

Impacts of tree species diversity on litter decomposition in northern temperate forests of Wisconsin, USA: a multi-site experiment along a latitudinal gradient

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Abstract Over the past decade an increasing amount of research has sought to understand how the diversity of species in an ecosystem can influence fluxes of biologically important materials, such as the decomposition of organic matter and recycling of nutrients. Generalities among studies have remained elusive, perhaps because experimental manipulations have been performed at relatively small spatial scales where site-specific variation generates patterns that appear idiosyncratic. One approach for seeking generality is to perform parallel experiments at different sites using an identical species pool. Here we report results from a study where we manipulated the diversity of leaf litter from the same six dominant tree species in the litter layer of three forested ecosystems. These ecosystems spanned a 300 km latitudinal transect in Wisconsin, USA, and were characterized by a large gradient in temperature

and moisture, and thus, rates of decomposition. After allowing combinations of one, two, four, and six species of leaf litter to decompose for 1 year, we found that increasing leaf litter richness led to slower rates of decomposition and higher fractions of nitrogen lost from litter. Across all sites, climate and initial litter chemistry explained more of the variation in decomposition rates than did litter richness. Effects of leaf litter diversity were non-additive, meaning they were greater than expected from the impacts of individual species, and appeared to be strongly influenced by the presence/absence of just 1–2 species (*Tilia americana* and *Acer saccharum*). The rate of decomposition of these two species was highly site-specific, which led to strong negative effects of litter richness only being observed at the southernmost sites where *T. americana* and *A. saccharum* decomposed more quickly. In contrast, litter diversity increased nitrogen loss at the northernmost sites where decomposition of *T. americana* was notably slowed. Our study shows that species diversity affected at least one of the two litter processes at each site along this 300-km gradient, but the exact nature of these effects were spatially variable because the performance of individual species changed across the heterogeneous landscape.

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Introduction

Over the past century, a variety of human activities have accelerated rates of species extinction to 2–3 orders of magnitude higher than ambient levels recorded in the fossil record (Tilman et al. 1994; Pimm et al. 1995; Ricciardi and Rasmussen 1999; Sala et al. 2000; Jablonski 2004). While the causes of modern extinction are relatively well understood (Vitousek et al. 1997; Rozenwieg 2001; Sax and Gaines 2003; Thomas et al. 2004), the community and ecosystem-level consequences of extinction have only recently become a major research agenda in ecology (Tilman 1999; Naeem 2002; Hooper et al. 2005; Srivastava and Vellend 2005). Since the 1990s, a body of empirical work has coalesced to formalize the hypothesis that loss of diversity can alter the rates of fundamental ecosystem processes such as primary production, nutrient cycling, and decomposition of organic matter (Chapin et al. 1998; Schlapfer and Schmid 1999; Hooper et al. 2005). But even while this hypothesis has been tested by a growing number of studies, the generality of empirical results is a currently topic of considerable debate (Huston 1997; Schwartz et al. 2000; Wardle et al. 2000; Srivastava and Vellend 2005).

Part of the current debate stems from the fact that most empirical studies have been performed at relatively limited spatial and temporal scales (Bengtsson et al. 2002; Cardinale et al. 2004). Indeed, the bulk of experimental manipulations of diversity have been performed in small plots or micro/mesocosms that have been intentionally homogenized in space and time to minimize experimental ‘noise’ (Cardinale et al. 2000, 2004; Loreau et al. 2001; Schmid et al. 2001; Symstad and Tilman 2001; Symstad et al. 2003; Hooper et al. 2005). Yet, it is the inherent heterogeneity of ecosystems in both space and time that is typically invoked to explain why biodiversity exists in the first place (Hanski 1989; Caswell and Cohen 1991; Hastings 1991; Kolasa and Pickett 1991; Tilman and Kareiva 1997; Chesson 2000; Amarasekare 2003). Not surprisingly, a growing body of theory suggests that loss of diversity can have variable impacts on ecosystem processes from one location to the next (Cardinale et al. 2000, 2004; Mouquet et al. 2002; Loreau

et al. 2003), and identifying why these impacts change across spatially heterogeneous landscapes has become one of the major challenges in this field.

Within the field of biodiversity–ecosystem functioning research, the majority of attention has been given to how aboveground plant diversity impacts biomass production and nutrient stocks—especially in temperate grasslands (see Hooper et al. 2005 for a review). Much less work has tested the hypothesis that plant diversity can influence the decomposition of dead organic matter in spite of the fact that an estimated 90% of above ground NPP eventually enters the detrital food chain (Coleman and Crossley 1996). One of the mechanisms through which aboveground diversity can influence belowground functioning is through the diversity of leaf litter inputs (Wardle et al. 2004). Of the studies that have focused on the impacts of plant diversity on leaf litter decomposition, results have proven to be equivocal. A number of studies have found that litter mixtures decompose in a manner that is disproportionate to the rates expected from single species (Blair et al. 1990; Fyles and Fyles 1993; McTiernan et al. 1997; Salamanca et al. 1998; Hector et al. 2000), but the direction of these effects, and the role that diversity itself plays in mediating non-additive rates of decomposition rates is unclear (Wardle and Nicholson 1996; Wardle et al. 1997). Gartner and Cardon (2004) recently summarized the existing experimental evidence and concluded that leaf litter decomposition rates may increase, decrease, or remain constant as the number of species contributing litter to an organic pool increases. Is this variability due to inherent differences in the species pools or types of ecosystems that have been studied? Or does the effect of diversity depend on the specific environmental context of the study (Hattenschwiler et al. 2005)? These are questions that must be addressed if we are to have any hope of predicting how plant diversity might impact fundamental ecosystem processes such as decomposition and the recycling of biologically important nutrients.

One approach that can help us better understand the spatially-explicit impacts of biodiversity is to perform identical manipulations using the

same species pool at multiple sites spanning a defined environmental gradient. Here we report the results of an experiment that used a standard assay to compare how the diversity of leaf litter from six common tree species influenced rates of decomposition in three deciduous forest sites, which spanned a distinct gradient in soil moisture and temperature (two variables known to regulate decomposition). We addressed two questions: (1) Does leaf species richness have any general effect on leaf litter processes—i.e. a main effect on decomposition and/or N-loss data for data pooled among all sites, and (2) Is the effect of litter richness on litter decomposition consistent among sites? We show there are main effects of leaf litter richness on decomposition and N-loss, but these are driven by a subset of just 1 or 2 species that exhibit considerable site-specific variation. Our study is valuable because it shows that the nature of biodiversity effects depends on how the performance of individual species changes across a heterogeneous landscape.

Methods

Study sites

The study was performed at three sites spanning a 300 km north–south transect in central Wisconsin (Fig. 1), which differ in several physical and chemical features thought to influence rates of leaf decomposition. The most southern site was

located in the University of Wisconsin Arboretum (ARB), a 510 ha restored agricultural area that contains 75 year old mixed, deciduous, and pine forests as well as prairies and savannahs. The mid-latitude site was located in Keller Whitcomb Creek Woods State Natural Area (KW), a 40 ha site that includes a range of northern dry-mesic to mesic mixed forests. The most northern site was located in Germain Hemlocks State Natural Area (GH), a 36 ha site primarily consisting of old-growth northern mesic forest. Air and soil temperatures at these sites are inversely related to latitude, which also leads to a large gradient in snowfall and the number of days of snow cover (Table 1). Therefore, these sites represent an axis of heterogeneity in at least two abiotic variables known to exert strong control over decomposition.

Decomposition assay

We used a standardized assay to examine how mixtures of leaf litter from six tree species influenced rates of leaf litter decomposition at each of the three study sites: trembling aspen (*Populus tremuloides*), American basswood (*Tilia americana*), red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), red pine (*Pinus resinosa*), and eastern white pine (*Pinus strobus*). All of these species are common throughout Wisconsin, and are dominant components of the woody flora at each study site. In the fall of 2003 we collected senesced litter from three different forested locations near Madison, WI. These collections

Fig. 1 Field site locations in central Wisconsin. The University of Wisconsin Arboretum (ARB), Keller Whitcomb Creek Woods State Natural Area (KW) and Germain Hemlocks State Natural Area (GH). See text for site descriptions. Rates of loss of organic matter in standardized litterbags containing leaf litter from 1, 2, 4, and 6 species of trees were monitored over the course of 1 year in three blocks at each site (see inset)

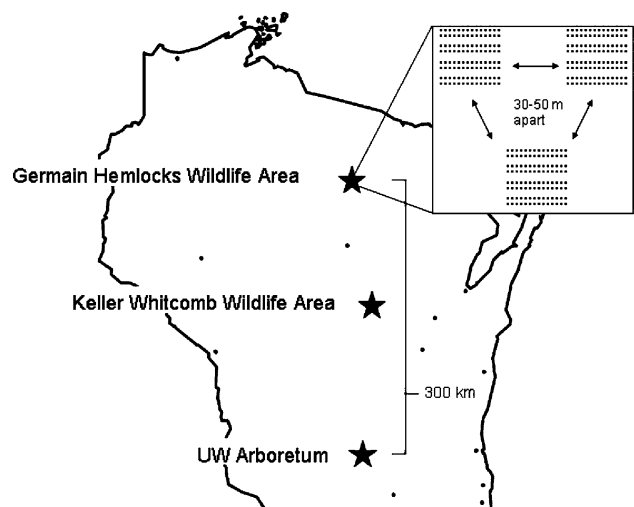


Table 1 Summary characteristics of the three study sites

Variable	ARB	KW	GH
Latitude	43°02'28" N	44°35'23" N	45°43'35" N
Longitude	89°25'16" W	89°03'51" W	89°36'28" W
Precipitation (in)	39.38	31.52	32.03
Snowfall (in)	29.60	45.20	149.00
Duration of snow cover (d)	48	70	134
Mean daily temp. (°C)	8.33	6.78	3.94
Mean daily max. temp. (°C)	13.61	12.67	10.61
Mean daily min. temp. (°C)	3.06	0.83	-2.72
Soil mean daily temp. (°C)	9.21	8.52	7.12
Soil mean daily max. temp. (°C)	10.19	9.79	7.99
Soil mean daily min. temp. (°C)	8.24	7.17	6.19
Mean daily potential evaporation (mm)	20.12	18.51	17.62
Soil pH	5.54 ± 0.04	4.03 ± 0.09	4.44 ± 0.12
SOM (%)	5.04 ± 0.32	3.89 ± 0.39	7.20 ± 0.51
Soil type	Fine silty over sandy or sandy skeletal, mixed, superactive, mesic Mollic Hapludalf	Coarse-loamy, mixed, superactive, frigid Haplic Glossudalf	Coarse-loamy, mixed, superactive, frigid Alfic Haplorthod

Precipitation, air temperature data, and potential evaporation data were obtained from the Midwestern Regional Climate Center, which has continuous monitoring weather stations located ~10 km from each field site. Data on soil temperatures were collected by burying HOBO temperature dataloggers in each block within a site. pH and soil organic matter (SOM, loss on ignition) were measured in 10 cm soil cores collected at three points within each block at the end of the study

were kept separate and used to make three replicates of each litter combination, each replicate was placed in one of three spatial 'blocks' within a site (see below). Litter was air dried at 25°C and 30% RH for 7 days. A subsample of each collection was freeze-dried to determine the moisture content of air-dried litter. Richness in the leaf packs was manipulated using a replacement-series design where initial mass was held constant (3.5 g of air-dried litter) in 15 cm × 15 cm fiberglass mesh bags (1 mm² pore size) containing all combinations of one, two, four, or six tree species (37 litter combinations in all). Care was taken to ensure that all litterbags received equal portions of constituting litter (e.g., the six species litterbags contained 0.58 g of each species).

In December 2003, three leaf packs of each litter combination were pinned to the soil surface at randomly selected locations in each of three blocks per site (999 litter bags total). The three blocks at a site were separated by 30–50 m to encompass a range of soil conditions, and to include the understory of as many of the focal tree species as a possible (Fig. 1). One randomly selected bag from each block was sampled on days 132, 236, and 338 of the study. Litterbags were freeze-dried and the

contents were sorted to separate grass and dirt from treatment litter. We were unable to identify decomposed litter to species level and weighed the total weight of all freeze-dried, decomposed litter. Mass loss values were calculated by weighing freeze-dried litter and comparing to initial dry mass. Initial dry mass values were calculated using initial air-dried weights and moisture correction factors for each species (the moisture content of air dried litter varied from 3% to 10%). Leaf litter was ground in a ball mill for analysis of total carbon (C) and nitrogen (N) on a Carlo Erba CNS analyzer. Final C and N concentrations were compared with initial C and N values to calculate the percentage change in C and N over the course of the experiment. Initial leaf litters were also analyzed for lignin content using an ANKOM A200 Fiber Analyzer.

Data analyses

Rates of leaf decomposition were calculated by fitting the mass of the three packs collected at a site through time to the function $y = e^{-kt}$, where y is the proportion of the initial mass remaining at time t , and k gives the decomposition rate as a proportion of mass loss per unit time. The single

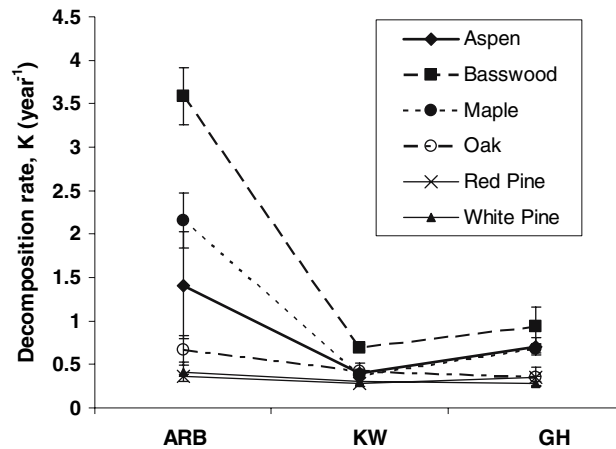


Fig. 2 Individual litter species decomposition rates across the three sites (ARB–UW Arboretum, KW–Keller Whitcomb, GH–Germain Hemlocks). ANOVA results

on decomposition: Site $F_{2,36} = 45.33$, $P < 0.001$, Species $F_{5,36} = 22.39$, $P < 0.001$, Site*Species $F_{10,36} = 9.67$, $P < 0.001$

exponential function we used to calculate k explained an average 92% of the variation in mass loss over time across all litter treatments. To test the hypothesis that effects of richness on decomposition rate differed among sites, we used ANOVA to model k as a function of leaf species richness, site location, and the richness \times site interaction. To account for the heterogeneity of variances that results from there being fewer species combinations at higher levels of richness, observations were weighted to be inversely proportional to the variance of each level of diversity within a site.

To determine whether rates of decomposition were additive or non-additive in leaf packs containing two or more species, we calculated the deviation in decomposition rate for each leaf pack from the value expected based on the mean of each single species treatment

$$D_k = (k_{\text{obs}} - k_{\text{exp}})/k_{\text{exp}}$$

$$k_{\text{exp}} = \frac{1}{N} \sum_{i=1}^N k_i$$

where k_{obs} is the measured rate of decomposition in a pack of N species, and k_i is the decomposition rate of the constituent single species packs (see Loreau 1998). To assess which of the six species contributed to non-additive decomposition rates,

we used ANOVA to model D_k as a function of the presence/absence of each species in a leaf pack. The percentage change in N content was analyzed in an identical manner to that described for k .

Within each site, we used simple regressions to test for relationships between decomposition and N loss with litter chemistry and leaf pack diversity. Across all sites we combined weather data with leaf chemistry data in multiple regression models to explain variation in decomposition rates, similar to Meentemeyer (1978).

Results

Leaf litter decomposition rates of the six tree species varied widely within and among study sites (Fig. 2). Variation in decomposition rates among single species packs was most notable at the southernmost site (ARB) where basswood and maple leaves had the highest mean rates of mass loss, followed by aspen and oak. Basswood had the lowest C:N ratio and lignin content and fastest decomposition rates, while white pine was the poorest quality litter and decomposed most slowly (Figs. 2, 3). The more labile species (basswood, maple, and to a lesser extent aspen) all exhibited large declines in rates of decomposition across the latitudinal gradient, each having substantially reduced k -values at the two most northerly sites (Fig. 2). Across all sites, variation

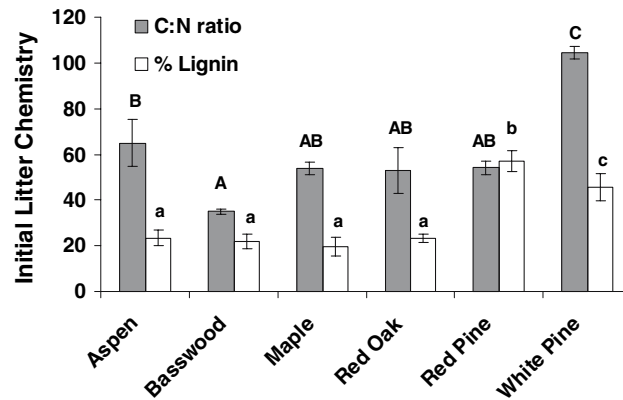


Fig. 3 Initial litter C:N and lignin (%) of individual litters. Different letters indicated significant differences within chemistry indices, Tukey $\alpha = 0.05$. Each bar represents the

average of three pooled (\pm S.E.M.) samples collected from three distinct forest stands

in single species decomposition rates was negatively related to initial litter lignin content ($k = 1.47 - 0.02 * \text{Initial \% lignin}$, Fig. 4) and initial C:N ($k = 1.64 - 0.01 * \text{Initial C:N}$, Fig. 4).

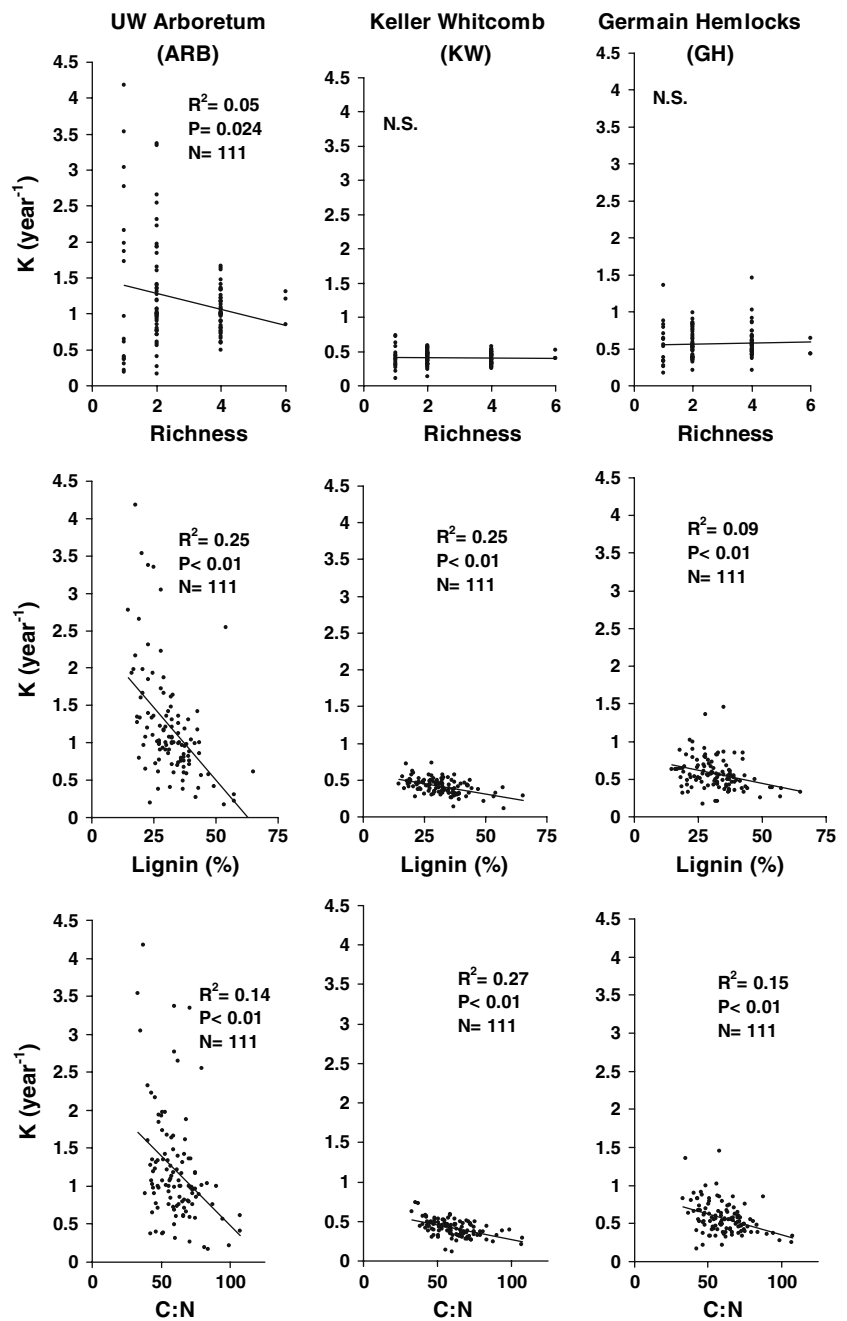
When averaged across all sites, k -values characterizing the proportion of mass loss per year decreased as richness increased from one to six species ($k = 0.83 - 0.04 * \text{richness}$, Table 2). There was, however, a significant richness \times site interaction (Table 2) indicating that the effect of leaf species richness on decomposition rates was heterogeneous among sites (Fig. 4). While leaf species richness had no impact on k -values at the two most northerly sites, decomposition rates at the most southerly site (ARB) decreased as leaf richness increased from one to six species (Fig. 4). The negative relationship between leaf litter richness and decomposition appeared to be influenced by the presence/absence of just two species. Results indicate that when either basswood or maple were present in the leaf packs, values of D_k were significantly more negative than when each species was absent (Table 3). Thus, rates of decomposition of leaf packs containing basswood or maple were disproportionately slow compared to decomposition of each species when alone (Fig. 5).

Leaf litter richness also had site-specific effects on the percentage of initial nitrogen remaining in leaf packs at the end of the experiment (Fig. 6). Across all sites, the percentage of initial N remaining decreased as diversity increased ($\% \text{ initial N remaining} = 10.73 - 2.01 * \text{richness}$,

Table 2). This was primarily due to a strong trend at the most northern site where the amount of initial N remaining decreased as richness increased from one to six litter species (Fig. 6). A similar negative relationship between richness and N content was noted at the mid-latitude site, however litterbags at the KW site experienced a net increase in N relative to initial values (Fig. 6). Analyses of $D_{\%N}$, which characterize the deviations in N content from that expected based on single species leaf packs, indicate that only the presence or absence of basswood in a leaf pack influenced the change in N content over the course of the experiment. Treatments that contained basswood retained less N than would be expected in mixtures compared to those without basswood (0.56 ± 0.35 and -2.01 ± 1.28 , respectively, Table 3). In contrast to the northern two sites, there was no effect of richness on the N content of leaf litter placed at the southernmost site (Fig. 6).

More often than not, initial litter chemistry explained much more of the variation in decomposition rates and initial N remaining than did litter diversity at each site (Figs. 4 and 6). Diversity and lignin were both weakly related to litter N remaining at the northern two sites where decomposition was slowest (Fig. 6). Meentemeyer (1978) correlated variation in regional decomposition rates using actual evapotranspiration and initial lignin concentrations. We used potential evaporation, initial lignin content, and litter richness in a mixed-model multiple regression to explain variation in

Fig. 4 Decomposition rates as a function of leaf litter richness, initial lignin content, and initial litter C:N ratio at each of the three study sites (left to right = south to north, see Fig. 1)



decomposition rates. The potential evaporation at each site explained most of the variation in decomposition rates (partial $r^2 = 0.27$, $P < 0.001$), followed by lignin (partial $r^2 = 0.08$, $P < 0.001$), and then richness (partial $r^2 = 0.01$, $P = 0.05$) with the whole model explaining roughly 36% of the variation in decomposition rates across all sites (total $R^2 = 0.36$, $P < 0.001$).

Discussion

In this study, we used a standard assay to manipulate the richness of leaf litter from six common tree species at three study sites spanning an environmental gradient. These three sites differed in overall rates of decomposition, most likely due to variation in several abiotic variables

Table 2 Litter treatment effects on decomposition and nitrogen flux

	Site	Litter richness	Site*Richness	Litter mix ID	Site*Mix ID
D.F.	2,327	1,327	2,327	30,176	60,176
Decomposition rate, <i>k</i>	149.06	4.29	4.43	2.99	1.60
	<i><0.001</i>	<i>0.039</i>	<i>0.013</i>	<i><0.001</i>	<i>0.010</i>
% Initial N remaining	255.52	3.96	1.04	5.78	2.20
	<i><0.001</i>	<i>0.047</i>	<i>0.354</i>	<i><0.001</i>	<i><0.001</i>

Results are given from a weighted ANOVA of site, mix identity, and species richness on decomposition and nutrient changes in leaf litter. D.F. indicates numerator and denominator degrees of freedom. *F* values are reported with *P* values directly below in italics

Table 3 Presence/absence of individual species effects on D_k and $D_{\%N}$

<i>K</i> -value	D.F.	D_k affected by spp. presence/absence
Site	2,258	2.59 <i>0.076</i>
Basswood	1,258	22.22 <i><0.001</i>
Site*Basswood	2,258	10.08 <i><0.001</i>
Maple	1,258	3.29 <i>0.071</i>
Site*Maple	2,258	5.76 <i>0.004</i>
% Initial N Remaining	$D_{\%N}$ affected by spp. presence/absence	
Basswood	1,248	4.1323 <i>0.0431</i>

No other significant effects.

Results from an ANOVA testing the influence of presence or absence of individual species on D_k and $D_{\%N}$ lost (the proportional deviation observed from the expected). D.F. indicates numerator and denominator degrees of freedom. *F* values are reported in the right column with *P* values directly below in italics

that are well-known to regulate rates of decomposition (namely temperature and moisture). In spite of manipulating the same species pool, we found that the effect of leaf litter richness on rates of decomposition and nitrogen loss from litter were qualitatively different among sites. Litter richness only impacted rates of organic matter loss at the most southern sites, which also had the fastest rates of overall decomposition. In contrast, leaf litter diversity only affected the loss of nitrogen from leaf packs at the more the northerly sites, which had the slowest rates of decomposition. These findings are consistent with a hypothesis that has received growing support,

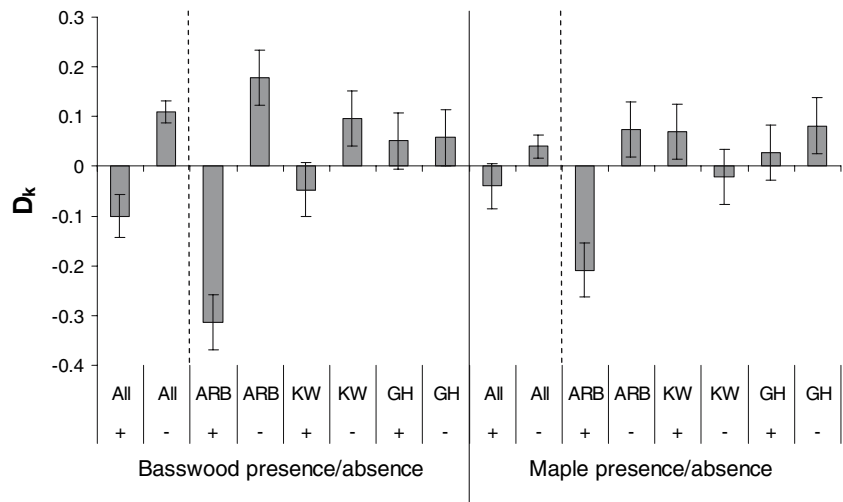
which is that the effects of biodiversity on ecosystem processes are likely to be spatially dynamic as the performance of individual species changes across a heterogeneous landscape (Hector et al. 1999; Bengtsson et al. 2002; Loreau et al. 2003; Symstad et al. 2003; Cardinale et al. 2004). Determining why diversity impacts processes in one site but not another now ranks among the primary challenges in this field.

Hypothesized mechanisms

It is well known that climate and litter chemistry influence litter decomposition (Swift et al. 1979); climate controls a large portion of regional variation in decomposition rates, while litter chemistry can explain much of the local variation. We were more interested in the effects of plant diversity on leaf litter decomposition, and used chemistry indices to help explain observed patterns, as well as to provide a reference with which to compare the effects of leaf litter richness. Overall, the relative importance of climate, litter chemistry, and richness on litter decomposition was: climate \gg litter chemistry $>$ litter richness.

In our study, we expected *k*-values to be independent of litter richness only if the rates of leaf decomposition in mixed species packs were predictable from the rates of single species leaf packs (i.e. additive). This is because the replacement-series design used in our experiment held total initial leaf mass constant across all levels of richness such that mass loss of a mixed species pack should be proportional to the average of the single-species packs under assumptions of additivity. Given this, any significant effects of leaf species richness on decomposition rate and N loss indicate

Fig. 5 D_k for decomposition rates when basswood and maple were present and absent. Error bars show standard errors. Across all sites, each (+) bar represent 144 samples, each (-) bar represents 135 samples. Within each site, each (+) bar represent 48 samples, each (-) bar represents 45 samples. See Table 3 for ANOVA results



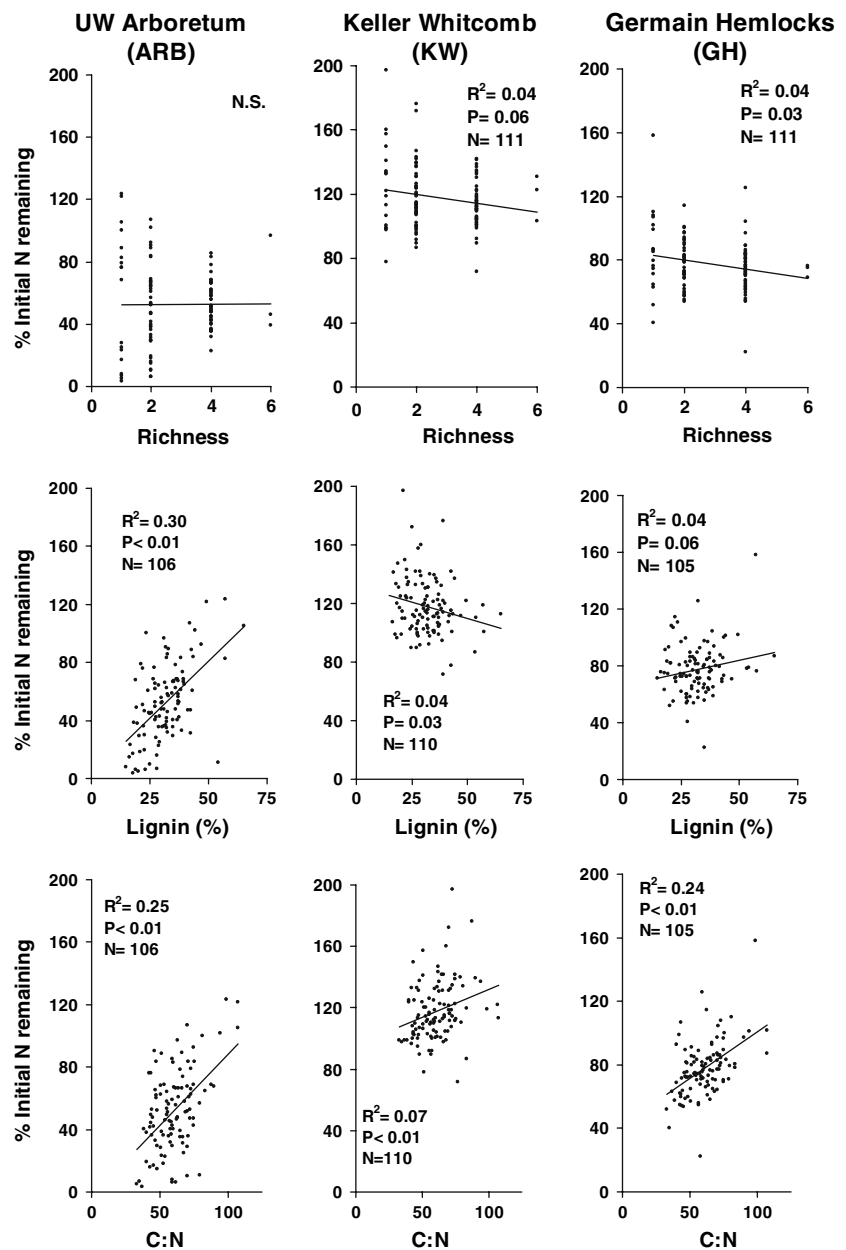
that litter decomposed more slowly, and lost more nitrogen than one would expect from single species packs. Supplemental analyses indicated that the presence or absence of basswood (and possibly maple) in a leaf pack was the best indicator of the response variables, and whenever basswood was present in a leaf pack, decomposition was slower and N loss faster than expected. This is conceptually analogous with what has been called the ‘sampling’ or ‘selection-probability’ effect of diversity where the traits of a single species can explain the average difference between species mono- and polycultures (Huston 1997; Loreau 2000). The fact that the results of our study can largely be explained by the presence/absence of one species raises two key questions: (1) Why did basswood generate non-additive effects on rates of decomposition and N-loss, and (2) why did the strength non-additivity for both decomposition and N-loss change among sites?

We can think of two non-mutually exclusive hypotheses that might help to explain non-additive rates of decomposition. First, basswood may have retarded the rates of decomposition of other leaf species when together. If this effect was proportional to decomposition rates, then it would have been more pronounced at the southerly site where decomposition of basswood and all other species was the fastest. We do not have the data to support or refute this hypothesis, but we think it is an unlikely explanation of the reported patterns. Of all the leaf litter species studied, basswood had the

lowest lignin content and the lowest C:N ratio. When coupled with the fact that it had the highest rates of decomposition of any single-species pack, this would argue that basswood is a high ‘quality’ resource for decomposers. There is little reason to expect that a high quality resource would retard the consumption of other resources...in fact, several studies have shown the opposite is true where nutrient rich leaf species facilitate the decomposition of lower quality resources (Fyles and Fyles 1993; Briones and Ineson 1996; McTiernan et al. 1997; Salamanca et al. 1998). This leads us to consider an alternative explanation, which is that recalcitrant litters tended to slow the decomposition of labile species. Recalcitrant litters may leach secondary metabolites and phenolics into surrounding litters and, in turn, retard decomposition rates (Fyles and Fyles 1993; Hoorens et al. 2002). It seems reasonable to expect that any form of inhibition, such as leaching of polyphenolics, would be proportional to decomposition rate, and therefore, be more pronounced at southerly sites where decomposition was comparably fast. In contrast, where rates of decomposition were slow (i.e. at northerly sites), antagonisms that generate non-additivity could be rendered insignificant or undetectable.

In comparison to decomposition, the effects of litter diversity on nitrogen dynamics has received little attention and, perhaps as a result, is more difficult to predict (Hobbie 2005). As observed in our study and elsewhere, N dynamics typically do not typically follow mass loss responses in mixed

Fig. 6 Percent change in N as a function of leaf litter richness, initial lignin content, and initial litter C:N ratio at each at the three study sites (left to right = south to north, see Fig. 1)



litter experiments (Gartner and Carton 2004). For example, similar to our findings at the northernmost site, Blair et al. (1990) found a negative relationship between leaf pack diversity and initial nitrogen remaining despite there being no relationship between diversity and decomposition in a temperate forest system. Our analysis of $D_{\%N}$ shows that the presence of basswood in a litter pack decreased the amount of initial N remaining (i.e. increased the amount of initial N lost),

suggesting that diversity effects may have resulted from high quality litter facilitating N loss from leaf mixtures (McTiernan et al. 1997).

Multiple mechanisms could account for the observed diversity effects on nitrogen loss at the northern sites and the absence of such an effect at the southern site. First, because decomposition rates at the ARB were at least twice that of the two northern sites, far greater mass loss could have masked any effect of diversity on nitrogen

fluxes. Alternatively, slow decomposition rates at northern latitudes may have increased soil carbon stores disproportionate relative to nitrogen stores causing soil microbes, fungi and arthropods to use nitrogen more completely. These possibilities are speculative, and since we do not have the data to test them direct, they warrant attention in future studies. It is, however, worth noting that both the mass loss and N patterns we observed follow predictions made by Hoorens et al. (2003), who argued that recalcitrant litters retard decomposition of labile species, while litters with high N content facilitate N loss.

It is well known that microbes influence decomposition and underground processes (Swift et al. 1979). Increased microbial diversity is likely to increase rates of leaf litter decomposition through functional niche complementarity (Loreau 2001). Litter from different species may be differentially processed by specific groups of fungi or bacteria. In addition, different soil communities develop under different aboveground communities, producing feedbacks that vary across environments (Wardle et al. 2004). We did not directly measure or experimentally control the microbial communities at our three sites. Therefore, the independent and interactive effects of leaf litter diversity and site on decomposition rates may also be due to unknown differences in microbial populations along a latitudinal gradient.

Implications

There are two potentially important implications of our study. The first is that biodiversity can have a qualitatively different impact on a given process at different sites. This is not to say there are no general effects of biodiversity on ecosystem functioning. In fact, to the contrary, we found that when data were pooled together and site-specific information ignored, there was a main effect of species diversity on both litter decomposition and N-loss. But obviously, the statistical interaction between species richness and study location indicates that key information is lost when one pools data across coarse scales of resolution and ignores site-specific differences. Our findings are comparable to, and compliment those of other projects that have manipulated plant diversity at multiple sites and found there are, in

fact, general effects on the production of above-ground plant biomass, but that these effects vary in form and magnitude among sites (e.g., Hector et al. 1999). However, an important advance of our work is that we performed parallel experiments using an identical pool of species common to all sites. Thus, our results further serve to highlight a growing sentiment among ecologists that for any single group of organisms, there may be general implications of species loss at larger scales of observation even though those effects are highly variable among sites (Cardinale et al. 2000; Bengtsson et al. 2002; Loreau et al. 2003). Not only does this emphasize the caution required in extrapolating the conclusions of experiments performed at single locations, it highlights the importance of multi-site comparative research.

A second implication of our study is that species diversity may influence different processes in different ways. Indeed, we found that decomposition and nitrogen loss responded to manipulations of diversity in exactly the opposite manner. During the first round of biodiversity–ecosystem function studies, a large number of projects have focused on response variables in isolation (primary production, decomposition, or N-cycling). This focus has served to muddle an obvious, but important point that species affect numerous processes simultaneously, and there is no empirical or theoretical reason to believe that different ecological processes should all respond to diversity in a qualitatively similar way. Had we studied just one dependent variable, we would have concluded that diversity has no impact on ecosystem functioning at some portion of the study sites. But by studying decomposition and N-loss in combination, we are left to conclude that diversity had some impact on litter processing across all sites.

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References

- Amarasekare P (2003) Competitive coexistence in spatially structured environments: a synthesis. *Ecol Lett* 6:1109–1122

- Bengtsson J, Engelhardt KAM, Giller PS, Hobbie SE, Lawrence D, Levine JM, Vila M, Wolters V (2002) Slippin' and slidin' between the scales: the scaling components of biodiversity–ecosystem functioning relations. In: Loreau MA, Inchausti P, Naeem S (eds) *Biodiversity and ecosystem functioning: synthesis and perspectives*, Oxford University Press, pp 209–220
- Blair JM, Parmelee RW, Beare MH (1990) Decay rates, nitrogen fluxes, and decomposer communities of single- and mixed-species foliar litter. *Ecology* 71:1976–1985
- Briones MJI, Ineson P (1996) Decomposition of Eucalyptus leaves in litter mixtures. *Soil Biol Biochem* 28:1381–1388
- Cardinale BJ, Ives AR, Inchausti P (2004) Effects of species diversity on the primary productivity of ecosystems: extending our spatial and temporal scales of inference. *Oikos* 104:437–450
- Cardinale BJ, Nelson K, Palmer MA (2000) Linking species diversity to the functioning of ecosystems: on the importance of environmental context. *Oikos* 91:175–183
- Caswell H, Cohen JE (1991) Disturbance, interspecific interaction and diversity in meta-populations. *Biol J Linn Soc* 42:193–218
- Chapin FS, Sala OE, Burke IC, Grime JP, Hooper DU, Lauenroth WK, Lombard A, Mooney HA, Mosier AR, Naeem S, Pacala SW, Roy J, Steffen WL, Tilman D (1998) Ecosystem consequences of changing biodiversity. *Bioscience* 48:45–52
- Chesson P (2000) General theory of competitive coexistence in spatially-varying environments. *Theor Popul Biol* 58:211–237
- Coleman DC, Crossley DA Jr (1996) *Fundamentals of soil ecology*. Academic Press, San Diego
- Fyles JH, Fyles IH (1993) Interaction of Douglas-fir with red alder and salal foliage litter during decomposition. *Can J For Res* 23:358–361
- Gartner TB, Cardon ZG (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos* 104:230–246
- Hanski I (1989) Metapopulation dynamics: does it help to have more of the same? *Trends Ecol Evol* 4:113–114
- Hastings A (1991) Structured models of metapopulation dynamics. *Biol J Linn Soc* 42:57–71
- Hattenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annu Rev Ecol Syst* 36:191–218
- Hector A, Beale AG, Minns A, Otway SJ, Lawton JH (2000) Consequences of the reduction of plant diversity for litter decomposition: effects through litter quality and microenvironment. *Oikos* 90:357–371
- Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-Danell K, Joshi J, Jumpponen A, Korner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinz A, Read DJ, Scherer-Lorenzen M, Schulze ED, Siamantziouras ASD, Spehn EM, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (1999) Plant diversity and productivity experiments in European grasslands. *Science* 286:1123–1127
- Hobbie SE (2005) Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. *Ecosystems* 8:644–656
- Hooper DU, Chapin FA, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setälä H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr* 75:3–35
- Hoorens B, Aerts R, Stroetenga M (2002) Litter quality and interactive effects in litter mixtures: more negative interactions under elevated CO₂? *J Ecol* 90:1009–1016
- Hoorens B, Aerts R, Stroetenga M (2003) Does initial litter chemistry explain litter mixture effects on decomposition? *Oecologia* 137:578–586
- Huston MA (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460
- Jablonski D (2004) Extinction: past and present. *Nature* 427:589–589
- Kolasa J, Pickett ST (1991) *Ecological heterogeneity*. Springer-Verlag, New York
- Loreau M (1998) Separating sampling and other effects in biodiversity experiments. *Oikos* 82:600–602
- Loreau M (2000) Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos* 91:3–17
- Loreau M (2001) Microbial diversity, producer–decomposer interactions and ecosystem processes: a theoretical model. *Proc R Soc Lond Ser B* 268:303–309
- Loreau M, Mouquet N, Gonzalez A (2003) Biodiversity as spatial insurance in heterogeneous landscapes. *Proc Natl Acad Sci* 100:12765–12770
- Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294:804–808
- McTiernan KB, Ineson P, Coward PA (1997) Respiration and nutrient release from tree leaf litter mixtures. *Oikos* 78:527–538
- Meentemeyer V (1978) Macroclimate and lignin control of litter decomposition rates. *Ecology* 59:465–472
- Mouquet N, Moore JL, Loreau M (2002) Plant species richness and community productivity: why the mechanism that promotes coexistence matters. *Ecol Lett* 5:56–65
- Naeem S (2002) Ecosystem consequences of biodiversity loss: the evolution of a paradigm. *Ecology* 83:1537–1552
- Pimm SL, Russell GJ, Gittleman JL, Brooks TM (1995) The future of biodiversity. *Science* 269:347–350
- Ricciardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. *Conserv Biol* 13:1220–1222
- Rosenzweig ML (2001) Loss of speciation rate will impoverish future diversity. *PNAS* 98:5404–5410
- Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson

- RB, Kinzig AP, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* 287:1770–1774
- Salamanca EF, Kaneko N, Natagiri S (1998) Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods. *Ecol Eng* 10:53–73
- Sax DF, Gaines SD (2003) Species diversity: from global decreases to local increases. *Trends Ecol Evol* 18:561–566
- Schlapfer F, Schmid B (1999) Ecosystem effects of biodiversity: a classification of hypotheses and exploration of empirical results. *Ecol Appl* 9:893–912
- Schmid B, Joshi J, Schlapfer F (2001) Empirical evidence for biodiversity–ecosystem functioning relationships. In: Kinzig AP, Pacala SW, Tilman D (eds) *Empirical progress and theoretical extensions. The functional consequences of biodiversity: monographs in population biology* 33. Princeton University Press, Princeton, NJ, pp 120–150
- Schwartz MW, Brigham CA, Hoeksema JD, Lyons KG, Mills MH, van Mantgem PJ (2000) Linking biodiversity to ecosystem function: implications for conservation ecology. *Oecologia* 122:297–305
- Srivastava DS, Vellend M (2005) Biodiversity–ecosystem function research: is it relevant to conservation? *Annu Rev Ecol Syst* 36:267–294
- Swift MJ, Heal OW, Anderson JM (1979) *Decomposition in terrestrial ecosystems*. University of California Press, Berkeley
- Symstad AJ, Tilman D (2001) Diversity loss, recruitment limitation, and ecosystem functioning: lessons learned from a removal experiment. *Oikos* 92:424–435
- Symstad AJ, Chapin FS, Wall DH, Gross KL, Huenneke LF, Mittelbach GG, Peters DPC, Tilman D (2003) Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. *Bioscience* 53:89–98
- Thomas CA, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. *Nature* 427:145–148
- Tilman D (1999) The ecological consequences of changes in biodiversity: a search for general principles. *Ecology* 80:1455–1474
- Tilman D, Kareiva P (1997) *Spatial ecology: the role of space in population dynamics and interspecific interactions*. Princeton University Press, Princeton
- Tilman D, May RM, Lehman CL, Nowak MA (1994) Habitat destruction and the extinction debt. *Nature* 371:65–66
- Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. *Science* 277:494–499
- Wardle DA, Nicholson KS (1996) Synergistic effects of grassland plant species on soil microbial biomass and activity: implications for ecosystem-level effects of enriched plant diversity. *Funct Ecol* 10:410–416
- Wardle DA, Bonner KI, Nicholson KS (1997) Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *Oikos* 79:247–258
- Wardle DA, Huston MA, Grime JP, Berendse F, Garnier E, Lauenroth WK, Setälä H, Wilson SD (2000) Biodiversity and ecosystem function: an issue in ecology. *Bull Ecol Soc Am* 81:235–239
- Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. *Science* 304:1629–1633