SUPPORTING INFORMATION FOR:

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

Guille Peguero, Jordi Sardans, Dolores Asensio, Marcos Fernández-Martínez, Albert Gargallo-Garriga, Oriol Grau, Joan Llusià, Olga Margalef, Laura Márquez, Romà Ogaya, Ifigenia Urbina, Elodie A. Courtois, Clément Stahl, Leandro Van Langenhove, Lore T. Verryckt, Andreas Richter, Ivan A. Janssens, Josep Peñuelas

SUPPLEMENTARY METHODS:

- **Nutrient pools**

We collected three random soil cores with a soil auger (4 cm diameter and 15 cm length, Van Walt, Haslemere, UK) and combined them for a single composite topsoil sample at the onset of the wet season for obtaining the nutrient status of each sampling point. We also collected the litter accumulated within a 20-cm PVC square. The soil samples were sieved to 2 mm and then freeze-dried (Cryodos 50, Telstar, Terrassa, Spain). The leaf litter was dried at 60 °C in a heater to a constant weight and then weighed. Soil and litter subsamples were pulverized in a ball mill (MM400, Retsch, Haan, Germany) for the analysis of elemental composition. Between 0.15 and 0.2 g of soil and 0.035 and 0.045 g of litter were weighed with a microbalance (MX5 Mettler Toledo, Columbus, USA), and the C and N concentrations were determined by combustion coupled to an isotope ratio mass spectrometer at the Stable Isotopes Facility (UC Davis, USA). P, K, Ca, Mg and Na concentrations were determined by diluting 0.25 g of soil and litter with an acid mixture of HNO_3 (60%) and H_2O_2 (30% p/v) and digested in a microwave oven (MARS Xpress, CEM Corporation, Matthews, USA). The digested solutions were then diluted to a final volume of 50 mL with ultrapure water and 1% HNO_3. Blank solutions (5 mL of HNO_3 with 2 ml of H_2O_2 with no sample biomass) were regularly analyzed. The concentrations of each element where determined using inductively coupled plasma/optical emission spectrometry (ICP-OES Optima 4300DV, Perkin-Elmer, Wellesley, USA). We used the standard certified biomass NIST 1573a to assess the accuracy of the biomass digestion and analytical procedures. The available P concentration in the topsoil was determined by the Olsen and Bray method (Pansu & Gautheyrou, 2006).

- **Activities of extracellular enzymes**

We measured the maximum potential activity of *β*-glucosidase, leucine and glycine aminopeptidases, and acid and alkaline phosphatases in the topsoil (0-15 cm) and the litter environment at each sampling point (enzymes referred as βgluc, leu, gly, acidP and alkP hereinafter) by means of colorimetric assay techniques using *p*-Nitrophenylphosphate and *p*-Nitroaniline derivate chromogenic substrates (pNP and pNA, respectively) following (German et al., 2012; Popova & Deng, 2010; Sinsabaugh et al., 1993) with some modifications.

Soil and litter samples were transported to the lab and stored in plastic zip bags at 4 ºC until analysis. Fresh soil was sieved to 2 mm; for each sample one part was used for enzyme analyses and the other part was dried 24h at 105ºC for gravimetric water content determination. Fresh litter was cut in 1 – 2 cm pieces prior to enzyme analyses, one part was dried 48h at 70ºC for water content determination.

For enzyme analyses, 2 g of soil or 1 g of litter were mixed with 60 ml of sodium acetate buffer (SA: 50 mM, pH 5, used for acidP and β-gluc) or 60 ml of tris-acetate buffer (TA: 50 mM, pH 8, used for alkP, leu and gly) in a 125-ml glass jar for 10 min using a 2.5 cm magnetic stir bar at a maximum speed. Aliquots (100 µl each) of the soil suspension or litter homogenate were taken during continuous mixing using a 20–200 µl multi-channel pipette with wide orifice tips and were placed into 96 clear wells microplate (3 replicate wells per sample). Subsequently, 100 µl of respective substrate (5mM for acidP, alkP and βgluc and 2mM for leu and gly) that was dissolved previously in its respective buffer was added to each microplate well. Plates were shaken 5 min in a microplate shaker at medium speed and incubated at 37 ºC for 2 h (acidP, alkP and βgluc) or 5 h (leu and gly). Soil/litter particles sank to the bottom of the wells during incubation. After incubation, 100 µl of the clean supernatant were transferred to a new microplate, where 5 µl of NaOH 1M was previously added to each microplate well to terminate the enzymatic reaction (for acidP, alkP and βgluc). Absorbance of the clean supernatant was measured at 405 nm using a microplate reader with an automixing feature (Tecan Sunrise, Tecan GmbH Grödig, Austria). For leu and gly activities no NaOH was added. The substrate controls (8 replicate wells per enzyme) were performed using the same procedure but the mixture in each well was 100 µl substrate plus 100 µl of the corresponding buffer. The sample controls (3 replicate wells per sample) were assayed similarly in different plates, where the mixture in each well was 100 µl sample plus 100 µl of the corresponding buffer. The average absorbance of the eight substrate controls plus the average absorbance of the 3 sample controls was subtracted from the average absorbance of the 3 readings for each analysis. Negative potential activities were considered to indicate that no enzyme was present, and were converted to zero values before further analyses. Enzyme activity in reaction mixtures, expressed as µmole of pNP or pNA g^{-1} soil / litter DW h^{-1}, was calculated using the absorbance readings against a calibration curve that was constructed using pNP and pNA standards (Sigma-Aldrich, Darmstadt, Germany).

- **Plant and animal community data**

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 for each plot. We used the fully resolved and dated Bayesian phylogeny for the tree species from French Guiana (Baraloto et al., 2012) to obtain Faith's D phylogenetic diversity index at the plot level. Leaf-litter quality in the organic horizon may be to some extent a legacy of the distribution of foliar traits in the canopy (Cornwell et al., 2008). We thus collated the species data for the six foliar functional traits in the BRIDGE database (Baraloto et al., 2012): foliar thickness, foliar toughness, chlorophyll concentration, C and N concentrations and the C:N mass ratio. We then calculated the community-weighted means of each trait for each plot and calculated three functional diversity indices (functional richness, evenness and diversity; see Table S1) standardized against 999 randomized null communities to control for differences in species richness among plots (Swenson, 2014).

We sampled the communities of arthropods in the litter surrounding each sampling point at the onset of the wet season. We collected all the litter inside four randomly placed 0.25-m^2 PVC squares and sifted it through a 0.7-cm mesh Winkler bag, carefully catching by hand or with entomological aspirators all escaping macrofauna. The litter siftate was then hung in Moczarsky traps for 48 h and then carefully revised for any remaining specimen, and the bulk communities were stored in ethanol (Agosti, Majer, Alonso, & Schultz, 2000). Each specimen was counted in the laboratory using a stereomicroscope and classified into 33 Order or sub-Order taxonomic categories covering all major lineages within Arthropoda.

Figure SM1: Annual Precipitation (a) precipitation (b) and mean temperature (c) during the driest quarter in French Guiana. The higher temperature in Paracou during the driest quarter entails a higher evaporative demand which is coupled with a lower amount of rainfall during the same period. Data from http://worldclim.org

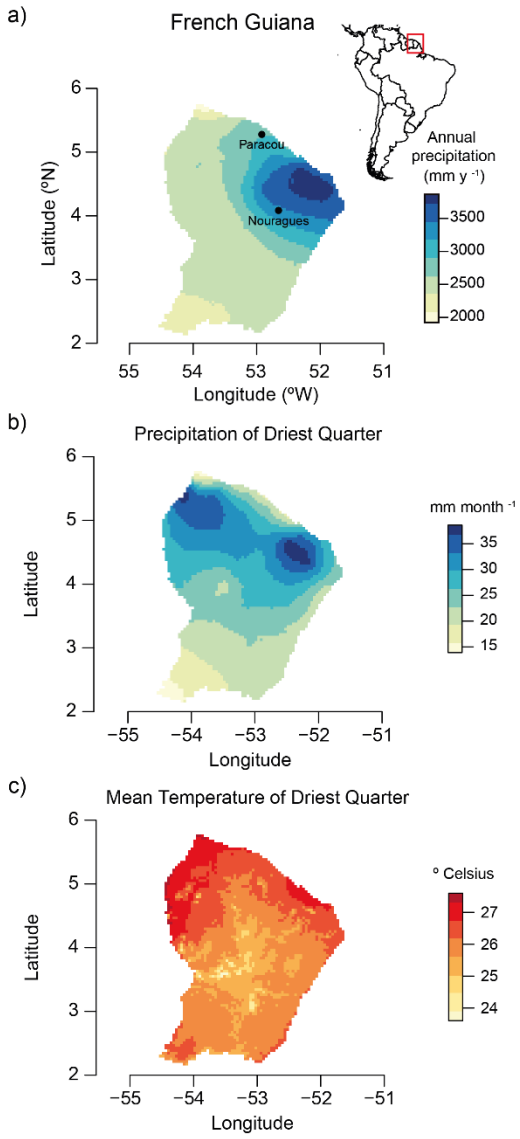


Figure SM2. Design of the sampling points within each experimental plot (a) and distribution of plots across the topographic levels in Nouragues (b) and Paracou (c). Red, green and blue squares show experimental plots located at bottom, slope and top positions across the topographic gradients, while yellow polygons depict long-term monitoring plots set up by hosting research organizations at each study site. Modified with permission from (Courtois et al., 2018).

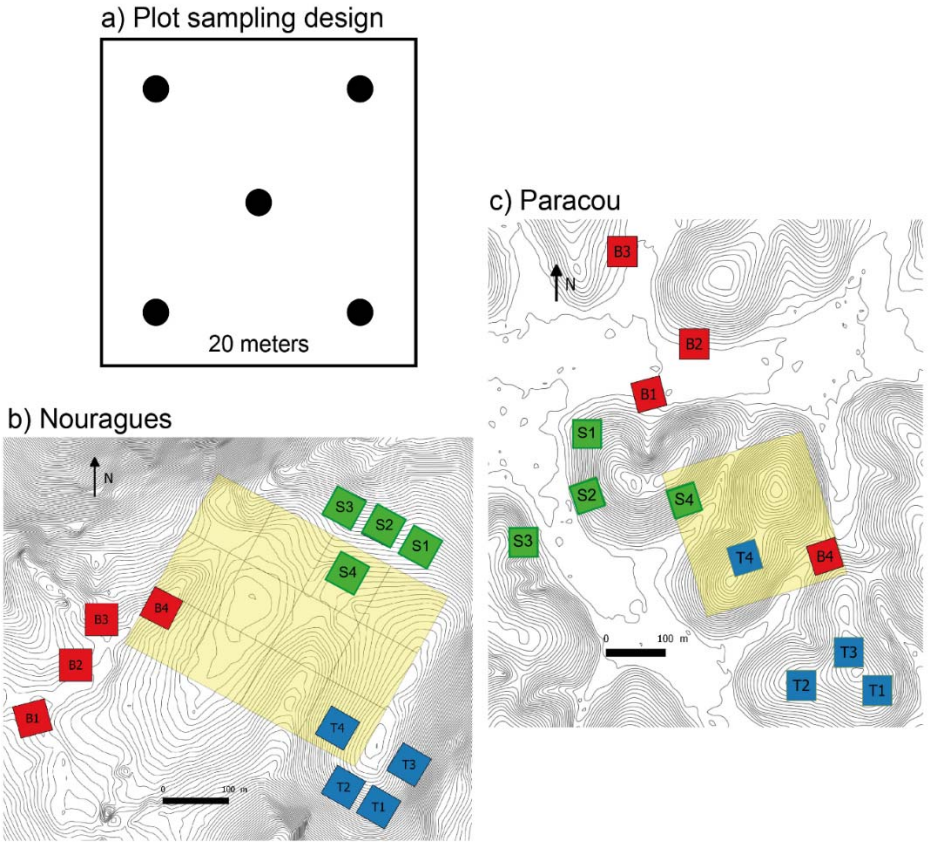


Table S1. Abbreviations and brief methodological description of the 44 environmental variables measured.

Compartment	Biological Component	Abbreviation	Variable Description	Unit	Method	References	Sampling level
Belowground (topsoil)	Biophysical	Csoil	Carbon content	%	Combustion coupled to an isotope ratio mass spectrometer	---	Sampling point (N=120)
		Nsoil	Nitrogen content	%	Combustion coupled to an isotope ratio mass spectrometer	---	
		Psoil	Phosphorus content	%	Acid digestion and ICP-OES	---	
		Ksoil	Potassium content	%	Acid digestion and ICP-OES	---	
		Nasoil	Sodium content	%	Acid digestion and ICP-OES	---	
		CNsoil	Carbon to Nitrogen ratio	Mass ratio	---	---	
		CPsoil	Carbon to Phosphorus ratio	Mass ratio	---	---	
		NPsoil	Nitrogen to Phosphorus ratio	Mass ratio	---	---	
		Polsen	Phosphorus available	ppm	Olsen	---	
		Pbray	Phosphorus available	ppm	Bray	---	
	Microbial community	leusoil	Leucine aminopeptidase activity	µmol pNA g^{-1} dry soil h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
		glysoil	Glycine aminopeptidase activity	µmol pNA g^{-1} dry soil h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
		alkPsoil	Alkaline Phosphatase activity	µmol pNP g^{-1} dry soil h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
		acidPsoil	Acid Phosphatase activity	µmol pNP g^{-1} dry soil h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
		βglucsoil	β-glucosidase activity	µmol pNP g^{-1} dry soil h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
Aboveground	Biophysical	litter	Accumulated litter pool	gr m^{-2}	PVC quadrat of 20*20 cm	---	
		Clitter	Carbon content	%	Combustion coupled to an isotope ratio mass spectrometer	---	
		Nlitter	Nitrogen content	%	Combustion coupled to an isotope ratio mass spectrometer	---	
		Plitter	Phosphorus content	%	Acid digestion and ICP-OES	---	
		Klitter	Potassium content	%	Acid digestion and ICP-OES	---	
		Mglitter	Magnesium content	%	Acid digestion and ICP-OES	---	
		Calitter	Calcium content	%	Acid digestion and ICP-OES	---	
		Nalitter	Sodium content	%	Acid digestion and ICP-OES	---	
		CNlitter	Carbon to Nitrogen ratio	Mass ratio	---	---	
		CPlitter	Carbon to Phosphorus ratio	Mass ratio	---	---	
		NPlitter	Nitrogen to Phosphorus ratio	Mass ratio	---	---	
	Microbial community	leulitter	Leucine aminopeptidase activity	µmol pNA g^{-1} dry litter h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
		glylitter	Glycine aminopeptidase activity	µmol pNA g^{-1} dry litter h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
		alkPlitter	Alkaline Phosphatase activity	µmol pNP g^{-1} dry litter h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
		acidPlitter	Acid Phosphatase activity	µmol pNP g^{-1} dry litter h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
		βgluclitter	β-glucosidase activity	µmol pNP g^{-1} dry litter h^{-1}	Colorimetric assay	Sinsabaugh et al. 1993	
	Animal community	abundance	Density of arthropods	individuals m^{-2}	Winkler litter sifter and Moczarsky extraction traps	Agosti et al. 2000	
		richness	Richness of arthropod communities	No. Orders	---	---	
	Plant community	SR	Richness of tree communities	No. Species	Species of all individuals >= 10 cm DBH	---	Plot (N=24)
		PD	Phylogenetic diversity	Million of years	Sum of total branch lengths connecting all species together	Faith, 1992	
		FRic	Functional Richness	Unitless	Standardized Effect Size (SES) of the hyper-volume of leaf traits	Swenson 2014	
		FEve	Functional Evenness	Unitless	SES of the regularity of species abundances across functional space	Swenson 2014	
		FDiv	Functional Divergence	Unitless	SES of the sum of average distances of species from the centroid	Swenson 2014	
		thickness	Leaf Thickness	mm	Community Weighted Mean (CWM) of leaf thickness	Baraloto et al. 2012	
		chlorophyll	Chlorophyll concentration	µg cm^{-2}	CWM of chlorophyll concentration	Baraloto et al. 2012	
		toughness	Leaf Toughness	N	CWM of Leaf Toughness	Baraloto et al. 2012	
		Cleaf	Leaf Carbon content	%	CWM of Leaf Carbon content	Baraloto et al. 2012	
		Nleaf	Leaf Nitrogen content	%	CWM of Leaf Nitrogen content	Baraloto et al. 2012	
		CNleaf	Leaf Carbon to Nitrogen ratio	Mass ratio	CWM of Leaf Carbon to Nitrogen ratio	Baraloto et al. 2012	

SUPPLEMENTARY RESULTS AND DISCUSSIONS

Figure S1. Principal component analysis (PCA) showing the distribution of all sampling points at Nouragues and Paracou stratified by topographic position (blue top, green slope and red bottom), along with the loadings of the 44 biotic and abiotic environmental variables (gray vectors). The contribution of soil fauna (mesofauna and meso- plus macrofauna) on the decomposition of three litter combinations are included in this analysis and highlighted in red for visualization. The inset shows the significant differences among topographies in the PC2 scores according to a linear mixed model. The effects of the soil fauna (mesofauna and meso- plus macrofauna) on the decomposition of the three litter combinations are included in the analysis and highlighted in red for visualization. Labels for the environmental vectors with the lowest loadings have been removed for clarity. See Methods and Table S1 for variable descriptions and abbreviations.

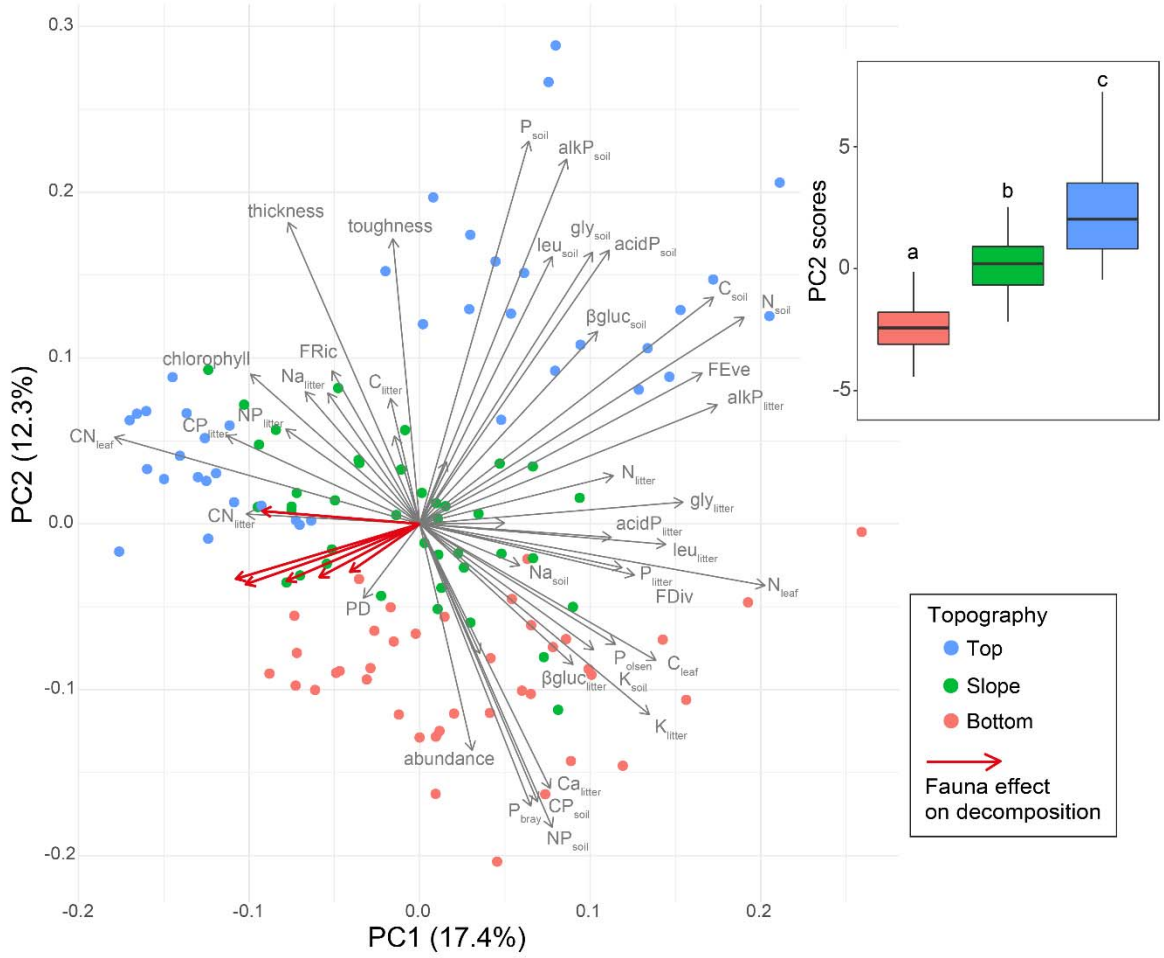


FIGURE S2. Principal component analysis (PCA) showing the distribution of all sampling points at Nouragues (blue) and Paracou (green) and the loadings of the 44 biotic and abiotic environmental variables (gray vectors). The inset shows the significant between-site differences on the scores of the first axis, which was mainly defined by nutrient-related variables. Labels for the environmental vectors with the lowest loadings have been removed for clarity. See Methods and Table S1 for variable descriptions and abbreviations.

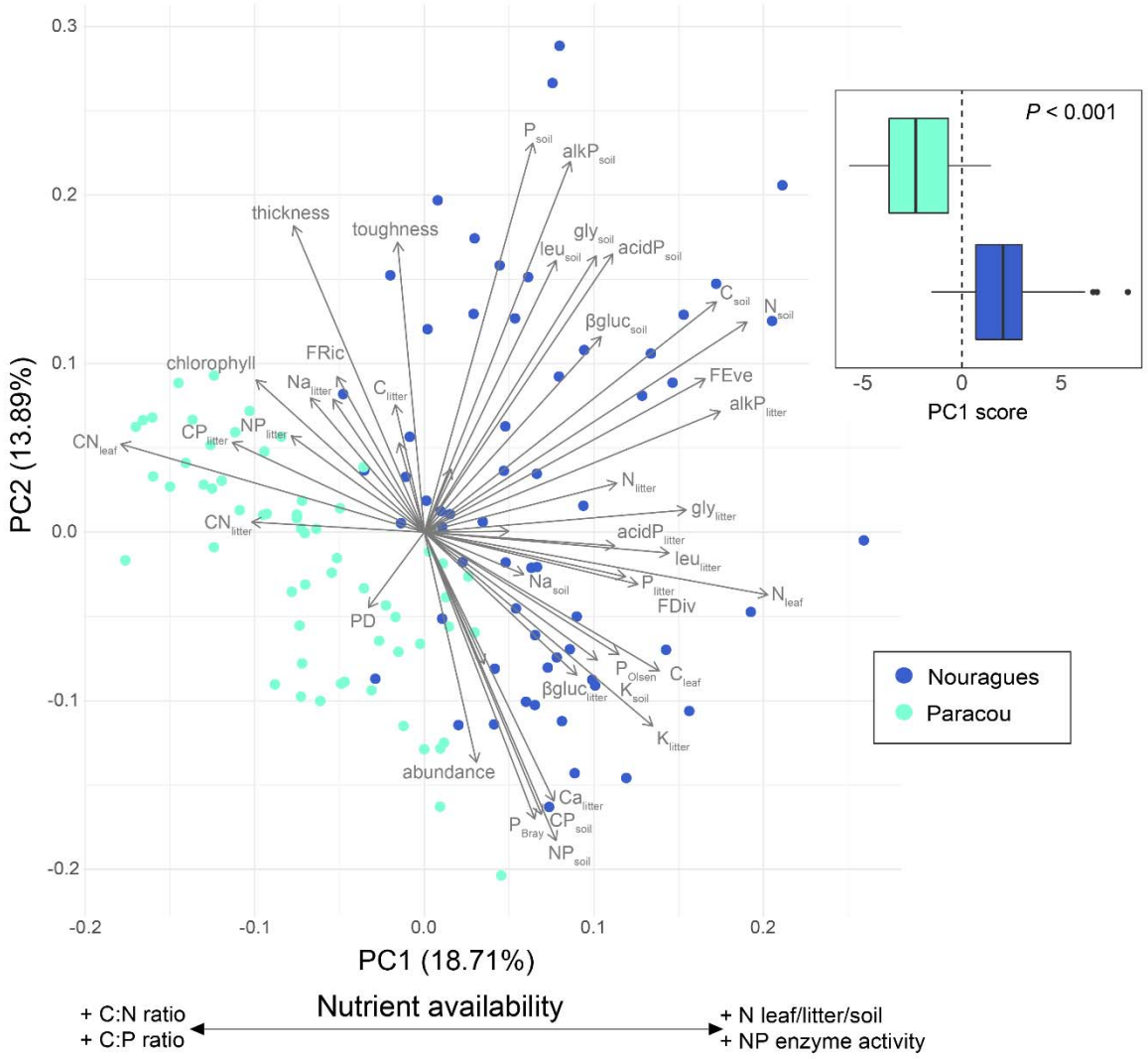
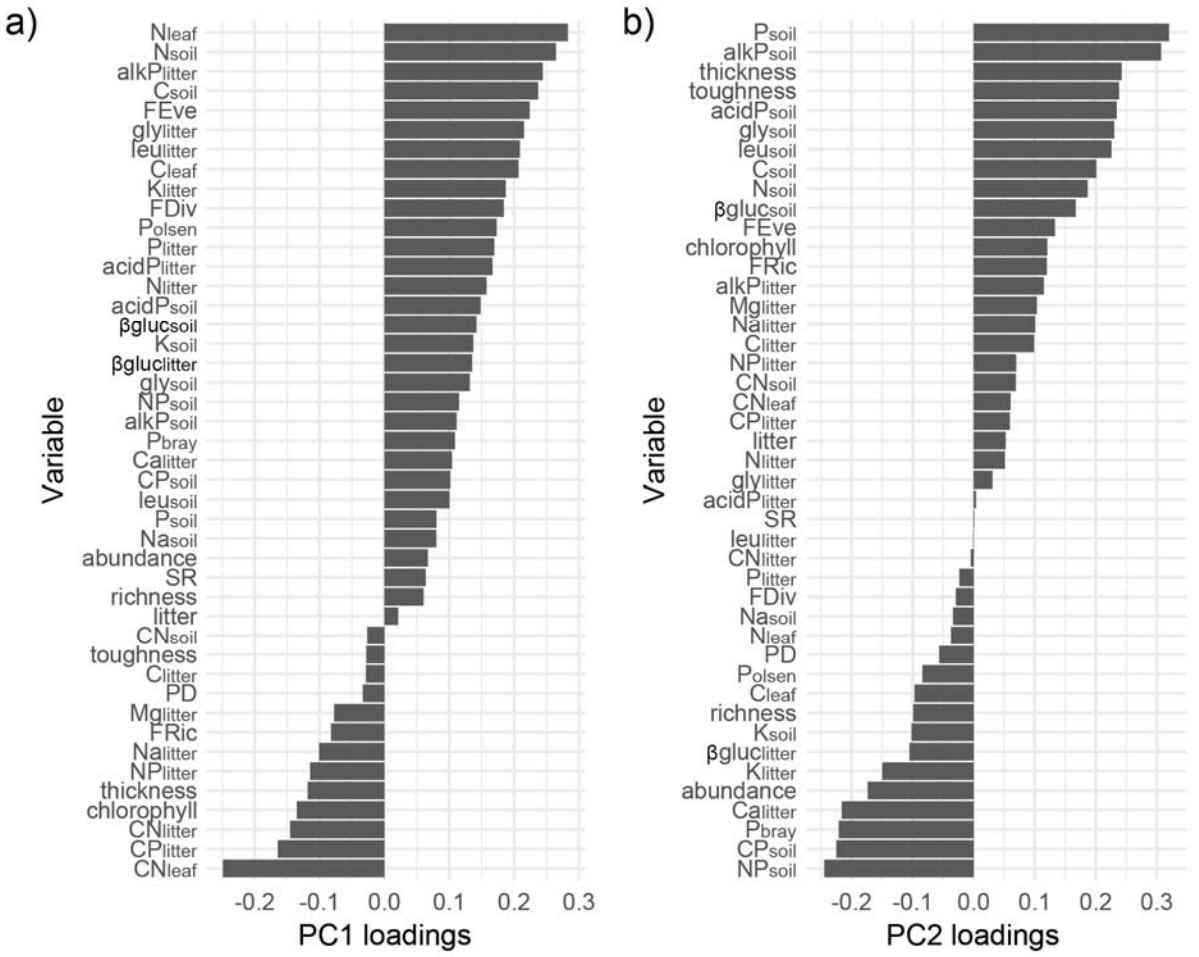


Figure S3. Loadings of environmental variables on first (a) and second (b) PCA axis including the 44 biotic and abiotic environmental variables described in table S1.



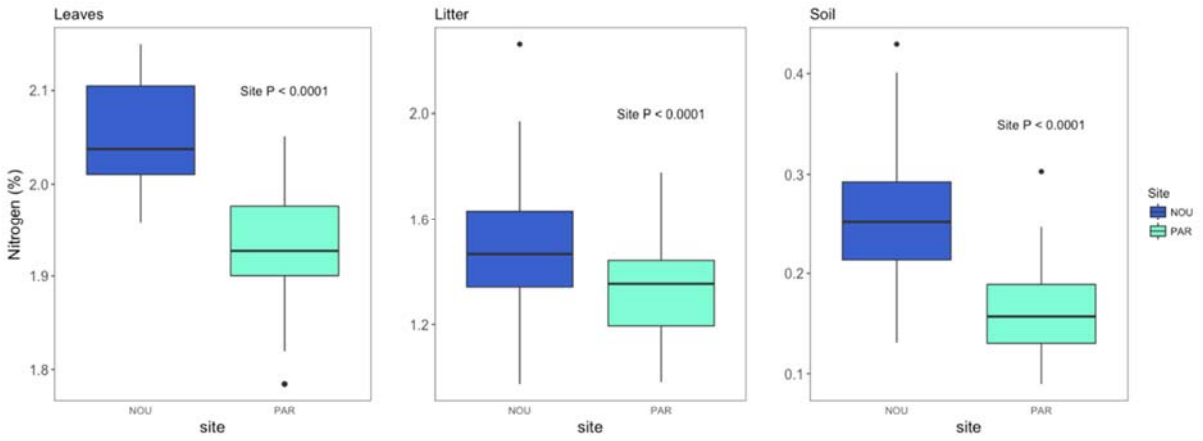
As seen in figure S2, the PC1 mainly captures variability in N contents in the canopy (leaf), litter and soil compartments as well as aminopeptidase and phosphatase activities in the litter, which increase towards the more fertile sampling points and decrease where CNP ratios in both canopy leaves and litter were greater.

On the other hand, environmental variables related to the second axis are associated to differences across the topographic relief. Plots in top locations tend to have greater total P content and phosphatase and peptidase activities in soil compared to bottom plots which have higher N:P and C:P ratios and also higher available P (e.g. Pbray), along with greater arthropod abundances.

Soils at the bottom parts at the mesoscale landscape relief are sandy podzols with great fractions of quartz coming from the degradation of the parent material. In contrast, toward the tops, clay content increases and particularly of iron and aluminum oxides (Fe^{3+}),

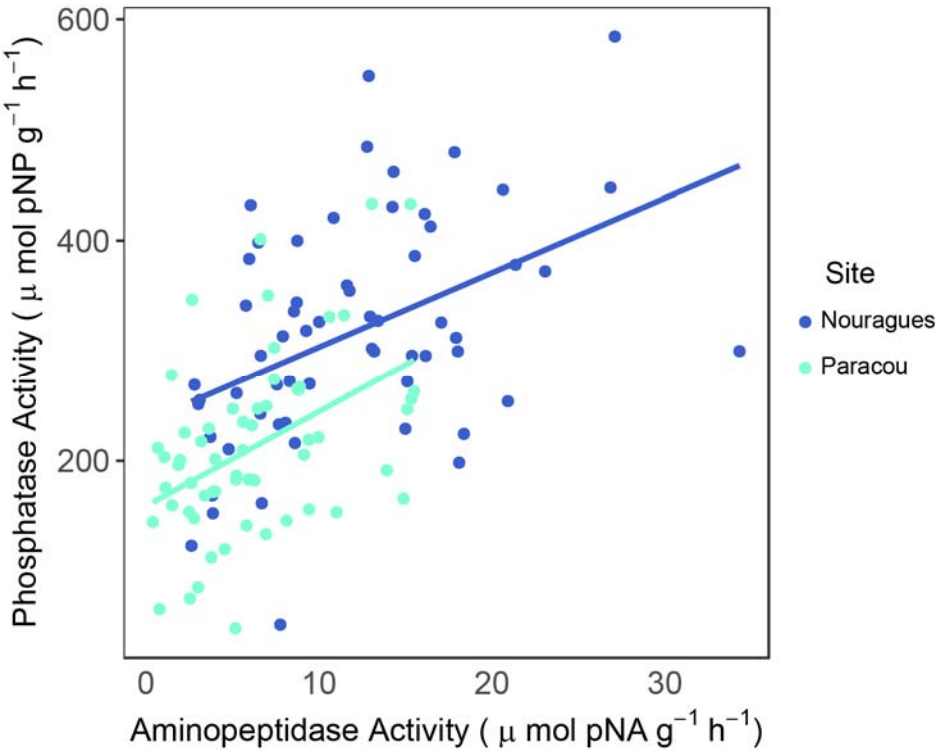
which have a greater capacity of absorbing and retaining P. This may explain the gradient of P-available/P-total from lowest values towards the top and consequently, it may determine the production of P-mining enzymes in the topsoil. Further detailed analyses of these topographic biogeochemical patterns are out of the scope of this contribution, but apparently are not major drivers of soil fauna effects on litter decomposition (red vectors in figure 2 and figure S1).

Figure S4. Total Nitrogen (N) concentration (% of dry mass) by sites in the canopy leaves, litter and soil compartments. The value for the canopy is a community mean of the N concentration in the leaves of each species and weighted by their abundance at each plot.



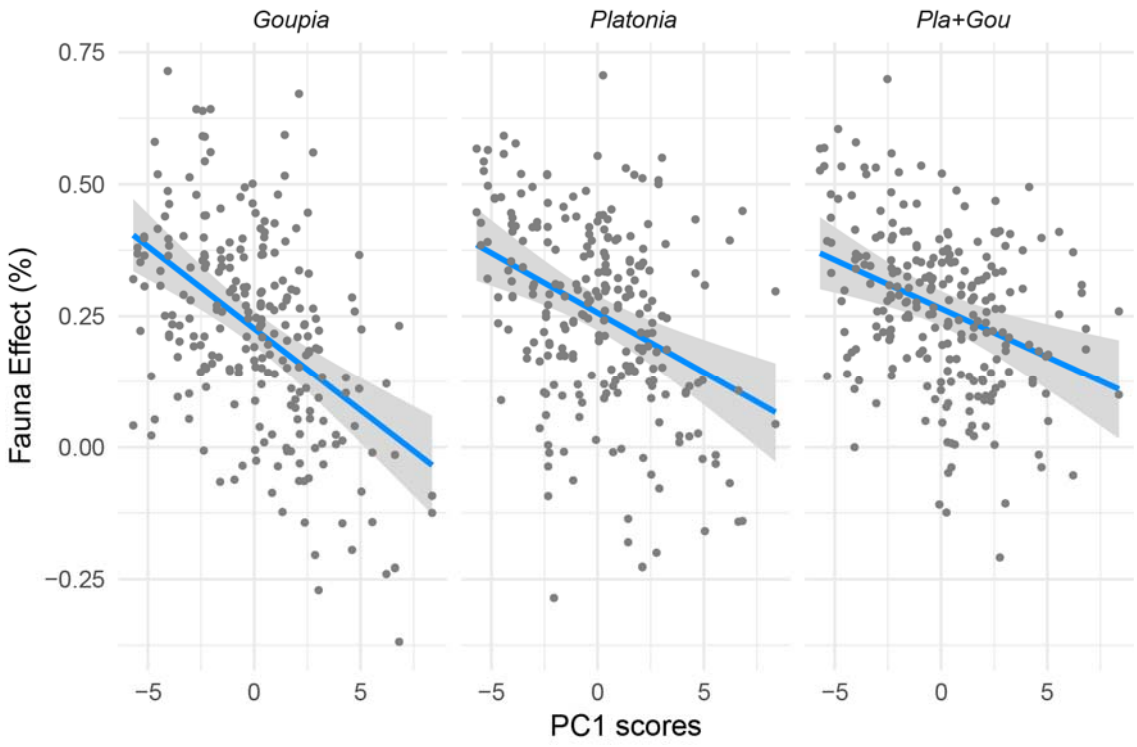
On average, Paracou has a comparatively poorer litter and topsoil environment, particularly in terms of total N concentration. This could partly be a legacy from the observed canopy-level differences in N concentration (Cornwell et al., 2008).

FIGURE S5. Relationship between N and P acquiring enzymatic activities in the litter from Nouragues (blue) and Paracou (light green). For N we measured Glycine and Leucine aminopeptidases and for P alkaline and acid phosphatases.



A linear mixed model to assess the relationship between total phosphatase with total aminopeptidase showed that in Paracou there was a significantly lower maximum potential of phosphatase activity (i.e. -70.95 ± 28.6 μmol pNP g^{-1} h^{-1}; $P < 0.05$). The activity of both kinds of enzymes was strongly correlated (slope 6.5 ± 1.6; $P < 0.0001$), however, their relationship did not differ between sites (site × aminopeptidase interaction $P = 0.82$, i.e. no slope differences between sites). This indicates that the relative nutritional demands are not stoichiometrically imbalanced in both sites.

FIGURE S6. Relationship between the microenvironment (PC1 scores) and the effect of fauna on decomposition for the three different litter combinations tested. According to a linear mixed model the mixture of *Platonia insignis* and *Goupia glabra* (*Pla+Gou*) had a significantly lower slope (see Table 2 PC1 × species interaction).



The general relationship between the PC1 scores, which mainly capture regional differences of nutrient availability in the litter microenvironment, and the fauna effect on litter decomposition denote that the importance of the latter is context-dependent. Here 'context' would mean not only large-scale environmental features such as climate (e.g. mean annual precipitation and temperature), but also microenvironmental characteristics such as the nutritional status of the microbial communities and the C to nutrient ratios of the surrounding litter pool. Overall, as the conditions for microbial decomposition become worse the relative contribution of soil fauna to decomposition increases. Furthermore, and as seen in previous studies (Hattenschwiler & Gasser, 2005), the contribution of soil fauna was greater in the more diverse litter mixture (the combination between the P-poor *Platonia* with the relatively richer *Goupia*), but the significant smaller slope of this mixture would denote that this litter diversity effect may reduce the strength of the context-dependency, that is decomposition is generally higher and less dependent of the microenvironment when different litter species are combined.

Table S2 Coefficients, significance and r^2 for the linear mixed model assessing the relationship between relative fauna effects on decomposition [decomposition with fauna – decomposition without fauna) / decomposition without fauna] with the environment, mesh size and litter combination.

Relative Fauna Effect (%) ~ PC1 + PC2 + mesh size + litter species + PC1:species + (1 | site:topo:plot:point)			
	Estimate ± s.e	t - value	Pr (>| t |)
Intercept	64.0 ± 5.2	12.2	< 0.001
PC1	-8.9 ± 1.7	-5.4	<0.001
PC2	-4.5 ± 1.6	-2.8	<0.01
Mesh size meso	-9.9 ± 4.0	-2.5	<0.05
Litter species Platonia	5.6 ± 4.9	1.2	0.25
Litter species Pla+Gou	3.4 ± 4.9	0.7	0.48
PC1 : Platonia	2.2 ± 1.7	1.4	0.18
PC1 : Pla+Gou	2.5 ± 1.7	1.5	0.12
Model r^2_m/ r^2_c	12.9 / 41.9		

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