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Nutrient scarcity strengthens soil fauna control over leaf litter decomposition in tropical rainforests

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Soil fauna is a key control of the decomposition rate of leaf litter, yet its interactions with litter quality and the soil environment remain elusive. We conducted a litter decomposition experiment across different topographic levels within the landscape replicated in two rainforest sites providing natural gradients in soil fertility to test the hypothesis that low nutrient availability in litter and soil increases the strength of fauna control over litter decomposition. We crossed these data with a large dataset of 44 variables characterizing the biotic and abiotic microenvironment of each sampling point and found that microbe-driven carbon (C) and nitrogen (N) losses from leaf litter were 10.1 and 17.9% lower, respectively, in the nutrient-poorest site, but this among-site difference was equalized when meso- and macrofauna had access to the litterbags. Further, on average, soil fauna enhanced the rate of litter decomposition by 22.6%, and this contribution consistently increased as nutrient availability in the microenvironment declined. Our results indicate that nutrient scarcity increases the importance of soil fauna on C and N cycling in tropical rainforests. Further, soil fauna is able to equalize differences in microbial decomposition potential, thus buffering to a remarkable extent nutrient shortages at an ecosystem level.

1. Introduction

More than 90% of the net primary production of global terrestrial ecosystems is channelled into the detrital food web [1], and soils store the majority of the Earth's organic carbon (C) [2]. Identifying the drivers of organic matter decomposition is therefore crucial to understanding and predicting global ecosystem functioning. Abiotic factors like climate and litter quality have traditionally been recognized as the dominant controls on decomposition at large spatial scales, while decomposer organisms would operate as additional, but secondary, local agents [3,4]. Recent evidence, however, indicates that the effect size of microbial biomass on decomposition rates can be equivalent to that of soil temperature and litter moisture, suggesting that biotic factors may

Table 1. Characteristics of the study sites. (Values are means ± s.e. ($n = 120$, except $n = 24$ for tree-community data). Elemental ratios are mass-based. AlkP refers to maximum potential activity of alkaline phosphatase in litter (μmol pNP g^{-1} h^{-1}). Tree species richness refers to the mean number of species per plot while functional richness is a unitless standardized effect size of the convex hull volume defined by six foliar traits. Between-site differences are based on linear mixed-effect models, with site and topography as fixed factors and sampling point within each plot as a random effect. See text and the electronic supplementary material for further details. $^{**}p < 0.01$; $^{***}p < 0.001$.)

	Nouragues	Paracou
coordinates	04°04′53″ N	05°16′38″ N
	52°41′13″ W	52°55′38″ W
soil type (FAO)	sandy podzols and acrisols	sandy podzols and acrisols
MAT (°C)	25.2	25.8
MAP (mm)	3280	2849
aboveground biomass (t ha^{-1})	423 ± 44	371 ± 20
litter pool (g m^{-2})	1259 ± 40	1265 ± 54
foliar N (%)	2.05 ± 0.01	1.93 ± 0.01***
litter N (%)	1.49 ± 0.03	1.32 ± 0.18**
foliar C : N	25.21 ± 0.09	26.28 ± 0.12***
litter C : N	33.53 ± 0.80	37.14 ± 0.70**
litter AlkP activity	73.73 ± 4.75	33.58 ± 2.29***
arthropod density (id m^{-2})	477 ± 28	536 ± 32
tree species richness	38 ± 2	32 ± 1**
tree functional richness	−0.09 ± 0.12	−0.11 ± 0.08

explain as much or even more variation than climate in multi-site comparisons, thus questioning such a hierarchical model of litter decomposition [5–7]. In addition, soil fauna has recently been reported to consistently increase the rates of litter decomposition across biomes by 37% [8] and losses in their functional diversity are expected to slow global cycling of C and nutrients [9]. Consequently, the role of biota (i.e. microorganisms and soil fauna) should attain a more central position in the biogeochemical models, to emphasize their ability to modulate the effects of the environment and a changing climate on organic matter decomposition [10–14].

Leaf litter fall is a dominant pathway for returning nutrients to the soil [15], and soil fauna plays a fundamental and often undervalued role in the litter decomposition process [9,16]. Assemblages of soil animals stimulate litter breakdown by a variety of interconnected mechanisms that alter the composition and performance of the microbial community, which ultimately transform complex plant-derived compounds into CO_2, mineral and organic nutrients and humus [12,13,16]. Despite their identification as key agents of organic matter decomposition, the interaction between soil fauna with litter traits, and particularly, with the soil microenvironment has remained elusive so far. A descriptive example is the hypothetical link between litter quality and the contribution of soil fauna to decomposition. Through selective feeding, soil invertebrates could preferentially increase the decomposition of litter with a low C to nitrogen (N) or C to phosphorus (P) ratio (C : N and C : P, respectively), i.e. litter with a high nutritional value [17,18]. Other studies, however, have suggested that the primary effect of soil fauna is precisely to promote the decomposition of low-quality litter [19–23]. Likewise, a landmark study documented that increasing diversity of leaf litter within a litterbag substantially enhanced the rate of disappearance of the more recalcitrant litter types, but only in the presence of soil fauna, suggesting that animals could stimulate the effects of litter diversity through a top-down mechanism [24]. Notwithstanding, evidence supporting this hypothesis is still sparse and comes from single-site or laboratory-based microcosm experiments [25,26], which may underestimate the large small-scale variability of decomposition rates in natural conditions [5,27]. Moreover, the nutritional status of the soil and the litter microenvironment may affect microbial communities and interact with soil fauna influencing its contribution to decomposition [28]. For instance, the decomposition of low-quality litter may be bottom-up controlled, especially in nutrient-poor environments, thus being more dependent on the fragmentation and the microbial stimulation driven by soil fauna [18,28–31]. Still, multi-site litterbag decomposition studies often fail to incorporate high enough within-site replicates along with data of environmental features like nutrient availability measured at the same spatial and temporal grain, therefore masking underlying local variability and hampering our ability to identify alternate regulatory factors [7].

We hypothesize that low nutrient concentrations in the litter substrate and in the surrounding litter and soil microenvironment should increase the importance of soil fauna promoting decomposition. To test this hypothesis, we conducted a litterbag experiment replicated at two rainforest sites in the Guiana shield (table 1), and additionally including a high within-site replication to take into account the natural biogeochemical variability typically associated with the topography in these nutrient-poor ecosystems [32,33]. To determine the contribution to the loss of litter mass by mesofauna alone and by meso- plus macrofauna (i.e. invertebrates with body widths smaller and larger than 2 mm, respectively

[3]), we used litterbags with three mesh sizes (hereafter referred to as microbes (less than 70 µm), mesofauna (less than 2 mm) and macrofauna (less than 7 mm) for clarity) and filled them with leaf litter substrates from two native tree species with contrasting C : P ratios and their combination: 1561 ± 337 for *Goupia glabra* Auble, and 2773 ± 307 for *Platonia insignis* Mart. [34] (hereafter referred as *Goupia* and *Platonia*). We focused on P because recent findings have indicated that this element is the predominant limiting nutrient for microbial decomposers in tropical forests [35,36]. Additionally, we also assessed the dependency of the effect of soil fauna on decomposition on a wide range of biotic and abiotic environmental factors, by compiling a unique dataset of 44 variables characterizing the below- and aboveground compartments (see the electronic supplementary material, table S1). These variables included soil and litter elemental compositions, activities of extracellular enzymes associated with CNP stoichiometry as indirect measures of the nutritional status of microbial communities [36,37], community-level metrics of functional foliar traits in tree canopies, and abundance and richness of the main orders of litter-dwelling arthropods. Importantly, all these potential regulatory factors were quantified—where appropriate—at the same spatial scale as our individual experimental observation unit (i.e. each block of litterbags). We included this environmental heterogeneity as a set of continuous covariates. Thus, we were able to test our hypothesis across the natural environmental gradients present in our study sites, from regional to local and to small within-plot spatial scales, and from low availability to extreme nutrient scarcity.

2. Material and methods

(a) Study sites and sampling design

This study was conducted in two primary tropical forests in French Guiana near the research stations of Nouragues (04° 04′53″ N, 52°41′13″ W) and Paracou (05°16′38″ N, 52°55′38″ W). Both sites have a mean annual temperature of 25.2 and 25.8°C and a tropical climate, with a wet season typically from December to June and a dry season from August to November. Rainfall at the annual scale is similar (2849 versus 3280 mm yr^{-1}), although Paracou has a more pronounced dry season owing to a higher evapotranspirational demand (mean precipitation and temperature during the driest quarter are 22.3 mm $month^{-1}$ and 26.3°C at Paracou versus 29.9 mm $month^{-1}$ and 25.7°C at Nouragues, respectively; electronic supplementary material, figure SM1). The bedrock at Paracou and Nouragues is Precambrian schist and Caribbean granite, respectively. Soil texture and biogeochemistry in tropical forests can fluctuate with topography owing to variations in drainage capacity and erosion, which are usually associated with topographic position. Soils between hills are nutrient-poor sandy podzols, with clay minerals (kaolinite) and oxides contents increasing towards the tops where acrisols dominate (O. Margalef *et al*. 2019, unpublished results). We established 12 plots of 0.25 ha at each site stratified by three topographic positions to account for this heterogeneity: at the top, at the middle and at the bottom between slopes (henceforth referred to as top, slope and bottom plots). We delimited a central 20 m quadrat in each plot where we marked five evenly spaced sampling points around which we focused all our measurements (electronic supplementary material, figure SM2). This design thus contained a total of 120 sampling points (2 sites × 3 topographic positions × 4 replicate plots per topography × 5 sampling points in each plot).

(b) Litterbag experiment

We assessed the contribution of invertebrate meso- and macrofauna (body widths smaller and larger than 2 mm, respectively) to the rates of litter mass loss using 10 × 10 cm polyamide litterbags differing in mesh size: 70 µm (PA-21-71 SEFAR NYTAL, Heiden, Switzerland) excluding both faunal groups but allowing microbes (i.e. fungi and prokaryotes) to decompose the litter substrates, and 2 mm (06-2000/53 SEFAR NYTEX, Heiden, Switzerland) and 7 mm (PE-01903-013 FIBERCORD, Alicante, Spain) allowing the entry of mesofauna and meso- plus macrofauna, respectively. The bottom layers of these latter litterbags with the largest opening size were made of 0.5 mm mesh [26] (06-500/38 SEFAR NYTEX) to prevent the loss of litter fragments. Each litterbag was filled with 2 g of dried leaf litter in three combinations: (i) only *Goupia*, (ii) only *Platonia*, and (iii) equal proportions by weight of both species. These native tree species were chosen because of their contrasting C : P and N : P ratios (1561 ± 337 and 36.9 ± 3.1 for *Goupia* versus 2773 ± 307 and 80.7 ± 1.3 for *Platonia*; mean ± s.e., data from [34]).

Freshly fallen leaf litter was collected with litter traps placed under trees in monocultured plantations established by the Center for the International Cooperation in Agronomic Research for the Development (CIRAD) in 1983–1984 near the Paracou research station. The traps were harvested monthly, and the plant material was dried at 40°C in a heater to a constant weight. The leaf litter was placed inside the litterbags and visually inspected. Any material in an advanced stage of degradation was discarded. All individually tagged litterbags were closed and fixed to the soil surface with stainless-steel staples and wire. Each block of nine litterbags (3 mesh sizes × 3 litter combinations) was tied with polyamide thread at each sampling point in November 2015 (end of the dry season) and retrieved in June 2016 (end of the wet season) in the same order as they were initially placed. All harvested litterbags were dried at 40°C in a heater to constant weight, root and soil residues were gently removed, litter fragments were identified to species for the *Goupia-Platonia* mixture and were then weighed. Owing to the high levels of litter mass lost after the near seven months incubation, we only could analyse the amount of C and N lost from a subset of the litterbags that contained enough litter remaining. Therefore, a subsample of litterbags representative of all site, topographic, mesh size and litter-composition combinations, along with five random samples of each litter type, were milled and analysed to obtain the initial and final C and N contents. Losses of these two elements from the litter were calculated as $100 \times [(M_i \times CN_i) - (M_f \times CN_f)]/(M_i \times CN_i)$, where M_i and M_f are the initial and final litter dry masses within a litterbag, respectively, and CN_i and CN_f are the initial and final C or N concentrations (% of litter dry mass), respectively [9]. Using C and N loss (%) allowed us to assess the potential effects of any possible inorganic contamination of the litter retrieved from the field [9], and thus to validate our analyses on litter mass lost.

(c) Environmental biotic and abiotic data

We compiled data for 44 variables describing the below- and aboveground biophysical and biological components surrounding each sampling point (i.e. block of litterbags) to identify the potential microenvironmental and biotic drivers behind the effect of fauna on decomposition (see the electronic supplementary material, table S1 and supplementary methods for detailed procedural descriptions). Briefly, we determined the concentrations of nutrients (C, N, P, potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na)) in the litter (organic horizon) and soil (0–15 cm depth) pools at each sampling point by means of coupled plasma/optical emission spectrometry. Additionally, the concentration of available P in the soil was determined by both the Olsen and Bray methods. We also

determined the activities of the extracellular enzymes β-glucosidase, leucine and glycine aminopeptidases and acid and alkaline phosphatases (henceforth referred to as βgluc, leu, gly, acidP and alkP, respectively) in the litter and soil at each sampling point by means of colorimetric assays. We sampled the communities of arthropods in the litter surrounding each sampling point by means of Winkler/Moczarsky traps and then classifying each collected specimen into 33 Order or sub-Order taxonomic categories covering all major lineages within Arthropoda. Finally, all trees (diameter at breast height ≥10 cm) within the 0.25 ha plots were mapped, tagged and identified to species or genus with herbarium vouchers for determining the tree species richness, phylogenetic diversity and three complementary indexes of functional trait diversity for each plot.

(d) Data analyses

All statistical analyses were carried out with R v. 3.4.3 [38]. The variation of litter mass lost from the litterbags after the incubation was assessed using a linear mixed model as implemented in the *lme4* package [39], including site, topography, mesh size, litter composition and the interaction between site and mesh size as fixed-effects terms. Sampling point was added as a random intercept term nested within plot, topography and site, thus representing the spatial structure of our experimental design. Higher-order interactions were sequentially removed when not significant ($p > 0.05$), additionally assessing the Akaike information criterion (AIC) and retrieving the coefficients of determination (r^2). Parameter-specific p-values for the mixed models were calculated by normal, Satterthwaite and Kenward-Rogers approximations to the number of degrees of freedom, and all approaches yielded qualitatively identical results. The same models were used for C and N losses, although the use of a subset of the litterbags for the C and N loss models resulted in not having enough within-plot replicates for all the plots and litter treatments, and so, it precluded the use of the same nested random effects structure of the original experimental design.

We determined the distribution of all environmental biotic and abiotic variables using a principal component analysis (PCA). We confirmed the apparent differences between sites and across topographic levels for the first and second PCA axes using a linear mixed model with the PC1 and PC2 scores as response variables. Then, we analysed the variation of the most relevant environmental variables, i.e. those with larger loadings on these first two axes of the PCA. The net effects of soil fauna on leaf litter decomposition were measured as the difference in mass loss between the litterbags with and without fauna access [34] but also as a relative effect (i.e. (decomposition with fauna – decomposition without fauna)/decomposition without fauna). Importantly, these net and relative fauna effects on decomposition were calculated with paired litterbags, therefore litterbags that were tied together and incubated in the same sampling point and only differing in the size of their mesh, which allows us to link the effect of fauna with the microenvironment. To visualize these fauna effects within the multivariate environmental space, we repeated this PCA including the six corresponding fauna-effect variables (two mesh sizes crossed with three litter combinations).

The relationship between the contribution of soil fauna to decomposition with the microenvironment was assessed using a linear mixed model with fauna effect (net or relative) as a response variable and the same random effects structure than the model of litter mass loss but replacing site and topographic categorical factors by the scores of each sampling point over the PC1 and PC2 (obtained from the PCA without fauna-effect variables included), as surrogates of variations in nutrient availability associated with the environment. This analytical approach allowed us to synthesize a complex multidimensional scenario of regional and topographically associated variation in the environment into a more tractable and interpretable output [18,40]. Furthermore, by including this environmental heterogeneity as continuous covariates, we were able to assess the effect of soil fauna on decomposition across the natural gradient of nutrient availability encompassed in our study sites. Finally, we additionally explored the potential contribution of the first six PCA axes (which together explained a cumulative proportion of variance of 58%) over the effects of the fauna on decomposition using automated model selection with the *dredge* function from the *MuMIn* package [41]. However, the subset of models with the lowest AIC only included PC1, therefore discarding all other axes.

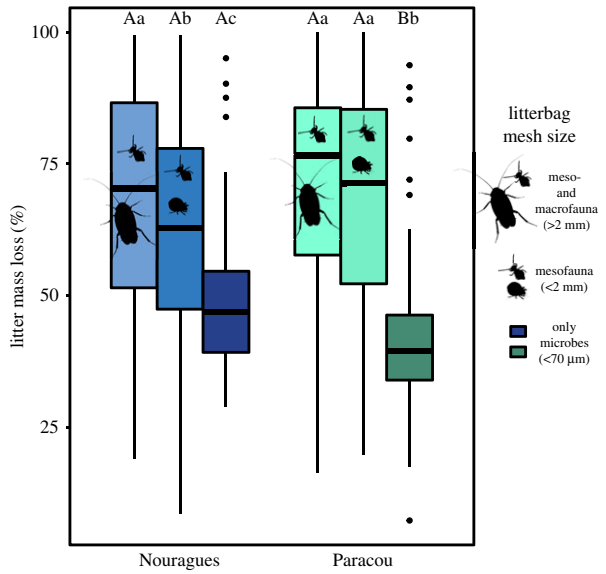

Figure 1. Variation in the loss of litter (as a percentage of initial dry mass) by site and litterbag mesh size. Different uppercase letters denote significant differences between sites for the same mesh size, and lowercase letters denote significant differences among mesh sizes within the same site and points indicate outliers. Among-group comparisons are the Tukey post hoc tests based on marginal means estimated from a linear mixed model. See table 2 for model output. (Online version in colour.)

3. Results

(a) Loss of litter mass and nutrients

After seven months of incubation, between 68 and 70% of the initial leaf litter mass was lost when meso- and macrofauna had access to the litterbags. However, in litterbags with the smallest mesh size (microbial decomposition only), litter mass loss dropped to 48% on average in Nouragues, and to only 40% in the more nutrient-poor site of Paracou (figure 1 and table 2, site × size interaction). Models assessing C and N losses yielded qualitatively similar results, although this between-site difference in microbial decomposition potential was even larger for N, being 18% lower at Paracou than at Nouragues (table 2, site × size interaction). The effect of soil fauna in Paracou, however, compensated this lower baseline of microbial decomposition, so that the loss rates of litter mass and nutrients were equalized between sites when both meso- and macrofauna had access to the litterbags (figure 1 and table 2). Additionally, the decomposition rates of the comparatively P-richer litter of *Goupia* and the P-poorer *Platonia* were unexpectedly similar, although the mass losses for the combination of the two species were larger (+3.4%), indicating that when mixed, both species decomposed faster (table 2,

Table 2. Coefficients, significance and r^2 for the linear mixed models used to evaluate the controls on losses of litter mass and nutrients and fauna effects on decomposition. (Losses of litter mass, carbon (C) and nitrogen (N) are percentages from initial dry mass and C and N contents, respectively. The fauna effect on decomposition is the difference between the loss of litter mass from the litterbags with meso- and macrofauna relative to the losses from the corresponding microbial-only litterbag (mesh sizes of 2 and 7 mm versus 70 µm, respectively; see Methods). Intercept group-level is Nouragues-bottom-macrofauna-*Goupia* for the models of litter mass loss ($n = 1080$) and C and N losses ($n = 206$), and the intercept for the fauna effect model ($n = 720$) is macrofauna-*Goupia*. The factor species denotes three litter combinations based on two species with contrasting C to phosphorus ratios. PC1 and PC2 are the scores of each sampling point for the first and second PCA axes, which encompass gradients of nutrient availability and topographic microenvironmental variation (figure 3; electronic supplementary material, figures S1 and S2). Models are linear mixed models, with sampling point as a random intercept nested within plot, topography and site, except for models of C and N loss, for which the lower number of samples precluded the inclusion of a random term. When applicable, marginal $r^2(r^2_m)$ values are associated with fixed factors while the conditional $r^2(r^2_c)$ additionally retain the random effects structure. Significant ($p < 0.05$) and marginally significant ($p < 0.1$) parameter coefficients are highlighted in bold and italics, respectively.)

variables		model			
		litter mass loss	C loss	N loss	fauna effect
intercept		**65.0 ± 2.3**	**71.9 ± 3.3**	**57.8 ± 3.9**	**22.6 ± 1.7**
site	Paracou	2.9 ± 2.4	−1.9 ± 3.5	3.5 ± 4.2	
topography	slope	0.6 ± 2.4	−0.5 ± 2.5	3.2 ± 2.9	
	top	*4.1 ± 2.4*	*4.3 ± 2.4*	**5.9 ± 2.9**	
size	mesofauna	**−5.3 ± 1.6**	*−6.0 ± 3.5*	−4.4 ± 4.1	**−4.1 ± 1.3**
	microbes	**−19.8 ± 1.6**	**−19.9 ± 3.5**	**−20.6 ± 4.2**	
species	*Platonia*	0.8 ± 1.1	−2.9 ± 2.4	0.9 ± 3.3	*3.0 ± 1.5*
	Platonia + *Goupia*	**3.4 ± 1.1**	**5.2 ± 2.4**	**9.1 ± 2.9**	**3.8 ± 1.5**
PC1 (nutrient availability)					**−3.1 ± 0.5**
PC2 (topography)					−0.7 ± 0.5
site × size	Paracou-Mesofauna	2.4 ± 2.2	6.1 ± 4.9	3.7 ± 5.8	
	Paracou-Microbes	**−10.3 ± 2.2**	**−10.1 ± 4.9**	**−17.9 ± 5.9**	
PC1 × species	*Platonia*				0.8 ± 0.5
	Platonia + *Goupia*				**1.3 ± 0.5**
model r^2_m/r^2_c		29.5/50.2	41.5	42.5	12.6/43.3

species). The position of the litterbags across the topographic levels did not appear to have a great influence beyond a marginal to significant trend to higher decomposition rates and N loss at the top plots (table 2, topography).

(b) Environmental variation between and within study sites

A PCA combining 44 potential regulatory controls with the effect of soil fauna on litter decomposition, measured as the difference in the loss of litter mass between the litterbags with and without faunal access [34], showed that the first two axes comprised 29.7% of the total variation between and within sites, underlining the high environmental heterogeneity at large and small spatial scales (figure 2; see the electronic supplementary material, table S1 for descriptions of the variables). Despite this variability, the clear separation of the sampling points at both sites indicated that PC1 captured regional-scale disparities mostly associated with nutrient-related variables in the litter layer. Conversely, PC2 mainly identified within-site soil-related variation linked with topographic position of sampling plots (see the electronic supplementary material, figure S1 and related discussion). Total N concentration in all compartments, foliar C : nutrient ratios in the canopy and litter and phosphatase and aminopeptidase activities in the litter were the most important variables in PC1 (electronic supplementary material, figure S3). Overall, the Nouragues site was richer in N in all compartments, from the canopy to the soil (table 1; electronic supplementary material, figure S4), whereas the higher litter C : nutrient ratios at Paracou suggested that the activity of microbial decomposers could be constrained to some degree.

Indeed, we also found that the activities of the extracellular aminopeptidases and phosphatases in the litter were lower at Paracou, indicating either a lower microbial biomass, restricted microbial performance [37], or lower substrate availability [42]. The stoichiometry of extracellular enzymes is a good indicator of the relative nutrient demands of microbial communities [36,37]. The relative allocation between N- and P-acquiring enzymes was similar at both sites, despite the lower activity of all extracellular enzymes at Paracou, suggesting that the microbial communities there were generally nutrient-limited instead of stoichiometrically unbalanced (electronic supplementary material, figure S5). In contrast with the organic horizon, enzymatic activity in the topsoil mostly varied across topographic levels, generally increasing towards the top as total nutrient concentrations did in that compartment (electronic supplementary material, figure S1).

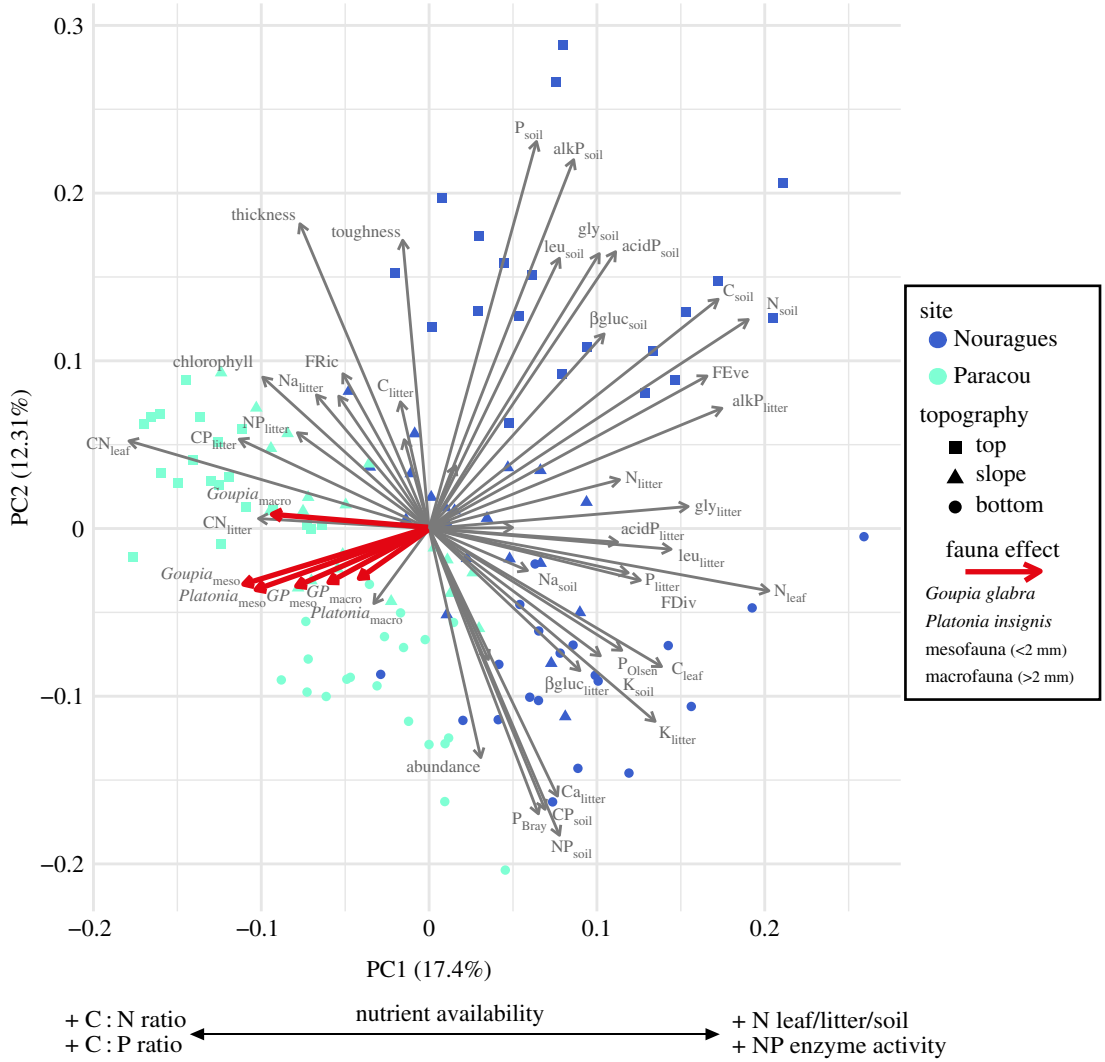

Figure 2. PCA showing the distribution of all sampling points at Nouragues (blue) and Paracou (green) and the loadings of the 44 biotic and abiotic environmental variables (grey vectors). The contribution of soil fauna (mesofauna and meso- plus macrofauna) on the decomposition of three litter combinations (*G. glabra*, *P. insignis* and both) are included and highlighted in red for visualization. PC1 axis was mainly defined by nutrient-related variables in the litter layer. Labels for the environmental vectors with the lowest loadings have been removed for clarity. See Methods; also electronic supplementary material, table S1 for variable descriptions and abbreviations, and figure S3 for the loadings of each variable on the axes. (Online version in colour.)

(c) Environmental dependency of the effect of fauna on decomposition

All net fauna effects appeared to consistently correlate with lower scores on the PC1 (figure 2, red vectors). Repeating the PCA excluding these fauna effect variables resulted in very subtle changes but a slight increase in the amount of total variance explained by PC1 and PC2 (32.6%, electronic supplementary material, figure S2). Indeed, the effect of soil fauna on decomposition was strongly and negatively correlated with the PC1, but not with the PC2 scores, indicating that the main drivers of the variation in the fauna effect on decomposition were the microenvironmental variables associated with differences in nutrient availability in the litter layer such as total N concentration, C : nutrient ratios and enzymatic activities (figure 3 and table 2). An equivalent model with relative fauna effects yielded qualitatively similar results (electronic supplementary material, table S2). The effect of the soil fauna was also larger in the mixed litter treatment (+3.8%) and was marginally larger (+2.9%) in the relatively P-poor litter species (*Platonia*, figure 4*a* and table 2, species). The relationship between this fauna impact on decomposition and the variation of the microenvironment (PC1 scores),

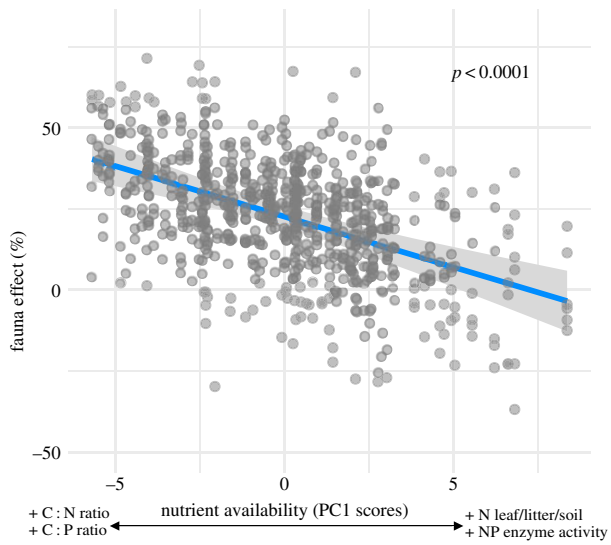

Figure 3. Relationship between the net absolute effect of soil fauna on decomposition (as the difference between the litter mass loss in the litterbags with meso- and macrofauna relative to the corresponding loss in the litterbags with only microbial access) with the PC1 scores of each sampling point as a proxy of the relative nutrient availability in the litter microenvironment. See table 2 for model outputs. (Online version in colour.)

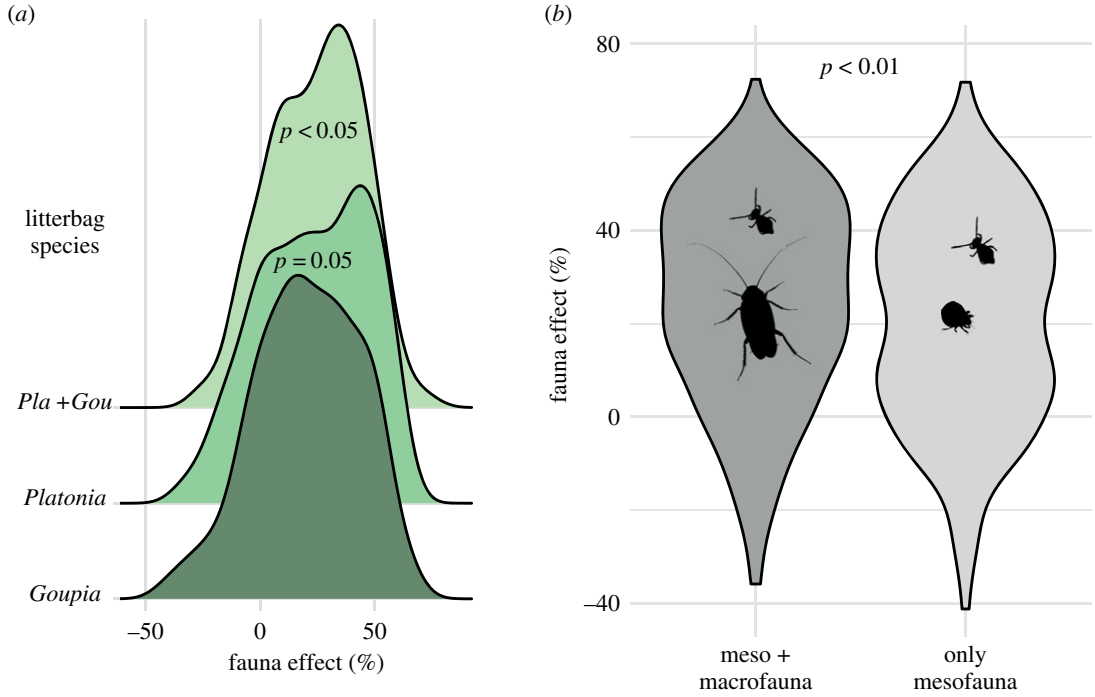

Figure 4. (*a*) Differences in the net absolute effect of soil fauna on the decomposition of three litter combinations differing in their C : P ratio. (*b*) The net absolute effect of soil mesofauna alone (less than 2 mm body width) versus the combined effect of the meso- plus macrofauna over litter decomposition. In both panels, the distribution of fauna effects is shown as a density function with highest or widest points having greater probabilities within each categorical group. See table 2 for model outputs. (Online version in colour.)

however, had a smoother, less negative slope for the mixed litter treatment, indicating that the combination of different litter substrates may have weakened the context-dependency of fauna effects on decomposition (table 2, PC1 × species; electronic supplementary material, figure S6). Finally, as anticipated in the analysis of litter mass loss, the net effect on decomposition was larger (+4.1%) for the complete community of soil fauna (i.e. meso- plus macrofauna) than for the mesofaunal component only, irrespective of the microenvironment and in all litter combinations (figure 4*b* and table 2).

4. Discussion

We here demonstrate that the strength of soil fauna control on litter decomposition is linked with its biotic and abiotic environment. The net contribution of soil fauna to litter mass loss increased as the conditions for microbial decomposition were more adverse, specifically when nutrient concentrations, and N in particular, were lower, not only in the litter substrate within each litterbag but also in the surrounding litter pool. This was consistent with the reduction in the activity of N- and P-acquiring extracellular enzymes in the litter layer, which were associated with stronger fauna effects on decomposition, thus providing additional support to the view that when the microbial communities inhabiting the organic horizon are relatively nutrient-limited, the facilitating role of soil fauna acquires a greater importance. Therefore, we found that soil fauna was able to minimize differences in litter decomposition buffering ecosystem-level nutrient shortages at regional scales. This supports recent findings challenging the long-standing view that biotic controls on decomposition would be subordinate to regional and global-scale features such as climate [6,7], and support propositions of local-scale variables regulating microbial activity as predominant drivers of decomposition [5].

Microbes are the ultimate agents responsible for the transformation of dead organic matter, mineralization to CO_2 and inorganic nutrients, and humus formation [12,13,16]. Nutrient availability rather than abundance of detritus *per se* is a main limitation to microbial growth and so of litter decomposition [35,36]. Microbial communities inhabiting environments differing in nutrient availability may face contrasting stoichiometric imbalances that can restrict their ability to decompose organic matter [35,43]. In low-nutrient environments (e.g. with high C : N ratios), microbes can adjust their metabolism to reduce their C-use efficiency while increasing their nutrient-use efficiency (i.e. the ratios of growth over organic C or nutrient uptake) to cope with the physiological challenges of resource imbalance [44,45]. Many direct and indirect animal-mediated processes may enhance nutrient supply, potentially stimulating microbial activity [12,13]. For example, the fragmentation and comminution of litter increases its surface area to mass ratio, making it more readily attacked by microbes [46,47]. The translocation and redistribution of freshly fallen litter across soil surfaces and depths together with modifications of aggregation properties and pore structure may likewise accelerate nutrient release [12,13]. Microbial inoculation and the preconditioning of litter during transit through animal guts may also facilitate decomposition [12,13], and importantly, this effect can be directly associated with initial litter quality [47,48]. In fact, Joly *et al*. found that the lower the initial litter quality, the greater the magnitude of microbial stimulation after invertebrate gut passage [48], and that the positive effect of soil fauna was mainly related with greater N release from faeces than from litter where this nutrient is more rapidly immobilized [47]. Direct grazing by soil fauna on living fungal hyphae, bacterial mat and microbial necromass may also alter density-dependent community functions such as substrate, enzyme and nutrient diffusion and exploitative and interfering competitive interactions affecting species

coexistence and thus the composition and performance of microbial communities [49,50].

The nutrients acquired by soil animals generally exceed their demands, and the surplus is excreted in easily available forms such as urea, ammonia, phosphate and other derivative forms [46]. At a macroscopic scale, it is well known that, through their dung and flesh, megafauna increases nutrient diffusion across the landscape with strong impacts on ecosystem functioning [51]. Likewise, soil fauna could improve the movement of nutrients across the litter–soil interface. Indeed, nutrient transfer between litter types, from the N pool in the soil or from microbial fixation, has been suggested as a widespread mechanism behind the diversity-function effects on decomposition [9,29,52]. We argue that soil fauna may play a role in these phenomena because they could locally enrich low-quality litter substrates by increasing nutrient diffusivity, thereby relaxing the stoichiometric constraints that may hinder their breakdown. If so, a low nutrient concentration in a particular litter substrate and in the associated microenvironment should increase the importance of the facilitation of nutrient mobility by soil fauna.

Previous studies have reported that soil fauna can strengthen the diversity-function effects on litter decomposition, increasing the rates of loss of litter mixtures with higher diversity [24,26]. Our results also indicated that soil fauna had a slightly larger effect in the litter mixture treatment. The stoichiometric heterogeneity of complex litter mixtures could better match the nutritional demands of litter-feeding animals, thereby stimulating its activity [25]. Synergistic diversity effects on decomposition have been found to be correlated with the stoichiometric dissimilarity of the litter mixture but only in the presence of soil fauna, and importantly, this relationship disappeared when the nutrient pool available in the microenvironment was experimentally increased in a fertilization experiment [26]. As suggested by these authors, microbial activity can be subsidized by nutrient uptake coming from other sources than the litter present in the litterbags. In the light of our findings, we add that soil fauna may be a key facilitator of this external flow of nutrients, which could be increasingly important for microbes as the nutrient content in the microenvironment decreases or the litter mixture become poorer or more unbalanced.

This study showed that the importance of soil fauna control on litter decomposition increases as nutrient availability in the microenvironment decreases, as well as that soil fauna can buffer ecosystem-level disparities in nutrient-related constraints on decomposition. In order to explore the mechanisms behind the above soil fauna effects, future studies could include some elements that would strengthen our conclusions. Microbial activity is regulated by several small-scale microclimate features that can strongly influence decomposition rates [5]. Among them, soil moisture is particularly important and very variable both at spatial (e.g. topographic) and temporal (e.g. daily to seasonal) scales. Continuous microclimate data logging coupled with longitudinal litterbag experiments may allow us to refine the linkages between soil fauna and the microenvironment and their combined effects over the decomposition of litter. Moreover, the measurement of litter mass loss can be influenced by inorganic contamination. Our analyses on C and N loss performed with a representative subset of litterbags, pointed out that, despite this potential noise, litter mass loss can be a rather good proximal variable of actual C and N cycling (table 2). We acknowledge, however, that fauna effects on decomposition should be ideally computed based on C and N losses. Overall, taking into account this in further fieldwork experiments, and perhaps coupled with laboratory-based microcosms, may help to figure out the mechanistic basis behind the observed findings.

Data accessibility. All data and code supporting the results presented in this contribution are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.r2r5964 [53].

Authors' contributions. G.P., I.A.J. and J.P. designed the study. G.P., D.A., A.G.-G., O.G., J.L., L.M., O.M., R.O., I.U., E.A.C., C.S., L.V.L. and L.T.V. performed field and/or laboratory work. G.P. compiled and analysed the data with advice of J.S., M.F.-M. and J.P. G.P. wrote the manuscript with substantial inputs of J.S., A.R., I.A.J. and J.P., and revisions of all co-authors.

Competing interests. We declare we have no competing interests.

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References

1. Cebrian J. 1999 Patterns in the fate of production in plant communities. *Am. Nat.* **154**, 449–468. (doi:10.1086/303244)
2. Crowther TW *et al.* 2016 Quantifying global soil carbon losses in response to warming. *Nature* **540**, 104–108. (doi:10.1038/nature20150)
3. Swift MJ, Heal OW, Anderson JM. 1979 *Decomposition in terrestrial ecosystems*. Berkeley, CA: University of California Press.
4. Cornwell WK *et al*. 2008 Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol. Lett.* **11**, 1065–1071. (doi:10.1111/j.1461-0248.2008.01219.x)
5. Bradford MA, Warren RJ, Baldrian P, Crowther TW, Maynard DS, Oldfield EE, Wieder WR, Wood SA, King JR. 2014 Climate fails to predict wood decomposition at regional scales. *Nat. Clim. Chang.* **4**, 625–630. (doi:10.1038/nclimate2251)
6. Bradford MA *et al*. 2017 A test of the hierarchical model of litter decomposition. *Nat. Ecol. Evol.* **1**, 1836–1845. (doi:10.1038/s41559-017-0367-4)
7. Bradford MA, Berg B, Maynard DS, Wieder WR, Wood SA. 2016 Understanding the dominant controls on litter decomposition. *J. Ecol.* **104**, 229–238. (doi:10.1111/1365-2745.12507)
8. García-Palacios P, Maestre FT, Kattge J, Wall DH. 2013 Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. *Ecol. Lett.* **16**, 1045–1053. (doi:10.1111/ele.12137)
9. Handa IT *et al*. 2014 Consequences of biodiversity loss for litter decomposition across biomes. *Nature* **509**, 218–221. (doi:10.1038/nature13247)
10. Schmidt MWI *et al*. 2011 Persistence of soil organic matter as an ecosystem property. *Nature* **478**, 49–56. (doi:10.1038/nature10386)

11. Soong JL, Nielsen UN. 2016 The role of microarthropods in emerging models of soil organic matter. *Soil Biol. Biochem.* **102**, 37–39. (doi:10.1016/j.soilbio.2016.06.020)
12. Filser J *et al*. 2016 Soil fauna: key to new carbon models. *Soil* **2**, 565–582. (doi:10.5194/soil-2-565-2016)
13. Grandy AS, Wieder WR, Wickings K, Kyker-Snowman E. 2016 Beyond microbes: are fauna the next frontier in soil biogeochemical models? *Soil Biol. Biochem.* **102**, 40–44. (doi:10.1016/j.soilbio.2016.08.008)
14. Kaiser C, Franklin O, Dieckmann U, Richter A. 2014 Microbial community dynamics alleviate stoichiometric constraints during litter decay. *Ecol. Lett.* **17**, 680–690. (doi:10.1111/ele.12269)
15. Schlesinger WH, Bernhardt ES. 2013 *Biogeochemistry: an analysis of global change*. Waltham, MA: Academic Press.
16. Bardgett RD, van der Putten WH. 2014 Belowground biodiversity and ecosystem functioning. *Nature* **515**, 505–511. (doi:10.1038/nature13855)
17. Zimmer M, Kautz G, Topp W. 2005 Do woodlice and earthworms interact synergistically in leaf litter decomposition? *Funct. Ecol.* **19**, 7–16. (doi:10.1111/j.0269-8463.2005.00926.x)
18. Fujii S, Cornelissen JHC, Berg MP, Mori AS. 2018 Tree leaf and root traits mediate soil faunal contribution to litter decomposition across an elevational gradient. *Funct. Ecol.* **32**, 840–852. (doi:10.1111/1365-2435.13027)
19. Van Wensem J, Verhoef HA, Van Straalen NM. 1993 Litter degradation stage as a prime factor for isopod interaction with mineralization processes. *Soil Biol. Biochem.* **25**, 1175–1183. (doi:10.1016/0038-0717(93)90212-T)
20. Tian G, Brussaard L, Kang BT. 1995 Breakdown of plant residues with contrasting chemical compositions under humid tropical conditions: effects of earthworms and millipedes. *Soil Biol. Biochem.* **27**, 277–280. (doi:10.1016/0038-0717(94)00182-Z)
21. Schädler M, Brandl R. 2005 Do invertebrate decomposers affect the disappearance rate of litter mixtures? *Soil Biol. Biochem.* **37**, 329–337. (doi:10.1016/j.soilbio.2004.07.042)
22. Yang X, Chen J. 2009 Plant litter quality influences the contribution of soil fauna to litter decomposition in humid tropical forests, southwestern China. *Soil Biol. Biochem.* **41**, 910–918. (doi:10.1016/j.soilbio.2008.12.028)
23. Riutta T, Slade EM, Bebber DP, Taylor ME, Malhi Y, Riordan P, Macdonald DW, Morecroft MD. 2012 Experimental evidence for the interacting effects of forest edge, moisture and soil macrofauna on leaf litter decomposition. *Soil Biol. Biochem.* **49**, 124–131. (doi:10.1016/j.soilbio.2012.02.028)
24. Hattenschwiler S, Gasser P. 2005 Soil animals alter plant litter diversity effects on decomposition. *Proc. Natl Acad. Sci. USA* **102**, 1519–1524. (doi:10.1073/pnas.0404977102)
25. Vos VCA, van Ruijven J, Berg MP, Peeters ETHM, Berendse F. 2013 Leaf litter quality drives litter mixing effects through complementary resource use among detritivores. *Oecologia* **173**, 269–280. (doi:10.1007/s00442-012-2588-1)
26. Barantal S, Schimann H, Fromin N, Hättenschwiler S. 2014 C, N and P fertilization in an Amazonian rainforest supports stoichiometric dissimilarity as a driver of litter diversity effects on decomposition. *Proc. R. Soc. B* **281**, 20141682. (doi:10.1098/rspb.2014.1682)
27. Waring BG. 2012 A meta-analysis of climatic and chemical controls on leaf litter decay rates in tropical forests. *Ecosystems* **15**, 999–1009. (doi:10.1007/s10021-012-9561-z)
28. Milcu A, Manning P. 2011 All size classes of soil fauna and litter quality control the acceleration of litter decay in its home environment. *Oikos* **120**, 1366–1370. (doi:10.1111/j.1600-0706.2010.19418.x)
29. St John MG, Orwin KH, Dickie IA. 2011 No 'home' versus 'away' effects of decomposition found in a grassland-forest reciprocal litter transplant study. *Soil Biol. Biochem.* **43**, 1482–1489. (doi:10.1016/j.soilbio.2011.03.022)
30. Perez G, Aubert M, Decaëns T, Trap J, Chauvat M. 2013 Home-field advantage: a matter of interaction between litter biochemistry and decomposer biota. *Soil Biol. Biochem.* **67**, 245–254. (doi:10.1016/j.soilbio.2013.09.004)
31. Lin H, He Z, Hao J, Tian K, Jia X, Kong X, Akbar S, Bei Z, Tian X. 2017 Effect of N addition on home-field advantage of litter decomposition in subtropical forests. *For. Ecol. Manage.* **398**, 216–225. (doi:10.1016/j.foreco.2017.05.015)
32. Luizao RCC, Luizao FJ, Paiva RQ, Monteiro TF, Sousa LS, Kruijt B. 2004 Variation of carbon and nitrogen cycling processes along a topographic gradient in a central Amazonian forest. *Glob. Chang. Biol.* **10**, 592–600. (doi:10.1111/j.1529-8817.2003.00757.x)
33. Werner FA, Homeier J. 2015 Is tropical montane forest heterogeneity promoted by a resource-driven feedback cycle? Evidence from nutrient relations, herbivory and litter decomposition along a topographical gradient. *Funct. Ecol.* **29**, 430–440. (doi:10.1111/1365-2435.12351)
34. Coq S, Souquet J-M, Meudec E, Cheynier V, Hättenschwiler S. 2010 Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana. *Ecology* **91**, 2080–2091. (doi:10.1890/09-1076.1)
35. Fanin N, Fromin N, Buatois B, Hättenschwiler S. 2013 An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. *Ecol. Lett.* **16**, 764–772. (doi:10.1111/ele.12108)
36. Camenzind T, Hättenschwiler S, Treseder KK, Lehmann A, Rillig MC. 2018 Nutrient limitation of soil microbial processes in tropical forests. *Ecol. Monogr.* **88**, 4–21. (doi:10.1002/ecm.1279)
37. Sinsabaugh RL, Follstad Shah JJ. 2012 Ecoenzymatic stoichiometry and ecological theory. *Annu. Rev. Ecol. Evol. Syst.* **43**, 313–343. (doi:10.1146/annurev-ecolsys-071112-124414)
38. R Core Team. 2016 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
39. Bates D, Mächler M, Bolker B, Walker S. 2014 Fitting linear mixed-effects models using lme4. *ArXiv pre-print*.
40. Ochoa-Hueso R. 2016 Nonlinear disruption of ecological interactions in response to nitrogen deposition. *Ecology* **97**, 2802–2814. (doi:10.1002/ecy.1521)
41. Barton K. 2018 MuMIn: multi-model inference. R package v. 1.40.4. See https://CRAN.R-project.org/package=MuMIn.
42. Margalef O *et al*. 2017 Global patterns of phosphatase activity in natural soils. *Sci. Rep.* **7**, 1–13. (doi:10.1038/s41598-017-01418-8)
43. Sterner RW, Elser JJ. 2002 *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton, NJ: Princeton University Press.
44. Mooshammer M *et al*. 2014 Adjustment of microbial nitrogen use efficiency to carbon: nitrogen imbalances regulates soil nitrogen cycling. *Nat. Commun.* **5**, 1–7. (doi:10.1038/ncomms4694)
45. Manzoni S, Taylor P, Richter A, Porporato A, Ågren GI. 2012 Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* **196**, 79–91. (doi:10.1111/j.1469-8137.2012.04225.x)
46. Chapin III F, Matson P, Mooney H. 2002 *Principles of terrestrial ecosystem ecology*. New York, NY: Springer.
47. Joly FX, Coq S, Coulis M, Nahmani J, Hättenschwiler S. 2018 Litter conversion into detritivore faeces reshuffles the quality control over C and N dynamics during decomposition. *Funct. Ecol.* **32**, 2605–2614. (doi:10.1111/1365-2435.13178)
48. Joly FX, Coulis M, Gérard A, Fromin N, Hattenschwiler S. 2015 Litter-type specific microbial responses to the transformation of leaf litter into millipede feces. *Soil Biol. Biochem.* **86**, 17–23. (doi:10.1016/j.soilbio.2015.03.014)
49. Buchkowski RW, Bradford MA, Grandy AS, Schmitz OJ, Wieder WR. 2017 Applying population and community ecology theory to advance understanding of belowground biogeochemistry. *Ecol. Lett.* **20**, 231–245. (doi:10.1111/ele.12712)
50. Crowther TW, Boddy L, Jones TH. 2011 Outcomes of fungal interactions are determined by soil invertebrate grazers. *Ecol. Lett.* **14**, 1134–1142. (doi:10.1111/j.1461-0248.2011.01682.x)
51. Doughty CE, Wolf A, Malhi Y. 2013 The legacy of the Pleistocene megafauna extinctions on nutrient availability in Amazonia. *Nat. Geosci.* **6**, 761–764. (doi:10.1038/ngeo1895)
52. Vitousek PM, Hobbie S. 2000 Heterotrophic nitrogen fixation in decomposing litter: patterns and regulation. *Ecology* **81**, 2366–2376. (doi:10.1890/0012-9658(2000)081[2366:HNFIDL]2.0.CO;2)
53. Peguero G *et al*. 2019 Data from: Nutrient scarcity strengthens soil fauna control over leaf litter decomposition in tropical rainforests. Dryad Digital Repository. (https://doi.org/10.5061/dryad.r2r5964)