

ORIGINAL ARTICLE

The Influence of Litter Diversity on Leaf Breakdown and the Colonization of Decomposer Communities in Subtropical Highland Grassland Streams

Renan de Souza Rezende¹  | Marcelo da Silva Moretti²  | Emanuel Rampanelli Cararo^{1,3}  | Walace Pandolpho Kiffer²  | Larissa Corteletti da Costa²  | Alan M. Tonin⁴  | José Francisco Gonçalves Junior⁴ 

¹Graduate Program in Environmental Science, Communitarian University of Chapecó Region, Chapecó, Santa Catarina, Brazil | ²Laboratory of Aquatic Insect Ecology, University of Vila Velha, Vila Velha, Espírito Santo, Brazil | ³Graduate Program in Ecology and Evolution, Federal University of Goiás, Goiânia, Goiás, Brazil | ⁴AquaRiparia/Limnology Laboratory, Department of Ecology, Institute of Biology, University of Brasília, Brasília, Federal District, Brazil

Correspondence: Renan de Souza Rezende (renandezende30@gmail.com)

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ABSTRACT

Litter diversity can enhance leaf breakdown through selective effects when driven by a particular species or specific leaf chemical characteristics. We analyzed the influence of leaf mixtures on leaf breakdown and associated decomposers in a highland grassland stream that receives low-diversity leaf inputs. We incubated leaves of *Ocotea diospyrifolia*, *Nectandra megapotamica*, *Myrcia eugenioides*, and *Miconia flammea* in coarse mesh litter bags containing a leaf mixture of the four species, as well as leaves of each species in single treatments (6 streams × 3 replicates × 5 treatments = 90 sampling units). We found that leaves in single treatments had higher breakdown rates than the mixed treatment, suggesting that increased litter diversity did not positively influence leaf breakdown in highland grassland streams. Adaptations of decomposer assemblages to poorly structured riparian vegetation may have outweighed the potential effects of litter diversity. We found higher importance of leaf quality, measured by nutrient content, in driving leaf breakdown, with the effect of litter diversity on leaf breakdown rates depending on individual leaf chemical characteristics. Despite the lack of litter diversity positive effects on breakdown rates, the mixture treatment showed higher diversity of fungi and invertebrate decomposers. The leaf mixture may have increased habitat structure but reduced the number of individuals due to increased resource heterogeneity, favoring specialists. Functional feeding groups exhibited the expected patterns for their feeding strategies, with shredders benefiting from litter diversity and scrapers preferring single leaf treatments. Overall, riparian vegetation diversity was not crucial for organic matter processing but positively influenced decomposer diversity.

RESUMO

A diversidade de detritos de plantas pode aumentar a decomposição foliar por meio de efeitos seletivos, quando impulsionada por uma espécie específica ou características químicas específicas das folhas. Analisamos a influência de misturas de folhas na decomposição foliar e nos decompositores associados em um riacho de campos subtropical de altitude que recebe baixa diversidade nas entradas de folhas. Incubamos folhas de *Ocotea diospyrifolia*, *Nectandra megapotamica*, *Myrcia eugenioides* e *Miconia*

flammea em sacos de malha grossa contendo uma mistura de folhas das quatro espécies, bem como folhas de cada espécie em tratamentos isoladas (6 riachos \times 3 réplicas \times 5 tratamentos = 90 unidades amostrais). Observamos que folhas isoladas apresentaram taxas de decomposição mais altas do que juntas, sugerindo que o aumento da diversidade de detritos não influenciou positivamente a decomposição foliar em riachos de campos de altitude. As adaptações das assembleias de decompositores a vegetações ripárias pouco estruturadas podem ter superado os potenciais efeitos da diversidade dos detritos foliar. Verificamos maior importância da qualidade das folhas, medida pelo teor de nutrientes, na determinação da decomposição foliar, comparados aos efeitos da diversidade de detritos nas taxas de decomposição, dependendo das características químicas individuais das folhas. Apesar da ausência de efeitos positivos da diversidade de detritos foliar nas taxas de decomposição, o tratamento misto mostrou maior diversidade de fungos e invertebrados decompositores. A mistura de folhas pode ter aumentado a estrutura do habitat, mas reduziu o número de indivíduos devido à maior heterogeneidade dos recursos, favorecendo especialistas. Os grupos funcionais de alimentação apresentaram padrões condizentes com suas estratégias alimentares, com trituradores beneficiando-se da diversidade de detritos e raspadores preferindo tratamentos com folhas únicas. No geral, a diversidade da vegetação ripária não foi crucial para a decomposição da matéria orgânica vegetal, mas influenciou positivamente a diversidade dos decompositores.

1 | Introduction

Studies assessing the importance of biodiversity in stream functioning have mostly focused on the effects of tree species diversity on regulating the dynamics of leaf breakdown (Vos et al. 2013; Alonso et al. 2021; Boyero et al. 2021) and the activity of basal consumers, such as decomposers (Cararo et al. 2023; Rabelo et al. 2023). However, despite evidence of positive effects of increased litter diversity on leaf breakdown rates and associated decomposers, the results are inconsistent across various environmental and biogeographical contexts (Wantzen et al. 2010; Boyero et al. 2017, 2021). The diversity of leaves in leaf patches, which vary between phytophysiognomies (Boyero et al. 2017), appears to play an important role in regulating the litter dynamics (Del Cid et al. 2023), and consequently, the leaf breakdown rates in tropical streams (Rezende et al. 2016, 2017). Decomposers, such as aquatic invertebrates and microorganisms, typically prefer leaves with specific characteristics, such as soft and high quality (Bruder et al. 2013; Arias-Real et al. 2018; Casotti et al. 2019), with high nitrogen and phosphorus contents, and/or low contents of total phenolics and tannins (Sena et al. 2020). In this context, leaf mixtures may enhance the average leaf breakdown rate through selective effects when driving the process of a particular species (Alonso et al. 2021; Rabelo et al. 2023) or a specific leaf chemical characteristic (Basaguren and Pe 2018; Balik et al. 2022).

Most studies addressing leaf diversity-breakdown relationships have been conducted in temperate streams (Bruder et al. 2013; Arias-Real et al. 2018), while less information is available for tropical (Moretti, Gonçalves, and Callisto 2007) and subtropical streams (Ferreira et al. 2016, 2020). In tropical and subtropical regions, tree taxonomic and functional diversity tends to be higher than in temperate areas, resulting in heterogeneous and well-structured leaf patches available year-long on streambeds (Tonin et al. 2017; Bambi et al. 2022; Del Cid et al. 2023). On the other hand, leaf palatability (Boyero et al. 2017; Cararo et al. 2023), which may positively affect decomposer diversity, tends to be lower in tropical than in temperate areas (Wantzen et al. 2010; Boyero et al. 2012). Therefore, decomposers in tropical and subtropical areas are less diverse but have a more diversified diet compared to temperate streams (Gonçalves, Graça, and Callisto 2006; Boyero et al. 2015). Considering only

tropical and subtropical regions, the diversity of both decomposer fungi (Barreto et al. 2023) and invertebrate shredders (Boyero et al. 2012; Ferreira et al. 2019) increases towards subtropical areas.

Highland grassland streams in subtropical or tropical regions of the Neotropics often exhibit well-structured and dense riparian forests but can also have riparian forests with low tree diversity or be completely devoid of trees in natural grassland areas (Rezende et al. 2021; Bacca et al. 2023; Da Silva et al. 2024). As a result, the reduction or absence of tree species could lead to lower litter standing stocks in those streams (Alvim et al. 2014; Bacca et al. 2023). Additionally, the low diversity in the riparian forests of highland grassland streams may reduce the quality of leaf mixtures (Rezende et al. 2016, 2021; Bacca et al. 2023) and, consequently, leaf breakdown rates (Alvim et al. 2014, 2015). Limited resource availability may also potentially increase competition among decomposer communities in highland grassland streams (Rezende et al. 2015; Cararo, Lima-Rezende, and Rezende 2023; Da Silva et al. 2024) due to: (i) low litter inputs (Rezende et al. 2016); (ii) low leaf retention because of low streambed heterogeneity (Nuven et al. 2022) and (iii) high temporal variation in stream environmental conditions because of the reduced protective riparian buffer in naturally open streams (Alvim et al. 2014). An increase in competition for resources may occur, especially if leaf inputs have high quality (Arias-Real et al. 2018; Sena et al. 2020; Da Silva et al. 2024).

Invertebrate communities in highland grassland streams have lower biomass and diversity than those in streams located in fragments of mixed ombrophilous forest (Galeti, Capitano, and Baldissera 2020; Bacca et al. 2023). A similar pattern was observed for the activity of microbial decomposers, which showed higher values in mixed ombrophilous forest streams and even in silviculture streams, that is, streams surrounded by monocultures of a tree species, when compared to highland grassland streams (Rezende et al. 2021). These findings highlight the important role of riparian forests in protecting stream functionality (Postel and Thompson Jr. 2005; Inhamuns, Rezende, and Coelho 2021; Ferreira et al. 2023), and in increasing taxonomic and functional diversity of leaf patches

for decomposer communities (Abelho 2016; Cavalcante et al. 2023).

Given the increasing threat to grasslands, particularly in highland areas, there is an urgent need for studies on the conservation of these regions (Overbeck et al. 2024; Straffelini, Luo, and Tarolli 2024). In this context, our aims in this study were: (i) to analyze the influence of leaf mixtures on breakdown rates and associated decomposers (invertebrates and aquatic hyphomycetes) in highland grassland streams that receive low-diversity leaf inputs; (ii) to assess how the richness, density, and biomass of invertebrates and aquatic hyphomycetes, respond to plant species in mixture and in single treatments; and (iii) to investigate whether variations between observed and expected values of leaf breakdown and associated decomposers were associated with the initial chemical characteristics of each species, such as initial carbon, nitrogen, and protein contents, to disentangle mechanisms behind the effects of mixtures on leaf breakdown. For this, we examined the mixture effects by comparing leaves of each species in the mixed and in single treatments.

We hypothesized that a high diversity of food resources would positively affect biological communities, accelerating ecological processes. We predicted that high diversity in the mixed treatment would positively affect both the richness and density of decomposers, resulting in faster leaf breakdown rates. The premises of the study were: (i) highland grassland streams have low tree diversity in riparian zones and leaf inputs of low palatability (Rezende et al. 2021; Bacca et al. 2023; Da Silva et al. 2024), (ii) leaf mixtures provide a complementary and more diverse food resource than single plant species (Tonin et al. 2021; Del Cid et al. 2023), and (iii) poorly structured riparian vegetation

in grassland streams may lead decomposer communities to use a more diverse array of litter (due to complementary food resources) compared to single plant species (Alvim et al. 2014; Rezende et al. 2016, 2021).

2 | Material and Methods

2.1 | Study Area

The study area encompassed six sampling sites distributed within the Campos de Palmas Wildlife Refuge, located in the state of Paraná, southern Brazil, within the Atlantic Forest Biome (see Figure 1). The vegetation in this region consists of three primary phytophysionomies: “Campos stricto sensu” (Steppe—open grasslands), “Campos úmidos” (Hygrophilous Steppe—moist grasslands), and “Floresta Ombrófila Mista” (Mixed Ombrophilous Forest). The study was conducted in streams within the Hygrophilous Steppe phytophysionomy.

The climate in the study area falls under the Cfa category in the Köppen climate classification, characterized as temperate subtropical (Alvares, Stape, and Sentelhas 2013). The mean annual air temperature is 16°C, ranging between 5°C and 27°C. Altitude ranges from 950 to 1370 m a.s.l. Monthly average precipitation throughout the year is 142 mm, with monthly variations between 105 and 182 mm, resulting in a total annual precipitation of 1700 mm. These climatological averages are based on data collected over a span of 38 years, from 1980 to 2018. Precipitation and air temperature data were obtained from a meteorological station with the identification number 2651035. This station is located at coordinates 26°21'58.3" S and 51°51'58.2" W, managed by the National Agency of Waters of Brazil, and the data are

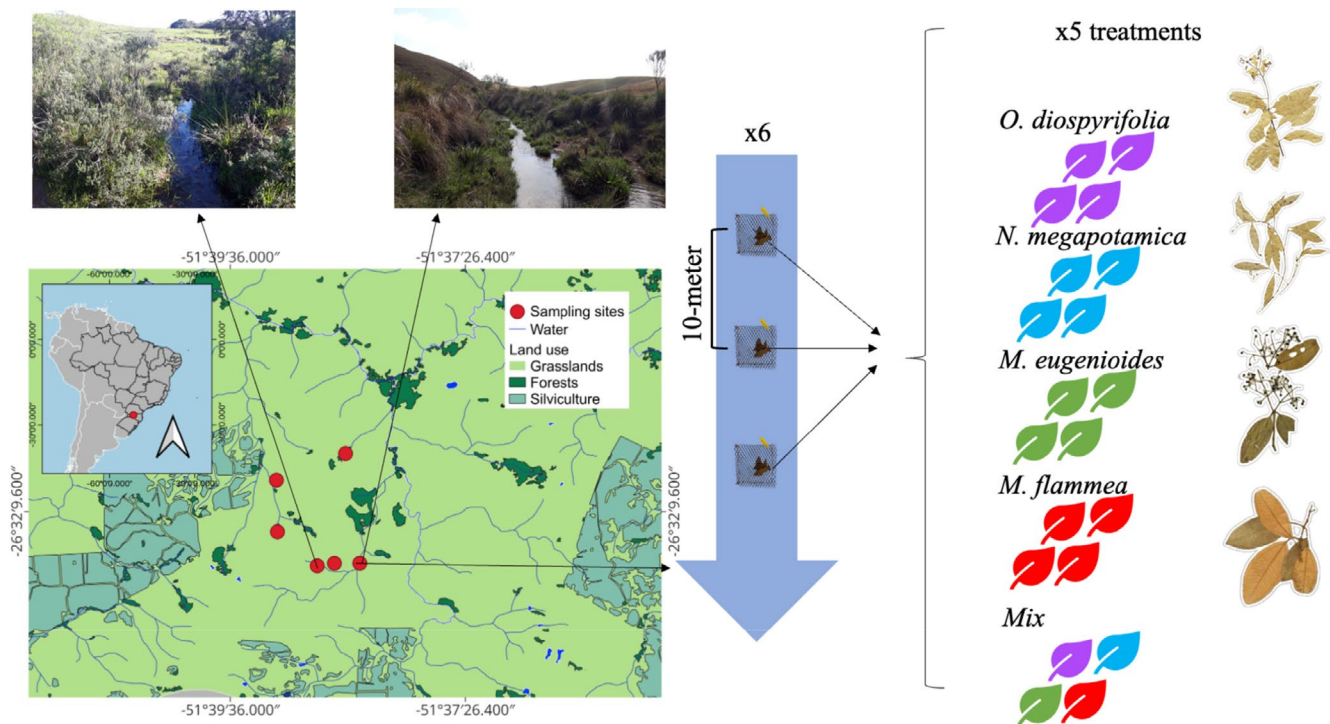


FIGURE 1 | Geographic location of the studied highland grassland streams in the Chopim River watershed, Paraná (South Brazil) and experimental design.

publicly accessible on the hidroweb website (<http://hidroweb.ana.gov.br/>).

2.2 | Study System

Ocotea diospyrifolia (Meisn.) Mez (Lauraceae), commonly known as “Canela,” is a medium to large-sized tree species native to the Brazilian Atlantic Forest. Individuals of this species show the tree bears small, yellowish-white flowers and are prized for their rugged, durable wood (Sobral et al. 2013). It has berries as fruits, characterized by the presence of a cupule, and its seed dispersal mechanism is primarily zoochory. Leaves of *O. diospyrifolia* have a thin blade form, with length ranging from 5.8 to 9.8 cm, width ranging from 1.4 to 2.4 cm, and a leaf area of 15 cm² (Giaretta and Peixoto 2015; Costa et al. 2019; Völtz and Blum 2020).

Nectandra megapotamica (Spreng.) Mez, known as “Canela-Preta” or “Canelinha,” is another tree species of the Lauraceae family which is native to South America, specifically Brazil, Argentina, and Paraguay. It has globose to elliptical fruits, and its dispersal mechanism is primarily zoochory. This species typically grows to 10–20 m in height and features yellowish-brown bark (Sobral et al. 2013). Leaves of *N. megapotamica* have a thin blade form, with length ranging from 5.5 to 11.2 cm, width ranging from 1.5 to 3.1 cm, and leaf area of 19 cm² (Giaretta and Peixoto 2015; Costa et al. 2019; Völtz and Blum 2020).

Myrcia eugenioides Cambess (O.Berg) Nied (Myrtaceae) is also native to the Brazilian Atlantic Forest. This species occurs as a small- to medium-sized tree or shrub (Santos, Sano, and Lucas 2019) and is known for its small, fragrant white or pale pink flowers. *Myrcia eugenioides* usually presents a berry-like fruit, globose or ellipsoid, with persistent calyx lobes, and the dispersal mechanism is primarily zoochory. Leaves of *M. eugenioides* have an oblong blade form, with length ranging from 10.1 to 14.3 cm, width ranging from 5.2 to 7.1 cm, and leaf area of 72 cm² (Giaretta and Peixoto 2015; Costa et al. 2019; Völtz and Blum 2020).

Miconia flammea Casar (Aubl.) DC (Melastomataceae) occurs in tropical South America, including the Amazon rainforest. This small tree or shrub is notable for its large, thin, with a distinctive veined pattern (The Brazil Flora Group et al. 2022). *M. flammea* produces clusters of small, white to pinkish flowers. It has berry-like fruits with two hemispherical seeds per fruit, and its dispersal mechanism is primarily zoochory. Leaves of *M. flammea* have an elliptical blade form, with length ranging from 12.5 to 19.2 cm, width ranging from 2.4 to 6.1 cm, and leaf area of 67 cm² (Giaretta and Peixoto 2015; Costa et al. 2019; Völtz and Blum 2020).

These species were chosen to represent the predominant vegetation found in riparian ecosystems, and due to their widespread distribution within the Neotropical region (Giaretta and Peixoto 2015; Costa et al. 2019; Völtz and Blum 2020). Leaves of these species were collected in Brasília (Central-west Brazil; *O. diospyrifolia*), Erechim (South Brazil, *N. megapotamica*), Vila Velha (South-east Brazil; *M. eugenioides*), and Manaus (North Brazil; *M. flammea*). Senescent leaves were collected using 1 m² nets with a mesh size of 0.5 mm, positioned 1 m above the

ground. The leaves were air-dried and stored in plastic bags until the start of the experiment.

2.3 | Experimental Design

For the leaf breakdown experiment, we used leaves of the four species in mixed and in single treatments. The experiment was conducted from October to December 2018, in the spring season of the southern hemisphere. Between October and December, the average air temperature was 18°C, and the mean precipitation was 148 mm.

Our experiment took place in six highland grassland streams categorized as Hygrophilous Steppe. Within each stream, we established three sampling points spaced at 10-m intervals. At each sampling point, we incubated litter bags containing a mixture of leaves of the four plant species (mixed treatment) as well as litter bags containing leaves of each species individually (single treatments). This setup resulted in a total of 90 litter bags, which were used as sampling units (6 streams × 3 replicates × 5 treatments; Figure 1).

2.4 | Water Physical and Chemical Parameters

We measured the physical and chemical water parameters at each sampling point of the studied streams during both the incubation and removal of the litter bags. The following parameters were measured in situ: stream depth (m) and width (m), water flow (cm³/s), velocity (m/s), temperature (°C), pH (measured using a pH meter, PHTEK, Curitiba, PR, BR), electrical conductivity (µS/cm; measured using a conductivity meter, Quimis, Diadema, SP, BR), dissolved oxygen (mg/L; measured using a Jenway 970 Dissolved Oxygen Meter, Staffordshire, OSA, UK), total dissolved solids (g/L), and turbidity (measured in Nephelometric Turbidity Units, NTU, using a multianalyzer model 85, YSI Incorporated). The concentrations of nitrate, ammonia (with a detection limit of 0.05 mg/L), and orthophosphate (with a detection limit of 0.015 mg/L) in the water were determined in the laboratory following Clesceri and Greenberg (1989).

The studied streams (water velocity: 2.76 ± 0.40 m/s; water flow: 0.51 ± 0.16 m³/s; mean ± SE) had acid waters (pH: 4.66 ± 0.28), high concentrations of dissolved oxygen (8.6 ± 0.40 mg/L), and lower temperatures (18°C ± 0.75°C). Stream waters also had low values of electrical conductivity (0.01 ± 0.01 mS/cm), turbidity (12.47 ± 9.02 NTU), and dissolved nutrients (nitrite [0.02 ± 0.02 mg/L], ammonia [0.22 ± 0.03 mg/L], and orthophosphate [6.65 ± 0.06 mg/L]).

2.5 | Leaf Chemical Characteristics

We evaluated the initial chemical composition of the leaves of each plant species by analyzing the carbon, nitrogen, and protein contents. For this, leaves (time 0) of each plant species were randomly selected from the initial pool of leaves. The total carbon content was determined using the combustion method (see Flindt et al. 2020). The nitrogen content

was determined using the Kjeldahl method as described by Cantarella and Trivelin (2001). The total protein content was determined using the spectrophotometric analysis described by Baerlocher (2020).

2.6 | Leaf Mass Loss and Associate Decomposers

We incubated the leaves in coarse mesh litter bags (20 mm mesh size, 30 × 30 cm) for 53 days on the streambed of the studied streams, at a depth of 0.2 to 0.5 m. Each litter bag contained 4 g (± 0.1) of air-dried leaves. Following removal from the streams, the litter bags were individually placed into insulated plastic bags and transported in thermal containers ($\pm 4^\circ\text{C}$) to the laboratory. In the laboratory, all leaves from the litter bags were washed with distilled water over a 120-mm mesh sieve. Invertebrates retained on the sieve were preserved in 70% ethanol for subsequent identification (Cummins, Merritt, and Andrade 2005; Cummins 1996; Hamada, Nessimian, and Querino 2014).

Individuals of each taxon were counted to determine invertebrate density and taxonomic richness (Table S1). Invertebrate biomass was determined by desiccation at 60°C for 72 h, followed by weighing (0.01 mg). The invertebrates were assigned to functional feeding groups (Cummins, Merritt, and Andrade 2005; Cummins 1996; Hamada and Ferreira-Keppeler 2012; Pérez 1988), and the occurrence (richness) and frequencies (density) of shredders and scrapers were determined to assess their direct impact on leaf breakdown.

From each sample, five leaves were randomly selected, and two sets of five leaf discs were cut (12 mm in diameter). For samples containing the mixed treatment, leaf discs were cut from the leaves of each plant species. These sets of leaf discs were used to determine the taxonomic composition (frequency and occurrence) of aquatic hyphomycetes associated with leaves (Table S2), through the identification of the conidia produced by sporulation (see Bärlocher 2005), and ash-free dry mass (AFDM) of the leaf discs (Graça, Barlocher, and Gessner 2005). Discs of the four leaf species of the mixture treatment were placed together for sporulation. The remaining leaves were oven-dried (60°C , 72 h), weighed (0.01 g), ignited (550°C , 4 h), and reweighed for AFDM estimation (see Barreto et al. 2023 for more information).

2.7 | Statistical Analysis

The normality of the data for leaf chemical characteristics (carbon, nitrogen, and protein) was tested using the Kolmogorov–Smirnov test, and when necessary, data were transformed using the natural logarithm (Ln). The chemical characteristics (dependent variable) among studied leaves (categorical variable) were compared by ANOVA (“aov” function from the “vegan” package, R software), followed by Tukey’s test for discrimination among the categorical variables (“lsmeans” function and package, R software).

We used the effect size analysis to check for differences in leaf breakdown (remaining mass), invertebrate metrics (richness,

density, and biomass), functional feeding groups (richness and abundance of shredders and scrapers), and aquatic hyphomycetes (richness and density) (dependent variables) among studied plant species (*O. diospyrifolia*, *N. megapotamica*, *M. eugenioides*, and *M. flammaea*) in single and mixed treatments (categorical variables). To compare leaf breakdown data, the mean remaining mass values for each species in the mixed treatment served as the control for estimating effect size and direction of leaf mass loss. For all other parameters (invertebrates, functional feeding groups, and aquatic hyphomycetes), the mean values of the mixed treatment were consistently used as the control.

The magnitude of effects (E++) was estimated using ratios (Response Ratio, also known as the Ratio of Means; for further details, see chapter 6 of Koricheva, Gurevitch, and Mengersen 2013) between the values of each treatment and the average control value for each replicate, following Correa-Araneda et al. (2020). The variables were log-transformed (Effect size = $[\log(\text{treatment}/\text{control})]$), for each replicate, to ensure consistent estimation of the magnitude of change from the null value (Koricheva, Gurevitch, and Mengersen 2013). In our case, we used the average values of each variable (for leaf breakdown, invertebrate, and aquatic hyphomycetes metrics) in the mixed treatment as the control for each plant species. As an additional control for leaf breakdown only, we used each leaf species in the single treatment compared to each respective species in the mixture. To quantify the uncertainty and provide more reliable estimates on the mean values and magnitude and direction of change for each treatment compared to the control (i.e., intervals that do not contain the value of 0), we used nonparametric bootstrapped with 95% confidence intervals (CI; by the basic method) as described by Davison and Hinkley (1997). We utilized the “boot” function from the “boot” package in R (Canty and Ripley 2016), conducting 1000 permutations to estimate the Bias-Corrected and Accelerated (BCa) minimum and maximum values for each treatment (Correa-Araneda et al. 2020; Rabelo et al. 2023). The incorporation of BCa in our analysis accounts for potential biases and skewness in the bootstrap distribution, enhancing the accuracy and robustness of our estimates for the minimum and maximum values associated with each treatment (for more see also; Correa-Araneda et al. 2020; Marsaro et al. 2023; Rezende et al. 2023).

In this experiment, instead of the conventional presentation of results (refer to Figures S3–S5), we opted for an unconventional approach to depict the results concerning remaining mass (for more see also; López-Rojo et al. 2019; Correa-Araneda et al. 2020; Rabelo et al. 2023; Rezende et al. 2023; Borges et al. 2024). Our interpretation hinges on discerning relationships between treatments and control, where a negative relationship indicates a species exhibiting lower remaining mass compared to the control (mixed treatment), implying a faster decomposition rate. Conversely, positive relationship suggests the inverse scenario.

An indicator analysis (Dufrêne and Legendre 1997) was employed to identify taxa (invertebrate and hyphomycete community, individually) that were characteristic of the respective leaf treatment using the “indval” function from the “vegan”

package. This analysis evaluates the frequency and occurrence of individuals within predefined groups, generating indicator values ranging from 0 (nonindicator) to 100 (perfect indicator). Significance was assessed via a Monte Carlo test with 1000 permutations, with a significance level set at $p < 0.05$. Only significant results were reported. Data visualization was conducted using the “ggplot2” and “ggstatsplot” functions and packages (Patil 2021). All statistical analyses were conducted in R (R Core Team 2023; version 4.3.0).

3 | Results

3.1 | Leaf Characterization

Leaves of *Ocotea diospyrifolia* had the highest carbon concentrations ($50.86\% \pm 0.4\%$; mean \pm SE), followed by *Nectandra megapotamica* ($49.90\% \pm 0.2\%$), *Myrcia eugenioides* ($48.05\% \pm 0.7\%$), and *Miconia flammea* ($46.03\% \pm 0.1\%$; ANOVA; $F_{(3,8)} = 216.2$; $p < 0.001$). Regarding nitrogen concentration, *O. diospyrifolia* also had the highest values ($4.8\% \pm 0.05\%$), followed by *M. flammea* ($1.2\% \pm 0.0007\%$), *M. eugenioides* ($1.8\% \pm 0.01\%$), and *N. megapotamica* ($1.4\% \pm 0.02\%$; ANOVA; $F_{(3,8)} = 8817$; $p < 0.001$). Finally, protein contents were highest in *O. diospyrifolia* ($30.1\% \pm 0.3\%$), followed by *M. eugenioides* ($11.4\% \pm 0.1\%$), *N. megapotamica* ($9.0\% \pm 0.1\%$), and *M. flammea* ($7.7\% \pm 0.004\%$; ANOVA; $F_{(3,8)} = 8801$; $p < 0.001$). Leaves of all plant species differed from each other in the comparisons of chemical characteristics (Table S1).

3.2 | Leaf Breakdown

The effect size between the mixed (all species indiscriminately) and single treatments on leaf remaining mass revealed that the breakdown rate of *N. megapotamica* ($E_{++} = 0.105$, CI 0.032 to 0.176) in the single treatment was significantly lower than the mixture, while *M. eugenioides* ($E_{++} = -0.285$, CI -0.352 to -0.228) showed the opposite pattern (i.e., significantly higher). Breakdown rates of *O. diospyrifolia* ($E_{++} = -0.045$, CI -0.093 to 0.000) and *M. flammea* ($E_{++} = -0.003$, CI -0.116 to 0.094) in single treatments did not differ from the mixture (Figure 2). The effect size on the leaf remaining mass of each species incubated in mixed and single treatments revealed that the breakdown rates of *O. diospyrifolia* ($E_{++} = -0.065$, CI -0.112 to -0.025) and *M. eugenioides* ($E_{++} = -0.128$, CI -0.183 to -0.075) were significantly higher in single treatments, while *N. megapotamica* ($E_{++} = -0.025$, CI -0.100 to 0.053) and *M. flammea* ($E_{++} = -0.100$, CI -0.208 to 0.009) showed no differences between mixed and single treatments (Figure 3a; Figure S3a).

3.3 | Invertebrates

M. eugenioides ($E_{++} = 0.371$, CI 0.029 to 0.732), *M. flammea* ($E_{++} = 0.525$, CI 0.188 to 0.916), and *O. diospyrifolia* ($E_{++} = 0.406$, CI 0.07 to 0.792) in single treatments had higher values of invertebrate richness than the mixture, while values found in *N. megapotamica* ($E_{++} = 0.280$, CI -0.039 to 0.606) did not differ (Figure 3b; Figure S3b). The mixture had the highest invertebrate density (Figure 3c; Figure S3c; range of

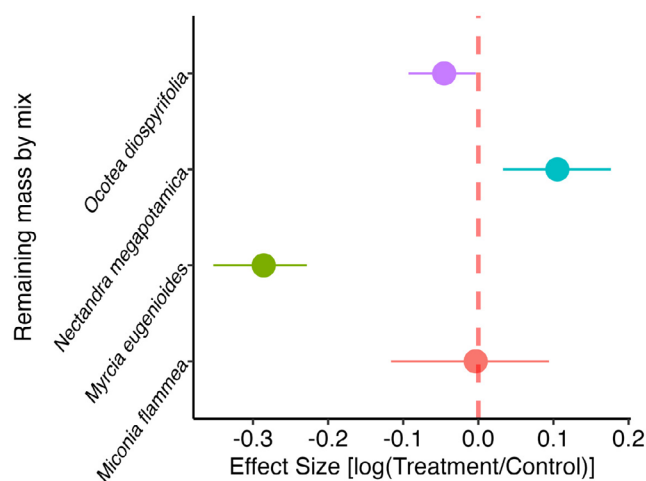


FIGURE 2 | Effect size and direction of mixture (dotted red line) effects on leaf remaining mass loss among leaves of *O. diospyrifolia*, *N. megapotamica*, *M. eugenioides*, and *M. flammea*. The values are expressed as log ratios between the mixture (control) versus single leaf treatments. Circles represent means, and whiskers indicate the upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Whiskers that do not touch the dashed line reject the null hypothesis (i.e., do not contain the value of 0), while whiskers that touch the dashed line do not reject the null hypothesis.

$E_{++} = -0.521$ to -0.832 , CI -1.160 to -0.124) and total biomass (Figure 3d; Figure S3d; range of $E_{++} = -0.885$ to -0.792 , CI -1.271 to -0.478) compared to all single treatments overall.

Values of shredders' abundance (Figure 4a; Figure S4a) and biomass (Figure 4b; Figure S4b) were lower in *N. megapotamica* ($E_{++} = -0.454$, CI -0.722 to -0.158 and $E_{++} = -0.341$, CI -0.581 to -0.062 , respectively), *M. flammea* ($E_{++} = -0.503$, CI -0.770 to -0.223 and $E_{++} = -0.748$, CI -1.061 to -0.436 , respectively), and *O. diospyrifolia* ($E_{++} = -0.592$, CI -0.890 to -0.275 and $E_{++} = -0.906$, CI -1.261 to -0.530 , respectively) than in the mixture. Again, values found in *M. eugenioides* ($E_{++} = -0.293$, CI -0.568 to 0.014 and $E_{++} = -0.301$, CI -0.588 to 0.033, respectively) showed no differences. Scrapers' abundance was higher in *M. eugenioides* ($E_{++} = 0.200$, CI 0.034 to 0.337) than in the mixture (Figure 4c; Figure S4c). No differences were observed in scrapers' biomass among all treatments (Figure 4d; Figure S4d; range of $E_{++} = -0.014$ to 0.122, CI -0.028 to 0.246). Larvae of Dryopidae (Coleoptera; indicator value = 0.98; $p = 0.001$) and Hydropsychidae (Trichoptera; indicator value = 0.38; $p = 0.015$) emerged as indicators of the invertebrate assemblages found in the mixture and *M. eugenioides*, respectively.

3.4 | Aquatic Hyphomycetes

Fungi richness was higher in *M. flammea* ($E_{++} = 0.144$, CI 0.031 to 0.239) than in the mixture, while values found in *N. megapotamica* ($E_{++} = 0.010$, CI -0.046 to 0.056), *M. eugenioides* ($E_{++} = 0.059$, CI -0.012 to 0.120), and *O. diospyrifolia* ($E_{++} = 0.029$, CI -0.041 to 0.078) did not differ from the mixture (see Figure 5a; Figure S5a). Conversely, *M. eugenioides* ($E_{++} = -0.636$, CI -0.923 to -0.342), *M. flammea* ($E_{++} = -0.741$, CI -1.041 to -0.415), and *O. diospyrifolia*

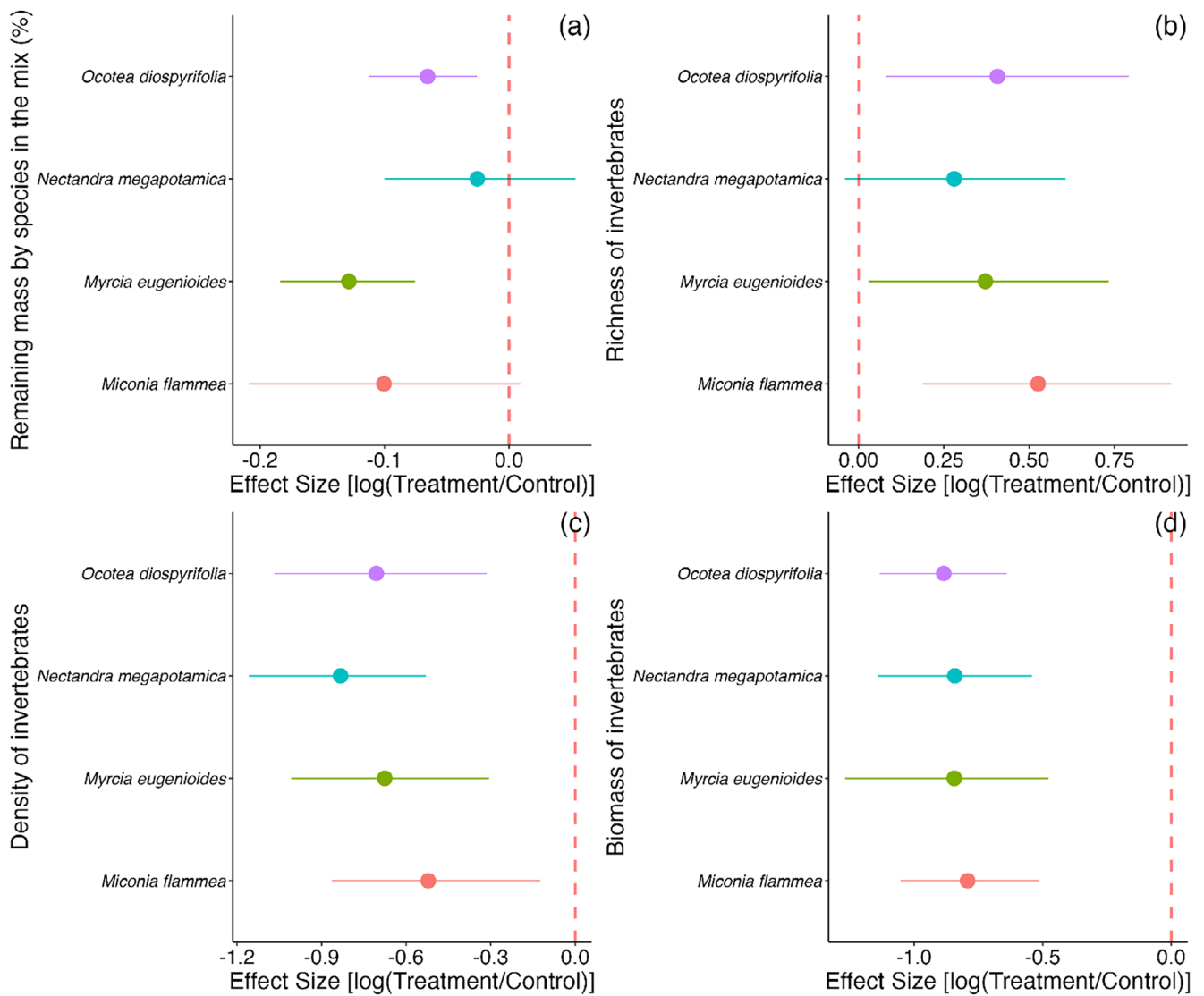


FIGURE 3 | Effect size and direction of mixture (dotted red line) effects on leaf remaining mass (a), and invertebrate richness (b), density (c), and biomass (d) among leaves of *O. diospyrifolia*, *N. megapotamica*, *M. eugenioides*, and *M. flammea*. The values are expressed as log ratios between each species in the mixed (control) versus single treatments for leaf mass loss. In comparisons with invertebrate data, values in the mixture (control) versus single treatments were used for log-ratios calculation. Circles represent means, and whiskers indicate the upper and lower bounds of 95% non-parametric bootstrapped confidence intervals. Whiskers that do not touch the dashed line reject the null hypothesis (i.e., do not contain the value of 0), while whiskers that touch the dashed line do not reject the null hypothesis.

($E_{++} = -0.389$, CI -0.662 to -0.061) had lower density values than the mixture. Fungal density in *N. megapotamica* ($E_{++} = -0.168$, CI -0.618 to 0.260) did not differ from the mixture. Additionally, *Triscelophorus deficiens* (indicator value = 0.41; $p = 0.032$) emerged as indicator species of aquatic hyphomycetes assemblages found in *M. flammea*, while *Lunulospora curvula* (indicator value = 0.38; $p = 0.023$) was the indicator species for *N. megapotamica*.

4 | Discussion

We investigated the effects of litter diversity on leaf breakdown rates and associated decomposers in highland grassland streams. Contrary to our hypothesis, we found that leaves in single treatments exhibited higher breakdown rates than in the mixture, suggesting that litter diversity did not stimulate leaf

breakdown rates in those streams. Adapting decomposer assemblages to streams with poorly structured riparian vegetation may have outweighed the potential effects of litter diversity on leaf breakdown. Our results also highlight the importance of leaf quality and nonshredding invertebrates, that is, leaf nutrient content and scrapers abundance, in driving leaf breakdown rates. Therefore, the influence of litter diversity on leaf breakdown is context-dependent, especially regarding leaf chemistry.

4.1 | Leaf Mixture Effects

We observed an increase in leaf breakdown rates in single treatments compared to both controls, that is, the mixture and each leaf species in the mixed treatment. The high breakdown rates in single treatments did not corroborate our hypothesis which predicted a positive effect of resource diversity on leaf

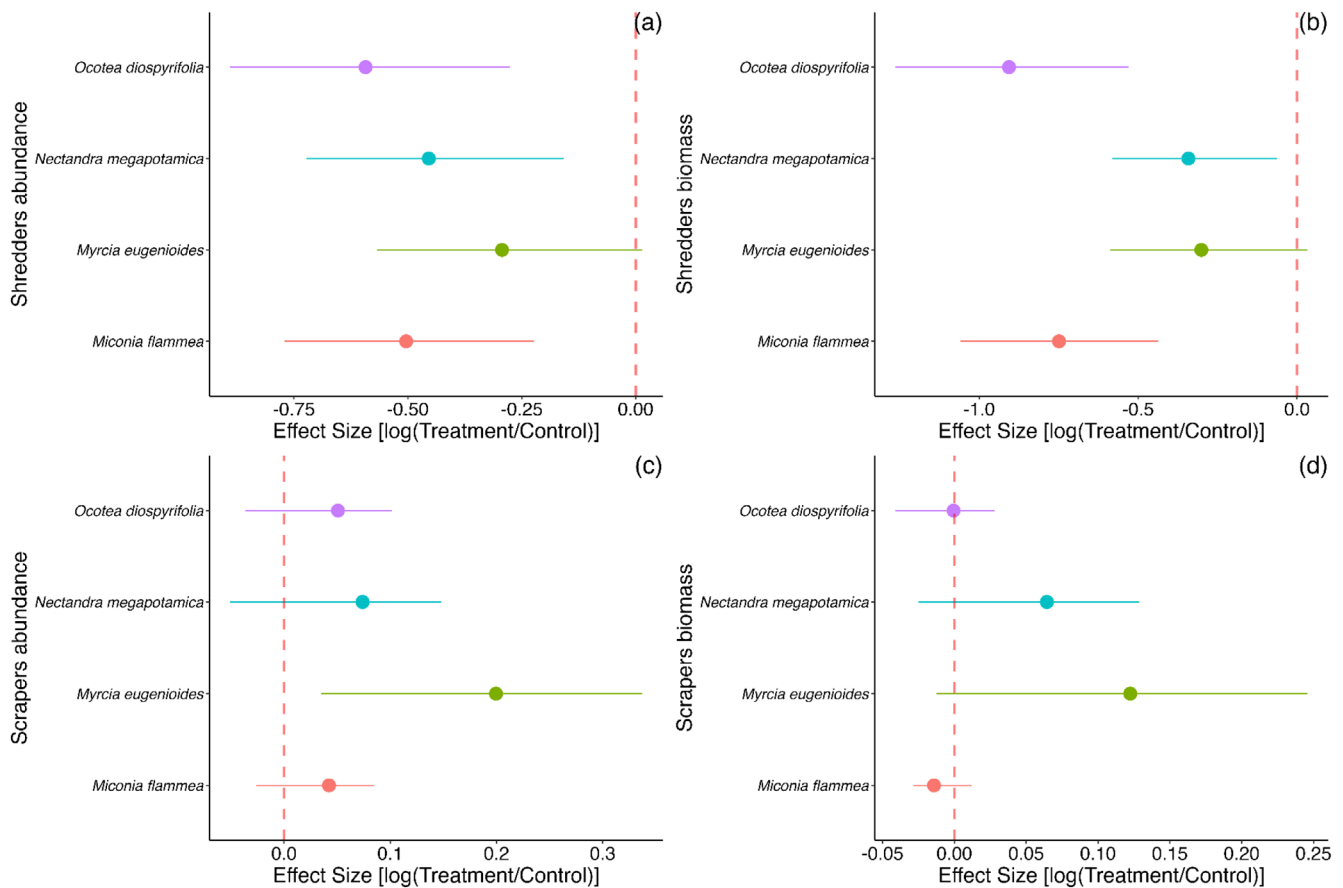


FIGURE 4 | Effect size and direction of mixture (dotted red line) effects on the abundance (a, c) and biomass (b, d) of invertebrate shredders and scrapers among leaves of *O. diospyrifolia*, *N. megapotamica*, *M. eugenioides*, and *M. flammea*. The values are expressed as log ratios between the mixture (control) versus single leaf treatments. Circles represent means, and whiskers indicate the upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Whiskers that do not touch the dashed line reject the null hypothesis (i.e., do not contain the value of 0), while whiskers that touch the dashed line do not reject the null hypothesis.

breakdown. The observed decrease in leaf breakdown rates in the mixed treatment, despite being observed in other studies (Moretti, Gonçalves, and Callisto 2007; Bruder et al. 2013), may be attributed to the progressive adaptation of the decomposer assemblages to the availability of low-diversity leaf patches on the streambeds (Alvim et al. 2014; Rezende et al. 2017; Biasi et al. 2019), as is regularly observed in highland grassland streams (Rezende et al. 2021; Bacca et al. 2023). In some subtropical streams, grasses are an important carbon source for aquatic hyphomycetes and shredders because their chemistry favors the activity of decomposers (Biasi et al. 2019). In general, this pattern may result from the “selection effect,” where the dominance of species with particular traits affects ecosystem processes (see also; Grime 1998; Loreau and Hector 2001; Fox 2005; Tonin, Hepp, and Gonçalves-Junior 2017).

The increase in breakdown rates in single treatments also highlights that leaf quality and specific chemical characteristics (e.g., nutrient contents, which were higher in *O. diospyrifolia* and *M. eugenioides*), instead of leaf identity or species composition (see Moretti, Gonçalves, and Callisto 2007; Bruder et al. 2013), driving leaf breakdown in highland grasslands streams. The inputs of *O. diospyrifolia* and *M. eugenioides* leaves, which have high nitrogen and protein contents, in contrast to low-quality leaves (such as *M. flammea* and *N. megapotamica*), are probably associated

with the high densities of decomposers that prefer to colonize high-quality leaves (Vos et al. 2013; Rezende et al. 2019). These results corroborate the strong relationship between terrestrial and aquatic ecosystems (Rezende et al. 2019) and give support to the importance of riparian vegetation diversity in driving ecosystem processes (Inhamuns, Rezende, and Coelho 2021; Nuven et al. 2022) because leaves with low stoichiometric ratios (e.g., C:N ratio) can accelerate organic matter processing (Rezende, Petrucio, and Gonçalves 2014; Cararo et al. 2023).

Nectandra megapotamica in the single treatment showed lower mass loss than the mixture and all species in the mixed treatment. This pattern suggests that the effect of diversity on leaf breakdown can vary among the plant species present in the mixture (Moretti, Gonçalves, and Callisto 2007; Vos et al. 2013). Leaves of *N. megapotamica* have antifungal and antimicrobial compounds (Costa et al. 2019), which may have detrained microbial conditioning and reduced leaf palatability to shredders (Casotti et al. 2019; Rezende et al. 2019), resulting in low leaf breakdown rates. Thus, depending on leaf quality, the “complementarity effect” may occur in the mixed treatment through resource partitioning or positive interactions that increase total resource use (Loreau and Hector 2001; Fox 2005). Therefore, the specific composition of riparian vegetation is crucial in determining the effects of taxonomic and functional diversity of leaf

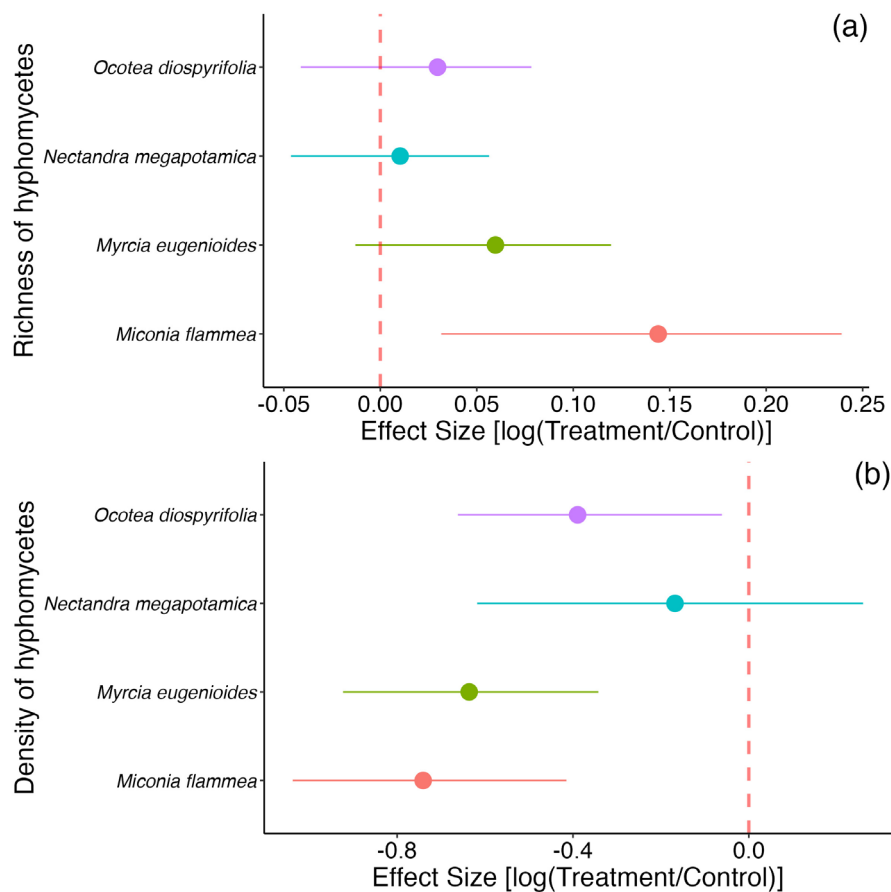


FIGURE 5 | Effect size and direction of mixture (dotted red line) effects on aquatic hyphomycetes density (a) and biomass (b) among leaves of *O. diospyrifolia*, *N. megapotamica*, *M. eugenioides*, and *M. flammea*. The values are expressed as log ratios between the mixture (control) versus single leaf treatments. Circles represent means, and whiskers indicate the upper and lower bounds of 95% nonparametric bootstrapped confidence intervals. Whiskers that do not touch the dashed line reject the null hypothesis (i.e., do not contain the value of 0), while whiskers that touch the dashed line do not reject the null hypothesis.

litter on the breakdown process and nutrient cycling (Boyero et al. 2021; Tonin et al. 2021; Bambi et al. 2022).

Regarding decomposer assemblages, we observed an increase in taxonomic richness in the mixed treatment compared to the single treatments, both for invertebrates (Bruder et al. 2013; Vos et al. 2013) and aquatic hyphomycetes (Kominoski et al. 2009; Lecerf et al. 2010). The diversity of decomposers is probably related to the high breakdown rates of *O. diospyrifolia* and *M. eugenioides* in the mixed treatment. On the other hand, decomposers' density and biomass values were lower in the mixed than in single treatments, suggesting the high competition among decomposers for high-quality leaves in the mixed treatment (Alvim et al. 2014; Rezende et al. 2016, 2021). Increasing leaf diversity can promote habitat structure, allowing many taxa to colonize the leaves (Nuven et al. 2022). However, it can also reduce density values, because the increase in resource heterogeneity favors specialist taxa (Alvim et al. 2014; Rezende, Petrucio, and Gonçalves 2014), which may consequently reduce biomass values (Tonin et al. 2014).

Shredders richness and biomass were higher in the mixed than single treatments, while scrapers had the opposite pattern. Because shredders directly use leaves as food source (Rezende, Petrucio, and Gonçalves 2014; Cararo et al. 2023), the

increase in litter diversity positively affects nutrient availability, making the leaf mixture more attractive to a high number of shredder taxa and resulting in high values of biomass (Bruder et al. 2013; Vos et al. 2013). Scrapers feed mainly on the biofilm that grows on leaf surface (Rezende et al. 2018; Loureiro et al. 2021). When feeding, they may also shred leaf litter indirectly (Rezende, Gonçalves Jr., and Petrucio 2010; Quintão, Rezende, and Júnior 2013). Because scrapers benefit from more stable substrates, that is, leaves with low breakdown rates that allow biofilm growth over longer periods (Rezende, Gonçalves Jr., and Petrucio 2010; Quintão, Rezende, and Júnior 2013; Loureiro et al. 2021), they preferred to colonize the leaves in single treatments.

4.2 | Decomposer Assemblages

The low abundance and biomass of shredders in treatments with higher breakdown rates (*M. eugenioides*, *M. flammea*, and *O. diospyrifolia* in single treatments) suggest that this group did not contribute substantially to leaf breakdown in highland grassland streams. An alternative inference could be that the low abundance and biomass in those leaves might also be due to the low quantity of remaining material. However, individuals of the family Dryopidae, which are assigned to the

functional feeding group of shredders (Cummins, Merritt, and Andrade 2005; Cummins 1996; Hamada and Ferreira-Keppler 2012; Pérez 1988), were indicators of the assemblages found associated with the mixed treatment. This taxon probably benefited from the high leaf diversity, resulting in higher abundances in the mixed than in single treatments (Bruder et al. 2013; Vos et al. 2013). Our results suggest that leaf mass loss was carried out by another functional feeding group, such as scrapers (Rezende, Gonçalves Jr., and Petrucio 2010; Quintão, Rezende, and Júnior 2013; Loureiro et al. 2021), or even by water physical abrasion (Ferreira et al. 2006; Nuven et al. 2022). This hypothesis can be corroborated by the high abundance of scrapers in assemblages associated with *M. eugenioides*. These findings could explain the high breakdown rates of leaves of this plant species compared to the mixed treatment. In addition, larvae of the family Hydropsychidae, which are assigned as gathering-collectors (Cummins, Merritt, and Andrade 2005; Cummins 1996; Hamada and Ferreira-Keppler 2012; Pérez 1988), are normally found in high abundances associated with leaves with high breakdown rates that produce high amounts of fine particles of organic matter (Serpa et al. 2020), such as *M. eugenioides* in this study.

High values of taxonomic richness and species-specific associations of aquatic hyphomycetes were found only in low-quality leaves (*M. flammaea* and *N. megapotamica*). Taken together with the low density of aquatic hyphomycetes in leaves that had high breakdown rates, these results suggest that leaf breakdown in highland grassland streams was driven by leaf chemical characteristics and abiotic fragmentation (Bruder et al. 2013; Rezende et al. 2021). Regarding the species that emerged as indicators of aquatic hyphomycetes assemblages, *L. curvula* is considered a common species in temperate and tropical streams (Biasi et al. 2019; Ferreira et al. 2019; Barreto et al. 2023), while *T. deficiens* is restricted to the Neotropical region. Both are tolerant and dominant species in Neotropical streams (Medeiros et al. 2015; Barreto et al. 2023). Those characteristics probably resulted in the high densities of these species found associated with leaves with low breakdown rates (*M. flammaea*, and *N. megapotamica*, respectively). In addition, our finding that two different species of aquatic hyphomycetes were indicators of assemblages found associated with low-quality leaves reinforces the importance of leaf traits for microbial colonization (Biasi et al. 2019; Rezende et al. 2019). It also suggests that species competition in aquatic hyphomycete assemblages found associated with high-quality leaves may even out the densities of different species (Rezende et al. 2019).

5 | Conclusion

The adaptation of decomposer assemblages to streams with poorly structured riparian vegetation may have outweighed the potential effects of litter diversity on leaf breakdown rates in this study. Our results emphasize the critical role of leaf quality and nonshredding invertebrates in driving leaf breakdown in highland grassland streams. Thus, the influence of litter diversity on leaf breakdown rates is context-dependent, especially concerning leaf chemistry. These findings highlight the importance of conserving riparian vegetation to sustain ecosystem functions in highland grassland streams, which

will likely encounter increased land use and climate change stress. Consequently, future research should aim to elucidate the mechanistic links between leaf litter diversity (size, density, and quantity) and chemical characteristics (stoichiometry) among decomposers adaptation, with a particular focus on shrub and grass species, mainly considering the impacts of climate change. Therefore, the specific composition of riparian vegetation is crucial in determining the effects of diversity on organic matter processing and nutrient cycling in highland grassland streams.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Dryad Digital Repository at <https://doi.org/10.5061/dryad.xwdbrv1q6>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.