4) Finally, breakdown rates were not only correlated with the characteristics of the invertebrate assemblage sampled from within the litter bags but

were also surprisingly related to the taxonomic richness of the invertebrates, especially shredders, present in the benthic habitat and the densities of the amphipod *Gammarus pulex* (Exp 1). This is the first time this link between *k* values in the hyporheic zone and benthic macroinvertebrate assemblages has been observed. Rulik et al. (2001) found a link between breakdown rates of buried leaf litter and invertebrate densities, that were much lower in the hyporheic zone (dominated by Diptera Chironomidae and Oligochaeta Tubificidae) than in the benthic layer (dominated by *Gammarus roeselii*). Similarly, Crenshaw & Valett (2002) observed a decrease in invertebrate densities from the surface (highest breakdown rates) to the hyporheic, and to parafluvial zones (lowest breakdown rates). Predictions made in the introduction of this article must therefore be rejected; we neither observe significant correlations between breakdown rates and interstitial characteristics nor with the land-use surrounding the streams (experiment 1, prediction 1), and we do not report significant differences between surface and interstitial breakdown rates in control riffles compared with the modified ones (experiment 2, prediction 2). Moreover, most of our observations support an increase in vertical connectivity between surface water and buried litter bags, for both water and invertebrates. On one hand, some of the fine particles were certainly washed out of the area during the burying of litter bags, resulting in a local increase in the hydraulic conductivity of the sediment. On the other hand, the presence of large, although mostly flat, litter bags in the substratum certainly modifies water circulation within the sediment per se, increasing again the exchange rates with the surface.

In conclusion, the benthic litter bag method therefore appears unreliable for the quantification of leaf litter breakdown rates within the stream sediment, especially in fine substratum or clogged conditions. Future research must include the elaboration of another method that would aim at reducing sediment disturbance during litter bag burial. This method must take into consideration the following three constraints that emerged out of this first study: (i) the structure that contains the litter must be small enough to avoid changes in sediment structure, (ii) the litter bag apertures must be large enough to allow invertebrates access to the litter, and (iii) the way litter bags will be incorporated into the sediment must reduce the loss of fine particles and the resulting changes in vertical connection. A possible answer to these technical constraints could lie in the use of small mobile piezometers allowing small litter bags to be introduced into the sediment without disturbing its structure. The size of the bags and the quantity of litter used for breakdown measurements must be carefully tested.

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