



# A comparison of decomposition rates and biological colonization of leaf litter from tropical and temperate origins

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**Abstract** Litter decomposition is an ecosystem process that allows energy and nutrients transfer from dead plant matter into detrital food webs. Several studies revealed that leaf litter decomposition rates differ across biomes, but the reasons for these differences are not yet clear. Here, we test the role of several leaf litter physical and chemical characteristics as predictors of decomposition rates and whether life forms (evergreen vs. deciduous) differ in decomposition rates. Leaves from dominant riparian trees were collected in four ecoregions (temperate deciduous forest, subtropical seasonal semideciduous forest, tropical Cerrado and subtropical dense ombrophylous forest) and their physical and chemical characteristics were measured. We then determined leaf litter decomposition, colonization by aquatic hyphomycetes and macroinvertebrates in a same stream (i.e., under the same environmental conditions), and consumption rates by shredders under laboratory conditions. Deciduous and evergreen tree species did not differ in the

measured litter characteristics, but deciduous leaves decompose faster than evergreens. Leaf litter from tree species from the four ecoregions differed in their physical and chemical characteristics. Litter from temperate deciduous species decomposed faster than litter from the other three ecoregions, generally supported higher aquatic hyphomycete species richness and sporulation rates and higher consumption rates by shredders. There were significant negative correlations between litter decomposition and associated biotic variables and litter initial lignin concentration and lignin:N ratio. We conclude that lignin concentration and lignin:N ratio are important predictors of litter decomposition, although other unmeasured parameters such as micronutrients may also play important roles in this key ecosystem process.

**Keywords** Aquatic hyphomycetes · Leaf traits · Litter breakdown · Shredder

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## Introduction

Riparian forests provide streams with large amounts of organic matter (Abelho 2001; Gessner et al. 2010). This organic matter, especially leaf litter, sustains aquatic food webs by promoting the incorporation of plant carbon (C) into secondary production through litter decomposition (Wallace et al. 1997; Hieber and

Gessner 2002). Leaf litter decomposition is mainly a biological process, carried out by microbial decomposers (mostly aquatic hyphomycetes) and shredding invertebrates (Hieber and Gessner 2002; Cornut et al. 2010). The rate at which litter decomposes depends on environmental factors, such as temperature (Follstad Shah et al. 2017; Amani et al. 2019) and dissolved nutrients (Woodward et al. 2012; Ferreira et al. 2015; Rosemond et al. 2015), being generally faster in warm and nutrient-rich waters.

Litter characteristics also exert strong control on litter decomposition (Gessner and Chauvet 1994; Ostrofsky 1997; Bakker et al. 2011; Frainer et al. 2015; Lin et al. 2019). Differences in litter characteristics among species result from plant different strategies to cope with their environmental challenges, ranging from slow-growth species that produce long-lived, low-quality leaves to fast-growing species that produce high-quality leaves ('leaf economics spectrum'; Wright et al. 2004).

The distribution of species along the leaf economics spectrum determines their decomposition rate and consequently, the rate at which nutrients cycle (Bakker et al. 2011; Lin et al. 2019). Most common intrinsic predictors of litter decomposition in streams include lignin, tannin and nitrogen (N) concentrations, and C:N ratio, with decomposition generally being faster for soft, nutrient-rich litter with low concentration of structural and secondary compounds (i.e., high-quality litter) than for more recalcitrant litter (Ostrofsky 1997; Lecerf and Chauvet 2008; Frainer et al. 2015; Jabiol et al. 2019; Zhang et al. 2019). High toughness and concentrations of structural and secondary compounds deter microbial colonization, biomass buildup and decomposer activity on the litter, and consequently invertebrate feeding activities (Motomori et al. 2001; Graça and Cressa 2010; Foucreau et al. 2013). Deterrents can also select litter decomposers and detritivores, as observed in eucalypt leaves with oil glands (Canhoto and Graça 1999) and in plant material with high concentration of structural compounds (Ferreira et al. 2016b; Jabiol et al. 2019), which may prevent feeding by large shredders while small shredders, such as chironomids, can feed avoiding oil gland and leaf veins (Callisto et al. 2007). Finally, the imbalance between high C:nutrient ratios on litter and low C:nutrient ratios on decomposers (Cross et al. 2003; Balseiro and Albariño 2006; Hladyz et al. 2009, 2011; Danger et al. 2016) may result in low

decomposers colonization and activity on nutrient-poor litter because of nutrient limitation (Gulis and Suberkropp 2003; Gulis et al. 2006; Greenwood et al. 2007). However, although shredders may prefer high-quality litter, in some cases they may exhibit high consumption of low-quality litter to guarantee ingestion of required nutrients ('compensatory feeding'; Albariño and Balseiro 2001; Flores et al. 2014).

Leaf litter characteristics and, consequently, litter decomposition may also differ among plant life forms (Cornelissen et al. 1999). Decomposition is generally slower in evergreen than in deciduous-tree litter in soils (Cornelissen et al. 1996; Cornwell et al. 2008; Godoy et al. 2010), which can be attributed to species position in the leaf economics spectrum. In contrast, a global review of litter decomposition in streams did not find significant differences between evergreen and deciduous tree species (Zhang et al. 2019).

Leaf litter quality may differ across biomes. Litter quality seems to be lower for tropical than for temperate tree species (García-Palacios et al. 2016; Boyero et al. 2017), likely due to nutrient-poor soils in tropical regions, which limits nutrient concentration in leaf litter (Ordoñez et al. 2008). Additionally, plants in nutrient-poor soils are generally better defended from herbivory with tougher leaves and higher concentrations of secondary compounds (Coley and Barone 1996; Silva and Batalha 2011a, b). However, how this difference in litter quality between tropical and temperate species translates into differences in decomposition rates is not yet clear. Large scale field studies found slower litter decomposition for tropical than temperate species incubated in soils in their 'home' region and when transplanted across regions (Makkonen et al. 2012), or no differences in litter decomposition between temperate and tropical species incubated in streams in their 'home' region (Boyero et al. 2016). Finally, global reviews of litter decomposition suggest a decrease in litter decomposition at the soil surface with increasing latitude (Zhang et al. 2008), or no difference in litter decomposition incubated in streams among climate zones (Zhang et al. 2019); it is not clear if the reviews considered litter incubated outside their 'home' region, but still this should represent a small fraction of the total sample size.

Finally, streams in temperate and tropical regions also differ in ambient conditions, which may affect litter decomposition. Tropical streams are warmer but

nutrient poorer than temperate streams (Boyero et al. 2011a) and many tropical streams have low abundance of shredders (Boyero et al. 2011b; Graça et al. 2015). Thus, leaf litter decomposition potential in tropical streams may be limited by suboptimal local factors (Gonçalves et al. 2006, 2007; but see Zhang et al. 2019). When litter quality was controlled by using the same litter species (*Alnus glutinosa*) across latitudes, Boyero et al. (2011b) found faster microbial-driven but slower invertebrate-driven litter decomposition in tropical streams compared with temperate streams.

Here, we determined the physical and chemical characteristics of leaf litter of riparian tree species ( $n = 30$ ) from four ecoregions (temperate deciduous forest, subtropical seasonal semideciduous forest, tropical Cerrado, and subtropical dense ombrophylous forest). We assessed litter decomposition and associated decomposers (colonization and activity) in a temperate stream (to control for variation in ambient conditions and shredder presence) to determine the potential decomposition of tropical and subtropical species compared with temperate species. We investigate whether leaf litter characteristics differ among ecoregions and between litter from evergreen and deciduous tree species. We predicted more recalcitrant litter for tropical/subtropical than temperate species, and for evergreen than deciduous species. Consequently, we predicted that litter decomposition and associated decomposers would differ among ecoregions and between evergreen and deciduous species with lower decomposer colonization and activity, and consequently slower litter decomposition, on litter from tropical/subtropical than temperate regions, and from evergreen than deciduous species. We also predicted that leaf litter decomposition and decomposer colonization and activity across species would be positively correlated with the leaf economics spectrum, with values being higher for high-quality litter (i.e., soft, nutrient-rich and with low concentration of structural and secondary compounds) than for recalcitrant litter.

## Materials and methods

### Forests and leaf litter characteristics

We collected leaf litter from 30 riparian tree species distributed by four ecoregions located in three climatic

regions: deciduous forest (temperate; 8 species), seasonal semideciduous forest (subtropical; 8 species), dense ombrophylous forest (subtropical; 6 species) and Cerrado (Brazilian savannah, tropical; 8 species) (Table 1). These species were the most common in riparian areas in the four locations.

The deciduous forest (DF) is located in Lousã mountain, central Portugal. The climate is temperate with average annual temperature of 16.1 °C and average annual rainfall of 922 mm. Rainfall is markedly seasonal with summer (June–September) average precipitation of ~ 10 mm and average temperature of 28 °C, and winter (December–March) with average precipitation of ~ 129 mm and average temperature of 4 °C (IPMA 2019). The mountain is largely covered with native species (*Castanea sativa*, *Quercus faginea*, *Q. robur*, *Arbutus unedo*), pine (*Pinus pinaster*) and eucalyptus (*Eucalyptus globulus*) plantations, and acacia (*Acacia dealbata* and *A. melanoxylon*) invaded areas.

The seasonal semideciduous forest (SD) is located in Paraná (south Brazil). The climate is humid subtropical with an average annual temperature of 21 °C and annual rainfall of 1500 mm, distributed throughout the year; the driest month is August (72 mm) and the rainiest month is January (170 mm) (INMET 2019). The region is heavily used for crop production leaving only 12% of the original plant cover.

The Cerrado (CE) is located in the mid-west Brazil; this is the second largest biome in South America, occupying an area of > 2 million km<sup>2</sup>. The climate is tropical with average annual temperature of 22–23 °C and annual rainfall of 1200–1800 mm (INMET 2019). Rainfall is seasonal, being higher in October–March (rainy season; average precipitation of 238 mm and temperature of 23 °C in Brasilia) and lower in April–September (dry season; average precipitation of 39 mm and temperature of 21 °C). The Cerrado is considered a biodiversity hotspot with high number of endemic species. It is also undergoing extreme habitat loss with > 50% of its original area used for cattle and crop production.

The dense ombrophylous forest (DO) is located in the coastal region of Paraná, southern Brazil. The climate is subtropical with an average annual temperature of 21 °C and annual rainfall of 2000–2200 mm, distributed in two seasons: a warm–rainy season from December to March (peak precipitation in January and

**Table 1** Riparian tree species providing leaf litter to this study, their life form, ecoregion and climate of origin

Tree species	No	Life form	Ecoregion	Climate
<i>Alnus glutinosa</i>	1	Deciduous	DF	Temperate
<i>Betula pendula</i>	2	Deciduous	DF	Temperate
<i>Castanea sativa</i>	3	Deciduous	DF	Temperate
<i>Ficus carica</i>	4	Deciduous	DF	Temperate
<i>Fraxinus</i> sp.	5	Deciduous	DF	Temperate
<i>Populus nigra</i>	6	Deciduous	DF	Temperate
<i>Quercus robur</i>	7	Deciduous	DF	Temperate
<i>Salix atrocinerea</i>	8	Deciduous	DF	Temperate
<i>Alchornea triplinervia</i>	9	Evergreen	SD	Subtropical
<i>Cabralea canjerana</i>	10	Deciduous	SD	Subtropical
<i>Cecropia Pachystachya</i>	11	Evergreen	SD	Subtropical
<i>Cedrela fissilis</i>	12	Deciduous	SD	Subtropical
<i>Cordia americana</i>	13	Deciduous	SD	Subtropical
<i>Ficus guaranitica</i>	14	Deciduous	SD	Subtropical
<i>Handroanthus heptaphyllus</i>	15	Deciduous	SD	Subtropical
<i>Luehea divaricata</i>	16	Deciduous	SD	Subtropical
<i>Calophyllum brasiliensis</i>	17	Evergreen	CE	Tropical
<i>Copaifera langsdorffii</i>	18	Deciduous	CE	Tropical
<i>Heteropterys anoptera</i>	19	Evergreen	CE	Tropical
<i>Hyeronima alchorneoide</i>	20	Evergreen	CE	Tropical
<i>Maprounea guianensis</i>	21	Evergreen	CE	Tropical
<i>Ormosia arborea</i>	22	Deciduous	CE	Tropical
<i>Protium spruceanum</i>	23	Deciduous	CE	Tropical
<i>Richeria grandis</i>	24	Deciduous	CE	Tropical
<i>Clusia criuva</i>	25	Evergreen	DO	Subtropical
<i>Guaraea macrophylla</i>	26	Evergreen	DO	Subtropical
<i>Inga edulis</i>	27	Deciduous	DO	Subtropical
<i>Miconia dodecandra</i>	28	Evergreen	DO	Subtropical
<i>Nectandra membranacea</i>	29	Evergreen	DO	Subtropical
<i>Quina glaziovii</i>	30	Evergreen	DO	Subtropical

DF Deciduous forest, SD Seasonal semideciduous forest, CE Cerrado, DO Dense ombrophylous forest

February: ~ 350 mm/month; 25 °C in February) and a cold–dry season from June to September (average precipitation: 80–150 mm/month; 17 °C in July) (INMET 2019). The preserved forest area is large, covering 65% of land area, and maintains great plant and animal diversity.

Freshly abscised leaves were collected from the ground in several consecutive visits to the forests and locally air-dried at room temperature for several weeks. Dried leaves were shipped to Portugal. For each species, we measured initial leaf toughness, specific leaf area and the concentration of nitrogen, phosphorus, carbon, lignin, polyphenols and tannins. For leaf toughness and specific leaf area

determinations, 10 leaf discs (12-mm diameter) were taken from individual, pre-soaked leaves, avoiding the central vein. Leaf toughness was estimated as the mass (g) needed to pierce the leaf disc using a penetrometer (Bärlocher et al. 2020). These leaf discs were oven-dried (105 °C, 48 h) and weighed to determine the specific leaf area (SLA) as the ratio of leaf disc area to its dry mass (mm<sup>2</sup>/mg).

For litter chemistry, three batches of leaves per species were ground in a Retsch ZM100 mill (Haan, Germany) to a fine powder (< 0.5 mm size), oven-dried (105 °C, 48 h) and analyzed as follow. Tannins were determined by the radial diffusion assay (Bärlocher et al. 2020), lignin by the Goering and

Van Soest method (Goering and Van Soest 1970) and polyphenols by the Folin-Ciocalteu method (Bärlocher et al. 2020). Regarding nutrients, phosphorus (P) was determined by basic digestion in the autoclave followed by the ascorbic acid method (Bärlocher et al. 2020), and nitrogen (N) and carbon (C) with a CNH autoanalyzer (IRMS Thermo Delta V advantage with a Flash EA, 1112 series; Thermo Fisher Scientific Inc., Waltham, MA, USA). All chemical parameters were expressed as % of dry mass (Bärlocher et al. 2020).

#### Leaf litter decomposition and associated macroinvertebrates

Portions of 2 g of air-dry leaves were weighed, sprayed with distilled water for softening (less susceptible to break due to handling) and enclosed in litter bags (18.5 × 13 cm). Two mesh sizes were used: fine mesh (0.5 mm) to allow microbial-driven decomposition and coarse mesh (5 mm) to allow decomposition by both microorganisms and invertebrate shredders. The largest shredders in the stream were cased caddisflies (Sericostomatidae and Limnephilidae) and blackflies (Tipulidae), all capable of passing through the 5 mm mesh and access litter in coarse mesh bags. Four leaf litter bags per mesh size and tree species were deployed in Cerdeira stream, on 9 May 2018 (240 bags in total). Although most of the annual litter inputs to local streams occur in autumn, litter production and accumulation in stream beds takes place year round (Abelho and Graça 1996) and invertebrate shredders are always present in the benthos (González and Graça 2003). Cerdeira stream is located within a deciduous forest in Lousã mountain, central Portugal (40°5' N, 8°11' W). The catchment (2.9 km<sup>2</sup>) is underlined by schist bedrock and nowadays does not have strong human activity. Stream water is circumneutral (pH: 6.5–6.8), well oxygenated (10.8 mg/L), and has low conductivity (30–32 µS/cm) and low nutrients concentration (50–76 µg N/L, 5–7 µg SRP/L) (Gulis et al. 2006; Ferreira et al. 2016a). During incubation, stream water temperature was 12.15 °C ± 0.84 (mean ± SD; HOBO Pendant UA-001–08, Onset Computer Corp., Bourne, MA, USA).

Litter bags were removed on 8 June 2018 (31 d later) and individually enclosed in plastic zip-lock bags for transport. In the laboratory, leaf litter was retrieved from the bags, gently rinsed with distilled water over a 0.5 mm mesh sieve to retain small litter

fragments and macroinvertebrates. Macroinvertebrates from coarse mesh bags were preserved in 80% ethanol for later identification and counting. Taxa richness was expressed as number of taxa/sample and abundance as number of individuals/sample. Litter was oven-dried (105 °C, 48 h) on pre-weighed aluminum pans, weighed for determination of dry mass (DM) remaining, and ignited (500 °C, 4 h). Ashes were weighed and ash-free dry mass (AFDM) remaining was determined as the difference between DM and ash mass. Results were expressed as percentage of AFDM lost: (initial AFDM–AFDM remaining)/initial AFDM × 100. The initial AFDM was estimated by multiplying the initial air DM by a conversion factor derived from extra sets of three bags for each mesh size and tree species for which AFDM was determined on day 0 as described above for the samples.

#### Aquatic hyphomycetes sporulation and community composition on leaf litter

Leaf litter portions (2 g) of the 30 tree species were enclosed in fine mesh bags (0.5 mm; 3 bags per species) and incubated in the same stream for 7 days (April 2018) to allow leaching of soluble compounds and microbial colonization. Incubated litter bags were transported to the laboratory, rinsed with distilled water and 5 discs (12-mm diameter) were cut and used to induce conidial production by aquatic hyphomycetes. Groups of 5 litter discs were allocated into 100-mL Erlenmeyer flasks with 25 mL of distilled water and incubated on an orbital shaker (100 rpm) for 48 h at 10 ± 1 °C under a 14 h light: 10 h dark photoperiod. After incubation, conidial suspensions were poured into 50-mL Falcon tubes, the flasks were rinsed twice with distilled water, the suspensions fixed with 2 mL of 37% formalin and the final volume adjusted to 30 mL with distilled water. Litter discs were saved, oven-dried (105 °C, 48 h), weighed, ignited (550 °C, 4 h) and reweighed to determine AFDM (mg) as described above.

When preparing filters for conidial identification and counting, 150 µL of 0.5% Triton X-100 were added to the suspensions and gently mixed with a magnetic stirring bar to ensure a uniform distribution of conidia. Aliquots of 10 mL were filtered through cellulose nitrate filters (25-mm diameter, 5-µm pore size; Sartorius Stedim Biotech GmbH, Göttingen,

Germany) with gentle vacuum and the filters were stained with 0.05% trypan blue in 60% lactic acid. Conidia were identified (Bärlocher et al. 2020) and counted at  $200\times$  magnification (Leica ICC50 W, Wetzlar, Germany). Aquatic hyphomycete species richness was expressed as the number of species/sample and sporulation rates as the number of conidia/mg litter AFDM/d.

#### Leaf litter consumption by shredders

From the same leaf litter used to induce sporulation, 20 additional discs were cut to measure feeding rates of shredders. As a model consumer, we used *Allogamus ligonifer* (Trichoptera, Limnephilidae) larvae, a common shredder in central Portugal. Similar-body mass larvae ( $0.017 \pm 0.008$  g; mean  $\pm$  SD) were collected in Múceres stream ( $40^{\circ}31'$  N,  $8^{\circ}11'$  W) on 7 May 2018. Specimens were placed in an aquarium with aerated stream water and mineral sediment in a temperature-controlled room set at  $10^{\circ}\text{C}$  with 14 h light: 10 h dark photoperiod for 7 days to allow acclimation to laboratory conditions. During acclimation, larvae were fed with a mixture of stream conditioned leaves.

Experimental arenas consisted of  $14.5$  cm height  $\times$   $8.5$  cm diameter plastic cups containing 200 mL of filtered stream water and 0.5 cm deep ( $\sim 27.5$  g) ignited stream sand. Each cup received one specimen and two litter discs, one disc was exposed to the shredder, while the other was enclosed in a fine mesh bag (not accessible to the larvae) and hung from the cup's edge to be used as a control for mass changes related to factors other than feeding. Cups (10 for each plant species, 300 in total) were incubated at  $10^{\circ}\text{C}$  under a 14 h light: 10 h dark photoperiod. The experiment was stopped when exposed discs from at least one species were reduced to nearly 50% of their initial area. Litter discs and uncased larvae were oven-dried ( $105^{\circ}\text{C}$ , 48 h) and weighed (mg). Consumption was calculated as the difference in mass between the control and exposed disc, divided by the larvae mass and elapsed time (g litter DM/g individual DM/d) (Graça and Cressa 2010).

#### Data analyses

The initial leaf litter physical and chemical characteristics (except tannins), and the biological parameters

associated with litter decomposition were compared (a) among the four ecoregions by one-way analysis of variance (ANOVA) followed by unequal Tukey's honest significant difference test, or by Fisher's test when Tukey's test did not identify differences among ecoregions, and (b) between evergreen and deciduous species by Student's *t*-test, or Mann–Whitney rank sum test in the case of non-normal data distribution. Tannins were compared among the four ecoregions by Kruskal–Wallis one-way ANOVA on ranks followed by Dunn's pairwise multiple comparisons. Ecoregion and life form were not crossed in the same analysis because evergreen and deciduous species were unevenly distributed across ecoregions, with the seasonal semideciduous forest having only two evergreen species and the deciduous forest having none. Pearson correlations were performed among leaf litter initial physical and chemical characteristics, leaf mass lost, macroinvertebrate and aquatic hyphomycete variables and leaf consumption rates by shredders considering all 30 species.

Variables not normally distributed (checked with Shapiro–Wilk's test) or heteroscedastic (checked with Bartlett's test) were transformed (details are given on tables with statistical results). Analyses were performed using Statistica 7.

## Results

#### Leaf litter characteristics

Leaf litter characteristics varied among tree species; differences were greater for P (range: 0.03–0.5% DM), C:P molar ratio (280–6500), N (0.4–4.6% DM) and toughness (40–291 g), and comparatively lower in terms of C (41–71% DM) and SLA (6–16 mm<sup>2</sup>/mg) (Table S1). Leaf litter toughness did not differ among ecoregions, while specific leaf area did, with values being lower for Cerrado (i.e., harder) than for seasonal semideciduous and dense ombrophylous forests (Tukey's test,  $p < 0.001$ ; Tables 2 and S2). Phosphorus concentrations were higher in seasonal semideciduous litter than in that from deciduous and dense ombrophylous forests (Tukey's test,  $p < 0.001$ ; Tables 2 and S2). Nitrogen concentrations were higher in seasonal semideciduous litter than in that from deciduous forest and Cerrado (Tukey's test,  $p < 0.001$ ; Tables 2 and S2). C:P and C:N ratios were

**Table 2** Physical and chemical characteristics of leaf litter from tree species from the four ecoregions used in the study (mean  $\pm$  SD;  $n = 8$  except for DO where  $n = 6$ )

Leaf litter characteristics	DF	SD	CE	DO	<i>p</i>
Toughness (g)	124.29 $\pm$ 31.98 <sup>a</sup>	85.13 $\pm$ 13.99 <sup>a</sup>	138.75 $\pm$ 25.98 <sup>a</sup>	98.51 $\pm$ 31.73 <sup>a</sup>	0.167
Specific leaf area (mm <sup>2</sup> /mg)	12.07 $\pm$ 5.52 <sup>ab</sup>	13.24 $\pm$ 2.07 <sup>b</sup>	9.55 $\pm$ 1.83 <sup>a</sup>	14.46 $\pm$ 7.15 <sup>b</sup>	< 0.001
Phosphorus (% DM)	0.16 $\pm$ 0.08 <sup>a</sup>	0.38 $\pm$ 0.12 <sup>b</sup>	0.21 $\pm$ 0.04 <sup>ab</sup>	0.06 $\pm$ 0.03 <sup>a</sup>	< 0.001
Nitrogen (% DM)	1.17 $\pm$ 0.22 <sup>ab</sup>	2.34 $\pm$ 0.27 <sup>c</sup>	0.76 $\pm$ 0.24 <sup>ab</sup>	2.21 $\pm$ 0.18 <sup>bc</sup>	< 0.001
Carbon (% DM)	50.30 $\pm$ 3.3 <sup>a</sup>	53.38 $\pm$ 6.08 <sup>a</sup>	54.38 $\pm$ 10.6 <sup>a</sup>	54.30 $\pm$ 4.21 <sup>a</sup>	0.438
C:P (molar ratio)	2763 $\pm$ 3198 <sup>a</sup>	525 $\pm$ 273 <sup>b</sup>	841 $\pm$ 339 <sup>ab</sup>	3819 $\pm$ 2260 <sup>a</sup>	< 0.001
C:N (molar ratio)	70.30 $\pm$ 11.68 <sup>a</sup>	31.15 $\pm$ 2.51 <sup>b</sup>	86.83 $\pm$ 11.9 <sup>ab</sup>	32.64 $\pm$ 2.31 <sup>a</sup>	< 0.001
Lignin (% DM)	31.77 $\pm$ 1.03 <sup>a</sup>	32.24 $\pm$ 0.93 <sup>a</sup>	36.70 $\pm$ 0.96 <sup>a</sup>	40.35 $\pm$ 1.53 <sup>a</sup>	0.169
Lignin:N (molar ratio)	36.85 $\pm$ 0.04 <sup>bc</sup>	16.85 $\pm$ 0.06 <sup>a</sup>	52.35 $\pm$ 0.02 <sup>c</sup>	19.31 $\pm$ 0.07 <sup>ab</sup>	< 0.001
Polyphenols (% DM)	9.62 $\pm$ 1.70 <sup>a</sup>	1.98 $\pm$ 0.80 <sup>b</sup>	5.05 $\pm$ 1.68 <sup>b</sup>	5.98 $\pm$ 0.84 <sup>b</sup>	< 0.001
Tannins <sup>a</sup> (%)	0.25 $\pm$ 0.03 <sup>a</sup>	0.08 $\pm$ 0.03 <sup>a</sup>	0.60 $\pm$ 0.18 <sup>b</sup>	*	0.007

DM dry mass, C carbon, P phosphorus, N nitrogen, DF Deciduous forest, SD Seasonal semideciduous forest, CE Cerrado, DO Dense ombrophylous forest

Ecoregions with the same letter do not significantly differ (one-way ANOVA followed by Tukey's test,  $p \geq 0.050$ , or Kruskal–Wallis one-way ANOVA on ranks for tannins followed by Dunn's pairwise multiple comparisons)

\*Below detection limit

<sup>a</sup>Tannins as tannic acid equivalent

lower in litter from semideciduous forest than in that from dense ombrophylous and deciduous forests (Tukey's test,  $p < 0.001$ ; Tables 2 and S2). Lignin:N ratios were higher in litter from deciduous forest and Cerrado than in that from seasonal semideciduous and dense ombrophylous forest (Tukey's test,  $p < 0.001$ ; Tables 2 and S2). Polyphenol concentrations were higher in litter from deciduous forest than in that from the other ecoregions (Tukey's test,  $p < 0.001$ ; Tables 2 and, S2), while tannins were higher in litter from Cerrado than in that from the other ecoregions (rank ANOVA,  $p = 0.007$ ). No significant differences were found for C and lignin concentrations among ecoregions (Tables 2 and S2). Deciduous and evergreen tree species did not differ in litter characteristics (Table 3).

### Leaf litter decomposition

After 30 days of incubation, leaf litter mass lost across tree species varied between 3 and 91% of initial AFDM for fine mesh bags and between 4 and 98% of initial AFDM for coarse mesh bags (Fig. 1; Table S3). There were differences in decomposition among ecoregions for fine and coarse mesh bags (one-way ANOVA,  $p < 0.001$ ; Table S4). Litter from deciduous

forest decomposed faster than that from the other ecoregions, in both mesh sizes (Fisher's test,  $p < 0.001$ ; Fig. 2a, c), and litter from semideciduous forest decomposed faster than that from dense ombrophylous forest in fine mesh bags (Fisher's test,  $p = 0.003$ ; Fig. 2a). Litter mass lost was significantly higher for deciduous than for evergreen species, in both mesh sizes (Student's *t*-test,  $p = 0.018$  and  $p = 0.031$ , respectively; Fig. 2b, d).

### Macroinvertebrates on leaf litter

Macroinvertebrate taxa richness and abundance in leaf litter across tree species varied between 0 and 8 taxa/sample and between 0 and 13 individuals/sample, respectively (Table S3). There were no differences in macroinvertebrate taxa richness and abundance among litter from the four ecoregions (one-way ANOVA,  $p = 0.682$  and  $p = 0.518$  respectively; Table S4; Fig. 3a, c). However, macroinvertebrate abundance, but not taxa richness (Student's *t*-test,  $p = 0.056$ ), was higher on deciduous than on evergreen species ( $p = 0.019$ ) (Fig. 3b, d).

**Table 3** Physical and chemical characteristics of leaf litter from the deciduous ( $n = 19$ ) and evergreen ( $n = 11$ ) tree species used in the study (mean  $\pm$  SD)

Leaf litter characteristics	Deciduous	Evergreen	$p$
Toughness (g)	122.18 $\pm$ 24.56	95.92 $\pm$ 27.21	0.366 (m)
Specific leaf area (mm <sup>2</sup> /mg)	12.40 $\pm$ 4.48	11.83 $\pm$ 3.02	0.698 (m)
Phosphorus (% DM)	0.23 $\pm$ 0.08	0.18 $\pm$ 0.05	0.316 (t)
Nitrogen (% DM)	1.41 $\pm$ 0.23	1.88 $\pm$ 0.23	0.224 (t)
Carbon (% DM)	51.29 $\pm$ 5.22	56.68 $\pm$ 7.81	0.575 (t)
C:P (molar ratio)	1710.55 $\pm$ 1772.08	2131.76 $\pm$ 942.63	0.282 (m)
C:N (molar ratio)	58.73 $\pm$ 6.77	53.29 $\pm$ 8.57	0.665 (t)
Lignin (% DM)	33.96 $\pm$ 1.09	36.60 $\pm$ 1.08	0.518 (m)
Lignin:N (molar ratio)	32.48 $\pm$ 0.05	31.57 $\pm$ 0.05	0.906 (t)
Polyphenols (% DM)	6.27 $\pm$ 1.24	4.54 $\pm$ 1.36	0.458 (m)
Tannins <sup>a</sup> (% DM)	0.18 $\pm$ 0.02	0.38 $\pm$ 0.14	0.505 (m)

DM dry mass, C carbon, P phosphorus, N nitrogen

Comparisons were made by Student's  $t$ -test (t) or Mann–Whitney (m) in the case of non-normal data distribution, and  $p$  values are shown

<sup>a</sup>Tannins as tannic acid equivalent

#### Aquatic hyphomycetes sporulation and community composition on leaf litter

We identified 24 species of aquatic hyphomycetes; species richness in leaf litter varied between 1 and 13 species/sample across tree species (Tables S3 and S5). Species richness differed among litter from the different ecoregions (one-way ANOVA,  $p < 0.001$ ; Table S4), with higher values for deciduous and seasonal semideciduous forests than for Cerrado and dense ombrophylous forest (Fig. 4a). Aquatic hyphomycetes species richness also differed between litter from evergreen and deciduous tree species (Mann–Whitney Rank Sum Test,  $p = 0.037$ ), with higher values for deciduous species (Fig. 4b).

The most frequent aquatic hyphomycete species in leaf litter were *Neonectria lugdunensis* (in 67% tree species), *Flagellospora curvula* (53%), *Anguillospora filiformis* (47%), *Articulospora tetracladia* (43%), *Lemonniera aquatica* (43%), and *Tetrachaetum elegans* (40%) (Table S5). Besides being frequent, these species also contributed the most to conidial production (at least 20% of conidial production in litter from at least one tree species), except for *A. tetracladia* that contributed with up to 5% of conidial production across tree species.

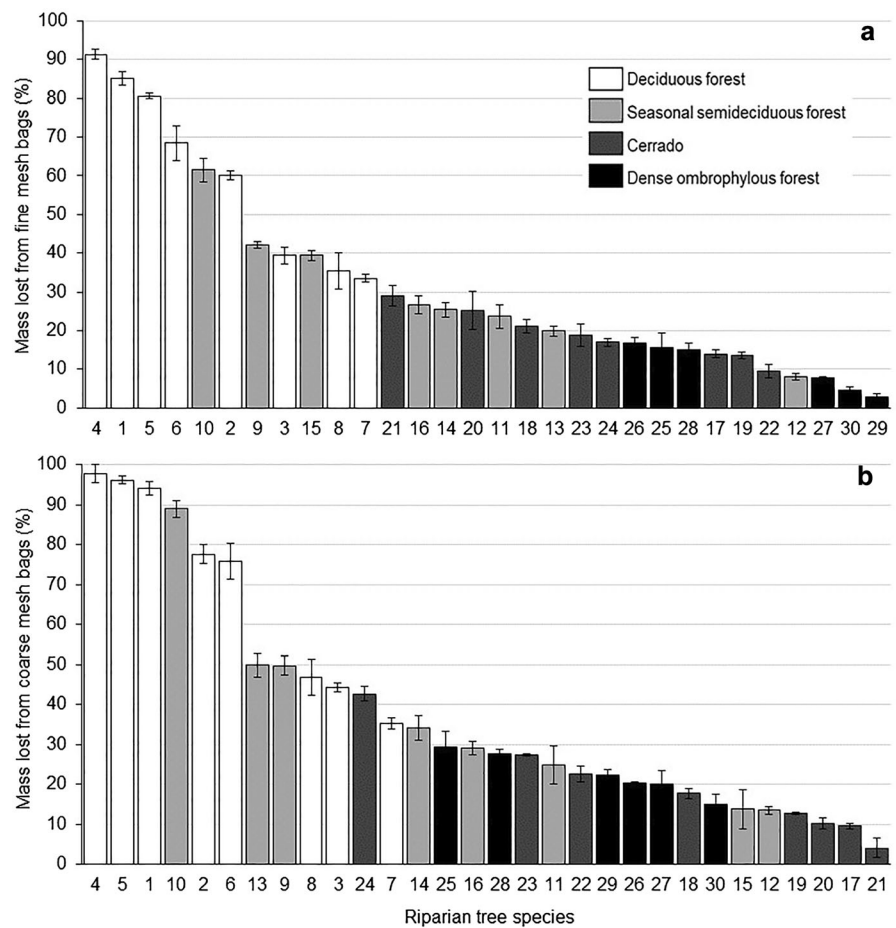
Sporulation rates by aquatic hyphomycetes varied between 0 and 435 conidia/mg litter AFDM/d across tree species (Table S3) and differed among litter from the four ecoregions (one-way ANOVA,  $p < 0.001$ ; Table S4), with higher values for litter from deciduous and seasonal semideciduous forests than for that from Cerrado and dense ombrophylous forest (Fisher's test,  $p \leq 0.025$ ) (Fig. 4c). Sporulation rates were also higher on deciduous than on evergreen species (Student's  $t$ -test,  $p = 0.016$ ) (Fig. 4d).

#### Leaf litter consumption by shredders

Leaf litter consumption by *A. ligonifer* ranged from 0.007 to 0.140 g litter DM/g individual/d across tree species (Table S3). Consumption rates differed among litter from the four ecoregions (one-way ANOVA,  $p = 0.044$ ; Table S4), being higher for litter from deciduous than for that from semideciduous forest and Cerrado (Fisher's test,  $p \leq 0.025$ ) (Fig. 5a). Consumption rates did not differ between evergreen and deciduous species (Student's  $t$ -test,  $p = 0.546$ ) (Fig. 5b).



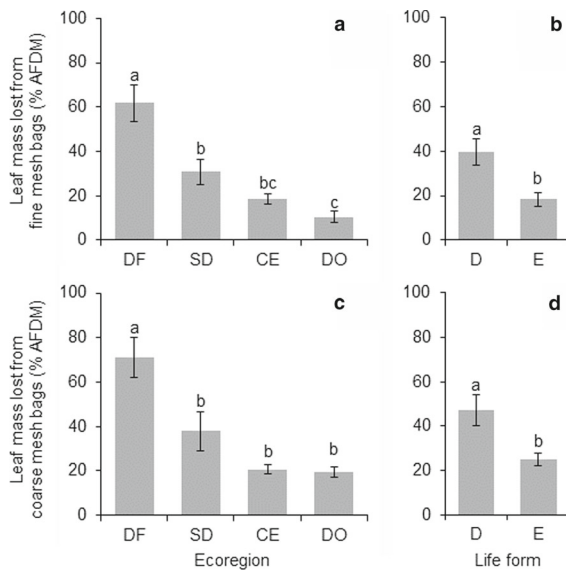
**Fig. 1** Mass lost from fine (a) and coarse (b) mesh bags for leaf litter from 30 tree species incubated in Cerdeira stream for 30 days (May 2018) (mean ± SE). Numbers identify tree species (see Table 1)



**Determinants of leaf litter decomposition**

Decomposition, in terms of percentage of leaf litter mass lost, was negatively correlated with lignin concentration (Pearson correlation,  $r = -0.59$  and  $p < 0.001$  for fine mesh bags,  $r = -0.40$  and  $p = 0.030$  for coarse mesh bags), lignin:N ratio ( $r = -0.63$  and  $p < 0.001$  for fine mesh bags,  $r = -0.53$  and  $p = 0.002$  coarse mesh bags) and tannins concentration ( $r = -0.38$  and  $p = 0.038$  for coarse mesh bags) (Table S6). Additionally, percentage of leaf litter mass lost was positively correlated with aquatic hyphomycetes species richness (Pearson correlation,  $r = 0.75$  and  $p = 0.016$  for fine mesh bags,  $r = 0.60$  and  $p < 0.001$  for coarse mesh bags), sporulation rates ( $r = 0.84$  and  $p < 0.001$  fine mesh bags,  $r = 0.65$  and  $p < 0.001$  for coarse mesh bags) and leaf consumption rates by shredders under laboratory conditions ( $r = 0.61$  and  $p < 0.001$  for coarse mesh

bags) (Table S6). Leaf litter mass lost in fine and coarse mesh bags were also positively correlated (Pearson correlation,  $r = 0.80$  and  $p = 0.002$ ) (Table S6). Aquatic hyphomycetes species richness was negatively correlated with lignin concentration (Pearson correlation,  $r = -0.56$  and  $p < 0.001$ ) and lignin:N ratio ( $r = -0.62$  and  $p < 0.001$ ) and positively correlated sporulation rates ( $r = 0.93$  and  $p < 0.001$ ) (Table S6). Sporulation rates were negatively correlated with lignin concentration (Pearson correlation,  $r = -0.58$  and  $p = 0.001$ ) and lignin:N ratio ( $r = -0.66$  and  $p < 0.001$ ) (Table S6). Leaf consumption rates by shredders were negatively correlated with lignin concentration (Pearson correlation,  $r = -0.42$  and  $p = 0.022$ ) and positively correlated with aquatic hyphomycete species richness ( $r = 0.46$  and  $p = 0.010$ ) and sporulation rates ( $r = 0.54$  and  $p = 0.002$ ) (Table S6).



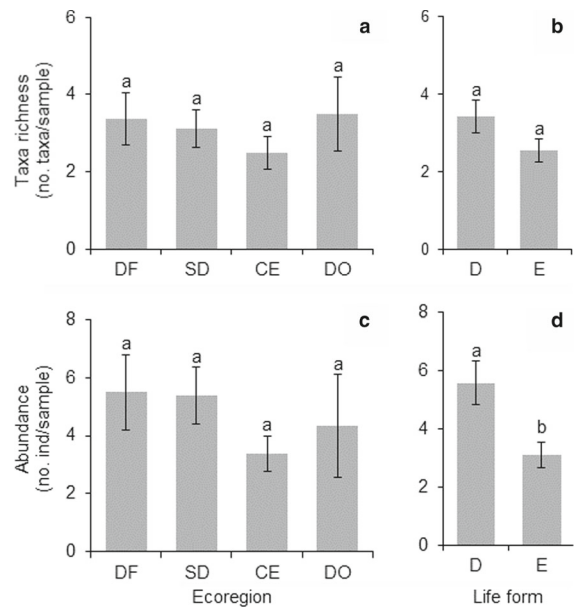
**Fig. 2** Leaf litter mass lost from tree species from four ecoregions and two life forms enclosed in fine mesh (0.5 mm; **a**, **b**) and in coarse mesh bags (5 mm; **c**, **d**) and incubated in Cerdeira stream for 30 days (May 2018) (mean  $\pm$  SE;  $n = 8$ , except for DO where  $n = 6$ ;  $n = 19$  for D and  $n = 11$  for E). DF, Deciduous forest; SD, Seasonal semideciduous forest; CE, Cerrado; DO, Dense ombrophylous forest; D, deciduous; E, evergreen. The same letter indicates no significant differences ( $p \geq 0.050$ ; one-way ANOVA followed by Fisher's test for ecoregion and Student's t-test for life forms)

## Discussion

We found that (a) leaf litter characteristics differed among the four tested ecoregions. We also found that (b) leaf litter decomposition and associated biological decomposers colonization and activity differed among ecoregions, with values being generally higher for litter from the temperate deciduous forest, followed by litter from the semideciduous forest, and lower for litter from the Cerrado and dense ombrophylous forest. We also found (c) higher decomposition and decomposers colonization and activity for leaf litter from deciduous than evergreen trees. Finally, (d) we identified key leaf litter chemical characteristics explaining the differences, as discussed below.

### Leaf litter characteristics differed among ecoregions

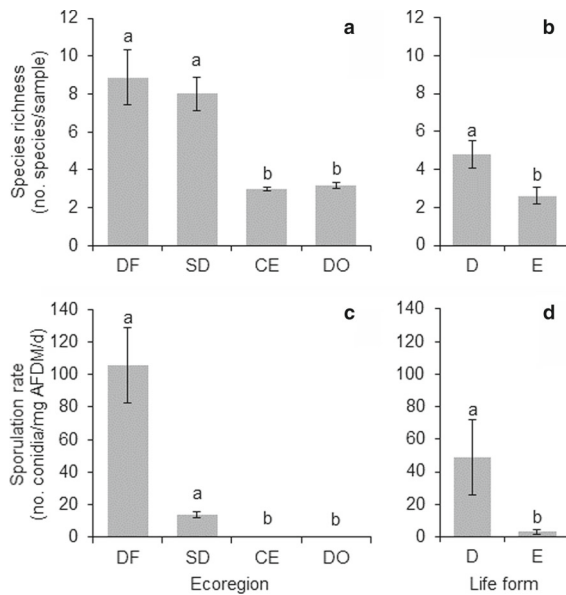
As predicted, we found significant differences in leaf litter characteristics among ecoregions. As expected, litter from tropical Cerrado had low quality, resulting



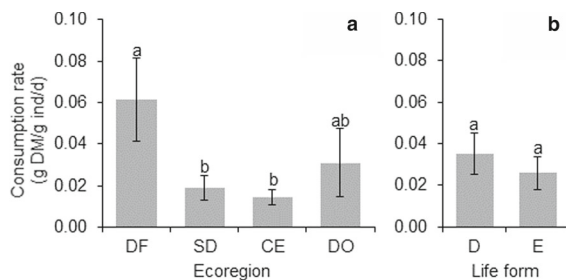
**Fig. 3** Taxa richness (**a**, **b**) and abundance (**c**, **d**) of macroinvertebrates associated with leaf litter of tree species from four ecoregions and two life forms enclosed in coarse mesh bags and incubated in Cerdeira stream for 30 days (May 2018) (mean  $\pm$  SE;  $n = 8$ , except for DO where  $n = 6$ ;  $n = 19$  for D and  $n = 11$  for E). DF, Deciduous forest; SD, Seasonal semideciduous forest; CE, Cerrado; DO, Dense ombrophylous forest; D, deciduous; E, evergreen. The same letter indicates no significant differences ( $p \geq 0.050$ ; one-way ANOVA followed by Fisher's test for ecoregion and Student's t-test for life forms)

from their lowest SLA and N concentration, and highest C:N and lignin:N ratios and tannin concentration. The Cerrado is characterized by low water availability and nutrient-poor soils (Ruggiero et al. 2002). Such stressing conditions favor plants with high investment in defenses against herbivory and water losses (Coley and Baronen 1996; Silva and Batalha 2011b). Such defenses may remain active against microbial and invertebrate consumption after senescence (Grime et al. 1996).

Surprisingly, leaf litter from the subtropical semideciduous forest had high quality, resulting from their tendency to show high SLA, the highest P and N concentrations, and the lowest C:P, C:N, lignin:N, polyphenolics and tannin concentrations. Our findings are consistent with the reported higher litter quality for semideciduous forests than Cerrado, which was attributed to differences in soil nutrients concentrations, which are higher in the former than the latter ecoregion (Miatto and Batalha 2016).



**Fig. 4** Species richness (a, b) and sporulation rate (c, d) of aquatic hyphomycetes associated with leaf litter of tree species from four ecoregions and two life forms enclosed in fine mesh bags and incubated in Cerdeira stream for 7 days (April 2018) (mean  $\pm$  SE;  $n = 8$ , except for DO where  $n = 6$ ;  $n = 19$  for D and  $n = 11$  for E). DF, Deciduous forest; SD, Seasonal semideciduous forest; CE, Cerrado; DO, Dense ombrophylous forest; D, deciduous; E, evergreen. The same letter indicates no significant differences ( $p \geq 0.050$ ; one-way ANOVA followed by Fisher's test for ecoregion and Student's t-test for life forms)



**Fig. 5** Consumption rates of leaf litter of tree species from four ecoregions (a) and two life forms (b) by the shredder *Allogamus lignifer* in a laboratory experiment (mean  $\pm$  SE;  $n = 8$ , except for DO where  $n = 6$ ;  $n = 19$  for D and  $n = 11$  for E). DF, Deciduous forest; SD, Seasonal semideciduous forest; CE, Cerrado; DO, Dense ombrophylous forest; D, deciduous; E, evergreen. The same letter indicates no significant differences ( $p \geq 0.050$ ; one-way ANOVA followed by Fisher's test for ecoregion and Student's t-test for life forms)

Unexpectedly, leaf litter from the temperate deciduous forest did not globally differ from litter from the Cerrado or dense ombrophylous forest. This contrasts with previous reports. For instance, Graça and Cressa

(2010) found tropical litter (from a seasonal forest) to have higher toughness than temperate litter, and Boyero et al. (2017) found a linear decrease in litter N:P ratio and tannin concentration and an increase in SLA with latitude.

Therefore, the results only partially agreed with our first prediction: there were indeed differences in the measured leaf litter characteristics among ecoregions, but these differences do not allow to say that litter is more recalcitrant for tropical/subtropical than temperate species. In the same way, unlike our prediction, litter from deciduous and evergreen species did not differ in any of the measured litter characteristics. Our findings should be interpreted with caution since leaf litter characteristics of the tested species may reflect both genotypic and environmental factors that influence phenotypic expression (e.g., nutrient concentration of leaves and leaf litter reflects soil nutrient availability; Biasi et al. 2017). Nevertheless, leaf litter originated from trees in their typical habitats.

Leaf litter from temperate deciduous forest decomposed faster than litter from subtropical and tropical species

We incubated leaf litter from 30 tree species originating from four different ecoregions under identical environmental conditions to investigate the relative importance of litter characteristics in controlling litter decomposition rates. We predicted that leaf litter decomposition and associated decomposers would differ among ecoregions with lower decomposer colonization and activity and, consequently, slower litter decomposition, on litter from tropical and subtropical regions than from the temperate deciduous forest. Our findings were partially consistent with our prediction: decomposition was faster for leaf litter from temperate deciduous forest than from the other three ecoregions, but litter from deciduous and seasonal semideciduous forests were similar in terms of aquatic hyphomycete and invertebrate colonization.

The observed higher colonization and activity of aquatic hyphomycetes and faster decomposition of leaf litter from the temperate deciduous forest could be attributed predominantly to lignin: microbial variables and litter decomposition were negatively correlated with litter lignin concentration and lignin:N ratio. These findings agree with previous studies that found lignin concentration to be an important factor

controlling decomposer colonization and activity on litter and, consequently, determining litter decomposition (Schindler and Gessner 2009; Jabiol et al. 2019). High lignin concentration may provide low-quality carbon or bind to nutrients making them unavailable (Jabiol et al. 2019). In fact, litter lignin concentrations may be a more important determinants of litter decomposition than litter nutrients concentrations if microbes can supply their needs by up taking nutrients from the stream water (Ferreira et al. 2016a; Jabiol et al. 2019).

Nevertheless, we also need to consider that other factors could be driving litter decomposition. Litter from the semideciduous forest was of apparently high quality (i.e., low toughness, high nutrients concentrations, low C:nutrient and lignin:N ratios, and low polyphenols and tannin concentrations), but still its decomposition was significantly slower than that of litter from the temperate deciduous forest (although generally no significant differences were found for decomposer colonization between deciduous and semideciduous ecoregions). Other authors found that micronutrients such as Mg, Mn and Ca correlated with decomposition rates and that tropical litter has lower Mg and Ca concentrations and higher Na concentration (and high condensed tannins and toughness related measurements) than temperate litter (Makkonen et al. 2012; García-Palacios et al. 2016; Vivanco and Austin 2019). We did not measure Mg, Ca or Na; however these micronutrients are essential for invertebrates and/or important in fungal enzymatic activity, stimulating litter decomposition (Hill et al. 2004; Maller et al. 2013; Li et al. 2020; Yue et al. 2020) and they should be considered in future research.

The faster decomposition of temperate deciduous litter could also result from home-field advantage as litter incubation took place in a single, temperate stream where decomposers are naturally exposed to litter from the deciduous forest, while litter from the other three ecoregions would be 'exotic' (Hunt et al. 1988; Strickland et al. 2009; Yeung et al. 2019). This is plausible and should be further investigated with simultaneous reciprocal experiments in which litter decomposition rates of local tree species are compared with those of species from other systems. The few studies that have so far explicitly addressed the home-field advantage hypothesis to explain litter decomposition in streams have found limited support for it (Fenoy et al. 2016; Yeung et al. 2019; Fugère et al.

2020). Also, a recent meta-analysis showed that overall leaf litter decomposition rates were similar for native and exotic species, although litter from exotic species decomposed faster than that of native species at warm-water streams and when litter was incubated in coarse mesh bags (allowing for the presence of macroinvertebrates), but not in cold-water streams and when litter was incubated in fine mesh bags (Kennedy and El-sabaawi 2017). Additionally, studies in terrestrial ecosystems revealed the same species ranking in litter decomposition at different environments (different seasons: Cornelissen et al. 1996; different climates: Cornelissen et al. 1999; Perez-Harguindeguy et al. 2000; different biomes: Makkonen et al. 2012), suggesting that decomposers may not have a preference for 'native' litter species and that litter characteristics moderating litter decomposition are mostly the same across environmental conditions. The extrapolation of this rationale to stream ecosystems is supported by García-Palacios et al. (2016) who found strong relationships between litter characteristics (i.e., elemental composition and ratios) and litter decomposition of local species across biomes for both terrestrial and stream ecosystems, and Yue et al. (2018) who found the same species ranking in litter decomposition in streams and terrestrial ecosystems. Also, most aquatic hyphomycete species are cosmopolitan (Duarte et al. 2016), and many species commonly found in temperate regions (and in the study stream) are also found in Brazilian streams (Gomes et al. 2016; Biasi et al. 2020). Thus, the lower microbial colonization of leaf litter from tropical and subtropical ecoregions is not probably a result from local aquatic hyphomycete species rejecting non-local litter.

Litter incubation in the stream was allowed for 30 days, which was obviously a short period for some species (15 species lost < 25% initial mass), but it allowed a comparison between the range of species considered that had decomposition rates from very slow to very fast (6 species lost > 75% initial mass). Longer incubation periods would have resulted in a complete litter mass loss for the fast decomposing species with concomitant decrease in the resolution at this end of the species decomposition spectrum. Also, Cornelissen et al. (1996) found the same species ranking in litter decomposition at different incubation durations in the soil (8 weeks: 15–48% mass loss, 12 weeks: 25–74% mass loss), suggesting that litter

characteristics moderating litter decomposition are the same over its decomposition process, even though litter characteristics change over it (García-Palacios et al. 2016). The species ranking we found in litter decomposition after 30 days is, thus, likely to have been maintained if a longer incubation period had been used.

Faster leaf litter decomposition for deciduous than evergreen species

As predicted, litter decomposition and associated decomposers differed between plant life forms with lower decomposer colonization and activity and, consequently, slower litter decomposition, on evergreen than on deciduous species in agreement with previous studies (Cornelissen et al. 1996; Cornwell et al. 2008). However, contrary to previous studies, differences in biotic colonization and decomposition of leaf litter between life forms cannot be attributed to differences in the measured litter characteristics between deciduous and evergreen species. Evergreen species predominate in our species sample from the subtropical dense ombrophylous forest (5/6) and the tropical Cerrado (4/8). This predominance may partially explain the lower litter mass lost, lower aquatic hyphomycete species richness and sporulation rates compared with litter from the subtropical semideciduous and the temperate deciduous forests. The reasons for the differences in litter decomposition between temperate deciduous versus tropical and subtropical, and between deciduous and evergreen may lay on unmeasured leaf litter characteristics including micro nutrients referred above. This subject needs further investigation.

## Conclusion

Leaf litter decomposition potential and thus, nutrient cycling, was faster for temperate deciduous species, which tended to support higher microbial colonization and activity and higher invertebrate feeding than tropical and subtropical species. Litter lignin concentration and lignin:N ratio seem to be the moderators of litter decomposition. This suggests that litter decomposition in tropical and subtropical streams may be limited by low litter quality (i.e., high lignin concentration and lignin:N ratio). The low carbon quality of

tropical and subtropical litter may further limit the possibility that microbes take advantage from dissolved nutrients, which are, nevertheless, at low concentrations in many streams of these regions.

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