Arch. Hydrobiol. **165** 4 493–513 Stuttgart, April 2006

Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves

Verónica Ferreira[1]*, **Manuel A. S. Graça**[1], **João L. M. P. de Lima**[2] and **Ricardo Gomes**[2]

Universidade de Coimbra

With 6 figures and 7 tables

Abstract: We evaluated the relative importance of current velocity and invertebrate activities in the breakdown rate of alder [*Alnus glutinosa* (L.) Gaertner] leaves. Decomposition experiments were carried out in artificial channels, where current velocity and shredder presence were manipulated, and in a 4th order stream, in both summer and autumn, where litter bags were incubated in several reaches differing in both depth and current velocity. Alder leaves incubated in artificial channels decomposed significantly faster in the presence of shredders than in their absence (k = 0.0368/d *vs.* k = 0.0210/d in low current and k = 0.0472/d *vs.* k = 0.0219/d in high current). However, current (up to 2.35 m/s) had no significant effect on decomposition rates. In channels without invertebrates, no significant differences in k values were found between coarse and fine mesh bags in high (0.20 m/s) and low (0.05 m/s) current. Leaves incubated in the stream during summer, in reaches with current velocity ranging from 0.003 to 1.185 m/s, did not differ in their decomposition rates (k = 0.0489/d to k = 0.0645/d). In autumn, leaves exposed to high current (1.228 m/s) had faster decomposition rate (k = 0.0417/d *vs.* k = 0.0136/d), which may be related to sediment transport during this time of the year or to the tendency for higher number of shredders in high current-shallow reaches.

Key words: alder leaves, shredders, current velocity, decomposition, stream.

Introduction

In low order streams running through forests, the major energy source is allochthonous organic matter, i. e. leaves and other organic material produced

[1] **Authors' addresses:** Departamento de Zoologia and IMAR-CIC, Universidade de Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal.

[2] Departamento de Engenharia Civil and IMAR-CIC, Universidade de Coimbra, 3030-219 Coimbra, Portugal.

* Corresponding author; E-mail: veronica@ci.uc.pt

DOI: 10.1127/0003-9136/2006/0165-0493 0003-9136/06/0165-0493 $ 5.25

by trees in the riparian zone (Vannote et al. 1980, Abelho 2001). In the water, litter decomposition starts immediately and usually proceeds in three overlapping phases: (1) leaching of soluble compounds, which can lead to the loss of up to 42 % of the initial leaf mass (reviewed by Abelho 2001); (2) microbial decomposition, which can be responsible for the loss of about 27 % of leaf mass (Hieber & Gessner 2002); and (3) biotic and physical fragmentation. Biotic fragmentation results from the feeding activities of invertebrates, mainly shredders, which can result in up to 64 % of mass loss (Graça 2001, Hieber & Gessner 2002).

Many studies on litter processing address the effect of microbes *vs.* invertebrates on litter decomposition, using fine and coarse mesh bags implanted in streams. These studies assume the differences in mass loss between bag types as a measure of invertebrate feeding since fine mesh bags exclude invertebrates. However, differences between bag types may also be due to physical fragmentation resulting from abrasion caused by current and transported sediments. This question can be addressed by isolating the effect of current velocity, using artificial channels where current velocity is controlled. This approach was used by Chergui & Pattee (1988), Canton & Martinson (1990), Rader et al. (1994) and Vingada (1995), who reported high decomposition rates in high current. The effect of invertebrate activity on leaf litter decomposition was also addressed by Cuffney et al. (1990), Stewart (1992), Jonsson et al. (2001) and Huryn et al. (2002), by comparing decay rates of leaf species in streams differing in invertebrate densities. However, the compared streams could also differ in other factors besides invertebrate densities.

The relative importance of physical fragmentation and invertebrate feeding activities in experiments with coarse mesh bags is therefore still unclear. Recently, litter breakdown has been proposed to be used as an assessment tool to assess the functional health of aquatic ecosystems (Pascoal et al. 2001, Gessner & Chauvet 2002, Pascoal et al. 2003, Dangles et al. 2004), and since this is usually done by using the litter bag approach it seems crucial to assess the sensitivity of this technique to physical fragmentation so that it would be possible to know to what extent decomposition rates reflect functional health and not physical conditions.

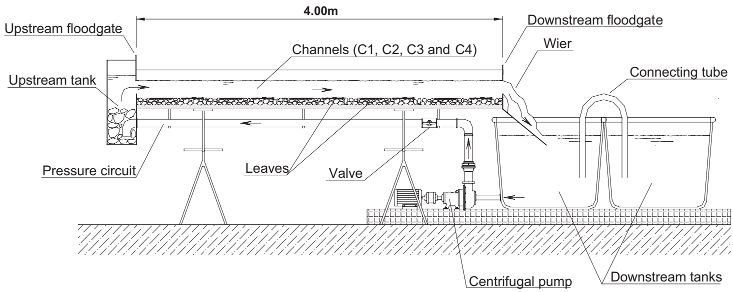
Our objectives with this study were: (1) to investigate the relative importance of physical fragmentation *vs.* invertebrate fragmentation in leaf litter decomposition experiments using the coarse-fine mesh bag approach, in artificial channels where current velocity and invertebrate densities were manipulated; (2) to assess the effect of increasing current velocities in leaf litter decomposition experiments, in artificial channels and (3) to evaluate the inter-habitat and temporal variability (depth and current velocity in summer and autumn) in leaf litter decomposition in a 4th order stream.

Methods

Experiment 1: Physical *vs.* shredder fragmentation in laboratory flumes

To assess the effects of current velocity and invertebrate feeding in leaves, 4 acrylic contiguous artificial channels were used (Fig. 1 a). The indoor channels had a total length of 4 m, width of 0.15 m [surface area (each) = $0.6\,m^2$] and height of 0.20 m (Fig. 1 b). The channels ended in two $0.5\,m^3$ fiberglass reservoirs, to which a centrifuge pump was connected (Fig. 1 a). The substrate was a mixture of stream gravel (2.5–15 cm size) and sand (0.9–2 mm grain size). The water used in the channels was collected from S. João stream (Lousã Mountain, Portugal; N 40° 05′ 59″, W 8° 14′ 02″) five days before the experiment started. By the middle of each experiment (day 8 or 15), $0.5\,m^3$ of stream water were added to compensate for evaporation. When collecting water from the stream, pH, conductivity, and temperature were measured *in situ* using field meters, and 1 L of stream water was collected, in acid washed plastic bottles, for nutrient analysis [NO_3^-, determined by ion chromatography (Dionex DX-120, Sunnyvale, CA), and SRP, determined by the ascorbic acid method (APHA 1995)]

1a



1b

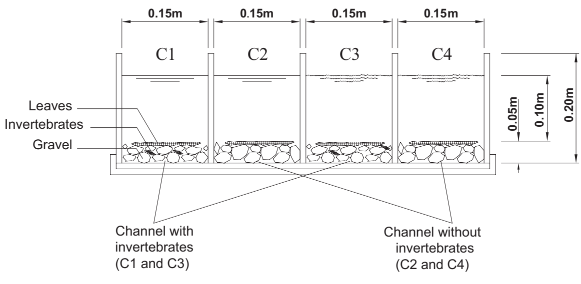


Fig. 1. Scheme of the flume used in the laboratory experiments (the arrows show the direction of flow in the hydraulic circuit; **a**) and cross-section of the channels (channels C1 and C2 had low current velocity while channels C3 and C4 had high current velocity; channels C1 and C3 had shredders; **b**).

and alkalinity determination (by titration to an end point of 4.5; APHA 1995). Current velocity was also measured in several zones of the stream with a current meter (VALEPORT 15277).

As an *inoculum* of aquatic fungi, conditioned leaves (approximately 20 g) were collected from the stream and placed in fine mesh bags in the upstream tank that delivered water to the channels (Fig. 1 a). Current velocity was set to match the maximum measured in zones of the stream where there was organic matter accumulation (0.20 m/s; channels C3 and C4) and to a low value (0.05 m/s; channels C1 and C2). Current velocity was regulated by acrylic barriers located upstream and downstream each channel (Fig. 1 a).

Alder [*Alnus glutinosa* (L.) GAERTNER] leaves were used as a decomposition substrate. Leaves were collected from the same group of trees at Varandas do Ceira (Portugal, N 40° 10′ 20″; W 8° 18′ 10″) just after abscission (4–12 November, 2002). They were air dried and stored dry until needed. Batches of 1 ± 0.25 g of dry leaves (average per bag = 0.85 g; average initial AFDM per bag = 0.71 g) were rehydrated and placed in fine mesh (FM; 10 × 15 cm, 0.5 mm mesh size) and coarse mesh (CM; 10 × 15 cm, 10 mm mesh size) bags. Six bags were set apart to determinate initial ash free dry mass (AFDM). Five fine mesh and 5 coarse mesh bags were placed in each of four artificial channels.

Individuals of the shredder *Sericostoma* sp. (Trichoptera: Sericostomatidae) were added in one low (C1) and one high (C3) current velocity channel, at densities similar to the ones observed in S. João stream, i. e. 100 individuals/channel. This species is generally present and abundant all over the year in the area. At our sampling site (S. João stream, Lousã Mountain), an annual mean of 6.3 g AFDM/m^2 of coarse particulate organic matter (CPOM) and annual mean density of 119 Sericostomatids/m^2 were reported by GONZÁLEZ & GRAÇA (2003). The number of invertebrates in the channels was a compromise between the value expected in terms of area of each channel (71 individuals per 0.6 m^2) and the number expected per amount of organic matter in each channel (138 individuals per 7.3 g AFDM). The density used in the channels was a lower consumer/resource ratio than observed in the stream. Food was therefore assumed not to be a limiting factor.

The animals were in the laboratory for at least one week before they were placed in the channels. In the laboratory they were maintained in plastic boxes, with aerated stream water and with stream sand in the bottom (0.9–2 mm grain size), exposed to a light-dark photoperiod of 12/12 h. During this time they were fed with conditioned alder leaves.

One fine mesh and one coarse mesh bag were retrieved from each channel at days 3, 7, 14, 21, and 28 (these bags will be referred to as Set I). To maintain the same amount of organic matter in the channels, each retrieved bag was replaced with a new one. The replacement bags were taken all at once at day 31. So, they were in water for 3, 10, 17, 24 and 28 days (these bags will be referred to as Set II). To maintain the density of invertebrates, pupae and empty cases were replaced weekly.

To assess remaining mass, leaf remains were placed in an oven at 70 °C for 72 h, weighed, ashed at 550 °C for 4 h and reweighed to calculate AFDM. At each sampling date, conductivity, temperature and discharge (volumetric method) were measured and

1 L of channel water was collected for nutrient analysis and alkalinity determination (as described above). The experiment was repeated three times (the 1st run on February, the 2nd on March and the 3rd on April, 2003), to generate replicates. Between each run, water, conditioned leaves and animals were changed and stones, sand and channels were cleaned.

Experiment 2: Effects of high current on leaves in advanced stage of decomposition

To assess the effects of increasing current velocity on mass loss, 52 fine mesh bags containing alder leaves (origin: Casa do Sal, Portugal, N 40° 6′ 5″; W 8° 13′ 49″; prepared as before) were placed in a low current stretch of the S. João stream, for 15 days to allow decomposition and softening. Bags were transported in an ice chest to the laboratory. The leaves were gently rinsed with tap water and transferred to 20 coarse mesh (10 mm mesh size, to allow physical abrasion by flowing water) and 20 fine mesh (0.5 mm mesh size, to minimize the effect of current velocity) bags and placed in the channels (5 of each type in each channel), which contained tap water but not sediments or invertebrates. The 12 samples left were used to calculate the initial AFDM of leaves placed in the channels.

These bags were retrieved all at once after 15 days (therefore after 1 month of decomposition). Leaf remains were rinsed with tap water, dried (70 °C for 72 h), weighed, ashed (550 °C for 4 h) and reweighed to calculate AFDM. The experiment was repeated 4 times (between May and August, 2004) with increasing current velocities; the 1st with 0.37–0.53 m/s, the 2nd with 0.60–0.68 m/s, the 3rd with 0.85–1.12 m/s and the 4th with 1.14–2.35 m/s (determined by the volumetric method).

Experiment 3: Stream inter-habitat and temporal variability

Decomposition rates of alder leaves were also measured under natural conditions in S. João stream, in both summer (July 2003) and autumn (November/December 2004). S. João stream is a 4th order stream that drains a small siliceous catchment (18 km^2) with *Pinus pinaster, Castanea sativa, Eucalyptus globulus, Acacia dealbata, Populus* spp. and *Salix* spp. in the riparian zone. The stream substrate is mainly composed of gravel and pebbles, and sand in depositional zones.

Alder leaves were collected from the same group of trees in Casa do Sal in 12–15 June 2003. Leaves were air dried and stored dry until needed. Batches of 2.75–2.85 g (summer) or 2.50–2.70 g (autumn) of dry leaves were rehydrated and allocated into coarse mesh bags. On June 28th (summer), six bags were placed in each of 18 stream reaches classified into 6 classes (3 replicate reaches per class) according to depth and current velocity (Table 1). In autumn (start on November 13th 2004), only 12 stream reaches classified into 4 classes were used (Table 1). On both dates, a group of six bags was taken to the stream and brought back to the laboratory to determine initial AFDM, taking into account losses due to handling. One bag from each reach (3 per class) was sampled after 2, 5, 12, 19, 26 and 33 (summer) or 8, 22, 29 and 35 (autumn) days in water. Conductivity, pH, temperature, depth and current velocity were measured using field meters; water was also taken for nutrient analysis as described above.

Table 1. Current velocity, depth and water chemical and physical parameters in S. João stream, in summer (July, 2003) and autumn (Nov./Dec., 2004). Values are means (with range in parenthesis).

Reach class	Current velocity (m/s)		Depth (m)	
Summer, 2003				
LS	Low	0.003 (0.000–0.021)	Shallow	0.15 (0.10–0.19)
LD	Low	0.006 (0.000–0.014)	Deep	0.37 (0.32–0.41)
MS	Medium	0.388 (0.232–0.559)	Shallow	0.17 (0.14–0.18)
MD	Medium	0.318 (0.236–0.408)	Deep	0.34 (0.27–0.40)
HS	High	1.185 (0.621–1.740)	Shallow	0.13 (0.09–0.18)
HD	High	0.670 (0.491–0.950)	Deep	0.34 (0.31–0.36)
Water parameters				
pH		7.1 (7.0–7.3)		
Conductivity (µS/cm)		47 (44–50)		
NO_3-N (µg/L)		179.3 (141.0–220.0)		
SRP (µg/L)		10.3 (6.4–14.5)		
Water temp. (°C)		16.4 (15.3–18.0)		
Autumn, 2004				
LS	Low	0.142 (0.086–0.198)	Shallow	0.10 (0.08–0.13)
LD	Low	0.145 (0.058–0.205)	Deep	0.30 (0.28–0.33)
HS	High	1.228 (0.857–1.577)	Shallow	0.16 (0.15–0.19)
HD	High	0.851 (0.626–1.333)	Deep	0.39 (0.35–0.47)
Water parameters				
pH		6.7 (6.6–6.7)		
Conductivity (µS/cm)		37 (36–37)		
NO_3-N (µg/L)		157.0 (138.8–168.6)		
SRP (µg/L)		12.4 (10.6–13.6)		
Water temp. (°C)		8.2 (7.5–9.8)		

After retrieval, each sample was processed as previously described, except that invertebrates were collected on a 0.5 mm sieve and preserved in 70 % ethanol for later identification. Once during the experiment, benthic macroinvertebrates were sampled from each reach, using a kick net (0.3 × 0.3 m opening and 0.5 mm mesh size; along one meter, for 30 seconds). Samples were stored in 4 % formalin, sorted and invertebrates preserved in 70 % ethanol for later identification. Invertebrates were identified to genus or species, except for Oligochaeta (family), Hidracarina, Ostracoda and Nemathelmintha (presence) and some Diptera (subfamily or tribe), and classified into 2 groups: shredders and non-shredders (Merrit & Cummins 1996, Tachet et al. 2000).

Data treatment

Experiment 1

Comparisons of water parameters of channels among the three experimental runs were made by ANOVA. When data were not normally distributed (Shapiro-Wilk's test) they were log transformed (Zar 1999).

Remaining mass along time was fitted to the negative exponential model $M_t = M_o \cdot e^{-kt}$, in which M_o is the initial mass, M_t is the remaining mass at time t, and k is the breakdown rate. For k per degree-day calculations, time (t) was substituted by the cumulative Celsius degrees at the sampling day. Slopes on ln-transformed data were compared using a 5-way ANCOVA with run, bag type, current velocity and shredders as categorical variables and time (or degree-days) as continuous variable followed by a Tukey's test.

Experiment 2

Mass loss (ln-transformed) of alder leaves incubated in coarse and fine mesh bags, at different current velocities, was compared using a 3-way ANCOVA with run and bag type as categorical variables and current velocity as continuous variable.

Experiment 3

Comparisons of stream water parameters between seasons were made by t test (Zar 1999). Breakdown rates (k) of alder leaves decomposing in S. João stream were calculated as before and related with current velocity by a linear regression model. Within each season, slopes on ln-transformed data were compared using a 3-way ANCOVA with current and depth as categorical variables and time as continuous variable, followed by a Tukey's test. For comparisons between seasons, slopes on ln-transformed data were compared using a 4-way ANCOVA with season, current and depth as categorical variables and degree-days as continuous variable, followed by a Tukey's test.

Comparisons of invertebrate numbers and taxa among the different reach classes in S. João stream were made by 2-way ANOVA with current and depth as categorical variables (benthic invertebrates) and 3-way ANOVA with current, depth and time as categorical variables (litter bag invertebrates). When data was not normally distributed (Shapiro-Wilk's test) they were log [or log $(x+1)$] transformed. Statistical analyses were done with STATISTICA 6 software.

Results

Experiment 1

Water parameters

Conditions in all measured water parameters were not statistically different among the 3 experimental runs (ANOVA, $p > 0.050$). However, temperature, conductivity and nitrogen concentration were higher in the channels than in the stream whereas the opposite was true for phosphate. Alkalinity and pH were similar between channels and stream (Table 2).

Current velocity in channels C1 and C2 was similar in all experimental runs (range = 0.04–0.05 m/s). Similarly, in channels C3 and C4 current velocities

Table 2. Channels and stream water chemical and physical parameters, over the three experimental runs (February–April, 2003). Values are means (with range in parenthesis). n, number of measurements.

	Channels			S. João stream		
	n	mean	range	n	mean	range
pH	8	7.1	6.9–7.4	5	6.9	6.7–7.2
Alkalinity (mg $CaCO_3$/L)	7	12.7	8.5–17.0	4	9.1	6.5–13.6
Conductivity (µS/cm)	21	63.3	40.1–93.0	6	36.5	35.0–39.2
NO_3-N (µg/L)	21	1270.9	246.7–3572.2	6	388.0	206.7–1164.7
SRP (µg/L)	21	4.4	0–11.8	5	8.2	2.0–13.7
Temperature (°C)	21	22.7	17.7–24.7	6	9.9	8.3–11.9

ranged from 0.17 to 0.25 m/s. The ratio between the high current velocity and the low current velocity channels was always 5. In the stream, in places were there was organic matter accumulation, the mean current velocity was 0.13 m/s (± 0.10), but it reached 1.94 m/s (± 1.06) in places were there was no organic matter accumulation.

Invertebrates

One hundred Sericostomatids were placed in channels C1 and C3 at the beginning of each experimental run. However, due to pupation, and in spite of larvae replacement during the experiment, some animals were lost and by the end of each run the final number of individuals in each channel varied between 46 and 71, being the difference between channels always ≤16 %.

Decomposition

Decomposition rates in channels were measured in two sets of leaves: leaves retrieved periodically from the channels (Set I) and leaves placed periodically in the channels and retrieved at once (Set II). Leaves in coarse mesh bags lost 65 (Set I)–69 (Set II) % of their initial AFDM after 28 days in low current + shredders channel, and 73 (Set I)–75 (Set II) % in high current + shredders channel. All the other bag types and channels had higher remaining leaf material after 28 days (58–67 %) (Fig. 2 and Table 3). Decomposition rates were significantly affected by shredder presence (5-way ANCOVA, $p < 0.001$) but not by current velocity (5-way ANCOVA, $p > 0.132$) (Table 4). In the presence of shredders (C1 and C3), decomposition rates were significantly higher in coarse mesh bags than in fine mesh bags (5-way ANCOVA, $p < 0.001$; Table 4).

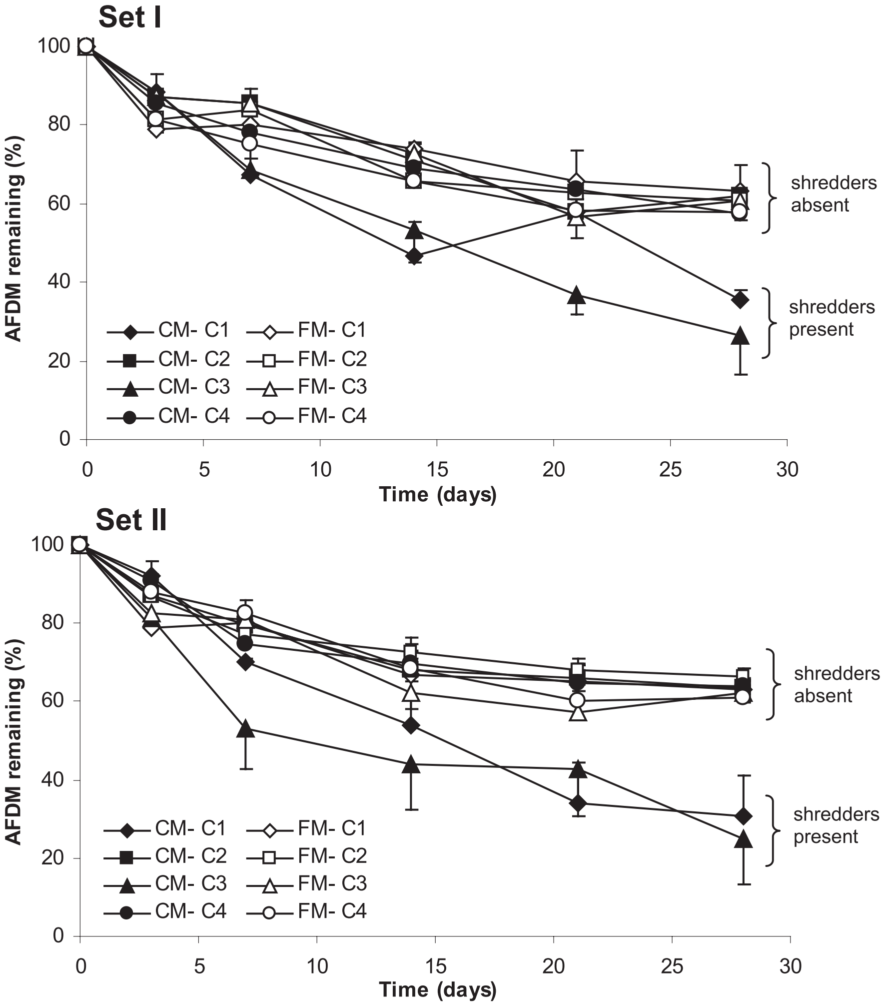


Fig. 2. Remaining mass (mean ± SE) of alder leaves, in two sets of leaves (see text), in coarse mesh (CM) and fine mesh (FM) bags in channels 1 (C1: low current + shredders), 2 (C2: low current – shredders), 3 (C3: high current + shredders) and 4 (C4: high current – shredders), on a per day basis.

Experiment 2

Decomposition

After 15 days in the stream, leaves lost around 50 % of their initial mass, primarily due to tissue softening by microorganisms since almost no invertebrates (except for some chironomid early stage larvae) were found inside mesh bags and no reduction in area was noticed. The remaining mass was consid-

Table 3. Breakdown rates of alder leaves incubated in the channels (February – April, 2003; experiment 1) and in S. João stream (in summer, 2003, and autumn, 2004; experiment 3), calculated on a per-day and on a per degree-day basis. Bag types with the same letter do not have significantly different *k* values (*, considering both seasons; Tukey's test $\alpha = 0.05$).

			Set I				Set II			
Bag type	Shredders	Current	k/day	R^2	k/degree-day	R^2	k/day	R^2	k/degree-day	R^2
Coarse	Yes	Low	0.0368 ac	0.7270	0.0016 ab	0.7434	0.0416 a	0.9771	0.0017 a	0.9636
Fine	Yes	Low	0.0191 b	−0.0133	0.0008 b	0.1393	0.0187 b	0.0561	0.0008 b	0.2289
Coarse	No	Low	0.0210 bc	0.7651	0.0009 b	0.7905	0.0196 b	0.7467	0.0008 b	0.8150
Fine	No	Low	0.0213 bc	0.4991	0.0009 b	0.5687	0.0166 b	0.5209	0.0007 b	0.6379
Coarse	Yes	High	0.0472 a	0.9962	0.0020 a	0.9945	0.0455 a	0.8462	0.0019 a	0.8626
Fine	Yes	High	0.0215 bc	0.8026	0.0009 b	0.8230	0.0212 b	0.6086	0.0009 b	0.6737
Coarse	No	High	0.0219 bc	0.7559	0.0009 b	0.8103	0.0184 b	0.7566	0.0008 b	0.8143
Fine	No	High	0.0238 bc	0.4107	0.0010 b	0.5044	0.0198 b	0.9084	0.0008 b	0.9331
			Summer, 2003				Autumn, 2004			
Reach class	Current	Depth	k/day	R^2	k/degree-day*	R^2	k/day	R^2	k/degree-day*	R^2
LS	Low	Shallow	0.0598 a	0.9395	0.0035 ab	0.9308	0.0136 a	0.9604	0.0017 a	0.9271
LD	Low	Deep	0.0645 a	0.8548	0.0028 a	0.9560	0.0147 a	0.7077	0.0018 a	0.7023
MS	Medium	Shallow	0.0546 a	0.9256	0.0037 ab	0.8482				
MD	Medium	Deep	0.0489 a	0.9664	0.0029 a	0.9180				
HS	High	Shallow	0.0497 a	0.9267	0.0032 a	0.9223	0.0417 b	0.6032	0.0051 b	0.5929
HD	High	Deep	0.0541 a	0.8326	0.0031 a	0.8309	0.0270 b	0.5314	0.0033 ab	0.5245

Table 4. Results of a 5-way ANCOVA (run, current velocity, shredders and bag type as categorical variables; time as continuous variable) on mass loss of alder leaves incubated in the artificial channels.

		Set I		Set II	
Effect	df	F ratio	p	F ratio	p
Intercept	1	707.268	<0.001	441.853	<0.001
Time	1	168.294	<0.001	118.430	<0.001
Run	2	1.428	0.490	5.685	0.058
Cvel	1	1.554	0.213	2.265	0.132
Shredders	1	12.552	<0.001	36.682	<0.001
Bag type	1	15.472	<0.001	24.512	<0.001
Run×Cvel	2	0.793	0.673	5.842	0.054
Run×Shredders	2	3.581	0.167	0.030	0.985
Cvel×Shreeders	1	0.010	0.920	1.258	0.262
Run×Bag type	2	5.361	0.069	0.978	0.613
Cvel×Bag type	1	0.529	0.467	0.887	0.346
Shredders×Bag type	1	28.972	<0.001	22.964	<0.001
Run×Cvel×Shredders	2	2.006	0.367	6.367	0.041
Run×Cvel×Bag type	2	0.006	0.997	4.780	0.092
Run×Shredders×Bag type	2	3.978	0.137	0.194	0.908
Cvel×Shredders×Bag type	1	1.105	0.293	1.769	0.184
Run×Cvel×Shredders×Bag type	2	1.494	0.474	5.408	0.067

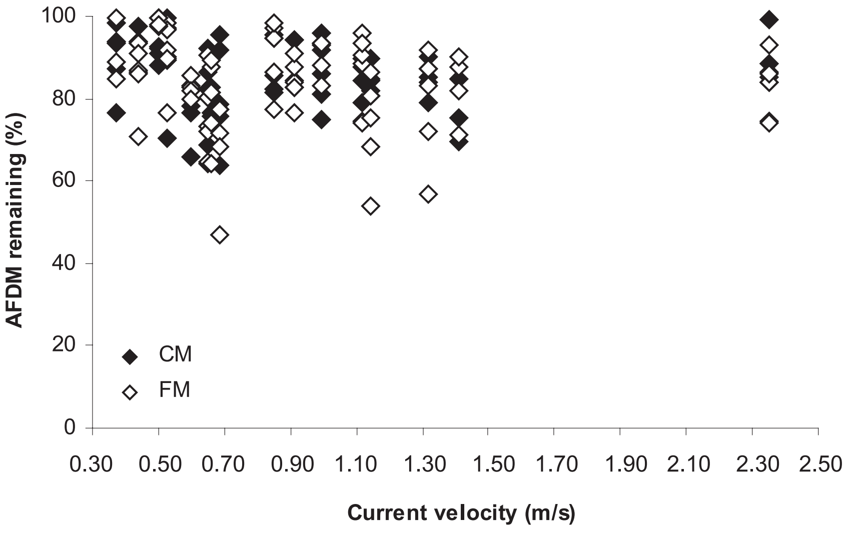


Fig. 3. Remaining mass of alder leaves incubated, in coarse mesh (CM) and fine mesh (FM) bags, in artificial channels with increasing current velocity.

Table 5. Results of a 3-way ANCOVA (run and bag type as categorical variables; current velocity as continuous variable) on mass loss of alder leaves in advanced stage of decomposition incubated in the artificial channels.

Effect	df	F ratio	p	p*
Intercept	1	516.159	<0.001	<0.001
Cvel	1	2.656	0.103	0.868
Run	3	57.269	<0.001	<0.001
Bag type	1	1.521	0.217	0.258
Run×Bag type	3	5.151	0.161	0.247

p: Considering all current velocities; p*: without considering 2.35 m/s.

ered the initial mass for channels experiment. After 15 days in the channels, there was still 47 (at 0.8 m/s)–99 (at 0.37, 0.53 and 2.35 m/s) % of mass remaining (Fig. 3). No effect of current velocity or bag type was detected on % mass remaining (3-way ANCOVA, $p = 0.103$ and 0.217, respectively; Table 5). Even when the channel with current of 2.35 m/s was not considered in the analysis, as it seemed to be an outlier, current velocity and bag type were considered to be unimportant to mass loss of alder leaves (3-way ANCOVA, $p = 0.868$ and 0.258, respectively; Table 5). However, as runs were carried out at different times, and without replication, a significant effect of the run was detected (3-way ANCOVA, $p < 0.001$; Table 5).

Experiment 3

Water parameters

Water in S. João stream was circumneutral, with low conductivity and low nutrient content, in both summer 2003 and autumn 2004. As expected, temperature was significantly lower in autumn (8.2 °C) than in summer (16.4 °C; t test, $p < 0.001$; Table 1). Current velocity and depth did not vary greatly in the experimental reaches during the experiment (Table 1).

Decomposition

Litter decomposition proceeded much faster in summer than in autumn (Fig. 4). After the 1st week in water, leaves incubated in summer had already lost 23–30 % of their initial AFDM while leaves incubated in autumn only lost 6–13 % of their initial AFDM, this loss being primarily due to leaching. After 5 weeks in water, only 12–22 % of the initial AFDM was still remaining in bags incubated in summer while 25–63 % was still left in bags incubated in autumn (Fig. 4).

In summer, breakdown rates varied between 0.0489/d and 0.0645/d, but there were no significant differences among reach classes (3-way ANCOVA, p

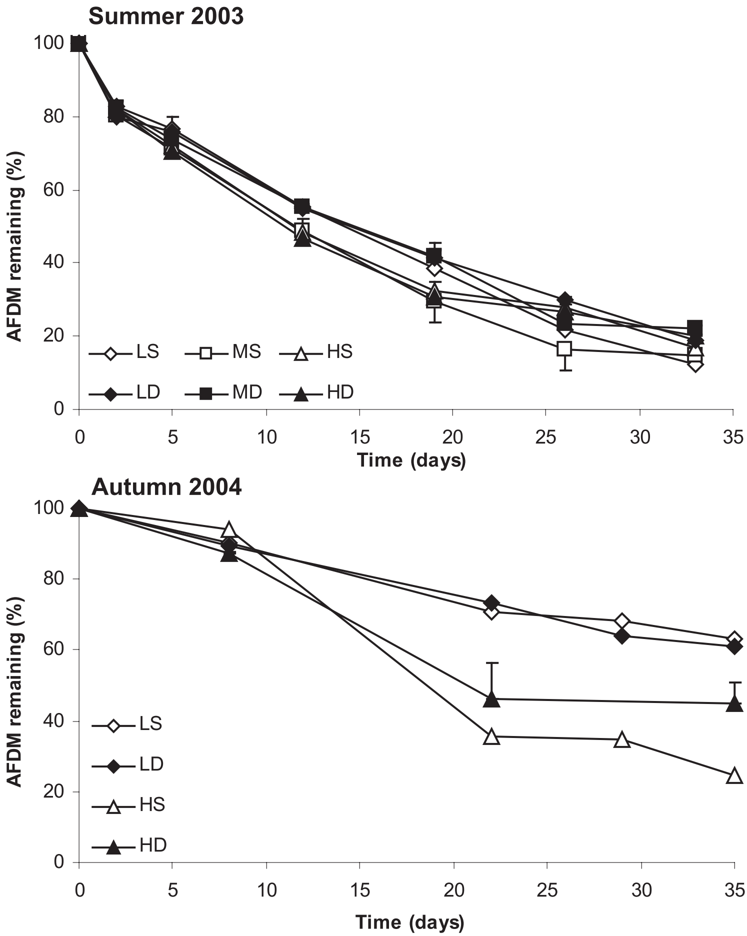


Fig. 4. Remaining mass (mean ± SE) of alder leaves, in S. João stream, in summer (July, 2003) and autumn (Nov./Dec., 2004), on a per day basis. See Table 1 for class definitions.

= 0.125 for current velocity and 0.109 for depth; Tables 3 and 6). However, in autumn, breakdown rates varied between 0.0136/d and 0.0417/d and significant differences were found between low current velocity and high current velocity reach classes (3-way ANCOVA, $p < 0.001$; Tables 3 and 6).

Although breakdown rates on a per day basis were much higher in summer than in autumn, comparisons between seasons must be done using breakdown rates on a per degree-day basis to account for differences in temperature between seasons. Significant differences were found between high current velocity-shallow reach class in autumn (HS, 2004) and low current velocity reach classes in autumn (LS and LD, 2004), deep reach classes in summer and high

Table 6. Results of a 3-way (velocity and depth as categorical variables; time as continuous variable) and 4-way ANCOVA (season, current velocity and depth as categorical variables; degree-days as continuous variable) on mass loss of alder leaves incubated in different reach classes in S. João stream in summer and autumn.

Effect	df	F ratio	p
Summer, 2003			
Intercept	1	924.171	<0.001
Time (days)	1	1239.243	<0.001
Cvel	2	4.153	0.125
Depth	1	2.574	0.109
Cvel×Depth	2	4.425	0.109
Autumn, 2004			
Intercept	1	38.965	<0.001
Time (days)	1	42.672	<0.001
Cvel	1	17.292	<0.001
Depth	1	0.856	0.355
Cvel×Depth	1	1.241	0.265
Summer *vs.* Autumn			
Intercept	1	360.040	<0.001
Degree-days	1	478.384	<0.001
Season	1	17.326	<0.001
Cvel	1	28.597	<0.001
Depth	1	1.185	0.276
Season×Cvel	1	13.936	<0.001
Season×Depth	1	0.874	0.350
Cvel×Depth	1	0.861	0.354
Season×Cvel×Depth	1	2.267	0.132

current velocity reach classes in summer (LD, MD, HD and HS, 2003); (4-way ANCOVA, $p < 0.001$; Tables 3 and 6). No relationship between breakdown rates of alder leaves and current velocity was found in summer (linear regression, $p = 0.976$, $R^2 < 0.001$), however, in autumn, breakdown rates were significantly related to current velocity (linear regression, $p = 0.017$, $R^2 = 0.966$).

Benthic and litter bag invertebrates

Between 60 (autumn) and 65 (summer) taxa were recovered from the stream benthos. Shredders corresponded to 12 (summer) – 14 % (autumn) of individuals. Leuctridae (Plecoptera) was the most abundant group (accounting for more than 50 % of total number of shredders in 61 % of reaches) on summer, and Nemouridae (Plecoptera) the most abundant (accounting for more than 50 % of total number of shredders in 75 % of reaches) on autumn. Generally, reaches did not differ in terms of total number of individuals and taxa and number of shredders and shredder taxa (2-way ANOVA, $p > 0.050$), except

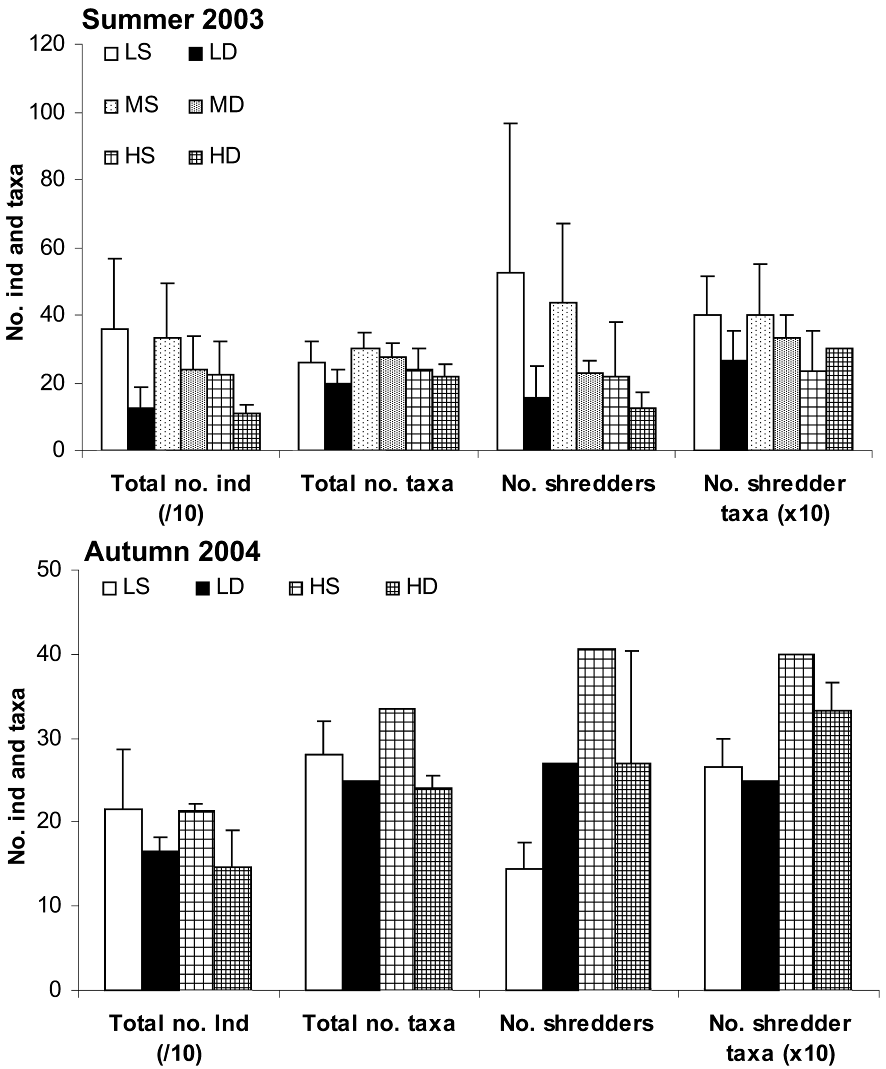


Fig. 5. Total number of individuals and taxa and number of shredders and shredder taxa (mean ± SE) per reach class in S. João stream, in summer (July, 2003) and autumn (Nov./Dec., 2004). See Table 1 for class definitions.

high current reaches in autumn which had significantly higher number of shredder taxa than low current reaches (2-way ANOVA, df = 1, F ratio = 8.01, $p = 0.030$) (Fig. 5).

Between 26 (autumn) and 60 (summer) taxa were recovered from litter bags. Shredders accounted for 7 (summer) – 40 % (autumn) of the total number of individuals being *Lepidostoma hirtum* (Lepidostomatidae, Trichoptera)

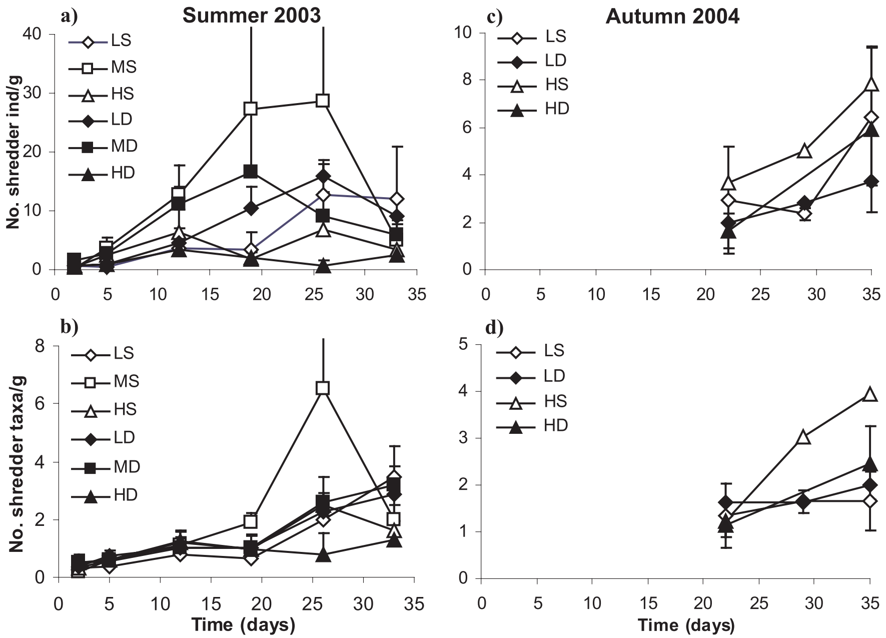


Fig. 6. Number of shredders (**a** and **c**) and shredder taxa (**b** and **d**) /g AFDM (mean ± SE) on alder leaves incubated in S. João stream, in summer (July, 2003) and autumn (Nov./Dec., 2004). The same pattern exists for total number of invertebrates and total number of taxa. See Table 1 for class definitions.

Table 7. Results of a 3-way ANOVA (current velocity, depth and time as categorical variables) on the number and taxa of invertebrates colonizing alder leaves incubated in different reach classes in S. João stream in summer.

	Total no. invertebrates	Total no. taxa	No. shredders	No. shredder taxa
Intercept	<0.001	<0.001	<0.001	<0.001
Cvel	<0.001	<0.001	<0.001	0.108
Depth	0.153	0.343	0.916	0.664
Time	<0.001	<0.001	<0.001	<0.001
Cvel×Depth	0.112	<0.001	0.115	0.495
Cvel×Time	<0.001	0.046	0.053	0.188
Depth×Time	0.391	0.441	0.326	0.336
Cvel×Depth×Time	0.475	0.729	0.867	0.711

the most abundant shredder in both seasons. In summer, the total number of individuals and shredders generally increased in leaf material up to day 26 (Fig. 6 a) while the total number of taxa and shredder taxa increased along the experiment with higher values in the last sampling date (Fig. 6 b). In autumn, invertebrates colonized litter bags only after 22 days of submersion, and the number of individuals and taxa increased along the experiment until the last sampling day (Fig. 6 c and d). In summer, medium current reaches had higher numbers of invertebrates and taxa than low and high current reaches (3-way ANOVA, $p < 0.001$; Table 7), this being particularly evident by day 26. In autumn, no significant differences were found among reach classes for the 4 invertebrate parameters (3-way ANOVA, $p > 0.050$).

Discussion

Our global objective was to assess the relative importance of current velocity in the decomposition of leaves. If current is a potential source of variability, then many results of decomposition studies could be influenced by the places where leaves are located during the experiments. This is particularly important if decomposition rates are going to be used as a functional measure of stream health condition (Pascoal et al. 2001, Gessner & Chauvet 2002, Pascoal et al. 2003, Dangles et al. 2004).

In the experimental channels, the presence of invertebrates caused a significant increase in mass loss of leaves (k CM bags > k FM bags), as predicted from the literature (e. g. Cuffney et al. 1990, Stewart 1992), whereas in experimental channels with no invertebrates, k values for leaves in coarse and fine mesh bags were similar, regardless current velocity. For fine mesh bags there was no difference in k values among the 4 channels. The absence of an effect of current velocity was probably due to the low range of values tested (0.05–0.20 m/s). Nevertheless, results from the channels experiments suggest therefore that for current velocity values up to 0.20 m/s, physical fragmentation can be considered unimportant in decomposition experiments, while shredder presence is a major factor controlling breakdown.

The k values obtained in the channels experiment (0.0166/d–0.0472/d) were higher than the ones obtained in other studies for the same leaf species, in Portugal (Canhoto & Graça 1996, Abelho 2001) and in other European countries (Chergui & Pattee 1990, Gessner et al. 1991, Baldy & Gessner 1997, Hieber & Gessner 2002), or similar leaves (Gessner et al. 1998, Robinson & Gessner 2000, Abelho 2001). These differences could be explained by higher water temperature observed in this study since it has been reported that high temperatures enhance leaching (Chergui & Pattee 1990), decomposition (Chergui & Pattee 1990, Irons et al. 1994, Jonson et al. 2001) and fungal growth and sporulation (Graça & Ferreira 1995, Chauvet & Suberkropp 1998).

It may be argued that current may be more important in the late stages of decomposition when leaves are more fragile due to the digestion of tissues by microbial enzymes (e. g. Chergui & Pattee 1988, Canton & Martinson 1990). However, in spite of the high current velocity values tested (up to 2.35 m/s; experiment 2), mass loss by alder leaves in advanced stages of decomposition was not related to current velocity and, once again, physical fragmentation was unimportant. Although there is no doubt that current should have an effect on the fragmentation of leaves in natural packs, it is plausible that leaves inside bags are unexposed to the direct effect of current. If this is the case, the fine-coarse mesh bag approach does indeed quantify the difference between invertebrate and microbial decomposition, but our ability to predict decomposition rates using this approach underestimates what happens under realistic field conditions.

In the field experiment carried out during summer no significant differences in k values ($k = 0.0489$/d–0.0645/d) were observed among reach classes differing in current velocity (range: 0.003–1.185 m/s) and depth (0.13–0.37 m). This absence of an effect of current velocity in litter breakdown was not a result of a compensation effect by invertebrate activity, in spite of differences in invertebrate numbers between different current velocity reach classes, as there were not higher numbers in low current and low numbers in high current velocity reach classes. In autumn, however, alder leaves incubated in high current velocity reach classes decomposed at higher rate than leaves in low current velocity reach classes. Since current velocity values were not much different between summer and autumn, for the same reach class, the difference observed in autumn could be due to an indirect effect related to a higher amount of transported sediments in the water during this season, caused by rains (absent in summer). The sediments could act as abrasive agents on the leaves. Heard et al. (1999) found mechanical abrasion to be an important contributor to organic matter processing in streams, though they evaluated the effect of coarse sediments. Another explanation for the difference could be the higher number of shredders taxa observed in fast-flowing stream sectors, which is consistent with previous studies indicating that, at river stretch level, shallow riffles had significantly higher coarse particulate organic matter and invertebrates, including shredders, than deeper sections (Graça et al. 2004).

The faster decomposition in summer ($k = 0.0489$/d–0.0645/d) than in autumn ($k = 0.0136$/d–0.0417/d) could be attributed to the temperature stimulation effect on leaching (Chergui & Pattee 1990) and on microbial activity (e. g. Graça & Ferreira 1995, Chauvet & Suberkropp 1998). Also, in summer it is expected to be food limitation for invertebrate shredders as much of the autumnal litter input was decomposed/washed downstream; the litter bags acted in this situation as food islands on which invertebrates probably fed at higher rates than in autumn when there is no food limitation. When the data were expressed on a per degree-day basis, no differences were found between seasons; only the high current-shallow reach class (HS, 2004) was significantly different from the majority of other reach classes.

Our results differ from other studies where an effect of current velocity on decomposition rates was found. Chergui & Pattee (1988) reported a fast breakdown rate of poplar leaves at a high current velocity (0.44 m/s) than in stagnant water, mostly as a consequence of microbial and invertebrate feeding. Canton & Martinson (1990) reported higher mass loss of willow leaves in artificial channels with higher (0.26 and 0.31 m/s) than with low (0.12 and 0.19 m/s) current velocity, but only after 6 weeks. Finally, Vingada (1995), using alder leaves also observed higher decomposition for coarse and fine mesh bags in an artificial channel with high current velocity (0.53 m/s) than in a tank with stagnant water.

Although in this study invertebrates appeared to have a central role in leaf decomposition, their importance is this process is also unclear. Rader et al. (1994) using chemical inhibitors to isolate the effect of current velocity and invertebrate activity concluded that neither base-flow current velocity nor shredders were important in the decomposition of sweet gum leaves and concluded that microbial degradation was the dominant factor controlling decomposition. On the other hand, Hieber & Gessner (2002) found that invertebrates were responsible for 64 % of mass loss in alder leaves. Cuffney & Wallace (1989) reported that in the absence of invertebrates (by excluding them with insecticide) the amount of CPOM accumulating in a stream reach increased because it was not being decomposed, which resulted in a decrease of the amount of FPOM exported. Other studies showed lower breakdown rates of leaves in streams with low number of invertebrates when compared to streams with high densities of invertebrates (Cuffney et al. 1990, Stewart 1992, Fabre & Chauvet 1998).

In conclusion, this study showed that current velocity (up to 2.35 m/s), by itself, did not affect the breakdown rates of alder leaves. However, the presence of fine sediments in water had the potential to amplify the effect of current velocity. On the other hand, unlike current, the presence of shredders increased the rate of mass loss of alder leaves. The main conclusion of this study was that the physical inter-habitat variability had a minor role in leaf breakdown in litter bags.

Acknowledgements

The authors are grateful to Mr. Joaquim Cordeiro for the assistance provided in the construction of the laboratory flumes and to Cláudia Mieiro and Andreia Ferreira for field support. The helpful comments provided by Dr. Vladislav Gulis, Dr. Steve Heard, Dr. Anne Robertson and an anonymous reviewer are gratefully acknowledged. This study was supported through the EU project “RivFunction” (EVKI-2001-00088) and through a grant by the Fundação para a Ciência e Tecnologia (Programa POCTI2010/SFRH/BD/11350/2002) to VF.

References

ABELHO, M. (2001): From litterfall to breakdown in streams: a review. – The Scientific World **1:** 656–680.

APHA (1995): Standard methods for the examination of water and wastewater, 19th ed. – EATON, A. E., CLESCERI, L. S. & GREENBERG, A. E. (eds), Washington, D.C., pp. 1–1050.

BALDY, V. & GESSNER, M. O. (1997): Towards a budget of leaf litter decomposition in a first- order woodland stream. – C. R. Acad. Sci. Paris. Sciences de la vie **320:** 747–758.

CANHOTO, C. & GRAÇA, M. A. S. (1996): Decomposition of *Eucalyptus globulus* leaves and three native leaf species (*Alnus glutinosa, Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. – Hydrobiologia **333:** 79–85.

CANTON, S. P. & MARTINSON, R. J. (1990): The effects of varying current on weight loss from willow leaf packs. – Freshwat. Biol. **5:** 413–415.

CHAUVET, E. & SUBERKROPP, K. (1998): Temperature and sporulation of aquatic hyphomycetes. – Appl. Environ. Mycrobiol. **64:** 1522–1525.

CHERGUI, H. & PATTEE, E. (1988): The effect of water current on the decomposition of dead leaves and needles. – Verh. Internat. Verein. Limnol. **23:** 1294–1298.

– – (1990): The influence of season on the breakdown of submerged leaves. – Arch. Hydrobiol. **120:** 1–12.

CUFFNEY, T. F. & WALLACE, B. J. (1989): Discharge- export relationships in headwater streams: the influence of invertebrates manipulations and drought. – J. N. Amer. Benthol. Soc. **8:** 331–341.

CUFFNEY, T. F., WALLACE, B. J. & LUGTHART, G. J. (1990): Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. – Freshwat. Biol. **23:** 281–299.

DANGLES, O., GESSNER, M. O., GUEROLD, F. & CHAUVET, E. (2004): Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. – J. Appl. Ecol. **41:** 365–378.

FABRE, E. & CHAUVET, E. (1998): Leaf breakdown along an altitudinal stream gradient. – Arch. Hydrobiol. **141:** 167–179.

GESSNER, M. O. & CHAUVET, E. (2002): A case for using litter breakdown to assess functional stream integrity. – Ecol. Appl. **12:** 498–510.

GESSNER, M. O., MEYER, E. & SCHWOERBEL, J. (1991): Rapid processing of fresh litter in an upland stream. – Verh. Internat. Verein. Limnol. **24:** 1846–1850.

GESSNER, M. O., ROBINSON, C. T. & WARD, J. V. (1998): Leaf breakdown in streams of an alpine glacial floodplain: dynamics of fungi and nutrients. – J. N. Amer. Benthol. Soc. **17:** 403–419.

GONZÁLEZ, J. M. & GRAÇA, M. A. S. (2003): Conversion of leaf litter to secondary production by a shredding caddis fly. – Freshwat. Biol. **48:** 1578–1592.

GRAÇA, M. A. S. (2001): The role of invertebrates on leaf decomposition in streams – a review. – Internat. Rev. Hydrobiol. **86:** 383–393.

GRAÇA, M. A. S. & FERREIRA, R. C. F. (1995): The ability of selected aquatic hyphomycetes and terrestrial fungi to decompose leaves in freshwater. – Sydowia **47:** 167–179.

Graça, M. A. S., Pinto, P., Cortes, R., Coimbra, N., Oliveira, S., Morais, M., Carvalho, M. J. & Malo, J. (2004): Factors affecting macroinvertebrate richness and diversity in Portuguese streams: a two- scale analysis. – Internat. Rev. Hydrobiol. **89:** 151–164.

Heard, S. B., Schultz, G. A., Ogden, C. B. & Griesel, T. (1999): Mechanical abrasion and organic matter processing in an Iowa stream. – Hydrobiologia **400:** 179–186.

Hieber, M. & Gessner, M. O. (2002): Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. – Ecology **83:** 1026–1038.

Huryn, A. D., Huryn, V. M. B., Arbuckle, C. J. & Tsomides, L. (2002): Catchment land-use, macroinvertebrates and detritus processing in headwater streams: taxonomic richness versus function. – Freshwat. Biol. **47:** 401–415.

Irons, J. G., Oswood, M. W., Stout, R. J. & Pringle, C. M. (1994): Latitudinal patterns in leaf litter breakdown: is temperature really important? – Freshwat. Biol. **32:** 401–411.

Jonsson, M., Malmqvist, B. & Hoffsten, P.-O. (2001): Leaf breakdown rates in boreal streams: does shredder species richness matter? – Freshwat. Biol. **46:** 161–171.

Merrit, R. W. & Cummins, K. W. (1996): An introduction to aquatic insects of North America. – Kendall/Hunt Publishing Company, Iowa, U. S. A., pp. 1–862.

Pascoal, C., Cássio, F. & Gomes, P. (2001): Leaf breakdown rates: a measure of water quality? – Internat. Rev. Hydrobiol. **68:** 407–416.

Pascoal, C., Pinho, M., Cássio, F. & Gomes, P. (2003): Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. – Freshwat. Biol. **48:** 2033–2044.

Rader, R. B., Mcarthur, J. V. & Aho, J. M. (1994): Relative importance of mechanisms determining decomposition in a southeastern blackwater stream. – Amer. Midl. Nat. **132:** 19–31.

Robinson, C. T. & Gessner, M. O. (2000): Nutrient addition accelerates leaf breakdown in an alpine springbrook. – Oecologia **122:** 258–263.

Stewart, B. A. (1992): The effect of invertebrates on leaf decomposition rates in two small woodland streams in southern Africa. – Arch. Hydrobiol. **124:** 19–33.

Tachet, H., Richoux, P., Bournaud, M. & Usseglio-Polatera, P. (2000): Invertébrés d'eau douce. Systématique, biologie, écologie. – CNRS Editions, Paris, pp. 1–588.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R. & Cushing, C. E. (1980): The river continuum concept. – Can. J. Fish. Aquat. Sci. **37:** 130–137.

Vingada, J. V. S. (1995): Decomposição de folhada num rio de montanha. Influência dos factores físicos e biológicos. – Master thesis. Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Coimbra, pp. 1–134

Zar, J. H. (1999): Biostatistical analysis. – Prentice-Hall International, Inc. USA.

Submitted: 27 May 2005; accepted: 6 January 2006.