

# Resource–consumer diversity: testing the effects of leaf litter species diversity on stream macroinvertebrate communities

JOHN S. KOMINOSKI\*<sup>†</sup> AND CATHERINE M. PRINGLE<sup>†</sup>

<sup>\*</sup>Department of Forest Sciences, University of British Columbia, Vancouver, BC, Canada

<sup>†</sup>Odum School of Ecology, University of Georgia, Athens, GA, U.S.A.

## SUMMARY

1. Understanding relationships between resource and consumer diversity is essential to predicting how changes in resource diversity might affect several trophic levels and overall ecosystem functioning.
2. We tested for the effects of leaf litter species diversity (i.e. litter mixing) on litter mass remaining and macroinvertebrate communities (taxon diversity, abundance and biomass) during breakdown in a detritus-based headwater stream (North Carolina, U.S.A.). We used full-factorial analyses of single- and mixed-species litter from dominant riparian tree species with distinct leaf chemistries [red maple (*Acer rubrum*), tulip poplar (*Liriodendron tulipifera*), chestnut oak (*Quercus prinus*) and rhododendron (*Rhododendron maximum*)] to test for additivity (single-species litter presence/absence effects) and non-additivity (emergent effects of litter species interactions).
3. Significant non-additive effects of litter mixing on litter mass remaining were explained by species composition, but not richness, and litter-mixing effects were variable throughout breakdown. Specifically, small differences in observed versus expected litter mass remaining were measured on day 14; whereas observed litter mass remaining in mixed-species leaf packs was significantly higher on day 70 and lower on day 118 than expected from data for single-species leaf packs.
4. Litter mixing had non-additive effects on macroinvertebrate community structure. The number of species in litter mixtures (two to four), but not litter species composition, was a significant predictor of the dominance of particular macroinvertebrates (i.e. indicator taxa) within mixed-species packs.
5. In addition, the presence/absence of high- (*L. tulipifera*) and low-quality (*R. maximum*) litter had additive effects on macroinvertebrate taxon richness, abundance and biomass. The presence of *L. tulipifera* litter had both positive (synergistic) and negative (antagonistic) effects on invertebrate taxon richness, that varied during breakdown but were not related to litter chemistry. In contrast, the presence/absence of *L. tulipifera* had a negative relationship with total macroinvertebrate biomass (due to low leaf mass remaining when *L. tulipifera* was present and higher condensed and hydrolysable tannins associated with leaf packs lacking *L. tulipifera*). Macroinvertebrate abundance was consistently lower when *R. maximum* was present, which was partially explained by litter chemistry [e.g., high concentrations of lignin, condensed tannins, hydrolysable tannins and total phenolics and high carbon to nutrient (N and P) ratios].

---

Correspondence: John Stephen Kominoski, Department of Forest Sciences, University of British Columbia, 3041-2424 Main Mall, Vancouver, BC V6T 1Z4, Canada. E-mail: jkominoski@gmail.com

6. The bottom-up effects of litter species diversity on stream macroinvertebrates and litter breakdown are different, which suggests that structural attributes of macroinvertebrate communities may only partially explain the effects of litter-mixing on organic matter processing in streams. In addition, stream macroinvertebrates colonising decomposing litter are influenced by resource diversity as well as resource availability. Broad-scale shifts in riparian tree species composition will alter litter inputs to streams, and our results suggest that changes in the diversity and availability of terrestrial litter may alter stream food webs and organic matter processing.

*Keywords:* additive effects, biodiversity, litter chemistry, non-additive effects, riparian, southern Appalachians

## Introduction

Widespread declines in species richness have prompted research exploring the relationship between biodiversity and ecosystem functioning (Schulze & Mooney, 1993; Kinzig, Pacala & Tilman, 2002; Loreau, Naeem & Inchausti, 2002; Hooper *et al.*, 2005). Earlier studies investigated the effects of terrestrial plant species richness on primary productivity (Naeem *et al.*, 1996; Tilman, Wedin & Knops, 1996; Hector *et al.*, 1999). More recently, attention has focused on the effects of terrestrial plant species diversity on leaf litter breakdown in both terrestrial (Gartner & Cardon, 2004; Hättenschwiler, Tiunov & Scheu, 2005; Ball *et al.*, 2008) and aquatic ecosystems (Swan & Palmer, 2004; LeRoy & Marks, 2006; Kominoski *et al.*, 2007; Lecerf *et al.*, 2007). Most primary production in temperate ecosystems enters food webs as detritus (McNaughton *et al.*, 1989; Cebrian, 1999), and the slow processing of detritus stabilises ecosystem energetics (Wetzel, 1995; Moore *et al.*, 2004). Therefore, detrital resource diversity may play an important role in structuring food webs that use detritus as a dominant energy source.

Detritus is a dominant basal resource in many aquatic ecosystems, and it contributes energy essential to maintaining diverse food webs (Wetzel, 1995; Hall, Wallace & Eggert, 2000). Allochthonous leaf litter (hereafter litter) entering forested, headwater streams supports several trophic levels and feeding guilds of macroinvertebrates (Wallace *et al.*, 1997, 1999; Hall, Likens & Malcom, 2001). Macroinvertebrate detritivores are dominant contributors to litter breakdown in streams (Wallace & Webster, 1996; Hieber & Gessner, 2002) and are prey for several levels of predators in aquatic ecosystems. The effects of litter

species diversity on in-stream breakdown have been shown to be largely non-additive, such that results from mixed-species litter are not predictable from single-species (Swan & Palmer, 2004; LeRoy & Marks, 2006; Kominoski *et al.*, 2007; Lecerf *et al.*, 2007). However, variation among macroinvertebrate communities appears to be explained more by differences in stream habitat than by litter species diversity (LeRoy & Marks, 2006). Further investigation into the relationships between resources and the diversity of macroinvertebrate consumers is needed to predict better which particular riparian tree species have the biggest structural and functional impacts on adjacent stream ecosystems.

Environmental changes are likely to affect biodiversity through non-random species losses and shifts in species composition (Vitousek *et al.*, 1997; Huston *et al.*, 2000; Loreau *et al.*, 2001; Tilman & Lehman, 2001; Ellison *et al.*, 2005), yet most experiments have been designed to measure the effects of random species loss. Such designs do not conclusively test the effect of the loss of a particular species and cannot separate the effects of species richness and composition (Huston, 1997; Drake, 2003). Therefore, the need to understand effects of non-random plant species losses (e.g., Ellison *et al.*, 2005) on litter breakdown dynamics requires experimental designs and statistical analyses that can test comprehensively for both individual litter species effects that are independent of other species (i.e. additivity) and emergent effects of litter species in mixed-species combinations (i.e. non-additivity) (see Kominoski *et al.*, 2007; Ball *et al.*, 2008). Understanding how non-random litter species losses will affect detritus-based stream ecosystems is vital as we continue to lose foundation species (i.e. dominant primary producers that have structural/functional

influence within ecosystems) and shift tree species composition in riparian forests (Orwig, Foster & Mausel, 2002; Ellison *et al.*, 2005).

Previously, we used a full-factorial experimental design to test for additive and non-additive effects of litter species diversity on breakdown dynamics in a headwater stream (Kominoski *et al.*, 2007). Overall, we found significant non-additive effects of litter species diversity on breakdown rates that were explained by litter species composition and marginally explained by litter species richness. However, the effects of litter species diversity on changes in litter chemistry throughout breakdown and on consumer biomass were less consistent. We observed non-additive effects of litter species diversity on litter nutrients and secondary compounds and additive effects on structural compounds. Non-additive effects of litter species diversity on bacterial and fungal biomass were seen during the early stages of breakdown, and additive effects on microbes during intermediate and later stages of breakdown were explained by the presence/absence of high- and low-quality litter species. There were no clear effects of litter species diversity on shredder biomass (Kominoski *et al.*, 2007).

Here, we tested further for the effects of litter species diversity on stream macroinvertebrate communities. We sought to determine how macroinvertebrates respond to litter species diversity and how the responses of macroinvertebrates may explain the effects of litter species diversity on litter mass remaining. As stream macroinvertebrates may contribute greatly to litter breakdown (Wallace & Webster, 1996; Hieber & Gessner, 2002), and because we observed non-additive effects of litter species composition on litter breakdown rates (despite largely additive effects on microbial biomass; Kominoski *et al.*, 2007), we made the following predictions:

1. Litter species diversity would have non-additive effects on macroinvertebrate assemblages (taxon diversity, abundance and biomass), similar to its effect on litter breakdown (Kominoski *et al.*, 2007).
2. Litter chemistry and mass remaining would explain non-additive effects of litter species composition on macroinvertebrates.
3. The effects of litter species diversity on macroinvertebrate communities would decline over time as the chemistry of litter species become homogeneous (*sensu* Moore *et al.*, 2004; Rier, Tuchman & Wetzel,

2005; Ardón & Pringle, 2008) and recalcitrant species persist relative to labile species.

Our experimental design allowed us to test how resource diversity, achieved by mixing litter species of different physical, chemical and biological properties, affects stream consumers. Specifically, we tested whether the independent role of single-species litter (additivity) or the emergent, interactive effects of litter mixing (non-additivity) explain patterns of macroinvertebrate communities.

## Methods

### Study site

Research was conducted at Coweeta Hydrologic Laboratory, Macon County, NC, U.S.A. (35°00'N, 83°30'W). Coweeta is a 2185 ha forested basin in the Blue Ridge physiographic province of the southern Appalachian Mountains (Swank & Crossley, 1988). Vegetation is mixed hardwood (dominated by *Quercus* spp., *Acer* spp. and *Liriodendron tulipifera* L.), with a dense understorey of *Rhododendron maximum* L. that contributes to year-round shading of streams, causing low in-stream autotrophic production (Webster *et al.*, 1997). Mean monthly air temperature ranges from 3 to 22 °C, and mean annual precipitation from 180 cm at low altitude to 250 cm at high altitude (Swift, Cunningham & Douglass, 1988). We selected a second-order reach of Ball Creek as our study location. Mean daily stream temperature and discharge of Ball Creek were measured continuously throughout the experiment.

### Leaf packs and experimental design

We selected four riparian tree species that are dominant and abundant at Coweeta [red maple *Acer rubrum* L. (A), tulip poplar *Liriodendron tulipifera* (L), chestnut oak *Quercus prinus* L. (Q), and rhododendron *R. maximum* (R)]. These species represent a range in initial litter chemistries and breakdown rates (Webster & Benfield, 1986; Kominoski *et al.*, 2007).

We collected freshly abscised leaves and air-dried them in the laboratory for 2 months during autumn 2003. Approximately 15 g of single- (richness level, 1) and mixed-species (richness level, two to four) litter, in equal proportions by mass, were placed in plastic mesh bags (19.1 × 38.1 cm, 5 × 5 mm mesh) after

initial determinations of dry mass. We used a randomised complete block design of 15 single- and mixed-species litter treatments, four replicate blocks and eight sampling dates. On 10 January 2004, we deployed leaf packs along a 75-m reach of Ball Creek. Additional leaf packs were transported back to the laboratory the same day and were used to estimate handling loss and initial litter chemistry. Fifteen leaf packs (representing all single- and mixed-species combinations) were grouped in arrays within each experimental block and secured to the stream bottom by using plastic ties with galvanised gutter nails. Litter was incubated in Ball Creek from 10 January to 31 July 2004. On each sampling day, arrays were randomly selected from in-stream blocks, and leaf packs were transported to the laboratory on ice and processed within 12 h.

Retrieved litter was rinsed over nested sieves (1 mm and 250  $\mu\text{m}$ ) to collect macroinvertebrates and remove sediments and debris. Litter was oven-dried at 60 °C for 24 h, and weighed. Ash-free dry mass (AFDM) on sampling days 14, 70 and 118 was determined as the difference between pre- and post-combustion (at 550 °C for 1 h). We report litter mass on each sampling day as percent AFDM remaining (as compared to breakdown rates reported in Kominoski *et al.*, 2007), which allowed us to test if litter-species-diversity effects were consistent across time (see Table S1 for litter mass data from all sampling days). Remaining litter in each leaf pack was milled with a Spex CertiPrep 8000-D Mixer Mill (Spex, Metuchen, NJ, U.S.A.) prior to chemical analyses.

#### Litter chemistry

Chemistry of single- and mixed-species litter was analysed on sampling days 14, 70 and 118 (see Table S1 for litter chemistry data from all sampling days). Litter carbon (C), nitrogen (N) and phosphorus (P) were measured as a percentage of litter mass (g AFDM). Litter C and N were measured with a Carlo Erba 1500N CHN Analyser (Carlo Erba, Milan, Italy). Litter for P-analysis was weighed into acid-washed, pre-ashed ceramic crucibles, combusted at 500 °C, acid-digested and analysed spectrophotometrically (ascorbic acid method; APHA, 1998). Cellulose, hemicellulose and lignin concentrations were analysed with an Ankom A200 Fibre Analyser (Ankom, Macedon, NY, U.S.A.). Tannin content was extracted

from ground litter with an acetone solution. Extracted tannin solution was further analysed for condensed tannins (CT), hydrolysable tannins (HT) and total phenolics (TP) using techniques described by Rossiter, Schultz & Baldwin (1988) and Hunter & Schultz (1995). Briefly, CT was measured by heat-extraction of tannin solution with *N*-butanol (Proanthocyanidin assay). The potassium iodate assay was used to measure HT, and TP was determined using the Folin-Denis assay.

#### Stream macroinvertebrates

Macroinvertebrates from single- and mixed-species litter were analysed on sampling days 14, 70 and 118 (S2). Macroinvertebrates were removed from all leaf packs and preserved in 95% ethanol. Individuals from two size classes (>1000  $\mu\text{m}$  and 250–1000  $\mu\text{m}$ ) were counted and identified to the lowest possible taxonomic level and assigned to a functional feeding group (Merritt & Cummins, 1996; Wallace *et al.*, 1999). Chironomidae were identified as Tanyptodinae and non-Tanyptodinae. A total of 118 genera from 50 families and 13 orders were identified (see Table S2). Length–mass regressions (Benke *et al.*, 1999) were used to estimate total macroinvertebrate biomass per leaf pack. Macroinvertebrate taxon diversity was analysed as richness (number of individual taxa), evenness, as well as Shannon's ( $H'$ ) and Simpson's diversity indices ( $D'$ ) for each leaf pack.

#### Statistical analyses

Individual litter AFDM remaining from single-species leaf packs was compared on sampling days 14, 70 and 118 using analysis of variance (ANOVA) and Tukey's HSD in SAS version 9.1 (SAS Institute Inc., Cary, NC, U.S.A.).

To test for additive and non-additive effects of litter species diversity on litter AFDM remaining and macroinvertebrate communities [taxon diversity (richness, evenness, Shannon's and Simpson's diversity indices), abundance and biomass], we used full-factorial ANOVA with type I sums of squares (SS) using PROC GLM in SAS (Kominoski *et al.*, 2007). Nested ANOVA was not used because it lacks the ability to differentiate statistically between additive and non-additive effects of species composition (Ball *et al.*, 2008). Note that species richness effects are

non-additive (emergent effects of species interactions) and species composition effects can be either additive or non-additive. For clarity, hereafter we define additive species composition effects as 'species presence/absence effects'. Using a full-factorial design of all combinations of four single-species litter allowed us to test additivity as an effect of species presence/absence (main effects) and non-additivity as species interactions above and beyond the presence/absence of individual species (diversity term). Time was treated as a discrete variable (e.g., Ball *et al.*, 2008), and block, time, the presence/absence of the four individual litter species (A, L, Q, R) to test for additive effects, and a diversity term (to test for non-additive effects) were added sequentially to the model. Interactions between time and block, time and species presence/absence, and time and diversity were added to the model. Each species presence/absence represented main effects, and the diversity term corresponded to either mixed-species richness (two to four) and/or composition effects ( $A \times L$ ,  $A \times Q$ ,  $A \times R$ ,  $L \times Q$ ,  $L \times R$ ,  $Q \times R$ ,  $A \times L \times Q$ ,  $A \times L \times R$ ,  $A \times Q \times R$ ,  $L \times Q \times R$ ,  $A \times L \times Q \times R$ ). A significant additive effect of diversity indicated that species presence/absence was significant. Since type I SS was used, species terms were sensitive to the order in which they were added to the model. Therefore, models were re-run with each species presence/absence term added first (to test for a significant additive effect of each species). A significant non-additive effect of diversity indicated that litter species diversity (richness and/or composition), but not species presence/absence, was significant. In this case, we re-ran the full-factorial analysis replacing diversity with richness. If litter species richness was significant we added a composition term to test for effects of litter species richness and composition. If litter species richness was not significant, non-additive effects were explained by litter species composition.

For all significant effects of litter species diversity (additive or non-additive) on macroinvertebrate assemblages (taxon diversity, abundance, biomass), we conducted simple linear regressions between macroinvertebrate data and litter chemistry and AFDM data using PROC REG in SAS.

We used an indicator taxa analysis (Dufrêne & Legendre, 1997) in PC-ORD (Version 4.10; MjM Software, Gleneden Beach, OR, U.S.A.) to determine the responses of specific macroinvertebrate taxa to

litter species diversity during breakdown. An indicator value (0–100) was generated for each taxon based on the product of its relative frequency and relative abundance. Monte Carlo tests (1000 randomisations) determined if indicator values were greater than expected by chance. Indicator taxa have both an indicator value  $>20$  and a  $P \leq 0.05$  (Dufrêne & Legendre, 1997).

Assumptions of normality of residuals were met for all analyses (Shapiro–Wilks test) through data transformations when necessary, and all analyses used an alpha (type I error rate) of 0.05.

## Results

### *Stream temperature and discharge*

Mean stream temperature increased steadily during the study and ranged from 1.5 to 16.3 °C, whereas mean discharge was variable, ranging from 0.11 to 1.1 m<sup>3</sup> s<sup>-1</sup> (Fig. 1).

### *Litter mass remaining*

Litter AFDM remaining varied among individual litter species (day 14:  $F_{3,9} = 24.6$ ,  $P < 0.001$ ; day 70:  $F_{3,9} = 26.7$ ,  $P < 0.001$ ; day 118:  $F_{3,9} = 14.0$ ,  $P = 0.001$ ) throughout the period (Fig. 2a). Although *Q. prinus* had the highest mass remaining after 14 days ( $P < 0.05$ ), *R. maximum* had higher mass remaining than all other litter species on sampling days 70 and 118 ( $P < 0.05$ ). On day 14, the AFDM remaining of *A. rubrum* was less than all other litter species ( $P < 0.05$ ), and *L. tulipifera* had less AFDM than *Q. prinus* ( $P < 0.05$ ). On day 70, AFDM of *A. rubrum* was less than *Q. prinus* ( $P < 0.05$ ). Although *L. tulipifera* and *A. rubrum* lost mass consistently over time, *Q. prinus* lost the greatest mass between 70 and 118 days of incubation. In addition, appreciable breakdown of *R. maximum* did not occur until after 70 days in the stream (Fig. 2a).

We observed non-additive effects of litter species diversity on litter AFDM remaining during breakdown, which were explained by species composition related to the presence/absence of *R. maximum* (Table 1; Fig. 2b). Observed versus expected AFDM remaining for mixed-species leaf packs became increasingly divergent during breakdown and varied among mixed-species litter (Fig. 2b). Specifically,

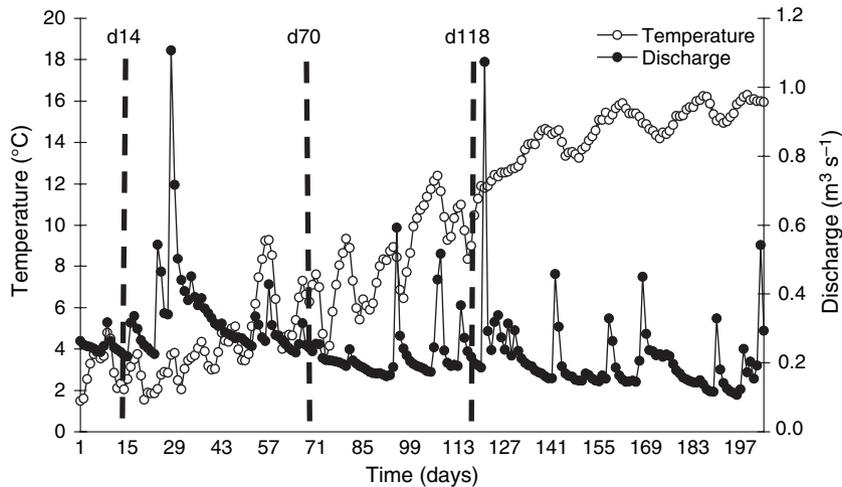


Fig. 1 Stream temperature and discharge from 10 January to 31 July 2004 in Ball Creek. Data are daily means. Vertical dashed lines indicate days during which leaf litter and macroinvertebrate samples were collected (14, 70 and 118). For clarity, SE not shown.

observed values were mostly similar to expected values on day 14, greater than expected values on day 70 and less than expected values on day 118. This means that AFDM remaining in mixtures became increasingly less predictable during breakdown, due to differences in AFDM among high- and low-quality litter species. Non-additive effects of litter mixing stimulated mass loss (i.e. synergistic effects) by day 70 and inhibited mass loss (i.e. antagonistic effects) by day 118 (Fig. 2b). The greatest differences between observed and expected litter AFDM remaining were seen for the four-species leaf pack (i.e. LAQR) on day 14, two-species leaf packs (i.e. LA, QR) on day 70 and three-species leaf packs (e.g., LQR, AQR) on day 118 (Fig. 2b).

#### Macroinvertebrate indicator taxa and diversity

Indicator taxa analyses showed an effect of litter species richness, but not composition, on the presence of macroinvertebrate taxa within leaf packs. Three shredder taxa, including the dominant *Tallaperla* sp., were associated with two- and three-species litter on days 14 and 70, whereas several predator taxa were associated with three- and four-species litter on day 118 (with the exception of the predator *Suwallia* sp., which was associated with two-species litter on day 14; Table 2).

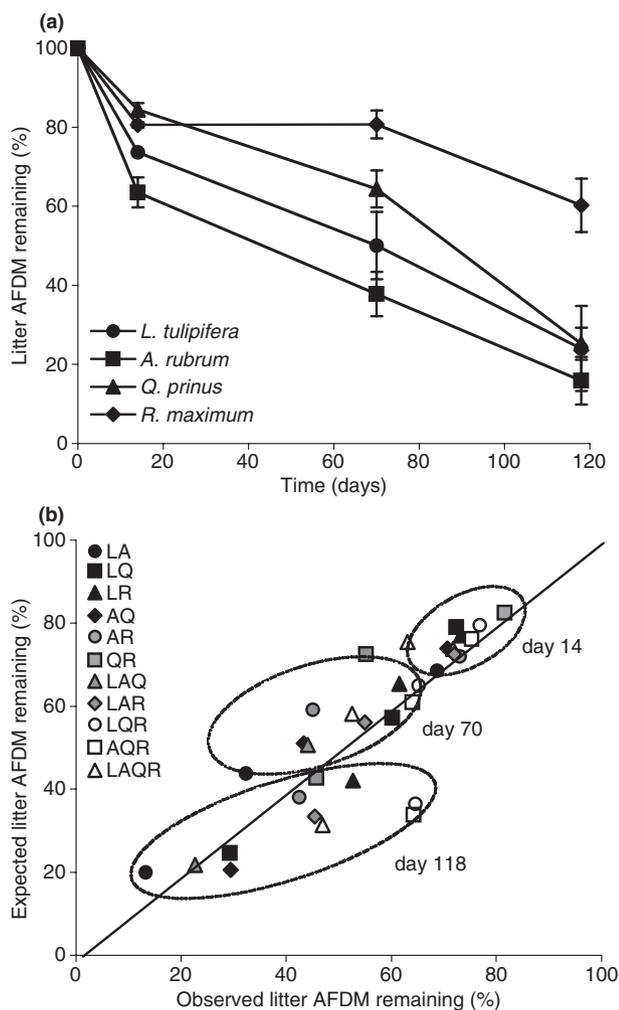
Presence/absence of *L. tulipifera* in litter mixtures had significant additive effects on taxon richness (Table 3). Macroinvertebrate taxon richness generally increased during breakdown and was greater in the presence of *L. tulipifera* during the early stages of

breakdown. By the intermediate stages of breakdown, taxon richness was greater in leaf packs that lacked *L. tulipifera* (Fig. 3). Additive effects on taxon richness were not related to litter chemistry or mass remaining ( $r^2 < 0.20$ ,  $P > 0.05$ ).

In contrast to macroinvertebrate taxon richness, litter species diversity had no effect on macroinvertebrate taxon evenness or diversity indices (Shannon's and Simpson's) during breakdown.

#### Macroinvertebrate abundance and biomass

We detected additive effects of *L. tulipifera* and *R. maximum* presence/absence on macroinvertebrate abundance and biomass. Additive effects of *R. maximum* presence/absence on macroinvertebrate abundance were consistent across time (Table 4) and resulted in lower macroinvertebrate abundance in single- and mixed-species leaf packs when rhododendron was present (Fig. 4a). In contrast, additive effects of *L. tulipifera* on macroinvertebrate biomass varied with time (Table 4), whereby biomass was lower during the intermediate and later stages of breakdown in leaf packs containing *L. tulipifera* (Fig. 4b). Additive effects of *R. maximum* on macroinvertebrate abundance were partially explained by litter nutrient content (N and P), structural compounds (lignin) and secondary compounds (condensed tannins, hydrolysable tannins, total phenolics; Table 5a). Similarly, additive effects of *L. tulipifera* on macroinvertebrate biomass were partially explained by litter secondary compounds (condensed tannins, hydrolysable tannins) but also by litter AFDM remaining (Table 5b).



**Fig. 2** Leaf litter ash-free dry mass (AFDM) remaining for single- and mixed-species leaf packs. (a) Bars represent mean mass remaining (+SE) for single-species leaf packs on each sampling day (14, 70, 118). Individual litter species are: red maple *Acer rubrum*, tulip poplar *Liriodendron tulipifera*, chestnut oak *Quercus prinus*, rhododendron *Rhododendron maximum*. (b) Scatter plots represent grand means of observed versus expected mass remaining for mixed-species leaf packs on each sampling day. Expected mass remaining for mixed-species packs was calculated as the average of individual species litter mass remaining from single-species leaf packs. Deviation from the 1 : 1 line indicates litter-species-diversity effects on mixed-species litter mass remaining. Species abbreviations are: (A) *A. rubrum*, (L) *L. tulipifera*, (Q) *Q. prinus*, (R) *R. maximum*.

## Discussion

Our results demonstrate that the effects of litter species diversity on macroinvertebrate communities (i.e. non-additive effects of litter species richness and additive effects of litter species presence/absence)

**Table 1** Results of ANOVA tests for additive (i.e. main effects terms; species presence/absence) and non-additive (i.e. Composition and Richness terms; species interactions) effects of litter species diversity on litter ash-free dry mass (AFDM) remaining using type I SS. *Acer rubrum* (A), *Liriodendron tulipifera* (L), *Quercus prinus* (Q), *Rhododendron maximum* (R). Species terms were affected by the order in which they were added to the model. Therefore, when results were additive, we re-ran analyses placing each species first in the order to ensure that overall model results were consistent. Model results reported here are main effects in alphabetical order (A, L, Q, R), as shown. Significant non-additive effects correspond to mixed-species composition effects (A × L, A × Q, A × R, L × Q, L × R, Q × R, A × L × Q, A × L × R, A × Q × R, L × Q × R, A × L × Q × R) and/or richness (two to four)

Litter AFDM	d.f.	SS	MS	F	P-value
Block	3	1438.9	479.6	3.67	0.01
Time	2	35 956.8	17 978.4	137.7	<0.01
A	1	4949.6	4949.6	37.9	<0.01
L	1	803.1	803.1	6.15	0.01
Q	1	344.5	344.5	2.64	0.11
R	1	9013.1	9013.1	69.1	<0.01
Composition	10	3144.2	314.4	2.41	<b>0.01</b>
Richness	1	32.1	32.1	0.25	0.62
Time × block	6	1849.1	308.2	2.36	0.03
Time × A	2	608.4	304.2	2.33	0.10
Time × L	2	10.3	5.1	0.04	0.96
Time × Q	2	47.5	23.7	0.18	0.83
Time × R	2	6062.5	3031.3	23.2	<0.01
Time × composition	20	2318.0	115.9	0.89	0.60
Time × richness	1	15.6	15.6	0.12	0.60
Error	124	16 186.4	130.5		
Total	155	82 780.1	37 849.3		

P-value for overall model results is given in bold.

were different than those measured for litter mass remaining (i.e. non-additive effects of litter species composition). Therefore, our hypothesis that litter species diversity would affect litter mass remaining and macroinvertebrates in leaf packs similarly, through non-additive effects of litter species composition, was not supported. Additive effects of litter species presence/absence on macroinvertebrates were partially explained by litter chemistry and mass remaining, and these effects varied but did not decline over time. To that end, our hypotheses that the effects of litter species diversity on macroinvertebrates would be reflected by litter chemistry and litter mass remaining, and that these effects would decline over time, were partially supported. Additive effects of litter species diversity were observed for both macroinvertebrates (this study) and microbes (Kominoski *et al.*, 2007). The presence of high- (e.g., *L. tulipifera*)

**Table 2** Results of indicator taxa analysis results for macroinvertebrates unique to different litter species richness levels and sampling days during breakdown in Ball Creek, Coweeta Hydrologic Laboratory, NC, U.S.A. Values represent indicator values (*I*-value), Monte Carlo *P*-values, litter species richness and sampling day (Day)

Indicator taxon	FFG	<i>I</i> -value	<i>P</i> -value	Litter species richness	Day
<i>Baetis</i> sp.	SC	35.3	0.02	4	14
<i>Ephemera</i> sp.	C-G	23.1	0.03	4	14
<i>Smothia</i> sp.	P	33.5	0.04	2	14
<i>Tallaperla</i> sp.	SH	43.4	0.02	2	14
<i>Pycnopsyche</i> sp.	SH	21.4	0.05	3	70
<i>Tallaperla</i> sp.	SH	45.0	0.05	2	70
Araneae	P	44.8	0.01	4	118
<i>Dicranota</i> sp.	P	37.4	0.03	4	118
<i>Hexatoma</i> sp.	P	41.5	0.01	4	118
Isopoda sp.	SH	25.0	0.03	3	118
<i>Stenus</i> sp.	P	18.7	0.05	3	118

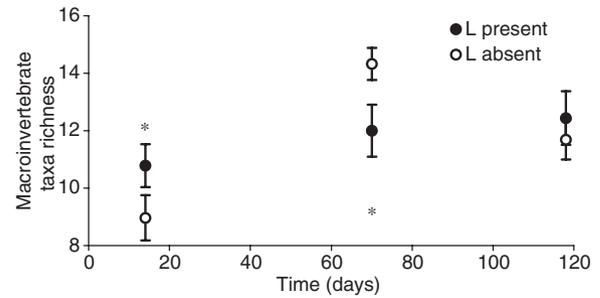
Functional feeding groups (FFG) were identified for each indicator taxon (C-G, collector-gatherer; P, predator; SC, scraper; SH, shredder).

**Table 3** Results of ANOVA tests for additive (i.e. main effects terms; species presence/absence) and non-additive (i.e. diversity term; species interactions) effects of litter species diversity on macroinvertebrate taxon richness (leaf pack<sup>-1</sup>) using type I SS. Remaining information as in Table 1

Taxon richness	d.f.	SS	MS	<i>F</i>	<i>P</i> -value
Block	3	18.9	6.3	0.31	0.82
Time	2	311.3	155.7	7.66	<.01
A	1	9.6	9.6	0.47	0.49
L	1	0.3	0.3	0.01	0.91
Q	1	9.5	9.5	0.47	0.50
R	1	0.1	0.1	0.01	0.94
Diversity	10	134.0	13.4	0.66	0.76
Time × block	6	266.3	44.4	2.18	0.05
Time × A	2	12.8	6.4	0.32	0.73
Time × L	2	130.2	65.1	3.20	<b>0.04</b>
Time × Q	2	0.7	0.4	0.02	0.98
Time × R	2	51.4	25.7	1.26	0.29
Time × diversity	20	494.1	24.7	1.22	0.25
Error	120	2438.4	20.3		
Total	173	3877.6	381.9		

*P*-value for overall model results is given in bold.

and low-quality (e.g., *R. maximum*) litter species had both inhibitory and stimulatory effects on macroinvertebrates during breakdown. In contrast, microbes appeared to respond more consistently to litter chemistry; higher bacterial and fungal biomass in



**Fig. 3** Main effects of *Liriodendron tulipifera* litter presence/absence on macroinvertebrate taxon richness (means ± SE). An asterisk denotes significant ( $P < 0.05$ ) differences between open and closed symbols on the same day.

the presence of *L. tulipifera* and lower microbial biomass in the presence of *R. maximum* (Kominoski *et al.*, 2007). There are several plausible explanations for differential effects of litter species diversity on litter mass remaining and consumers: (i) dynamic changes in litter mass and chemistry as well as consumer (macroinvertebrate and microbial) colonisation patterns occur at different time-scales; (ii) functional traits (e.g., labile versus recalcitrant) and resource roles of litter species (e.g., food, habitat, refugia) change during breakdown based on litter chemical integrity and mass remaining; and (iii) consumer resource requirements change throughout breakdown in accordance with life history traits, competition and resource availability.

Resource availability and differential resource utilisation among consumer groups may explain additive and non-additive responses by macroinvertebrates and microbes to litter species diversity. Litter mixtures containing labile and recalcitrant species can provide heterogeneous microhabitats that support different consumer communities (Hansen & Coleman, 1998; Kaneko & Salamanca, 1999). Our results show that dominant macroinvertebrate taxa from different functional feeding groups respond differently to litter species diversity. For example, we detected increased dominance of the shredder, *Tallaperla* sp., in two-species litter during early and intermediate stages of breakdown and multiple predator taxa in three- and four-species litter during later stages of breakdown. During the later stages of breakdown, most of the mass of labile litter species had probably gone, and recalcitrant litter, such as *R. maximum* remained in proportionally greater mass. In addition, occurrence of *R. maximum* was greater in

**Table 4** Results of ANOVA tests for additive (i.e. main effects terms; species presence/absence) and non-additive (i.e. Diversity term; species interactions) effects of litter species diversity on total macroinvertebrate abundance (individuals leaf pack<sup>-1</sup>) and biomass (mg leaf pack<sup>-1</sup>) using type I SS. Remaining information same as Table 1

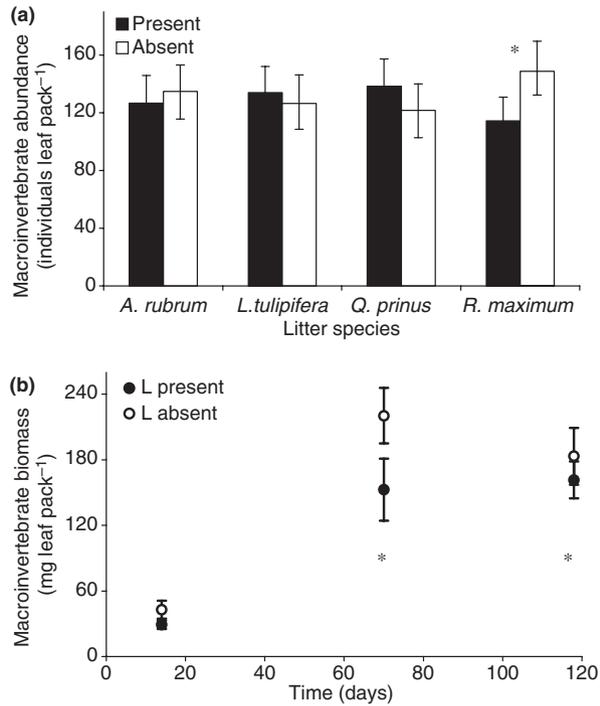
	d.f.	SS	MS	F	P-value
<b>Abundance</b>					
Block	3	25282	8427	0.73	0.53
Time	2	803 918	401 959	35.00	<0.01
A	1	1855	1855	0.16	0.69
L	1	1038	1038	0.09	0.76
Q	1	11 921	11 921	1.04	0.31
R	1	48 549	48 549	4.23	<b>0.04</b>
Diversity	10	124 201	12 420	1.08	0.38
Time × block	6	44 189	7364	0.64	0.70
Time × A	2	3967	1983	0.17	0.84
Time × L	2	5706	2853	0.25	0.78
Time × Q	2	22 909	11 454	1.00	0.37
Time × R	2	26 880	13 440	1.17	0.31
Time × diversity	20	289 092	14 454	1.26	0.22
Error	120	1 378 202	11 485		
Total	173	2 787 715	549 208		
<b>Biomass</b>					
Block	3	3.94	1.31	3.41	0.02
Time	2	8.27	4.14	10.75	<0.01
A	1	0.05	0.05	0.12	0.73
L	1	0.09	0.09	0.25	0.62
Q	1	0.27	0.27	0.69	0.41
R	1	0.13	0.13	0.35	0.56
Diversity	10	6.07	0.61	1.58	0.12
Time × block	6	17.79	2.97	7.71	<0.01
Time × A	2	0.41	0.20	0.53	0.59
Time × L	2	2.98	1.49	3.87	<b>0.02</b>
Time × Q	2	0.46	0.23	0.59	0.55
Time × R	2	0.36	0.18	0.46	0.63
Time × diversity	20	9.58	0.48	1.25	0.23
Error	120	46.17	0.38		
Total	173	96.56	12.52		

P-values for overall model results are given in bold.

more diverse mixed-species leaf packs, which may explain greater utilisation of this litter by non-shredder taxa (e.g., predators) during breakdown. In contrast, labile litter that supports high microbial biomass, such as *L. tulipifera* (Kominoski *et al.*, 2007), did not support high macroinvertebrate abundance and biomass when present in single- or mixed-species leaf packs. This is probably because there was too little *L. tulipifera* mass remaining to provide habitat and refugia for macroinvertebrates. Perhaps lower macroinvertebrate abundance and biomass associated with *L. tulipifera* litter facilitated higher microbial biomass during intermediate and later stages of breakdown (Kominoski *et al.*, 2007). In contrast, recalcitrant litter, such as *R. maximum*, offers less palatable food resources than labile litter and supports consistently lower microbial biomass (Kominoski *et al.*, 2007), which probably explains the

lower macroinvertebrate abundance and biomass associated with leaf packs containing *R. maximum* in this study. The presence of *R. maximum* in single- and mixed-species litter appears to inhibit bacterial and fungal biomass; whereas bacterial and fungal biomass in single- and mixed-species litter containing *L. tulipifera* was consistently higher (Kominoski *et al.*, 2007). To this end, we would expect that detritus containing a mixture of labile and recalcitrant litter species should support the highest abundance and biomass of macroinvertebrates (Hansen & Coleman, 1998; Kaneko & Salamanca, 1999), but this was not observed here.

Additive effects of litter species diversity on consumers (macroinvertebrates and microbes) may be explained by the increased similarity in chemistry among litter species during breakdown. Although variation in initial litter chemistry among species has



**Fig. 4** Main effects of litter species presence/absence on (a) macroinvertebrate biomass and (b) *Liriodendron tulipifera* litter on macroinvertebrate abundance (means  $\pm$  1SE;  $n = 8$  for 'present' and  $n = 7$  for 'absent'). Species abbreviations are: A, red maple, *Acer rubrum*; L, tulip poplar, *Liriodendron tulipifera*; Q, chestnut oak, *Quercus prinus*; R, rhododendron, *Rhododendron maximum*. An asterisk denotes significant ( $P < 0.05$ ) differences between open and closed symbols or open and shaded bars on the same day.

often been suggested to be a good predictor of litter breakdown (Webster & Benfield, 1986; LeRoy & Marks, 2006; LeRoy *et al.*, 2007), litter chemistry changes during breakdown. For example, secondary compounds rapidly leach from litter in streams (Ostrofsky, 1997; Rier *et al.*, 2005; Ardón & Pringle, 2008). Similarly, we observed declines in the concentrations of tannins and phenolics for all litter after 70 days (Kominoski *et al.*, 2007; See Table S1), which coincided with general increases in microbial (Kominoski *et al.*, 2007) and macroinvertebrate biomass (See Table S2). Given that the chemical characteristics of detritus change during breakdown (*sensu* Moore *et al.*, 2004), initial chemistry of single- and mixed-species litter may be inadequate to explain litter-species-diversity effects on macroinvertebrates throughout breakdown.

Although the non-additive effects of litter mixing on breakdown in streams appear to be predominately

**Table 5** Simple linear regressions relating effects of litter chemistry and ash-free dry mass (AFDM) remaining to (a) macroinvertebrate abundance (individuals leaf pack<sup>-1</sup>) in leaf packs where rhododendron, *Rhododendron maximum* (R) is present versus absent and (b) total macroinvertebrate biomass (mg leaf pack<sup>-1</sup>) in leaf packs where tulip poplar, *Liriodendron tulipifera* (L) is present versus absent. Comparisons were made for all single- and mixed-species litter where R and L were present and absent. Relationships explaining at least 20% ( $r^2 \geq 0.20$ ) of the variation in macroinvertebrate abundance and biomass are bolded and followed by a + or – to indicate the direction of the relationship. Carbon to nitrogen mass ratio (C : N) and carbon to phosphorus (C : P) were calculated as mass ratios. All litter chemistry values were calculated per g AFDM

a (Abundance)	R present		R absent	
	$r^2$	$P$ -value	$r^2$	$P$ -value
C : N	0.12	<0.01	<b>0.27–</b>	<0.01
C : P	0.04	0.04	<b>0.28–</b>	<0.01
% Cellulose	NS	NS	0.05	0.04
% Hemicellulose	0.07	0.01	NS	NS
% Lignin	<b>0.27+</b>	<0.01	NS	NS
% Condensed tannins	<b>0.42–</b>	<0.01	<b>0.25–</b>	<0.01
% Hydrolysable tannins	<b>0.35–</b>	<0.01	<b>0.27–</b>	<0.01
% Total phenolics	<b>0.35–</b>	<0.01	<b>0.27–</b>	<0.01
AFDM (g)	0.15	<0.01	0.17	<0.01

b (Biomass)	L present		L absent	
	$r^2$	$P$ -value	$r^2$	$P$ -value
C : N	0.09	<0.01	0.09	<0.01
C : P	0.05	0.03	0.07	0.02
% Cellulose	0.10	<0.01	NS	NS
% Hemicellulose	NS	NS	NS	NS
% Lignin	NS	NS	0.09	<0.01
% Condensed tannins	0.12	<0.01	<b>0.20–</b>	<0.01
% Hydrolysable tannins	0.06	0.01	<b>0.21–</b>	<0.01
% Total phenolics	0.13	<0.01	0.19	<0.01
AFDM (g)	<b>0.25–</b>	<0.01	<b>0.21–</b>	<0.01

NS, not significant ( $P > 0.05$ ).

explained by emergent effects of litter species interactions (Swan & Palmer, 2004; LeRoy & Marks, 2006; Kominoski *et al.*, 2007; Lecerf *et al.*, 2007; this study), our results suggest that these patterns are not completely explicable by resource–consumer diversity relationships. Here, we have shown that litter species diversity has different bottom-up effects on stream consumers and litter breakdown dynamics, indicating that resource and consumer diversity may only

partially explain the dynamics of organic matter processing in streams. To that end, further studies that explore the relative contributions of abiotic and biotic factors to biodiversity effects on ecosystem processes may lead to a broader mechanistic understanding of biodiversity–ecosystem functioning relationships.

### Acknowledgments

We thank Langley Amburn, Erica Chiao, Wyatt Cross, Sue Eggert, Eric Lunz, Tom Maddox and Julie Small for laboratory assistance. Mark Hunter and Star Scott provided assistance with litter chemistry. Carri LeRoy provided statistical assistance. Wyatt Cross, Ashley Helton, Cecil Jennings, Carri LeRoy, Chris Swan, Bruce Wallace, and the Pringle and Rosemond labs at the University of Georgia provided critical comments on the manuscript. Two anonymous reviewers greatly improved earlier versions of the manuscript. Research was funded by the National Science Foundation Coweeta LTER Project (DEB-0218001).

### References

- APHA (1998) *Standard Methods for the Examination of Water and Wastewater*, 20th edn. American Public Health Association, Washington, DC.
- Ardón M. & Pringle C.M. (2008) Do secondary compounds inhibit microbial- and insect- mediated leaf breakdown in a tropical rainforest stream, Costa Rica? *Oecologia*, **155**, 311–323.
- Ball B.A., Hunter M.D., Kominoski J.S., Swan C.M. & Bradford M.A. (2008) Consequences of non-random species loss for decomposition dynamics: experimental evidence for additive and non-additive effects. *Journal of Ecology*, **96**, 303–313.
- Benke A.C., Huryñ A.D., Smock L.A. & Wallace J.B. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular emphasis on the southeastern United States. *Journal of the North American Benthological Society*, **18**, 308–343.
- Cebrian J. (1999) Patterns in the fate of production in plant communities. *American Naturalist*, **154**, 449–468.
- Drake J.M. (2003) Why does grassland productivity increase with species richness? Disentangling species richness and composition with tests of overyielding and superyielding in biodiversity experiments. *Proceedings of the Royal Society of London, B*, **270**, 1713–1719.
- Dufrène M. & Legendre P. (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, **67**, 345–366.
- Ellison A.M., Bank M.S., Clinton B.D. *et al.* (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment*, **3**, 479–486.
- Gartner T.B. & Cardon Z.G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos*, **104**, 230–246.
- Hall R.O., Wallace J.B. & Eggert S.L. (2000) Organic matter flow in stream food webs with reduced detrital resource base. *Ecology*, **81**, 3445–3463.
- Hall R.O., Likens G.E. & Malcom H.M. (2001) Trophic basis of macroinvertebrate production in 2 streams at the Hubbard Brook Experimental Forest. *Journal of the North American Benthological Society*, **20**, 432–447.
- Hansen R.A. & Coleman D.C. (1998) Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (Acari: Oribatida) in litterbags. *Applied Soil Ecology*, **9**, 17–23.
- 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.
- Hector A., Schmid B., Beierkuhnlein C. *et al.* (1999) Plant diversity and productivity experiments in European grasslands. *Science*, **286**, 1123–1127.
- Hieber M. & Gessner M.O. (2002) Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, **83**, 1026–1038.
- Hooper D.U., Chapin F.S. III, Ewel J.J. *et al.* (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.
- Hunter M.D. & Schultz J.C. (1995) Fertilization mitigates chemical induction and herbivore responses within damaged oak trees. *Ecology*, **76**, 1226–1232.
- Huston M.A. (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia*, **110**, 449–460.
- Huston M.A., Aarssen L.W., Austin M.P. *et al.* (2000) No consistent effect of diversity on plant productivity. *Science*, **289**, 1255.
- Kaneko N. & Salamanca N. (1999) Mixed leaf litter effects on decomposition rates and soil arthropod communities in an oak-pine forest stand in Japan. *Ecological Research*, **14**, 131–138.

- Kinzig A.P., Pacala S.W. & Tilman D. (2002) *The Functional Consequences of Biodiversity: Empirical Progress and Theoretical Extensions*. Princeton University Press, Princeton.
- Kominoski J.S., Pringle C.M., Ball B.A., Bradford M.A., Coleman D.C., Hall D.B. & Hunter M.D. (2007) Nonadditive effects of litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology*, **88**, 1167–1176.
- Lecerf A., Risonoveanu G., Popescu C., Gessner M.O. & Chauvet E. (2007) Decomposition of diverse litter mixtures in streams. *Ecology*, **88**, 219–227.
- LeRoy C.J. & Marks J.C. (2006) Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. *Freshwater Biology*, **51**, 605–617.
- LeRoy C.J., Whitham T.G., Wooley S.C. & Marks J.C. (2007) Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. *Journal of the North American Benthological Society*, **26**, 426–438.
- Loreau M., Naeem S., Inchausti P. *et al.* (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*, **294**, 804–808.
- Loreau M., Naeem S. & Inchausti P. (2002) *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives*. Oxford University Press, Oxford.
- McNaughton S.J., Oesterheld M., Frank D.A. & Williams K.J. (1989) Ecosystem-level patterns of primary productivity and herbivory in terrestrial habitats. *Nature*, **341**, 142–144.
- Merritt R.W. & Cummins K.W. (1996) *An Introduction to the Aquatic Insects of North America*, 3rd edn. Kendall/Hunt, Dubuque, IA.
- Moore J.C., Berlow E.L., Coleman D.C. *et al.* (2004) Detritus, trophic dynamics and biodiversity. *Ecology Letters*, **7**, 584–600.
- Naeem S., Håkansson K., Lawton J.H., Crawley M.J. & Thompson L.J. (1996) Biodiversity and plant productivity in a model assemblage of plant species. *Oikos*, **76**, 259–264.
- Orwig D.A., Foster D.R. & Mausel D.L. (2002) Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. *Journal of Biogeography*, **29**, 1475–1487.
- Ostrofsky M.L. (1997) Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *Journal of the North American Benthological Society*, **16**, 750–759.
- Rier S.T., Tuchman N.C. & Wetzel R.G. (2005) Chemical changes to litter from trees grown under elevated CO<sub>2</sub> and the implications for microbial utilization in a stream ecosystem. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 185–194.
- Rossiter M.C., Schultz J.C. & Baldwin I.T. (1988) Relationships among defoliation, red oak, phenolics, and gypsy moth growth and reproduction. *Ecology*, **69**, 267–277.
- Schulze E.D. & Mooney H.A. (1993) *Biodiversity and Ecosystem Function*. Springer-Verlag, Berlin.
- Swan C.M. & Palmer M.A. (2004) Leaf diversity alters litter breakdown in a Piedmont stream. *Journal of the North American Benthological Society*, **23**, 15–28.
- Swank W.T. & Crossley D.A. Jr (1988) *Forest Hydrology and Ecology at Coweeta*. Springer-Verlag, New York.
- Swift L.W., Cunningham G.B. & Douglass J.E. (1988). Climatology and hydrology. In: *Forest Hydrology and Ecology at Coweeta* (Eds W.T. Swank & D.A. Crossley Jr), pp. 35–55. Springer-Verlag, New York.
- Tilman D.G. & Lehman C. (2001) Human-caused environmental changes: impacts on plant diversity and evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5433–5440.
- Tilman D.G., Wedin D. & Knops J. (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, **379**, 718–720.
- Vitousek P.M., Mooney H.A., Lubchenco J. & Melillo J.M. (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494–499.
- Wallace J.B. & Webster J.R. (1996) The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology*, **41**, 115–139.
- 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.
- Wallace J.B., Eggert S.L., Meyer J.L. & Webster J.R. (1999) Effects of resource limitation on a detrital-based ecosystem. *Ecological Monographs*, **69**, 409–442.
- Webster J.R. & Benfield E.F. (1986) Vascular plant breakdown in fresh-water ecosystems. *Annual Review of Ecology and Systematics*, **17**, 567–594.
- Webster J.R., Meyer J.L., Wallace J.B. & Benfield E.F. (1997) Organic matter dynamics in Hugh White Creek, Coweeta Hydrologic Laboratory, North Carolina, U.S.A. *Journal of the North American Benthological Society*, **16**, 74–78.
- Wetzel R.G. (1995) Death, detritus, and energy flow in aquatic ecosystems. *Freshwater Biology*, **33**, 83–89.

### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Litter mass remaining and chemistry from single- and mixed-species litter during breakdown

**Table S2.** Abundance and biomass of individual macroinvertebrate taxa and functional feeding groups in single- and mixed-species litter during breakdown

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

*(Manuscript accepted 2 February 2009)*