doi: 10.1111/j.1365-2435.2011.01903.x

Litter diversity, fungal decomposers and litter decomposition under simulated stream intermittency

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Summary

1. The drying of stream channels resulting from flow interruption is expected to increase as a consequence of climate change. Implications for aquatic organisms and processes are profound. We assessed whether riparian diversity can partially buffer against consequences of drying on fungal decomposers and leaf litter decomposition, an important ecosystem process.

2. Our central hypothesis was that during dry periods recalcitrant leaf litter with high water-holding capacity would extend the window of opportunity for microbial activity in less recalcitrant litter when both litter types are mixed, and that this would lead to a positive litter diversity effect on decomposition. To test for such interactive effects, we conducted a diversity experiment in a Mediterranean stream, in which alder and oak litter, and a mixture of both, was subjected to various drying regimes differing in intensity and timing.

3. Drying regime affected both fungal decomposers and the decomposition rate of alder litter. Effects were observed both immediately and 3 weeks after stream flow resumed. Small differences in the timing of the dry period influenced both decomposition rate and measures of fungal performance (i.e. biomass and sporulation activity). Litter mixing, in contrast, had no effect on either decomposition or fungal decomposers, although mixing increased moisture retention in alder litter as required for the mechanism hypothesized to lead to a diversity effect.

4. Given the contrasting traits of the litter types used in the experiment, our results imply that riparian tree diversity is unlikely to buffer against increased frequencies of stream flow disruption expected in the face of climate change. It appears, however, that the precise timing of dry periods and high-flow events will strongly influence the extent to which stream food webs can exploit the resources supplied by riparian vegetation in the form of leaf litter.

Key-words: aquatic hyphomycetes, biodiversity and ecosystem functioning, decomposition, drought, litter diversity, litter traits, stream intermittency

Introduction

An important fraction of the world's streams and rivers are subjected to dry periods that cause occasional disruption of flow (Larned *et al*. 2010). The resulting flow intermittency involves contraction and fragmentation of running waters and temporary loss of aquatic habitat (Lake 2003). Climate change is an important factor likely to alter stream flow in the future, exacerbating both the spatial and temporal extent of intermittency in different climatic zones around the world (Milly, Dunne & Vecchia 2005). Particularly susceptible are Mediterranean streams, where climate change is projected not only to increase the extent of intermittency (Milly, Dunne & Vecchia 2005; IPCC 2007), but also to induce shifts from currently perennial to intermittent flow.

Changes in flow regimes have direct impacts on aquatic communities and processes (e.g. Boulton 2003; Lake 2003) and can also affect the density, composition and dominance

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patterns of riparian vegetation (Poff & Zimmerman 2010). Such changes in the structure of riparian communities affect both the amount and quality of leaf litter delivered to stream channels. The potential repercussions for stream communities and ecosystem processes are large, as allochthonous litter is a main source of carbon and nutrients for stream food webs and metabolism (Wallace *et al.* 1997; Webster 2007; Tank *et al.* 2010). Key in this respect is the utilization of leaf litter by decomposers and the resultant litter decomposition.

If riparian vegetation changes, the question arises to what extent litter species identity and diversity will affect stream communities and processes. The role of diversity in sustaining ecosystem processes in general has received great attention in recent years (Loreau *et al.* 2001; Hooper *et al.* 2005). However, although this includes experiments assessing diversity effects on decomposition (Gartner & Cardon 2004; Hättenschwiler, Tiunov & Scheu 2005; Kominoski *et al.* 2010; Lecerf & Richardson 2010), the significance of litter diversity for decomposition has not been resolved (Gessner *et al.* 2010). In part, this is because mechanisms behind the effects of litter diversity observed in various studies have rarely been specifically examined (but see McArthur *et al.* 1994; Schimel & Hättenschwiler 2007).

Diversity effects are particularly likely to arise when the species within a community contrast in their functional traits (Hillebrand & Matthiessen 2009; Gessner *et al.* 2010). In the case of litter decomposition, relevant litter traits include nutritional quality and properties that influence microenvironmental conditions afforded to decomposers. The significance of litter traits for microenvironmental conditions is unclear, but it could be tested and be particularly relevant in intermittent streams. As stream channels are drying, microenvironmental conditions in litter packs retained in the channels fundamentally change, leading to strong declines in decomposition rates (Herbst & Reice 1982; Maamri *et al.* 1998; Langhans & Tockner 2006; Leberfinger, Bohman & Herrmann 2010). However, different litter species differ greatly in their water-holding capacity (Dirks *et al.* 2010). Therefore, in litter mixtures subject to desiccation, the presence of species with high water-holding capacity might slow the loss of moisture in adjacent species with low water-holding capacity (Wardle *et al.* 2003). This would extend the window of decomposer activity during dry periods and enhance survival of microbes until flow resumes (Langhans & Tockner 2006), notwithstanding the fact that some aquatic decomposers can occur in terrestrial environments as well (Sanders & Webster 1978; Sridhar & Bärlocher 1993). Litter-mixing effects on decomposition of individual species can thus arise.

Particularly important drivers of litter decomposition in streams are a group of microfungi known as aquatic hyphomycetes (Gessner *et al.* 2007; Krauss *et al.* 2011). Their role is twofold: They directly degrade leaf litter, and they stimulate litter consumption by detritivores. This stimulation occurs because fungi produce biomass rich in nutrients and because they enzymatically change the physical and chemical properties of decomposing litter, thereby enhancing litter quality and palatability for consumers (Gessner *et al.* 2007). Unlike invertebrates, which also can be important for litter decomposition in streams (Graça 2001; Hieber & Gessner 2002), fungi are intimately associated with their substrate. As a consequence, they cannot evade desiccation when stream flow recedes and water levels drop. This makes fungal decomposers particularly vulnerable to desiccation stress accompanying stream intermittency.

This study aimed at assessing litter mixture effects on decomposition under simulated stream intermittency. By determining litter mass loss, fungal biomass and sporulation activity in mixed- and single-species litter under different drying regimes in a field experiment, we tested whether (i) aquatic fungi and litter decomposition are affected by drying resulting from stream intermittency; (ii) litter mixing alleviates effects on fungal biomass and activity induced by desiccation stress; and (iii) any effect of litter mixing, desiccation and the interaction of both factors on fungi translates into effects on litter decomposition. Effects on decomposers and decomposition during dry periods might extend well beyond the time when flow resumes because microbial decomposers and litter-consuming invertebrates might have to recolonize the litter, resume growth or both. Therefore, assessments of drying effects need to consider both immediate and propagated effects.

Materials and methods

STUDY SITE

The study was conducted from 22 November 2007 to 10 January 2008 in a third-order stream (Table 1) of the Pyrenees in south-western France (42°28′21″N, 02°47′58″E). The catchment was dominated by evergreen oak (*Quercus ilex* L.). Black alder (*Alnus glutinosa* (L.) Gaertn.) and several other deciduous tree species were prominent in the riparian zone. The stream bed consisted of very coarse substratum or bedrock, and the stream water was circumneutral with low nutrient concentrations (Table 1).

Table 1. Physicochemical characteristics of the investigated stream and its catchment during the study period

Parameter	Mean	SD	Dates	*N*
Slope (%)	2·7	–	–	–
Catchment size (km^2)†	22·4	–	–	–
Altitude (m a.s.l.)†	175	–	–	–
Water temperature (°C)	6·3	2·1	CR	6
Air temperature (°C)	7·1	3·8	CR	8
pH	7·9	0·1	5	3
Conductivity ($\mu S\ cm^{-1}$)	187·4	14·5	5	3
Alkalinity (mM)	0·74	0·039	2	2
O_2 ($mg\ L^{-1}$)	11·1	0·7	5	3
$N\text{-}NH_4^+$ ($\mu g\ L^{-1}$)	9·6	8·7	4	3
$N\text{-}NO_2^-$ ($\mu g\ L^{-1}$)	0·1	0·2	4	3
$N\text{-}NO_3^-$ ($\mu g\ L^{-1}$)	51·7	89·5	4	3
$P\text{-}PO_4^{3-}$ ($\mu g\ L^{-1}$)	9·1	4·6	4	3

CR, continuous record; *N*, number of sampling sites.
†Derived from a 1:25 000 map for the downstream end of the study reach.

EXPERIMENTAL PROCEDURES

Alder (*A. glutinosa*) litter was collected upon abscission in the winter of 2006 under several trees located near Gibel, France (43°17′35″N, 1°40′51″E), whereas oak (*Q. ilex*) litter was collected weekly in early summer 2006 from nets installed under trees in a forest near Puéchabon, France (43°44′30″N, 3°35′40″E). The two species contrast in regard to various litter traits, including lignin content (Gessner & Chauvet 1994) and water-holding capacity, potentially resulting in distinct decomposition rates and rates of drying following exposure to air (Dirks *et al.* 2010). Litter packs (6·00 ± 0·03 g; min–max range) for exposure in the field were constructed from dried (40 °C) litter, which were weighed to the nearest 0·01 g, wetted and placed in tetrahedral mesh bags. The tetrahedral shape and mesh size (4 mm) of the bags ensured access of a natural decomposer community to the litter and minimized artefacts resulting from unnatural moisture retention in the bags during dry periods. Leaching losses of litter upon wetting were determined by soaking dried and weighed litter batches in running tap water for 24 h, retrieving them and drying them to constant weight at 65 °C. Conversion factors to account for differences in residual moisture between litter dried at 40 and 65 °C were determined from the same litter batches.

The field study was set up in a randomized complete block design with each of eight stream riffles serving as a separate block. To simulate stream intermittency, we removed litter bags from the stream channel in the middle of the experiment and placed them for 7 days on the stream bank (Langhans & Tockner 2006), where they were protected from precipitation by translucent plastic sheets installed just above the litter bags. The 7-day duration was chosen to ensure that monospecific packs of alder leaves were completely dried, while oak leaves and mixed litter packs still retained some moisture. The required time was determined in a pilot study in which we used identical techniques as in the main experiment. Alder litter exposed in mixtures with oak in this pilot experiment lost an average of 33% less humidity than when exposed alone. The opposite effect was observed for oak litter, which lost an average of 43% more humidity when exposed together with alder litter than when exposed alone (ANOVA: $F_{1,12} = 7{\cdot}7$, $P = 0{\cdot}017$).

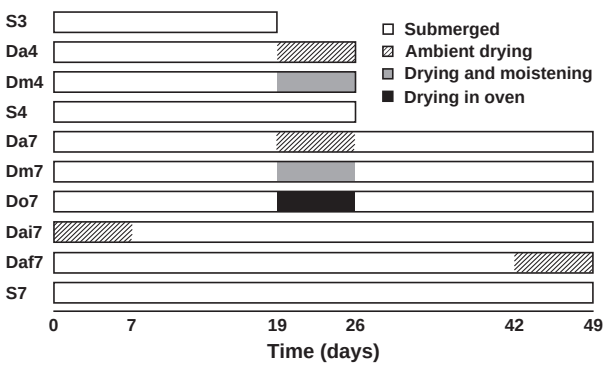

Fig. 1. Design and time course of the reported field experiment testing for the effects of drying on litter decomposition and associated fungal biomass and sporulation activity. Treatment codes include a capital letter to distinguish between treatments involving drying (*D*) from continuously submerged treatments (*S*), a lower case letter indicating the intensity of drying (a, drying under ambient conditions; m, drying and moistening; and o, drying in oven), a lower case letter denoting the timing of the dry period (i, initial; f, final) and the number of weeks of field exposure.

Litter was retrieved from the field and moved between the stream channel and bank according to various drying regimes (Fig. 1). Immediate effects of desiccation were assessed by comparing litter retrieved from the field just before (S3 = submergence for 3 weeks) and after drying on the stream bank under ambient conditions for an additional week (Da4) to simulate stream intermittency. An additional treatment involved submergence for 3 weeks followed by moistening during placement on the banks for 1 week (Dm4). It aimed at testing for the effect of slowed humidity loss and involved spraying litter at 2-day intervals, each time with *c.* 0·33 L of filtered stream water (GF/F filters; Whatman, Kent, UK) per litter bag. Control litter bags remained submerged in the stream during the whole 4-week period (S4).

A second set of treatments (Fig. 1) aimed at assessing how drying effects might propagate after stream flow had resumed. Litter bags of these treatments were returned to their original positions in the stream after the simulated intermittency, with control bags again remaining submerged in the stream during the whole experiment of 7 weeks (S7). These treatments allowed us to compare responses to drying under ambient conditions (Da7) with those after drying and moistening (Dm7) and after forced drying for 48 h in an oven (40 °C) in the middle of the dry period (Do7). Finally, we included two additional treatments, in which litter was submerged in the stream and exposed on the stream bank for the same durations as the litter in the drying treatment under ambient conditions (Da7; i.e. 6 weeks + 1 week), but with the dry period on the stream bank occurring at the beginning (Dai7) or end (Daf7) of the experiment. These treatments served as controls to assess the effect of interrupted submergence (i.e. submergence for 2 × 3 weeks vs. a continuous 6-week period) and of the timing of the dry period.

LITTER MASS REMAINING

The litter retrieved from the field was separated by leaf species and gently cleaned with tap water. Leaf discs (14 mm diameter) were cut immediately after cleaning and were preserved for analyses of fungal sporulation activity and biomass. The central vein of alder leaves was avoided, but this was not possible for oak leaves because of their small size. The remaining litter material was dried to constant mass (65 °C) and weighed to the nearest 0·01 g.

FUNGAL SPORULATION ACTIVITY

Fungal sporulation activity was assessed by identifying and counting aquatic hyphomycete spores released from 10 leaf discs per sample and litter species during short-term laboratory incubations (Gessner, Bärlocher & Chauvet 2003). Leaf discs were placed in a Petri dish containing 20 mL of filtered (GF/F filters) stream water. Sporulation was induced by gentle shaking on an orbital shaker at 10 °C. Leaf discs were removed after 24 h and frozen at −20 °C. The spore suspension was transferred to a plastic tube and preserved with 3 mL of 37% formaldehyde. The Petri dish was rinsed with 2 mL of distilled water to collect spores adherent to the Petri dish. The rinsing water was added to the tubes, and the volume was adjusted with distilled water to a total of 50 mL. One mL of Triton X-100 (0·01% solution) was later added before stirring the suspension for at least 20 min and filtering 5 or 10 mL through a membrane filter (5 μm pore size; Millipore, Cork, Ireland), staining the spores with 0·1% Trypan blue in 60% lactic acid and identifying and counting 200 of them at 200× in randomly chosen microscopic fields. The entire filter was scanned if there were < 200 spores. Daily spore biomass production was

calculated based on species-specific spore biovolumes (Bärlocher & Schweizer 1983) of the six fungal species dominating the community in terms of spore numbers (accounting for >99% of all spores) and an average dry-mass density of 500 fg μm^{-3} (Findlay & Arsuffi 1989). Spore production was then expressed on a mycelial biomass basis (ng spore biomass per mg mycelial biomass per day).

FUNGAL BIOMASS

Biomass of fungal mycelium was derived from ergosterol measurements (Gessner & Newell 2002). Freeze-dried leaf discs were immediately weighed (±0·05 mg) and preserved in methanol/KOH at −20 °C. Lipids were extracted at 80 °C for 30 min under constant stirring. The extracts were transferred to solid-phase extraction cartridges (Sep-Pak®, Waters, Milford, MA, USA; Vac RC, tC18, 500 mg), acidified (pH 2–3) and passed through the cartridges by applying a gentle vacuum. Before eluting ergosterol with isopropanol, the cartridges were rinsed and dried under a stream of air. Extraction efficiency of ergosterol was routinely monitored by running standards in parallel (Fluka, Neu-Ulm, Germany). Ergosterol in the eluate was purified and quantified by high-pressure liquid chromatography (HPLC; detection wavelength: 282 nm, flow rate: 1·5 mL s^{-1}, column temperature: 33 °C, injection volume: 20 μL). Mycelial biomass was calculated from ergosterol content by applying a mean conversion factor of 5·5 μg ergosterol per mg of fungal dry mass (Gessner & Chauvet 1993).

STATISTICAL ANALYSES

Separate statistical analyses were performed to test for immediate and propagated effects of simulated stream intermittency. We used litter mass loss, fungal biomass and spore biomass production rate as response variables in ANOVAs using Type I sums of squares with drying regime, litter species and number of litter species as fixed factors. Model assumptions were tested on models omitting the four-way interaction because the full model did not have sufficient degrees of freedom. Fungal biomass data were square-root transformed, spore biomass production rate was $\log(Y + 1)$ transformed and litter mass remaining data were subjected to a Box-Cox transformation, where $Y' = (Y^3 - 1)/3$. Block (i.e. stream riffle) was treated as a random factor. *F*-ratios and probability values were calculated based on the error structure defined by the mixed model analysis (Quinn & Keough 2002; Chapter 10) as detailed in Tables 2 and 3. Pair-wise differences between treatments were assessed with Tukey's HSD test following the ANOVAs. All statistical analyses were performed with the software R, version 2.11.1 (R Development Core Team 2010), including the package 'MASS' (Venables & Ripley 2002) for Box-Cox transformations of the litter mass loss data.

Results

LITTER MASS REMAINING

Alder litter had lost about 28% of the initial dry mass just before and after the dry period on the stream bank imposed after 3 weeks (Fig. 2a). An average of 57% of the initial dry mass was lost by the end of the experiment (Fig. 3a). Initial leaching during 24 h accounted for 16·6 ± 1·1% (mean ± 95% CI) of the loss (data not shown). Mass loss of oak litter was small, amounting to an average of 12% just before and after the dry period (Fig. 2d), and to 21% at the end of the experiment (Fig. 3c). Initial leaching accounted for 2·9 ± 0·5% (mean ± 95% CI) of the oak mass loss.

Litter mass loss was unaffected by litter mixing in any of the 10 drying treatments and either litter species (Tables 2 and 3). Drying regimes produced immediate effects on litter mass loss, which also differed between leaf species (Table 2). Exposure of alder litter on the stream bank halted mass loss or even slightly increased mass, regardless of whether or not litter was moistened (S3 vs. Da4 and Dm4; $P = 0{\cdot}012$ and $P = 0{\cdot}063$, respectively; Fig. 2a), whereas an additional 4% of the initial mass was lost when litter remained continuously submerged (S3 vs. S4; $P < 0{\cdot}001$). Qualitatively, oak litter showed similar patterns as alder, but differences were small and only significant between litter dried on the stream bank and continuously submerged (Da4 vs. S4; $P = 0{\cdot}006$; Fig. 2d).

Effects on litter mass loss caused by drying after 3 weeks propagated till the end of the experiment (Fig. 3a,c, Table 3). In particular, continuously submerged alder litter (S7) lost more mass than alder litter subjected to drying (Fig. 3a), especially when drying was forced during the dry period (Do7; $P = 0{\cdot}004$). The same tendency was apparent for oak litter (Fig. 3c), although differences among drying treatments were exceedingly small (S7 vs. Do7; $P = 0{\cdot}33$).

Timing of litter exposure on the stream bank had a large effect on alder mass loss (Fig. 3a). Drying on the stream bank in the last week of the experiment (Daf7) reduced mass loss compared to drying in both the first week (Dai7; $P < 0{\cdot}001$) and the middle of the experiment (Da7; $P < 0{\cdot}001$). Oak litter showed again the same tendencies (Fig. 3c), but differences among drying periods were very small (Da7 vs. Daf7 and Dai7; $P = 0{\cdot}026$ and $P = 0{\cdot}15$, respectively).

FUNGAL BIOMASS

Fungal biomass in decomposing litter ranged from 3·3 to 63·4 mg g^{-1} dry mass in alder and between 11·2 and 58·2 mg g^{-1} in oak litter (Figs 2b,e and 3b,d). The presence of oak leaves in litter mixtures did not significantly influence fungal biomass in alder litter (Figs 2b and 3b), nor did the presence of alder affect fungal biomass in oak litter in any of the 10 drying regimes we applied (Figs 2e and 3d, Tables 2 and 3).

Drying regimes produced differences in fungal biomass in alder but not oak litter immediately after the experimentally imposed drying period (Fig. 2b,e, Table 2). Fungal biomass decreased by 73% when alder litter was moistened during the drying period (S3 vs. Dm4; $P < 0{\cdot}001$), remained unchanged when the litter was not moistened (S3 vs. Da4; $P = 0{\cdot}93$) and increased nearly threefold when the litter remained continuously submerged (S3 vs. S4; $P < 0{\cdot}001$).

Propagated effects of drying treatments on fungal biomass were apparent in both alder and oak litter 3 weeks after the drying period (Fig. 3b,d, Table 3). However, these differences were less clear-cut, partly owing to considerable

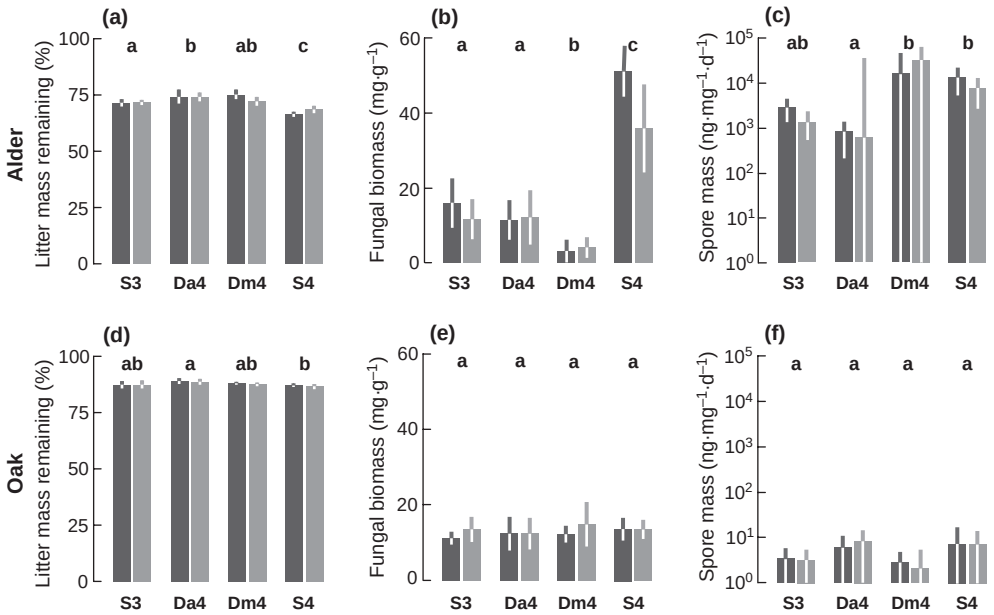

Fig. 2. Immediate effects of drying on alder and oak mass remaining (a and d), fungal biomass (b and e), and fungal spore mass (c and f). Dark grey bars indicate litter decomposing in monospecific litter packs; light grey bars denote individual litter species sorted from mixtures. Values are means ± 95% CI. Treatment codes as in Fig. 1.

Table 2. Results of statistical analyses of the immediate effects of drying. Italics indicate random terms. Significant probability values are highlighted in bold. Data on fungal biomass, spore biomass production and litter mass remaining were analysed after square-root, log(Y + 1) or Box-Cox transformations, respectively

No.	Source of variation	No. denominator term	Fungal biomass				Spore biomass production rate				Litter mass remaining			
			d.f.	SS	*F*	*P*	d.f.	SS	*F*	*P*	d.f.	SS ($\times 10^9$)	*F*	*P*
1	*B*		*7*	*24·5*			*7*	*23·8*			*7*	*1·5*		
2	D	3	3	92·1	51·2	**< 0·001**	3	34·5	2·5	0·08	3	10·1	25·8	**< 0·001**
3	*D × B*		*21*	*12·6*			*21*	*95·3*			*21*	*2·9*		
4	S	5	1	0·6	0·6	0·47	1	1089·7	439·4	**< 0·001**	1	341·7	2628·7	**< 0·001**
5	*S × B*		*7*	*7·6*			*7*	*17·4*			*7*	*0·9*		
6	M	7	1	0·1	0·1	0·80	1	1·3	0·9	0·37	1	0·2	0·7	0·43
7	*M × B*		*7*	*5·3*			*7*	*9·8*			*7*	*1·6*		
8	D × S	9	3	88·7	48·3	**< 0·001**	3	54·9	16·5	**< 0·001**	3	2·6	5·3	**0·007**
9	*D × S × B*		*21*	*12·9*			*21*	*23·3*			*21*	*3·4*		
10	D × M	11	3	3·0	2·2	0·12	3	1·3	0·3	0·81	3	0·7	1·6	0·22
11	*D × M × B*		*21*	*9·5*			*21*	*27·7*			*21*	*3·2*		
12	S × M	13	1	1·5	4·4	0·07	1	2·9	1·6	0·24	1	0·001	< 0·1	0·91
13	*S × M × B*		*7*	*2·3*			*7*	*12·3*			*7*	*0·7*		
14	D × S × M	15	3	2·0	1·1	0·37	3	3·5	1·0	0·40	3	0·8	2·8	0·07
15	*D × S × M × B*		*21*	*11·7*			*16*	*17·9*			*21*	*2·0*		
	Total		126	274·2			122	1415·4			127	372·8		

No. denominator term, number of the term (first column) used as the denominator to calculate the *F*-ratio; B, block; D, drying regime; S, litter species; M, litter mixing.

variation among replicates for some of the treatments. Forced drying of litter during the dry period (Do7) led to reduced fungal biomass 3 weeks after resubmergence of alder litter (Do7 vs. S7; P = 0·019), but not when the litter was moistened during the drying period or when it dried naturally on the stream bank (Da7 and Dm7 vs. S7; Fig. 3b; $P \geq 0{\cdot}34$). Propagated effects on fungal biomass in oak litter were different (Fig. 3d): moistening tended to increase biomass, although the difference was not significant (Dm7 vs. S7; P = 0·082), whereas forced drying and exposure on the stream bank without moistening had no effect (Da7 and Do7 vs. S7; $P \geq 0{\cdot}89$).

Timing of the dry period affected fungal biomass developing in both litter species, although the effect was less pronounced in oak (Fig. 3b,d). Exposure on the stream bank during the last week of the experiment (Daf7) increased both

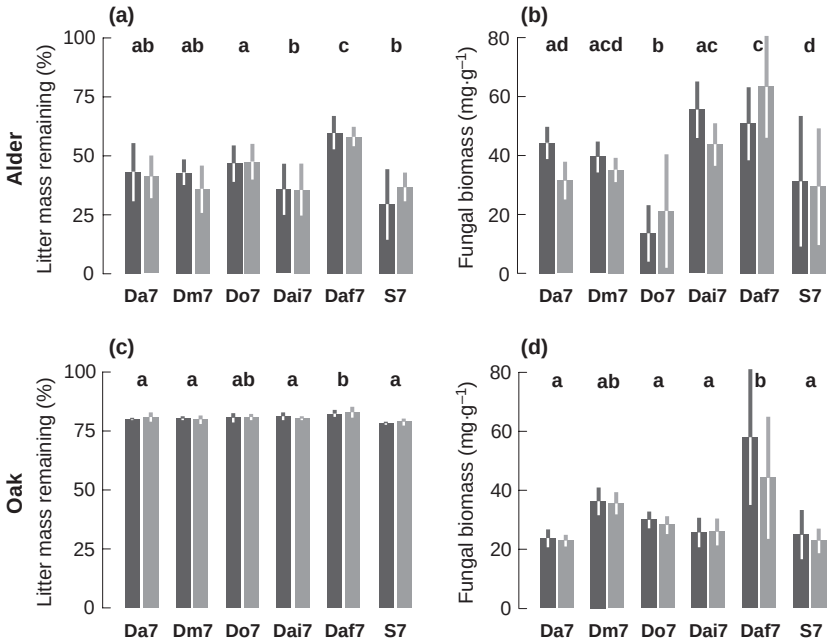

Fig. 3. Effects of drying on alder and oak mass remaining (a and c) and fungal biomass (b and d) 3 weeks after resubmerging litter in the study stream (i.e. propagated effects). Dark grey bars indicate litter decomposing in monospecific litter packs; light grey bars denote individual litter species sorted from mixtures. Values are means ± 95% CI. Treatment codes as in Fig. 1.

Table 3. Results of statistical analyses of the propagated effects of drying. Italics indicate random terms. Significant probability values are highlighted in bold. Data on fungal biomass and litter mass remaining were analysed after square-root or Box-Cox transformation, respectively

			Fungal biomass				Litter mass remaining			
No.	Source of variation	No. denominator term	d.f.	SS	*F*	*P*	d.f.	SS ($\times 10^9$)	*F*	*P*
1	*B*		*7*	*6·4*			*7*	*9·2*		
2	D	3	5	118·8	9·0	**< 0·001**	5	33·3	6·4	**< 0·001**
3	*D × B*		*35*	*92·0*			*35*	*36·3*		
4	S	5	1	10·1	4·4	0·07	1	768·7	4037·1	**< 0·001**
5	*S × B*		*7*	*16·0*			*7*	*1·3*		
6	M	7	1	1·7	2·4	0·16	1	0·0	0·3	0·60
7	*M × B*		*7*	*4·8*			*7*	*0·9*		
8	D × S	9	5	54·9	9·1	**< 0·001**	5	5·9	7·2	**< 0·001**
9	*D × S × B*		*33*	*40·0*			*31*	*5·0*		
10	D × M	11	5	2·9	1·7	0·16	5	0·4	0·6	0·74
11	*D × M × B*		*33*	*11·2*			*25*	*3·7*		
12	S × M	13	1	< 0·1	< 0·1	0·87	1	0·3	1·1	0·34
13	*S × M × B*		*7*	*2·4*			*7*	*2·2*		
14	D × S × M	15	5	9·2	2·1	0·10	5	0·2	0·3	0·92
15	*D × S × M × B*		*28*	*24·8*			*17*	*2·4*		
	Total		180	395·1			159	869·8		

No. denominator term, number of the term (first column) used as the denominator to calculate the *F*-ratio; B, block; D, drying regime; S, litter species; M, litter mixing.

the mean and variability in fungal biomass compared to drying periods at the beginning (Dai7) or in the middle of the experiment (Da7).

FUNGAL SPORULATION ACTIVITY

Sporulation activity on alder and oak litter differed greatly (Fig. 2c,f). Numbers of spores produced per mg mycelial biomass per day ranged from $2{\cdot}5 \times 10^3$ to $1{\cdot}6 \times 10^5$ (treatment means) on alder and from 9·6 to 34·8 on oak litter, corresponding to $6{\cdot}6 \times 10^2$–$3{\cdot}5 \times 10^4$ and 2·5–12·0 ng per mg mycelial biomass per day respectively.

Mixing leaf species had no significant effect on the total spore biomass produced per day on either alder or oak litter immediately before or after the drying period (Fig. 2c,f, Table 2; $P = 0{\cdot}37$).

In spite of considerable variation among replicate samples, sporulation of aquatic hyphomycetes also significantly differed among drying treatments, with alder and oak litter showing different patterns (Fig. 2c,f, Table 2). Highest sporulation rates occurred on continuously submerged litter (S4), especially on alder litter. Exposure on the dry stream bank (Da4) and moistening litter during the drying period (Dm4) had minor effects on sporulation (Fig. 2c,f). Sporulation activity was dominated by three fungal species (*Flagellospora curvula* Ingold, *Lemonniera terrestris* Tubaki and *Lemonniera aquatica* de Wildeman), which accounted for over 99% of the spore biomass produced on the two litter types.

Discussion

LITTER MIXTURE EFFECTS

A clear result of the present study is the striking similarity in all response variables observed between leaves decomposing in single-species vs. mixed-species litter bags. This is compelling evidence that any interaction between litter species that might have occurred failed to induce diversity effects, i.e. failed to lead to either an increase or decrease in decomposition rates in litter mixtures. Unlike most other litter mixture experiments conducted to date (but see e.g. Schindler & Gessner 2009; Hoorens, Coomes & Aerts 2010), we were able to sort the leaves retrieved from the field by species and to analyse decomposition rates and variables describing fungal performance separately for the two litter types. Therefore, we can preclude that the observed lack of diversity effects was caused by a balance between negative and positive effects on the two species included in the mixtures.

This outcome of our experiment is unexpected given that the two leaf species chosen, alder and oak, starkly contrasted in resource quality, especially in terms of nitrogen content (high in alder) and toughness (high in oak owing to a high lignin content; Gessner & Chauvet 1994). The potential significance of contrasting species traits for diversity effects has been pointed out previously (e.g. Hillebrand & Matthiessen 2009; Gessner *et al.* 2010). However, this idea is not clearly supported by our results, consistent with a few experimental tests conducted in a forest (Fyles & Fyles 1993), a grassland (Hoorens, Aerts & Stroetenga 2003) and, to some extent, a stream (Schindler & Gessner 2009), even though litter diversity effects on decomposition have been repeatedly detected in both aquatic (Gessner *et al.* 2010; Kominoski *et al.* 2010; Lecerf & Richardson 2010) and terrestrial ecosystems (Gartner & Cardon 2004; Hättenschwiler, Tiunov & Scheu 2005; Gessner *et al.* 2010).

Some evidence exists that diversity is particularly important to maintain ecosystem process rates under stressful conditions such as pathogen load (Maron *et al.* 2011), periodic disturbance (Cardinale & Palmer 2002) or drought (Mulder, Uliassi & Doak 2001). In the present study, we tested a mechanism potentially causing diversity effects on residual moisture during dry periods (Wardle *et al.* 2003; Hättenschwiler, Tiunov & Scheu 2005), with consequences on litter decomposition. Specifically, we hypothesized that recalcitrant litter with high water-holding capacity would serve as a moisture reservoir (Dirks *et al.* 2010) that prolongs decomposer activity in easily degradable leaves under desiccation stress and thus extends the window of opportunity for decomposers of alder litter in mixtures with oak. This mechanism was clearly not supported by our results, although our pilot study showed that the presence of oak leaves indeed retarded the desiccation of alder leaves. Importantly, the negative outcome was not caused by a lack of statistical power, as our experiment with a replication level of eight (i.e. 8 blocks) produced nearly identical mean responses in single-species and mixed-species litter.

EFFECTS OF DRYING REGIME

Lack of influence of litter drying on litter mixture effects does not mean that decomposition and fungal performance were unaffected by desiccation. In fact, influences of drying regimes on alder were immediately apparent after the experimentally imposed drying, and these effects also propagated until 3 weeks after resubmergence of the litter in the stream channel. The delayed decomposition and lower fungal biomass that we observed as an immediate response of alder litter to desiccation reflect the fact that decomposition rates are typically much higher in aquatic than terrestrial settings (Boulton & Lake 1992; Langhans *et al.* 2008; Larned *et al.* 2010; Leberfinger, Bohman & Herrmann 2010). That similar effects on oak litter were not apparent was expected, because fungal colonization and decomposition had not sufficiently advanced to detect them (for differences between the two litter species, see Gessner & Chauvet 1994).

The severe drying imposed in one of our treatments (Do7) had negative long-term consequences on fungal biomass especially in alder litter. Because mycelia and spores of the primary microbial decomposers of leaves in streams (i.e. aquatic hyphomycetes; Gessner *et al.* 2007; Krauss *et al.* 2011) are thin-walled and delicate, these fungi were likely to be impeded during the drying period, requiring at least partial recolonization and resumption of growth after resubmergence of the litter in the stream (Bärlocher 2009). This would explain the observed lag of fungal development 3 weeks later. In contrast, less severe desiccation (Da7 and Dm7), which better reflects natural conditions, probably allowed fungal colonies to quickly resume activity following resubmergence, thus limiting propagation of drying effects. This interpretation is in line with the observations that aquatic hyphomycetes in leaves can persist in humid terrestrial environments (Sanders & Webster 1978; Sridhar & Bärlocher 1993) and that the total duration, but not frequency, of desiccation events affects fungal biomass and litter decomposition in streams (Langhans & Tockner 2006). Duration of the drying–rewetting cycles tested by Langhans & Tockner (2006) ranged from 1 to 7 days for both dry and wet periods, suggesting that aquatic fungi can regain activity within 1 day after stream flow resumes.

EFFECTS OF FLOW

An unanticipated result emerging from a suite of controls we had included in our experiment revealed that seemingly minor differences in the timing of drying can be important for both fungal biomass development and litter decomposition in streams. This is suggested by our observation that mass loss was lowest and fungal biomass highest in litter transferred to the stream bank 1 week before the experiment was terminated (Daf7). The observed acceleration of litter mass loss in the stream during the last week (S7) may be partly attributable to increased shear stress during high stream flow resulting from a rainstorm in the catchment (A. Bruder, pers. obs.). Based on exponential decay rates calculated for alder litter bags submerged under base flow conditions (i.e. S3, S4 and Daf7 corrected for mass loss owing to leaching; Petersen & Cummins 1974), average litter mass loss after 7 weeks is projected to amount to 44·0 ± 1·4% (mean ± 95% CI), whereas alder litter retrieved from the stream after 7 weeks experienced a mass loss of 67·5 ± 6·6%, i.e. 23·5% more. Thus, the observed litter mass loss of alder at the last sampling date was clearly higher than expected, implying that factors other than biological decomposition were influential in the last week.

However, mechanical particle losses cannot readily explain the sharp decline in fungal biomass observed at the last sampling date, especially in oak leaves (Dai7 and S7 vs. Daf7). One other possible mechanism is that litter-consuming invertebrates used the litter bags as a flow refuge (see Winterbottom *et al*. 1997). This could have led to aggregation and thus enhanced feeding pressure on the litter-associated fungi, which are preferred food for litter-consuming stream detritivores and thus are selectively removed (Arsuffi & Suberkropp 1985; Graça 2001). Although not straightforward, this interpretation is supported by the fact that fungal biomass patterns were broadly similar on oak and alder leaves, despite very different decomposition stages at the time of sampling, and that the differences among treatments (Dai7 and S7 vs. Daf7) were more pronounced for oak litter, which was likely to provide a better refuge than the softer and partly decomposed alder litter. If this interpretation is correct, then part of the acceleration of litter mass loss might indeed have been owing to litter consumption by invertebrates as well.

Overall, our results provide evidence that hydrological conditions such as stream intermittency and timing of high-flow events influence fungal biomass and litter decomposition more than does mixing of litter species with contrasting traits. In extreme meteorological conditions, which are projected to become more common in the future, the combined effects of dry periods and floods might increase the risk of washing resources for food webs downstream before they can be utilized (Webster 2007), reducing biomass at all trophic levels (Wallace *et al*. 1997). This risk is likely to increase as climate change affects the flow regime of future and currently intermittent streams (Milly, Dunne & Vecchia 2005). Although litter-mixing effects on decomposition have repeatedly been demonstrated (Gessner *et al*. 2010), our results suggest that high species richness of litter derived from riparian vegetation is unlikely to serve as an effective buffer against such negative influences of drying on litter resource use in stream food webs that rely on riparian litter inputs.

Acknowledgements

We thank J. Jabiol, J. Cornut, S. Lamothe and D. Lambrigot for field and laboratory assistance; D. Hohmann for identifying and counting fungal spores; D. Steiner for assistance with ergosterol extractions; and V. Acuña and three anonymous reviewers for constructive comments that improved earlier versions of the manuscript. This research was funded by the Swiss National Science Foundation (SNF grant 31ED30-114213) through the European Science Foundation's (ESF) EUROCORES programme EuroDIVERSITY, which supported BioCycle as a collaborative research project. BioCycle has been endorsed by DIVERSITAS as contributing to its biodiversity research priorities. The work presented in this paper conforms to local, national and international legal requirements.

References

Arsuffi, T.L. & Suberkropp, K. (1985) Selective feeding by stream caddisfly (Trichoptera) detritivores on leaves with fungal-colonized patches. *Oikos*, **45**, 50–58.

Bärlocher, F. (2009) Reproduction and dispersal in aquatic hyphomycetes. *Mycoscience*, **50**, 3–8.

Bärlocher, F. & Schweizer, M. (1983) Effects of leaf size and decay rate on colonization by aquatic Hyphomycetes. *Oikos*, **41**, 205–210.

Boulton, A.J. (2003) Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages. *Freshwater Biology*, **48**, 1173–1185.

Boulton, A.J. & Lake, P.S. (1992) Benthic organic matter and detritivorous macroinvertebrates in two intermittent streams in south-eastern Australia. *Hydrobiologia*, **241**, 107–118.

Cardinale, B.J. & Palmer, M.A. (2002) Disturbance moderates biodiversity-ecosystem function relationships: experimental evidence from caddisflies in stream mesocosms. *Ecology*, **83**, 1915–1927.

Dirks, I., Navon, Y., Kanas, D., Dumbur, R. & Grünzweig, J.M. (2010) Atmospheric water vapor as driver of litter decomposition in Mediterranean shrubland and grassland during rainless seasons. *Global Change Biology*, **16**, 2799–2812.

Findlay, S.E.G. & Arsuffi, T.L. (1989) Microbial growth and detritus transformations during decomposition of leaf litter in a stream. *Freshwater Biology*, **21**, 261–269.

Fyles, J.W. & Fyles, I.H. (1993) Interaction of Douglas-fir with red alder and salal foliage litter during decomposition. *Canadian Journal of Forest Research*, **23**, 358–361.

Gartner, T.B. & Cardon, Z.G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos*, **104**, 230–246.

Gessner, M.O., Bärlocher, F. & Chauvet, E. (2003) Qualitative and quantitative analyses of aquatic hyphomycetes in streams. *Freshwater Mycology* (eds C.K.M. Tsui & K.D. Hyde), pp. 127–157. Fungal Diversity Press, Hong Kong.

Gessner, M.O. & Chauvet, E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology*, **59**, 502–507.

Gessner, M.O. & Chauvet, E. (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*, **75**, 1807–1817.

Gessner, M.O. & Newell, S.Y. (2002) Biomass, growth rate, and production of filamentous fungi in plant litter. *Manual of Environmental Microbiology* (eds C.J. Hurst, R.L. Crawford, G. Knudsen, M. McInerney & L.D. Stetzenbach), pp. 390–408. ASM Press, Washington, DC.

Gessner, M.O., Gulis, V., Kuehn, K.A., Chauvet, E. & Suberkropp, K. (2007) Fungal decomposers of plant litter in aquatic ecosystems. *Environmental and Microbial Relationships* (eds C.P. Kubicek & I.S. Druzhinina), pp. 301–324. Springer, Berlin.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. & Hättenschwiler, S. (2010) Diversity meets decomposition. *Trends in Ecology & Evolution*, **25**, 372–380.

Graça, M.A.S. (2001) The role of invertebrates on leaf litter decomposition in streams – a review. *International Review of Hydrobiology*, **86**, 383–393.

Hättenschwiler, S., Tiunov, A.V. & Scheu, S. (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 191–218.

Herbst, G. & Reice, S.R. (1982) Comparative leaf litter decomposition in temporary and permanent streams in semi-arid regions of Israel. *Journal of Arid Environments*, **5**, 305–318.

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

Hillebrand, H. & Matthiessen, B. (2009) Biodiversity in a complex world: consolidation and progress in functional biodiversity research. *Ecology Letters*, **12**, 1405–1419.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J. & Wardle, D. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Hoorens, B., Aerts, R. & Stroetenga, M. (2003) Does initial litter chemistry explain litter mixture effects on decomposition? *Oecologia*, **137**, 578–586.

Hoorens, B., Coomes, D. & Aerts, R. (2010) Neighbour identity hardly affects litter-mixture effects on decomposition rates of New Zealand forest species. *Oecologia*, **162**, 479–489.

IPCC (2007) *Climate Change 2007: Synthesis Report. Contributions of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Core Writing Team, R.K. Pachauri & A. Reisinger). IPCC, Geneva, Switzerland.

Kominoski, J.S., Hoellein, T.J., Leroy, C.J., Pringle, C.M. & Swan, C.M. (2010) Beyond species richness: expanding biodiversity-ecosystem functioning theory in detritus-based streams. *River Research and Applications*, **26**, 67–75.

Krauss, G.-J., Solé, M., Krauss, G., Schlosser, D., Wesenberg, D. & Bärlocher, F. (2011) Fungi in freshwaters: ecology, physiology and biochemical potential. *FEMS Microbiology Reviews*, **35**, 620–651.

Lake, P.S. (2003) Ecological effects of perturbation by drought in flowing waters. *Freshwater Biology*, **48**, 1161–1172.

Langhans, S.D. & Tockner, K. (2006) The role of timing, duration, and frequency of inundation in controlling leaf litter decomposition in a river-floodplain ecosystem (Tagliamento, northeastern Italy). *Oecologia*, **147**, 501–509.

Langhans, S.D., Tiegs, S.D., Gessner, M.O. & Tockner, K. (2008) Leaf-decomposition heterogeneity across a riverine floodplain mosaic. *Aquatic Sciences*, **70**, 337–346.

Larned, S.T., Datry, T., Arscott, D.B. & Tockner, K. (2010) Emerging concepts in temporary-river ecology. *Freshwater Biology*, **55**, 717–738.

Leberfinger, K., Bohman, I. & Herrmann, J. (2010) Drought impact on stream detritivores: experimental effects on leaf litter breakdown and life cycles. *Hydrobiologia*, **652**, 247–254.

Lecerf, A. & Richardson, J.S. (2010) Biodiversity-ecosystem function research: insights gained from streams. *River Research and Applications*, **26**, 45–54.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D.A. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*, **294**, 804–808.

Maamri, A., Chauvet, E., Chergui, H., Gourbière, F. & Pattee, E. (1998) Microbial dynamics on decaying leaves in a temporary Moroccan river. I – Fungi. *Archiv für Hydrobiologie*, **144**, 41–59.

Maron, J.L., Marler, M., Klironomos, J.N. & Cleveland, C.C. (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters*, **14**, 36–41.

McArthur, J.V., Aho, J.M., Rader, R.B. & Mills, G.L. (1994) Interspecific leaf interactions during decomposition in aquatic and floodplain ecosystems. *Journal of the North American Benthological Society*, **13**, 57–67.

Milly, P.C.D., Dunne, K.A. & Vecchia, A.V. (2005) Global pattern of trends in streamflow and water availability in a changing climate. *Nature*, **438**, 347–350.

Mulder, C.P.H., Uliassi, D.D. & Doak, D.F. (2001) Physical stress and diversity–productivity relationships: the role of positive interactions. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 6704–6708.

Petersen, R.C. & Cummins, K.W. (1974) Leaf processing in a woodland stream. *Freshwater Biology*, **4**, 343–368.

Poff, N.L. & Zimmerman, J.K.H. (2010) Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. *Freshwater Biology*, **55**, 194–205.

Quinn, G.P. & Keough, M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Sanders, P.F. & Webster, J. (1978) Survival of aquatic hyphomycetes in terrestrial situations. *Transactions of the British Mycological Society*, **71**, 231–237.

Schimel, J.P. & Hättenschwiler, S. (2007) Nitrogen transfer between decomposing leaves of different N status. *Soil Biology & Biochemistry*, **39**, 1428–1436.

Schindler, M.H. & Gessner, M.O. (2009) Functional leaf traits and biodiversity effects on litter decomposition in a stream. *Ecology*, **90**, 1641–1649.

Sridhar, K.R. & Bärlocher, F. (1993) Aquatic hyphomycetes on leaf-litter in and near a stream in Nova Scotia, Canada. *Mycological Research*, **97**, 1530–1535.

Tank, J.L., Rosi-Marshall, E.J., Griffiths, N.A., Entrekin, S.A. & Stephen, M.L. (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society*, **29**, 118–146.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*. Springer, Berlin.

Wallace, J.B., Eggert, S.L., Meyer, J.L. & Webster, J.R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science*, **277**, 102–104.

Wardle, D.A., Nilsson, M.C., Zackrisson, O. & Gallet, C. (2003) Determinants of litter mixing effects in a Swedish boreal forest. *Soil Biology & Biochemistry*, **35**, 827–835.

Webster, J.R. (2007) Spiraling down the river continuum: stream ecology and the U-shaped curve. *Journal of the North American Benthological Society*, **26**, 375–389.

Winterbottom, J.H., Orton, S.E., Hildrew, A.G. & Lancaster, J. (1997) Field experiments on flow refugia in streams. *Freshwater Biology*, **37**, 569–580.

Received 15 March 2011; accepted 18 July 2011
Handling Editor: Ken Thompson