Ecology, 88(5), 2007, pp. 1167–1176
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NONADDITIVE EFFECTS OF LEAF LITTER SPECIES DIVERSITY ON BREAKDOWN DYNAMICS IN A DETRITUS-BASED STREAM

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Abstract. Since species loss is predicted to be nonrandom, it is important to understand the manner in which those species that we anticipate losing interact with other species to affect ecosystem function. We tested whether litter species diversity, measured as richness and composition, affects breakdown dynamics in a detritus-based stream. Using full-factorial analyses of single- and mixed-species leaf packs (15 possible combinations of four dominant litter species; red maple [*Acer rubrum*], tulip poplar [*Liriodendron tulipifera*], chestnut oak [*Quercus prinus*], and rhododendron [*Rhododendron maximum*]), we tested for single-species presence/absence (additive) or species interaction (nonadditive) effects on leaf pack breakdown rates, changes in litter chemistry, and microbial and macroinvertebrate biomass. Overall, we found significant nonadditive effects of litter species diversity on leaf pack breakdown rates, which were explained both by richness and composition. Leaf packs containing higher litter species richness had faster breakdown rates, and antagonistic effects of litter species composition were observed when any two or three of the four litter species were mixed. Less-consistent results were obtained with respect to changes in litter chemistry and microbial and macroinvertebrate biomass. Our results suggest that loss of litter species diversity will decrease species interactions involved in regulating ecosystem function. To that end, loss of species such as eastern hemlock (*Tsuga canadensis*) accompanied by predicted changes in riparian tree species composition in the southeastern United States could have nonadditive effects on litter breakdown at the landscape scale.

Key words: Acer rubrum; *composition; detritus-based ecosystem; ecosystem functioning;* Liriodendron tulipifera; *nonadditive effects;* Quercus prinus; Rhododendron maximum; *richness; species diversity; streams.*

Introduction

Increasing global species declines have stimulated research exploring the relationship between biodiversity and ecosystem function (Schulze and Mooney 1993, Kinzig et al. 2002, Loreau et al. 2002). Most of these studies have been conducted in terrestrial ecosystems and have focused primarily on effects of plant species diversity on net primary production (Naeem et al. 1996, Tilman et al. 1996, Hooper and Vitousek 1997, Hector et al. 1999, Tilman 1999) and effects of litter species diversity on breakdown dynamics (Taylor et al. 1989, Blair et al. 1990, Wardle et al. 1997, Kaneko and Salamanca 1999, Hector et al. 2000, Hättenschwiler et al. 2005). Similar research in aquatic ecosystems is lacking (Gessner et al. 2004, Giller et al. 2004). For example, only a few studies (Leff and McArthur 1989, McArthur et al. 1994, Swan and Palmer 2004, LeRoy and Marks 2006) have investigated the relationship between litter species diversity and breakdown dynamics in streams. Given that many stream food webs are dependent upon allochthonous litter as a source of energy, nutrients, and habitat (Vannote et al. 1980, Wallace et al. 1997, 1999), understanding the relationship between litter species diversity and breakdown dynamics in streams draining forested watersheds is of considerable ecological importance.

Differences in litter chemistry account for high interspecies variation in breakdown rates. Previous studies examining structural and chemical composition of decomposing litter found that species containing higher initial C:N (Melillo et al. 1982, Webster and Benfield 1986), tannin (Gallardo and Merino 1992, Ostrofsky 1997), and lignin concentrations (Meentemeyer 1978) had slower breakdown rates than less recalcitrant species. However, while several studies have examined changes in chemistry of single-species litter throughout decomposition (Petersen and Cummins 1974, Suberkropp et al. 1976, Chauvet 1987, Hunter et al. 2003), this information is lacking for mixed-species assemblages. Understanding changes in mixed-species litter chemistry during breakdown may provide insights

Manuscript received 24 April 2006; revised 2 November 2006; accepted 6 November 2006. Corresponding Editor: D. A. Wardle.

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TABLE 1. Initial chemistry (mean ± SE) of single-species litter.

Species	C:N	Lignin	CT	HT	Phenolics
Liriodendron tulipifera	56.3 ± 0.4	12.5 ± 0.4	11.4 ± 2.0	19.5 ± 0.3	23.8 ± 1.0
Acer rubrum	93.7 ± 1.8	9.8 ± 0.2	12.6 ± 1.6	19.3 ± 4.1	16.3 ± 5.2
Quercus prinus	61.6 ± 1.3	14.4 ± 0.5	4.7 ± 1.4	13.7 ± 1.4	18.9 ± 3.3
Rhododendron maximum	162.7 ± 4.8	10.6 ± 0.1	10.5 ± 3.6	14.1 ± 3.2	20.3 ± 3.6

Notes: Values represent concentrations as a percentage of AFDM (g/g AFDM). Abbreviations are: CT, condensed tannins; HT, hydrolyzable tannins. We conducted this study in a second-order reach of Ball Creek, a headwater stream at Coweeta Hydrologic Laboratory in Macon County, North Carolina, USA.

into mechanisms by which litter species interactions affect breakdown dynamics (Moore et al. 2004).

Microbial conditioning and subsequent invertebrate consumption of litter contribute to breakdown (Swift et al. 1979, Webster and Benfield 1986). Bacteria and fungi differ in their contribution to breakdown, with fungi being apparently more important than bacteria in both terrestrial (Hendrix et al. 1986, Blair et al. 1990, Bailey et al. 2002) and aquatic (Findlay and Arsuffi 1989, Gessner and Chauvet 1994, Baldy et al. 1995, Hieber and Gessner 2002) ecosystems. Invertebrates are dominant processors of litter in detritus-based ecosystems (Seastedt 1984, Cuffney et al. 1990), and litter species diversity can influence invertebrate composition, abundance, feeding activity, and growth rates (Hansen and Coleman 1998, Hansen 1999, Swan and Palmer 2006). Comparing the trophic dynamics of bacteria, fungi, and invertebrates in response to litter species diversity may help predict effects of changes in riparian tree species diversity on the functioning of detritus-based ecosystems.

Traditionally, studies investigating effects of species diversity on ecosystem function have measured species richness as a surrogate for species diversity; however, species diversity can be defined as species richness, evenness, functional traits, and composition or interactions among species (Chapin et al. 2000, Hooper et al. 2005). Yet studies attempting to identify the causal mechanisms behind how species diversity affects ecosystem function have often not separated the confounding effects of species richness and composition (Huston 1997, Drake 2003). One solution is to investigate single-species presence/absence vs. mixed-species richness and composition effects using full-factorial experimental designs to test additivity of litter species diversity. Here, we used full-factorial analyses of variance to test additivity of litter species diversity (richness and composition) on breakdown dynamics (leaf pack breakdown rates, changes in litter chemistry, and effects on microbial and macroinvertebrate biomass) in a detritus-based stream. This design allowed us to address the following questions: (1) Are effects of litter species diversity on breakdown dynamics additive? (2) If nonadditive effects (interactions above and beyond main effects of species presence or absence) exist, are they explained by species richness or composition? (3) If species composition effects exist, which species interactions are driving nonadditive effects?

METHODS

Study site

We conducted this study in a second-order reach of Ball Creek, a headwater stream at Coweeta Hydrologic Laboratory in Macon County, North Carolina, USA (35°00′ N, 83°30′ W; see Plate 1). Coweeta is a 2185-ha forested basin in the Blue Ridge physiographic province of the southern Appalachian Mountains (Swank and Crossley 1988). Vegetation at Coweeta is mixed hardwood (dominated by *Quercus* spp., *Acer* spp., and *Liriodendron* spp.), with a dense understory of *Rhododendron maximum* that provides year-round shading of streams. Mean monthly air temperature ranges from 3° to 22°C, and mean annual precipitation ranges from 180 cm at low elevations to 250 cm at high elevations (Swift et al. 1988). Mean daily stream temperature along the study reach of Ball Creek during this study ranged from 1.3° to 16.6°C.

Leaf packs and experimental design

We selected four dominant riparian tree species (Swank and Crossley 1988) in the Coweeta basin (tulip poplar, *Liriodendron tulipifera* [L]; red maple, *Acer rubrum* [A]; chestnut oak, *Quercus prinus* [Q]; and rhododendron, *Rhododendron maximum* [R]) that represented a range of initial litter chemistries (Table 1). Webster and Waide (1982) found the relative contribution of annual litter fall at Coweeta for these species as follows: 9.1% (L), 4.8% (A), 19.0% (Q), 11.6% (R). Our experimental design was a randomized complete block. All 15 possible single- and mixed-species combinations (L, A, Q, R, LA, LQ…LAQR; sensu Jonsson and Malmqvist 2000) were crossed with nine levels of exposure time (i.e., nine sampling dates) and were replicated at four stream locations (blocks), giving a total of 540 experimental units ($15 \times 9 \times 4$). Note that although time was an experimental factor, this was not a repeated-measures design. Because of the destructive nature of sampling leaf packs, distinct, individually randomized experimental units were used at each time point.

In autumn 2003, we collected freshly abscised leaves and then air-dried them. Each leaf pack comprised ~15 g total litter, with individual species in mixed-species packs being represented in equal mass propor-

tions; litter was encased in plastic, mesh bags (19.1 cm × 38.1 cm, 5 × 5 mm mesh) after initial dry mass determinations. On 10 January 2004 we deployed 480 leaf packs (15 treatments × 4 blocks × 8 sampling dates); the remaining 60 leaf packs were transported back to the laboratory the same day and were used to measure initial litter chemistry and handling loss. Packs within each experimental block were grouped in arrays of 15 treatments and secured to the stream bottom using plastic ties with galvanized gutter nails. Arrays were randomly retrieved from blocks 7, 14, 28, 70, 118, 169, 183, and 190 d after deployment. Packs were transported on ice to the laboratory and processed within 12 h of retrieval.

After retrieval, litter was rinsed over nested sieves (1 mm and 250 μm) to collect macroinvertebrates and remove sediments and debris. Five leaf disks (1.2 cm diameter) were sampled from a representative composite of litter species in each leaf pack for both fungal and bacterial analyses. An additional five leaf disks were taken to estimate mass of leaf disks removed for fungal and bacterial analyses. Remaining litter was oven-dried at 60°C for 24 h, ground using a Wiley Mill, and then ball-milled using a Spex CertiPrep 8000-D Mixer Mill (Spex, Metuchen, New Jersey, USA) prior to litter chemistry analyses.

Litter mass loss and chemistry

Ash-free dry mass (AFDM) was determined as the difference between pre- and post-combustion (at 550°C for 1 h) mass of oven-dried (60°C) litter. Mass loss was measured by dividing the retrieved AFDM by the initial (i.e., day 0) AFDM. Litter carbon and nitrogen concentrations were measured using a Carlo Erba 1500N CHN Analyzer (Carlo Erba, Milan, Italy), cellulose, hemicellulose, and lignin concentrations using an Ankom A200 Fiber Analyzer (Ankom, Macedon, New York, USA), and condensed tannins, hydrolyzable tannins, and total phenolics using techniques described by Rossiter et al. (1988) and Hunter and Schultz (1995).

Biota

We measured biota biomass on sampling days 14, 70, and 118. We estimated bacterial cell density using epifluorescent microscopy (Porter and Feig 1980). Briefly, the five 1.2 cm diameter leaf disks from each replicate leaf pack were preserved at 4°C in a 0.2-μm filtered solution of 5% formaldehyde (Velji and Albright 1986). Next, bacterial cells were separated from leaf disks by sonication (Weyers and Suberkropp 1996), and cells were stained with 2 mL of 10 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) solution for an incubation period of 10 min. Samples were filtered and mounted on glass microscope slides (Velji and Albright 1986), then kept in the dark and refrigerated until counted. We counted cells from 10 random fields per slide using 1000× epifluorescent microscopy. Bacterial cell biovolumes were estimated using geometric shapes (Bratbak 1985, Psenner 1993, Wetzel and Likens 2000), and total bacterial carbon was estimated by multiplying cell biovolumes by 5.6×10^{-13} g C/μm^3 (Bratbak 1985).

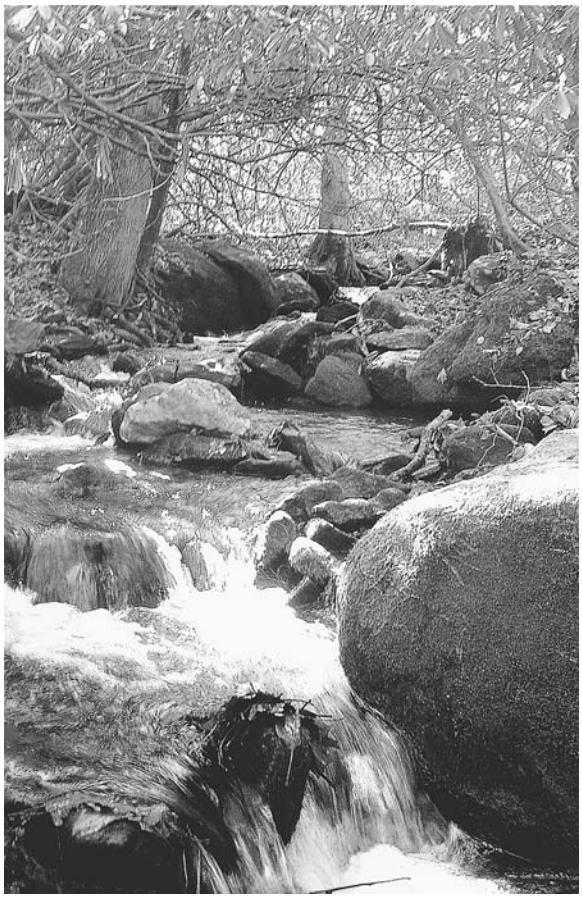

PLATE 1. A second-order reach of Ball Creek, a headwater stream at Coweeta Hydrologic Laboratory, Macon County, North Carolina, USA. Photo credit: J. S. Kominoski.

Leaf disks for fungal biomass estimates were preserved at 4°C in 99.9% high-pressure liquid chromatography (HPLC)-grade methanol (Newell et al. 1988). Ergosterol, a surrogate for fungal biomass (Gessner and Chauvet 1993), was extracted by refluxing for 30 min at 80°C in alcoholic base (5 mL of 4% potassium hydroxide [KOH] in methanol added to 25 mL methanol). Samples were partitioned into pentane and evaporated to dryness at 30°C using N_2 gas. Dried samples were re-dissolved in 2 mL of methanol, sonicated, filtered (0.45 μm), and stored at 4°C. Ergosterol was measured on an RP-10 (Shimadzu, Columbia, Maryland, USA) column using an HPLC by comparing absorbance at 282 nm after separation from other lipids (Suberkropp and Weyers 1996). Ergosterol concentrations were converted to fungal biomass using the ratio of 5.5 μg ergosterol per 1 mg fungal dry mass (Gessner and Chauvet 1993).

Macroinvertebrates were separated into two size classes (>1 mm and 250 μm–1 mm) and preserved in 95% ethanol. Organisms were identified to lowest

possible taxonomic level and were assigned to functional feeding groups (Merritt and Cummins 1996, Barbour et al. 1999, Wallace et al. 1999). Chironomidae were separated into Tanypodinae and non-Tanypodinae. Length–mass regressions (Benke et al. 1999) were used to estimate macroinvertebrate biomass.

Statistical analyses

To estimate leaf pack breakdown rates, we used an exponential model to incorporate litter mass remaining from all nine sampling dates into a single model. We assumed the relationship $E(\text{AFDM}_t) = \theta e^{-kt}$, where $E(\text{AFDM}_t)$ is the expected (i.e., population mean) proportion of ash-free dry mass (AFDM) on day t, k is the breakdown rate, and θ is the initial mean AFDM at time 0. To simplify model fitting and statistical inference, we linearized this relationship and fit models of the form $\ln_e(\text{AFDM}_t) = \varphi - kt + \varepsilon_t$, where $\varphi = \log(\theta)$, and ε_t is a mean 0, constant variance error term. Note that we allow $\varphi \neq 0$ (i.e., $\theta \neq 1$) and also allow it to differ across experimental conditions to account for nonconstant handling loss. In addition, the coefficient on time, k, which represents the breakdown rate, was also allowed to differ across experimental conditions to assess litter species diversity effects on this parameter. The effects of species diversity on the breakdown rate k (which appear as interactions between the continuous variable time and individual species presence/absence indicators in the linearized model) are of primary interest in the analysis rather than the species diversity effects on the initial mean AFDM parameter φ (which appear as main effects and interactions among the species presence/absence indicators not involving time in the linearized model). The model assumed that the error variance was proportional to the square of time and was therefore fit with weighted least squares. This assumption was made to account for nonconstant variance observed in the residuals.

Litter chemistry variables were modeled similarly to AFDM. In each case, the chemical component and time were each transformed to make their relationship approximately linear so that a model similar to that used for AFDM could be used, whereby a constant (through time) rate of change parameter appeared as the slope on (transformed) time. That is, we fit models of the form $f_1(y) = \beta_0 + \beta_1 f_2(t) + \varepsilon$ and modeled species diversity effects on the rate of change parameter β_1. Here, f_1 and f_2 are transformations of the chemistry variable y and time t, respectively.

To test whether leaf pack breakdown rates and rates of change in litter chemistry depended upon species diversity or single-species presence/absence, we used full-factorial models for the rate of change parameter (the coefficient on the continuous variable time: k in the models for AFDM and β_1 in the litter chemistry models). That is, the rate parameter was modeled in terms of main effects A, L, Q, R and interactions 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, where here the main effects corresponded to dummy variables for presence/absence of the four single species. The influence of diversity on the rate of change was tested through a joint test of significance ($P < 0.05$) of the two- and higher-way interaction terms, which we refer to as the test for nonadditivity. A significant nonadditivity test means that individual species presence/absence in leaf packs is insufficient to explain differences in the rates of change, but it is the combinations of species that occur, or species diversity, that is crucial. Eight outliers (having external studentized residuals > 3) were omitted from the analysis, and six leaf packs were lost during the study. This changed our total degrees of freedom to 525 of the 540 possible experimental units.

When species effects were additive, we reported marginal means for presence and absence of each species and whether the presence/absence effect was significant. To investigate whether this nonadditivity was due to species richness (the number of species present in mixed-species packs, 2, 3, or 4) or species composition (which particular species are present together in mixed-species packs), we decomposed treatment effects (differences among the 15 different leaf pack types, corresponding to 14 degrees of freedom in the analysis of variance) into effects of individual species (4 df, 1 for each species), richness effects (accounting for 2 df), and composition effects (interactions among species not explained by richness, accounting for 8 df). When richness alone was insufficient to explain litter species diversity effects, we reported the particular species interactions that were found to be significant (see *Results*).

In contrast to AFDM and the litter chemistry variables, which were measured on nine sampling dates, biota data were collected on only three sampling dates. This limited sampling precluded the modeling of biota variables via a function of time. Therefore, biota data were analyzed at each sampling date using three separate models.

Data were transformed when necessary to meet assumptions of homoscedasticity. All analyses were conducted using PROC GLM or MIXED at $\alpha = 0.05$ in SAS version 9.1 (SAS Institute, Cary, North Carolina, USA).

Results

Litter breakdown and chemistry

In Fig. 1 we show observed breakdown rates for each leaf pack and, for mixed-species packs, the expected breakdown rates based on the monocultures (sensu Wardle et al. 1997). Differences in breakdown rates are apparent between the different litter species in monocultures; however, mixed-species packs appeared to behave idiosyncratically (Fig. 1). In contrast, using a full-factorial ANOVA we detected significant nonadditivity of litter species diversity effects on leaf pack breakdown rates ($F_{10,490} = 1.93$, $P = 0.039$; Table 2), which was composed of marginally significant species

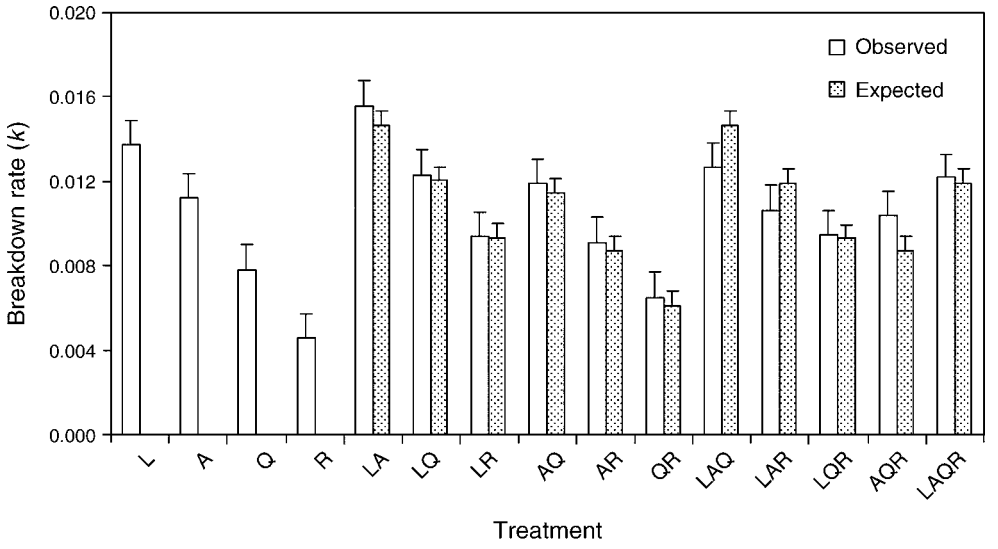

FIG. 1. Single- and mixed-species leaf pack breakdown rates (k). Expected values for mixed-species rates are the means of monocultures. Error bars represent +SE. We conducted this study in a second-order reach of Ball Creek, a headwater stream at Coweeta Hydrologic Laboratory in Macon County, North Carolina, USA. Species abbreviations are: L, tulip poplar, *Liriodendron tulipifera*; A, red maple, *Acer rubrum*; Q, chestnut oak, *Quercus prinus*; and R, rhododendron, *Rhododendron maximum*.

richness ($F_{2,490} = 2.86$, $P = 0.059$; Table 2) and composition effects ($F_{8,490} = 1.70$, $P = 0.096$; Table 2). This nonadditivity could not be adequately explained by litter species richness alone. Although higher litter species richness was associated with faster breakdown rates (Fig. 2), there were also antagonistic interactions between certain species, whereby the combined presence of A with L, A with Q, and L with Q resulted in mean breakdown rates that were lower than those resulting from the combined additive effects of the two species (Fig. 3a–c).

Effects of litter species diversity on rates of change for C:N and phenolics were nonadditive (time × diversity terms, $F_{10,494} = 6.43$, $P < 0.0001$ and $F_{10,489} = 2.70$, $P = 0.003$, respectively), whereas the effect of species diversity on rate of change for lignin was additive (time × diversity term, $F_{10,470} = 0.79$, $P = 0.640$). The significant litter species diversity effect for C:N was composed of both significant species richness and composition effects ($F_{2,494} = 3.72$, $P = 0.025$ and $F_{8,494} = 7.11$, $P < 0.001$, respectively), while composition, but not richness, accounted for the diversity effect

TABLE 2. ANOVA results for test of litter species diversity effects above and beyond the main effects of species presence/absence on leaf pack breakdown rates (k).

Source	Interpretation	df	Type I SS	MS	F	P
Block	Variability in θ due to blocks	3	0.001	<0.001	0.120	0.951
Treatment	Variability in θ due to treatments	14	0.031	0.002	0.780	0.687
Time	Constant term in the factorial model for k	1	4.148	4.148	1473.030	<0.001
Time × block	Variability in k due to blocks	3	0.005	0.002	0.600	0.618
Time × A	Variability in k due to presence/absence of species A	1	0.043	0.043	15.330	<0.001
Time × L	Variability in k due to presence/absence of species L	1	0.077	0.077	27.240	<0.001
Time × Q	Variability in k due to presence/absence of species Q	1	0.001	0.001	0.490	0.486
Time × R	Variability in k due to presence/absence of species R	1	0.080	0.080	28.420	<0.001
Time × diversity†		10	0.054	0.005	1.930	0.039
Time × richness	Nonadditivity of species effects on k decomposed into	2	0.016	0.008	2.860	0.058
Time × composition	species richness and species composition effects	8	0.038	0.005	1.700	0.096
Error		490	1.379	0.003		
Total		525	5.821			

Notes: Litter species diversity effects are time × diversity, composed of effects of species richness (time × richness) and species composition effects (time × composition). Species abbreviations are: L, tulip poplar, *Liriodendron tulipifera*; A, red maple, *Acer rubrum*; Q, chestnut oak, *Quercus prinus*; and R, rhododendron, *Rhododendron maximum*. Presence/absence of species is indicated as time × A, time × L, time × Q, and time × R. A significant ($\alpha = 0.05$) time × diversity effect indicates nonadditivity of litter species interactions. The initial mean ash-free dry mass at time 0 is θ.

† Note that a Type I (sequential) F test is used here, so this effect represents treatment differences in k above and beyond those already accounted for in the model; that is, above and beyond main effects of time × A, time × L, time × Q, and time × R on k.

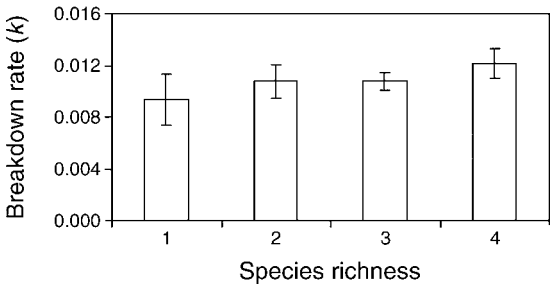

FIG. 2. Mean leaf pack breakdown rates (k), defined as ash-free dry mass loss per day, for each level of litter species richness: 1 ($n = 16$), 2 ($n = 24$), 3 ($n = 16$), and 4 ($n = 4$). "Species richness" refers to the actual number of leaf litter species present in a leaf pack. The sample size refers to the number of replicates comprising each species richness level. Error bars represent ±SE.

on phenolics (composition term, $F_{8,489} = 3.03$, $P = 0.003$). Higher litter species richness (>1 species) was associated with lower rates of change in C:N. A three-way interaction between A, L, and Q and a two-way interaction between Q and R were found ($F_{1,494} = 14.60$, $P < 0.001$ and $F_{1,494} = 7.68$, $P = 0.006$, respectively) in which Q and R had positive, but antagonistic effects on the C:N rate of change through time. In addition, L and Q had positive effects on C:N rate of change that were additive in the absence of A, but antagonistic when species A was present. Species A had a positive effect on this parameter only when both L and Q were absent; otherwise, the presence of A reduced the rate of change in the C:N ratio. For phenolics, three-way interactions among A, L, and Q and among L, Q, and R were found to affect the rate of change ($F_{1,489} = 4.12$, $P = 0.043$ and $F_{1,489} = 5.28$, $P = 0.022$, respectively). Essentially, there is no simple pattern to describe how the rate of change in phenolics depended upon the presence/absence of the four species. The presence of *Q. prinus* significantly increased the rate of change in lignin concentrations ($F_{1,470} = 9.02$, $P = 0.003$).

Biota

For bacterial biomass we detected nonadditivity of litter species diversity on day 14 ($F_{10,42} = 2.05$, $P = 0.052$) and additivity on days 70 and 118 (diversity terms, $F_{10,34} = 0.71$, $P = 0.710$ and $F_{10,41} = 1.36$, $P = 0.230$, respectively). The nonadditive litter species diversity effect on day 14 was explained by composition ($F_{8,42} = 2.09$, $P = 0.06$) rather than richness. A significant three-way interaction between A, L, and Q ($F_{1,42} = 3.72$, $P = 0.06$) occurred, which resulted in lower bacterial biomass than would have been expected under additivity. When diversity effects were simply additive, there were clear effects of species presence or absence on bacterial biomass. That is, on days 70 and 118 presence of R inhibited bacterial biomass ($F_{1,34.3} = 18.74$, $P < 0.001$; $F_{1,41.2} = 46.36$, $P < 0.001$, respectively; Fig. 4a), while presence of L enhanced bacterial biomass on day 70 ($F_{1,34.2} = 6.77$, $P = 0.014$; Fig. 4a).

As for bacteria, there was a nonadditive effect of litter species diversity on fungal biomass on day 14 ($F_{10,21.1} = 2.82$, $P = 0.022$) and additive effects on days 70 and 118 ($F_{10,23} = 1.99$, $P = 0.084$ and $F_{10,30.5} = 0.36$, $P = 0.950$, respectively). The nonadditive diversity effect on day 14 was the result of composition ($F_{8,17.3} = 3.15$, $P = 0.022$) rather than richness. Significant two-way interactions between A and R ($F_{1,3.25} = 9.85$, $P = 0.046$) and L and R ($F_{1,7.15} = 5.80$, $P = 0.046$) were found, whereby interactions resulted in lower fungal biomass than would have been expected under conditions of additivity. For

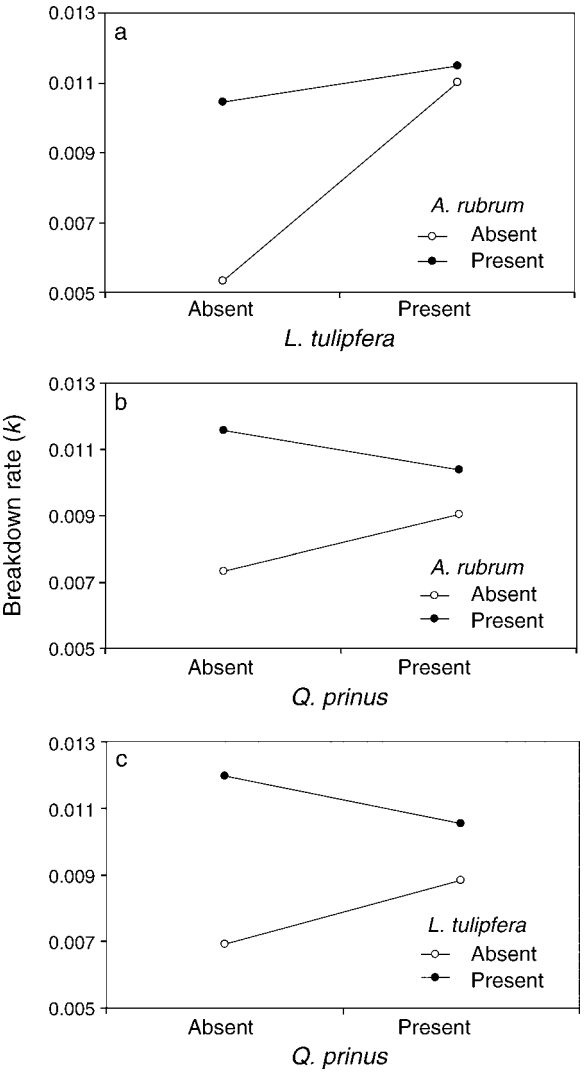

FIG. 3. Profile plots (interaction plots) of nonadditive effects of litter species interactions on mean breakdown rates: (a) A × L (*Acer rubrum* × *Liriodendron tulipifera*); (b) A × Q (*A. rubrum* × *Quercus prinus*); and (c) L × Q (*L. tulipifera* × *Q. prinus*). Convergent (antagonism) as opposed to divergent (synergism) lines are indicated, whereas additivity would be represented by parallel lines.

day 70, the presence of L enhanced fungal biomass ($F_{1,14} = 5.59$, $P = 0.032$), while presence of Q and R inhibited fungal biomass ($F_{1,14} = 5.17$, $P = 0.051$ and $F_{1,14} = 34.52$, $P = 0.0003$, respectively; Fig. 4b). Presence of R also inhibited fungal biomass on day 118 ($F_{1,14} = 30.23$, $P < 0.001$).

In contrast to bacteria and fungi, there was neither significant litter species diversity nor litter species presence/absence effects on macroinvertebrate shredder biomass. Shredder biomass values, across all leaf packs, were 19.7 ± 3.3, 99.6 ± 21.7, and 83.7 ± 16.0 mg/g litter AFDM (mean ± SE) for days 14, 70, and 118, respectively.

Discussion

We observed overall significant nonadditive effects of litter species diversity (both richness and composition) on leaf pack breakdown rates. Less-consistent results were obtained with respect to changes in litter chemistry and microbial and macroinvertebrate biomass. Both leaf pack breakdown rates and changes in litter C:N were affected by species richness; however, leaf pack breakdown rates increased with increasing species richness, whereas rates of change in litter C:N were not different among leaf packs containing more than one species. Interactions between *L. tulipifera* and *A. rubrum* and each with either *Q. prinus* or *R. maximum* resulted in decreased rates of change of litter C:N and phenolics concentrations and slower breakdown rates than expected under additivity. However, *R. maximum* did not have a significant effect on leaf pack breakdown rates. The presence of *L. tulipifera* was also associated with higher bacterial and fungal biomass during intermediate stages of breakdown, whereas bacterial and fungal biomass were consistently lower in the presence of *R. maximum*, and the presence of *Q. prinus* increased the rate of change of litter lignin concentrations and inhibited fungal biomass during intermediate stages of breakdown. However, neither presence of *R. maximum* or *Q. prinus* (or low microbial biomass) explained breakdown rates per se. Finally, it is possible that nonadditive effects of litter species diversity on bacterial and fungal biomass on day 14 could be due to low macroinvertebrate shredder biomass, whereas higher macroinvertebrate shredder biomass on days 70 and 118 may have resulted in top-down control of microbial biomass.

Results suggest that complex litter species interactions drive patterns of breakdown dynamics. Mixing litter of different chemical and physical properties alters the resource quality and physical habitat complexity within leaf packs, resulting in changes in breakdown rates and decomposer abundance and activity (Hansen and Coleman 1998, Hansen 1999, Hector et al. 2000, Swan and Palmer 2006). More recalcitrant litter, such as *R. maximum*, may alter the physical structure and subsequent breakdown of the entire leaf pack; however, we did not observe a significant effect of *R. maximum*

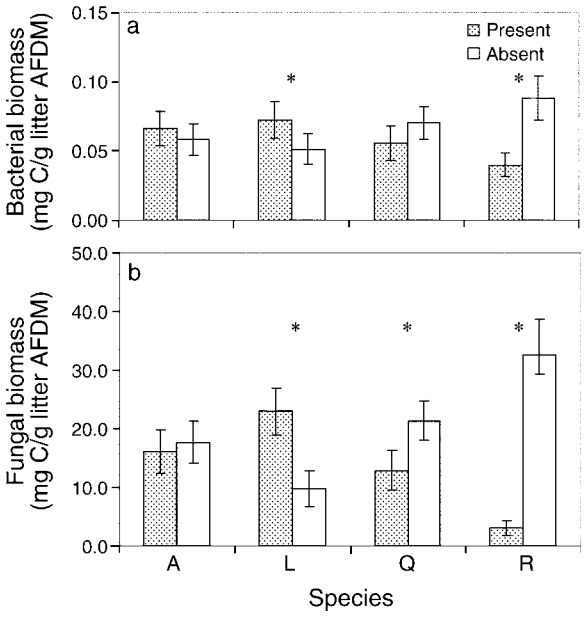

FIG. 4. Main effects of litter species presence/absence on (a) bacterial biomass and (b) fungal biomass on day 70 (mean ± SE; AFDM, ash-free dry mass). An asterisk denotes significant differences ($P < 0.05$) between bars. Species abbreviations are: L, tulip poplar, *Liriodendron tulipifera*; A, red maple, *Acer rubrum*; Q, chestnut oak, *Quercus prinus*; and R, rhododendron, *Rhododendron maximum*.

presence/absence or species interactions with *R. maximum* on leaf pack breakdown rates. In our study, mixed-species leaf packs were composed of chemically and structurally diverse litter, which affected changes in litter chemistry and microbial but not macroinvertebrate biomass. Presence of lower-quality (*Q. prinus* and *R. maximum*) and higher-quality (*L. tulipifera*) litter in leaf packs appeared to inhibit and stimulate microbial biomass, respectively. However, we observed both synergistic and antagonistic effects of litter mixing on breakdown rates due to litter species richness and composition, respectively. It is possible that microbial and shredder activity or diversity, rather than biomass, are better explanatory variables of nonadditive effects of litter species diversity on observed breakdown rates (Jonsson et al. 2001). However, shredder diversity in our study stream was dominated by only two taxa *Tallaperla* sp. and *Lepidostoma* sp.; all other shredder taxa were rare. It is also possible that macroinvertebrate shredders mediated the observed effects of presence of low-quality litter on microbial biomass. Macroinvertebrate shredders may have selectively consumed the high-quality, and presumably microbially conditioned, litter in mixed-species packs when low-quality litter was present (Arsuffi and Suberkropp 1985), which could explain the apparent inhibition of low-quality litter on microbial biomass but lack of subsequent inhibition on breakdown rates.

Our results expand on existing data sets that have related litter species diversity to breakdown dynamics. One study in a mid-Atlantic (Maryland, USA) stream found additive effects of litter species diversity on breakdown rates during autumn (Swan and Palmer 2004), whereas we observed nonadditive effects of litter species diversity on breakdown rates. Aside from explanations provided by Swan and Palmer (2004; e.g., temperature), differences between the two studies may be explained by differences in litter species assemblages. Swan and Palmer (2004) assembled leaf packs using litter from eight common riparian tree species, such as slippery elm and black willow, which are not common to Coweeta watersheds. McArthur et al. (1994) suggested that the presence of a recalcitrant litter species inhibited microbial activity and subsequent breakdown of a fast-decomposing species. Although we observed inhibitory effects of recalcitrant species, such as *Q. prinus* and *R. maximum* on microbial biomass, *R. maximum* did not contribute to antagonistic, nonadditive effects of litter species diversity on leaf pack breakdown rates. In addition, we found significant effects of litter species diversity (specifically composition) and litter species presence/absence on bacterial and fungal biomass, which have not been reported previously for litter mixtures. Fungal diversity has been linked to litter diversity (Bärlocher and Graça 2002, Laitung and Chauvet 2005), and although fungal diversity and litter breakdown rates are not directly related (Bärlocher and Graça 2002, Dang et al. 2005), there is evidence of indirect effects of fungal diversity on litter breakdown rates via macroinvertebrate consumers (Lecerf et al. 2005). However, while fungal biomass and breakdown rates are positively correlated for single-species leaf packs in streams (Gessner and Chauvet 1994), the mechanisms linking litter species diversity, bacterial and fungal biomasses, and mixed-species leaf pack breakdown rates remain unclear and will be investigated further. Lastly, a recent laboratory study found that feeding activity and secondary production of the macroinvertebrate shredder *Tallaperla maria* was affected by litter species composition and not richness per se (Swan and Palmer 2006). *Tallaperla* sp. and *Lepidostoma* sp. were dominant shredders in our study stream (J. S. Kominoski, *unpublished data*); however, our in situ study found no effects of litter species presence/absence or species interactions on total shredder biomass. The absence of an effect of litter species diversity on shredder biomass could be explained by the absence of crayfish from leaf packs. Crayfish are important shredders in Ball Creek (Schofield et al. 2001), but were likely excluded from our leaf packs due to mesh size.

Our results suggest that loss of riparian tree species diversity will affect stream ecosystem functioning by reducing dynamic interactions that naturally occur during breakdown of mixed-species leaf packs. Of current concern in North American forests is the loss of eastern hemlock (*Tsuga canadensis*) due to introduction of the hemlock woolly adelgid (*Adelges tsugae*), a rapidly infesting insect from which the hemlock is undefended (Orwig et al. 2002). Another plant pathogen concern is "sudden oak death," which has recently been found in non-oak hosts, such as rhododendron (Garboletto et al. 2003). In forests of the southeastern United States, eastern hemlock and rhododendron are dominant, low-quality riparian species (Webster and Benfield 1986). In the absence of rhododendron, eastern hemlock is predicted to be replaced by tulip poplar (Ellison et al. 2005), a high-quality species (Webster and Benfield 1986). Also, a loss of rhododendron could be followed by an increase in red maple, which exhibits high understory tolerance and is predicted to increase in dominance over the next century (Abrams 1998). Results from our study suggest that decreases in tree species diversity and changes in species composition in riparian forests of the southeastern United States could have nonadditive effects on litter breakdown in streams at the landscape scale.

Species losses and extinctions are predicted to be nonrandom (Huston et al. 2000, Tilman and Lehman 2001). Therefore, experimental designs that randomly select mixed-species interactions at different levels of richness do not completely test effects of biodiversity loss on ecosystem function. If we can predict which species we expect to lose given specific environmental changes, knowing how those species interact with other species to influence ecosystem function is of paramount importance. Our results suggest that litter species diversity drives species interactions involved in regulating ecosystem functioning. Given that riparian ecosystems on a global scale are subject to environmental changes, there is a need for additional studies to test the effects of declines in litter species diversity on in-stream breakdown dynamics using dominant riparian tree species assemblages in other regions of the world.

Acknowledgments

This research was funded by the National Science Foundation Coweeta LTER Project, DEB-9632854. Staff at Coweeta provided logistical support. The Institute of Ecology Analytical Chemistry Laboratory assisted in litter chemical analyses. We thank L. Amburn, J. Drake, W. Duncan, S. Dye, S. Eggert, J. French, C. Frost, F. Golley, C. Hall, R. Hall, C. Jensen, E. Lunz, D. McDuffey, J. Meyer, G. Peltier, G. Person, the Pringle and Rosemond lab groups, S. Scott, C. Swan, K. Suberkropp, and J. Todd for field, laboratory assistance, and comments. This manuscript was greatly improved by two anonymous reviews.

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