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Plant Litter from Rare Species Increases Functional Diversity and Decomposition of Species Mixtures

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ABSTRACT

Biodiversity loss is altering key ecosystem processes as primary production and decomposition, however, the after-life effects of plant diversity (species-mixing effects) on instream organic matter (litter) decomposition is still under debate. Available evidence of litter species-mixing effect (or the lack of) comes from studies using dominant plant species, despite rare species comprising the majority of species in an ecosystem and can contribute to ecosystem functions or in the provisioning of essential elements. Here, we simulated different extinction scenarios of plant from rare species by incubating leaf litter in artificial channels located within a tropical stream. We thus, assessed whether the loss of litter from rare plant species alters functional diversity (resource dissimilarity) and litter quality (resource concentration) of species mixtures and change decomposition, N loss and fungal biomass production. We show that the loss of litter from rare plant species reduced the functional diversity of litter mixtures and consequently, reduced decomposition, N loss and fungal biomass production. Although species lost also changed the nutritional quality of litter mixtures (resource concentration), it did not affect decomposition or N loss but fungal biomass production. Also, when only similar rare species were present, processes were reduced to higher rates than in the scenario with only dissimilar rare species (except for N loss). Our findings reveal the relevance of litter from rare plant species to key ecosystem processes related to carbon and nutrient flow in tropical streams, especially when dissimilar traits are added to litter pools.

Key words: dominant species; species diversity; biodiversity-ecosystem functioning; nitrogen cycling; fungal biomass production; tropical stream; functional traits.

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HIGHLIGHTS

- Rare species loss reduces the functional diversity of mixtures but not their quality
- Functional diversity decreases reduced decomposition, N loss and fungal biomass
- Dissimilar traits from rare species are critical to instream ecosystem processes

INTRODUCTION

The alarming loss of biodiversity across multiple trophic levels and ecosystems has motivated thousands of experimental studies primarily focused on the diverse effects of plants on key ecosystem processes such as primary productivity and decomposition (Caliman and others 2010; Cardinale and others 2011; Tilman and others 2012). Mounting evidence has accumulated for how the diversity of living plants is fundamental to support ecosystem functions (Cardinale and others 2011; Tilman and others 2012). However, the after-life effects of plant diversity (species-mixing effects) on organic matter (litter) decomposition remains an open question, especially in stream ecosystems where divergent effects were commonly reported. For example, a recent meta-analysis found no litter species-mixing effect on decomposition in streams (Mori and others 2020), which is contrasting to previous and recent studies supporting the role of litter functional diversity (quantified as functional types or species traits) to decomposition (Handa and others 2014; Boyero and others 2021).

Importantly, most studies of litter species-mixing effect on decomposition have used only dominant species from communities, despite the greater probability of rare species (that is, those with limited geographical range and/or low abundance in the local community; Rabinowitz 1981) disappearing from communities (Wardle 2016; Dee and others 2019). As a result, available evidence of diversity effects (or the lack of) on decomposition are from experiments using unrealistic extinction scenarios or assuming uniform biomass across species (but see Gonçalves and Canhoto 2009; Swan and others 2009), despite different species contributions to litterfall in forests and riparian ecosystems (Zhang and others 2014; Tonin and others 2021). For instance, previous studies ignore that few dominant species produce the largest amount of litterfall, while many rare species contribute with only modest quantities of litter (Tonin and others 2021), although with potentially dissimilar and complementary traits. It is therefore important to understand whether these issues lead to under- or overestimation of diversity effect on instream decomposition and unravel the mechanisms behind the observed effects.

In theory, we can expect to find species mixtures with higher functional diversity when rare species add dissimilar traits to litter mixtures and lower functional diversity when rare species are mostly similar to overall mixtures. Possible mechanisms underlying the functional diversity effect on decomposition include nutrient transfer from litter of a nutrient-rich to nutrient-poor species (Handa and others 2014) and divergent use of resources that are complementary in their nutritional composition (that is, litter with different concentration of nutrients and different degrees of toughness) (Vos and others 2013), which translates into higher consumption by decomposers. However, diversity effects on decomposition may occur not only due to the functional diversity of litter mixtures, but due to differences in average litter quality of mixtures (resource concentration effect, that is, mixtures with higher concentration of nutrients or higher toughness), as appear to operate at higher latitudes (Boyero and others 2021). Consequently, the integration of both metrics to describe species mixtures (functional diversity and average quality of litter) provide a powerful tool to investigate whether and how rare species influence diversity effects on instream decomposition, which remains unexplored.

Tropical streams have a primer relevance in this context because their riparian forests harbor a high diversity of tree species, most of which are rare species (Slik and others 2015), are historically understudied ecosystems and are experiencing rapid species loss (Ramírez and others 2008). Such losses not only reduce species diversity but may change the functional diversity of litter species mixtures (dissimilarity in species traits) that end up in forest soils and streams through alterations in species composition (Gessner and others 2010). It is often assumed that losing a rare species has weaker effect than losing a dominant one to ecosystem functions given the low representativeness of a rare species in a community (that is, mass ratio hypothesis; Grime 1998). However, previous studies pointed to the disproportionately negative effect of losing rare species to resistance to invasions (for example, Lyons and Schwartz 2001), nutrient cycling (for example, Marsh and others 2000) and ecosystem multifunctionality (Soliveres and others 2016), although its effects on litter decomposition remain largely unknown. In contrast to the most previous studies that focused on litter mass loss only, we also investigate diversity effects on in-stream processes as an indicator of nutrient transformations (immobilization and mineralization) and biological activity of decomposers (fungal biomass production). While nutrient transformations are essential to stream biota (Wallace and others 1997), especially in detritus-based systems where most energy comes in the form of terrestrial litter, fungi are efficient decomposers and indicators of greater biological activity on plant litter (Krauss and others 2011).

Here, we assessed how the loss of litter from rare plant species influenced three key processes in stream ecosystems (litter decomposition, litter N loss and fungal biomass production), and how that effect depended on whether the lost rare species were functionally similar or dissimilar to dominant species. We did so through an experiment conducted in 20 artificial channels located within a tropical stream and simulating different extinction scenarios that are described below. We hypothesized that (i) the loss of litter from rare plant species would decrease functional diversity of whole litter mixtures and reduce decomposition, N loss and fungal biomass production, and (ii) the loss of litter from rare plant species that are functionally dissimilar to dominant species would have greater influence on these processes than the loss of litter from rare species that are functionally similar. Although we anticipate differences in average litter quality of the mixtures (that is, resource concentration) with changes in species composition, we expect these differences would be of lower importance than the functional diversity of mixtures due to the major influence of dominant species on resource concentration (Tonin and others 2021).

METHODS

Study site

The experiment was carried out in artificial channels set up at the Cabeça-de-Veado stream (15° 53′ 22.15″ S and 47° 50′ 34.10″ W, 1079 m asl), which is located within a preserved area at the Ecological Station of the Botanical Garden of Brasília, Federal District, Brazil. The riparian forest is typical of the Cerrado biome, mostly composed of evergreen species, with dense vegetation cover (Tonin and others 2020) and high species diversity (ca. 111 plant species; Bambi and others 2016). The climate is seasonal (savanna climate, Aw, according to the Köppen-Geiger classification), with a dry season (May to September), a rainy season (October to April) and two transitional seasons: dry–wet (September to October) and wet-dry (April to May; Eiten 1972).

The experimental period (August to October 2016) encompassed the transition between the dry and the rainy season and part of the rainy season; this coincides with the period of highest litterfall and litter storage and fastest litter decomposition in streams of the Cerrado savanna biome (Bambi and others 2017; Tonin and others 2017, 2020). During the experimental period, stream water temperature was 21.1 ± 0.1 °C, conductivity 5.5 ± 0.2 $\mu S\ cm^{-1}$, pH 6.8 ± 0.1, water flow 0.96 ± 0.06 $m^3\ s^{-1}$ and dissolved oxygen 6.6 ± 1.3 $mg\ L^{-1}$ (Table S1). Stream water was nutrient-poor (28.7 ± 1.7 $\mu g\ L^{-1}$ for dissolved inorganic nitrogen (N) and 20.2 ± 1.2 $\mu g\ L^{-1}$ for orthophosphate; Tonin and others 2020).

Dominant and Rare Plant Species

We selected four dominant and four rare species based on the composition of litter mixtures entering the stream. Dominant species considered were those that together contributed with greater than 50% of total litterfall biomass—and thus, are major drivers of litterfall chemistry (Tonin and others 2021)—, whereas rare species had an individual contribution of less than 1% to total litterfall biomass—and thus, less likely to drive litterfall chemistry (Figure S1). The selected dominant species were *Protium spruceanum* (Benth.) Engl., *Calophyllum brasiliense* Cambess., *Ormosia arborea* (Vell.) Harms and *Hyeronima alchorneoides* Allemão, which have chemical and structural litter traits similar of riparian forests of the Cerrado biome (that is, moderate N and phosphorus [P] concentrations and high toughness) (Tonin and others 2021). The selected rare species were *Maprounea guianensis* Aubl., *Tapirira guianensis* Aubl., *Hymenaea courbaril* L. and *Copaifera langsdorffii* Desf., which represented two distinct groups: species with similar (*H. courbaril* and *C. langsdorffii*) or dissimilar (*M. guianensis* and *T. guianensis*) chemical and structural traits to dominant species. Species selection was based on litter traits that are usually most relevant for litter decomposition worldwide, including the Cerrado biome (that is, N and P concentrations and leaf toughness; Gonçalves and others 2007; Zhang and others 2019), using a database of more than 30 plant species from riparian forests of the Cerrado biome.

Litter was collected in the riparian forest of the experimental stream, air-dried at the lab and stored until used. Litter sub-samples were oven-dried

(60 ºC, 72 h), ground in a ball mill to powder and analyzed for the initial concentration of carbon, C), N and P. The concentrations of C and N were obtained from total combustion at 950 °C of one sample (100 mg; accuracy of 0.01 mg) per species in an elemental analyzer (Leco Corporation—TruSpec Micro CHN628), which detects C and N in the form of CO_2 and N_2 by means of infrared cells and thermal conductivity, respectively. The P concentration was obtained from three replicates of each species, using the ascorbic acid method (Flindt and Lillebo 2005). Leaf toughness was estimated from five measurements (in different parts of the leaf mesophyll) using five leaves of each species with a penetrometer, according to the method of Graça and Zimmer (2005).

Litter Quality and Functional Diversity of Mixtures

Litter quality and functional diversity of species mixtures were quantified for each replicate (as detailed below) and used as continuous predictors in linear models to indicate resource concentration or resource dissimilarity, respectively.

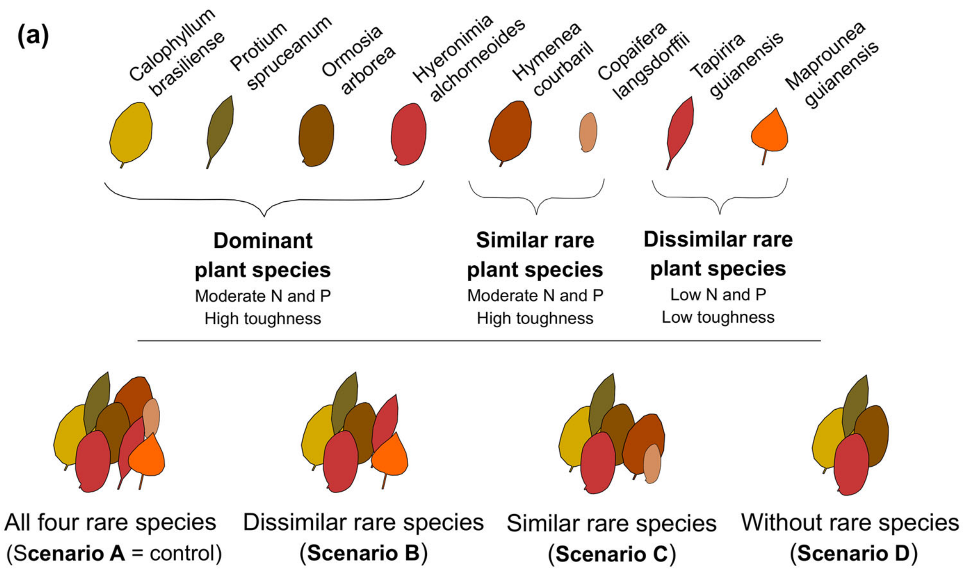
We used the community-weighted mean (CWM) traits of different species mixtures to quantify the average litter quality of mixtures. CWM is often used to describe the functional composition of communities which varies according to the abundances of species (Ricotta and Moretti 2011), but we rather used species dry mass in the mixture as widely used in decomposition studies (García-Palacios and others 2017). First, we calculated the CWM for each trait individually (N and P concentrations and toughness) using the functcomp function from the FD package. Second, we conducted a principal component analysis (PCA; PCA function, FactoMineR package) with the CWM values of each trait after z-score standardization. Finally, litter quality of species mixtures was expressed as the scores of the first PCA axis (hereafter CWM 1), which summarized 89% of total variance of traits (Figure 2a). CWM 1 was positively correlated with N and P concentration and toughness, indicating that resource concentration increases with CWM 1 scores (Figure 2a).

Due to the limitations of categorical classifications (for example, lack of variation within categorical groups; Ricotta 2005), we used the Rao's quadratic entropy index to determine the functional diversity of litter mixtures. The index was calculated using the same three litter traits (N and P concentrations and toughness) weighted by the initial dry mass of each species in the sample, incorporating both the functional dissimilarity between species and their relative biomass (Epps and others 2007). Rao's quadratic entropy index was calculated using all traits simultaneously rather than for each trait individually due to the former approach is more appropriate when there are more species than traits in the dataset (see Laliberte and Legendre 2010), as in our study. It was done using the dbFD function and FD package (Laliberte and Legendre 2010). To explore whether the loss of litter from rare plant species decreases functional diversity of whole litter mixtures we compared 95% confidence intervals for the functional diversity of litter mixtures across the four scenarios. Confidence intervals were based on 1,000 resamples using the BCa method with the boot function and package (Davison and Hinkley 1997; Canty and Ripley 2020). All analyses were performed using R v. 4.0.3, R Core Team 2020).

Experimental Design

We set four different scenarios of rare species loss using litter from all selected dominant and rare species (Figure 1a). All scenarios contained the same four dominant species, but differed in the presence of rare species and/or in the proportion of litter from each species: in scenario A (used as a control), there was no loss of rare species (so all four species were present); in scenario B, two rare species were lost (those functionally similar to the dominant ones; that is, *H. courbaril* and *C. langsdorffii*); in scenario C, two rare species were lost (those functionally dissimilar to the dominant ones; that is, *M. guianensis* and *T. guianensis*); and, in scenario D, all rare species were lost (that is, there were only dominant species) (Figure 1a, S2; Table 1).

Litter decomposition was evaluated using fine-mesh litter bags (250 μm), which allowed colonization and biological degradation of litter only by microorganisms. Although detritivore animals are responsible for a large fraction of litter mass loss in several regions worldwide (and recent evidence pointed to the greatest effect of detritivore diversity loss in tropical streams; Boyero and others 2021), they are mostly absent or have a minor role to decomposition in Cerrado streams, where most decomposition is mediated by microorganism (for example, Gonçalves and others 2007; Boyero and others 2011). The experiment consisted of 20 litter bags (20 × 13 cm), containing 5.0 ± 0.3 g of litter from eight, six or four species (Figure 1a), with a 4:1 biomass ratio between dominant and rare species (except in scenario D where there were only



(b) Artificial channels within the stream

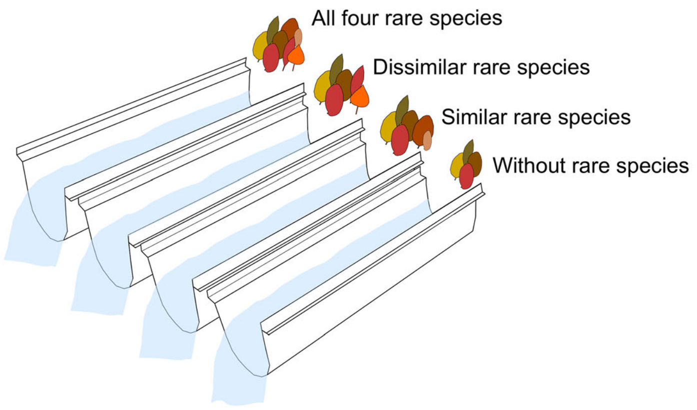


Figure 1. Experimental design representing **a** dominant, similar and dissimilar rare plant species used, their functional traits and the four extinction scenarios of rare species loss (A, B, C and D) and **b** exemplification of how the four scenarios were distributed in one set of artificial channels located within the stream.

Table 1. Litter traits of the eight plant species used in the experiment according to functional group category (dominant species or rare species with similar or dissimilar traits in relation to the dominant species)

Functional group	Plant species	C	N	P	C:N	C:P	N:P	Toughness
Dominant	*Hyeronima alchorneoides*	48.24	0.83	0.12 ± 0.02	58	428 ± 85	7 ± 1	69 ± 3
Dominant	*Calophyllum brasiliense*	53.05	0.65	0.11 ± 0.01	82	472 ± 16	6 ± 1	279 ± 10
Dominant	*Ormosia arborea*	52.83	1.24	0.16 ± 0.01	43	335 ± 6	8 ± 1	238 ± 17
Dominant	*Protium spruceanum*	43.94	0.81	0.11 ± 0.01	54	391 ± 42	7 ± 1	84 ± 11
Rare (similar)	*Hymenaea courbaril*	51.81	0.82	0.26 ± 0.02	63	205 ± 16	3 ± 1	169 ± 13
Rare (similar)	*Copaifera langsdorffii*	53.76	1.07	0.20 ± 0.01	50	271 ± 9	5 ± 1	132 ± 6
Rare (dissimilar)	*Tapirira guianensis*	45.43	0.72	0.06 ± 0.01	63	742 ± 114	12 ± 2	34 ± 3
Rare (dissimilar)	*Maprounea guianensis*	51.40	0.60	0.06 ± 0.01	86	913 ± 43	11 ± 1	61 ± 10

Values are averages ± SE for all litter traits, except C, N and C:N mass ratio that were estimated using one replicate (see methods for details).

Table 2. Results of linear models testing for the effect of litter quality (expressed as the first axis of a PCA using the Community Weighted Mean Values for litter traits, CWM 1), functional diversity (FD, calculated using Rao's quadratic entropy) and their interaction on decomposition, nitrogen (N) loss and fungal biomass production

	df	F-value	p-value
Decomposition			
Intercept	1	492.25	
CWM 1	1	0.47	0.5004
FD	1	4.50	**0.0499**
CWM 1 × FD	1	> 0.01	0.9749
N loss			
Intercept	1	170.70	
CWM 1	1	0.10	0.7497
FD	1	14.60	**0.0015**
CWM 1 × FD	1	0.16	0.6904
Fungal biomass			
Intercept	1	311.89	
CWM 1	1	7.24	**0.0160**
FD	1	68.52	$<$ **0.0001**
CWM 1 × FD	1	0.80	0.3831

Bold values indicate statistically significant differences

dominant species). The 4:1 biomass ratio between dominant and rare species corresponds approximately to the proportion found in the period of the year when litterfall is greatest (that is, dry season and the transition of dry–wet seasons), which coincided with the experimental period (see above; Figure S3). Thus, litter bags of scenarios A, B and C contained 4 g of litter from dominant species (1 g per species) and 1 g of litter from rare species (0.25 g per species in scenario A and 0.50 g per species in scenarios B and C); and scenario D contained 5 g of litter from dominant species only (1.25 g per species). Only intact leaves (not broken or with any sign of decomposition) were used in the experiment and the number of leaves used to each species was kept constant within replicates and scenarios to homogenize litter mixtures.

Litter bags were deployed inside artificial channels (PVC channels of 0.5 length × 0.15 height × 0.15 width m), which were distributed in five sections along a 150 m stream reach. Each section had a set of four channels, one for each treatment. Each channel received three litter bags, one for each recovery time (30, 60 and 90 days of instream incubation), but for this study, only litter bags recovered after 90 days were used (Figure 1b). The use of artificial channels was intended to homogenize the environmental conditions commonly found in field experiments—for example, type of substrate, depth, current, accumulation of fine sediments, presence of other organic materials—and to impede cross-contamination between scenarios. Channels were placed inside the stream in non-sinuous areas to reduce the accumulation of sediment, with regular water flow and with a depth of no more than 40 cm. Channels were covered at the top and closed upstream with a 0.2 cm screen to prevent the entrance of litter other than the experimental one. The experiment was monitored weekly to ensure the homogeneity of environmental conditions inside the channels (for example, water flow and oxygenation) and to prevent the accumulation of organic material upstream of the channels.

Laboratory Procedures

Litter bags were recovered from the field and transported to the laboratory packed in a thermal container with ice. In the laboratory, litter was cleaned with distilled water and two sub-samples were collected: four discs (8 mm in diameter) of each dominant species and 1–2 discs of each rare species present in the mixture, keeping the 4:1 ratio between dominant and rare species. Discs of the first sub-sample were freeze-dried for 48 h and used to estimate fungal biomass, through the concentration of ergosterol of the dominant species and each one of the rare species in the mixture. Ergosterol was quantified by extraction in methanol/KOH solution at 80°C, filtration with SEP-PACK® columns (Waters, VAC RC tC18 500 mg) using vacuum, isopropanol elution and quantification in a high-performance liquid chromatograph (Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC) (Gessner 2005). The extraction efficiency was monitored using an ergosterol standard (Ergosterol ≥ 95% [HPLC], Sigma®), quantified in parallel. Discs of the second sub-sample were oven-dried (60°C, 72 h) and weighed (accuracy of 0.01 mg) to estimate their dry mass. They were then ground separately in a ball mill to powder to quantify final C and N concentrations (as above).

Response Variables

Three response variables were quantified for the entire mixture (mean values of dominant and rare species present in each sample): litter decomposition, litter N loss and fungal biomass production. Litter decomposition was estimated as litter C loss, that is, the difference between initial and final C concentrations (Ci and Cf, respectively), weighted by litter mass loss, that is, the difference between initial and final DM (Mi and Mf, respectively): C loss (prop.) = [(Mi × Ci) (Mf × Cf)]/(Mi × Ci). The use of C to estimate the decomposition allowed to correct for potential inorganic contamination of litter during the experiment, similar to the use of ash-free dry mass but with greater precision in the estimates (cf. Handa and others 2014). Similarly, the N loss was estimated through the difference between initial and final N concentrations (Ni and Nf, respectively), weighted by litter mass loss: N loss (prop.) = [(Mi × Ni) − (Mf × Nf)]/(Mi × Ni). Positive N loss values indicate N decreased during litter incubation, while negative values indicate N increased. Fungal biomass production was estimated as the concentration of ergosterol (in µg) divided by litter discs dry mass (in grams).

Statistical Analysis

We used linear models to test whether functional diversity (resource dissimilarity) and litter quality (resource concentration) of species mixtures or their interaction affect litter decomposition, N loss and fungal biomass production. The models were performed using the gls function (generalized least squares) and the restricted maximum likelihood method (REML), both from the nlme package (Pinheiro and others 2020).The stream section—to which artificial channels were distributed—was treated as a random factor in previous models, but discarded in the final models because both residuals and Akaike information criterion of this model indicated no spatial dependency or need of this component (Table S2). Residuals of each model were explored visually with graphical tools (for example, boxplots and scatterplots), and no violation of the assumptions of linear models was detected (Zuur and others 2009) after the incorporation of variance components in some models (*varIdent*; Zuur and others 2009; Table S2).

To test whether the loss of functionally dissimilar rare species had greater influence on decomposition, N loss and fungal biomass production of litter mixtures than the loss of functionally similar rare species, we quantified the magnitude of such effects. For decomposition and fungal biomass production, the magnitude of effects was calculated through the ratio between the values of each scenario in relation to the control values (that is, scenario A, with all four rare species present), for each replicate; for the N loss, this was calculated by subtracting the values of each scenario from the control values (due to the presence of both positive and negative values), for each replicate. We then calculated non-parametric 95% bootstrapped confidence intervals (as detailed above) for each ratio or difference to test whether these intervals contained a value of either one (for decomposition and fungal biomass production) or zero (N loss)—that is, the null expectation that the decomposition, N loss or fungal biomass production of different scenarios is similar to what would be expected based on the control scenario. The use of this analytical technique eliminates the need to meet assumptions of parametric models (for example, normal distribution and homogeneity of variance), and facilitates the interpretation of the results (Wood 2005). All analyses were performed using R v. 4.0.3 ® Core Team 2020).

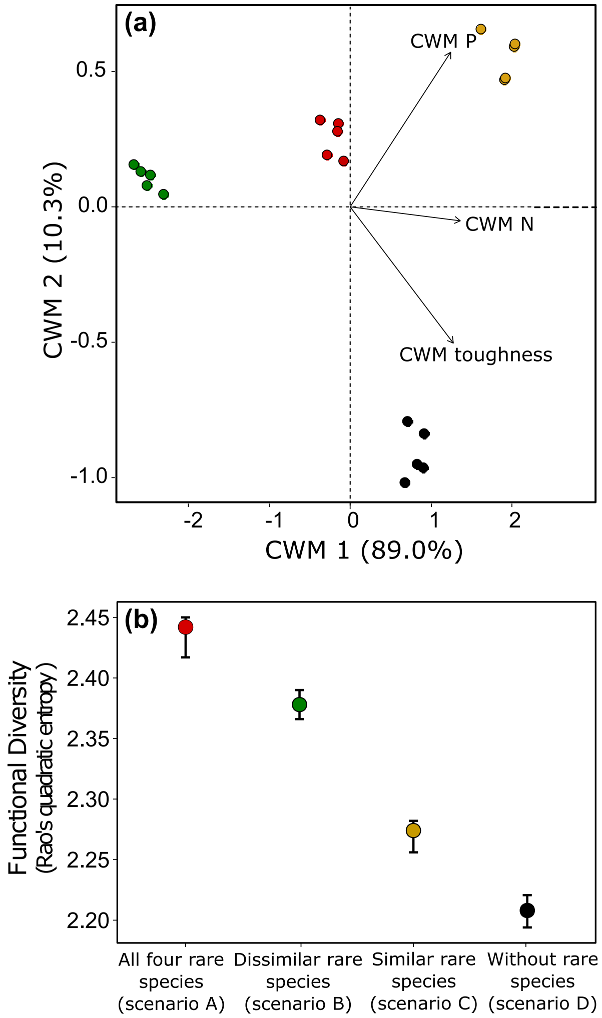


Figure 2. Litter quality (represented by the first axis of a principal component analysis of community-weighted mean [CWM] traits: nitrogen [N] and phosphorus [P] concentrations and toughness (**a**); and functional diversity (as the confidence intervals of Rao's quadratic entropy index; **b** of plant mixtures in the four scenarios of rare species loss.

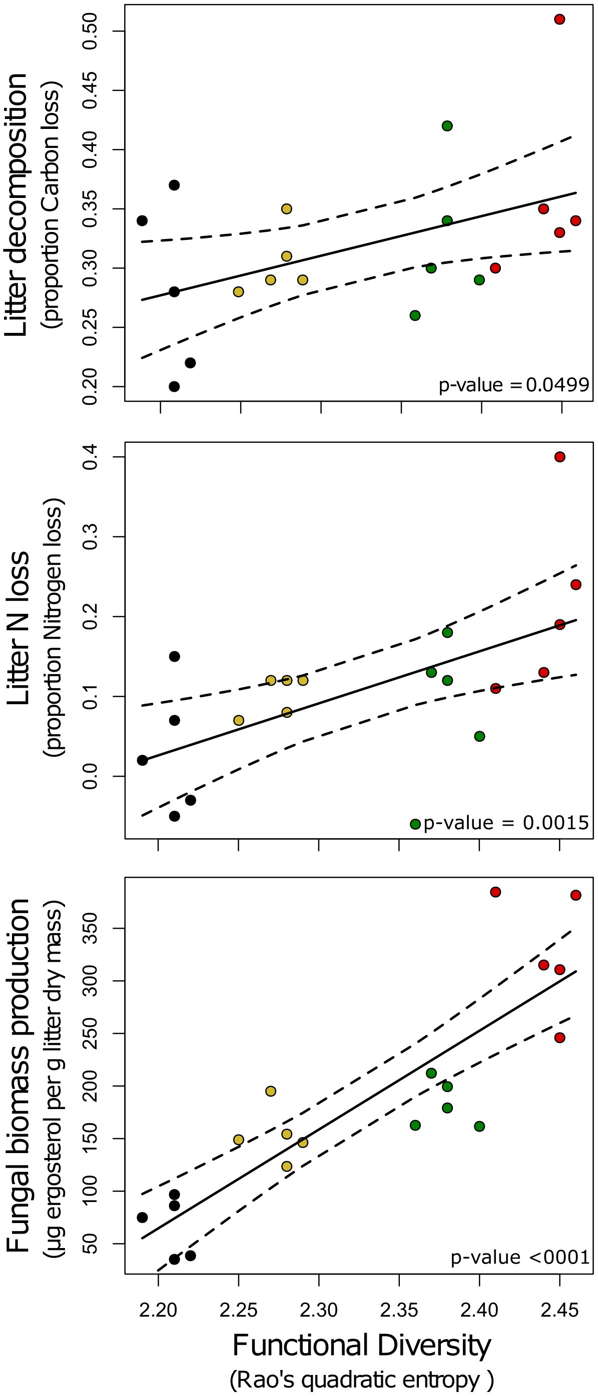


Figure 3. Linear models showing the relationship between functional diversity (Rao's quadratic entropy) of plant litter mixtures and the three processes: litter decomposition, litter nitrogen (N) loss and fungal biomass production. Circles colors represent scenarios that lost rare species: B (green; dissimilar rare species present), C (yellow; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species). Continuous lines are the fit of linear models and dashed lines the 95% confidence intervals.

RESULTS

Confidence intervals of functional diversity index for each scenario evidenced non-overlapping values for the four scenarios and showed that the loss of litter from rare plant species reduced functional diversity of whole litter mixtures by 3 to 11%, on average (Figure 2b).

After 90 days of incubation of litter mixtures in the stream, the average ± SE values of mixtures were: 0.318 ± 0.015 (proportion) for litter decomposition, 0.108 ± 0.023 (proportion) for N loss and 182.6 ± 22.8 µg ergosterol g^{-1} litter dry mass. Using linear models, we observed that an increase in the functional diversity of litter mixtures resulted in a consistent increase in litter decomposition, N loss and fungal biomass production (Figure 3; Table 2); whereas increased litter quality (that is, resource concentration) reduced fungal biomass production, although did not affect decomposition or N loss (Figure 4; Table 2). We did not find any interaction between functional diversity and litter quality in the models (Table 2). The presence of all four rare species in the litter mixtures (scenario A) increased decomposition (by 19% or 84 ± 53 mg C g^{-1}), N loss (18% or 182 ± 68 mg N g^{-1}) and fungal biomass production (80% or 261 ± 23 µg ergosterol g^{-1} DM) when comparing to the scenario without rare species (scenario D; Figure 5; Table S3). These results support the relevance of functional diversity and of rare species to these processes.

To assess the relative importance of similar versus dissimilar rare species in litter mixtures we compared bootstrapped confidence intervals of these two scenarios and their differences in relation to the control (all rare species present). The presence of only dissimilar rare species (scenario B) did not change decomposition to the rates observed in the presence of all rare species, but (ii) reduced N loss (on average, 13% or 130 ± 77 mg g^{-1}) and fungal biomass production (43% or 144 ± 18 µg g^{-1}) (Figure 5; Table S3). In contrast, all processes were reduced in the presence of only similar rare species (scenario C) to similar rates (N loss by 14% or 62 ± 35 mg g^{-1}) or even higher (decomposition by 11% or 112 ± 49 mg g^{-1} and fungal biomass by 53% or 174 ± 17 µg g^{-1}) than those observed in the presence of only dissimilar rare species (Figure 5; Table S3). These results suggested that the presence of dissimilar rare species (rather than similar ones) in litter mixtures was critical to the maintenance of these process.

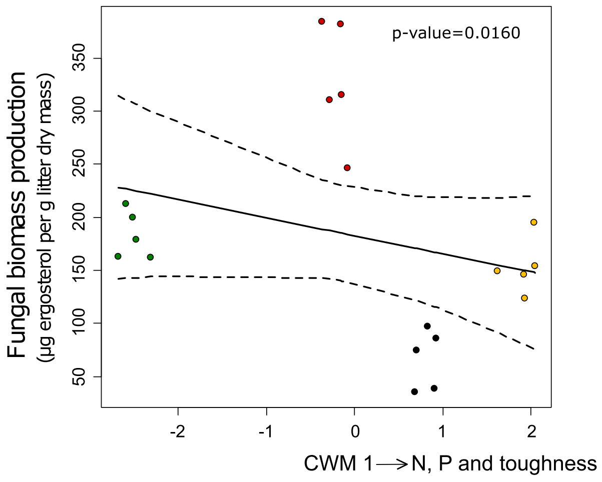
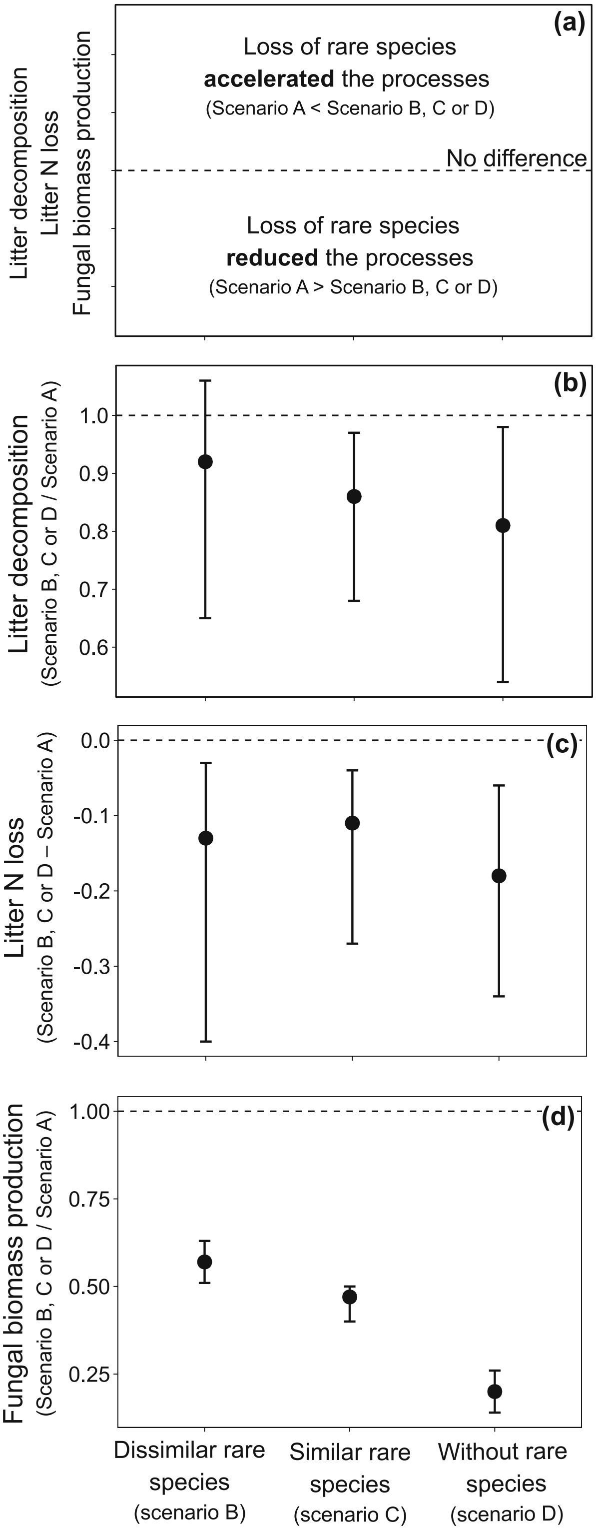


Figure 4. Relationship between litter quality of plant litter mixtures (expressed as the first axis of a PCA using the community weighted mean values for litter traits, CWM 1; Figure 2a) and fungal biomass production. Scores of CWM 1 were positively related to nitrogen (N) and phosphorus (P) concentrations and toughness. *P* value was obtained from a linear model testing for the effect of litter quality, functional diversity and their interactions on the fungal biomass production of litter mixtures (Table 2). Circles colors represent scenarios that lost rare species: B (green; dissimilar rare species present), C (yellow; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species). Continuous line is the fit of the linear model and dashed lines are the 95% confidence intervals.

Discussion

Plant species diversity effects on litter decomposition have been demonstrated using a variety of approaches in both observational and experimental studies (see Cardinale and others 2011; Mori and others 2020). However, considering that most studies were performed outside tropical areas, that is, in relatively low diverse forests with few numbers of rare plant species (for example, Swan and Palmer 2004), it is unsurprising that the role of these rare species has been seldom explored. Here, using artificial channels located within a tropical stream, we showed that losing litter from rare species reduces functional diversity of litter mixtures and inhibits several key ecosystem processes, namely litter decomposition, litter N loss and fungal biomass production. While the functional diversity of mixtures was important to all three instream processes, the quality of litter mixtures was relevant only to fungal biomass production, which is reduced by resource concentration (that is, higher N P and toughness). Also, our results



◀**Figure 5.** Ratio (for litter decomposition and fungal biomass production) or the relative difference (for litter N loss) between scenarios that lost rare species (B, C or D) and the scenario with all four rare species (A): **a** Expected responses of processes to rare species loss; **b** litter decomposition; **c** litter N loss; and **d** fungal biomass production. Circles are means and vertical lines denote upper and lower limits of 95% non-parametric bootstrapped confidence intervals. The dashed line denotes the value of one (for litter decomposition and fungal biomass production) or zero (for litter N loss), that is, the null expectation that the values of processes in the scenarios that lost rare species are not different from the scenario with all four rare species.

evidenced that the presence of litter from functionally dissimilar rare species in litter mixtures maintained or caused less reduction on the above-mentioned processes (except for N loss).

Our finding that rare species influence key ecosystem processes contradicts the mass ratio hypothesis (Grime 1998), which states that species in a community have an effect on the ecosystem that is proportional to their mass. Thus, we could expect no effect of rare species presence or their functional traits on ecosystem processes due to their low representativeness in terms of litter mixture total mass (that is, one quarter of total mass) (Tardif and others 2014). However, we showed that even a relatively small decrease in the average functional diversity of litter mixtures (RaoQ from 2.44 to 2.21)—for example, through the removal of rare species—was sufficient to considerably reduce the studied processes. Other studies have also found a decrease in the functional diversity (or richness, specialization and originality) with the loss of rare species in tallgrass prairies in the North America (Jain and others 2014), stream fish in the Brazilian Amazon, rainforest trees in French Guiana and birds in the Australian wet tropics (Leitão and others 2016). Similarly, others have found a reduction in key ecosystem processes—for example, productivity and nutrient cycling of terrestrial ecosystems—with the loss of rare species (Dee and others 2019).

Positive effects of functional diversity on instream litter decomposition have been previously reported in field, laboratory and mesocosm experiments (for example, Handa and others 2014; Oliva and others 2019; López-Rojo and others 2018; Boyero and others 2021), and on N loss in field experiments (Handa and others 2014). These results contrast with others showing weak or no evidence of litter trait dissimilarity effects on decomposition (for example, Hoorens and others 2003; Schindler and Gessner 2009; Frainer and others 2015) and on N loss (Frainer and others 2015). Inconsistent responses to litter functional diversity could be due to (i) its short-term effect that may disappear with time (Patoine and others 2017) and/or (ii) the different or arbitrary choice of functional diversity metrics across studies. For example, some studies have used functional diversity based on a single litter trait or on a categorical classification such as functional groups (Hoorens and others 2003; Schindler and Gessner 2009; Oliva and others 2019). Although positive effects of functional diversity on decomposition using these approaches have been observed (for example, Oliva and others 2019), they may not be adequate because they do not consider the complexity of multiple litter traits that potentially influence decomposition (Epps and others 2007).

Increased decomposition and N loss with greater functional diversity of mixtures may be due to nutrient transfer from nutrient-rich (dominant and similar rare species in our study) to nutrient-poor species (dissimilar rare species), which is promoted by leaching or fungi growing on litter (for example, Handa and others 2014). In streams, aquatic hyphomycetes dominate fungal communities in terms of biomass (Krauss and others 2011) and are capable of extending their hyphae to acquire resources from considerable distant litter (Ritz 2006). Laboratory studies using isotopes supported the occurrence of nutrient transfer between chemically distinct litter types (for example, Lummer and others 2012), which was also observed in a large-scale experimental field study using litter mixtures of N-fixing and rapidly decomposing deciduous plants (Handa and others 2014). In our study, carbon of high quality (that is, litter of lower toughness) from dissimilar rare species may have favored microbial growth, which in turn increased the demand for nutrients and promoted the transfer of N from neighboring dominant species (and/or rare species functionally similar to the dominant ones) (Lummer and others 2012). In this context, the increase of fungal biomass production with functional diversity suggests these fungi benefited from the variability of resources via complementarity or facilitation mechanisms—as previously observed (for example, Santoja and others 2017).

It is important to recognize that our experimental design did not fully allow disentangling species richness from functional diversity in different scenarios of rare species loss. For instance, the scenario with greatest functional diversity has also more species, while lower functional diversity occurred when fewer species were present. This could indicate that the enhanced processes were due to increased species richness of mixtures; however, this is unlikely, as all processes increased consistently and linearly across the functional diversity gradient even when species richness was the same. Also, recent studies support the use of functional diversity rather than species richness as a more important predictor of litter decomposition (Handa and others 2014; Boyero and others 2021), as well as of other ecosystem processes (Cadotte and others 2011).

In line with previous BEF studies (for example, Marsh and others 2000; Lyons and Schwartz 2001; Soliveres and others 2016), our study provides support that functionally unique rare species may contribute disproportionately to ecosystem functioning. Despite the rapid biodiversity decline faced by the tropics, BEF research has mostly neglected this region (Clarke and others 2017). This lack of knowledge in such rapidly growing research topic is surprising, considering that rare species are more vulnerable to disturbances (Lavergne and others 2005) and predominantly support distinct sets of traits capable to influence the functioning of ecosystems (Mouillot and others 2013). In consequence, there is a crucial need to a better integration of BEF research into the tropical context, especially to understand the role of rare species in such diverse ecosystems.

We provide novel evidence that litter from rare plant species is capable of accelerating key ecosystem processes related to carbon flow in tropical streams, especially when species provide dissimilar traits to litter pools. Our findings have important management implications and relevance to restoration of riparian forests in the tropics if the aim is to prioritize efforts and resources in the maintenance of species with greater functional role to ecosystems. Although such findings have emerged from studies in tropical streams, they are not limited to these ecosystems considering that the diversity of rare species is often greater than that of dominant species, and litter traits of species greatly differ within ecosystems (Homeier and others 2021). Also, our results provide empirical support for using trait-based approaches rather than species identity when modeling the effects of environmental changes such as species extinction or invasions on ecosystems.

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