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Nutrient enrichment does not affect diet selection by a tropical shredder species in a mesocosm experiment

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ABSTRACT

The decomposition of plant litter is a fundamental ecological process in small forest streams. Litter decomposition is mostly controlled by litter characteristics and environmental conditions, with shredders playing a critical role. The aim of this study was to evaluate the effect of leaf species (*Maprounea guianensis* and *Inga laurina*, which have contrasting physical and chemical characteristics) and water nutrient enrichment (three levels) on leaf litter chemical characteristics and fungal biomass, and subsequent litter preference and consumption by *Phylloicus* sp. (a typical shredder in tropical streams). *Maprounea guianensis* leaves had lower lignin and nitrogen (N) concentrations, higher polyphenols concentration and lower lignin:N ratio than *I. laurina* leaves. Phosphorus concentrations were higher for both leaf species incubated at the highest water nutrient level. Fungal biomass was higher on *M. guianensis* than on *I. laurina* leaves, but it did not differ among nutrient levels. Relative consumption rates were higher when shredders fed on *M. guianensis* than on *I. laurina* leaves, due to the lower lignin:N ratio and higher fungal biomass of *M. guianensis*. Consumption rates on *M. guianensis* leaves were higher for those exposed to low water nutrient levels than for those exposed to moderate water nutrient levels. Feeding preferences by shredders were not affected by leaf species or nutrient level. The low carbon quality on *I. laurina* leaves makes it a less attractive substrate for microbial decomposers and a less palatable resource for shredders. Changes in litter input characteristics may be more important than short-term nutrient enrichment of stream water on shredder performance and ecosystem functioning.

1. Introduction

Plant litter is energetically important for shaded headwater streams, as primary production is low and unable to provide energetic support for aquatic trophic webs (heterotrophic metabolism; Vannote et al., 1980; Wallace et al., 1997). Leaves are the main source of energy and nutrients for these ecosystems (60–80% of allochthonous organic matter; Esteves and Gonçalves, 2011; Bambi et al., 2017). The energy and nutrients contained in leaf litter are incorporated into aquatic food webs through leaf decomposition (Gessner et al., 1999; Hieber and Gessner, 2002). Leaf decomposition is mainly a biological process mediated by the activity of microbial decomposers and invertebrate shredders (Gessner et al., 1999; Hieber and Gessner, 2002; Baldy et al., 2007). Shredder activity on leaves is especially promoted by microbial conditioning that increases leaf softness and nutrient concentration (Graça et al., 2001; Graça and Cressa, 2010). The activity of both biological groups, and the rate at which litter decomposes and nutrients cycle, is controlled by leaf characteristics and environmental conditions (Boyero et al., 2016; Zhang et al., 2019).

Leaf characteristics such as toughness (Berg and McClaugherty, 2003; Zhang et al., 2019), concentrations of lignin (Schindler and Gessner, 2009; Jabiol et al., 2019), polyphenols (Moretti et al., 2009), nitrogen (N) and phosphorus (P) (García-Palacios et al., 2016), and nutrient ratios such as carbon:N (Chauvet et al., 2016) are important moderators of leaf decomposition. Soft leaves, with high nutrients concentrations (e.g. N and P) and low concentrations of structural (e.g. lignin) and secondary (e.g. polyphenols) compounds, are generally colonized faster and sustain higher microbial and shredder activity than more recalcitrant leaves (Gessner and Chauvet, 1994; Gulis and Suberkropp, 2003; Ferreira et al., 2012). Therefore, leaves of high

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nutritional quality decompose faster than low quality leaves (Gessner and Chauvet, 1994; Ferreira et al., 2006a, 2012; Zhang et al., 2019).

Among the environmental factors that most strongly affect decomposers activity and leaf decomposition are nutrient concentrations in stream water (Graça et al., 2015). Moderate nutrient enrichment of oligotrophic streams generally stimulates microbial and shredder activity on leaves (Gulis and Suberkropp, 2003; Ferreira et al., 2006b; Greenwood et al., 2007; Medeiros et al., 2015) and leaf decomposition rates (Woodward et al., 2012; Ferreira et al., 2015; Rosemond et al., 2015). Leaf decomposition mediated by shredders is particularly stimulated by nutrient enrichment (Gulis et al., 2006; Tant et al., 2015).

Leaf characteristics, water nutrient concentration and shredder presence often interact to control leaf decomposition (Kominoski et al., 2015; Tant et al., 2015; Jabiol et al., 2019). Tropical streams generally have low nutrient concentrations water, receive recalcitrant leaf inputs and have a low abundance of shredders (Gonçalves et al., 2006, 2007; Wantzen and Wagner, 2006; Boyero et al., 2012; Rezende et al., 2018). Nevertheless, even if shredders have low abundance in tropical streams, their biomass can attain high values making them important players on leaf decomposition (Tonin et al., 2014).

Within the shredders' guild, the genus *Phylloicus* Müller, 1880 (Trichoptera: Calamoceratidae) has a wide distribution in the neotropics (Prather, 2003), being the most abundant shredder genus in tropical and subtropical streams (Wantzen and Wagner, 2006; Tonello et al., 2016). *Phylloicus* sp. larvae tend to use leaves with a higher nutrient concentration for food and leaves with a higher concentration of lignin and polyphenols for the construction of their cases (Rincón and Martínez, 2006; Moretti et al., 2009; Rezende et al., 2018).

In this study we assessed the effects of leaf characteristics (two leaf species with contrasting initial characteristics) and water nutrient concentrations (three levels) on microbial leaf colonization and feeding preferences and consumption rates of *Phylloicus* sp. We predicted that: i) Higher dissolved nutrient availability would stimulate microbial colonization and decomposition of leaf litter (Gomes et al., 2017), ii) *Phylloicus* sp. would have higher consumption rates and prefer to feed on leaves with higher nutritional value (Moretti et al., 2009), and iii) Higher dissolved nutrient availability would stimulate the rate of leaf fragmentation by *Phylloicus* sp. due to increased microbial conditioning (Greenwood et al., 2007).

2. Methods

2.1. Leaf collection and microbial conditioning in the stream and in the laboratory

Senescent leaves of *Maprounea guianensis* Aubl. and *Inga laurina* (Sw.) Willd. were collected in August 2017, in the Ecological Station of the Botanical Garden of Brasília (JBB) and in the Fazenda Água Limpa (FAL) conservation unit of the University of Brasília, Federal District, Brazil, respectively. These are tree species common in the riparian vegetation of the Brazilian Cerrado and were selected for having contrasting leaf characteristics. *Maprounea guianensis* has lower concentrations of lignin (mean 24 % dry mass, DM), cellulose (16 % DM), N (0.76 % DM) and P (0.01 % DM) and lower lignin:N ratio (31) than *I. laurina* (45 % DM, 32 % DM, 1.05 % DM, 0.09 % DM and 36, respectively) (Gomes et al., 2016). Due to the lower concentration of structural compounds, *M. guianensis* is likely of better nutritional quality for shredders when compared with *I. laurina*, despite its lower nutrient concentration. Leaves were air-dried at room temperature in the dark and stored in paper boxes until used.

Air-dry leaves were enclosed in fine mesh bags (10 × 15 cm, 0.5 mm mesh) and incubated in the Cabeça de Veado stream (15°53′22.15″ S, 47°50′34.10″ W), located at JBB, in February 2018 to allow microbial conditioning. This stream water is generally warm (20.0 ± 0.9 °C), has circumneutral pH (7.0 ± 0.8) and moderate oxygenation (7.5 ± 3.0 mg O_2 L^{-1}); values are mean ± SE (n = 24) between September 2010 and August 2012 (Bambi et al., 2017). After seven days incubation, the litter bags were collected, placed individually in plastic bags and taken to the laboratory in ice boxes. Previous studies have found that the seven-days incubation period is enough to guarantee microbial colonization of the substrates (Rezende et al., 2015, 2018; Martins et al., 2020).

In the laboratory, leaves were gently rinsed with distilled water to remove sediments and distributed by three containers with media (25 L) of different N and P concentrations: low NP concentration corresponding to filtered water from Cabeça de Veado stream (18 μg P L^{-1} and 142 μg N L^{-1} ; N:P molar ratio: *17:1*), moderate NP concentration (100 μg P L^{-1} and 1390 μg N L^{-1}; *31:1*) and high NP concentration (980 μg P L^{-1} and 13,860 mg N L^{-1}; *31:1*). The N:P molar ratios were the same for the moderate and high nutrient concentrations, which only differed in the nutrients concentration. The moderate NP concentration was achieved by adding 0.5 g MOPS buffer, 0.55 mg K_2HPO_4 and 10 mg KNO_3 per liter of distilled water. The high NP concentration was achieved by adding 0.5 g MOPS buffer, 5.5 mg K_2HPO_4 and 100 mg KNO_3 per liter of distilled water. Solutions were neutralized with KOH, and 75.5 mg $CaCl_2$ and 10 mg $MgSO_4.7H_2O$ were added inside a flux chamber. The media were aerated continuously (1.5 L min^{-1}) and replaced every two days by fresh media. Containers were located in a temperature-controlled room (20 °C), with a 12 h light:12 h dark photoperiod, and leaf incubations lasted for one week. This laboratory incubation was aimed at exposing microbial decomposers to different nutrient concentrations, which would likely stimulate fungal biomass buildup, leaf nutrient enrichment and leaf maceration by microbial enzymes at higher nutrient concentrations (Gulis and Suberkropp, 2003). After incubation, leaf discs (12-mm diameter) were cut with a cork borer, avoiding main veins, lyophilized and stored to be used in experiments with *Phylloicus* sp. Lyophilization keeps the leaf discs nutritional properties while allowing the determination of their exact initial dry mass.

2.2. Chemical and microbiological litter characterization

After incubation in the laboratory, leaves from all six treatments (2 leaf species × 3 nutrient levels) were characterized regarding their chemical characteristics. Leaves were oven dried (60 °C for at least 72 h and until they were completely dry), ground to fine powder and used to determine the concentrations of total polyphenols by the Folin-Ciocalteau method (Bärlocher and Graça, 2005), lignin by the gravimetric method (Gessner, 2005a), total P by acid digestion with hydrochloric acid followed by the ascorbic acid method (Flindt and Lillebø, 2005), and carbon and N using an elemental analyzer (model Truspec CHN628, Leco Instruments Ltda, São José, Michigan, USA 2013). Concentrations were expressed as % dry mass (DM).

Sets of 3 leaf discs per treatment were frozen at − 20 °C, lyophilized for 24 h, weighed (± 0.01 mg) to determine DM and used for ergosterol extraction, as a surrogate for fungal biomass, by the solid phase extraction method (Gessner, 2005b). Determination of ergosterol concentration in the extract was made by high-performance liquid chromatography and results were expressed as μg ergosterol g^{-1} DM (Gessner, 2005b).

2.3. Collection of Phylloicus sp. individuals

Phylloicus sp. individuals were collected at Capetinga stream (15°57′40″ S, 47°56′33″ W), located at FAL, through an active search. The abundance of *Phylloicus* sp. larvae is higher in this stream than in other streams of the region (e.g. Cabeça de Veado Stream), which allowed the collection of enough similar sized individuals in a single occasion. The Capetinga stream is generally characterized by warm water (18 ± 1 °C), circumneutral pH (7 ± 1), moderate oxygenation (7.5 ± 2 mg L^{-1}) (values are mean ± SE (n = 24) between September 2010 and August 2012; Bambi et al., 2017), and low nutrient concentration (30 μg P L^{-1} and 160 μg N L^{-1}). Individuals with cases of similar size (~ 1 cm wide) were selected, placed in an ice box and taken to the laboratory.

Individuals were placed in an aquarium, with continuously aerated mineral water and a layer of stream sediment at the bottom. The aquarium was kept in a temperature-controlled room (20 °C), with a 12 h light: 12 h dark photoperiod, to allow acclimatization of individuals to the laboratory conditions for 48 h. Individuals' interocular distance was measured with the aid of an electronic magnifying glass with a millimeter eyepiece and used to estimate their initial biomass with power regression model (r^2: 0.66; Martins et al., 2014).

2.4. Leaf consumption experiment by shredders

Twelve experimental containers (300 mL) were established for each of the six treatments (12 containers × 2 leaf species × 3 water nutrient levels, 72 containers in total; Fig. 1). Each container received 300 mL of mineral water (Martins et al., 2017), which was diluted 100× to show low electrical conductivity (20.38 μS cm^{-1}), circumneutral pH (7.37) and the following chemical composition (sorted by increasing concentration): 0.002 mg L^{-1}, 0.008 mg L^{-1}, 0.009 mg L^{-1}, 0.009 mg N L^{-1}, 0.010 mg F L^{-1}, 0.011 mg L^{-1}, 0.017 mg L^{-1}, 0.021 mg SO_4 L^{-1}, 0.018 mg P L^{-1}, 0.148 mg K L^{-1}. The water was continuously aerated during the experiment (375 mL min^{-1}). The bottom of each container was covered with stream sediment previously ignited in a muffle furnace (550 °C, 24 h). Containers were displayed in a temperature-controlled room (20 °C), with a 12 h light:12 h dark photoperiod.

Each container received one shredder individual, three pre-weighed leaf discs from one of the six treatments and one fine mesh bag (70 × 50 mm; 0.5 mm mesh) with three leaf discs from the same treatment, which was hanged from the container's edge and was inaccessible to the shredder, to serve as a control for mass loss due to microbial activity (Fig. 1). The experiment lasted until approximately 50 % of the initial leaf mass from one of the treatments was consumed (7 days). Individuals and the remaining mass of exposed and control discs were collected, oven dried (60 °C, 72 h) and weighed to determine final mass. Leaf consumption rates were determined by the formula: ((exposed discs initial DM – exposed discs final DM) – (control discs initial DM – control discs final DM)) / individual DM /day. Results were expressed as mg leaf DM mg^{-1} individual DM day^{-1}. Leaf mass loss in control discs was expressed as percentage of initial DM.

2.5. Leaf feeding preferences by shredders

Twelve containers as those described in the 'Leaf consumption' section above were used. Each container received one shedder individual and six pre-weighed leaf discs, one from each treatment. Each container also received six fine-mesh litter bags (70 × 50 mm; 0.5 μm mesh opening) with one disc from each treatment, which were hung from the container's edge to serve as a control for mass loss due to microbial activity (Fig. 2). The experiment lasted until approximately 50 % of the initial mass of at least one disc was consumed (7 days). Individuals and the remaining mass of exposed and control discs were collected, oven dried (60 °C, 72 h) and weighed to determine the final mass. The leaf consumption rates and leaf mass loss in control discs were determined as described above.

2.6. Statistical analysis

The two-way factorial generalized linear mixed-effects analysis (*glmer* function in *lme4* package) was performed for testing the effect of leaf litter species (*M. guianesnis* vs. *I. laurina*), water nutrient level (low vs. moderate vs. high) and their interaction (explanatory variable) on leaf litter consumption by *Phylloicus* sp. larvae and decomposition by the microbial community (response variables; Bates et al., 2015). We performed a random effect GLMM analysis considering the microcosm identity (each container) to account for the specific features of each container, and contrast analysis by pairwise post hoc tests using multcomp (Hothorn et al., 2014) to interpret which of the factors is different from the others. The p-values were obtained by likelihood ratio tests (Chi-square distribution) of the full model against a partial model without the explanatory variables. All models were tested for error distribution by *hnp* function and package and corrected for over or under dispersion. The error distributions with the best fit for all models were the Gaussian family. We ran factorial generalized linear models (GLMs; Zuur and Ieno, 2016) and contrast analyses using *multcomp* (Hothorn et al., 2014) to test comparisons between initial chemical characteristics of leaf litter species, water nutrient concentrationand fungal biomass. All analyzes were performed using R software (R Core Team, 2020).

3. Results

3.1. Leaf litter characteristics and fungal biomass

Lignin concentrations differed between leaf species, being ~ 2× higher for *I. laurina* leaves (Table 1). Polyphenols concentrations also differed between leaf species, being ~ 4× higher for *M. guianensis* leaves

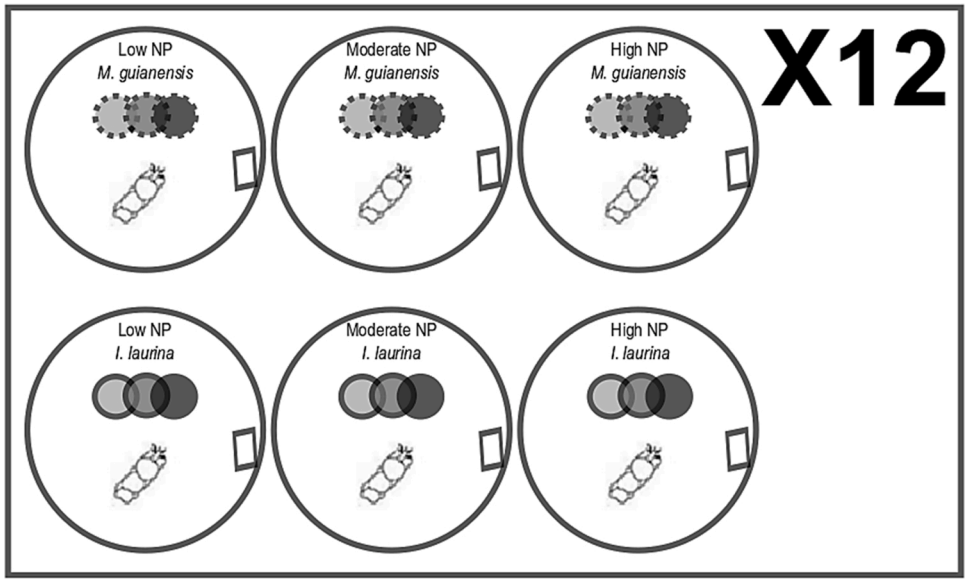

Fig. 1. Experimental design of the leaf consumption experiment. Twelve containers were set for each of six treatments crossing two leaf species of contrasting characteristics (*Maprounea guianesnis* and *Inga laurina*) and three nutrient levels (Low NP, Moderate NP and High NP) to which leaves had been previously exposed (72 containers in total). Each container received one shredder individual (*Phylloicus* sp.), three leaf discs that were accessible to the shredders, and one fine mesh litter bag with leaf discs protected from shredder access that was hung on the container's edge.

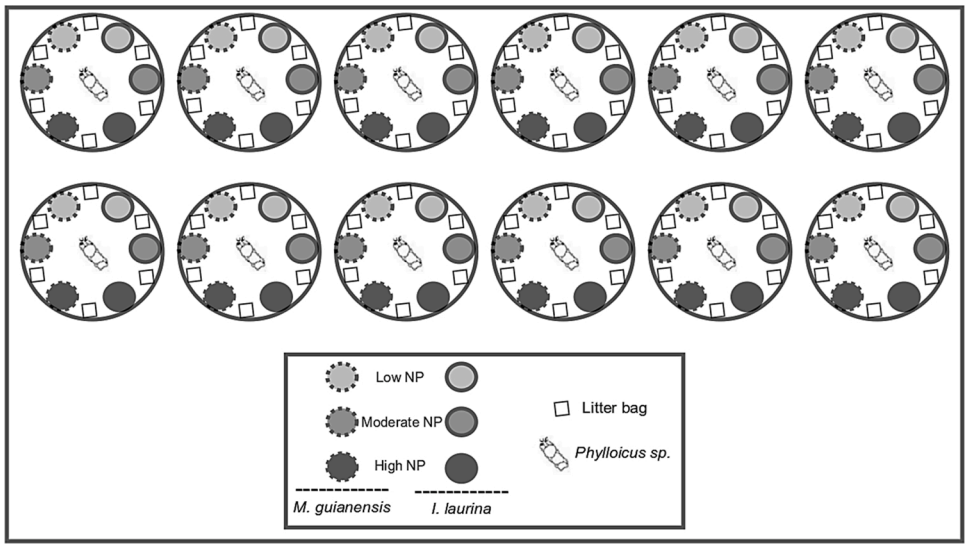

Fig. 2. Experimental design of the leaf feeding preferences experiment. Each of 12 containers received one shredder individual (*Phylloucus* sp.), one leaf disc of each of six treatments, crossing two leaf species of contrasting characteristics (*Maprounea guianesnis* and *Inga laurina*) and three nutrient levels (Low NP, Moderate NP and High NP) to which leaves had been previously exposed, and six litter bags (one per litter treatment) with one leaf disc each protected from shredder access that was hung on the container's edge.

Table 1
Chemical characterization (means ± SE) of *Maprounea guianensis* and *Inga laurina* leaves after being incubated in the stream for one week followed by one week incubation in the laboratory under three different water nutrient conditions (Low, Moderate and High water NP concentrations). Comparisons between leaf species and among nutrient levels were done by factorial GLM (treatments with the same letter do not significantly differ, $p \geq 0.050$). DM, dry mass; N, nitrogen; P, phosphorus.

Leaf species	Nutrient level	Lignin (% DM)	Polyphenols (% DM)	N (% DM)	P (% DM)	N:P	Lignin:N
M. guianensis	Low NP	26.7^{a} ±1.6	30.3^{a}±2.0	1.01^{b}±0.03	0.05^{a}±0.01	23.1^{b}±3.8	23.2^{a}±1.5
	Moderate NP	22.3^{a}±1.2	31.9^{a}±1.6	0.89^{a}±0.01	0.06^{a}±0.01	17.6^{ab}±5.0	25.1^{a}±0.8
	High NP	22.8^{a} ±1.2	27.4^{a}±1.6	1.06^{b}±0.01	0.13^{b}±0.03	9.4^{a}±1.9	21.5^{a}±1.4
I. laurina	Low NP	48.5^{b}±3.5	5.0^{b}±0.1	1.56^{c}±0.002	0.06^{a}±0.01	27.1^{b}±1.0	31.0^{b}±1.7
	Moderate NP	47.3^{b}±2.5	9.4^{b}±1.5	1.51^{c}±0.01	0.09^{a}±0.02	10.2^{ab}±3.2	31.3^{b}±1.9
	High NP	48.7^{b}±4.6	8.4^{b}±0.7	1.63^{c}±0.07	0.15^{b}±0.01	19.9^{ab}±3.0	30.3^{b}±4.0

(Table 1). No effects of water nutrient level or of the interaction between leaf species and water nutrient level were found for lignin or polyphenols concentrations (Table 2).

Nitrogen concentrations were affected by leaf species and water nutrient level (Table 2). N concentrations were ~ 1.6× higher for *I. laurina* than for *M. guianensis* leaves, but effects of nutrient level were found only for *M. guianensis* leaves with N concentration ~ 16 % lower for the moderate than for the other two nutrient levels (Table 1). P concentrations were affected only by water nutrient level (Table 2), being ~ 2× higher for leaves in the high-water nutrient level than for leaves in the other two nutrient levels (Table 1). N:P ratios were affected by water nutrient level and the interaction between factors (Table 2), being ~ 2.5× higher for *M. guianensis* at the low than at the high nutrient level (Table 1). Lignin:N ratios differed between leaf species (Table 2), being ~ 25 % higher for *I. laurina* (Table 1).

Fungal biomass associated with the leaves after being incubated in the laboratory at three water nutrient levels differed between leaf species, being ~ 3× higher on *M. guianensis* than on *I. laurina* (Table 2, Fig. 3). No effects of water nutrient level or of the interaction between factors were found for fungal biomass (Table 2).

3.2. Leaf consumption by shredders

Relative consumption rates of *Phylloicus* sp. larvae were affected by leaf species and the interaction between leaf species and water nutrient level (Table 3). Consumption rates on *M. guianensis* leaves were higher for those exposed to low water nutrient levels than for those exposed to moderate water nutrient levels (Fig. 4a, Table 4), while consumption rates on *I. laurina* leaves were not affected by water nutrient levels. Consumption rates were higher for *M. guianensis* than for *I. laurina* in the low and high water nutrient levels, while no effect of leaf species was

Table 2
Summary table of factorial GLM analyses performed on initial chemical characteristics and fungal biomass of *Maprounea guianensis* and *Inga laurina* leaves (Leaf species) after being incubated in the stream for one week followed by one week incubation in the laboratory under three different water nutrient concentrations (Nutrient level).

Source of variation	DF	Resid. Dev	DF	F	p
Lignin					
Leaf species	1	283.9	16	124.727	**<0.001**
Nutrient level	2	279.4	14	0.097	0.907
Leaf species × Nutrient level	2	278.8	12	0.014	0.986
Polyphenols					
Leaf species	1	136.20	16	369.442	**<0.001**
Nutrient level	2	102.73	14	2.767	0.102
Leaf species × Nutrient level	2	72.56	12	2.495	0.124
N					
Leaf species	1	0.112	16	405.927	**<0.001**
Nutrient level	2	0.048	14	8.443	**0.005**
Leaf species × Nutrient level	2	0.045	12	0.481	0.629
P					
Leaf species	1	0.037	16	2.187	0.164
Nutrient level	2	0.011	14	14.224	**<0.001**
Leaf species × Nutrient level	2	0.011	12	0.151	0.861
N:P					
Leaf species	1	1095.6	16	0.825	0.381
Nutrient level	2	625.4	14	7.448	**0.007**
Leaf species × Nutrient level	2	378.7	12	3.908	**0.049**
Lignin:N					
Leaf species	1	186.6	16	18.805	**<0.001**
Nutrient level	2	170.7	14	0.575	0.577
Leaf species × Nutrient level	2	166.0	12	0.169	0.846
Ergosterol					
Leaf species	1	1583.4	16	189.148	**<0.001**
Nutrient level	2	1299.8	14	1.315	0.304
Leaf species × Nutrient level	2	1293.6	12	0.028	0.971

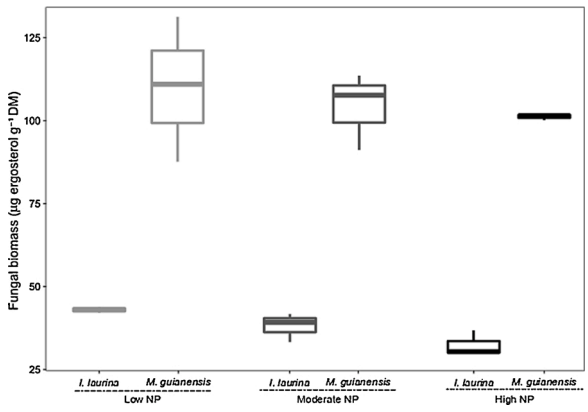

Fig. 3. Fungal biomass associated with two leaf species (*Inga laurina*) and *Maprounea guianesnis*) previously exposed to three water nutrient levels (Low NP, Moderate NP and High NP). Boxes represent the quartiles, the bold line represents the median and the vertical line represents the upper and lower limits.

found for the moderate water nutrient level (Fig. 4a, Table 4). Leaf species, water nutrient level and the interaction between factors did not affect leaf mass loss by microbial activity (Table 3, Fig. 4b).

3.3. Leaf feeding preferences by shredders

Leaf species, water nutrient level and the interaction between factors did not affect feeding preferences by *Phylloicus* sp. larvae (Table 3, Fig. 5a). Leaf mass loss in fine mesh bags was not affected by leaf species, water nutrient level or the interaction between leaf species and nutrient level (Table 3, Fig. 5b).

4. Discussion

Most leaf chemical characteristics measured (except P concentration), and leaf-associated fungal biomass were affected by leaf species, while only leaf N and P concentrations and N:P ratio were affected by water nutrient level. Consequently, stronger effects of leaf species than of water nutrient level were found on microbial-driven leaf mass loss and leaf consumption by shredders.

Leaf species naturally differ in their characteristics (Ostrofsky, 1997; Jabiol et al., 2019), which control microbial colonization and establishment on leaf litter (Gessner and Chauvet, 1994; Canhoto and Graça, 1999). Microbial colonization and their activities are generally promoted in litter with high nutrient concentration and low concentration of structural and secondary compounds (Gessner and Chauvet, 1994; Gulis and Suberkropp, 2003; Ferreira et al., 2012). Among the various leaf characteristics measured, lignin concentration and lignin:N ratio were the most important ones controlling leaf colonization by microorganisms and its subsequent microbial decomposition and consumption by *Phylloicus* sp. larvae. The lower lignin concentration and lignin:N ratio of *M. guianensis* leaves than of *I. laurina* leaves likely facilitated fungal biomass accumulation. Consumption rates by *Phylloicus* sp. larvae were sensitive to the lower lignin concentration and lignin:N ratio and higher fungal biomass accumulation on *M. guianensis* leaves than of *I. laurina* leaves, on low and high water nutrient levels, but not on moderate nutrient levels. The low consumption rate of *Phylloicus* sp. larvae on *M. guianensis* leaves exposed to moderate nutrient levels may result from their lower N concentration. When given a choice between both leaf species no preference was found. The lack of feeding preferences by *Phylloicus* sp. larvae was unexpected since the same shredder species showed feeding preferences in another study, although the contrasted leaf species differed between studies (Reis et al., 2019). Litter decomposition is generally slower when lignin concentration is high, likely due to a limitation of microbial and invertebrate activity by low

Table 3
Simplified two-way factorial generalized linear mixed-effects analysis performed on leaf consumption by shredders and mass remaining in control leaf discs for *Maprounea guianensis* and *Inga laurina* leaves (Leaf species) after being incubated in the stream for one week followed by one week incubation in the laboratory under three different water nutrient concentrations (Nutrient level).*, see Table 4 for multiple comparisions.

Model = Mass consumption ~ Plant species * Nutrient level (1 | Microcosm identity)								
GLMM	Df	AIC	BIC	logLik	Deviance	Chisq Chi	Df residual	Pr(>Chisq)
Leaf consumption experiment								
Leaf relative consumption rate								
Null model	5	3.55	14.19	3.22	−6.45			
Leaf species	8	−4.62	12.39	10.31	−20.62	14.18	58	0.003
Null model	4	−6.37	2.14	7.18	−14.37			
Nutrient level	8	−4.62	12.39	10.31	−20.62	6.26	59	0.181
Null model	3	2.33	8.71	1.83	−3.67			
Leaf species × Nutrient level	8	−4.62	12.39	10.31	−20.62	16.96	61	0.005*
Mass loss in control leaf discs								
Null model	5	409.08	420.40	−199.54	399.08			
Leaf species	8	411.70	429.80	−197.85	395.70	3.38	58	0.336
Null model	4	405.30	414.35	−198.65	397.30			
Nutrient level	8	411.70	429.80	−197.85	395.70	1.59	59	0.810
Null model	3	405.56	412.35	−199.78	399.56			
Leaf species × Nutrient level	8	411.70	429.80	−197.85	395.70	3.86	61	0.570
Leaf feeding preferences experiment								
Leaf relative consumption rate								
Null model	5	−90.86	−81.20	50.43	−100.86			
Leaf species	8	−88.52	−73.06	52.26	−104.52	3.66	58	0.301
Null model	4	−94.78	−87.05	51.39	−102.78			
Nutrient level	8	−88.52	−73.06	52.26	−104.52	1.74	59	0.784
Null model	3	−92.98	−87.18	49.49	−98.98			
Leaf species × Nutrient level	8	−88.52	−73.06	52.26	−104.52	5.54	61	0.354
Mass loss in control leaf discs								
Null model	5	597.82	609.13	−293.91	587.82			
Leaf species	8	600.98	619.08	−292.49	584.98	2.84	58	0.417
Null model	4	596.25	605.30	−294.12	588.25			
Nutrient level	8	600.98	619.08	−292.49	584.98	3.27	59	0.513
Null model	3	595.40	602.19	−294.70	589.40			
Leaf species × Nutrient level	8	600.98	619.08	−292.49	584.98	4.43	61	0.490

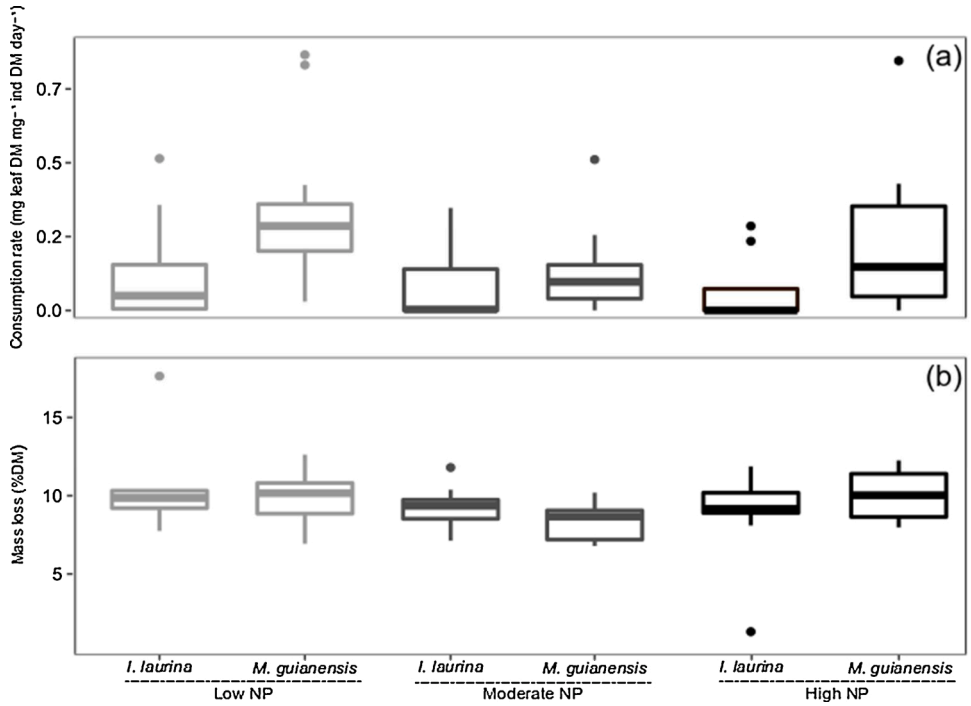

Fig. 4. Relative consumption rate of *Phylloicus* sp. larvae (a) and mass loss in control leaf discs protected from shredder access (b) of two leaf litter species (*Inga laurina* and *Maprounea guianensis*) previously exposed to three water nutrient levels (Low NP, Moderate NP and High NP). Boxes represent the quartiles, the bold line represents the median, the vertical line represents the upper and lower limits and circles the outliers.

Table 4
Results of contrast analysis by pairwise post hoc tests between leaf species (*Maprounea guianensis* and *Inga laurina*), nutrient level (low, moderate and high) and their interactions on leaf consumption by *Phylloicus* sp. larvae.

Species	Nutrient level	Contrast			Estimate	SE	df	t.ratio	p.value
I. laurina	.	High	×	Moderate	−0.011	0.057	56	−0.196	0.999
I. laurina	.	Low	×	High	0.017	0.054	56	0.313	0.999
I. laurina	.	Moderate	×	Low	−0.005	0.056	56	−0.105	1.000
M. guianensis	.	High	×	Moderate	0.045	0.056	56	0.807	0.948
M. guianensis	.	Low	×	High	0.076	0.052	56	1.452	0.622
M. guianensis	.	Moderate	×	Low	−0.122	0.054	56	−2.272	**0.017**
.	High	*M. guianensis*	×	*I. laurina*	0.242	0.102	56	2.376	**0.011**
.	Low	*M. guianensis*	×	*I. laurina*	0.245	0.090	56	2.710	**0.052**
.	Moderate	*M. guianensis*	×	*I. laurina*	0.068	0.094	56	0.727	0.957

quality carbon (Lecerf and Chauvet, 2008; Jabiol et al., 2019; Zhang et al., 2019). This implies that, although *I. laurina* leaves had higher N concentration than *M. guianensis* leaves, its higher concentration of structural compounds likely limited nutrient use, while at the same time provided low quality carbon (Gessner et al., 1999; Jabiol et al., 2019).

Increases in water nutrient enrichment generally stimulate decomposer colonization of leaf litter, biomass buildup and nutrient accumulation (Gulis and Suberkropp, 2003; Gulis et al., 2006; Kominoski et al., 2015). However, effects of water nutrient enrichment on leaf characteristics and fungal biomass buildup were mild, with the most remarkable effect being an increase in P concentration on leaves of both species incubated at high nutrient levels. There are two non-exclusive explanations for the lack of stronger effects of nutrient enrichment in our case. Effects of increases in water nutrient concentration on litter colonization and decomposition depend on litter characteristics. For instance, water nutrient enrichment has stronger effects on leaves with lower (< 15 % DM) than higher lignin concentration (Jabiol et al., 2019), while both leaf species used here had quite high lignin concentration (> 22 % DM). Also, leaves were exposed to the three nutrient levels in the laboratory for only seven days, which may not have been enough for nutrient effects to become evident in these high-lignin leaf species. We also need to consider that leaves were incubated in a specific stream (Cabeça de Veado) and received a specific microbial inoculum, which is adapted to the low nutrient levels of that stream. Incubation of leaves in the laboratory at different nutrient levels had an impact on leaf quality and microbial decomposition, but the microbial community likely remained the same. So, we may be observing the response of *Phylloicus* sp. to a very specific microbial community. Furthermore, consumption rates and not feeding preferences of *Phylloicus* sp. larvae were affected by higher increases in P concentration only on *M. guianensis* leaves. This contradicts the idea that due to their high body P concentration *Phylloicus* sp. larvae may be less sensitive to changes in litter P concentration (González et al., 2014).

Phylloicus sp. consumption rates were higher on the leaf species with lower lignin concentration and lignin:N ratio (*M. guianensis*) than on the more recalcitrant leaf species (*I. laurina*), in agreement with our prediction. However, water nutrient enrichment had only mild effects on leaf characteristics, consumption and no effects on feeding preferences by *Phylloicus* sp. larvae, contradicting our prediction. Thus, we observed that nutrient enrichment was not effective in influencing the diet selection by a tropical shredder species in a mesocosm experiment. Yet, our results need to be interpreted with caution since the study was performed under laboratory conditions with no replication, used a single stream as the source of the microbial inoculum, and addressed the

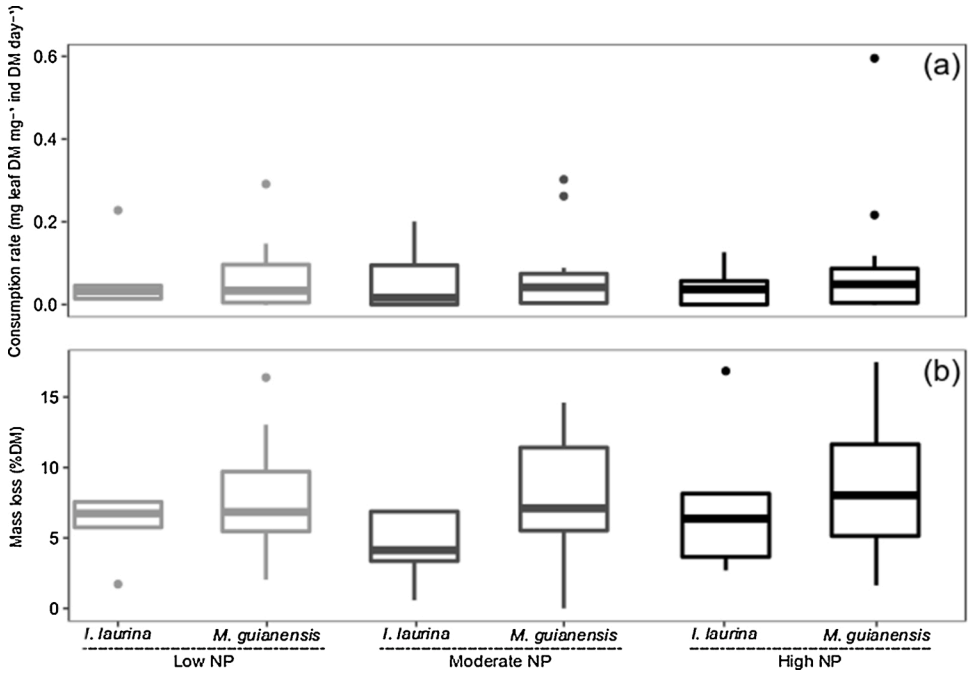

Fig. 5. Relative consumption rate of *Phylloicus* sp. larvae in the feeding preference experiment (a) and mass loss in control leaf discs protected from shredder access (b) of two leaf litter species (*Inga laurina* and *Maprounea guianensis*) previously exposed to three water nutrient levels (Low NP, Moderate NP and High NP). Boxes represent the quartiles, the bold line represents the median, the vertical line represents the upper and lower limits and circles the outliers.

combined effects of a reduced number of leaf species and water nutrient levels on a single Cerrado/Brazilian Savanna shredder species.

Authorship statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Limnologica Journal.*

Authorship contributions

V. Ferreira and J. F. Gonçalves Jr. conceived the study. G. Sena and V. Ferreira performed the experiments. G. Sena wrote the article, with contributions from V. Ferreira, R. S. Rezende and J. F. Gonçalves Jr.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.limno.2021.125883.

References

Baldy, V., Gobert, V., Guérold, F., Chauvet, E., Lambrigot, D., Charcosset, J.Y., 2007. Leaf litter breakdown budgets in streams of various trophic status: effects of dissolved inorganic nutrients on microorganisms and invertebrates. Freshw. Biol. 52, 1322–1335.

Bambi, P., Rezende, R.S., Feio, M.J., Leite, G.F.M., Alvin, E., Quintão, J.M.B., Araújo, F., Gonçalves Jr., J.F., 2017. Temporal and spatial patterns in inputs and stock of organic matter in savannah streams of central Brazil. Ecosystems 20, 757–768.

Bärlocher, F., Graça, M.A.S., 2005. Total phenolics. In: Graça, M.A.S., Bärlocher, F., Gessner, M.O. (Eds.), Methods to Study Litter Decomposition. A Practical Guide. Springer, Dordrecht, The Netherlands, pp. 97–100.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.

Berg, B., McClaugherty, C., 2003. Decomposer organisms. In: Berg, B., McClaugherty, C. (Eds.), Plant Litter. Springer, Berlin, Heidelberg, pp. 31–48.

Boyero, L., Pearson, R.G., Dudgeon, D., Ferreira, V., Graça, M.A.S., Gessner, M.O., Boulton, A.J., Chauvet, E., Yule, C.M., Albariño, R.J., Ramírez, A., Helson, J.E., Callisto, M., Arunachalam, M., Chará, J., Figueroa, R., Mathooko, J.M., Gonçalves Jr., J.F., Moretti, M.S., Chará-Serna, A.M., Davies, J.N., Encalada, A., Lamothe, S., Buria, L.M., Castela, J., Cornejo, A., Li, A.O.Y., M'Erimba, C., Villanueva, V.D., Zúñiga, M.C., Swan, C.M., Barmuta, L.A., 2012. Global patterns of stream detritivore distribution: implications for biodiversity loss in changing climates. Glob. Ecol. Biogeogr. 21, 134–141.

Boyero, L., Pearson, R.G., Hui, C., Gessner, M.O., Pérez, J., Alexandrou, M.A., Graça, M. A.S., Cardinale, B.J., Albariño, R.J., Arunachalam, M., Barmuta, L.A., Boulton, A.J., Bruder, A., Callisto, M., Chauvet, E., Death, R.G., Dudgeon, D., Encalada, A.C., Ferreira, V., Figueroa, R., Flecker, A.S., Gonçalves Jr., J.F., Helson, J., Iwata, T., Jinggut, T., Mathooko, J., Mathuriau, C., M'Erimba, C., Moretti, M.S., Pringle, C.M., Ramírez, A., Ratnarajah, L., Rincon, J., Yule, C.M., 2016. Biotic and abiotic variables influencing plant litter breakdown in streams: a global study. Proc. R. Soc. Lond. Biol. 283, 20152664.

Canhoto, C., Graça, M.A.S., 1999. Leaf barriers to fungal colonization and shredders (*Tipula lateralis*) consumption of decomposing *Eucalyptus globulus*. Microb. Ecol. 37, 163–172.

Chauvet, E., Ferreira, V., Giller, P.S., McKie, B.G., Tiegs, S.D., Woodward, G., Elosegi, A., Dobson, M., Fleituch, T., Graça, M.A.S., Gulis, V., Hladyz, S., Lacoursière, J.O., Lecerf, A., Pozo, J., Preda, E., Riipinen, M., Rîşnoveanu, G., Vadineanu, A., Vought, L.B.-M., Gessner, M.O., 2016. Litter decomposition as an indicator of stream ecosystem functioning at local-to-continental scales: insights from the European RivFunction project. Adv. Ecol. Res. 55, 99–182.

Esteves, F.A., Gonçalves Jr., J.F., 2011. Etapas do metabolismo aquático. In: Esteves, F.A. (Ed.), Fundamentos de Limnologia. Interciência, Rio de Janeiro, pp. 119–124.

Ferreira, V., Graça, M.A.S., de Lima, J.L., Gomes, R., 2006a. Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves. Arch. Hydrobiol. 165, 493–513.

Ferreira, V., Gulis, V., Graça, M.A.S., 2006b. Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecology 149, 718–729.

Ferreira, V., Encalada, A.C., Graça, M.A.S., 2012. Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. Freshw. Sci. 31, 945–962.

Ferreira, V., Castagneyrol, B., Koricheva, J., Gulis, V., Chauvet, E., Graça, M.A.S., 2015. A meta-analysis of the effects of nutrient enrichment on litter decomposition in streams. Fungal Biol. Rev. 90, 669–688.

Flindt, M.R., Lillebø, A.I., 2005. Determination of total nitrogen and phosphorus in leaf litter. In: Graça, M.A.S., Bärlocher, F., Gessner, M.O. (Eds.), Methods to Study Litter Decomposition: A Practical Guide. Springer, The Netherlands, pp. 115–120.

García-Palacios, P., Shaw, E.A., Wall, D.H., Hättenschwiler, S., 2016. Temporal dynamics of biotic and abiotic drivers of litter decomposition. Ecol. Lett. 19, 554–563.

Gessner, M.O., 2005a. Proximate lignin and cellulose. In: Graça, M.A.S., Bärlocher, F., Gessner, M.O. (Eds.), Methods to Study Litter Decomposition: A Practical Guide. Springer, The Netherlands, pp. 115–120.

Gessner, M.O., 2005b. Ergosterol as a measure of fungal biomass. In: Graça, M.A.S., Bärlocher, F., Gessner, M.O. (Eds.), Methods to Study Litter Decomposition: A Practical Guide. Springer, The Netherlands, pp. 189–195.

Gessner, M.O., Chauvet, E., 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75, 1807–1817.

Gessner, M.O., Chauvet, E., Dobson, M., 1999. A perspective on leaf litter breakdown in streams. Oikos 85, 377–384.

Gomes, P.P., Medeiros, A.O., Gonçalves Jr., J.F., 2016. The replacement of native plants by exotic species may affect the colonization and reproduction of aquatic hyphomycetes. Limnologica 59, 124–130.

Gomes, P.P., Ferreira, V., Tonin, A.M., Medeiros, A.O., Gonçalves Jr., J.F., 2017. Combined effects of dissolved nutrients and oxygen on plant litter decomposition and associated fungal communities. Microb. Ecol. 75, 854–862.

Gonçalves Jr., J.F., França, J.S., Medeiros, A.O., Rosa, C.A., Callisto, M., 2006. Leaf breakdown in a tropical stream. Int. Rev. Hydrobiol. 91, 164–177.

Gonçalves Jr., J.F., Graça, M.A.S., Callisto, M., 2007. Litter decomposition in a Cerrado savannah stream is retarded by leaf toughness, low dissolved nutrients and a low density of shredders. Freshw. Biol. 52, 1440–1451.

González, A.L., Romero, G.Q., Srivastava, D.S., 2014. Detrital nutrient content determines growth rate and elemental composition of bromeliad-dwelling insects. Freshw. Biol. 59, 737–747.

Graça, M.A.S., Cressa, C., 2010. Leaf quality of some tropical and temperate tree species as food resource for stream shredders. Int. Rev. Hydrobiol. 95, 27–41.

Graça, M.A.S., Cressa, C., Gessner, M.O., Feio, M.J., Callies, K.A., Barrios, C., 2001. Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. Freshw. Biol. 46, 947–957.

Graça, M.A.S., Ferreira, V., Canhoto, C., Encalada, A.C., Guerrero-Bolaño, F., Wantzen, K. M., Boyero, L., 2015. A conceptual model of litter breakdown in low order streams. Int. Rev. Hydrobiol. 100, 1–12.

Greenwood, J.L., Rosemond, A.D., Wallace, J.B., Cross, W.F., Weyers, H.S., 2007. Nutrients stimulate leaf breakdown rates and detritivore biomass: bottom-up effects via heterotrophic pathways. Oecology 151, 637–649.

Gulis, V., Suberkropp, K., 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw. Biol. 48, 123–134.

Gulis, V., Ferreira, V., Graça, M.A.S., 2006. Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. Freshw. Biol. 51, 1655–1669.

Hieber, M., Gessner, M.O., 2002. Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83, 1026–1038.

Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S., 2014. Multcomp: Simultaneous Inference in General Parametric Models. R package version, pp. 1–3.

Jabiol, J., Lecerf, A., Lamothe, S., Gessner, M.O., Chauvet, E., 2019. Litter quality modulates effects of dissolved nitrogen on leaf decomposition by stream microbial communities. Microb. Ecol. 77, 959–966.

Kominoski, J.S., Rosemond, A.D., Benstead, J.P., Gulis, V., Maerz, J.C., Manning, D.W., 2015. Low-to-moderate nitrogen and phosphorus concentrations accelerate microbially driven litter breakdown rates. Ecol. Appl. 25, 856–865.

Lecerf, A., Chauvet, E., 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in a stream. Basic Appl. Ecol. 9, 598–605.

Martins, R.T., Melo, A.S., Gonçalves Jr., J.F., Hamada, N., 2014. Estimation of dry mass of caddisflies *Phylloicus elektoros* (Trichoptera: calamoceratidae) in a Central Amazon stream. Zoologia (Curitiba) 31, 337–342.

Martins, R.T., Melo, A.S., Gonçalves Jr., J.F., Campos, C.M., Hamada, N., 2017. Effects of climate change on leaf breakdown by microorganisms and the shredder *Phylloicus elektoros* (Trichoptera: calamoceratidae). Hydrobiologia 789, 31–44.

Martins, R.T., Gonçalves Jr., J.F., Campos, C.M., Lopes, A., Piedade, M.T.F., Hamada, N., 2020. Leaf consumption by invertebrate aquatic shredders in the Amazon: effects of climate change and microbial conditioning. Limnology 21, 257–266.

Medeiros, A.O., Callisto, M., Graça, M.A.S., Ferreira, V., Gonçalves Jr., J.F., 2015. Microbial colonization and litter decomposition in a Cerrado stream is limited by low dissolved nutrient concentration. Limnetica 34, 283–292.

Moretti, M.S., Loyola, R.D., Becker, B., Callisto, M., 2009. Leaf abundance and phenolic concentrations codetermine the selection of case-building materials by *Phylloicus* sp. (Tri- choptera, Calamoceratidae). Hydrobiologia 630, 199–206.

Ostrofsky, M.L., 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. J. N. Am. Benthol. Soc. 16, 750–759.

Prather, A.L., 2003. Revision of the neotropical caddisfly genus *Phylloicus (Trichoptera: calamoceratidae)*. Zootaxa 275, 1–214.

R Core Team, 2020. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reis, D.F., Machado, M.M.D., Coutinho, N.P., Rangel, J.V., Moretti, M.S., Morais, P.B., 2019. Feeding preference of the shredder *Phylloicus* sp. For plant leaves of *Chrysophyllum oliviforme* or *Miconia chartacea* after conditioning in streams from different biomes. Braz. J. Biol. 79, 22–28.

Rezende, R.D.S., Leite, G.F.M., de Lima, A.K.S., Silva Filho, L.A.B.D., Chaves, C.V.C., Prette, A.C.H., Freitas, J.S., Gonçalves Jr., J.F., 2015. Effects of density and predation risk on leaf litter processing by *Phylloicus* sp. Aust. Ecol. 40, 693–700.

Rezende, R.S., Leite, G.F.M., Ramos, K., Torres, I., Tonin, A.M., Gonçalves Jr., J.F., 2018. Effects of litter size and quality on processing by decomposers in a Cerrado aquatic system. Biotropica. 50, 578–585.

Rincón, J., Martínez, I., 2006. Food quality and feeding preferences of *Phylloicus*sp. (Trichoptera: Calamoceratidae). J. N. Am. Benthol. Soc. 25, 209–215.

Rosemond, A.D., Benstead, J.P., Bumpers, P.M., Gulis, V., Kominoski, J.S., Manning, D. W.P., Suberkropp, K., Wallace, J.B., 2015. Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. Science 347, 1142–1145.

Schindler, M.H., Gessner, M.O., 2009. Functional leaf traits and biodiversity effects on litter decomposition in a stream. Ecology 90, 1641–1649.

Tant, C.J., Rosemond, A.D., Helton, A.M., First, M.R., 2015. Nutrient enrichment alters the magnitude and timing of fungal, bacterial, and detritivore contributions to litter breakdown. Freshw. Sci. 34, 1259–1271.

Tonello, G., Naziloski, L.A., Tonin, A.M., Restello, R.M., Hepp, L.U., 2016. Effect of *Phylloicus* on leaf breakdown in a subtropical stream. Limnetica 35, 243–252.

Tonin, A.M., Hepp, L.U., Restello, R.M., Gonçalves Jr., J.F., 2014. Understanding of colonization and breakdown of leaves by invertebrates in a tropical stream is enhanced by using biomass as well as count data. Hydrobiologia 740, 79–88.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37, 130–137.

Wallace, J.B., Eggert, S.L., Meyer, J.L., Webster, J.R., 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277, 102–104.

Wantzen, K.M., Wagner, R., 2006. Detritus processing by invertebrate shredders: a neotropical–temperate comparison. J. N. Am. Benthol. Soc. 25, 216–232.

Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., Mckie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, V., Graça, M.A. S., Fleituch, T., Lacoursière, J.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.-M., Chauvet, E., 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science 336, 1438–1440.

Zhang, M., Cheng, X., Geng, Q., Shi, Z., Luo, Y., Xu, X., 2019. Leaf litter traits predominantly control litter decomposition in streams worldwide. Glob. Ecol. Biogeogr. 28, 1469–1486.

Zuur, A.F., Ieno, E.N., 2016. A protocol for conducting and presenting results of regression-type analyses. Methods Ecol. Evol. 7, 636–645.