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C, N and P fertilization in an Amazonian rainforest supports stoichiometric dissimilarity as a driver of litter diversity effects on decomposition

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Plant leaf litter generally decomposes faster as a group of different species than when individual species decompose alone, but underlying mechanisms of these diversity effects remain poorly understood. Because resource C : N : P stoichiometry (i.e. the ratios of these key elements) exhibits strong control on consumers, we supposed that stoichiometric dissimilarity of litter mixtures (i.e. the divergence in C : N : P ratios among species) improves resource complementarity to decomposers leading to faster mixture decomposition. We tested this hypothesis with: (i) a wide range of leaf litter mixtures of neotropical tree species varying in C : N : P dissimilarity, and (ii) a nutrient addition experiment (C, N and P) to create stoichiometric similarity. Litter mixtures decomposed in the field using two different types of litterbags allowing or preventing access to soil fauna. Litter mixture mass loss was higher than expected from species decomposing singly, especially in presence of soil fauna. With fauna, synergistic litter mixture effects increased with increasing stoichiometric dissimilarity of litter mixtures and this positive relationship disappeared with fertilizer addition. Our results indicate that litter stoichiometric dissimilarity drives mixture effects via the nutritional requirements of soil fauna. Incorporating ecological stoichiometry in biodiversity research allows refinement of the underlying mechanisms of how changing biodiversity affects ecosystem functioning.

1. Introduction

Litter decomposition is a key process in the regulation of carbon and nutrient cycling, and it is controlled by multiple factors, including climate, soil, litter quality (i.e. chemical and physical characteristics of litter) and soil biota [1–3]. Among these, litter chemical quality explains most of the variation in leaf litter decomposition rates at the global scale [4], with consistent litter chemical quality driven differences among litter types across large environmental gradients [5]. While innumerable studies assessed decomposition using litter species individually, it was only recently more widely recognized that mixtures of litter species together decomposed faster [6,7]. These mixture effects are mostly synergistic, with up to 65% higher litter mass loss in mixtures compared with the arithmetic mean of the component species decomposing singly [6]. Mass loss rates in litter mixtures can even exceed those of the most rapidly decomposing single litter species [8], referred to as ‘transgressive overyielding’ in the biodiversity–ecosystem functioning literature. Such large diversity effects on decomposition are an important component in biogeochemical cycling, yet the underlying mechanisms of the documented litter mixture effects are still poorly established [9].

Recently, Handa *et al.* [10] proposed a general mechanism for positive litter mixture effects on decomposition with an experimental study across five

biomes and distinct aquatic and terrestrial ecosystems. They found evidence for N transfer, from N-rich litter of N-fixing plants to litter of deciduous plants of lower N concentration, that correlated with faster decomposition in litter mixtures containing both of these two plant functional types. However, they pointed out that the apparent N transfer did not simply depend on the [N] gradient between different litter types, as other plant functional types of similar and even larger differences in [N] showed no such pattern [10]. Rather, Handa *et al.* [10] argued that the N transfer depended on other litter quality properties such as the relative availability of additional resources like carbon (C) that are required by decomposer organisms at the same time. Accordingly, the direction and amount of particular resources transferred from one litter type to another may be determined by decomposers in order to improve their energetic and nutritional balance. This is in line with the theory of ecological stoichiometry stating that the ratios of elements, and particularly that of the key elements C, N and P controls growth and activity of decomposers to an important degree [11–13]. C:N:P stoichiometry of microbial decomposer communities is much narrower than that of their plant litter substrates [14], and the combined use of different elements provided by different litter types in mixtures should stimulate decomposer growth and would lead to faster decomposition of litter mixtures. Consequently, the divergence in litter stoichiometry of component species should be a strong predictor of diversity effects on litter mixture decomposition [15]. However, only very few studies explicitly tested the effect of stoichiometric, or more generally, functional divergence of litter mixtures on decomposition [8], and only one of them created a gradient of litter mixture C:N:P stoichiometry [16].

According to the theoretical prediction developed above, Hättenschwiler & Bracht Jørgensen [16] reported increasing litter mass loss with increasing dissimilarity in C:N:P stoichiometry of litter mixtures of tropical tree species. Interestingly, the positive correlation between stoichiometric dissimilarity and litter mass loss was observed in the presence of soil macrofauna, but not when fauna was excluded. This result suggests that fauna plays a key role in litter diversity effects on decomposition, at least in this neotropical study system. Although decomposition is largely driven by microorganisms, fauna can contribute significantly [17], particularly in tropical rainforests [3], and litter-feeding fauna has to deal with similar stoichiometric constraints as microbial decomposers do [18]. Because fauna is more mobile than microorganisms, they can more easily switch between individual leaves of different plant species which might explain a stronger impact of stoichiometric dissimilarity of litter mixtures on mixture decomposition when fauna is present [16]. However, owing to limiting data, the importance of stoichiometric heterogeneity in mixtures of different litter species for decomposers and for litter decomposition is at present difficult to evaluate. Moreover, the positive correlation between stoichiometric dissimilarity of litter mixtures and their decomposition in the study by Hättenschwiler & Bracht Jørgensen [16] was relatively weak compared with the positive effect of the availability of labile C in litter mixtures. It is reasonable to expect that the strength of the effects of C:N:P dissimilarity on litter mixture decomposition may depend on the overall availability (including external resources) of any of the three elements considered.

The experimental manipulation of C, N and P availability through fertilization combined with a gradient of litter

inherent C:N:P stoichiometry created by a wide range of different litter mixtures would provide a straightforward test of the relative importance of the absolute and relative availability of different resources. We expect that the external addition of C, N and P would diminish the importance of litter mixture stoichiometric heterogeneity for decomposers, but we are not aware of any previous test of this expectation. We designed a decomposition experiment in the Amazonian rainforest of French Guiana using different leaf litter types from six tropical tree species that vary widely in initial C:N, C:P and N:P ratios. From these six leaf litter types, a total of 14 different litter mixtures were constructed in order to create a large gradient of stoichiometric dissimilarity. The litter mixtures decomposed in the field within a fully factorial C (cellulose), N (urea) and P (phosphate) fertilization experiment [19]. Because of the supposed importance of soil fauna in dealing with stoichiometrically contrasted resources, we also included a fauna treatment in our study. We hypothesized that: (i) synergistic litter mixture effects on decomposition increase with stoichiometric dissimilarity of leaf litter mixtures, (ii) synergistic litter mixture effects in response to stoichiometric dissimilarity are mostly driven by the more mobile fauna, and (iii) C, N and P fertilization diminishes the importance of stoichiometric dissimilarity and hence neutralizes the synergistic litter mixture effects.

2. Material and methods

(a) Study site and fertilization design

The experiment was carried out in an undisturbed Amazonian rainforest at the Paracou experimental station in French Guiana (5°18' N, 52°53' W). The site is characterized by a tropical wet climate with an average annual temperature of 25.7°C (1971–2001) and an average annual precipitation of 3041 mm (1971–2001). Intra-annual variation is small for temperature (less than 2°C in monthly averages), but considerable for precipitation, with less rainfall (less than 100 mm per month) during two rather dry periods in March and from mid-August to mid-November [20]. The soil of the study area is characterized as acrisol developed over a Precambrian metamorphic formation called the Bonifacio-series. The tree species richness is around 140 species per hectare with a mean density of 620 individual trees per hectare (individuals of a diameter more than 0.1 m at breast height) [21].

A full factorial fertilization experiment with C, N and P additions in all possible combinations, including a control treatment (no fertilization) was initiated in April 2009 [19]. These treatments were replicated in each of a total of five blocks arranged in a randomized complete block design. Blocks were separated among each other by 50–300 m in a rather homogeneous area of about 2.5 ha on even terrain. Individual treatment plots within blocks covered an area of 30.25 m² (5.5 × 5.5 m), with at least 5 m distance between neighbour plots.

Five random soil samples (top 8 cm) per plot within each block were collected before fertilizer application in February 2009. Average values across blocks were 4.4 ± 0.2 for pH, 74.2 ± 5.5% for the sand fraction, 19.7 ± 3.9% for the clay fraction, 22.1 ± 5.4 mg total C g⁻¹ dry soil, 1.5 ± 0.3 mg total N g⁻¹ dry soil and 0.1 ± 0.027 mg total P g⁻¹ dry soil (see [22] for detailed soil characterization).

Fertilizers were added in the form of cellulose for C (commercial substrate Waterspare, Celliob industry, France), as coated urea ((NH₂)₂CO) for N, and as [KH₂PO₄] for P [19]. The amount of fertilizers were chosen in order to allow for comparison among different fertilization studies in tropical rainforests [23–25], with 1405 kg C ha⁻¹ yr⁻¹, 130 kg N ha⁻¹ yr⁻¹ and 69 kg P ha⁻¹ yr⁻¹.

These amounts correspond roughly to half (C), double (N) and 50-fold (P) of the total natural annual input via leaf litter fall at our study site [21,26]. Starting in April 2009, fertilizers were applied twice a year during the drier periods of the year (from mid-August to mid-November and in March–April) in order to avoid lateral and vertical wash off during heavy rainfalls during the wet seasons.

(b) Plant material

Leaf litter from the six tree species *Carapa procera* (Aublet), *Goupia glabra* (Aublet), *Hymenaea courbaril* (Linnaeus), *Platonia insignis* (Martius), *Simarouba amara* (Aublet) and *Vochysia tomentosa* (G. Mey) were chosen based on their wide variation in C : N, C : P and N : P ratios. (See the electronic supplementary material, appendix 1, for more details on litter quality.) Freshly fallen leaf litter was collected for each species in a tree plantation adjacent to the natural rainforest as described in more details in [19].

(c) Stoichiometric dissimilarity of litter mixtures

Litter mixtures were constituted from the six above-mentioned litter species and comprised mixtures of two, three, four and all six species in addition to all single species treatments. Species composition in mixtures was determined so as to maximize the stoichiometric dissimilarity between the least and the most dissimilar mixture (see below). In addition, litter species were equally often represented within each level of litter species richness: each species was present exactly in two 2-species, two 3-species and two 4-species mixtures, additionally to the single 6-species mixture. This equilibrated design allows us to disentangle the effects of species number from that of mixture composition and to explore the effects of the presence/absence of each litter species within mixtures.

The stoichiometric dissimilarity of litter mixtures was determined using the Rao's quadratic entropy [15,27] that integrates the average pairwise distances between n component species of mixtures as well as species relative abundances computed as follows:

$$\text{Rao}_{ij} = \sum_{i=1}^n \sum_{j=1}^n \rho_i \rho_j d_{ij},$$

where ρ_i and ρ_j are the relative abundance by biomass of species i and j , respectively, and d_{ij} , the stoichiometric dissimilarity coefficient based on Euclidean distance between two species i and j . Euclidean distances were calculated from the C : N, C : P and N : P ratios for all possible combinations of the six-litter species in order to determine the specific mixtures used in our experiment according to the two criteria defined above (wide dissimilarity gradient and equal species representation). The selected 14 different litter mixtures (six 2-species, four 3-species, three 4-species and one 6-species mixtures) represented a range of stoichiometric dissimilarity with Rao values ranging from 0.01 to 0.54 (electronic supplementary material, appendix 2).

(d) Litterbag construction

Two different types of 0.15×0.15 m litterbags were constructed in order to allow or prevent the access of fauna. Mesh bags of $68 \mu\text{m}$ (68PES4/135, DIATEX, St-Genis-Laval, France) were used to exclude meso- and macrofauna, and 8 mm mesh bags (F.1004, DIATEX, St-Genis-Laval, France) to allow fauna access to the litter material. To avoid losses of litter fragments passing the large mesh width litterbags, the soil surface-facing side of the litterbag was made of 0.5 mm mesh. The fauna community at our study site is roughly characterized by an abundance of about 650 individuals m^{-2} of microbio-detritivorous mesofauna dominated by acari (96%), and of about 1750 individuals m^{-2} of detritivorous macrofauna with isoptera (34%) and diptera (26%) as the two largest groups [10]. Each litterbag was filled

with 8 g of air-dried litter, with mixtures containing equal mass proportions of the component species. One of each of the 40 different litterbag types ((6 monospecific + 14 mixtures) \times 2 mesh sizes) was placed randomly in each of the treatment plots of all five blocks (see above), yielding a total of 1600 litterbags (40 litterbag types \times 8 fertilization treatments \times 5 blocks). Litterbags were fixed with wire directly on the soil surface from which the naturally occurring litter was previously removed between 3 and 11 September 2009 (one month before the second fertilization event). All litterbags were retrieved after a total of 158 days of field exposure between 9 and 17 February 2010, in the same order as they had been placed in the field. Upon harvest, remaining litter was gently rinsed with tap water to remove adhering plant root parts, invertebrates and soil particles, dried at 65°C to constant weight and weighed to determine the remaining litter mass.

(e) Data analysis

Litter mass loss was expressed as a percentage of initial litter dry mass (oven-dry mass was determined from sub-samples of the initial air-dried litter material). Litter mass loss data were log transformed before analyses to meet the requirements of heteroscedasticity and normality. Expected mass loss (E) of litter mixtures was calculated as the mean mass loss of the component species decomposing singly in the corresponding fertilizer and fauna treatments. The resulting five data points (one replicate from five blocks) for expected mass loss for each treatment combination were averaged for robust comparisons with observed litter mixture mass loss. Paired Student's t -test was used to test whether observed mass loss differed from expected mass loss across the entire dataset. The relative mixture effect for each individual litterbag was calculated as the ratio of ((observed – expected)/expected) mass loss, according to Wardle *et al.* [28].

We used one-sample Student's t -tests for each type of litter mixture (in absence and in presence of fauna) to test whether relative mixture effects were significantly different from zero in the control treatment (without any fertilization). One-sample Student's t -tests were also used to test whether relative mixture effects for each different type of mixtures in presence and in absence of fauna significantly differed from 0 across all fertilization treatments. We used an analysis of variance (type I) to test for the effects of fertilization (all fertilizer treatments included as individual levels of this factor), fauna presence/absence and litter species diversity, their interactions and block effects on observed mass loss. Litter species richness (from one to six species) and species composition (the 20 different litter mixtures) were not considered independent of one another (the sum of these two terms corresponds to the total litter diversity effect). The same model was run for the relative mixture effects (with species richness levels from two to six species). To investigate the effects of litter species presence within mixtures combined with C, N and P fertilization, we used an analysis of variance with the presence/absence of each species in mixtures (comparison of all mixtures that included a given litter species with all mixtures that did not include this species), the presence/absence of C, N and P fertilizer (comparison of relative mixture effects in the plots that received a given fertilizer with that of plots that did not receive this fertilizer), and block as factors for each of the two mesh sizes of litterbags separately. The two- and three-way interactions between C, N and P fertilization were included but not four-way interactions between species presence/absence and C, N and P fertilization. If these interactions were not significant ($p > 0.05$), they were removed from the model and the analysis was run again until all remaining terms of interaction were significant. Tukey post hoc tests were used to explore multiple comparisons of interaction terms. Relationships between stoichiometric dissimilarity and the relative mixture effects were explored with linear regression analyses for the control treatment

Table 1. Analysis of variance to test the effects of litter diversity (decomposed in species richness (Sr) and species composition (Sc)), presence/absence of fauna (F), fertilization treatments (Fz) and their interactions, on observed mass loss and on relative mixture effects. (Levels of significance are indicated as asterisks. Third-order interactions between species richness (or species composition), fauna and fertilization were removed because they were not significant. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.)

	mass loss			relative mixture effect		
	d.f.	F-value	p-value	d.f.	F-value	p-value
species richness (Sr)	4	39.21	***	3	1.66	0.17
species composition (Sc)	15	62.61	***	10	2.31	*
fauna (F)	1	1080.08	***	1	248.97	***
fertilization (Fz)	7	67.17	***	7	4.9	***
Sr × F	4	15.32	***	3	1.59	0.19
Sc × F	15	24.46	***	10	2.06	*
Sr × Fz	28	0.52	0.98	21	0.47	0.98
Sc × Fz	105	1.42	**	70	0.8	0.88
F × Fz	7	9.37	***	7	2.56	*
block	4	13.22	***	4	25.9	***

and for each fertilizer treatment, as well as for the overall mean across all fertilizer treatments. All statistical analyses were run using the R software [29]. Levels of significance are indicated as * $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$.

3. Results

(a) Litter diversity, fertilization and fauna effects on litter mass loss

Litter mass loss was affected by all three main factors; litter diversity, fauna presence and fertilization (table 1). The presence of fauna had the strongest effect among all factors and interactions and led to a 46% greater mass loss than when fauna was excluded. Both components of litter diversity, species richness and species composition, had significant effects on mass loss, and both terms interacted significantly with the presence of fauna (table 1). Litter mass loss increased with species richness in the presence of fauna, but was not affected by species richness when fauna was excluded. However, the species richness effect was exclusively the result of a lower mass loss of single litter species (average mass loss of 48%), compared with litter mixtures, without any differences among the treatments with 2, 3, 4 and 6 litter species (average mass loss of 56%). The strong, mostly fauna-driven, effect of litter mixing on decomposition resulted in a significantly higher observed mass loss compared to the expected mass loss calculated from the component species decomposing singly (figure 1; paired t -test between observed and expected mass loss: $t_{1257} = 23.2^{***}$). The litter species composition effect remained significant irrespective of whether fauna had access or not, but the different single litter species and mixtures responded differently to fauna presence. Litter mass loss was higher with fertilization, but the fertilization effect depended on litter species composition and on the presence of fauna (table 1). The magnitude of fertilization effects on mass loss differed according to the different single litter species and mixtures. Fauna presence generally increased the fertilization effect on litter mass loss,

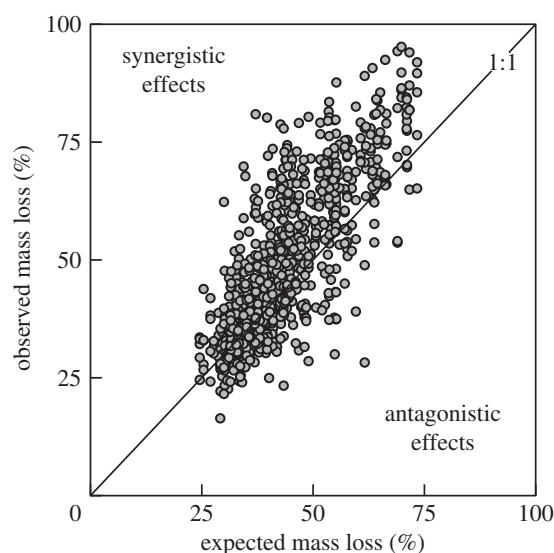


Figure 1. Observed mass loss of litter mixtures as a function of expected mass loss (calculated as the average mass loss based on component species decomposing singly) across all treatments (litter diversity, absence/presence of fauna and CNP fertilization) in an Amazonian rainforest. The 1:1 line defines identical observed and expected mass loss values. Each point represents an individual litterbag.

independently of litter species composition (the third-order interaction was not significant).

(b) Litter diversity, fertilization and fauna effects on relative mixture effects

The higher observed mass loss of mixtures than expected when species decompose singly (figure 1) resulted in an overall relative mixture effect of +10.5% across all litter mixtures and across all treatments. However, the relative mixture effect varied significantly among different litter mixtures (species composition effect, table 1), between fauna treatments and among fertilization treatments (table 1 and figure 2). Again, fauna had by far the strongest effect among the different factors

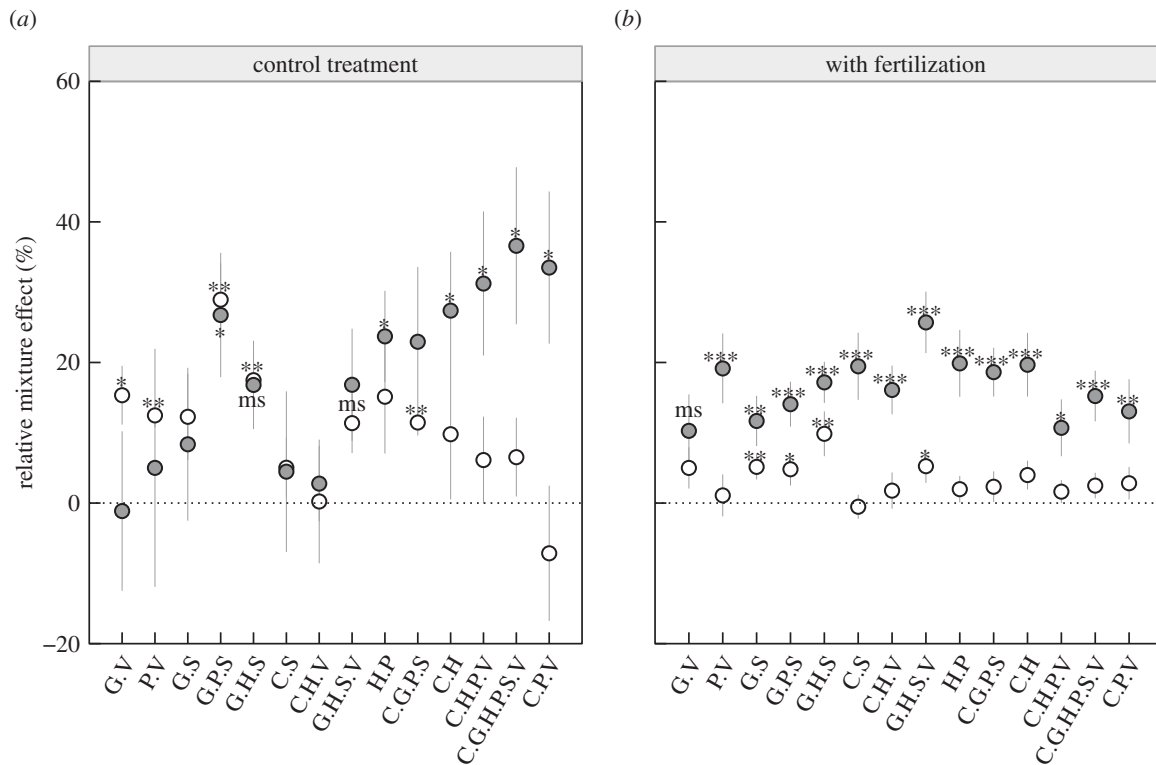


Figure 2. Relative mixture effects ((observed mass loss – expected mass loss)/expected mass loss) \times 100 without and with fauna for each individual litter mixture (mean \pm s.e.) in the control treatment without fertilization ((a), $n = 5$) and across all fertilization treatments ((b), $n = 35$). Litter mixtures are arranged from left to right according to increasing soil fauna effects in the control treatment. Grey circles indicate data in the presence of fauna (corresponding to coarse mesh bags) and white circles indicate data in the absence of fauna (corresponding to fine mesh bags). Asterisks indicate a significant relative mixture effect, i.e. a significant deviation from 0 ($*p < 0.05$, $**p < 0.001$, $***p < 0.0001$); 'ms' indicates marginally significant values ($0.05 < p < 0.06$). C, *Carapa*; G, *Goupia*; H, *Hymeneae*; P, *Platonia*; S, *Simarouba*; V, *Vochysia*.

and it interacted significantly with litter species composition and fertilization.

Across all litter mixtures and fertilization treatments, the average relative mixture effect was +16.7% in the presence of fauna compared to only +4.3% in its absence. According to the significant species composition \times fauna interaction, the fauna effect depended on the species composition of litter mixtures (table 1 and figure 2). In the presence of fauna, litter mixtures that contained *Hymeneae* and *Simarouba* leaf litter, slightly but significantly increased the overall positive mixture effects by 3.2% and 1.8% on average, respectively (table 2). The same trend, close to significance, was also observed for the presence of *Platonia* litter. Without fauna, litter mixtures with *Carapa* and *Goupia* leaf litter had the greatest influence on mixture effects (table 2). When *Carapa* was included, the relative mixture effects were weaker (+2.4% on average), compared with the mixtures that did not contain *Carapa* (+6.2% on average). By contrast, when *Goupia* litter was included in the mixtures, the relative mixture effects were higher (+6.2% on average) than in its absence (+2.3% on average).

The addition of C, N and P fertilizers modified the relative mixture effects also in interaction with fauna (table 1 and figure 2). Relative mixture effects ranged from -3.4% (N treatment) to +5.2% (C N P treatment) without fauna and from +11.2% (C N treatment) to +23.7% (C treatment) with fauna.

When fauna did not have access to decomposing litter, N supply significantly altered relative mixture effects (table 2). This N effect was negative, i.e. the relative mixture effects were smaller with N fertilization compared with treatments without N (figure 3). When N was added singly, the average relative mixture effect shifted to clearly antagonistic effects (-3.4% on

average) that were significantly lower than zero ($t_{69} = -2.9^{**}$). These antagonistic effects with N fertilization were somewhat counteracted by an additional fertilization with P or C, when fauna was absent (significant interaction term of N with P and C fertilization, table 2). The interaction between *Hymeneae* litter and C fertilization (table 2) revealed that without C, the presence of *Hymeneae* in mixtures significantly increased mixture effects (from +2.3% without *Hymeneae* to +5.9% with *Hymeneae* in all non-C fertilized plots) while it tended to decrease mixture effects with C fertilization.

N inhibition of the relative mixture effect persisted when soil fauna had access to the litterbags. The size of the N effect was similar compared to the treatment without fauna (figure 3), with a mean relative mixture effect that decreased from 18.4% without N fertilization to 14.9% on average across all N-fertilized plots (table 2 and figure 3). Unlike in the treatment without fauna, an additional fertilization with P or C did not counteract the negative N fertilization effect on relative mixture effects (non-significant interaction terms, table 2). Fertilization with P only did not change the relative mixture effects on mass loss (table 2 and figure 3) but the effect of P fertilization was influenced by the presence of *Platonia* litter in the presence of fauna (table 2). Without P, mixture effects were higher when *Platonia* was in the mixture (+20% on average) compared with mixtures without *Platonia* (+14.7%), whereas the presence of *Platonia* significantly decreased relative mixture effects with P fertilization (from +18.6% to +13.7%). Unlike in the treatments without fauna access, C fertilization significantly increased the relative mixture effect from +15.5% without C fertilization to +17.9% with C fertilization (table 2 and figure 3). This C fertilization

Table 2. Analysis of variance to test for the effects of the presence of each litter species and of the occurrence of C, N or P fertilization on relative mixture effects within each fauna treatment. (Fine mesh bags excluded meso- and macrofauna, whereas coarse mesh bags allowed meso- and macrofauna access. Levels of significance are indicated as asterisks. An initial model was computed including all interactions among C, N and P supply and second-order interactions between species and each resource (C or N or P). Only interactions that accounted for significant variation in relative mixture effects were kept in the final model. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.)

without fauna				with fauna			
	d.f.	F-value	p-value		d.f.	F-value	p-value
<i>Carapa</i>	1	12.36	***	<i>Carapa</i>	1	0E + 00	0.99
<i>Goupia</i>	1	6.78	**	<i>Goupia</i>	1	0.3	0.59
<i>Hymenea</i>	1	1.67	0.19	<i>Hymenea</i>	1	9.25	**
<i>Platonia</i>	1	0.53	0.47	<i>Platonia</i>	1	3.43	0.06
<i>Simarouba</i>	1	0.65	0.42	<i>Simarouba</i>	1	6.46	*
<i>Vochysia</i>	1	0.94	0.33	<i>Vochysia</i>	1	0.19	0.66
C	1	0.08	0.77	C	1	9.19	**
N	1	11.38	***	N	1	7.6	**
P	1	0.71	0.4	P	1	0.01	0.92
C × N	1	12.7	***	<i>Platonia</i> × C	1	5.42	*
N × P	1	6.29	*	<i>Platonia</i> × P	1	7.9	**
<i>Hymenea</i> × C	1	7.93	**	block	4	17.75	***
block	4	38.54	***				

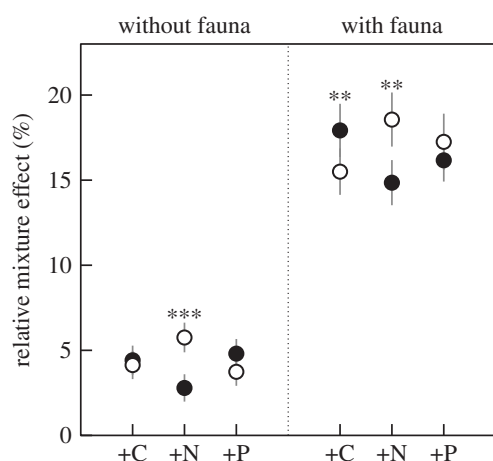


Figure 3. Relative mixture effects from the factorial-C N P fertilization experiment without and with fauna (mean \pm s.e.). Relative mixture effects were calculated as (observed mass loss – expected mass loss)/expected mass loss \times 100. Dark circles represent data from fertilized plots and white circles represent data from plots that were not fertilized with C, N or P, respectively. Asterisks denote significant differences in relative mixture effects on mass loss from plots with or without fertilization with a particular fertilizer (** $p < 0.001$, *** $p < 0.0001$).

effect interacted with the presence of *Platonia* (table 2). The response to C fertilization was substantially higher in the absence of *Platonia* (+20.4% without *Platonia* to +15.4% with *Platonia* in all C fertilized treatments).

(c) Stoichiometric dissimilarity of litter mixtures

Without fauna, the relative mixture effects did not correlate with the stoichiometric dissimilarity of litter mixtures, regardless of the fertilization treatment (data not shown). However, in the presence of fauna and for non-fertilized litter mixtures, the

relative mixture effects significantly increased with the stoichiometric dissimilarity of the litter mixtures ($r^2 = 0.52$, $p = 0.005$; figure 4; excluding the *Platonia*–*Vochysia* mixture that appeared as an outlier). The correlation remained significant when the outlier *Vochysia*–*Platonia* mixture was included ($r^2 = 0.29$, $p = 0.049$). The positive correlation between relative mixture effects and stoichiometric dissimilarity disappeared with fertilization (figure 4 for the average relative mixture effects across all fertilization treatments), except for the C N P treatment for which the relative mixture effects correlated marginally negatively with stoichiometric dissimilarity of the mixture ($r^2 = 0.20$, $p = 0.057$, data not shown).

4. Discussion

We observed clear and mostly synergistic litter mixture effects on decomposition (figures 1 and 2), in line with the majority of litter diversity experiments in various ecosystem types [6,7] and a recent study across a latitudinal gradient [10]. Mass loss of litter mixtures was up to 51% higher than expected from the respective litter species decomposing singly, with an overall average stimulation of 10.5%. These litter mixture effects depended on species composition, but were unrelated to the number of litter species included in the mixtures in accord with previous studies [8,10,16,30–32]. In line with our hypothesis, soil fauna played a key role as a driver of litter mixture effects (figure 2), with 16.7% more rapid decomposition of litter mixtures than expected, compared with 4.3% when fauna was excluded. Independently of litter diversity, the strong impact of soil fauna on decomposition (previously documented at our study site [33,34]) is generally in line with the idea that fauna contributes comparatively more to decomposition in tropical ecosystems than in ecosystems at higher latitudes [3,17,35]. The contribution of soil fauna to litter

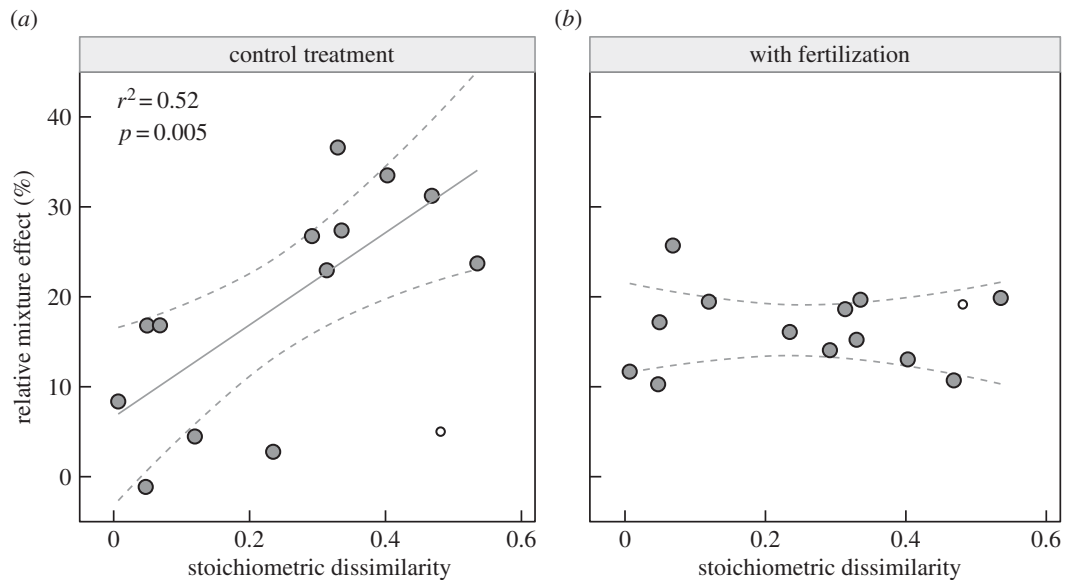


Figure 4. Mean relative mixture effect as a function of litter mixture stoichiometric dissimilarity in presence of fauna in the unfertilized control treatment ((a), $n = 5$), and in all fertilization treatments ((b), $n = 35$). Stoichiometric dissimilarity was calculated as the Rao index from initial leaf litter C : N, C : P and N : P ratios of the individual species contained in mixtures. The solid line indicates the linear regression line (if slope is significantly different from zero) and dashed lines illustrate 95% CIs of regression lines. The mixture *Platonia*–*Vochysia* (small white circle) was excluded from calculations (see text).

mixture decomposition has rarely been considered in previous studies, but the few extra-tropical studies specifically testing for soil fauna effects found that non-additive effects on litter mixture decomposition were mostly attributed to the presence of fauna [36–40]. Fauna could reinforce the litter mixtures effects directly through higher litter consumption in mixtures (owing to complementary uptake of resources from a mixed diet [36] and/or as a result of diluted inhibitory compounds [41]) and/or indirectly by altering the microbial decomposer activity.

In support of complementary uptake of different resources by litter-feeding fauna in litter mixtures, and in line with our initial hypothesis, we reported increasing relative mixture effects with increasing stoichiometric dissimilarity of litter mixtures in the presence of fauna (figure 4). This positive correlation suggests that a greater heterogeneity of leaf litter C : N : P stoichiometry in mixtures improves fauna access to an equilibrated availability of C, N and P, consequently leading to a greater resource use and therefore accelerated litter mass loss. In line with our study, Liu *et al.* [42] used paired litter mixtures of grassland species that differed in N and P concentrations and showed that initial differences in N and P content partly explain synergistic effects in a field experiment including soil fauna (body width of less than 1 mm).

We acknowledge that the leaf litter varies in many other traits than their C : N : P stoichiometry, to which fauna might be sensitive as well. For example, Hättenschwiler & Bracht Jørgensen [16] showed that C quality of litter mixtures explained more variation in mixture decomposition than stoichiometric dissimilarity. Moreover, when fauna was excluded in our study, we could not confirm the positive correlation between stoichiometric dissimilarity of litter mixtures and mixture effects on decomposition. Microbial decomposers may not respond to stoichiometric dissimilarity at the comparatively large spatial scale of leaf litter mixtures even though they show essentially the same discrepancy between resource stoichiometry and their own biomass stoichiometry as litter-feeding fauna [14,18]. In a laboratory experiment, Vos *et al.* [43] showed that litter mixture effects in the

presence or absence of one species of macrodetritivore depended on different aspects of nutritional dissimilarity of mixtures. Litter mixture effects caused by the microdetritivore community (including soil mesofauna and also microorganisms) correlated with P dissimilarity, whereas mixing effects caused by macro-detritivore addition correlated with N dissimilarity. Thus, the extent to which litter quality explained mixture effects depended on the type of nutrient limitation of decomposer organisms [43]. In our study site, we showed that litter decomposition was co-limited by N and P both in the absence or presence of soil fauna [19]. However, we reported a stronger N P fertilization effect on litter mass loss in the presence of soil fauna than when fauna was excluded, suggesting that soil fauna might be more strongly limited by N and P than microorganisms. The extent to which the different components of decomposer community was N P co-limited in our study may partly explain why mixture effects correlated with stoichiometric dissimilarity only in the presence of soil fauna.

By adding the elements C, N and P with our fertilization treatments, we could test our prediction that the importance of litter mixture stoichiometric dissimilarity on relative mixture effects diminishes when these resources are no longer limiting. According to our hypothesis, the positive relationship between the stoichiometric dissimilarity and relative mixture effects on mass loss disappeared under any of the fertilization treatments (figure 4b). This suggests that decomposers took up at least part of these key resources independently of tree leaf litter, and complementary resource uptake from stoichiometrically dissimilar litters was then apparently less important. Such compensation by external resource availability is clearly supported by N fertilization that reduced the positive mixture effects on decomposition irrespective of the fauna treatment (figure 3). The only other two studies exploring interactive effects of litter diversity (though with only few mixtures and without considering stoichiometric dissimilarity) and nutrient fertilization we are aware of showed contrasting results. In an experiment in a temperate stream ecosystem using three different litter species [44], fertilization with N P

compensated resource heterogeneity within mixtures of litter, leading to the suppression of mixture effects under fertilization. However, in contrast to our study, these authors reported antagonistic effects under reference conditions (without fertilization), suggesting an entirely different mechanism underlying mixture effects and the impact of fertilization than in our study. In a Patagonian forest, Vivanco & Austin [45] reported faster decomposition than expected for a three-species litter mixture with N fertilization. They did not observe this positive litter mixture effect without N fertilization, but since they studied only one single litter mixture it is difficult to generalize from these results.

Compared to fertilization with N, the addition of C and P in our study had a less clear impact on overall litter mixture effects (figure 3). Fertilization with C increased overall litter mixture effects when fauna was present, but had no effect when fauna was excluded. It has previously been suggested that litter-feeding fauna at our study site might be limited by C availability and that the rate of decomposition would increase with increasing labile C [34]. In support of this hypothesis, fauna appeared to have preferentially used the cellulose added in our fertilization experiment, as cellulose addition decreased the absolute litter mass loss in the presence of fauna [19]. Here, we show that at the same time fauna stimulated the mixture effects when cellulose was added, suggesting stronger complementary resource use by litter-feeding fauna when C is less limiting. However, the cellulose effect was independent of stoichiometric dissimilarity of litter mixtures, but it was weaker in the presence of *Platonia* leaf litter (table 2). *Platonia* is particularly rich in labile C [16] which may suggest a relatively stronger effect of cellulose when the litter mixture's inherent availability of labile C is lower (i.e. in absence of *Platonia*).

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5. Conclusion

We showed that litter diversity, fauna and C N P fertilization interactively influenced litter decomposition. Litter diversity (mainly through a species composition effect) clearly increased litter decomposition in this highly diverse ecosystem, with soil fauna as the main driver of mixture effects. Importantly, litter mixture effects driven by soil fauna were predictable from C : N : P stoichiometry traits of litter species. The positive correlation between relative mixture effects and stoichiometric dissimilarity disappeared with C N P fertilization, supporting the prediction that litter stoichiometric dissimilarity drives mixture effects via the nutritional requirements of litter-feeding fauna. Our results show that integrating ecological stoichiometry in the study of how changing biodiversity affects ecosystem processes provides a conceptual framework for a mechanistic understanding of biodiversity effects across trophic levels.

Data accessibility. Data are available via the Dryad Repository (doi:10.5061/dryad.7gm28). Litter chemical composition of the six tropical tree species and stoichiometric dissimilarity of the litter mixtures are available in the electronic supplementary material.

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