

Leaf litter species evenness influences nonadditive breakdown in a headwater stream

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Abstract. Species loss directly affects the magnitude and stability of various ecosystem processes, and species composition can drive this phenomenon. Much of the evidence that species loss affects ecosystem processes comes from experiments where species richness was manipulated while holding abundance/biomass of individual species constant. Given that species rarely coexist in equal proportions, neglecting evenness might under/overestimate the role of important species combinations. We examined leaf litter breakdown in a small stream based on species-specific input rates of leaf litter from the four dominant species (*Liriodendron tulipifera*, *Fraxinus pennsylvanica*, *Fagus grandifolia*, *Quercus prinus*), comprising 71% of inputs over peak leaf fall, for a full-factorial litter mixture study. Our experimental approach departed from previous mixture studies in that while we created all two-, three-, and four-species combinations holding species-specific mass constant, we also created a complementary set of mixtures that reflected the natural proportion we estimated from the survey. We found that species richness and evenness alone did not explain variation in breakdown rate, but an interaction between the two did, and mixtures reflecting ambient evenness lost mass nearly 33% faster than single species treatments. Analysis of individual treatments revealed that the emergent effect of mixing species was nearly twice as common in uneven vs. even mixtures. The compositional effects of litter diversity on breakdown uncovered in previous studies might be more pronounced if evenness, and not just richness, is considered when evaluating the role of species loss in these ecosystems.

Key words: composition; ecosystem functioning; evenness; *Fagus grandifolia*; *Fraxinus pennsylvanica*; headwater stream; leaf litter breakdown; *Liriodendron tulipifera*; nonadditive effects; *Quercus prinus*; richness.

INTRODUCTION

The worldwide decline of species is associated with changes in ecosystem processes (Schulze and Mooney 1993, Kinzig et al. 2002, Loreau et al. 2002). More than 85% of primary production in temperate ecosystems enters the detrital pool (McNaughton et al. 1989, Cebrian 1999), and decay of this material (i.e., leaves, woods, flowers, and so on) supports soil formation and provides energy to detritus-based food webs (e.g., streams; Wallace et al. 1999). Given substantial interspecific variation in detrital chemistry, especially among leaf litter (Webster and Benfield 1986, Ostrofsky 1997), ecologists are questioning how loss of plant species might not only alter rates of primary production, but also organic matter decomposition (Gartner and Cardon 2004, Swan and Palmer 2004, Hättenschwiler et al. 2005, Kominoski et al. 2007, Lecerf et al. 2007). The results of experiments manipulating leaf litter species loss and

estimating rates of litter decay in both soil and stream ecosystems are complex. When effects of litter diversity on decay occur, leaf species composition, not species richness per se, is often responsible for emergent effects of litter mixing (Gartner and Cardon 2004, Hättenschwiler et al. 2005, Swan and Palmer 2006b; but see Kominoski et al. 2007). By embracing new insights into how multi-species litter assemblages interact, ecologists are beginning to change their view of litter decomposition processes across ecosystem types.

Litter-mixing studies have documented positive, negative, and sometimes no effect of leaf diversity on decay (see reviews by Gartner and Cardon 2004, Hättenschwiler et al. 2005). The rationale for performing such studies stems from the observation that interspecific variation in litter chemistry can drive litter decomposition rate (e.g., C:N, secondary compounds; Webster and Benfield 1986). Litter assemblages on the forest floor or in water bodies adjacent to forests reflect forest plant community composition (Johnson and Covich 1997, Swan and Palmer 2004), and thus loss of tree species translates into loss of litter species and their specific qualities. Consumers known to strongly regulate

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litter decay respond to litter mixtures in a number of ways related to litter chemistry (Hättenschwiler and Bretscher 2001, Hättenschwiler and Gasser 2005, Swan and Palmer 2006b): (1) they might acquire complementary resources from different species, increasing decay of the mixture beyond that predicted from each leaf species in isolation, (2) preferential resource use may result in avoidance of refractory species in a mixture as the consumer feeds on more labile species, resulting in overall slower decay of the mixture compared to that predicted by each leaf species alone, and (3) consumers may respond similarly to each leaf species in a mixture as when alone, resulting in no overall effect of mixing. Experimental evidence for some of these patterns often indicate the presence of a few key leaf species as being important to explain how diversity changes rates of litter decomposition (Gartner and Cardon 2004, Hättenschwiler and Gasser 2005, Swan and Palmer 2006b).

Litter-mixing studies repeatedly reveal that leaf species composition is most often more important than richness (Gartner and Cardon 2004). Often a very labile (e.g., poplar; Kominoski et al. 2007) or refractory (e.g., sycamore; Swan and Palmer 2006b) species in the community drives nonadditive effects of mixing. Therefore, if such a key species is more/less common in the community, holding evenness unnaturally high may under- or overestimate the effect of litter species diversity on breakdown rates. While the effect of plant evenness has been experimentally manipulated and shown to be important in how species loss regulates rates of primary production and likelihood of species invasibility (Wilsey and Potvin 2000, Wilsey and Polley 2002, Smith and Knapp 2003, Smith et al. 2004, Kirwan et al. 2007), plant evenness has not been investigated in the context of litter decomposition (but evenness of litter consumers have; Dangles and Malmqvist 2004, McKie et al. 2008). In light of accumulating evidence that leaf species loss influences rates of litter breakdown (Gartner and Cardon 2004, Hättenschwiler et al. 2005), our perspective on litter-mixing studies may need to be updated to embrace species evenness as a controlling factor on how diversity changes rates of decomposition.

In this study, we asked (1) does decay of diverse litter assemblages depend on the relative abundance of species in the mixtures? (2) for a specific community (i.e., litter species mixture), do decay rates vary for mixtures constructed evenly or not? and (3) does evenness change the incidence of nonadditive decay of mixtures for the same forest community? We approached answering these questions with a combination survey and field experiment. We determined the relative abundance (as biomass) of litter species delivered to a headwater stream through leaf fall. Using this information, we performed a field experiment where the breakdown rate of the dominant four litter species were examined alone and in all two-, three- and four-species combinations, and where each individual leaf species in mixtures were either equally abundant (e.g., the mixture had maximum

evenness), or reflected the natural relative abundance (e.g., uneven).

METHODS

Study site

We carried out the study at an unnamed, spring-fed headwater stream draining into the Patapsco River, located in Howard County, Maryland, USA (39°13'48" N, 76°44'24" W, elevation 97 m above sea level; see Plate 1). This Piedmont watershed is >99% forested and exhibits a typical riffle-run geomorphology, with nearly 100% streambed coverage of litter during peak leaf fall (early October through early November). The overstory riparian community is dominated by oaks and beeches (*Quercus prinus*, *Quercus rubra*, *Fagus grandifolia*), ash (*Fraxinus pennsylvanica*) and poplar (*Liriodendron tulipifera*). The understory is predominately witchhazel (*Hamamelis virginiana*) and dogwood (*Cornus florida*). Over the 200-m study reach, stream width ranged from 0.2 to 1.5 m, and depth 2 to 15 cm. Discharge was typically low, averaging 0.001 m³/s. Mean water temperature over the course of the study (December–April) was 8.1°C, pH 8.3, conductivity 146.5 μS, NO₃ 1.1 mg/L, and PO₄ 0.1 mg/L.

Litter input survey

Litter inputs to the stream were carried out over the four-week period (9 October–6 November) of peak leaf fall in 2006. Ten litter baskets (0.42 × 0.55 m) were placed ~10 m apart streamside and sampled weekly for all organic matter inputs (Swan and Palmer 2004). Leaves were separated by species, dried to a constant weight at 60°C, and ash-free dry mass (AFDM) determined after combustion at 550°C for 45 minutes. We then calculated species-specific daily input rates of litter to the stream (g AFDM·m⁻²·d⁻¹).

Litter breakdown study

Litter breakdown rates were estimated using a standard litter-bag method whereby pre-weighed amounts of litter were placed in mesh bags, replicate bags placed in the stream, and the bags retrieved over time to determine the mass remaining (Benfield 2006). Litter treatments for the present study included the four dominant species identified in the survey (*Liriodendron tulipifera*, *Fraxinus pennsylvanica*, *Fagus grandifolia*, *Quercus prinus*) alone, and every two-, three- and four-species combination. We created two treatments for each combination: one “even” treatment where each leaf species was represented equally by biomass (e.g., a 50:50 mix for a two-species combination), and one “uneven” treatment where each species was represented by its relative abundance determined by the litter input survey. For example, *Liriodendron tulipifera* comprised 35% of the inputs among the dominant species, while *Fagus grandifolia* only 28%. Therefore, a mixture of the two species reflected this difference in representation, i.e., 35%/(35% + 28%) = 56% for *L. tulipifera*, and thus 44%

TABLE 1. Litter mixture treatment summary, and relative abundance of species for even and uneven treatments.

Richness	Mixture species	Even	Uneven
2	LT FP	50:50	58:42
2	LT FG	50:50	56:44
2	LT QP	50:50	76:24
2	FP FG	50:50	47:53
2	FP QP	50:50	69:31
2	FG QP	50:50	71:29
3	LT FP FG	33:33:33	40:29:31
3	LT FP QP	33:33:33	49:40:16
3	LT FG QP	33:33:33	47:38:15
3	FP FG QP	33:33:33	40:33:17
4	LT FP FG QP	25:25:25:25	35:28:25:12

Notes: Species abbreviations are as follows: LT, *Liriodendron tulipifera*; FP, *Fraxinus pennsylvanica*; FG, *Fagus grandifolia*; QP, *Quercus prinus*. The order of the relative proportion of each species under "Uneven" reflects the order of species listed in the "Mixture" column. For example, the mixture LT FP is 58% LT and 42% FP.

for *F. grandifolia*. This resulted in four single-species, 11 even mixtures, and 11 uneven mixtures for a total of 26 litter treatments (Table 1).

Freshly senesced leaf litter of each species was collected during leaf fall from the forest immediately adjacent to the stream during the same period the litter input survey was being conducted. Samples (20 g fresh mass) were set aside to determine initial fiber content (lignin, Goering and Van Soest [1970]; cellulose, Association of Official Analytical Chemists [2000]), nutrient content (N, P and K; Association of Official Analytical Chemists [2000]; Cumberland Valley Analytical Services, Hagerstown, Maryland, USA) and AFDM. The average percentage AFDM across the four species was 85%, and varied <1% across species. Each leaf species was added to leaf packs as fresh mass, and when in combination, all species were mixed together before placing in a 7 × 11 mm mesh bag. Packs were 6.0 ± 0.4 g (mean fresh mass ± range) of leaf litter, closed at each end with a plastic tie, and labeled. All leaf packs were tethered to galvanized wire, 10–20 per line separated by ~0.5 m, and staked to the bottom of the stream on 4 December 2006. Three replicate bags were collected from each combination after 14, 30, 58, 92, and 142 days. A total of 390 leaf packs were placed along the 200-m stream reach.

Sampling involved cutting the plastic tie attaching each pack to the wire line and releasing it into a plastic bag. Each bag was placed on ice and transported to the laboratory (<10 minutes travel time). Leaf packs were kept cold until processed, which occurred within 24 h. Leaf packs were emptied into a 500- μ m sieve and gently rinsed. Remaining litter was transferred to a pre-weighed aluminum tin, dried at 60°C for at least 24 h, then combusted at 550°C to determine AFDM.

Data analysis

Patterns in litter chemistry between litter species were analyzed using a one-way ANOVA for each constituent

(lignin, cellulose, N, P, K). Post hoc comparisons between litter species were evaluated using Tukey's hsd.

We tested for differences between breakdown rates of the four dominant species with an indicator variables regression analysis, which is essentially a one-way ANCOVA with day as a covariate and relaxing the assumption of identical slopes across the four species. Breakdown of leaf litter generally follows a negative exponential process (Jenny et al. 1949, Olson 1963, Webster and Benfield 1986), so the natural logarithm of the fraction of AFDM remaining was tested as a function of days in the stream and the interaction between day and leaf species identity. Preliminary examination of the decay curves suggested that a negative exponential decay process described mass loss well. A significant interaction term in the regression analysis was taken as evidence that breakdown differed between at least two species. The breakdown rate (k) was estimated from the output of the model using the ESTIMATE statement in SAS (version 9.1; SAS Institute 2003). Comparisons of breakdown rates between species were carried out upon obtaining a significant day × leaf species interaction, and the P values adjusted for inflation of error using the false discovery rate (FDR) method (Benjamini and Hochberg 1995).

The effect of leaf litter diversity on breakdown was tested similarly between three treatments: all single species, all mixtures with maximum evenness (hereafter "even"), and all mixtures with lower evenness (hereafter "uneven"). We termed this set of three treatments the "evenness" treatments. After a significant day × evenness interaction was obtained, breakdown rate (k) was estimated as above, and two comparisons made: breakdown of even vs. single species, and breakdown of uneven vs. single species. Again, the FDR method was used to adjust the P values since these comparisons were not independent.

We used indicator variables regression to test the relative effects of species richness, evenness and composition. The natural log of mass fraction remaining was tested as a function of days in the stream, the interaction of day with richness, evenness (even, uneven), richness × evenness, and composition nested within richness × evenness. All factors were treated as fixed in the analysis. Output was used to estimate breakdown of each mixture × evenness combination, and orthogonal comparisons made between even and uneven treatments within each of the 11 mixtures.

We predicted that mixing species in proportions reflecting availability should result in more instances of nonadditivity in breakdown. We used the mixture analysis approach of Cornell (2002) and recently employed by Kirwan et al. (2007) to test if mixing species explained more variation in mass loss than that of leaf species alone. Briefly, this is a general linear model where the species composition for each leaf pack is indicated using a matrix with a column for each leaf species with values identifying the initial proportion of

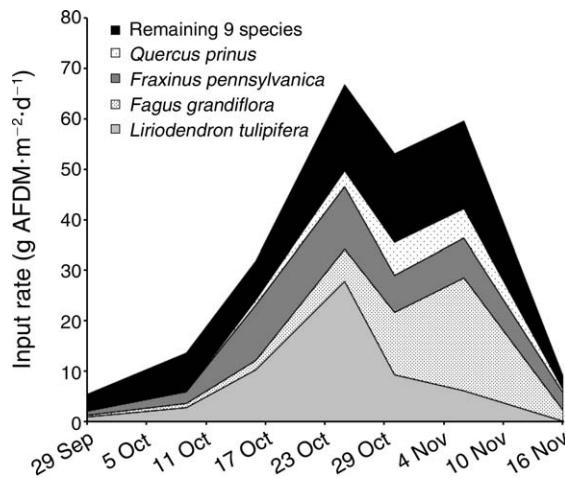


FIG. 1. Litter input rate over leaf fall, 2006. The bottom four areas are the dominant four species used in the breakdown study. The solid black area at the top is the sum of the inputs of the remaining nine species identified in the survey. AFDM is ash-free dry mass.

each species in the leaf pack, and zeros if a species is not included in the mixture. Thus, initial proportions for each species in a particular mixture were either identical for the “even” treatment (e.g., a three species mixture was comprised of 33% of each species by mass), or were the actual proportions estimated from the litter input survey for the “uneven” treatments. The natural log of mass fraction remaining was made a function of the interaction between day and the presence/absence of each species and all interactions. Significant interaction terms based on these initial proportions indicate nonadditive (i.e., multiplicative) effects of mixing species (Cornell 2002, Kominoski et al. 2007). We predicted that the instance of nonadditivity should be more common for uneven mixtures than in even mixtures. Therefore we ran two analyses: one with the single-species treatments and all even mixtures, and one with the single species treatments and all uneven mixtures.

Analyses were carried out in SAS (version 9.1). Assumptions of normality of residuals were met (Shapiro-Wilkes test), however we did observe unequal variances among some mixture treatments. To address this, we grouped the residual variances by mixture treatments using the GROUP option in PROC MIXED using the method of (Littell et al. 1996), and denominator degrees of freedom adjusted using the Satterthwaite approximation.

TABLE 2. Fiber and nutrient content of the four dominant litter species used in the breakdown study.

Species	Lignin (%)	Cellulose (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)
<i>L. tulipifera</i>	19 ^a ± 0.3	22 ^a ± 0.6	0.77 ^a ± 0.02	0.04 ^a ± 0.002	0.66 ^a ± 0.01
<i>F. pennsylvanica</i>	15 ^b ± 0.3	26 ^b ± 0.6	0.91 ^b ± 0.02	0.08 ^b ± 0.002	0.27 ^b ± 0.01
<i>F. grandifolia</i>	9 ^c ± 0.3	54 ^c ± 0.6	0.87 ^b ± 0.02	0.05 ^a ± 0.002	0.22 ^c ± 0.01
<i>Q. prinus</i>	25 ^d ± 0.3	25 ^b ± 0.6	0.75 ^a ± 0.02	0.03 ^c ± 0.002	0.46 ^d ± 0.01

Notes: Values are the mean ± SE ($n = 3$). Estimates with the same letter are not significantly different ($P > 0.05$) following a one-way ANOVA and applying Tukey's hsd to comparisons.

RESULTS

Patterns of litter inputs

Litter inputs were estimated from 29 September 2006 through 16 November 2006, and peaked at 66.8 g AFDM·m⁻²·d⁻¹. Inputs rose sharply after the 10 October sampling, and declined after 6 November (Fig. 1). This period was taken as peak leaf fall, accounting for 88.1% of the total litter inputs for the entire sampling period (Fig. 1). The composition of leaf species during that time was used to calculate the composition of the uneven mixtures. Of the 13 leaf species identified in the litter traps, four comprised 71% of the total inputs (Fig. 1), and were used in the mixture study. These species exhibited an appreciable range in litter quality (i.e., fiber components, nutrients; Table 2), and were: *Liriodendron tulipifera* (LT, 25% of total inputs, 35% of the four dominant species); *Fagus grandifolia* (FG, 20% of the total, 28% of the dominants); *Fraxinus pennsylvanica* (FP, 18% of the total, 25% of the dominants); and *Quercus prinus* (QP, 8% of the total, 12% of the dominants).

Litter breakdown study

Individual leaf species exhibited significant differences in litter breakdown rate (significant day × leaf species interaction, $F_{3,46.1} = 14.6$, $P < 0.0001$), and predictable patterns across species (Fig. 2A). *Liriodendron tulipifera* lost mass ~43% faster than *Fraxinus pennsylvanica*, 2.4 times that of *Quercus prinus*, and more than 4 times faster than *Fagus grandifolia*. *F. pennsylvanica* was the second fastest decaying species, breaking down significantly faster than *Q. prinus* (1.7 times) and *F. grandifolia* (2.9 times).

We tested whether species occurring alone, mixed evenly, or unevenly decayed at different rates, regardless of composition, to determine if species evenness influenced breakdown. After learning there was an effect (day × evenness interaction; $F_{2,316} = 4.6$, $P = 0.01$), we determined that unevenly mixed litter lost mass ~33% faster than single-species litter irrespective of species composition (Fig. 2B; $P = 0.009$). However, as is consistent with many previous studies, even mixtures did not decay differently than single-species treatments (Fig. 2B; $P = 0.18$).

A nested analysis was carried out on all litter treatments with two or more species to determine the interactive effect of evenness, richness and species composition. Results revealed that richness and even-

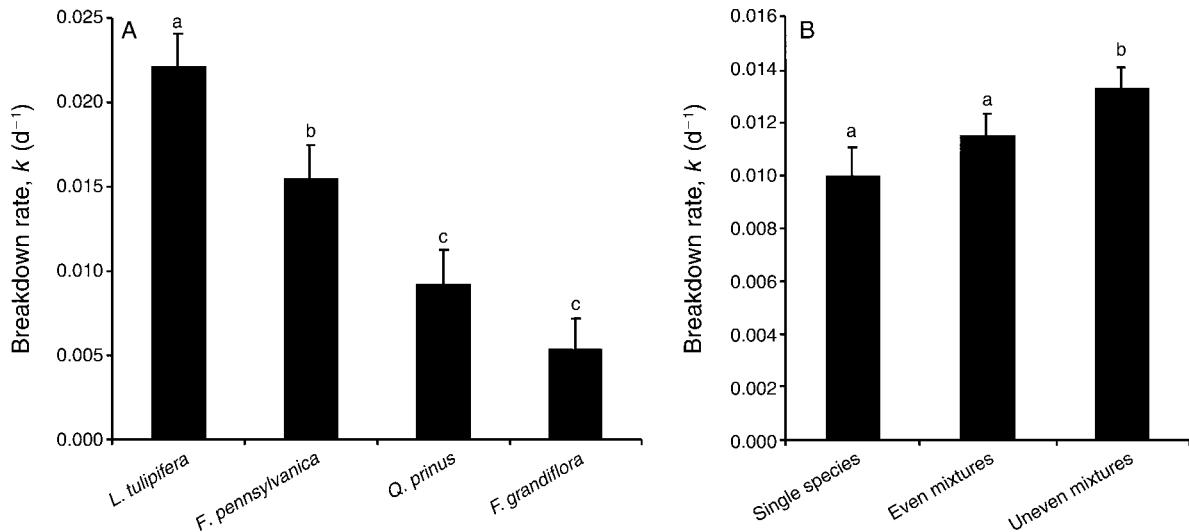


FIG. 2. Breakdown rate (\pm SE) of (A) each leaf species alone and (B) all single-species, all mixtures where species within a mixture were equally represented (e.g., even), and all mixtures where species reflect proportions estimated from the input survey (e.g., uneven). For (A), bars with identical letters are not significantly different ($\alpha = 0.05$). For (B), comparisons are restricted to single-species vs. even mixtures, and single-species vs. that of uneven mixtures. Bars with identical letters are not significantly different ($\alpha = 0.05$).

ness alone did not significantly explain variation in mass loss, but their interaction did (Table 3). Species composition was also significant when nested within both evenness and richness (Table 3), indicating that breakdown differed between even and uneven mixtures of the same species composition.

Four of the 11 species combinations exhibited differences in breakdown rates between even and uneven mixtures (Fig. 3). Three of these species were mixtures of two species, and the other a three species mixture (LT FP FG, Fig. 3). The magnitude of the difference between even and uneven treatments of the same species composition varied from more than 52% slower (FG QP, Fig. 3) to more than 80% faster decay of the uneven treatment compared to the even (LT QP, Fig. 3). The most refractory species was *Fagus grandifolia*, and when it dominated the two-species mixture with *Q. prinus*, mass loss was significantly slower than the corresponding even mixture by 52%. *Liriodendron tulipifera* was the dominant species contributing leaf litter to the stream (Fig. 1) and the fastest to lose mass when alone (Fig. 2A). The two mixtures showing the largest positive effect of evenness were the species mixed with *Fraxinus*

pennsylvanica (29% increase over even), and mixed with *Quercus prinus* (80% over even, Fig. 3). The significant increase in breakdown of uneven mixtures over single-species litter (regardless of composition; Fig. 2B) was likely driven by these two combinations.

Tests of nonadditivity were carried out to determine if the emergent effect of mixing species was more common for uneven mixtures compared to even. The mixture analysis identified three mixtures (LT FP, LT FP FG, all four species) where mixing species explained more variation in breakdown than the presence of each species alone ($P < 0.05$, Table 4). However, mixing effects were more common when the same analysis was performed on the uneven mixtures of the same species. Six of the 11 mixtures exhibited significant nonadditivity: LT FP, LT QP, FG QP, LT FP FG and LT FP QP, $P < 0.05$; FP QP, $P = 0.055$ (Table 4). Thus, the emergent effect of mixing species was nearly twice as common when species combinations were assembled based on their relative abundance to the stream during leaf fall.

DISCUSSION

We demonstrated that acknowledging evenness of leaf species mixtures was more important than species richness alone to litter breakdown rates in a detritus-based headwater stream. We measured species-specific litter inputs to a forested headwater stream, and learned there was interspecific variation in biomass of litter reaching the streambed. Results from our litter breakdown experiment revealed that species evenness interacted strongly with richness to explain variation in breakdown dynamics. Furthermore, we determined that the incidence of nonadditivity in litter mixing, that is

TABLE 3. Results of the nested analysis of leaf species richness, evenness, and leaf species composition on mass loss.

Source	df	F	P
Day	1, 243	474.0	<0.001
Day \times richness	2, 200	0.3	NS
Day \times evenness	1, 263	0.9	NS
Day \times richness \times evenness	2, 201	4.7	0.010
Day \times composition (richness \times evenness)	16, 130	9.0	<0.001

Note: NS, not significant.

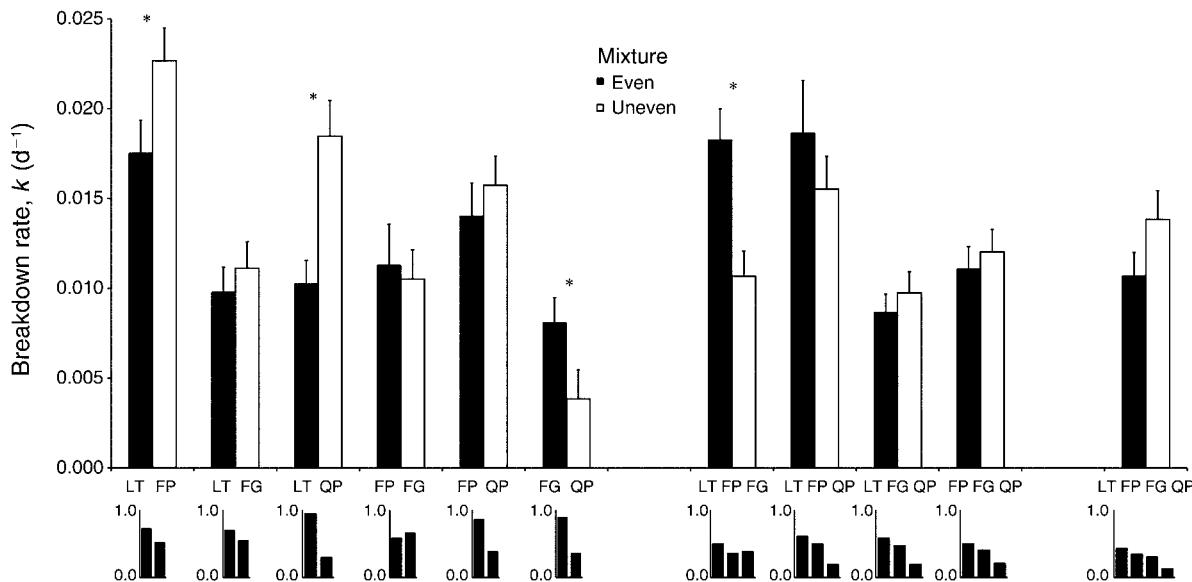


FIG. 3. Breakdown rate (+SE) of each mixture, when species were equally represented within a mixture (e.g., even; solid bars), and when the proportional representation of each species matched that estimated from the input survey (e.g., uneven; open bars). Significant differences ($P \leq 0.05$) between even and uneven treatments within the same species combination are indicated by asterisks. Species abbreviations are as follows: LT, *Liriodendron tulipifera*; FP, *Fraxinus pennsylvanica*; FG, *Fagus grandifolia*; QP, *Quercus prinus*. To aid interpretation, the relative abundance of species within the uneven mixtures, which is identical to that reported in Table 2, is given below each species combination, where the bar to the left corresponds to the species labeled first, the second bar the second species, and so on.

when breakdown of mixtures departs significantly from the breakdown of species alone, was more common in our uneven treatments compared with the identical analysis carried out on the even treatments. These data suggest three important conclusions: (1) the current assumptions regarding the role of leaf litter diversity on organic matter breakdown should be updated to consider the relative abundance of litter species in the system, (2) the role of dominant species may be more important than previously thought, and (3) the effect of

litter species loss on organic matter dynamics might be stronger than currently reported.

Consistent with other studies in soil and stream ecosystems, litter species richness alone did not explain significant variation in litter breakdown rate. Species rarely occur in equal proportions, and there is evidence from other systems that the relative abundance of species strongly influences interspecific interactions (Wilsey and Potvin 2000, Wilsey and Polley 2002, Smith and Knapp 2003, Smith et al. 2004, Kirwan et al. 2007).

TABLE 4. Results of the mixture analysis to determine if mixing species resulted in explaining more variation in loss rate than by each component species alone.

Source	Even			Uneven		
	df	F	P	df	F	P
Day × LT	1, 150	75.0	<0.001	1, 59.6	118.4	<0.001
Day × FP	1, 29	35.6	<0.001	1, 74.4	42.2	<0.001
Day × FG	1, 150	22.1	<0.001	1, 86.8	14.9	0.001
Day × QP	1, 148	37.3	<0.001	1, 84.8	27.7	<0.001
Day × LT × FP	1, 150	5.3	0.023	1, 128.0	27.3	<0.001
Day × LT × FG	1, 37	0.0	NS	1, 70.7	0.2	NS
Day × LT × QP	1, 138	0.7	NS	1, 53.1	9.9	0.003
Day × FP × FG	1, 73	1.7	NS	1, 110.0	0.5	NS
Day × FP × QP	1, 152	2.0	NS	1, 74.4	3.8	0.055
Day × FG × QP	1, 138	0.0	NS	1, 112.0	5.3	0.023
Day × LT × FP × FG	1, 170	4.3	0.041	1, 90.2	8.9	0.004
Day × LT × FP × QP	1, 141	2.3	NS	1, 126.0	9.8	0.002
Day × LT × FG × QP	1, 107	0.1	NS	1, 106.0	0.9	NS
Day × FP × FG × QP	1, 154	0.1	NS	1, 116.0	0.7	NS
Day × LT × FP × FG × QP	1, 150	5.9	0.017	1, 115.0	2.4	NS

Notes: Species abbreviations are as follows: LT, *Liriodendron tulipifera*; FP, *Fraxinus pennsylvanica*; FG, *Fagus grandifolia*; QP, *Quercus prinus*. The results (df, F, and P) are given for both the analysis of even mixtures and uneven mixtures.



PLATE 1. First-order tributary to the Patapsco River in Maryland, USA, draining a diverse deciduous forest. Accumulations of leaf litter delivered to the channel in autumn undergo breakdown through late May. Photo credit: C. Swan.

In diversity–function studies, the more uneven the community, the more the performance of the community matches the performance of the dominant species in monoculture (Smith and Knapp 2003, Smith et al. 2004, Kirwan et al. 2007). In light of this, species loss might be disproportionately more/less important depending on the relative abundance of the species in question (Hooper et al. 2005). Experiments explicitly addressing the role of species evenness and richness are less common than those addressing richness alone (see Wilsey and Potvin 2000, Wilsey and Polley 2002, Smith and Knapp 2003, Smith et al. 2004, Kirwan et al. 2007), but might prove integral to understanding the consequences of species loss in systems where composition is reported to play a very large role, as is the case in detritus-based ecosystems (Gartner and Cardon 2004, Hättenschwiler et al. 2005). In this study, only after incorporating relative abundance into the experimental design of the mixture treatments (e.g., ambient species evenness), was species richness a significant explanatory variable. On average, diverse litter mixtures reflecting relative abundance of species decayed 33% faster than single-species treatments, whereas a significant effect of the mixtures with equal species representation was not found. Furthermore, nonadditive effects of mixing on decay were more common for uneven than even mixtures.

Our field survey identified four dominant leaf species contributing the majority of litter to the stream during leaf fall. Furthermore, these species spanned an appreciable range in decomposability, with *Liriodendron*

tulipifera the most labile, and *Fagus grandifolia* and *Quercus prinus* the least labile, despite appreciable variation in litter chemistry. Richness and evenness alone did not explain variation in litter breakdown, but the interaction between both factors did. This was revealed in four of the 11 mixtures. In two, LT FP and LT FG, *L. tulipifera* was the dominant species and, given it lost mass the fastest of all four species, its dominance perhaps led to overall faster breakdown in these two mixtures compared to when species were equally represented. *F. grandifolia* was more common in the inputs compared to *Q. prinus* and when mixed together to reflect this, the former had more than twice the initial mass compared to *Q. prinus*. Since *F. grandifolia* was the slowest to breakdown, this supports the result that the even mixture of FG QP was faster to decay than the uneven where *F. grandifolia* dominated. The notion that the decomposability of the dominant species helps elucidate why relative abundance is important to decay fails, however, to explain why the mixture of LT FP FG decayed faster when species were equally represented. This occurred despite the initial proportions for the uneven treatment were not that different from equal (i.e., 40:29:31 vs. 33:33:33). Furthermore, there were instances when uneven mixtures exhibited high dominance (e.g., FP QP, LT FP QP) and, given that the dominant species were faster decomposers (i.e., *L. tulipifera*, *F. pennsylvanica*), we might have expected that the uneven mixtures should have decayed faster than their even counterparts. While this was not the case, there were nonadditive effects of

mixing on breakdown revealed for these two uneven combinations, but not for the even.

One set of mechanisms as to why diverse litter assemblages should decay nonadditively stems from how consumers can respond to interspecific variation in litter quality (Hättenschwiler and Bretscher 2001, Swan and Palmer 2006b). Microbial consumers (e.g., fungi) and invertebrate detritivores might respond positively or negatively (or not at all) to litter mixtures as mixed-resources offer a suite of complementary nutrients, or a mixture of inhibitory compounds (Blair et al. 1990). Such mechanisms are hard to distinguish from that of consumers responding to a single, overwhelmingly labile or refractory species in the mixture. Kominoski et al. (2007) report some of the strongest effects of mixing on both breakdown and microbial biomass for mixtures containing *Liriodendron tulipifera*, a very labile species, and *Rhododendron maximum*, a very refractory species. Labile species might induce faster growth of fungi and/or higher abundances of invertebrates, resulting in higher microbial biomass and invertebrate abundance on adjacent, less labile species in the mixture, accelerating loss rate (Kaneko and Salamanca 1999). The opposite might occur when especially refractory species (e.g., oaks) lead to a reduction in microbial biomass and/or invertebrate abundance, reducing the capacity of these two groups to degrade labile litter. For example, McArthur et al. (1994) suggested the presence of *Quercus nigra* reduced mass loss of more labile *Liquidambar styraciflua* in floodplain soils. Nilsson et al. (1999) concluded that evergreen litter high in secondary compounds reduced mass loss of moss and other evergreen species, presumably by reducing microbial activity. Swan and Palmer (2006b) reported that inhibition of mixing litter on breakdown was mediated by preferential feeding by an aquatic detritivore, *Caecidotea communis*, as it avoided *Platanus occidentalis* in mixtures. In the present study, we found that mixtures reflecting ambient proportions of litter species in mixtures amplified effects of species composition, resulting in the observed effects of diversity on organic matter processing more often than traditional experimental approaches reveal.

Complex interactions between invertebrate detritivores and microbial decomposers may have developed over the course of the study. Swan and Palmer (2006a) showed that the presence of the leaf-chewing stonefly *Tallaperla maria* induced nonadditive mass loss in litter mixtures that were not revealed in absence of the consumer, suggesting the interaction between microbial and invertebrate decomposers is important to how litter diversity alters decay. Consumers might change microbial degradative ability, reducing it via direct consumption of microbial biomass or stimulating it by keeping microbial populations in log-phase growth (Ribblett et al. 2005). These interactions might become amplified as mixtures of leaf species offer a range in resource qualities. The disproportionate availability of leaf

species in combination with variation in quality could explain why uneven mixtures resulted in more instances of nonadditive decay.

This study supports previous work demonstrating that tree species loss can result in altered breakdown dynamics via changes in leaf litter species availability (Gartner and Cardon 2004, Swan and Palmer 2004, Hättenschwiler et al. 2005, Kominoski et al. 2007, Lecerf et al. 2007). While effects of richness per se are rare, the role of the relative availability of species (e.g., evenness) was more important in this study and critical to understanding the full scope of potential species loss and relative species composition in our system. This might have substantial implications for situations when the dominant tree species in a stand might be at risk of extinction. For example, in many north-facing slopes in the northeastern United States, the eastern hemlock is in decline due to uncontrolled infestation by the hemlock woolly adelgid. It is predicted that this dominant, foundation species (Ellison et al. 2005) will be replaced by deciduous tree species (Orwig et al. 2002). Such predicted shifts in species dominance might, given our results, suggest a different role litter diversity plays in organic matter dynamics. We suggest that how we view species loss in systems relying on leaf litter inputs warrants further exploration. For example, how does tree species richness and evenness vary across the landscape? What will the outcome of species loss be if the dominant is a refractory species vs. a labile species? Do we need to re-evaluate the relative importance of microbial and invertebrate decomposers in mediating nonadditive breakdown dynamics (e.g., Hättenschwiler and Gasser 2005) if these organisms are faced with different proportions of litter species in mixtures? Results from this work indicate that the role of leaf diversity on litter breakdown might be underestimated.

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