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Linkages between below and aboveground communities: Decomposer responses to simulated tree species loss are largely additive

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ABSTRACT

Inputs of aboveground plant litter influence the abundance and activities of belowground decomposer biota. Litter-mixing studies have examined whether the diversity and heterogeneity of litter inputs affect decomposer communities in ways that can be predicted from monocultures. They have mainly attempted to detect non-additive effects of litter mixing, although individual species effects (additivity) as well as species interactions (non-additivity) may alter decomposition rates. To determine potential impacts of plant species loss on aboveground-decomposer linkages, we assessed both additive and non-additive effects of litter mixing on decomposer communities. A full-factorial litterbag experiment with leaves from four deciduous tree species was conducted, to assess responses of bacteria, fungi, nematodes, and microarthropods. Data were analyzed using a statistical method that first looked for additive effects based on the presence or absence of species and then any significant species interactions. We observed almost exclusively additive effects of all four litter species on decomposer biota, with each species exerting effects on different aspects of the community. These results imply that the consequences of species loss for the decomposer community will be largely predictable from knowledge of single species litter dynamics. The two species at opposite ends of the quality spectrum exerted the most effects. High-quality *Liriodendron tulipifera* supported a more diverse arthropod community and drove bottom-up effects on the decomposer food web. Low-quality *Rhododendron maximum* had negative effects on most groups of biota. Litter of mid-quality species exerted fewer effects. The influence of litter species richness on the Tylenchidae (nematodes) was the only non-additive effect of litter mixing. Together, these data demonstrate an effect of plant community composition on decomposer biomass, abundance, and diversity, confirming a link between above and belowground communities. We were able to identify the species to which the decomposer community is most sensitive, aiding predictions of the consequences of the loss of these dominant species on the decomposer community, with potential feedbacks for organic matter and nutrient turnover.

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1. Introduction

Decomposer biota, including microbes and invertebrate fauna, play a pivotal role in litter decomposition and through their feeding activity drive the amount and timing of organic matter turnover and mineral nutrient availability (Seastedt, 1984; Beare et al., 1992; Hunter et al., 2003). Control over the availability of resources for plant productivity forms a feedback from belowground systems to aboveground processes and communities (De Ruiter et al., 2005). Recently, there has been increasing interest in effects that operate in the opposite direction. That is, how aboveground systems affect belowground communities and processes (Scheu et al., 2003; Wardle et al., 2004; Bardgett et al., 2005; De Deyn and Van der Putten, 2005; Wardle, 2006; Nilsson et al., 2008). With this focus in mind, much research has been conducted to determine how plant communities might affect soil processes and decomposer communities. Since a major influence of plants on the soil system is through litter (e.g. Negrete-Yankelevich et al., 2008), there has been a large focus specifically on the effects of altered plant litter characteristics on decomposer communities, through litter quality

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(Saetre and Bååth, 2000; Gonzalez and Seastedt, 2001), species and functional diversity (Bardgett and Shine, 1999; Wardle et al., 2003; Milcu et al., 2006; Wardle et al., 2006), and resource heterogeneity (Blair et al., 1990; Hansen, 1999; St John et al., 2006).

From this interest, many litter-mixing studies have been conducted to determine whether decomposer communities and their impacts on decay dynamics differ under multi-species mixtures when compared to monocultures (reviewed by Gartner and Cardon, 2004). Additive effects on biota result from the independent influence of individual litter species, where diverse litter mixes may support abundant and diverse decomposer communities that are simply combinations of those that occur in litter monocultures (Johnson et al., 2006). Specifically, if decay dynamics in mixtures are the sum of their parts, biota of single litters can be used to predict biota colonizing multi-species litter layers. Alternatively, non-additive effects emerge if decomposer communities in mixture are not simple averages of those in monoculture. That is, they are unpredictable based solely on studying litter monocultures. A number of studies have attempted to identify non-additive effects of litter mixing on a variety of decomposition parameters, both in terrestrial (reviewed by Gartner and Cardon, 2004; Hättenschwiler et al., 2005) and aquatic (Lecerf et al., 2005; LeRoy and Marks, 2006; Swan and Palmer, 2006) systems. Results vary among studies (see Gartner and Cardon, 2004), perhaps in part because of differences in the parameters measured. Biotic assessments vary from measurements of abundance, density, biomass, or activity and describe various different groups of decomposers. Additionally, studies have also been conducted under a variety of plant richness levels and covering different time spans (e.g. those reviewed by Gartner and Cardon, 2004).

Under scenarios of global environmental change, many systems are at risk of losing dominant plant species (Grime, 1997; Vitousek et al., 1997; Loreau et al., 2001; Ellison et al., 2005). While previous litter-mixing studies have explored consequences of species loss, they generally tested for non-additivity, where interactions among species are the focus. However, individual (additive) effects based on the identity of species may also have a major impact on ecosystem processes (Gross and Cardinale, 2005; Schläpfer et al., 2005). That is, the loss of a particular species from a system may have a large impact on the decomposer community, even if its role in mixture dynamics is additive. Statistical methods used in litter-mixing studies to test for non-additive effects commonly are not designed to also test whether individual species have significant additive effects in mixture (see Ball et al., 2008); that is, whether species are functionally redundant or not. To predict accurately the consequences of species loss for ecosystem functioning, it is necessary to consider both additive and non-additive effects of species loss, reflecting either an independent influence of species on ecosystem functioning (additivity) or emergent dynamics that arise due to species interactions (non-additivity).

To determine the potential consequences of species loss on decomposer communities, we conducted a three-year, full-factorial litter-mix study in a southeastern US temperate forest. We used leaf litter from four co-dominant tree species, which differed markedly in initial chemical quality and so might each be expected to have pronounced effects on decomposer communities (Wardle and Lavelle, 1997). To obtain a comprehensive understanding of the decomposer community responses to litter mixing, we measured many commonly studied groups of decomposers over the course of two years: microbes, nematodes, microarthropods, and small macroarthropods. To analyze these data, we used a statistical model that sequentially tests first for additive effects of the loss of each component litter species, then whether any of the remaining variance is explained by interactions among the litter species. Significant interactions are indicative of non-additivity and were explored using post hoc analyses to determine whether non-additive effects were explained by richness and/or composition (Mikola et al., 2002; Drake, 2003). The strength of the approach is that we can first ask whether loss of a particular species is likely to affect community structure (Ball et al., 2008). If it does, we can then ask whether its loss is likely to be additive or whether the consequence of its loss will be dependent (i.e. non-additive) on the presence of some or all of the other species in the community. We hypothesized that, given the gradient in initial litter quality, structure, and decomposition rate, there would be compositional effects of litter mixing on the decomposer community, suggesting a feedback between aboveground plant communities and belowground communities. Specifically, we hypothesized that (1) both high- and low-quality litters will influence decomposer communities, due to the fact that high-quality litter (with high nutrient content and low secondary metabolites) should provide a better resource to support a larger decomposer community (Wardle et al., 2006) and low-quality litter with lower nutrient content and more structural compounds (e.g. lignin) should provide a poor resource but more habitat complexity (Hansen and Coleman, 1998) for the decomposer community. We also hypothesized that (2) individual species effects will be non-significant (i.e. neither significantly additive or non-additive and hence functionally redundant) when in mixture with species of similar quality, but will support a significantly larger and more diverse decomposer community when in mixture with litter species of markedly different quality (sensu Wardle, 2002). Given the high degree of variation in observations of additive or non-additive effects of litter mixing on decomposer biota (Gartner and Cardon, 2004), we felt that we could not reliably hypothesize whether significant species effects in mixture will likely be due to species identity or interactions.

2. Materials and methods

2.1. Study site

The experiment was conducted at Coweeta Hydrologic Lab (US Forest Service) in the southern Appalachians near Otto, North Carolina, USA (35°00′N, 83°30′W; elevation 1300 m). The area is a deciduous hardwood forest, comprised largely of oaks (Quercus spp.), tulip poplar (Liriodendron tulipifera), maples (Acer spp.), birches (Betula spp.), and riparian stands of eastern hemlock (Tsuga canadensis), with an abundant evergreen understory comprised mainly of Rhododendron maximum (rhododendron) and Kalmia latifolia (mountain laurel). The mean annual temperature over the duration of the experiment was 14 °C. Mean monthly temperature varied with season, and followed the same annual pattern of peak temperatures in July–August and minimum temperatures in December–February (National Climatic Data Center, Appendix 1 (supplementary material – online)). The mean annual rainfall was approximately 1700 mm, generally with moderate levels of precipitation (approx. 12 cm month^{-1}) except during late summer peaks and autumnal minimums. The study was conducted in Watershed 20 on Ball Creek, which drains into Coweeta Creek, a tributary of the Little Tennessee River. A tropical storm in September 2004 temporarily flooded the low areas of the riparian zone.

2.2. Experimental design

Litters from four co-dominant tree species were used: L. tulipifera L. (tulip poplar, L), Acer rubrum L. (red maple, A), Quercus prinus L. (chestnut oak, Q), and R. maximum L. (rhododendron, R). The litters from these species cover a range of chemical compositions and decay rates in monoculture (Table 1; Ball et al., 2008). Senesced leaves of each species were collected in October 2003 and air-dried

Table 1
Initial litter chemistries and decay rates for four co-dominant tree species in monoculture. Values represent mean ± 1 Standard Error ($n = 4$). From Ball et al. (2008).

	% N	% C	% P	C/N	% Lignin	k (day^{-1})
L. tulipifera	0.95 ± 0.04	47.9 ± 0.60	0.04 ± 0.002	1:50:1197	8.58 ± 0.36	0.00099
A. rubrum	0.70 ± 0.06	49.8 ± 0.95	0.03 ± 0.009	1:71:1658	9.15 ± 0.42	0.00097
Q. prinus	1.25 ± 0.09	50.1 ± 1.15	0.05 ± 0.004	1:40:1001	13.55 ± 0.37	0.00092
R. maximum	0.55 ± 0.08	48.9 ± 1.08	0.02 ± 0.004	1:89:2444	12.54 ± 1.15	0.00086

at room temperature in paper bags in the lab for one week. Litters were placed in litterbags in all possible combinations (15 in total) of the four species, where each species in any one combination was equally represented in mass. Litterbags (15 cm × 15 cm) were constructed from 1 mm nylon mesh and heat-sealed at the edges. Each litterbag contained 5 g of leaves. Four replicate blocks were established along a 30 m reach of Ball Creek, with two blocks on each side of the stream. Blocks were approximately 5 m from the stream edge and 10 m from the neighboring block. The four study species co-occurred in each block. On November 17, 2003, one set of all 15 combinations was placed in each of the four blocks for each of 7 collection dates across 2.5 years: 0, 92, 181, 273, 365, 730, and 911 days. At each collection date, one set from each replicate plot was randomly chosen for processing, and litterbags were transported back to the laboratory on ice.

When bags were returned to the lab, five leaf cores (13 mm dia.) were taken for each of bacterial and fungal biomass analyses. Leaf disks for bacterial analysis were preserved in filtered (0.2 μm) 3.7% formaldehyde and stored at 4 °C. Leaf disks for fungal biomass were stored in 99.9% high-pressure liquid chromatography (HPLC)-grade methanol at 0 °C. To estimate the dry weight of the punches used in these assays, another five disks from each bag were taken. The average dry mass of the disks was used to estimate the weight of the ten disks used for microbial analysis. After punches were removed for microbial analysis, half of the litter was then taken from each sample bag to be used in gravimetric extraction in water via Baermann funnels for 48 h (Tarjan, 1949). Nematodes were harvested and preserved in 4% formaldehyde. Remaining litter in bags was placed on Tullgren funnels for seven days for dry heat and light extraction of arthropods (Macfadyen, 1953); those micro and small macroarthropods collected were preserved in 70% ethanol.

Bacterial cells were removed from leaf disks by sonication (Weyers and Suberkropp, 1996), and subsamples of the suspension were stained with a 1:1 proportion of sample to 10 μg mL^{-1} DAPI (4′6-dia-midino-2-phenylindole; Velji and Albright, 1993). Samples were incubated for 10 min prior to vacuum filtration onto black 0.2 μm membrane filters (supported by a 0.45 μm backing filter), then slide mounted and stored in the refrigerator in the dark until counted. Cells were enumerated using epifluorescent microscopy (1000×) by counting ten random fields and categorizing cells into shape (coccoid or rod) and size class (small and large). Cell biovolumes were calculated using equations for the geometric shape size classes (Wetzel and Likens, 2000), and total bacterial C was estimated using the conversion factor of 5.6×10^{-13} g C μm^{-3} (Bratbak, 1985). Bacterial C was then expressed as amount per g Ash Free Dry Mass (AFDM; the mass of combustible, organic content of litter).

Ergosterol, an estimate of fungal biomass (Gessner and Chauvet, 1993), was extracted from leaf disks by refluxing for 30 min at 80 °C in 25 mL methanol with an alcoholic base KOH (Weyers and Sub-erkropp, 1996). Samples were then partitioned into pentane and the pentane evaporated to dryness at 30 °C under a stream of N$_2$ gas. Samples were redissolved in 1 mL methanol, filtered through a 0.45 μm Acrodisc filter, and stored at 0 °C. Ergosterol concentration was measured by HPLC at 282 nm on an RP-10 column (Shimadzu, Columbia, MD, USA). Ergosterol concentration was converted to fungal C using the conversion factor 5.5 μg ergosterol g^{-1} fungal dry mass (Gessner and Chauvet, 1993), assuming a 43% C content of fungal dry mass (Baldy et al., 1995; Baldy and Gessner, 1997). Fungal C was then expressed as amount per g AFDM.

Nematodes were identified to feeding group (Yeates et al., 1993) and expressed as number per g AFDM (Ball et al., 2008). We also counted tardigrades and copepods, two groups of biota extracted by Baermann's funnels that are not often included in decomposition studies. Micro and macroarthropods were identified to the order level, or lower when possible, and expressed in the same manner. Abundance and taxa richness were recorded.

2.3. Data analyses

All statistical analyses were conducted in S-Plus 7.0 (Insightful Corp., Seattle, USA). An Analysis of Variance (ANOVA), using Type I sums of squares (SS), was performed to test for additivity and non-additivity of litter species effects. Following the methodology of Ball et al. (2008), we used a statistical model that first identified whether there was a significant influence of the presence of each litter species (additivity), then tested for the presence of any significant interactions of species (non-additivity) beyond what was explained by the presence or absence of a species. With this approach, we are able to test first whether a litter species influences the decomposer community, then ask whether its effect is largely additive or if it depends on the presence of other litter species. The emphasis is therefore on the litter species, rather than diversity *per se* as with other models (e.g. Schmid et al., 2002). Block, time, and the presence/absence of each of the four species were added sequentially as terms to the model. Block had four levels; time had six levels for microarthropod data (days 92, 181, 273, 365, 730, and 911) and four for bacteria, fungi, and nematode data (days 92, 181, 365, and 730). The term representing each litter species had two levels, "present" or "absent", so that each of the treatments that contained a species (both monocultures and mixtures) was compared to the treatments where the species was absent. A species interaction term (SpInt) was then included to test for non-additivity. This term had 11 levels, each representing one of the specific litterbag multi-species combinations. Lastly, interactions between time and block, the species, and SpInt terms were included. Time (days) was analyzed as a discrete, rather than continuous, factor to test whether the relative effects of species loss were consistent or different across time.

A significant SpInt term (and/or its interaction with time) indi-cates a significant non-additive interaction among species, due to richness or composition that is not explained by simple presence or absence of individual species. To explore potential richness effects we replaced the SpInt term with a Richness term, composed of four levels (1–4 species). In the absence of a significant effect of Richness or its interaction with time, a significant SpInt term must arise through non-additive composition effects. If a Richness term is significant, a Composition term, with 11 possible levels and thereby equivalent to the SpInt term, can be added to the model, while retaining Richness, to evaluate if both non-additive richness and composition effects manifest. Non-additive composition effects can be further explored to determine which of the species were inter-acting. If the SpInt term was not significant, the model was re-run with each of the four species' presence/absence terms added first because, given that Type I (Sequential) SS was used, the F-values of

the species terms were sensitive to the order in which they were added. A significant species presence/absence term indicated significant additive effects of that species on decay dynamics (i.e. species identity mattered).

Data were transformed when necessary to meet the assumptions of normality of variance. Arthropod abundance data were square root-transformed, while nematode abundance and microbial biomass data were $\ln(x+1)$-transformed. Richness values were not transformed.

3. Results

3.1. Microbes

There were no significant non-additive effects of plant litter on bacterial biomass, given that the SpInt term and its interaction with time were not significant, but there were significant additive effects of composition based on the presence/absence of *R. maximum* (Table 2). The manner in which *R. maximum* had its effect was marginally dependent on time. Specifically, bacterial biomass was significantly lower in the presence of *R. maximum* after 6 months of decomposition (Fig. 1a). Similarly, there were only significant additive effects on fungal biomass. These effects were based on the presence/absence of *R. maximum* and *L. tulipifera* (Table 2) and were time-dependent. The presence of *L. tulipifera* generally increased fungal biomass, whereas that of *R. maximum* decreased it. For both species, the difference in fungal biomass was initially small, increased in the early stages of decomposition, then decreased later in time (Fig. 1b). The ratio of fungal:bacterial biomass (F:B) was also driven by additive effects, but only *R. maximum* had significant effects on this variable, and its effect changed throughout time (Table 2, Fig. 1c).

3.2. Nematodes, tardigrades and copepods

Variation in nematode abundance was driven by additive composition effects based on the presence (or absence) of certain litter species, but the litter species responsible varied with nematode feeding group (Table 2). All litter species except *A. rubrum* influenced one or more feeding groups. Interestingly, the presence of those species that did have a significant effect decreased nematode abundance. The exception was the increase in abundance of omnivorous nematodes (OM) when *L. tulipifera* was present (Fig. 2). Conversely, *A. rubrum* was the only species whose presence consistently increased nematode abundance (Fig. 2a), but this influence was not significant ($P > 0.1$ for all feeding groups). The additive effects of composition were significant for fungal feeders (FF), bacterial feeders (BF), and omnivores (OM). *L. tulipifera* was involved in all of these, and its effects were time-dependent (Fig. 2b), while those of *R. maximum* and *Q. prinus* were generally constant and could be pooled across time (Fig. 2a). Predatory nematodes are the only group not influenced by litter species.

For "plant-feeding" nematodes (family Tylenchidae), there were non-additive interactions among litter species that were time-dependent. Non-additivity was due to an effect of litter species richness, not composition (Table 2). Single- and four-species litter mixtures contained more plant feeders than did two- and three-species mixtures at 365 days, but by 730 days plant-feeding nematodes in three- and four-species mixtures were significantly lower in abundance than the other litter richness levels.

There were no effects of composition or richness on tardigrade abundance ($P > 0.1$ for all additive and non-additive main effects), but there were additive effects on copepod abundance driven by *L. tulipifera*, *Q. prinus* and *R. maximum*. *L. tulipifera* had a positive effect on copepod abundance, while *Q. prinus* and *R. maximum* had negative impacts (data not shown). The way in which *L. tulipifera*

Table 2
Summary of significant effects of four leaf litter species on the decomposer community of a southern Appalachian riparian zone. An effect or interaction was considered significant if $P < 0.05$, but marginally significant interactions are also included.

	df	SS	MS	F	P
Bacterial biomass:					
R. maximum	1	0.58	0.58	5.50	0.020
R. maximum × day	3	0.77	0.26	2.42	0.068
Fungal biomass:					
L. tulipifera × day	4	7.17	1.79	4.56	0.002
R. maximum	1	12.3	12.3	31.3	0.000
R. maximum × day	4	3.52	0.88	2.24	0.066
Fungi:bacteria ratio					
R. maximum × day	3	7.44	2.48	3.38	0.020
Bacterial feeding nematode abundance:					
L. tulipifera × day	3	13.49	4.50	5.90	0.001
R. maximum	1	7.28	7.28	9.56	0.002
Fungal-feeding nematode abundance:					
L. tulipifera × day	3	12.4	4.14	3.19	0.025
Q. prinus × day	3	10.4	3.47	2.67	0.049
Plant-feeding nematode (Tylenchidae) abundance:					
Richness × day	6	27.4	4.57	4.98	0.000
Omnivorous nematode abundance:					
L. tulipifera × day	3	6.43	2.14	3.05	0.030
R. maximum	1	6.31	6.31	8.99	0.003
Predatory nematode abundance:					
No significant effects					
Copepod abundance:					
L. tulipifera × day	3	4.19	1.40	3.40	0.019
Q. prinus × day	3	5.89	1.96	4.78	0.003
R. maximum	1	1.81	1.81	4.41	0.037
Tardigrade abundance:					
No significant effects					
Total microarthropod abundance:					
A. rubrum	1	16.7	16.7	4.48	0.035
R. maximum × day	5	93	18.6	4.98	0.000
Oribatid abundance:					
A. rubrum	1	11.8	11.8	5.59	0.019
R. maximum × day	5	55	11.0	5.21	0.000
Mesostigmata abundance:					
L. tulipifera × day	5	14.6	2.92	2.50	0.032
R. maximum × day	5	15.2	3.0	2.60	0.026
Prostigmata abundance:					
R. maximum × day	5	5.4	1.08	2.38	0.039
Collembola abundance:					
No significant effects					
Total microarthropod richness:					
Q. prinus	1	10.7	10.7	4.47	0.035
R. maximum × day	5	37.4	7.49	3.14	0.009

and *Q. prinus* exerted their effect changed through time (Table 2), where at day 365 there were twice as many copepods in the presence of *L. tulipifera* or in the absence of *Q. prinus* (and no significant difference between their presence and absence at the other sampling dates; data not shown).

3.3. Arthropods

The most abundant microarthropods found in all samples were Collembola and the three suborders of Acarina. Twenty-two other taxa were found in various samples throughout the sampling dates (Appendix 2 (supplementary material – online)). While our mesh size excludes biota wider than 1 mm, many taxa commonly considered to be macroarthropods were present (e.g. spiders, ants,
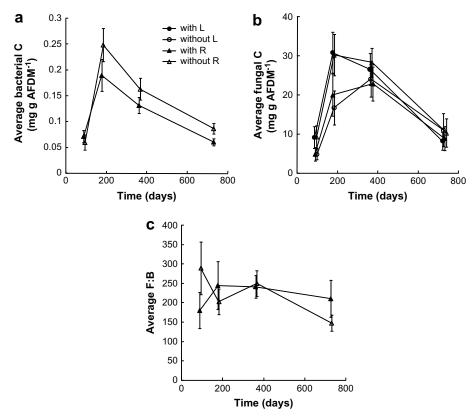
Fig. 1. Significant additive effects ($P < 0.05$) of leaf litter species on (a) bacterial biomass, (b) fungal biomass, and (c) ratio of fungi:bacteria biomass over two years of decomposition. Letters refer to the genus of each of the tree species: *Liriodendron tulipifera* (L) and *Rhododendron maximum* (R). Solid symbols represent all treatments that contained that species, and open ones include all treatments that did not. Values are means ± 1 SE; $n = 4$.
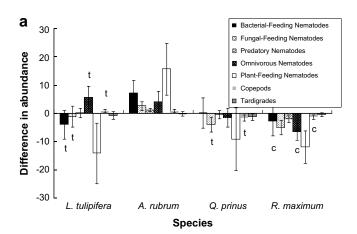
millipedes, and centipedes), as well as Enchytraeids (Phylum Annelida). Additionally, fauna taxa covering all basic functional roles were present in the litterbags (e.g. microbivores, shredders, predators). Statistical analyses were conducted on total abundance of all taxa, and also focused on the taxa that were most abundant in all samples (Acarina and Collembola). Other microarthropod taxa were not as abundant as these groups, so were not analyzed individually, but are included in analysis of total arthropod abundance. Total arthropod abundance was driven by additive effects (Table 2). *A. rubrum* and *R. maximum* had the greatest effects on total arthropod abundance. *A. rubrum* increased total abundance, as well as Oribatid abundance (Fig. 3a). The presence of *R. maximum* decreased abundance of all taxa except Oribatid mites which, overall were significantly more abundant in its presence (Fig. 3). For all effects driven by *R. maximum* that were time-dependent (Fig. 3), there was initially lower abundance in its presence, then higher abundance later in the decomposition process, only to return to lower abundance by the final sampling date (though this pattern is not very accentuated for Prostigmata; Fig. 3b). *L. tulipifera* presence had an initial positive effect on Mesostigmata, but then its effects were relatively neutral (Fig. 3b). Of the abundant taxa, the Collembola were the only taxa not significantly influenced by species composition.

Microarthropod taxa richness was affected by the presence of individual litter species (Table 2). At most sampling dates, richness significantly declined in the presence of *R. maximum* (Fig. 3b) but increased with *Q. prinus* (Fig. 3a).

4. Discussion

Our results demonstrate a link between the aboveground plant community and the decomposer biota: leaf litter species composition affected abundance, biomass, and diversity of the decomposer biota. Effects of the four leaf litter species on the decomposer community were largely additive, with each species exerting effects on different aspects of the community and at different stages of decomposition (summarized in Table 3). Overall, the two species at opposite ends of the quality spectrum, *L. tulipifera* and *R. maximum*, affected the abundance and biomass of more groups of the biota, suggesting an important role for these two species in shaping the decomposer community. *R. maximum* had negative impacts on microbial biomass and the abundance of most groups of arthropods and nematodes (at various sampling dates). *L. tulipifera* provided a rich resource that appeared to create a bottom-up template for the decomposer community (Mikola and Setala, 1998; Scheu and Schaefer, 1998; Chen and Wise, 1999). Specifically, the presence of *L. tulipifera* led to larger biomass in the lower trophic level (microbes) and greater abundance in the higher trophic level (Mesostigmata mites, omnivorous nematodes), but decreased abundance of taxa mid-level in the food web (microbivorous nematodes; Table 3). The presence of *Q. prinus* had moderate effects on the decomposer community, decreasing the abundance of copepods and fungal-feeding nematodes but increasing arthropod richness. Its influence on the arthropod community could be due to its high nitrogen content or structural features that create domatia for soil-dwelling organisms (Hansen, 1999). *A. rubrum* did not exert as great an effect on the biota as did the other litter species, save for its positive influence on Oribatid and total arthropod abundance (Table 2). Together, these data demonstrate an influence of all four dominant tree species on the decomposer community. Each tree species is likely important for maintenance of the current decomposer community (i.e. their effects are not redundant). Our data suggest that loss of any of these species will alter the decomposer community structure, but that the decomposer community will be most altered by loss of the two
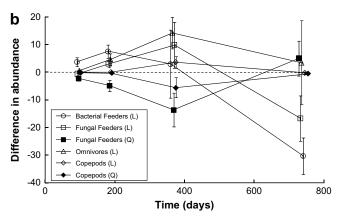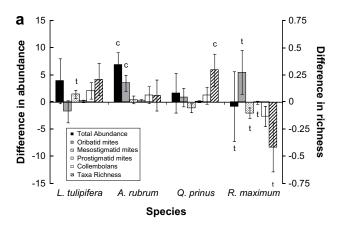
Fig. 2. Significant additive effects of leaf litter species identity on nematode communities over two years of decomposition. Values on the *y*-axis are the average difference in numerical abundance between treatments where each leaf litter species is present and those where it is not. The bar graph (a) represents pooled values across time. Letters above bars denote significant effects at *P* < 0.05; "c" indicates effects that were consistent over time, and "t" indicates effects that interacted with time. Species whose effects interacted with time (*Liriodendron tulipifera* (L) and *Quercus rubrum* (Q)) are shown in the line graph (b), where each taxon was enumerated at the same sampling dates; points were deviated for clarity. See Appendix 3 (supplementary material – online) for average abundances in the presence and absence of each species. Values are means ± 1 SE; *n* = 16 for (a) and *n* = 4 for (b).

Fig. 3. Significant additive effects of leaf litter species on arthropod communities over two years of decomposition. Values on the *y*-axis are the average difference in numerical abundance between treatments where each leaf litter species is present and those where it is not. Pooled values across time are shown in (a); letters above bars denote significant effects at *P* < 0.05, where "c" indicates effects that were consistent over time, and "t" indicates effects that interacted with time. Metrics involving abundance of individuals correspond to the left *y*-axis, whereas the taxa richness and Shannon index correspond to the right *y*-axis. Species whose effects interacted with time (*Liriodendron tulipifera* (L), and *Rhododendron maximum* (R)) are shown in the line graph (b), where each taxon was enumerated at the same sampling dates; points were deviated for clarity. See Appendix 4 (supplementary material – online) for average abundances in the presence and absence of each species. Values are means ± 1 SE; *n* = 24 for (a) and *n* = 4 for (b).

species at opposite ends of the quality spectrum, *L. tulipifera* and *R. maximum*, and most resistant to loss of *A. rubrum*.

The manner in which litter species interacted with time to drive the decomposer community did not appear to be dependent on season. For example, the manner in which total arthropod abundance varies through time in the presence of *R. maximum* (Fig. 3b) does not correspond to differences in temperature or moisture at, or directly preceding, each sampling date (Appendix 1 (supplementary material – online)). Though climate has been demonstrated to influence decomposer communities (e.g. Seastedt and Crossley, 1980; Gonzalez and Seastedt, 2001), the changes in abundance and biomass of decomposer taxa we observed may better correspond to different stages of decomposition (Anderson, 1975). However, climatic influences are likely the cause of significant interactions of time with block, due to the tropical depression that flooded two of the blocks just prior to the 365-day sampling. Given our statistical approach, the variation caused by time and block was removed before the investigation of species effects, so the flood did not compromise our ability to test for litter species effects. In addition, that we did identify significant compositional effects suggests that species' effects were not masked by the influence of the tropical storm; a common phenomenon in our study system.
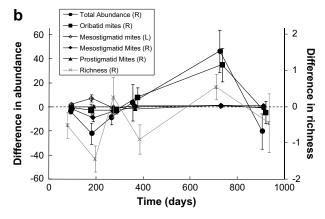
In accord with previous studies (Wardle et al., 2003; De Deyn et al., 2004), we observed a stronger response of the lower trophic groups (e.g. microbial consumers and microbial-feeding invertebrates) to litter mixing than we observed in the predators (Table 2). There were no significant effects of litter species or their interactions on predatory nematode abundance, and a marginally significant *P*-value for the Prostigmata and Mesostigmata (frequently grouped as predators; Coleman et al., 2004). The influence of *L. tulipifera* and *R. maximum* on lower trophic levels, in addition to *L. tulipifera*'s bottom-up effects on all levels, suggests that the consequences of the loss of these three species will be through alterations in the basal resource (i.e. food quality) of the decomposer food web. It is interesting to note that the presence of *Q. prinus*, and *A. rubrum* to a lesser extent, influenced microbial-feeding invertebrates, but not microbial biomass. This suggests that the role of mid-quality species in shaping the decomposer community may lie in generating habitat heterogeneity (e.g. Hansen and Coleman, 1998), as opposed to providing a food resource, making it functionally distinct from the high- and low-quality litter species.

Mixing of litters with different chemical and physical (habitat) structure is expected to provide a heterogeneous resource for

Table 3

Summary of direction of significant effects of four leaf litter species on the decomposer community of a southern Appalachian riparian zone. An effect or interaction was considered significant if $P < 0.05$, but marginally significant interactions are also included. Arrows denote direction of effect (i.e. increase or decrease in magnitude) when each tree species was present. A "t" denotes an effect that interacted with time, and a dash a non-significant effect.

	L. tulipifera	A. rubrum	Q. prinus	R. maximum
Microbes				
Bacterial biomass	–	–	–	↓ᵗ
Fungal biomass	↑ᵗ	–	–	↓ᵗ
Nematodes				
Bacterial feeders	↓ᵗ	–	–	↓
Fungal feeders	↓ᵗ	–	↓ᵗ	–
Tylenchidae	–	–	–	↓
Omnivores	↑ᵗ	–	–	↓
Predators	–	–	–	–
Crustaceans				
Copepods	↑ᵗ	–	↓ᵗ	↓
Tardigrades	–	–	–	–
Microarthropods				
Oribatida	–	↑	–	↑ᵗ
Mesostigmata	↑ᵗ	–	–	↓ᵗ
Prostigmata	–	–	–	↓ᵗ
Collembola	–	–	–	–
Total abundance	–	↑	–	↓ᵗ
Total richness	–	–	↑	↓ᵗ

decomposer biota, leading to non-additive interactions among litter species on biota (e.g. De Deyn et al., 2004). However, in contrast to many previous studies (reviewed by Gartner and Cardon, 2004), we observed very little evidence for significant non-additive effects of litter mixing on the decomposer community. The only group for which we saw non-additive effects was the Tylenchidae (Fig. 3). The abundance of these nematodes was high in litters containing 1 or 4 tree species and low in litters containing 2 or 3 tree species. More research would be necessary to determine why this pattern might be occurring, but it is worth noting that although named plant feeders, the Tylenchidae can feed upon microbes and algae (Yeates and Coleman, 1982; Coleman et al., 2004). Given the absence of plant roots in our litterbags, presumably the richness effects on these nematodes arose through changes in the basal decomposer community, which may constitute their food resource.

Litterbag mesh size is known to influence decomposition dynamics through restriction of access of larger biota (e.g. macroarthropods) and microclimate modification (Bradford et al., 2002; Hunter et al., 2003). Further, it has been demonstrated that functional dissimilarity of macrofauna influences decomposition (Heemsbergen et al., 2004). Though our litterbags excluded larger fauna, we still observed the presence of all basic functional groups over a wide variety of taxa, including those typically considered to be macrofauna (Appendix 2 (supplementary material – online)). The largely additive influence of litter species holds across all of these taxa and functional groups (save for one taxa of nematode, the Tylenchidae). The presence of larger fauna, were the mesh size larger, may have led to altered abundances of the taxa we identified here. We cannot envisage why, however, any exclusion of macrofauna and/or microclimate effects of litterbags would influence our observation of largely additive, and not non-additive, litter species effects. Our use of a more conservative analytical model more likely explains our results. Specifically, detecting additive and non-additive effects in litter-mixing studies is sensitive to the analytical techniques used (see Ball et al., 2008). Some authors compare observed communities with those that would be expected based on the monocultures of each species involved in the mixture, where

significant deviations from the expected suggest non-additive effects on communities (e.g. Blair et al., 1990; Hansen, 1999). It is possible that additional non-additive effects would have been detected in our study had we employed such methodologies. Using a full-factorial design and a statistical model that incorporates all data, our analysis is more conservative than most previous methods (Ball et al., 2008). As such it asks whether additive or non-additive diversity effects are likely to be dominant and illustrates that additive effects are dominant in our system, supporting other studies that emphasize the importance of species identity (reviewed by Hooper et al., 2005). A dominance of additive effects in explaining variation in the decomposer community suggests that the consequences of species loss may be predictable from monocultures. That is, we need only to know the properties of each individual species, rather than requiring new information on interactive effects, to predict the outcome of tree species loss on decomposer communities. However, if tree species influence decomposer communities in ways other than through litter (Vivanco and Austin, 2008), the predictability may not hold with species loss. In this experiment, the litters were all incubated in a mixed-species forest, and with species loss, the decomposer species available to colonize the litter may differ from the current assemblage.

The ability to predict consequences of changes in tree species abundance is valuable given that the abundance of dominant species is likely to change in temperate forests experiencing global change pressures (Orwig and Foster, 1998; Ellison et al., 2005). For example, in our study region disease dynamics are predicted to potentially increase *L. tulipifera* and *R. maximum* abundance (Orwig and Foster, 1998; Ellison et al., 2005) or decrease *Q. prinus* and *R. maximum* abundance (Rizzo et al., 2002). In addition, *A. rubrum* is generally predicted to increase (Fei and Steiner, 2007). While the last of these will likely have little influence on the decomposer biota (unless it replaces the other co-dominants), our results suggest that the impact of diseases which alter *L. tulipifera* and *R. maximum* abundance will likely have major impacts on decomposer community structure. Given the importance of the belowground subsystem to nutrient cycling, this may have substantial effects on ecosystem productivity, nutrient retention and structure (e.g. van der Heijden et al., 2008). Future research should focus on whether the potential changes in decomposer communities with tree species loss might themselves further alter plant communities through feedbacks on nutrient availability. Overall, our data suggest that tree species loss, predicted to affect this system through introduced pests and pathogens, will likely alter the decomposer biota community, potentially changing the way in which organic matter and nutrients are processed in the forest floor.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2009.02.025.

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