



Evenness modulates effects of the loss of plant litter from rare species on fungal decomposers in tropical streams

Raiane S. Rabelo^{a,*}, Alan M. Tonin^a, Adriana Oliveira Medeiros^b, Luz Boyero^{c,d}, José F. Gonçalves Júnior^a

^a Department of Ecology, Institute of Biology, Univ. of Brasília (UnB), Brasília, Federal District, Brazil

^b Environmental Microbiology Lab, Institute of Biology, Federal Univ. of Bahia, Salvador, Bahia, Brazil

^c Faculty of Science and Technology, Univ. of the Basque Country (UPV/EHU), 48940, Leioa, Spain

^d IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

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ABSTRACT

While most studies evaluating plant species diversity effects on in-stream fungal decomposers have focused on dominant species, our study simulated different rare species extinction scenarios. We assessed whether the loss of these species altered the fungal biomass and aquatic hyphomycete sporulation, diversity and taxonomic composition in two experiments: even experiment, where we used the same biomass for all species (i.e., even litter mixtures); and natural proportion experiment, where we kept proportions of plant species as found in stream leaf litter (i.e., natural litter mixtures). We found that the loss of litter from rare plant species reduced fungal biomass and the reduction was greater for natural litter mixtures, indicating that evenness modulated the effect of diversity on fungal biomass, possibly through resource dissimilarity. Our findings reveal the relevance of litter from rare plant species and of the maintenance of natural species proportions in tropical riparian forests for the functioning of stream ecosystems.

1. Introduction

Tropical streams are highly diverse ecosystems, but they are suffering rapid loss of species diversity in their riparian forests due to anthropic disturbances (Ramírez et al. 2008). Rare species – i.e., those with a geographical range limited and/or low abundance in the community (Rabinowitz 1981) – are more vulnerable than dominant species to anthropogenic impacts on ecosystems, and thus their loss commonly drives changes in diversity (Wardle 2016; Dee et al. 2019). The loss of rare species can be expected to have low impact on ecosystems, due to their low abundance (see Dee et al. 2019). However, some studies indicate that, when rare species with unique functional roles are lost, food webs and key ecosystem processes such as decomposition and nutrient cycling can be seriously altered (Marsh et al. 2000; Bracken and Low 2012; Rabelo et al. 2022).

In this context, rare plant species may add unique traits to litter mixtures and increase their functional diversity, that is, the variation in species trait values. This may affect both organic matter processing and decomposer organisms (resource dissimilarity effects; Rabelo et al.

2022). Numerous mechanisms have been put forward to explain positive effects of functional diversity related to the organic matter processing such as nutrient transfer among distinct litter species via fungal hyphae or leaching (Handa et al. 2014) and complementary resource use by consumers (i.e., litter with different nutrients concentration or toughness; Chapman et al. 2013; Vos et al. 2013; Handa et al. 2014). Besides being able to increase the functional diversity of litter mixtures, rare plant species can change the average litter quality of mixtures, although they have low representativeness in terms of biomass in litter mixtures. This may also affect decomposer organisms (resource concentration effects, that is, mixtures with higher concentration of nutrients or higher toughness; Rabelo et al. 2022).

The aquatic hyphomycetes include a variety of fungal species commonly associated with litter in streams (Krauss et al. 2011) and they are main agents of litter breakdown in tropical streams (Gonçalves et al. 2007; Graça et al. 2016). Even though aquatic hyphomycetes have a high degree of functional redundancy (Gessner et al. 2007), some studies have indicated species preferences for specific litter substrates, which are mainly determined by differences in chemical and structural traits.

* Corresponding author.

E-mail address: raiane.serejo@yahoo.com (R.S. Rabelo).

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The litter that is soft (low toughness) and has high concentrations of nutrients is generally preferred by these fungi (e.g., [Gulis 2001](#); [Gomes et al. 2016](#)). In addition, studies in terrestrial (e.g., [Chapman et al. 2013](#)) and aquatic (e.g., [Kominoski et al. 2009](#); [Jabiol and Chauvet 2012](#)) ecosystems indicate that more functionally diverse litter mixtures support more diverse fungal communities, as they favor the colonization of fungal species with different functional traits or energy needs. Fungal communities can also benefit from more heterogeneous resources through complementarity use of resources, which may optimize nutrient acquisition and contribute to the increase of fungal biomass and activity (e.g., [Bardgett and Shine 1999](#); [Chapman et al. 2013](#)).

Plant species evenness in litter mixtures influences the heterogeneity and overall quality of litter resources to consumers (e.g., nutrient contents, phenolics and structural compounds), and thus also can affect decomposer organisms (e.g., [Goncalves and Canhoto, 2009](#); [Pereira and Ferreira 2022](#)). Despite that, in most biodiversity-ecosystem function (BEF) experimental studies conducted in the tropics, litter mixtures involve higher species evenness than is normally encountered in nature. In general, these studies equate diversity to species richness (see [Hill-Brand et al. 2008](#)), without taking evenness into account. However, leaf litter in tropical streams is often dominated by a few species, with the rest being rare species ([Tonin et al. 2021](#)). Consumption by shredders seems not to be influenced by the litter species evenness in these streams ([Sena et al. 2020](#)). In contrast, the effect of litter species evenness on aquatic hyphomycetes in tropical streams still is an understudied question. Thus, it is important to investigate how the species evenness can influence the diversity effects of litter mixtures on these fungal decomposers in these streams ([Swan et al. 2009](#); [Liu et al. 2020](#)).

Here, we assess the effect of different aspects of litter diversity (including functional diversity and species evenness) on fungal colonization by manipulating the presence/absence of rare species functionally similar or dissimilar to dominant species and the species evenness (in terms of biomass) in litter mixtures placed within artificial channels in a tropical stream. We hypothesized that (1) the loss of litter from rare plant species would reduce fungal biomass, diversity and activity (i.e., sporulation rate) if litter species lost are in similar proportions to other species (even litter mixtures) – because of a greater concentration of elements and compounds (resource concentration) – and are functionally dissimilar to dominant species (resource dissimilarity; [García-Palacios et al. 2017](#)). In contrast, we expected (2) weaker or no effect on fungal variables when litter species lost are in smaller proportion (natural litter mixtures) or are functionally similar to dominant species.

2. Methods

2.1. Study site

We conducted two experiments at the Cabeça-de-Veado stream (15° 53' 22.15" S and 47° 50' 34.10" W, 1079 m asl.), located in a permanent preservation area of the Ecological Station of the Botanical Garden of Brasília, Federal District, Brazil. The climate of the region is tropical savanna (Aw), characterized by two seasons, wet (October–April) and dry (May–September). The experiments were carried out from August to October 2016, during the dry-wet transition and part of the wet season, which are the periods of higher litterfall, litter storage and litter breakdown in streams of the Brazilian savanna biome or 'Cerrado' ([Bambi et al. 2016a](#); [Tonin et al. 2019](#)).

The riparian forest of the stream presents perennial vegetation, high floristic diversity (ca. 111 plant species; [Bambi et al. 2016b](#)) and dense vegetation cover (70–95%; [Ribeiro and Walter 2008](#)). During the experimental period the stream water presented circumneutral pH (6.79 ± 0.12), low conductivity ($5.51 \pm 0.18 \mu\text{S cm}^{-1}$), good oxygenation ($6.62 \pm 1.3 \text{ mg L}^{-1}$), stable temperature ($21.15 \pm 0.01 \text{ }^\circ\text{C}$) and low discharge ($0.96 \pm 0.06 \text{ m}^3 \text{ s}^{-1}$; [Rabelo et al. 2022](#)). The concentration of dissolved nutrients was low ($28.7 \pm 1.7 \mu\text{g L}^{-1}$ and $20.2 \pm 1.2 \mu\text{g L}^{-1}$ of dissolved inorganic nitrogen [N] and orthophosphate, respectively;

[Tonin et al. 2019](#)).

2.2. Dominant and rare plant species

Four dominant and four rare species were selected from those present in the riparian forest. The selection was made based on the composition of terrestrial litterfall of the experimental stream throughout one year, studied previously (September 2010 to August 2011; [Rabelo et al. 2022](#)). Dominant species considered were those with the highest proportions in biomass that together contributed >50% of the total litterfall biomass, whereas rare species had an individual contribution of less than 1% to the total. Dominant species with high toughness and moderate nutrient concentrations (i.e., N and phosphorus [P]) were chosen to represent the chemical and structural traits of dominant species in the Cerrado ([Tonin et al. 2021](#), [Fig. 1A](#); [Table S1](#)). Rare species were selected to represent two distinct groups: species with either similar or dissimilar chemical and structural traits (i.e., toughness and nutrient concentrations) to dominant species ([Fig. 1A](#); [Table S1](#)). The dominant species selected were *Protium spruceanum* (hereafter *Protium*), *Calophyllum brasiliense* (*Calophyllum*), *Ormosia arborea* (*Ormosia*) and *Hyeronima alchorneoides* (*Hyeronima*); similar rare species were *Hymenaea courbaril* (*Hymenaea*) and *Copaifera langsdorffii* (*Copaifera*), and dissimilar rare species were *Maprounea guianensis* (*Maprounea*) and *Tapirira guianensis* (*Tapirira*). For more detailed information on dominant and rare species selection and their chemical and structural traits, see [Rabelo et al. \(2022\)](#).

2.3. Experimental design

The experiments were named even experiment and natural proportion (hereafter NatProp) experiment. They were simultaneous and simulated the loss of rare plant species from litter mixtures. In even experiment, the abundances of different dominant and rare species were kept even in terms of biomass; in NatProp experiment, their proportions were determined by a litterfall survey from the experimental stream (cf., [Rabelo et al. 2022](#)).

Four litter mixtures made of different combinations of dominant and rare species were used in the experiments, in order to simulate different scenarios of rare species loss. All scenarios contained the four dominant species mentioned above, plus different numbers and combinations of rare species: scenario A had four rare species, with no species loss (control); scenario B had two rare species (*M. guianensis* and *T. guianensis*) and represented the loss of rare species functionally similar to dominant species; scenario C had two rare species (*H. courbaril* and *C. langsdorffii*) and represented the loss of rare species functionally dissimilar to dominant species; and scenario D had no rare species ([Fig. 1A](#); [Table S1](#)).

Whole leaves of each species were collected from natural litterfall in the study stream, air-dried and stored until used. Then, they were weighed and introduced into fine-mesh litterbags (0.25 mm), which allowed colonization and biological degradation only by microorganisms. For even experiment, we used 60 litterbags (20 × 13 cm), 15 for each scenario. Each dominant and rare litter species was represented equally by biomass (as mentioned above). That is, each litterbag contained a total of $5.0 \pm 0.3 \text{ g}$ – $0.63 \pm 0.3 \text{ g}$ per species in scenario A, $0.83 \pm 0.3 \text{ g}$ per species in scenarios B and C and $1.25 \pm 0.3 \text{ g}$ per species in scenario D. Litterbags were distributed in 20 PVC artificial channels (0.5 × 0.15 × 0.15 m), which were placed along a 150 m stretch of the stream in five groups of four channels (sections), with each channel within a group representing a different scenario ([Fig. 1B](#)). Each channel received three litterbags, one for each incubation period (20, 40 and 68 days; [Fig. 1B](#)). There was thus a total of five replicated litterbags per scenario for each incubation period.

For NatProp experiment, methods were similar, except for the litter and the incubation periods. Litter proportions followed a 4:1 ratio, with $4 \pm 0.3 \text{ g}$ in total from dominant species ($1 \pm 0.3 \text{ g}$ per species) and $1 \pm 0.3 \text{ g}$ in total from rare species ($0.25 \pm 0.3 \text{ g}$ per species in scenario A

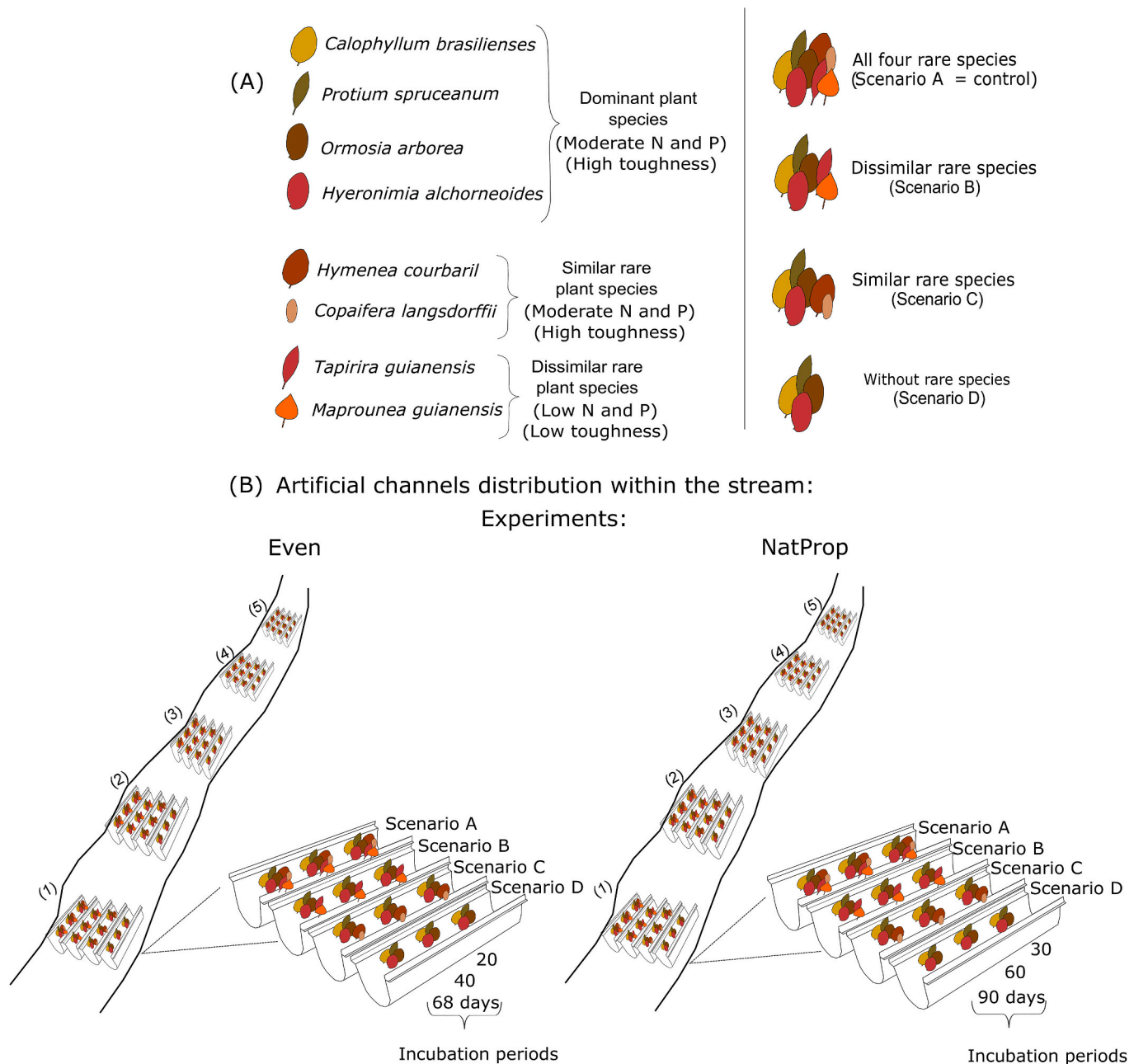


Fig. 1. Experimental design representing (A) dominant, similar and dissimilar rare plant species used, their functional traits and the four extinction scenarios of rare species loss (A, B, C and D), and (B) organization of litterbags of each scenario in the artificial channels, even and natural proportion (NatProp) experiments, incubation periods and distribution of the channels composing for each experiment a total of five sections with four channels along the stream.

and 0.50 ± 0.3 g per species in scenarios B and C); the exception was scenario D, where there were only dominant species (1.25 ± 0.3 g per species). Incubation periods in this case were 30, 60 and 90 d (Fig. 1B).

We used artificial channels to homogenize the environmental conditions found in field as type of substrate, depth, current, accumulation of fine sediments and presence of other organic materials. The channels were placed in areas non-sinuuous, with regular water flow and with a depth of around 40 cm, being covered at the top and closed upstream with a screen (0.2 cm) to prevent the entrance of litter other than the experimental one.

2.4. Laboratory procedures

At each retrieval time, litterbags were collected and transported to the laboratory within a thermal container with ice. In the laboratory, the

litter was washed carefully with distilled water. Two sub-samples of discs (8 mm in diameter) were taken for the even (2 discs per species in each mixture) and NatProp (4 discs per dominant species and 1–2 discs per rare species in each mixture, maintaining the 4:1 ratio) experiments, except for the last incubation period (i.e., 68 or 90-days period of even and NatProp experiment, respectively), when three sub-samples were taken.

The discs of the first sub-sample were used to induce sporulation by shaking (60 rpm) the leaf discs in 50 mL of distilled water for 48 h in a 12:12 h light regime with constant temperature (20 °C), conditions like those observed in the stream. After 48 h of agitation, the water was collected and spores fixed with 4% formalin. Aliquots of the conidial suspensions were filtered (Millipore, pore size 5 μ m, Billerica, MA, USA), and filters were stained with 0,05% of cotton blue (Sigma-Aldrich, Steinheim, Germany) in 60% lactic acid (Vetec, Rio de Janeiro, Brazil)

and observed with a compound microscope at 400× magnification (Olympus B×43). Conidial morphology was used to identify aquatic hyphomycete species (Bärlocher 2020) using taxonomic keys (Gulis et al. 2005).

The discs of the second sub-sample were oven-dried (60 °C, 72 h) and weighed to determine dry mass (DM; accuracy 0.01 mg). The discs of the third sub-sample (taken only for the last period) were freeze-dried for 48 h and used to estimate fungal biomass, through concentration of ergosterol of the dominant species group 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 high-performance liquid chromatograph (Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC) (Gessner 2020). The remaining litter was oven-dried (60 °C, 72 h), weighed to determine DM and added to the DM of the discs taken from the sub-samples to determine the final DM of the mixture: final DM = litter DM + (disc DM × 2 or 3).

2.5. Data analysis

2.5.1. Litter quality and functional diversity of mixtures

Litter quality and functional diversity of species mixtures were calculated for all replicates (see below) and used as continuous predictors in linear models to indicate resource concentration or resource dissimilarity effects, respectively.

Community-weighted mean (CWM) traits of different species mixtures were used to quantify the average litter quality of mixtures. Trait values were based on measurements by Rabelo et al. (2022) from litter collected in the riparian forest of the study stream. The traits measured were N and P concentrations and toughness (see Rabelo et al. (2022) for details on measurement procedures). For abundance values, we used species dry mass in the mixture as used in decomposition studies (García-Palacios et al. 2017). First, we calculated the CWM for each trait individually using the `functcomp` function from the `FD` package (Lavorel et al. 2008). Then, we conducted a principal components analysis (PCA; `PCA` function, `FactoMineR` package; Lê et al. 2008) using the CWM values of all the traits measured. Before conducting the PCA, we applied standardization using Z-scores. Litter quality of species mixtures was assessed by retaining the first PCA axis (hereafter CWM 1).

Categorical classifications (including our scenarios of rare species loss described above) have several limitations, such as the lack of variation within categorical groups (Ricotta 2005). Therefore, Rao's quadratic entropy index was used to determine the functional diversity of litter mixtures (Epps et al. 2007). This was calculated using the same above-mentioned litter traits (N and P concentrations and toughness) weighted by initial dry mass of each species in the sample, using the `dbFD` function in `FD` package (Laliberté and Legendre 2010). Rao's quadratic entropy index is commonly used in multi-trait instead of single-trait approaches, which is more appropriate when there are more species than traits in the dataset, as in our study (Laliberté and Legendre 2010).

We compared 95% confidence intervals for the functional diversity of litter mixtures across the four scenarios to assess whether the loss of litter from rare plant species decreases the functional diversity of whole litter mixtures. Confidence intervals were based on 1000 resamples using the `BCa` method with the `boot` function and package (Davison and Hinkley 1997; Canty and Ripley 2020).

2.5.2. Calculation of response variables

The percent of litter mass loss from the mixture was calculated using the initial (M_i) and final (M_f) DM of litter: $(M_i - M_f)/M_i \times 100$. Fungal biomass was estimated as the concentration of ergosterol (in μg) divided by litter disc dry mass (in g; Gessner and Chauvet 1993); then, it was quantified for the entire mixture (i.e., mean values of dominant and rare species present in each sample). The sporulation rate from the mixture was expressed as the number of spores per mg of DM per day (conidia

$d^{-1} \text{ mg}^{-1} \text{ DM}$) (Bärlocher 2020). Aquatic hyphomycete diversity in the mixture was assessed using the Simpson diversity index (Magurran 2010) and calculated using the diversity function of the `vegan` package (Oksanen et al. 2018).

2.5.3. Assessing the importance of the loss of litter from rare plant species and evenness for the fungal colonization and the role of litter quality and functional diversity

To test the effects of rare species loss scenarios, evenness (even and `NatProp` experiments) and their interaction on fungal parameters (fungal biomass, aquatic hyphomycete sporulation rate and diversity) and the role of litter quality (resource concentration) and functional diversity (resource dissimilarity), we used two alternative approaches. First, we fitted a linear model with the scenario, evenness, and their interaction as predictor variables. Secondly, we fitted a model with litter quality, functional diversity, evenness, and all interactions. We did not fit all variables into a single model because we evaluated collinearity between the continuous (litter quality and functional diversity) and categorical (scenario) predictor variables using linear models (Zuur et al. 2009) and collinearity was detected. After a significant interaction between scenario, litter quality or functional diversity and evenness was obtained, we used separate linear models for both experiments to examine how responses differed between them. Moreover, after a significant effect was obtained, the relationships between fungal biomass, aquatic hyphomycete sporulation rate and diversity and litter mass loss were investigated using linear models.

We used the `gls` or `lme` function of the `nlme` package (Pinheiro et al. 2018) to fit all models. The best models were selected based on the Akaike Information Criterion (AIC), and when required we used a variance component (`varIdent`; Zuur et al. 2009), and/or the different stream sections or incubation periods were treated as random factors. Residuals of each model were visually explored using graphical tools (e.g., boxplots and scatterplots) to check for violations of the assumptions of linear models, and no violation was detected (Zuur et al. 2009). We used the `rsquared` function of the `piecewiseSEM` package to calculate pseudo-R² of models (Lefcheck 2016) and explored pairwise multiple comparisons between scenarios with Tukey tests using the `glht` function of the `multcomp` package (Hothorn et al. 2008).

In addition, non-metric multidimensional scaling (NMDS; `metaMDS` function of the `vegan` package) was used to visualize the effects of scenario and evenness (categorical predictor variables) on the composition of the aquatic hyphomycete communities, where abundances (spore number) of hyphomycete species was the multivariate response variable (Bray-Curtis dissimilarity matrix). One sample was considered outlier by visual exploration and therefore removed. Then, a permutational analysis of variance (PERMANOVA; `adonis` function of the `vegan` package) was performed to test the differences observed between scenario, evenness, and their interaction. All analyses were performed using the software R version 4.1.3 (R Development Core Team, 2022).

3. Results

3.1. Litter quality and functional diversity of mixtures

The first PCA axis explained most of the variance of the mean trait values of litter mixtures (84.1%). The CWM 1 was positively loaded by higher concentrations of N, P and toughness (Fig. 2A). The litter mixtures of the scenario with presence of only dissimilar rare species (scenario B; on the N and P-poor and with low toughness end) and of the scenario with presence of only similar rare species (scenario C; on the N and P-rich and with high toughness end) were the two extremes along CWM 1 (Fig. 2A).

Litter mixtures in both experiments showed reduced functional diversity with the loss of litter from rare plant species (Fig. 2B). The confidence intervals of the functional diversity index for each scenario evidenced non-overlapping values for the four scenarios in `NatProp`

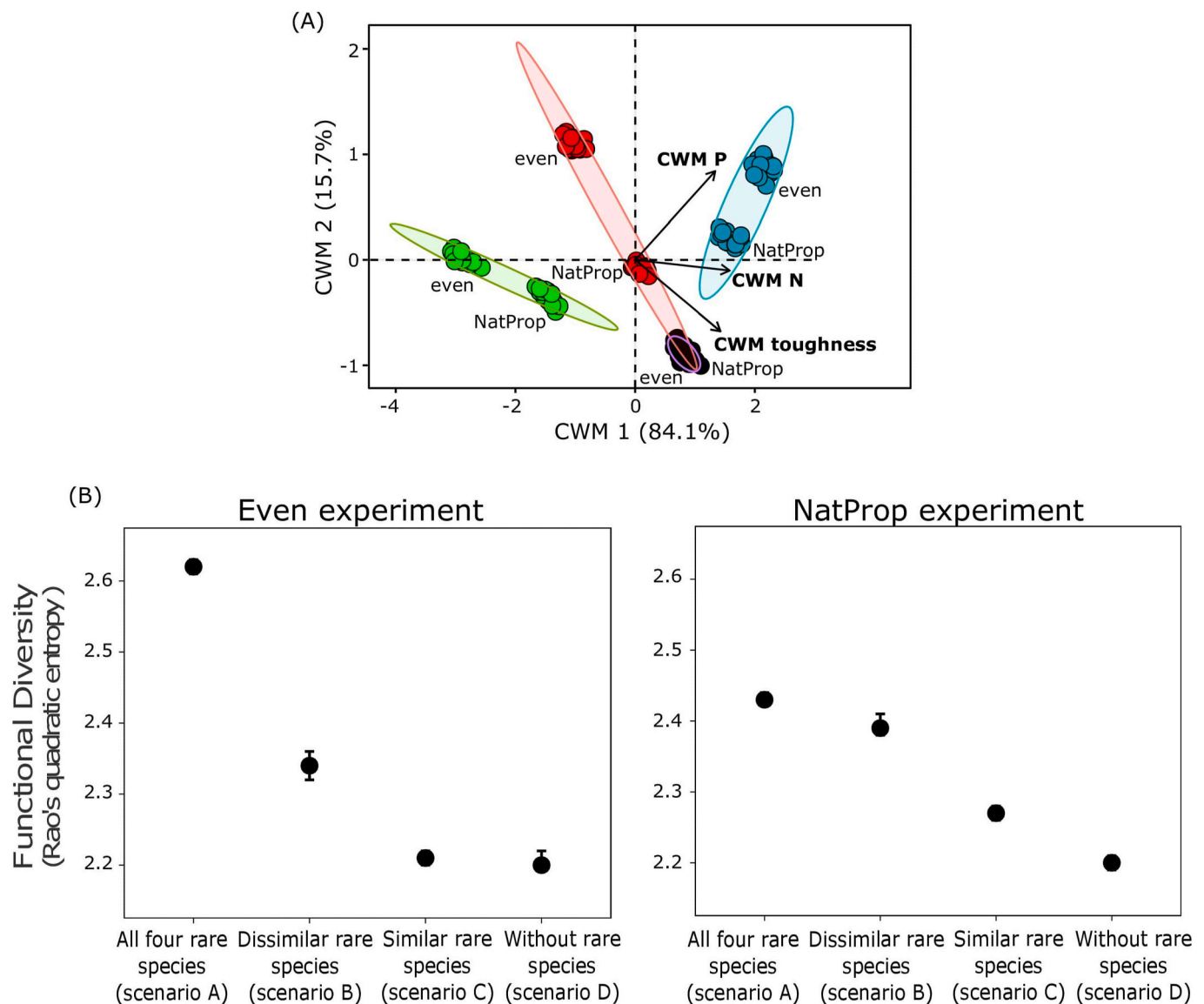


Fig. 2. Litter quality and functional diversity of plant mixtures in the four scenarios of rare species loss in even and natural proportion (NatProp) experiments. The quality (A) was represented by the first axis of principal component analysis of community-weighted mean (CWM) traits: nitrogen (N) and phosphorus (P) concentrations and toughness, and the functional diversity (B) by confidence intervals of Rao's quadratic entropy index. Circles colors (A) represent scenarios that lost rare species: B (green; dissimilar rare species present), C (blue; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species).

experiment (Fig. 2B). Even experiment values were overlapping only for the scenarios with presence of only similar rare species (scenario C) and without rare species (scenario D; Fig. 2B).

3.2. Effects of the loss of litter from rare plant species and evenness on fungal decomposers

Using linear models, we found that the scenarios of rare plant species loss significantly explained variation in fungal biomass. However, we did not find an effect on aquatic hyphomycete sporulation rate or diversity (Fig. 3; Table 1). The presence of rare plant species, especially the dissimilar ones, tended to increase fungal biomass – being the increase significant for mixtures with the presence of only dissimilar (scenario B; on average by more than two times higher) or only similar rare species (scenario C; 95%) when comparing to mixtures without rare species (scenario D; Fig. 3A). An increase in the fungal biomass resulted in a significant increase in litter mass loss (Fig. S1).

We did not find an effect of litter evenness alone on fungal biomass,

aquatic hyphomycete sporulation rate or diversity (Tables 1 and 2). However, there was a significant interaction between scenario and evenness for fungal biomass (Table 1). While in even experiment the presence of only dissimilar (scenario B) or similar (scenario C) rare species increased fungal biomass by 65 and 78%, respectively, when compared to mixtures without rare species (scenario D), in NatProp experiment fungal biomass was more than twice higher in scenarios B and C compared to D (Fig. 4).

We found no clear pattern related to the decline of functional diversity observed in rare species loss scenarios for fungal biomass in even experiment, but we did in NatProp experiment, although scenarios B and C did not differ significantly (Fig. 4). Mixtures with presence of all four rare species (scenario A) had the highest fungal biomass: 79% higher compared to mixtures with presence of only dissimilar rare species (scenario B), more than twice higher than mixtures with presence of only similar rare species (scenario C) and four times higher than mixtures without rare species (scenario D; Fig. 4).

We identified ten species of aquatic hyphomycetes on decomposing

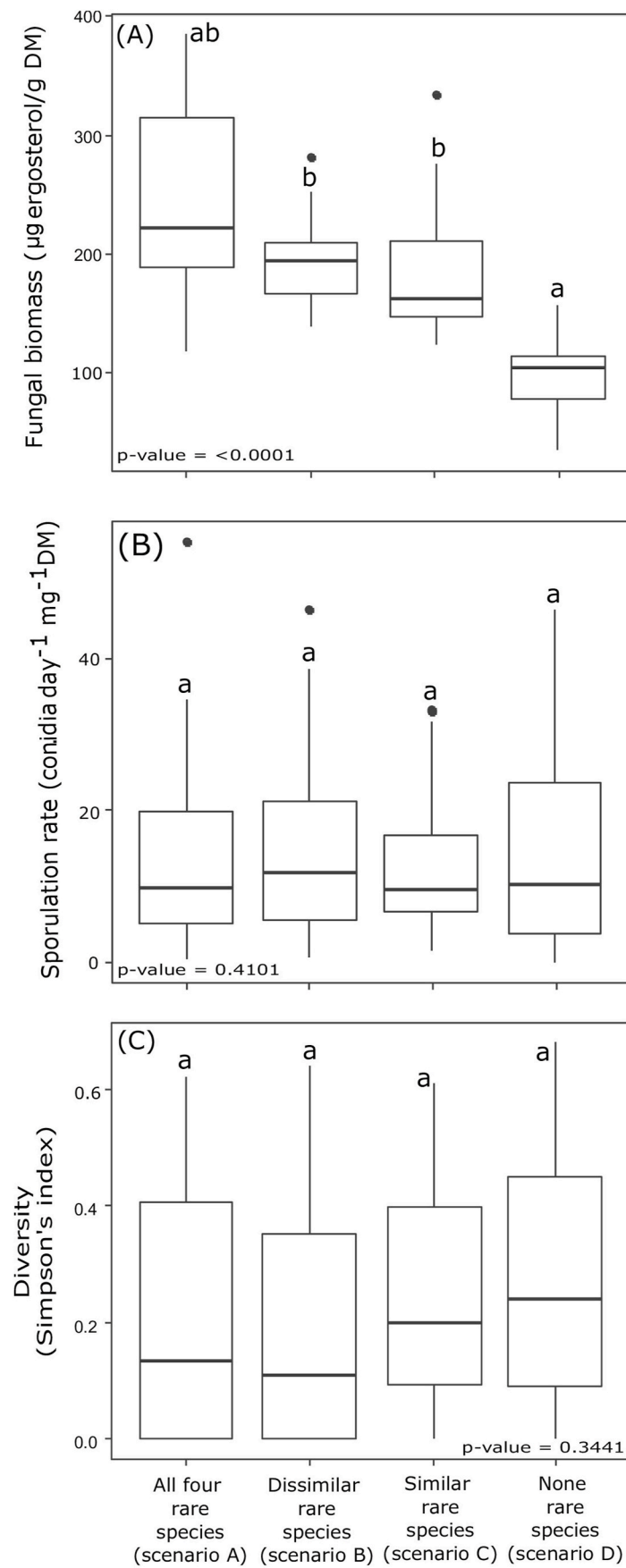


Fig. 3. Effect of the scenarios of rare species loss (A, B, C and D) on the (A) fungal biomass and aquatic hyphomycete (B) sporulation rate and (C) diversity (Simpson's index). Different letters indicate statistically significant differences ($p < 0.05$).

Table 1

Results of linear models testing for the effect of the scenarios of rare species loss: B (dissimilar rare species present), C (similar rare species present) or D (without rare species) and the scenario A (with all four rare species), evenness (even and natural proportion experiments) and their interaction on the fungal biomass and aquatic hyphomycete sporulation rate and diversity (Simpson's index). Bold values indicate statistically significant differences.

	df	F-value	p-value
Fungal biomass			
Intercept	1	685.13	
Scenario	3	20.30	<.0001
Evenness	1	0.06	0.8074
Evenness * Scenario	3	14.41	<.0001
Sporulation rate			
Intercept	1	14.44	
Scenario	3	0.97	0.4101
Evenness	1	0.03	0.8675
Evenness * Scenario	3	0.08	0.9697
Diversity			
Intercept	1	20.36	
Scenario	3	1.12	0.3441
Evenness	1	0.00	0.9640
Evenness * Scenario	3	1.13	0.3403

litter: *Alatospora acuminata*, *Anguillospora filiformis*, *Anguillospora longissima*, *Colispora curvata*, *Flagellospora curvula*, *Aquanectria submersa*, *Lunulospora curvula*, *Triscelophorus acuminatus*, *Triscelophorus monosporus* and an unidentified species (Fig. S2). Most variation in community composition (93%) was not explained by our predictor variables (Table 3). Evenness significantly explained variation in community composition (although it only explained 5%; Fig. S3a; Table 3), while scenario explained no variation (Fig. S3b; Table 3). There was no effect of the interaction between scenario and evenness (Table 3).

Litter mixtures in NatProp experiment were colonized by only five species of aquatic hyphomycetes, while mixtures in even experiment had higher species richness (i.e., nine species; Fig. S2). Four species were shared by the two experiments – *F. curvula*, *A. submersa*, *L. curvula* and *T. monosporus*. *T. acuminatus* was recorded only in NatProp experiment, while *A. acuminata*, *A. filiformis*, *A. longissima*, *C. curvata* and an unidentified species were found only in even experiment (Fig. S2). *F. curvula* was the dominant species both in even and NatProp experiment (mean \pm SE of 80.8 ± 3.2 and $80.5 \pm 2.8\%$, respectively). *A. submersa* was the second most abundant species in even experiment ($10.8 \pm 1.6\%$), whereas *L. curvula* was in NatProp experiment ($9.3 \pm 1.9\%$; Fig. S2).

3.3. The roles of litter quality and functional diversity of mixtures

Using linear models, we found that the increase in litter mixture functional diversity (resource dissimilarity) resulted in an increase in fungal biomass but had no effect on other fungal parameters (Fig. 5;

Table 2

Results of linear models testing for the effect of the functional diversity (FD, calculated using Rao's quadratic entropy), litter quality (expressed as the first axis of a PCA using the Community Weighted Mean Values for litter traits, CWM 1; Fig. 2a), evenness (even and natural proportion experiments) and their interaction on fungal biomass and aquatic hyphomycete sporulation rate and diversity (Simpson's index). Bold values indicate statistically significant differences.

	Fungal biomass			Sporulation rate			Diversity		
	df	F-value	p-value	df	F-value	p-value	df	F-value	p-value
Intercept	1	675.65		1	15.40		1	19.52	
FD	1	22.97	<.0001	1	0.04	0.8352	1	0.29	0.5889
CWM 1	1	3.00	0.0927	1	0.76	0.3848	1	2.56	0.1128
Evenness	1	0.00	0.9749	1	0.03	0.8716	1	0.00	0.9502
FD*CWM 1	1	0.16	0.6957	1	1.65	0.2011	1	0.23	0.6311
FD*Evenness	1	28.40	<.0001	1	0.81	0.3690	1	0.93	0.3382
CWM 1*Evenness	1	11.88	0.0016	1	0.45	0.5061	1	3.54	0.0625

Table 2). Similarly, we found that the litter quality (resource concentration) influenced only fungal biomass, but its effect was dependent upon evenness of litter mixtures (Table 2). Litter quality reduced fungal biomass in both experiments, but it had a much weaker effect on fungal biomass in NatProp experiment than in even experiment (r^2 values of 0.02 and 0.32, respectively; Fig. 6A and B). Functional diversity interacted with evenness to affect fungal biomass: the increase in functional diversity resulted in a great increase in fungal biomass in NatProp experiment ($r^2 = 0.75$), whereas it had no effect on fungal biomass in even experiment (Fig. 6C and D). Litter quality and functional diversity did not interact to affect any fungal parameter (Table 2).

4. Discussion

Most studies evaluating plant litter diversity effects on fungal decomposers in streams have addressed this issue by focusing either on random species loss, or the loss of dominant species, which contribute most to ecosystem processes according to the mass ratio hypothesis (Grime 1998). Here, we have focused on the loss of rare species that co-occur with dominant species in tropical streams. In particular, we investigated whether the observed effects of their loss depend on whether experiments assume uniform biomass across plant litter species in mixtures or whether species proportions found in natural communities are considered. In line with another recent study (Rabelo et al. 2022), we found that the loss of rare plant species from litter mixtures reduces fungal biomass, particularly when the lost species are functionally dissimilar species. Here, we showed that the loss of these species does not directly affect other fungal parameters in tropical streams (i.e., aquatic hyphomycete sporulation rate, species diversity and taxonomic composition). Also, our results evidenced that, while evenness alone has weak or no effect on fungi, it interacts with the diversity to affect fungal biomass: the reduction in fungal biomass due to the loss of rare species was higher in litter mixtures in NatProp experiment (i.e., natural litter mixtures), which was caused mainly by resource dissimilarity.

Despite the lower proportion of rare plant species in litter mixtures, their loss can change the average litter quality and functional diversity in these mixtures (see Rabelo et al. 2022), which may explain the effects of the loss of plant litter from rare species on fungal decomposers. Differences in average litter quality in mixtures can affect decomposer organisms via resource concentration effects, while differences in functional diversity can determine the availability of complementary resources and thus affect fungi through resource dissimilarity effects (Handa et al. 2014; García-Palacios et al. 2017). While we expected a greater effect of rare species loss from mixtures in even experiment (i.e., even litter mixtures), due to the higher proportional biomass that these species have in those mixtures, which is known to influence the fungal colonization process (mass ratio hypothesis; Grime 1998), we found the opposite: fungal biomass was more affected (reduced) when rare species were lost from natural litter mixtures. Despite that, litter quality had a weaker effect on fungal biomass in these mixtures, corroborating our

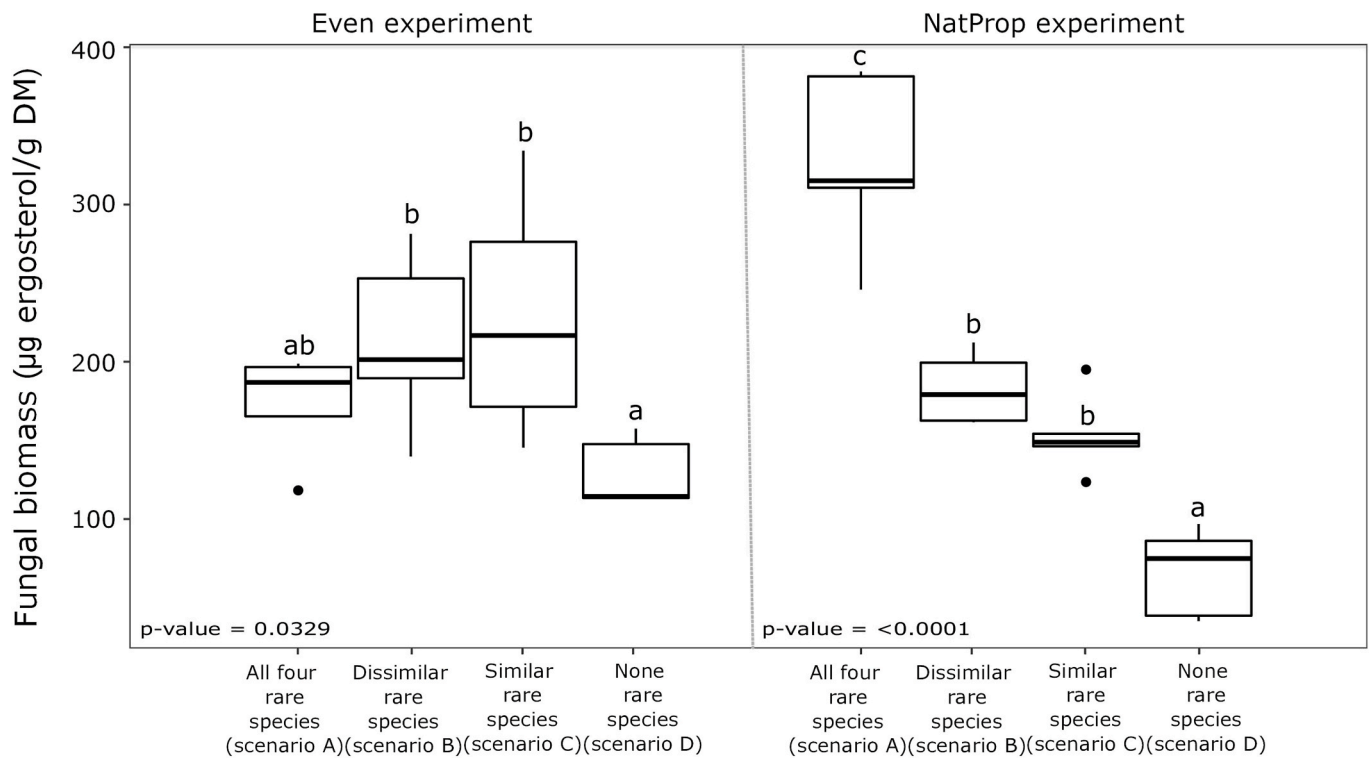


Fig. 4. Effect of the scenarios of rare species loss (A, B, C and D) on fungal biomass in even and natural proportion (NatProp) experiments. Different letters indicate statistically significant differences ($p < 0.05$).

Table 3

Results of PERMANOVA analysis testing for the effect of the scenarios of rare species loss: B (dissimilar rare species present), C (similar rare species present) or D (without rare species) and the scenario A (with all four rare species), evenness (even and natural proportion experiments) and their interaction on aquatic hyphomycete composition. Bold values indicate statistically significant differences.

	df	SS	R ²	F	Pr > F ^a
Scenario	3	0.15	0.01	0.29	0.994
Evenness	1	0.97	0.05	5.66	0.003
Evenness*Scenario	3	0.40	0.02	0.77	0.662
Residual	111	19.07	0.93		
Total	118	20.59	1.00		

Abbreviations: SS: mean sum of squares.

^aSignificance values based on 999 permutations.

hypothesis. On the other hand, functional diversity explained most variation in fungal biomass in natural litter mixtures, whereas it had no effect on fungal biomass in even litter mixtures. Thus, functional diversity was the key factor for the higher reduction in fungal biomass due to rare species loss in natural litter mixtures compared to even litter mixtures. Mixtures reflecting ambient evenness may have a higher occurrence of positive non-additive effects, and thus favor more microbial growth (Swan et al. 2009; Liu et al. 2020), which may explain the results found. For example, fungi can benefit from the higher resource variability via nutrient transfer by fungal hyphae, which can contribute to their growth and activity (Bardgett and Shine 1999; Chapman et al. 2013).

Even though species evenness differs significantly in the litterfall from tropical streams (i.e., few species provide most litterfall and many species provide only small amounts of litter; Tonin et al. 2021), studies considering the effect of evenness on decomposers still are scarce in these ecosystems. Since decomposers show substrate preferences (Gulis 2001; Gomes et al. 2016), aquatic fungi would be affected by species evenness in decomposing litter (Goncalves and Canhoto, 2009; Pereira

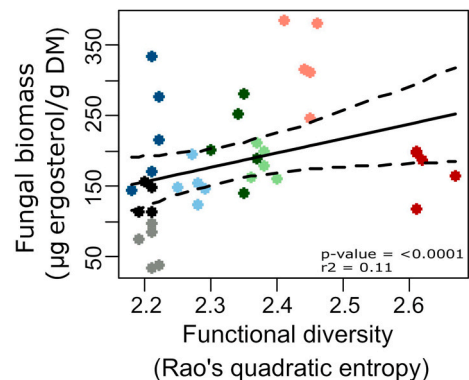


Fig. 5. Linear model showing the relationship between functional diversity (Rao's quadratic entropy) of plant litter mixtures and fungal biomass. Circles' colors represent scenarios that lost rare species: B (green; dissimilar rare species present), C (blue; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species). Darker and lighter colors represent even and natural proportion (NatProp) experiments, respectively. Continuous line is the fit of the linear model, and dashed lines are the 95% confidence intervals.

and Ferreira 2022). The interaction effect of diversity and evenness in litter mixtures on growth of fungal biomass found in our study confirms the important effect of species evenness on these fungi.

According to the mass ratio hypothesis, most plant diversity studies have focused on dominant species and their resource concentration effects (Hillebrand et al. 2008; Dee et al. 2019). Our results show that the loss of plant litter from rare species may substantially affect decomposers in tropical streams through resource dissimilarity effects. However, this is most likely to be observed when plant litter species proportions found naturally in the ecosystem are considered. In contrast, most experimental studies evaluating the impact of plant species

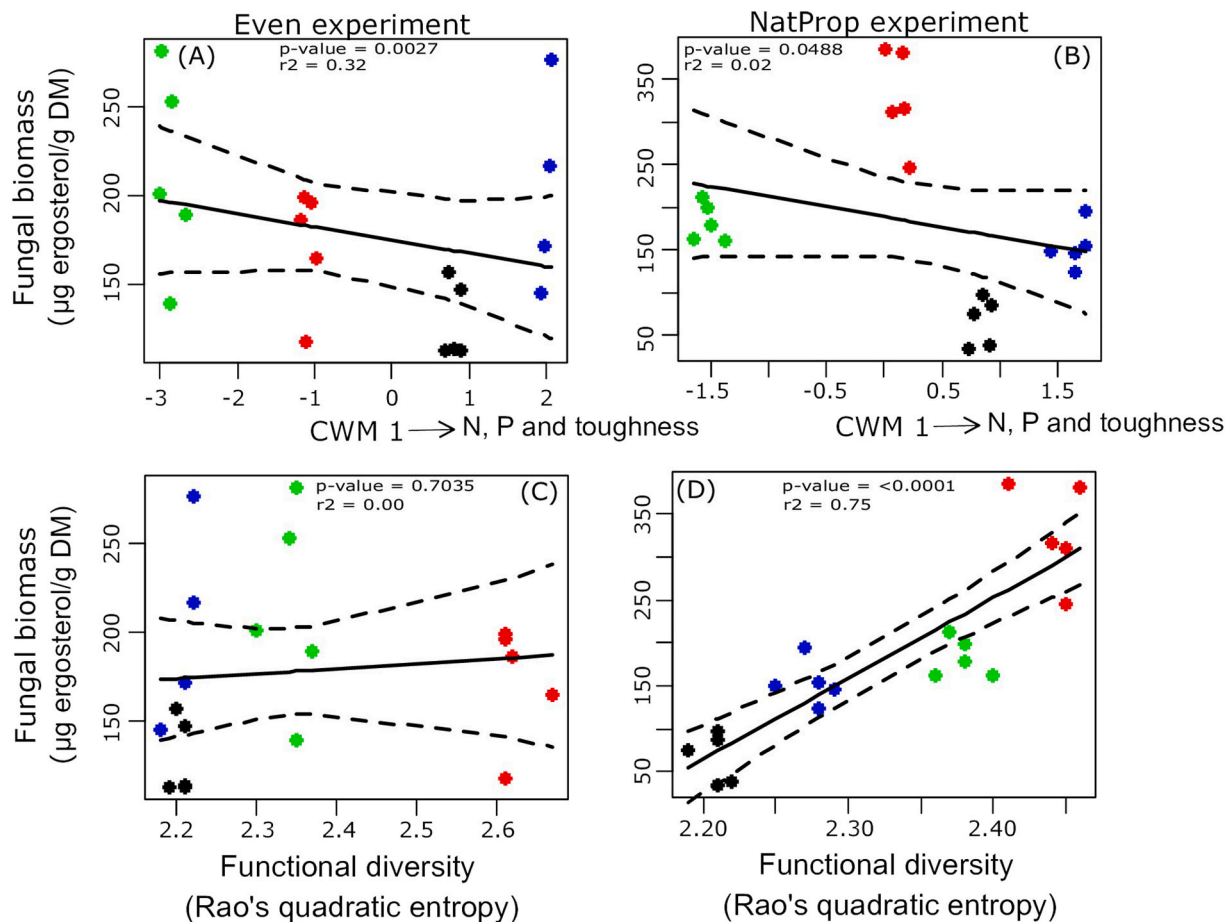


Fig. 6. Relationship between litter quality (expressed as the first axis of a PCA using the community weighted mean values for litter traits, CWM 1; Fig. 2A) and functional diversity (calculated using Rao's quadratic entropy index) of plant litter mixtures and fungal biomass in even (A, C) and natural proportion (NatProp; B, D) experiments. Scores of CWM 1 were positively related to nitrogen (N) and phosphorus (P) concentrations and toughness. Circles' colors represent scenarios that lost rare species: B (green; dissimilar rare species present), C (blue; 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 are the 95% confidence intervals.

diversity on decomposers in streams have simulated overly simplified conditions of this reality (Wardle 2016), despite the high diversity of plant species commonly found on litter in tropical streams (see Wantzen 2008). Therefore, it is imperative that future studies evaluating plant litter diversity effects on decomposer organisms simulate the real conditions observed in ecosystems.

Higher fungal biomass did not lead to higher reproduction of aquatic hyphomycetes in our study. There is often a significant correlation between maximum fungal biomass developing on litter and maximum sporulation throughout decomposition (Maharning and Bärlocher 1996). However, we note that we assessed fungal biomass only in the last experimental period.

4.1. Evenness affects the composition of the aquatic hyphomycete communities of litter mixtures

Evenness explained variation in aquatic hyphomycete community composition in our study, as previously observed (Goncalves and Canhoto, 2009; Pereira and Ferreira 2022), although it had a weak effect. Differences in community composition between even and natural litter mixtures may be due to differences in the overall quality of litter mixtures. By mixing litter species at different proportions, fungal communities are altered due to changes in the contents of phenolics, structural compounds, and nutrients in the mixture. Litter quality may act as an environmental filter, benefiting some fungal species (Gulis 2001), potentially leading to their dominance by competitive exclusion (Tretton

et al. 2004). We found that even litter mixtures had higher aquatic hyphomycete species richness than natural litter mixtures. This may have occurred because mixtures with increasing evenness have greater distribution of traits such as nutrient availability, levels of phenolic compounds, and contrasting structural traits (Hillebrand et al. 2008), which enhance the range of niches available to decomposers.

5. Conclusions

Aquatic fungi exert primary biological control on litter decomposition in tropical streams, since shredder invertebrates are scarce in these ecosystems (Goncalves et al. 2007; Boyero et al. 2011). Therefore, the basis for understanding litter decomposition in tropical streams can lie principally in assessing the effects of different factors on these organisms. Our study showed that the loss of rare plant species from litter mixtures in tropical streams affected the production of fungal biomass (a key fungal parameter; Krauss et al. 2011), but it did not affect other parameters. Moreover, we showed the importance of plant species evenness on fungal biomass through its interaction with diversity. Thus, taking evenness into account seems important to understand the effects of plant species diversity on fungal decomposers in tropical streams. Our study provides support for the importance of rare plant species for stream ecosystem functioning, an aspect rarely considered (Hillebrand et al. 2008). We thus suggest the necessity of further research on rare plant species to support conservation and management decisions aimed at mitigating environmental impacts on stream ecosystems.

Authors' contributions

JFGJr, AMT and RSR conceived the ideas and designed the methodology; AOM identified aquatic hyphomycetes; RSR and AMT analyzed the data; RSR led the writing of the manuscript with substantial inputs from AMT, JFGJr, AOM and LB. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jose Francisco Goncalves Junior reports financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico. Jose Francisco Goncalves Junior reports financial support was provided by Fundação de Amparo à Pesquisa do Distrito Federal.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.funeco.2023.101275>.

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