



# Tree species richness affects litter production and decomposition rates in a tropical biodiversity experiment

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We report data on leaf litter production and decomposition from a manipulative biodiversity experiment with trees in tropical Panama, which has been designed to explore the relationship between tree diversity and ecosystem functioning. A total of 24 plots (2025 m<sup>2</sup>) were established in 2001 using six native tree species, with 1-, 3-, and 6-species mixtures. We estimated litter production during the dry season 2005 with litter traps; decomposition was assessed with a litter bag approach during the following wet season.

Litter production during the course of the dry season was highly variable among the tree species. Tree diversity significantly affected litter production, and the majority of the intermediate diverse mixtures had higher litter yields than expected based on yields in monoculture. In contrast, high diverse mixtures did not show such overyielding in litter production. Litter decomposition rates were also highly species-specific, and were related to various measures of litter quality (C/N, lignin/N, fibre content). We found no overall effect of litter diversity if the entire litter mixtures were analyzed, i.e. mixing species resulted in pure additive effects and observed decomposition rates were not different from expected rates. However, the individual species changed their decomposition pattern depending on the diversity of the litter mixture, i.e. there were species-specific responses to mixing litter. The analysis of temporal C and N dynamics within litter mixtures gave only limited evidence for nutrient transfer among litters of different quality.

At this early stage of our tree diversity experiment, there are no coherent and general effects of tree species richness on both litter production and decomposition. Within the scope of the biodiversity-ecosystem functioning relationship, our results therefore highlight the process-specific effects diversity may have. Additionally, species-specific effects on ecosystem processes and their temporal dynamics are important, but such effects may change along the gradient of tree diversity.

A major research effort launched by ecologists in the last decade seeks an understanding of the relationship between species diversity and ecosystem functioning, i.e. the activities, processes, or properties of ecosystems that are influenced by their biota (Schulze and Mooney 1993, Kinzig et al. 2002, Loreau et al. 2002, Scherer-Lorenzen et al. 2005a). Scientifically, this field has largely been developed through the use of model systems, both mathematical and real ones. For very practical reasons, the experimental model systems were small in size, short-lived and even-aged, mainly herbaceous assemblages or microbial microcosms. The majority of those studies suggest that ecosystem function is

positively related to biodiversity and that it reaches an asymptote at half, or less, the background diversity level. In most cases, above-ground biomass accumulation or productivity was used as a response variable to changing levels of plant diversity, but several studies also examined litter decomposition, mineralisation and soil nutrient dynamics or soil diversity (recently over-viewed by Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006). Two major groups of underlying mechanisms have been identified. First, niche differentiation among coexisting species with divergent traits, which decreases interspecific competition, can raise average rates of productivity and nutrient retention as

diversity increases (“complementarity effect”). Second, the probability to include species with specific traits that exert strong effects on ecosystem processes increases along the gradient of diversity (“sampling/selection effect”, Loreau and Hector 2001).

Experiments in forest ecosystems have almost been absent, although forests (including plantations) cover over 30% of the Earth’s surface (Anonymous 2001), produce 65% of the annual carbon fixation (net primary production, Lieth 1975), and store more than 80% of the biomass carbon of the planet (Watson et al. 2000). In addition, they are facing even larger changes in their biological diversity than herbaceous vegetation (Anonymous 2000). It is thus a key ecological question to ask if tree diversity influences functioning of forest ecosystems.

An experimental manipulation of the producer-level in forests, i.e. trees, obviously is a difficult and long-lasting task and only recently attempts in this direction have been made (Scherer-Lorenzen et al. 2005b). The experiment by Ewel et al. in the tropics of Costa Rica (Ewel et al. 1991) has often been mentioned as the first manipulative diversity experiment showing diversity effects on biogeochemistry. However, this experiment was designed to explore the possibilities to develop sustainable agroecosystems for the humid tropics mimicking structural diversity of successional communities, and not to study the interaction of species and ecosystem functioning per se. Clear effects on soil chemistry were only detectable between maize monocultures and highly-diverse (>100 species) treatments consisting of herbaceous and woody plants. Positive mixture effects on biomass production and nutrient cycling have been reported from afforestation experiments in Costa Rica, for example, comparing monocultures with 4-species mixtures (Byard et al. 1996, Stanley and Montagnini 1999, Montagnini 2000). In contrast, mixture-experiments from forestry sciences are mostly done with one- and two-species assemblages of economically important species (Kelty 1992, Pretzsch 2005) and are thus of limited value to explore the biodiversity-ecosystem functioning relationship. With reforestation plots of one to eighteen species, the Sardinilla experiment where this study was carried-out, is part of a new generation of tree diversity experiments specifically designed to examine the relationship between ecosystem function and tree diversity (Scherer-Lorenzen et al. 2005b). This tropical plantation is part of a global network of forest diversity experiments with sites in Germany, Finland and Borneo where a variety of ecosystem processes and properties are monitored in response to changing levels of tree species richness.

Litter production and subsequent decomposition are key processes in carbon and nutrient cycling of forest ecosystems: the physical and chemical breakdown of organic matter during decomposition releases both

CO<sub>2</sub> and nutrients, the latter being again available for plant nutrition (Swift et al. 1979). The decomposition rate of leaf litter is influenced by its physico-chemical properties (e.g. content of lignin and other phenolic compounds, lignin/nitrogen ratios, C/N ratios, physical leaf toughness, physical barriers on the leaf surface), but also by the composition of soil organisms and microclimatic conditions (Swift et al. 1979, Coûteaux et al. 1995, Hättenschwiler and Vitousek 2000). Therefore, litter decomposition and the turnover of labile soil organic matter could be affected by plant species diversity due to differences among species in litter quantity and quality, in timing of litter inputs, but also due to different microclimatic conditions within a stand (Prescott 2002, Hättenschwiler 2005, Hättenschwiler et al. 2005). These differences may also result in changes of the abundance and composition of the soil fauna and microbial community (De Deyn and Van der Putten 2005). In three current reviews of litter decomposition studies across different vegetation types, Gartner and Cardon (2004), Hättenschwiler (2005), and Hättenschwiler et al. (2005) showed that synergistic diversity effects are known for roughly half of all litter mixtures studied, i.e. decomposition rates of litter mixtures are higher than expected based on decay rates of single-species litter. Purely additive effects are reported for a third of all cases, and even antagonistic effects can be observed in the remaining 20% of mixtures. Similarly, nitrogen dynamics in mixed litters are also often non-additive and idiosyncratic, with decreasing or increasing N mineralisation and/or immobilisation. As one of several potential underlying mechanisms for such synergistic effects, nutrient transfer from high-quality litter to low-quality, recalcitrant litter has been suggested (Seastadt 1984). The relationship between litter species richness and decomposition rates or nitrogen dynamics is thus difficult to predict with the identity of the species within a mixture and their functional traits possibly being more important than the number of species. From a biodiversity – ecosystem functioning perspective, a strong limitation of all these studies, however, is the use of mostly two-species litter mixtures and their corresponding single species. Additionally, species-specific differences in decomposition rates within mixtures have rarely been studied, although opposite diversity effects within the same litter mixture might be more common than usually believed (Hättenschwiler and Gasser 2005).

Here, we report results on leaf litter production and decomposition when native tree diversity is manipulated at the plot level within one site. Specifically, we were testing the following hypotheses:

1. Tree species of similar age differ in their temporal and total litter production due to species-specific differences in growth rate and biomass allocation.

2. Community litter production increases along the gradient of tree diversity due to increasing trait differences among species in mixtures, which may result in resource use complementarity and higher productivity.
3. Litter decomposition rates differ among tree species due to species-specific litter chemistry, with faster decomposition at low C/N and lignin/N ratios.
4. Increasing tree diversity results in enhanced litter decomposition rates because the occurrence of highly degradable litter favours the breakdown of more recalcitrant litter in mixtures.
5. Species-specific decomposition rates change in litter mixtures of varying composition and diversity because of differences in litter chemistry of the admixed species.
6. Within decomposing litters, N concentrations change over time, with nitrogen concentrations increasing in poor-quality litter while decreasing in high-quality litter.

To our knowledge, the study presented here is the first one carried out in the tropics that (1) directly assesses whether decomposition rates in litter mixtures can be predicted from single-species decay rates over a long gradient of tree diversity, that (2) uses loading ratios of the component species determined by the natural abundances in litterfall, that (3) focuses on temporal dynamics of both litter mass loss and measures of litter quality, and that (4) analyzes component litters in the mixtures separately and compares decay of each species in the mixture to the decay of each species in monoculture. All these different aspects have been put forward as research priorities in recent reviews of diversity effects on litter decomposition (Gartner and Cardon 2004, Hättenschwiler 2005, Hättenschwiler et al. 2005).

## Material and methods

### Experimental site

The experimental site is located in Sardinilla, Central Panama (9°19'30"N, 79°38'00"W), approximately 50 km north of Panama City. The elevation is around 70 m a.s.l., mean annual precipitation is 2350 mm, with 25–50 mm per month during the dry season (January – March), and 250 mm per month during the rainy season (May – November). Daily and seasonal temperatures are relatively constant throughout the year, with annual mean daily maximum temperatures of 33.1°C and annual mean daily minima of 21.7°C. The soil is a typic Tropadalf (Alfisols) with high clay content of about 65%, underlying geology belonging to the Gantuncillo formation is characterised by silt and

clay stones formed during the middle to upper Eocene (Potvin et al. 2004). The original forest, classified as semideciduous lowland forest, was logged in 1952/53. The site was used for agriculture during two years, and then converted into pasture by seeding grasses.

### Experimental design

The Sardinilla experiment is a permanent large-scale facility to study the links between biodiversity and ecosystem functioning. It consists of 12 ha of two fully established forest plantations with varying tree species richness (1, 3, 6 species and 6, 9, 18 species, respectively for the old and young plantations) and composition (Scherer-Lorenzen et al. 2005b). The results presented here come from the older plantation, set up in July 2001. A total of 24 plots were established using six native tree species representing a range of relative growth rates (RGR). These species are the fast-growing species *Luehea seemanii* (Ls, Tiliaceae) and *Cordia alliodora* (Ca, Boraginaceae), two moderately fast growing species *Anacardium excelsum* (Ac, Anacardiaceae) and *Hura crepitans* (Hc, Euphorbiaceae) and two slower growing species *Cedrela odorata* (Co, Meliaceae) and *Tabebuia rosea* (Tr, Bignoniaceae) (RGR on nearby Barro Colorado Island (BCI): 9.1% and 7.0%; 5.9% and 4.9%; 2.3% and 3.4%, S. Lao pers. comm.). The site includes (1) twelve monocultures (each of the six species replicated twice), of which only one replicate was used in this study (see below); (2) six plots of different three-species mixtures, each triplet containing one species of our three categories of growth rates, the three species were randomly drawn without replacement from the species pool with the constraint that all species occur three times; and (3) six replicates of the six-species mixture (Table 1). Diversity treatments were randomly distributed to plots. Each of the plots is 45 × 45 m, further divided into four subplots of 22.5 × 22.5 m, and seedlings were planted with 3 m spacing (225 trees per plot, equivalent to a stem density of 1111 ha<sup>-1</sup>, which is the commercially prevalent planting density in Central America, allowing for comparisons with other plantations). For the whole experiment a total of 5400 trees were planted. Seedlings were bought from a local reforestation company, Geoforestal, and were around 50 cm in height at planting. At the time of data collection for this study after four years of growth, trees had a mean height of 284 cm, with single individuals reaching up to 10 m in height, and *Hura crepitans* and *Luehea seemanii* were reproductive. As demonstrated by Hector et al. (2002), comparisons of mixture productivity with that of the best monoculture provide a conservative way to estimate superior performance of a mixture. In keeping with these analytical guidelines we designed our

Table 1. Experimental design with list of tree species in the different afforestation plots. Letters in parentheses are abbreviations for species names used throughout the text. Plot denotes the plot identification code used in the Sardinilla experiment.

Plot	One species
Ae 1	<i>Anacardium excelsum</i> (Ae)
Ca 1	<i>Cordia alliodora</i> (Ca)
Co 1	<i>Cedrela odorata</i> (Co)
Hc 1	<i>Hura crepitans</i> (Hc)
Ls 1	<i>Luehea seemanii</i> (Ls)
Tr 2	<i>Tabebuia rosea</i> (Tr)
Three species	
T 1	<i>Cordia alliodora</i> <i>Cedrela odorata</i> <i>Hura crepitans</i>
T 2	<i>Anacardium excelsum</i> <i>Luehea seemanii</i> <i>Tabebuia rosea</i>
T 3	<i>Anacardium excelsum</i> <i>Cedrela odorata</i> <i>Luehea seemanii</i>
T 4	<i>Cedrela odorata</i> <i>Hura crepitans</i> <i>Luehea seemanii</i>
T 5	<i>Cordia alliodora</i> <i>Hura crepitans</i> <i>Tabebuia rosea</i>
T 6	<i>Anacardium excelsum</i> <i>Cordia alliodora</i> <i>Tabebuia rosea</i>
Six species	
A 1 to A 6	<i>Anacardium excelsum</i> <i>Cordia alliodora</i> <i>Cedrela odorata</i> <i>Hura crepitans</i> <i>Luehea seemanii</i> <i>Tabebuia rosea</i>

experiment to take into account the best monocultures of each species. Both monocultures of *Cordia alliodora* failed to establish successfully in 2001 and were replanted in 2002. However this second planting was also plagued with high mortality (more than 85%) suggesting that the environmental conditions of Sardinilla might be outside the domain of tolerance of *Cordia*. Because of the very sparse canopy of both *Cordia* monocultures we decided not to collect litter in these plots. Thus only five species were left at the 1-species level.

### Litter production and litter quality

A total of 204 rectangular litter traps of 1 m<sup>2</sup> each were distributed within the study area, with 12 traps per plot and three per subplot. The traps were placed at random within the subplots at 1 m from nearest tree to the

random location, with the constraint that in multi-species mixtures each species was near a trap in equal frequency. Litter collection was started when trees became deciduous and was carried out during 12 weeks in the dry season of 2005, i.e. from 1 February until 27 April. This time interval covered the peak of litter shedding, although some species (e.g. *Anacardium*) also shed leaves, to a lesser degree, all year round. The traps were collected on a bi-weekly basis. The litter from all traps per plot was pooled and separated to species. All samples were dried to constant weight for a period of 48 h at a temperature of 70°C.

Sub-samples of leaves from all species in all plots from the second sampling date (4 March) were grounded down to powder for analyzing total carbon and nitrogen, and fibre, lignin and cellulose concentrations. C and N were measured with a Flash EA 1112 Series elemental analyzer at the laboratories of the Inst. of Plant Sciences, ETH Zurich. Fibre, cellulose and lignin concentrations could only be measured on two samples per species, from one monoculture and one six-species plot (or from one three-species and one six-species plot for *Cordia alliodora*). They were analyzed by the acid detergent fibre procedure described in Gessner (2005), which consists of determining the residual weight of samples following successive removal of various tissue constituents by extraction of compounds soluble in an acid detergent. The fractions resulting from this method are referred as proximate cellulose and lignin, because they do not necessarily match concentrations of these substances as defined chemically (Gessner 2005). These analyses were done at the laboratories of the Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science & Technology (EAWAG), Kastanienbaum.

### Litter decomposition

We adopted a litter bag approach in order to evaluate the rate of leaf litter decomposition, measured as litter mass loss during a period of four months. Flat 1-mm-mesh nylon bags of 25 × 25 cm in size were filled with 10 g of dry litter and placed on the soil surface in the centre of the plots. To ensure good contact to the soil, bags were pinned with nails on the surface and care was taken to ensure that vegetation did not begin to grow beneath the bags. The proportion of species within each bag was not equal, but derived from data of litter production from its respective mixture, i.e. representing the abundance of litter species within each mixture. Because no litter from *Cordia alliodora* monocultures was available (see above), we took litter of this species from various multi-species mixtures, mixed it and placed it in the plots where *Cordia* has been planted in monoculture. The bags were retrieved

every four weeks (except the last time step with 4.5 weeks) during a 116-day period from 9 May until 2 September 2005, corresponding to the wet season. At each of those four time steps, we sampled six replicate bags in the six monocultures, two replicates in the six three-species plots, and one replicate in the six six-species plots, yielding a total of 216 litter bags. With this design, every litter species was sampled in six replicates at each diversity level and time step, ensuring equal sample sizes on a species level.

After retrieval, the remaining litter was cleaned from roots which had grown into the bag, and separated according to species, which was possible even with small fragments of litter due to marked morphological differences among species. The litter was dried and analyzed for weight, ground down to powder and analyzed for total C and N.

### Statistical analysis

Effects of species identity on total litter production were tested with one-way analysis of variance (ANOVA), with a subsequent stepwise multiple comparison test (Tukey's HSD). The effects of tree diversity and species identity on the temporal pattern of litter fall were analyzed with repeated measures ANOVA, with tree diversity, and species identity nested within diversity as the between-subject factors, and time, time  $\times$  diversity, and time  $\times$  species(diversity) as within-subject factors. The effects of diversity and of time  $\times$  diversity were tested against the type III mean squares of species(diversity), and of time  $\times$  species(identity), respectively, as the appropriate error terms. The effects of tree diversity and tree species identity on decomposition and on C and N concentrations of the litter, and their changes during time were analyzed with a similar repeated measures ANOVA. The initial time step (time = 0) was excluded from the analysis of mass loss data. All data were log-transformed to meet assumptions of ANOVA.

To detect synergistic effects of litter production in species mixtures, we compared observed and expected litter production by calculating the proportional deviation indices  $D_T$  and  $D_{max}$  (Wardle et al. 1997, Hector et al. 2002) as follows:

$$D_T = (OE_T)/E_T \quad (1)$$

$$D_{max} = (OE_{max})/E_{max} \quad (2)$$

O is the observed litter production in mixture, and  $E_T$  is the sum of monoculture litter yields ( $E_i$ ) of the component species divided by the number of species ( $n_i$ ) in the mixture.  $E_{max}$  is the monoculture litter yield of the highest yielding component species. In analogy to the terminology used for total aboveground biomass production (Hector et al. 2002), non-transgressive and transgressive overyielding of litter production would be

indicated by  $D_T > 0$  and  $D_{max} > 0$ , respectively. Overyielding occurs if species are complementary in their resource use, while competition would either result in  $D_T, max \approx 0$  if there are compensatory tradeoffs among species or even in  $D_T, max < 0$  with interference competition. We excluded *Cordia alliodora* from these calculations because it failed to establish in monoculture, i.e. its litter production in certain mixtures was not counted and  $n_i$  was reduced by one.

We also examined the relationship between the standing aboveground tree biomass in each plot and litter production. Tree biomass was determined from species-specific allometric relationships between diameter at breast height and total aboveground dry weight. These equations were obtained by harvesting five individuals per species in 2001 from an adjacent plantation (L. Coll, C. Potvin, C. Messier and S. Delagrangé unpubl.). In addition, we determined the influence of the size of neighbour trees on litter production within a plot in the three- and six-species plots. We estimated the basal area (BA,  $m^2$ ) of the nine nearest trees around each litter trap (the "target" tree where the trap has been installed, see methods, plus the eight surrounding trees) with measurements of diameter at breast height (DBH) as:

$$BA = 3.142(DBH/2)^2 \quad (3)$$

To account for differences in distance from the litter trap to the neighbouring trees, we standardized BA values by dividing them by trap-tree distance. We tested the correlation between neighbor size and litter production both with the nearest "target" tree (1m distance to trap) and with the summed standardized basal area of all nine nearest trees (Spearman's rank correlation coefficient).

Decomposition rates of single-species litter of the six tree species were obtained by fitting a single exponential decay model to the changes of litter dry weight over time (Wieder and Lang 1982). This model corresponds well with current understanding of the decomposition process, with initially rapid loss of soluble and easily degraded compounds, and slower loss of more recalcitrant materials. For each species, we calculated the decomposition rate constant,  $k$ , from the decay model:

$$X = 100e^{-kt} \quad (4)$$

where X is the proportion of initial mass remaining at time  $t$ ;  $k$  is the exponential decay coefficient; and  $t$  is time in days. The model was fitted with the restriction that at time = 0 all of the initial litter was present ( $X_0 = 100$ ) (Wieder and Lang 1982), and with the stipulation that  $k$ -values could not be  $< 0$  (Wieder and Wright 1995). Litter turnover times were calculated as time to 95% litter decomposition using  $3/k$  (Didham 1998).

Differences between species-specific decay rates in litter monocultures were tested with an analysis of

covariance using time as covariate, excluding time = 0 since for all treatments at time = 0 the mean percent remaining is 100 (Wieder and Lang 1982). A significant time by species interaction indicates that litter decomposition rates, i.e. k-values, differ among species.

We calculated predicted litter mass loss of multi-species mixtures for each sampling date based on measured decomposition rates in monocultures of each component species and their proportions within each individual mixture at the start of the litter bag experiment. The differences between observed and predicted decomposition at the three- and six-species level were tested with two-tailed t-tests.

Finally, we analyzed the relationship between measures for litter quality and decomposition rates using Kendall's rank correlation coefficient. Analyses were done with SPSS 13.0.1. and SAS 9.1.

## Results

### Litter production

During the collection period, litter production of *Cordia alliodora*, *Cedrela odorata* and *Hura crepitans* peaked in February and early March, and continuously declined until the end of April 2005. In contrast, *Tabebuia rosea* peaked rather late in the dry season (early April). Litter production of *Anacardium excelsum* and *Luebea seemanii* showed two peaks in February/March and April (Fig. 1A). Consequently, our repeated measure analysis revealed significant effects of species

identity, time and time  $\times$  species interaction (Table 2). Total litter production, accumulated at the end of the dry season 2005, also differed among the six tree species (marginal significant effect of species( diversity)  $F_{14,36} = 1.82$ ;  $p = 0.073$ ) and varied more than four-fold between the least productive species *Cedrela odorata* ( $17.7 \text{ g m}^{-2}$ ) and the most productive species *Luebea seemanii* ( $79.6 \text{ g m}^{-2}$ , Fig. 1B).

Tree diversity treatments differed significantly in litter production (Fig. 2A, Table 2), with similar temporal variations in all treatments (non-significant time  $\times$  diversity interaction). Species richness had also a significant effect on total litter production, summed at the end of April ( $F_{2,36} = 11.06$ ;  $p < 0.001$ , Fig. 2B). There was a trend for an unimodal distribution of total litter fall with increasing tree species richness with an approximately two-fold higher production at the three-species level ( $57.5 \text{ g m}^{-2}$ ) than at the one- or six-species level ( $27.5$  and  $31.7 \text{ g m}^{-2}$ , respectively, Fig. 2B). Variability within each treatment was high, especially in the three-species mixtures with one plot dominated by high litter production of *Luebea seemanii*. Even in the six-species plots, which in contrast to the one- and three-species mixtures had all the same species composition, high variability was observed with one plot having double litter production than the next lower one.

The majority of the three-species mixtures had higher litter yields than expected based on average monoculture yields, with  $D_T$  values  $> 0$  (Table 3). However, compared with the most productive monoculture, only two mixtures showed overyielding in litter

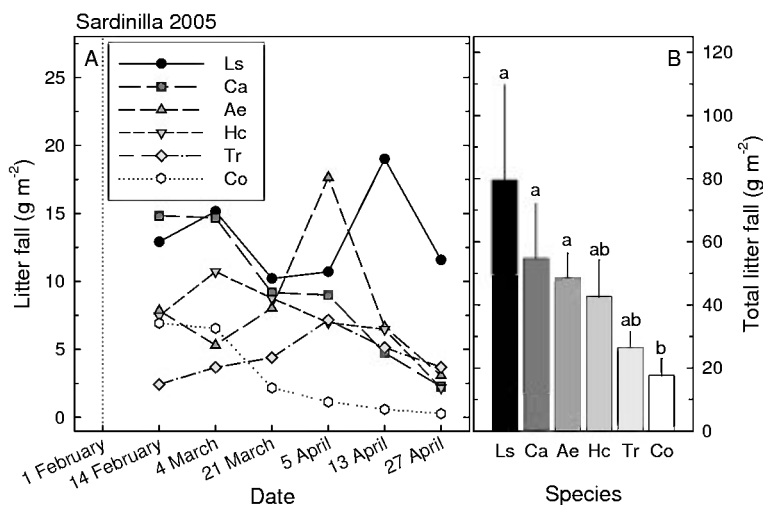


Fig. 1. (A) Temporal dynamics of litter production of the six tree species during the dry season of 2005. Data are means of standardized litter fall values of all mixtures ( $n = 10$ ). Note that *Cordia alliodora* (Ca) was not present in all plots due to high mortality ( $n = 3$ ). Error bars were omitted to improve readability. Dotted line indicates start of the collection period (1 February). (B) Total litter production by species, means  $\pm$  SE. For species abbreviations: see Table 1.

Table 2. Repeated measures analysis of variance for diversity and species-effects on litter production. Data have been log-transformed. Significant effects at  $p < 0.05$  are in bold, those at  $p < 0.1$  are in italics.

Source of variation	DF	MS	F	p
Between subject				
Diversity	2	2.664	5.63	<b>0.016</b>
Species(Diversity)	14	0.473	1.99	<i>0.051</i>
Within subject				
Time	5	0.472	14.25	<b>&lt;0.001</b>
Time × Diversity	10	0.073	0.98	0.473
Time × Species(Diversity)	70	0.075	2.26	<b>&lt;0.001</b>
Residual	170	0.033		

Greenhouse-Geisser epsilon=0.548; Huynh-Feldt epsilon=0.881.

production ( $D_{\max} > 0$ ), while another two mixtures showed no deviation from expected litter yields ( $D_{\max} \approx 0$ ), and one mixture had lower yields than expected. At the six-species level with identical species combinations, two plots showed non-transgressive overyielding, and only one plot showed transgressive overyielding, while the majority of plots had lower litter production than expected (Table 3).

In contrast to our expectations, litter production was neither correlated to standing aboveground tree biomass per plot (Spearman's rank correlation coefficient  $r_s = 0.255$ ,  $p = 0.323$ ) nor to distance-standardized basal area of the single nearest individual around the litter traps ( $r_s = -0.378$ ,  $p = 0.226$ ). However, litter production was positively correlated with standardized basal area of the nine trees surrounding each trap ( $r_s = 0.594$ ,  $p = 0.042$ ).

## Litter decomposition

Litter mass loss of the six tree species in monoculture decreased exponentially throughout the decomposition period of 116 days (Fig. 3), as shown by the very good statistical fit of the single exponential decay model with  $r^2$ -values between 0.896 and 0.978 (Table 4). The decay model, however, underestimated litter mass loss at the last sampling date for all species. Decomposition rates in monocultures differed significantly between species (ANCOVA, time × species interaction:  $F_{5,132} = 11.822$ ;  $p < 0.001$ ): *Hura crepitans* had much higher decay rates ( $k = 7.337 \text{ year}^{-1}$ ) than the other five species, leading to only 1.4% of litter remaining after 116 days, while the other species had between 31.5 and 61.6% of initial litter mass after this time period. Litter turnover times, calculated as time to 95% litter decomposition from the single exponential decay model, varied more than five-fold between the most easily decomposable litter of *Hura* ( $3/k = 0.41$  years) and the most recalcitrant litter of *Anacardium* ( $3/k = 2.28$  years, Table 4). The other four species had similar turnover times between 1.05 and 1.68 years.

Decomposition was not affected by diversity, i.e. decomposition rates were the same for one-, three- or six-species litter mixtures if the entire litter mixtures were considered (Fig. 4 right panels, "observed" values, Table 5). Mixing litter did also not result in any synergistic or antagonistic effects, so that observed decomposition rates did not differ from expected rates that were calculated based on monospecific decomposition rates and the abundance of each litter species

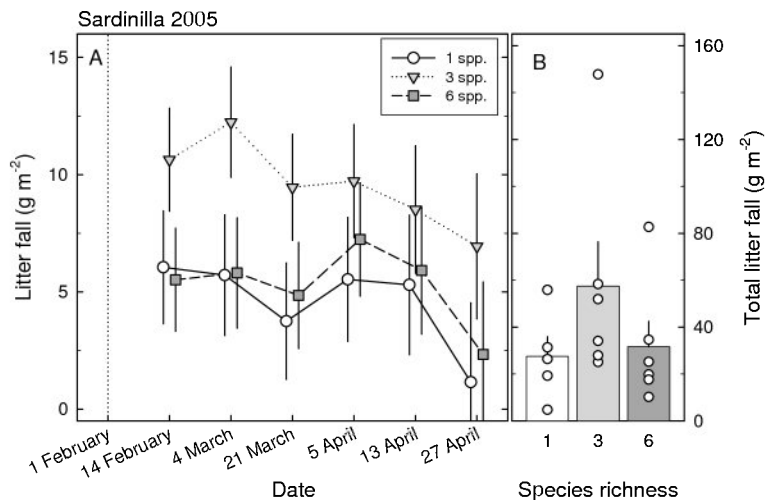


Fig. 2. (A) Temporal dynamics of litter production of the diversity treatments during the dry season of 2005. Data are means  $\pm$  SE for each diversity level. Data points are slightly staggered to improve readability. Dotted line indicates start of the collection period (1 February). (B) Total litter production by tree diversity, means  $\pm$  SE. Single data points represent values for each mixture.

Table 3. Deviation from expected litter production for each species mixture. The index  $D_T$  uses the average monoculture litter production of the component species for comparison with the observed litter production, while  $D_{max}$  is based on the highest yielding monoculture species (see methods for calculations). In plots with *Cordia alliodora* (Ca), the indices were calculated excluding this species because of failure in monoculture. Species abbreviations are given in Table 1.

Diversity	Plot	Mixture	$D_T$	$D_{max}$
3	T1	Ca, Co, Hc	0.48	-0.08
3	T2	Ae, Ls, Tr	2.91	1.65
3	T3	Ae, Co, Ls	-0.09	-0.50
3	T4	Co, Hc, Ls	1.19	0.05
3	T5	Ca, Hc, Tr	0.39	0.20
3	T6	Ae, Ca, Tr	0.18	0.09
6	A1	Ae, Ca, Co, Hc, Ls, Tr	-0.36	-0.69
6	A2	Ae, Ca, Co, Hc, Ls, Tr	-0.63	-0.82
6	A3	Ae, Ca, Co, Hc, Ls, Tr	0.26	-0.38
6	A4	Ae, Ca, Co, Hc, Ls, Tr	1.52	0.24
6	A5	Ae, Ca, Co, Hc, Ls, Tr	-0.09	-0.55
6	A6	Ae, Ca, Co, Hc, Ls, Tr	-0.28	-0.65

within mixtures (Fig. 4 right panels, “observed” vs. “predicted” values,  $t$ -tests: all non-significant).

Because we were able to separate litter mixtures to species, we could follow species-specific decomposition rates within each litter mixture. The ANOVA revealed a significant effect of species(diversity) and time  $\times$  species(diversity) for litter decomposition. This suggest that the decomposition is affected not only by species identity but that species-specific decomposition also varied among different diversity treatments, i.e. litter mass loss of a species was not the same in monoculture than in mixture (Fig. 4 left panels, Table 5). This effect of species(diversity) may be best understood when examining the % litter remaining after 116 days with the species ordering neatly according to their decomposition rate constants. Furthermore, the species with the highest decay rate, *Hura crepitans*, decomposed fastest in monocultures and slowest in mixture and shows a pattern very distinctive of the other species (Fig. 4 left panels). Conversely, the most recalcitrant species, *Anacardium*, decomposed the fastest in the six-species plots. Fig. 4 also shows that individual species responses to the diversity treatments varied through time: at the onset of the experiment *Cordia alliodora*, for example, decomposed slower with increasing diversity, but this trend reversed during the course of the experiment.

The six tree species differed in their initial chemical composition of leaf litter (Table 4), and decomposition was highly dependent on those measures of litter quality: with increasing C/N ratio decomposition slowed down and leveled off at C/N ratios  $>40$ , which occurred with only one species, *Anacardium excelsum* (Fig. 5A with data after 116 days of decomposition, Kendall's rank correlation coefficient  $\tau = 0.44$ ,  $p < 0.001$ ). This correlation was equally strong at the earlier sampling dates ( $\tau$  between 0.42 and 0.5, all with  $p < 0.001$ ). Litter decay was positively correlated with initial nitrogen concentra-

tion of fallen litter at all time steps, also levelling off at N concentrations below 1.2% with *Anacardium* (data not shown). The ratio of proximate lignin to nitrogen was negatively correlated with decomposition rate: the higher this ratio, the slower was decomposition (Fig. 5B,  $\tau = 0.81$ ,  $p < 0.001$ ).

The chemical composition of the litter changed during the decomposition process (significant time effect for %N, %C and C/N ratio in the repeated measures analysis, Table 6), with a slight initial increase in the C/N ratio, and a subsequent continuous decrease in all species, except for *Cordia alliodora*, which did not show the initial increase in C/N (data not shown). This pattern was caused by an initial decrease in nitrogen

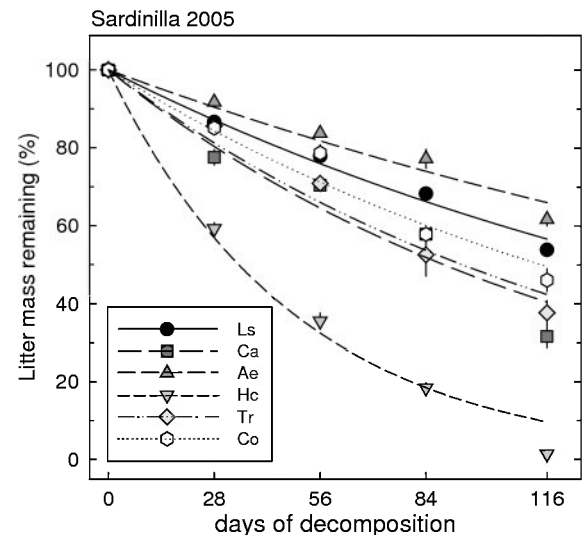


Fig. 3. Temporal dynamics of litter decomposition of single-species litter from the six tree species. Means  $\pm$  SE from six litter bags per species and time. Lines are single exponential decay functions, fitted to the single data points per species and time step.



Table 4. Decomposition constants and initial litter quality of studied species.  $k$  year<sup>-1</sup>: decomposition rate constants, calculated from a single exponential decay model for the six different tree species when single-species litter was decomposed ( $n = 24$ , total number of litter bags retrieved, six per time step and species);  $t^2$ : proportion of variance explained by these decay models;  $3/k$ : litter turnover time (time to 95% decomposition, in years);  $N$  (%): nitrogen concentration;  $C/N$ : C to N ratio, fibre (%): acid detergent fibre (ADF) in percent of dry weight; cellulose (%): acid detergent cellulose (ADC) in percent of dry weight; lignin (%): acid detergent lignin (ADL) in percent of dry weight; lignin/N: lignin to N ratio. Measures of litter quality are from the first sampling date (4 March 2005) of freshly fallen litter in all plots ( $n = 10$  per species, except for *Cordia alliodora* with  $n = 3$ ). Fibre and lignin analyses were done on samples from one monoculture and one six-species plot (or samples from one three-species and one six-species plot for *Cordia*). All values are means  $\pm$  SE.

Species	$k$ year <sup>-1</sup>	$t^2$	$3/k$ (years)	N (%)	C/N	fibre (%)	cellulose (%)	lignin (%)	lignin/N
<i>Anacardium excelsum</i>	1.31 $\pm$ 0.07	0.90	2.28	1.01 $\pm$ 0.08	46.5 $\pm$ 3.1	77.2 $\pm$ 3.4	34.3 $\pm$ 0.7	38.2 $\pm$ 2.7	47.4 $\pm$ 7.8
<i>Cordia alliodora</i>	2.85 $\pm$ 0.18	0.91	1.05	1.88 $\pm$ 0.27	23.0 $\pm$ 3.2	41.4 $\pm$ 2.2	19.1 $\pm$ 0.8	19.1 $\pm$ 2.2	13.7 $\pm$ 1.6
<i>Cedrela odorata</i>	2.23 $\pm$ 0.15	0.91	1.35	1.28 $\pm$ 0.03	36.0 $\pm$ 0.7	53.6 $\pm$ 3.7	21.6 $\pm$ 1.7	28.5 $\pm$ 2.9	23.1 $\pm$ 0.7
<i>Hura crepitans</i>	7.34 $\pm$ 0.29	0.98	0.41	1.82 $\pm$ 0.05	23.4 $\pm$ 0.7	25.6 $\pm$ 1.3	17.4 $\pm$ 2.4	7.7 $\pm$ 0.9	4.0 $\pm$ 0.3
<i>Luehea seemanii</i>	1.79 $\pm$ 0.07	0.96	1.68	1.42 $\pm$ 0.05	33.5 $\pm$ 1.2	54.9 $\pm$ 1.6	24.4 $\pm$ 0.4	29.9 $\pm$ 1.5	22.5 $\pm$ 1.1
<i>Tabebuia rosea</i>	2.70 $\pm$ 0.18	0.90	1.11	1.92 $\pm$ 0.08	24.2 $\pm$ 1.0	45.7 $\pm$ 6.2	24.7 $\pm$ 4.3	19.8 $\pm$ 2.9	10.1 $\pm$ 1.2

concentration within the first 28 days of decomposition, followed by a continuous increase (Fig. 6). Carbon concentrations, however, decreased continuously (Fig. 6). In contrast, litter from *Cordia* showed no initial decline in N concentration and C concentration remained constant over 56 days, followed by a sharp decline. Litter species richness had no effect on temporal C and N dynamics (Table 6), but species differed significantly among each other and these differences varied over time (significant time  $\times$  species( diversity) interaction, Table 6). For instance, the fastest decomposing species, *Hura crepitans*, had higher %N values in pure litter bags than in mixed bags at intermediate time intervals. In contrast, *Cedrela odorata*, a species with intermediate litter quality and decomposition rates (Table 4, Fig. 3 and 5) generally had lower N concentrations in pure than in mixed litter bags.

## Discussion

### Litter production

Our sampling period for litterfall certainly covered the peak of annual litter production, as comparisons with data from litterfall dynamics of natural tropical moist forests from nearby sites (Barro Colorado Island, Pipeline Road) confirm (Wieder and Wright 1995, Santiago and Mulkey 2005). Although some litter fall generally occurs during the whole year and can even peak during the rainy season in the case of unusual severe windstorms (Wieder and Wright 1995), litter production quickly increases after the onset of the dry season, and declines towards the transition between dry and wet season. Our maximum dry season litterfall data from one three-species plot (Fig. 2) was 12.3 g m<sup>-2</sup> week<sup>-1</sup>, which is approximately one half to one third of mature forests (Wieder and Wright 1995, Santiago and Mulkey 2005), but remarkably high for a plantation of only five years. The mean weekly litterfall, however, was only 3.2 g m<sup>-2</sup> week<sup>-1</sup> and thus about only a tenth of values observed for mature forests on Barro Colorado Island. In other similarly young plantations of neotropical native species at La Selva Biological Station in Costa Rica with higher precipitation and less pronounced dry season, weekly litter production ranged between 8.3 and 14.2 g m<sup>-2</sup> week<sup>-1</sup> during a 12-week-period at the time of lowest rainfall (calculated from Montagnini et al. 1993). However, stem density was 2500 ha<sup>-1</sup> in that afforestation and thus more than two times denser than ours. Our litterfall data are also lower than values reported by Ewel (1976) for four-year-old secondary succession stands with woody species in Guatemala (mean weekly litterfall: 11.5 g m<sup>-2</sup> week<sup>-1</sup>), again presumably due to lower stem density in our experimental plots. However, other

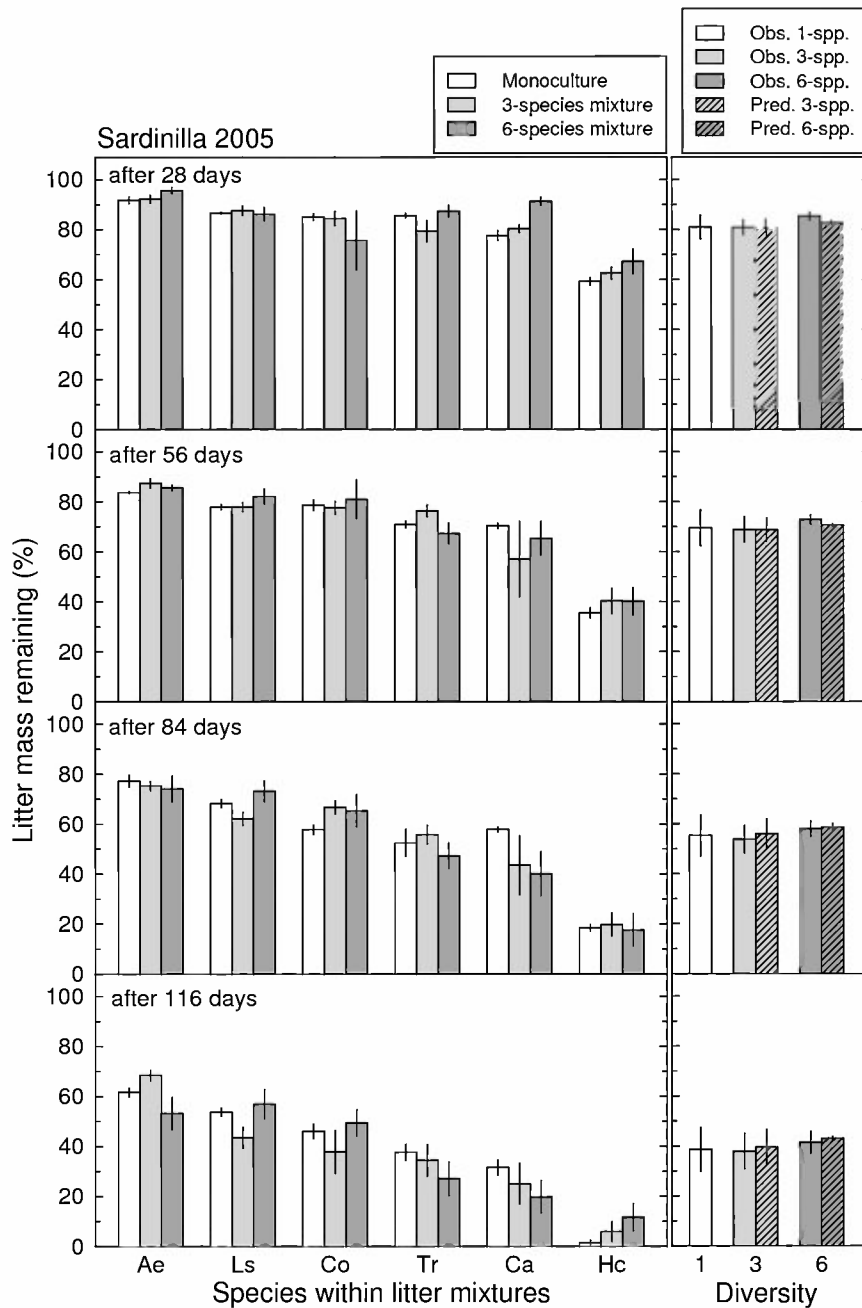


Fig. 4. Left panels: litter decomposition, separated by species within mixtures. Bars indicate means  $\pm$  SE. Right panels: litter decomposition of entire mixtures, predicted values are calculated from single-species decomposition rates and abundance of species within mixtures (see methods section for details).

factors make direct comparisons of litter fall data from several locations difficult because it will also vary with climate and soil fertility, especially with P concentrations (Vitousek 1984).

There were large differences among our six species in the temporal dynamics of litter production and total

litter fall at the end of the dry season, as expected (hypothesis 1). This is consistent with results from other studies, looking at litter fall dynamics of various native species in plantation forests. For instance, Montagnini et al. (1993), Montagnini (2000), and Stanley and Montagnini (1999) reported significant

Table 5. Repeated measures analysis of variance for effects of tree diversity and species identity on litter decomposition. Data have been log-transformed. Significant effects at  $p < 0.05$  are in bold.

Source of variation	DF	MS	F	p
Between subject				
Diversity	2	0.061	0.06	0.946
Species(Diversity)	15	1.099	14.16	<b>&lt;0.001</b>
Within subject				
Time	3	5.921	121.96	<b>&lt;0.001</b>
Time $\times$ Diversity	6	0.040	0.15	0.988
Time $\times$ Species(Diversity)	45	0.268	5.51	<b>&lt;0.001</b>
Residual	352	0.041		

Greenhouse-Geisser epsilon = 0.580; Huynh-Feldt epsilon = 0.705.

differences in peaks of leaf litter fall or standing leaf biomass among native species in four young plantations with four species each in Costa Rica.

There is much less information, however, whether the combination of species with such pronounced differences in litter production results in any positive or negative diversity effects on litter fall. Inferring from the data on standing leaf biomass by Montagnini (2000) and Stanley and Montagnini (1999), mixing four species did not result in increased leaf biomass compared to the corresponding monocultures. From forestry experiments, positive mixture effects on total aboveground biomass production – and thus also on litter production, given the generally good fit between net primary production and fine litter fall in tropical trees (Jordan 1983) – have been found for some combinations of species, while not for others. Those combinations with synergistic effects of mixing species are characterized by species with different traits related to resource acquisition, such as stratified mixtures of fast-growing, shade-intolerant species in the overstorey and slower growing, shade-tolerant in the understorey (Kelty 1992, Scherer-Lorenzen et al. 2005a). Such trait

differences may result in resource use complementarity and can explain higher productivity of mixed versus pure stands (Hector 1998). Our three- and six-species mixtures theoretically fulfill those requirements since they were composed of fast-, moderately fast-, and slow-growing species. In fact, tree diversity significantly affected litter production, and five of our six three-species mixtures had higher litter yields than expected based on the mean monoculture yield (non-transgressive overyielding). Even two mixtures out-performed their respective best monoculture (transgressive overyielding), suggesting the existence of resource use complementarity among species, as hypothesized. A recent analysis of individual tree growth also demonstrated complementarity in our mixed plantings (C. Porvin and N.J. Gorelli unpubl.). Such synergy is illustrated by the most productive mixture that had a litter production 2.6 times higher than the highest yielding monoculture (*Luehea seemanii*). That mixture developed a stratified canopy with the fast-growing species *Luehea seemanii* in the upper canopy, the moderately fast growing species *Anacardium excelsum* in the middle, and the slower growing species *Tabebuia rosea* at the lowest level. These three species have the highest RGR within our categorization of growth rates, respectively, which might explain the success of this species combination. Other mixtures yet did not show such a pronounced stratification.

In contrast, only two of the six-species plots had higher litter yields than expected based on the average monoculture performance, and only one out-performed the *Luehea seemanii* monoculture. In most cases, litter yields were lower than expected. The mechanisms responsible for positive diversity effects on ecosystem productivity and related properties (complementarity, facilitation, sampling effects for highly productive species) are all expected to asymptotically increase with diversity (Hooper et al. 2005), both acting in isolation or in conjunction (Schmid et al. 2002). There is currently

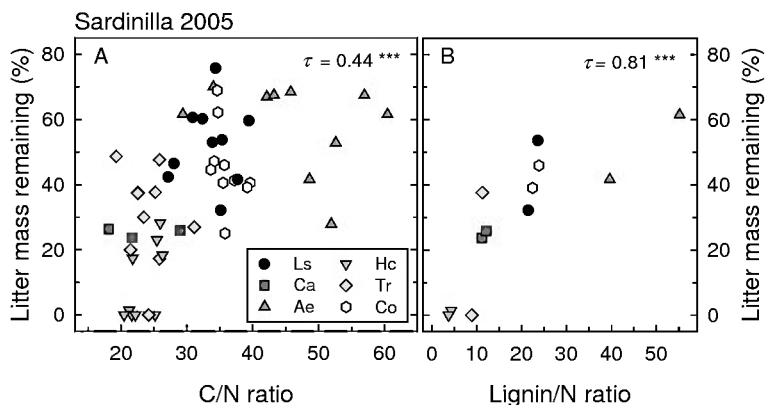


Fig. 5. The relationship between C/N ratio of leaf litter (A) or lignin/N ratio (B) and decomposition after 116 days.

Table 6. Repeated measures analysis of variance for effects of tree diversity and species identity on litter chemistry during decomposition. Data have been log-transformed. Significant effects at  $p < 0.05$  are in bold.

Source of variation	N (%)				C (%)				C/N			
	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
Between subject												
Diversity	2	0.037	0.09	0.913	2	0.008	0.71	0.507	2	0.074	0.16	0.852
Species(Diversity)	15	0.407	125.76	<0.001	15	0.011	14.54	<0.001	15	0.460	120.41	<0.001
Within subject												
Time	4	0.090	37.28	<0.001	4	0.052	91.90	<0.001	4	0.254	95.10	<0.001
Time × Diversity	8	0.002	0.46	0.883	8	0.002	1.52	0.169	8	0.003	0.42	0.903
Time × Species(Diversity)	60	0.005	2.21	<0.001	60	0.001	1.78	0.001	60	0.006	2.26	<0.001
Residual	276	0.002			280	0.001			280	0.001		

Greenhouse-Geisser epsilon  $N(\%) = 0.971$ ,  $C(\%) = 0.575$ ,  $C/N = 0.556$ ; Huynh-Feldt epsilon  $N(\%) = 1.290$ ,  $C(\%) = 0.740$ ,  $C/N = 1.268$ .

no coherent theory to explain an optimum type of diversity effect on productivity – or indices comparing performance in mixture and monoculture – as found in our study. It could result from a change in predominance of sampling or complementarity effects at intermediate diversity to predominance of interference competition among species at higher diversity levels. Alternatively, a particular complementary mixture at the intermediate level, as our Ae-Ls-Tr combination mentioned above, could be diluted at higher diversity with other, less complementary species (A. Hector, pers. comm.). However, our gradient of species richness is rather short, and the second plantation established two years later with maximum diversity of 18 species will offer the possibility to examine the shape of the diversity – productivity relationship over a longer gradient.

Another important aspect of our litter production data of the six-species mixtures is the high variability despite their identical species composition. Environmental factors such as soil heterogeneity have been advocated to such variation (Huston and McBride 2002). Previous analyses have confirmed the importance of such small-scale heterogeneity for ecosystem properties at our experiment, e.g. of topographic position for height and diameter growth of trees during the first years (Scherer-Lorenzen et al. 2005b, C. Healey and C. Potvin unpubl.). All six species grew better on the top of a hill than in depressions with a few days of waterlogging, although the maximum difference between both topographic positions was less than 5 m. It is worth noting that the six-species plot which is outperforming the *Luehea* monoculture is located adjacent to the best three-species mixture.

In summary, we do have indications for synergistic mixture effects on litter production at the intermediate diverse level and for competitive effects at the highest diversity, although the possibilities for analyses based on the comparison of performance in monoculture and mixture remain limited because the loss of *Cordia* in pure stands. Additionally, because tree biomass (and litter) production is responsive to soil conditions, environmental heterogeneity may also act as a “hidden treatment” (Huston and McBride 2002).

## Litter decomposition

Our observed litter decomposition rates of six native species during the wet season of 2005, expressed as exponential decay  $k$ -values of  $1.314 - 7.337 \text{ k year}^{-1}$ , were similar to those reported from mixed litter of natural mature forest on nearby Barro Colorado Island (mean of  $2.994 \text{ k year}^{-1}$  for five consecutive wet seasons, Wieder and Wright 1995). These values are also equivalent to decomposition rates from other tropical forests, which are typically  $> 1.0 \text{ k year}^{-1}$

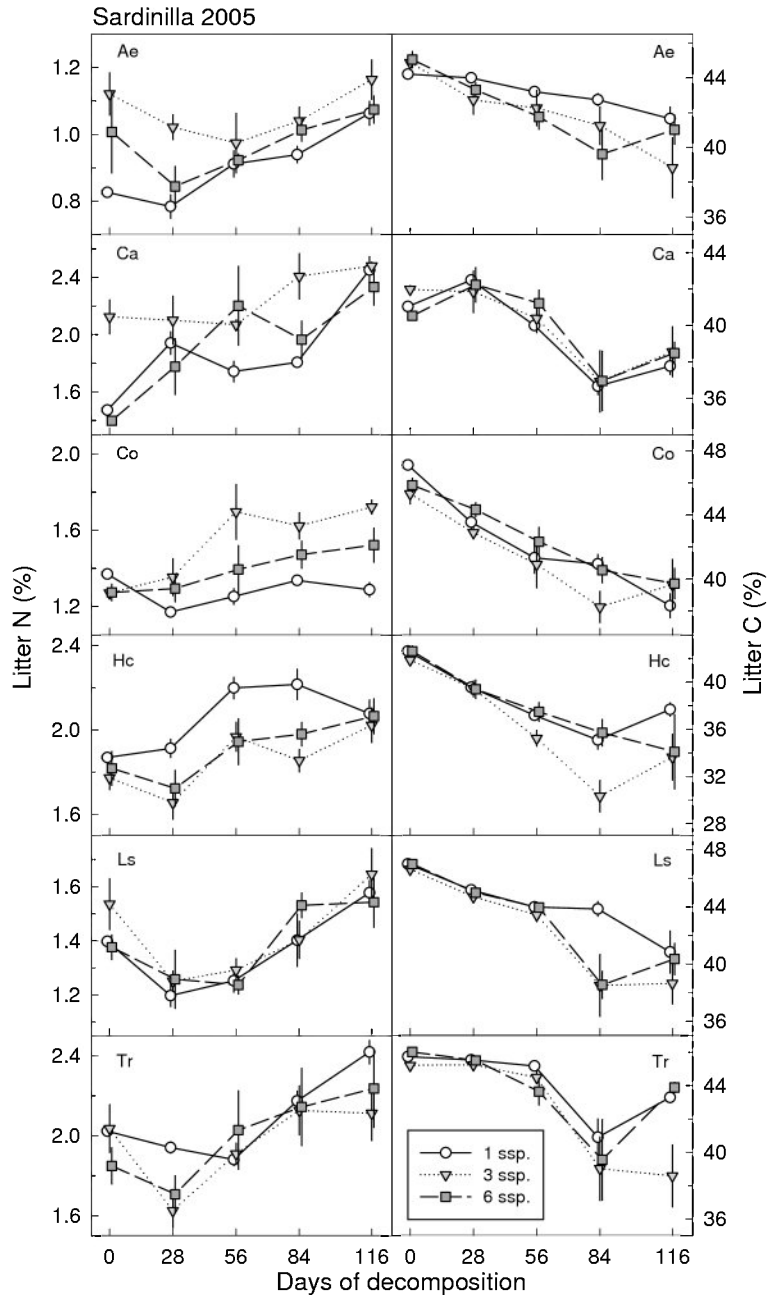


Fig. 6. Species-specific changes in litter nitrogen (left panels) and carbon concentration (right panels) during the decomposition process, separated by diversity treatments. Means  $\pm$  SE. Note different scaling for each species for both N and C percent values.

e.g. from the Biological Dynamics of Forest Fragments Project in Central Amazonia, Brazil (Didham 1998). Consequently, mean turnover times of litter as predicted from decay  $k$ -values were also in the range of other tropical forest types, although these predictions from early stages of decay have serious limitations due to the altered patterns of long-term decomposition (Prescott 2005). Thus, despite clear differences in

microclimatic conditions between mature forests and our five-year old plantation due to lower canopy height and closure, average litter decomposition rates were very similar to undisturbed mature forests.

Litter decomposition research has focused mainly on single-species decomposition, and only about 30 studies exist that explicitly address the question whether litter mixtures have different decomposition patterns than the

component litter species decaying alone (Gartner and Cardon 2004, Hättenschwiler 2005, Hättenschwiler et al. 2005). Tropical forests are critically under-represented in these studies with only two by Byard et al. (1996) and Montagnini et al. (1993). The latter study, for instance, compared two indigenous tree species of Costa Rica decomposing in monoculture and in a 1:1 mixture over a period of four months. In one species, decomposition rate was significantly higher in mixture than in the pure treatment, while there was no difference in the other species. A similar long gradient of species diversity as used in our study was analyzed by Hättenschwiler and Gasser (2005) who found that litter decay of three out of six temperate tree species increased with increasing diversity, while the other species showed no response to changes in litter diversity. The responsive species were those that had the most-recalcitrant litter with higher lignin/N ratios. Analyzed across all treatments (litter diversity and presence/absence of macrofauna) and with the entire mixtures, observed litter decomposition was significantly higher than expected based on decay rates with single litters after 204 days of exposure. In contrast, during the initial period of 92 days without addition of fauna, mixing did not result in higher decomposition rates (Hättenschwiler 2005). We also found no overall effect of litter diversity if the entire litter mixtures were analyzed, i.e. mixing species resulted in pure additive effects, in contrast to our hypothesis 4. However, the individual species changed their decomposition pattern depending on the diversity of the litter mixture, i.e. there were species-specific responses to mixing litter, as expected (hypothesis 5), and as reported in most studies that also separated litter species within mixtures (reviewed by Hättenschwiler et al. 2005).

The correlations between the measures of litter quality we used (data shown for C/N ratio, lignin/N ratio) and mass loss clearly underline the well known importance of the chemical composition of litter for decomposition (Swift et al. 1979, Coûteaux et al. 1995, Hättenschwiler and Vitousek 2000). These and other measures of litter quality are often highly autocorrelated among each other and no single quality factor controls decomposition (Prescott 2005). Therefore, we do not discuss whether one measure of litter decomposition is better than the other to predict litter decay. However, it became clear that chemical differences are to a large part responsible for the differences in decay rates observed between litter species, and hence our different tree species can be reasonably well grouped along the quality – decomposition trajectory. Nevertheless, some species had distinct decomposition rates besides having similar values for one measure of litter quality, e.g. *Hura crepitans* vs. *Tabebuia rosea* for C/N ratio. However, other quality measures may then be different; in this case the two species differed in fibre content with

much lower values for *Hura*, which decomposed much faster than *Tabebuia*. Within species, however, differences in chemical composition did not result in changes in decay rates. This was true for species with either small intraspecific differences in litter quality, such as *Cedrela odorata*, or large differences, such as *Anacardium excelsum* for C/N ratio for instance. Thus, a variety of chemical and physical litter traits that differ between tree species are responsible for the highly significant effects of species identity for the decomposition process (hypothesis 3).

Nutrient transfer among litter types is often discussed as an underlying mechanism for observed diversity effects on decomposition (Hättenschwiler et al. 2005). Such transfer could occur via leaching or fungal hyphae and microbes, for instance, and would alleviate nutrient limitation of decomposition of poor quality litter. Seastadt (1984) suggested that high-quality litter with high decay rates enhance decomposition of more recalcitrant, poor-quality litters. For example, Wardle et al. (1997) found that synergistic mixture effects causing enhanced decomposition rates were more likely when litter of plants with high N concentrations were mixed, and these effects were stronger in mixtures of dissimilar litter types. In consequence, when each species in litter mixtures is analysed separately, nitrogen concentrations may increase in the poor-quality litter while decreasing in the high-quality litter (Briones and Ineson 1996, McTiernan et al. 1997, Salamanca et al. 1998). However, litter gains and losses of N, i.e. N immobilisation and mineralisation, also occur in pure litters and depend on its initial C/nutrient ratios (Swift et al. 1979). The nutrient-transfer hypothesis is also challenged by the results of Hoorens et al. (2003), who have shown that the difference in initial single litter chemistry parameters (e.g. C, N, P, phenolic compounds) cannot explain non-additive effects in litter mixtures. In contrast to the findings of Hättenschwiler (2005), in our study the three species with the lowest litter quality and slowest decay rates (Ac, Ls, Co) showed no clear pattern across the gradient of diversity, i.e. their decay was not enhanced by adding litter of higher quality, although litter N concentrations did indeed increase in *Cedrela odorata* when it occurred in mixture with either three or six species. The two species with intermediate decay rates (Ca, Tr) decomposed slightly faster in presence of other litters, although in *Cordia alliodora*, the trend reversed during the time course of the experiment, and no clear pattern concerning N concentrations emerged. The most rapidly decomposing species, *Hura crepitans*, decomposed slower in mixture with other species, and its %N values decreased in presence of other, more recalcitrant litters. Thus, our data only partially support the nutrient transfer hypothesis. Although intuitively plausible, the existence of this mechanism for litter-mixing effects seems to be rather uncommon and its

importance for litter-species interactions might have been overestimated in the past. While it may occur occasionally, other mechanisms such as effects of specific compounds (e.g. polyphenols), altered microenvironmental conditions, or interactions across trophic levels have to be considered in explaining non-additive litter mixture effects (Hättenschwiler et al. 2005). However, inferring from element concentration data on nutrient transfer among litters is equivocal and only isotope studies, e.g. with  $^{15}\text{N}$  labelled litters, would give direct support for or against this hypothesis. Following the rationale of the nutrient transfer mechanism, litter diversity effects should largely depend on the specific quality of the litters to be mixed, and on certain key traits that have strong impacts on the decomposition process (e.g. inhibitory effects through polyphenols, Hättenschwiler and Vitousek 2000). Therefore, it should be difficult to find a general and robust relationship between litter diversity and decomposition rates. These species-specific effects and their modulation by diversity underlie the difficulty to predict decomposition processes and their consequences for biogeochemical cycles in mixtures of varying diversity from those patterns observed in monoculture.

The effect of tree species diversity and composition on nutrient cycling might be better predictable from the litter mass and its nutrient content, than from litter decomposition rates obtained from standard litter bags, as pointed out by Prescott (2005). Our approach of adjusting the amount of each litter species in the litter bags to species-specific litter production within each community is one step to mimic decomposition processes in a more realistic setting, and it will help to calculate community-specific nutrient budgets in the future. However, it may mask species-specific responses to litter mixing that could emerge if abundance of each litter species were equal, as done in most litter mixing studies. For instance, within the different three-species combinations, our most recalcitrant species, *Anacardium*, had proportions between 0.17 and 0.74 of the whole litter mixture. On the other hand, the proportions of most rapidly decomposing species, *Hura*, were much less variable (0.38 to 0.43). Salamanca et al. (1998) could demonstrate that the mixing ratio (0.25; 0.5; 0.75 in two-species mixtures) can influence mass loss of both the individual litter species within a mixture, and of the entire mixture, with different effects depending on the litter quality of the species. Additionally, our litter bags were not placed all together in a common homogeneous environment, but in the corresponding plots with their specific microenvironmental conditions influenced by the tree species composition and topographic location. Thus, our species-specific responses might also be related to variations in the microenvironment that may level out or enhance responses seen under more standardized

conditions applied in most other studies (Briones and Ineson 1996, Salamanca et al. 1998, Hättenschwiler and Gasser 2005). To test this assumption, an interesting follow-up experiment would be to monitor species-specific litter decay at constant mixing ratios within a uniform environment.

## Conclusion

From a biodiversity – ecosystem functioning perspective, two aspects of our work deserve special attention: First, the species used in our experiment showed marked differences in temporal variability of litter production and in total litter fall throughout the whole dry season. Litter decomposition rates were also significantly different among species, strongly related to measures of litter quality, and species-specific decomposition varied among different diversity treatments, i.e. litter mass loss of a species was not the same in monoculture than in mixture. That is, the identity of species does matter for nutrient cycling. Second, we found significant effects of tree species richness on total litter production with indications for overyielding in litter yields at the intermediate diverse level. In contrast, litter decomposition and C and N dynamics were not affected by tree species richness, and there were no synergistic or antagonistic effects on decomposition when litter diversity increased from one to three and six species. Thus, effects of tree species richness on productivity (indicated by leaf litter production) and nutrient cycling (indicated by decomposition as one important aspect) are decoupled at this early stage of our tree diversity experiment, and given the limitations of our methodologies (no litter collection throughout the whole year, litter decomposition trials for 116 days only, confounding effects of environmental heterogeneity). Taken together, these results highlight the importance of species-specific effects and the temporal and spatially dynamic nature of litter inputs and decomposition processes, with complex consequences for biogeochemical cycles along a gradient of tree diversity beyond those reported here: While our decomposition experiment started at a fixed time with a constant total litter mass, we expect that variation in the timing and amount of litter production will lead to temporal changes in the physico-chemical environment of the litter layer and activity of the decomposer community that differ from those observed in monocultures. In addition, while our study covered only one period of high litterfall, the two-year study by Montagnini et al. (1993) demonstrated that interspecific differences in litterfall may also change during different years. Thus, interannual variability adds to the complexity of tree species effects on litter production and decomposition. Finally, in diverse stands, litter will not homogeneously be mixed on the forest floor so that local

patches of highly degradable or more recalcitrant litter will form a spatial mosaic with small-scale differences in nutrient dynamics (Burghouts et al. 1998), further modulated by species-specific nutrient uptake patterns of the plants. Such diversity effects are rarely covered by standard litter bag approaches, asking for more complex and comprehensive experiments mimicking more closely natural variability in litter production and the processes involved in decomposition, mineralisation and formation of soil organic matter.

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