UNIVERSIDADE DE BRASÍLIA

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

DETERMINAÇÃO DE CARACTERÍSTICAS FITOQUÍMICAS E A DECOMPOSIÇÃO FOLIAR EM RIACHOS TROPICAIS

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TESE DE DOUTORADO

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Ao meu pai Nilson,
aos meus avós Dona Glorinha e Seu Elísio
e à minha sobrinha Dulcy.

"Se eu vi mais longe,
foi por estar sobre ombros de gigantes."

Issac Newton

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CAPÍTULO 3 – LITTER TRAITS AND STREAM FLOW DRIVE LEAF BREAKDOWN IN TROPICAL STREAMS

RESUMO

O estudo do processo de decomposição do detrito foliar de origem alóctone é fundamental para a compreensão do fluxo de energia e manutenção do metabolismo em riachos florestados. O dossel da floresta ripária reduz a entrada de luz nos riachos e, consequentemente, a sua produtividade primária. Dessa forma, a principal fonte de energia para os riachos florestados vem majoritariamente da entrada e posterior decomposição de detritos foliares oriundo das florestas ripárias. Os detritos foliares que adentram nestes ecossistemas podem ser retidos e acumulados no leito dos riachos onde sofrem lixiviação de compostos solúveis em água e são colonizados por micro-organismos. Por fim, os invertebrados fragmentam as folhas para utilizá-las como recurso alimentar e/ou abrigo, aumentando drasticamente as taxas de decomposição foliar, mostrando a importância dos fragmentadores para o funcionamento dos riachos. No **Capítulo 1** aborda atributos físicos e químicos de detritos foliares de espécies de florestas ripárias da Amazônia, da Mata Atlântica e do Cerrado. O **Capítulo 2** tratou da co-limitação da quantidade e da qualidade dos detritos foliares para a biomassa de fragmentadores em um riacho amazônico. O **Capítulo** 3 relatou a influência de detritos foliares de origens e características físicas e químicas distintas e a decomposição em riachos tropicais.

Palavras-chave: Matéria orgânica; neotropical; decomposição foliar; ciclagem de nutrientes; funcionamento do ecossistema.

ABSTRACT

The study of the decomposition process of allochthonous leaf litter is essential for understanding the energy flow and metabolism maintenance in forested streams. The riparian forest canopy reduces the entry of light into streams and, consequently, their primary productivity. Thus, the main source of energy for forested streams comes from the entry and subsequent decomposition of leaf litter from riparian forests. The leaf litter that enters these ecosystems can be retained and accumulated in the streambed where they suffer leaching of water-soluble compounds and are colonized by micro-organisms. Finally, invertebrates fragment the leaves to use them as a food resource and/or shelter, drastically increasing the rates of leaf decomposition, showing the importance of the shredders for the functioning of the streams. In **Chapter 1**, addresses physical and chemical attributes of leaf debris from riparian forest species in the Amazon, Atlantic Forest and Cerrado. **Chapter 2** dealt with co-limitation of quantity and quality of leaf litter for shredder biomass in an Amazonian stream. **Chapter 3** reported the influence of leaf litter of different origins and physical and chemical characteristics and decomposition in tropical streams.

Keywords: Organic matter; neotropical; leaf decomposition; nutrient cycling; ecosystem functioning.

INTRODUÇÃO GERAL

Os riachos florestados são as menores seções de uma bacia hidrográfica, entretanto, representam em grande parte a bacia de drenagem em termos de extensão, fluxo de água, ciclagem de nutrientes, sedimentos e material orgânico (Cummins, 1977). Em particular, os riachos florestados são fortemente influenciados pela vegetação ripária adjacente, que reduz a produção autótrofa pelo sombreamento e contribui amplamente na entrada de matéria orgânica alóctone (Vannote et al., 1980; Webster e Meyer, 1997). Aproximadamente 90% do material vegetal produzido em florestas ripárias entram nesses riachos diretamente (aporte vertical) ou indiretamente (aporte lateral) na forma de matéria orgânica morta (Tank et al., 2010). Dentro do riacho, a matéria orgânica pode ser transportada a jusante ou retida em rochas ou troncos, formando o estoque bêntico (Tank et al., 2010; Rezende et al., 2016). Esta matéria orgânica é constituída majoritariamente por detritos foliares, tornando uma das principais fontes de energia para comunidades heterotróficas através de sua decomposição (Esteves e Gonçalves, 2011; Bambi et al, 2017; Marks, 2019).

O estudo do processo de decomposição do detrito foliar de origem alóctone (oriundo das florestas ripárias) é fundamental para a compreensão do fluxo de energia e manutenção do metabolismo em riachos florestados (Benfield, 1997; Wallace et al., 1997). O modelo tradicional de decomposição de detritos foliares enfatiza os seguintes eventos: lixiviação de componentes químicos, colonização microbiana e colonização por invertebrados. Estes eventos não devem ser vistos de forma sucessiva, mas como eventos que ocorrem concomitantemente e interagem durante todo o processo de decomposição (Gessner et al., 1999). A lixiviação de compostos químicos solúveis em água ocorre pela ação da abrasão física da água, que pode ter uma grande influência na perda de massa dos detritos devido ao fluxo da água (Nuven et al., 2022). Os microrganismos são

representados principalmente por fungos (em particular hifomicetos aquáticos) e bactérias (Gessner et al., 1999) e desempenham um papel essencial no condicionamento de detritos antes que sejam utilizados por fragmentadores (Gessner e Chauvet, 1994). Os hifomicetos aquáticos produzem as enzimas que degradam os principais polissacarídeos dos tecidos foliares (Suberkropp e Chauvet 1995; Graça et al., 2001) tornando o detrito foliar mais palatável para os invertebrados fragmentadores (Suberkropp e Chauvet, 1995). Além destes compartimentos tradicionais da decomposição, outros fatores bióticos e abióticos que são dependentes da escala também agem na decomposição de detritos foliares (Graça et al., 2015; Marks, 2019). O processo de decomposição foliar em riachos florestados pode ser regido também pelas interações entre fatores do clima e do solo que influenciam as características das plantas, a disponibilidade de detritos foliares, a química e o fluxo da água dos riachos e a diversidade e o metabolismo de fragmentadores e decompositores microbianos (Reich e Oleksyn 2004, Graça et al., 2015; Tonin et al., 2017).

A absorção de nutrientes pelas plantas é um processo complexo que envolve a absorção de nutrientes essenciais majoritariamente oriundos do solo pelas raízes, em seguida do transporte por toda a planta e incorporação nos tecidos vegetais para seu crescimento e desenvolvimento (Reichardt e Timm, 2020). Características do solo (ex: textura, composição química e acidez) juntamente com o clima e o metabolismo vegetal influenciam as concentrações nutricionais nos tecidos das plantas (Weiher et al., 1999; Ordoñez et al., 2009). O *trade-off* entre as variações ambientais e os processos metabólicos vegetais produzem um espectro de características físicas e químicas das plantas: em um extremo deste espectro estão as características vegetais mais associadas à aquisição de recursos e, no outro, as características mais associadas à manutenção desses recursos (Wright et al. 2004). Por exemplo, plantas oriundas de solos pobres em nutrientes

tem geralmente menos concentração nutricional em seus tecidos e maior investimento em defesa contra a herbivoria (Haridasan, 2008; Miatto et al., 2016), enquanto em solos ricos em nutrientes as plantas tendem a possuir maior concentração de nutrientes nos tecidos e maior investimento em crescimento vegetal (Reich e Oleksyn 2004; Wright et al. 2004). Assim, riachos florestados que percorrem solos pobres em nutrientes provavelmente serão dominados por detritos foliares com menor concentração de nutrientes e maior concentração de compostos recalcitrantes (ex: lignina, celulose e fenóis) (Boyero et al., 2017), pois algumas das características físicas e químicas de folhas verdes são conservadas em folhas senescentes (Killingbeck, 1996), que se tornam detritos foliares posteriormente. Além das características do solo, a composição e distribuição das espécies de plantas nos ecossistemas terrestres também são determinados pelo clima (Reich e Oleksyn 2004; Wright et al. 2004), isto resulta em um gradiente latitudinal de características dos detritos foliares de florestas ripárias em escala global, com mais detritos de maior recalcitrância em direção às zonas tropicais (Boyero et al., 2017).

Tais características físicas e químicas intrínsecas dos detritos foliares são um dos principais controladores da atividade de decompositores microbianos e da fragmentação por invertebrados, e consequentemente, da decomposição de detritos foliares em riachos florestados (Cornwell et al., 2008; Boyero et al., 2016; Zhang et al., 2019). Estas características dos detritos foliares se referem a um conjunto de atributos químicos e morfológicos intrínsecos, comumente referido como 'qualidade do detrito', no qual detritos foliares de maior qualidade (ex: detritos com maiores concentrações de nutrientes e menores teores fenólicos e lignina) oferecem maior palatabilidade e valor nutricional com menos toxicidade para animais detritívoros e decompositores microbianos do que detritos de qualidade inferior (ex: detritos mais duros e com maior concentração de compostos recalcitrantes) (Gessner et al., 1999; García-Palacios et al., 2015, Graça et al.,

2015). A lignina e a celulose são constituintes estruturais essenciais das paredes celulares das plantas (Gessner, 2020). Juntas fornecem rigidez e resistência às paredes celulares vegetais, permitindo que elas resistam ao estresse mecânico, protegendo contra patógenos e herbívoros e mantendo sua integridade estrutural (Gessner, 2020). Consequentemente, os detritos foliares ricos destes compostos tendem a ser altamente refratários, conduzindo à lenta decomposição (Graça et al., 2015; Zhang et al., 2019). Além desses compostos estruturais, os compostos secundários, compostos fenólicos, também podem estar presentes nos detritos foliares. Os compostos fenólicos são um grupo de metabólitos secundários das plantas que são produzidos como defesa química contra insetos, radiação ultravioleta e infecções microbianas (Lill e Marquis 2001; Gould e Lee 2002) e podem permanecer na folha após a senescência e afetar negativamente a colonização dos organismos decompositores (Bärlocher e Graça, 2005; Moretti et al., 2009). Ademais, os tecidos foliares e, consequentemente, os detritos contém quantidades variáveis de nitrogênio (N), fósforo (P) e outros macronutrientes essenciais necessários para o crescimento das plantas (Reichardt e Timm, 2020), estimulando também a taxa de decomposição (Graça et al., 2015), pois são elementos igualmente indispensáveis para o metabolismo dos organismos decompositores (Madan e Thind, 1998; Gessner et al., 1999). Além desses atributos físicos e químicos, as razões elementares (principalmente C:N, C:P e N:P) dos detritos foliares tem sido proposta para prever a limitação de nutrientes em ecossistemas aquáticos (Danger, 2020). Isso ocorre porque o excesso de Carbono (C) pode impor uma limitação direta de outros elementos aos consumidores (Elser et al., 2012).

Além da influência que o solo e o clima têm sobre as características das plantas em florestas ripárias (Cornwell et al., 2008; Boyero et al., 2017), estes fatores ambientais podem também controlar a quantidade de detritos que adentram os riachos (Rezende et

al., 2016; Tonin et al., 2017) e os níveis de nutrientes dissolvidos na água (Graça et al., 2015). A dinâmica da entrada de detritos foliares na interface floresta ripária e riachos nos biomas tropicais é controlada por fatores climáticos (Tonin et al., 2017). As florestas ripárias de biomas tropicais apresentam mudanças sazonais naturais na quantidade de detritos foliares que entram nos riachos (Rezende et al., 2016; Sales et al., 2015). A Amazônia e o Cerrado apresentam uma sazonalidade marcante na dinâmica da entrada de detritos foliares, relacionando à precipitação como fator limitante (Tonin et al., 2017). A precipitação também influencia a profundidade da água, tendo impacto direto na quantidade de estoque bêntico disponível para os decompositores (Tonin et al., 2017). Grande parte da biomassa de detritos são oriundos de poucas espécies dominantes (Bambi et al., 2017; Tonin et al., 2021). Isso faz com que a composição de espécies de plantas de florestas ripárias também influencie a dinâmica de entrada de detritos foliares, repercutindo na decomposição (Gonçalves e Callisto, 2013; Bambi et al., 2017). Além disso, essas variações sazonais na entrada de detritos também sugerem uma mudança na qualidade dos detritos que adentram os riachos (Gonçalves e Callisto, 2013; Tonin et al., 2017), pois a quantidade de detritos foliares que entram nos riachos dependem do período de senescência foliar e da influência das variações ambientais que podem mudar a fenologia de múltiplas espécies de plantas presentes na floresta ripária (Reich, 1995; Gonçalves e Callisto, 2013; Tonin et al., 2021). No Cerrado, as estações mais secas apresentam detritos com menos nutrientes e compostos estruturais e mais compostos fenólicos, enquanto as estações mais úmidas os detritos são ricos em nutrientes, porém com menos polifenóis (Tonin et al., 2021). A senescência foliar permite a remobilização eficiente de nutrientes das folhas para outras partes da planta, servindo como um mecanismo de sobrevivência durante condições ambientais adversas, como seca ou estresse hídrico (Taiz et al., 2017). Dessa forma, comparado às folhas verdes os detritos

foliares são um recurso de menor qualidade (maior razão C:nutrientes), tornando os nutrientes um fator limitante para os decompositores (Elser e Bennett, 2011; Danger et al., 2020).

Além da influência ambiental sobre a dinâmica de detritos foliares, fatores físicos e químicos dos riachos também agem sobre a decomposição (Gulis e Suberkropp, 2003; Graça et al., 2015; Leite et al., 2016). A concentração de nutrientes dissolvidos pode estimular a atividade fúngica (Ferreira et al., 2006; Sena et al., 2021), como também afetar as enzimas fúngicas juntamente com o pH da água (Clivot et al., 2013). É previsto que aumentos na temperatura da água possam estimular a atividade fúngica e o acúmulo de biomassa (Chandrashekar et al., 1991; Ferreira e Chauvet, 2011) e consequentemente a ingestão de detritos por invertebrados detritívoros (Azevedo-Pereira et al., 2006). Como também, em riachos tropicais, variações da condutividade elétrica da água podem influenciar a dinâmica de populações de insetos fragmentadores (Leite et al., 2016). Apesar de, no geral, os fragmentadores (importante guilda de insetos para o processo de decomposição; Graça et al., 2001) serem encontrados em menor abundância em riachos tropicais (mais adaptados a temperaturas mais baixas; Boyero et al., 2011; Prather, 2003), podem atingir biomassa e tamanho do corpo elevados, tornando-os atores importantes na decomposição de detritos foliares (Martins et al., 2015; Tonin et al., 2014). Os fragmentadores podem acelerar a decomposição de detritos (Graça et al., 2015; Moulton et al., 2010), principalmente de detritos com menos compostos refratários e com maior conteúdo nutricional (detritos de maior preferência alimentar pelos fragmentadores; Rincón & Martínez, 2006; Biasi et al., 2019; Sena et al., 2020). Embora se credite uma maior importância da atividade fúngica para a decomposição nos trópicos (Boyero et al., 2011), os fragmentadores empenham um papel importante para a ciclagem de matéria e energia em riachos florestados (Graça et al., 2001).

Desse modo, estudos que verifiquem a diversidade de características físicas e químicas de detritos foliares de florestas ripárias tropicais e a repercussão da disponibilidade de detritos sobre a decomposição são necessários para o entendimento da importância de toda esta dinâmica complexa em riachos tropicais. Este processo envolve variações ambientais, influências fisiológicas e filogenéticas de espécies vegetais, mudanças na comunidade decompositora e as repercussões na ciclagem de matéria e energia (Graça et al., 2015).

OBJETIVO & ESTRUTURA DA TESE

O objetivo geral desta tese foi determinar características fitoquímicas de detritos foliares oriundos de zonas ripárias tropicais e a influência da variação da quantidade e da qualidade (características físicas e químicas) de detritos no processo de decomposição em riachos tropicais. O **Capítulo 1** aborda a exploração da variação da concentração e proporções de C, N e P, atributos físicos e defensivos de detritos foliares de espécies arbóreas representativas de zonas ripárias da Floresta Amazônica, Mata Atlântica e Cerrado. Foi analisado um conjunto de dados com informações de sete características químicas e duas físicas de detritos foliares de 68 espécies de plantas coletadas em 11 florestas ripárias em áreas preservadas de três biomas Neotropicais. O **Capítulo 2** trata da avaliação da co-limitação da quantidade e da qualidade dos detritos foliares no estoque bêntico para a biomassa de detritívoros ao longo do tempo um riacho amazônico. Foi realizado um experimento de campo relacionando a dinâmica do estoque bêntico em termos de quantidade e qualidade dos detritos (nas razões C:N, C:P e N:P) com a biomassa dos fragmentadores detritívoros associados ao longo do tempo. O **Capítulo** 3 relata a influência de detritos foliares de origens e características físicas e químicas distintas sobre a decomposição e repercussões na comunidade decompositora. Realizado experimentos

utilizando um folhas de uma espécie exótica e de uma espécie nativa em dois riachos de condições de referência com características distintas.

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CAPÍTULO 1 - DIVERGENT LITTER TRAITS OF RIPARIAN PLANT SPECIES BETWEEN HUMID AND DRIER BIOMES WITHIN THE TROPICS

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ABSTRACT

Riparian forests provide abundant plant litter – mostly in the form of dead leaves (hereafter litter) – for both forest soils and adjacent stream ecosystems, supporting terrestrial and aquatic detritus-based food webs. Although the fate of litter is predominantly dependent on its chemical and physical traits, there is limited availability of data on those traits over large spatial scales or empirical comparisons of traits across tropical biomes. We filled this gap by exploring the differences and similarities of nine litter traits and their dependence on phylogenetics for 68 plant species from riparian forests across three continental-scale, South American biomes: Amazon, Atlantic Forest and Cerrado. All three biomes produced litter with similar percentages of carbon (C) and phosphorus (P), C:P mass ratios, specific leaf area and toughness. However, litter from the driest biome (Cerrado) was better defended chemically (higher phenolic content) and had lower nutritional quality (higher C:nitrogen [N] mass ratio) but showed lower nutritional limitation (lower N:P mass ratio) than litter from more humid biomes. We found no phylogenetic signal for traits after constructing a phylogenetic tree across all biomes, suggesting that trait differences across biomes were environmentally determined. However, a strong phylogenetic signal was observed for P in the Atlantic Forest, which indicates that closely related species have similar %P in that biome. Our findings suggest that litter from more humid biomes was higher in nitrogen, although more phosphorus-limited, than litter produced in drier climates such as that of the Cerrado biome.

Keywords: plant detritus; litterfall; neotropical; litter decomposition; nutrient cycling; ecosystem functioning.

INTRODUCTION

Plant litter from riparian forests feeds heterotrophic communities of stream ecosystems, and its decomposition influences carbon and nutrient cycles in aquatic and terrestrial ecosystems (Marks, 2019; Pausas & Bond, 2020). This important process is controlled by a series of biological and environmental factors, but leaf litter (hereafter litter) traits are often considered the most important factors for predicting decomposition in terrestrial and aquatic ecosystems (Cornwell et al., 2008; Zhang et al., 2019). Litter traits refer to a set of intrinsic chemical and morphological attributes of litter, quantified in terms of palatability, toxicity, and nutritional value for detritivorous animals and microbial decomposers (Gessner, Chauvet, & Dobson, 1999). This set of attributes is commonly referred to as 'litter quality', in which litter of higher quality (e.g., higher nutrient concentrations and lower phenolic and lignin contents) are generally more attractive and sustains higher activity of detritivores and decomposers than litter of lower quality (García-Palacios et al., 2015; Graça et al., 2015).

Leaf traits may indicate trade-offs between available resources, plant metabolism and plant- influenced ecosystem processes (Weiher et al., 1999; Craine et al., 2001). Several leaf traits co-vary, producing a trait spectrum: at one extreme are the traits associated with resource acquisition, and at the other are traits associated with resource maintenance (Wright et al., 2004), resulting in overall litter trait syndromes (Boyero et al., 2017). While previous studies pointed to a strong influence of edaphic factors on traits of green leaves (Reich & Olesksym, 2004; McGroody, Daufresne & Hedin, 2004), some of these traits are conserved in senescent leaves (Killingbeck, 1996). Climate also drives the distribution and composition of plant species across terrestrial ecosystems (Reich & Olesksym, 2004; Wright et al., 2004), resulting in a latitudinal gradient of riparian plant litter traits at a global scale, with high recalcitrance toward tropical zones (Boyero et al.,

2017). This global spatial variation can influence the traits that affect forest soils and/or end up in streams (Ordoñez et al., 2009; Marks, 2019). In this context, litter from the tropics tends to be tougher, more chemically defended (e.g., high phenolic content), and more P-limited (higher N:P ratio) than litter from other regions (Boyero et al., 2017). However, litter traits may also be driven by species' phylogenetic relatedness, which means closely related species are expected to be more similar in terms of litter traits than distantly related species due to the conservation of traits during the evolution of species lineages (Cadotte et al., 2017; LeRoy et al., 2020).

Riparian forests of neotropical biomes such as Amazon, Atlantic Forest, and Cerrado produce large amounts of litter annually, which are subsequently decomposed in soils and streams (Tonin et al., 2017). However, the lack of a comparison of litter traits for riparian tree species from different tropical biomes has led to generalizations that tropical litter is of lower quality (García-Palacios et al., 2015; Boyero et al., 2017) despite the diversity of climates, plant species and biomes within the tropics. For example, the litter traits most relevant to carbon and food web dynamics, such as the content of nutrients in plant litter, were not comprehensively reported at the biome level. However, this information is essential to understanding ecosystem functions across and within regional scales.

We explored a dataset of litter traits – C, macronutrients (N, P, and their mass ratios), and physical and chemical defenses – of the most representative litter entering streams at multiple sites across three continental-scale South American biomes: Amazon, Atlantic Forest and Cerrado. We aimed to assess the dissimilarity of litter traits from these three tropical biomes and their influences on litter decomposition. We hypothesized that (i) litter from the Amazon and Atlantic Forest biomes would be richer in nutrients than those from the Cerrado biome because the high humidity year-round in the former biomes

stimulates and enhances the recycling of soil nutrients (Luizao, 1989; Boeger, Wisniewski & Reissmann, 2005; Grau et al., 2017); (ii) litter from the Cerrado biome (those from riparian forests) would be more refractory (higher C:N and C:P mass ratios and with tougher tissues) than those from the Amazon and Atlantic Forest biomes due to the harsh climatic and edaphic factors for plants found in the Cerrado, such as low pH, low fertility and reduced water availability in some periods of the year (Kraus et al., 2004; Haridasan, 2008; Miatto, Wright & Batalha, 2016); (iii) Variations in such plant litter traits should be driven by environmental variation, not being a result of phylogenetic relatedness of species occurring in different biomes or in the same biome Boyero et al., 2017).

METHODS

Study sites

We studied riparian forests of low-order streams located inside preserved areas (1st-3rd order reaches; hereafter sites) in three South American biomes (Figure 1): (i) Amazon (2 sites), (ii) Cerrado (3 sites), and (iii) Atlantic Forest (6 sites). Sites in the Amazon were located in nonflooded forests in the central and northern Amazon with equatorial climate, tall trees, and evergreen canopy. Cerrado sites were located in the Brazilian Central Plateau with a tropical wet–dry climate, where streams drain through dense corridors of evergreen forests known as gallery forests. Atlantic Forest sites were located in inland and coastal areas of Brazil, spanning a large latitudinal gradient in climate (~ 30 degrees of latitude), from subtropical to equatorial, comprising tropical and subtropical rainforests, Araucaria forests and seasonal tropical forests.

The dataset analyzed comprises information from litter for 68 plant species collected across 11 riparian forests within preserved areas (reference conditions) of three neotropical biomes within the tropics: the Amazon (Cantá and Manaus), the Cerrado

(Patrocínio, Brasília and Palmas) and the Atlantic Forest (Erechim, Florianópolis, Varzedo, Parnamirim, Palotina and Santa Leopoldina). We analyzed seven chemical traits (%C, %N, %P, %Phenols, C:N, C:P, and N:P ratios) and two physical traits of the litter (toughness and specific leaf area).

Field sampling protocol

The sampling sites consisted of a 100 m stretch of each forest stream with the most extensive vegetation cover and without apparent anthropogenic impact. Litterfall was sampled using suspended litter traps (1 m^2, 10 mm mesh) fixed 1.5 m high on both streambanks to optimize the sampling effort. Fallen leaves were collected every 15 days to avoid leaching or decomposition. Recently fallen leaves were sampled during the period of maximum litterfall in the year, which was defined according to recent literature (Tonin et al., 2017) and/or the expertise of local researchers. Litter was transported to the laboratory and oven-dried (60 °C, 72 h) for chemical analyses; litter used to determine specific leaf area (SLA) and toughness was air-dried only. All litter samples from the same site were pooled and homogenized. Then, the litter for the 5-10 most representative tree species in terms of dry mass were separated and identified to the lowest possible taxonomic level.
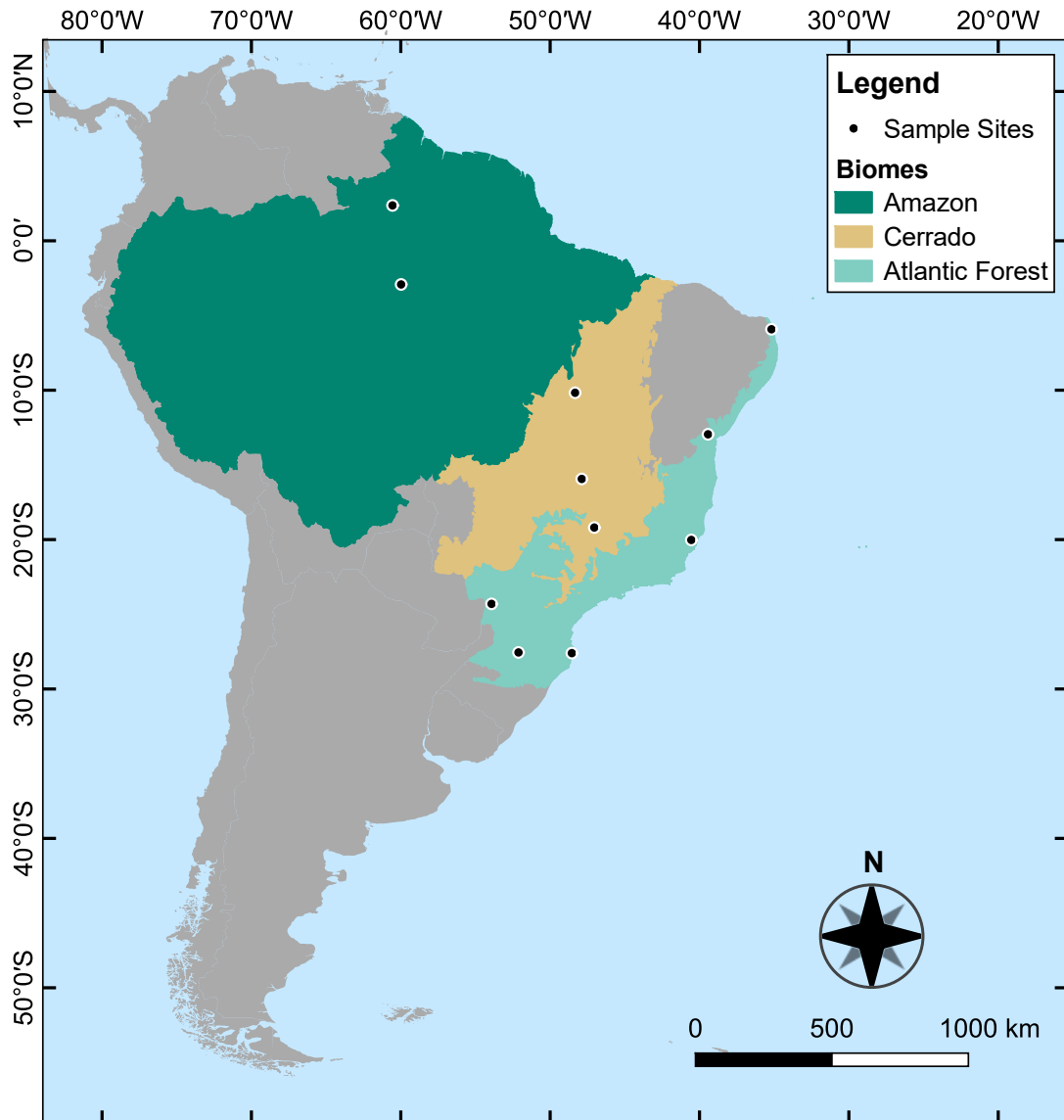
Figure 1. Location of litter collection sites at 11 riparian forests distributed in three Neotropical biomes: Amazon Forest (dark green area), Atlantic Forest (blue area) and Cerrado Neotropical savanna (orange area).

Physical and chemical characterization of litter traits

Twenty grams of litter from each was ground in a vibratory ball mill (Fritsch Pulverisette, Model 0, Idar-Oberstein, Germany) for the chemical analyses. The C and N concentrations (% of dry mass) were analyzed by total combustion (950 °C) in an elemental analyzer (Leco Instruments Ltda, Model Truspec CHN628, San José, Michigan, USA). The P concentration was quantified spectrophotometrically (measuring

absorbance at 882 nm) using the ascorbic acid method after combustion (550°C for 4 h) and digestion (with hydrochloric acid) of litter samples (Flindt et al., 2020). Total phenols were determined using the Folin-Ciocalteau method (Bärlocher & Graça, 2020). All chemical analyses were run using triplicates of 100-mg portions of litter powder from each species. Entire leaves were used for the determination of SLA and toughness. SLA, a proxy for litter toughness (that is, higher SLA values are usually found for softer litter; Boyero et al., 2017), was estimated using 10-20 leaf discs (12 mm diameter) from different parts of the leaves and from different leaves of the same species. Each leaf disc was then weighed (0.01 mg) to determine its mass. SLA was obtained through the quotient of leaf disc area to leaf dry mass (in grams). Litter toughness was estimated with a penetrometer, which measures the pressure (in $kgF\ cm^{-1}$) necessary to pierce the tissue of a leaf with a steel rod (1.55-mm diameter) (Boyero et al. 2011). The litter toughness of a species was the average of 10-20 measurements made on different leaves.

Data analysis

We analyzed the physical and chemical litter traits of plant species from biomes using principal component analysis (PCA; 'factoextra' and 'FactoMineR' packages) on standardized data ('scale' function). To explore how litter traits differed among biomes (Amazon, Atlantic Forest and Cerrado), we calculated the nonparametric 95% confidence intervals (CI) for litter traits from each biome (based on 1000 resamples with the BCa method) using the 'boot' function and package (Davison & Hinkley, 1997; Canty & Ripley, 2016). In addition to providing information about data variability/dispersion, CIs can express the statistical significance of tests regarding comparisons of means (Wood, 2005). This technique avoids meeting the assumptions for parametric models (for example, normal distribution and homogeneity of variance) and facilitates interpretation (Carpenter & Bithell, 2000; Wood, 2005).

We performed a hierarchical agglomerative clustering analysis to assess trait similarity from different species across sites and biomes using a Euclidean distance matrix calculated with the average method. We used the k-means method to define the number of clusters, minimizing a criterion known as inertia or within-cluster sum-of-squares (Steinley, 2006). Traits were standardized before the analysis. All analyses were performed using R v. 4.2.1 (R Core Team, 2022).

Finally, we tested the phylogenetic signal of each litter trait to investigate whether such traits might be determined by the species' phylogenetic relationship. The phylogenetic signal has been commonly used to investigate the tendency for related species to have more similar trait values with each other than with random species drawn from a phylogenetic tree (Blomberg & Garland, 2002). Therefore, we used the most complete dated mega-tree (i.e., GBOTB.extended.tre) available as a backbone to generate the phylogenetic relationships for the 62 species of our dataset using the R package 'V.Phylomaker' (Jin & Qian, 2019) (Figure S1). We also reconstructed phylogenetic trees separately for each biome (Amazon, Atlantic Forest, and Cerrado). The mega-tree found in GBOTB.extended.tre is a combination of GBOTB (GenBank taxa with a backbone provided by Open Tree of Life) for seed plants (Smith & Brown, 2018) and the pteridophyte clade of phylogeny found in Zanne et al. (2014). This mega-tree includes 74,533 species of vascular plants from 479 families. The 'phylo.maker' function generates phylogenetic hypotheses under three scenarios depending on how the new tips are bonded to nodes. Here, we used scenario 1, in which a new tip is bonded to the genus basal node (details in Jin & Qian, 2019). To maximize species match, we checked our species list and standardized the spelling and nomenclature of species with GBOTB.extended.tre tips. We determined the presence of a phylogenetic signal (i.e., a significant result) and quantified the strength of the phylogenetic signal (i.e., the effect

size, Nakagawa & Cuthill, 2007) of litter traits using Pagel's λ in the R package 'phytools' (Revell, 2012). We calculated the Pagel's λ index for all species and for each biome (Amazon, Atlantic Forest, and Cerrado). Pagel's λ indicates the correlation between species traits and phylogeny. Under a Brownian motion model (BM), species traits diverge from their ancestors at a continuous rate and randomly throughout the evolution time (Felsenstein, 1985); Pagel's λ is expected to be equal to 1 (strongly influenced by phylogeny), whereas values of 0 imply phylogenetic independence (Pagel, 1999). However, Pagel's λ can also adopt values >1 in cases in which traits are more similar than predicted by BM (Freckleton, Harvey & Pagel, 2015).

RESULTS

Sixty-one percent of the variability in litter traits was retained in the first two components of the PCA (33% of PC1 and 28% of PC2, Figure 2). The C:N mass ratio (r = 0.91), phenols (r = 0.62) and toughness (r = 0.59) were positively correlated with PC1, while N (r = -0.92) and SLA (r = -0.82) were negatively correlated with PC1 (Figure 2). C:P (r = 0.94) and N:P (r = 0.76) mass ratios were positively correlated with PC2, while P (r = -0.20) was negatively correlated with PC2. Litter from the Amazon tended to be positively associated with N, C:P, N:P and SLA; litter from the Cerrado tended to be positively associated with C, P, C:N, phenols, and toughness, while litter from the Atlantic Forest presented much greater variability than litter from other biomes and tended to be positively associated with several traits (Figure 2).
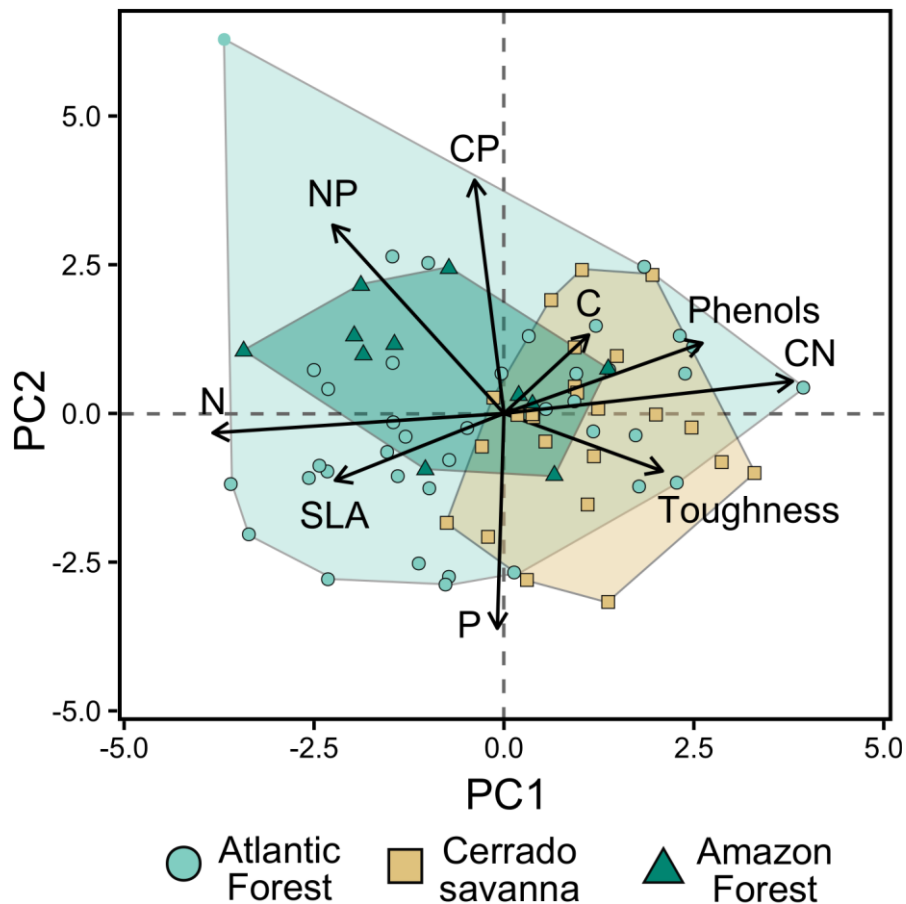
Figure 2. Principal component analysis (PCA) of litter traits [nitrogen (N); phosphorus (P) and carbon (C) and phenol concentrations; N:P, C:N, C:P ratios; toughness and specific leaf area (SLA)] and plant species litter from Amazon Forest (dark green triangle); Atlantic Forest (blue circle) and Cerrado Neotropical savanna (orange square).

When we analyzed each litter trait individually, we found that the litter samples from the three biomes had similar concentrations of C and P, C:P mass ratio, SLA and toughness, as supported by confidence intervals (Figure 3A, C, E, H). However, litter from the Amazon and the Atlantic Forest showed 87% more N and higher N:P mass ratios than litter from the Cerrado (1.21-177, 14.2-48.3 confidence intervals; 1.17-1.54, 20-40; 0.81-1.05, 12.6-18.7, respectively) (Figure 3B, F), suggesting a higher P limitation in the former and in the most humid biomes (N:P mass ratio > 16; Figure 3F). We found a higher C:N mass ratio and phenolic content in litter from the Cerrado (51.3-65.8, 30.5-45.0) than

in litter from the Amazon (32-48, 12-23) and the Atlantic Forest (32-47, 14-22) (Figure 3D, G).

The cluster analysis with the 62 species from different sites across biomes aggregated the species into five groups, which were related to different litter traits. The first group (red color in Figure 4) was related to high N:P and C:P ratios and was composed of only one species from the Atlantic Forest (Myrceugenia miersiana Gardn.) The second group (yellow color) was related to high SLA values and was composed of one species from the Cerrado (Copaifera langsdorffii Desf.). The third group (green color) was related to high P content and was composed of five species from the Atlantic Forest and three species from the Cerrado. The fourth group (blue color) was associated with high values of N, toughness, and C:N ratio and was composed of two species from the Amazon Forest, seven species from the Atlantic Forest and 27 species from the Cerrado. Finally, the fifth group (pink color) was related to low phenolic and C contents and was composed of six tree species from the Amazon Forest and 16 species from the Atlantic Forest (Figure 4).

No phylogenetic signals were found for most traits using the reconstructed tree for all three biomes or using one separate tree for each biome. However, P showed a strong phylogenetic signal for the Atlantic Forest, as indicated by significance tests for Pagel's λ ($\lambda = 0.709$, P = 0.045, S1). We then reconstructed the ancestral P content in litter from the Atlantic Forest, which provides evidence that the evolution of this trait may have resulted in species with low P content but also species with intermediate to high P content (Figure 5). For example, closely related species of the genera Myrceugenia, Eugenia, Myrcia, and Miconia showed low P contents. In contrast, the genera Nectandra, Ocotea, Virola, and Xylopia showed intermediate to high P contents.
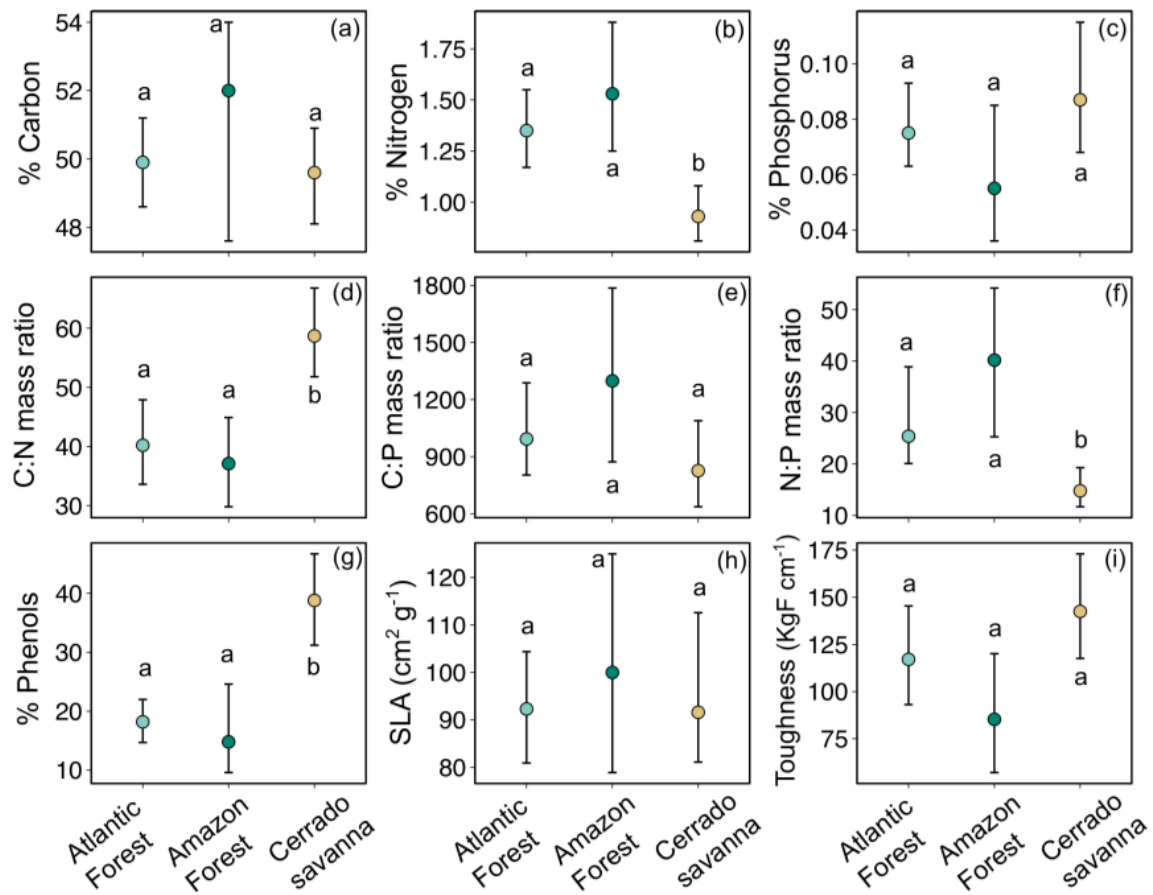
Figure 3. Concentration of carbon (A; [C]), nitrogen (B; [N]) and phosphorus (C; [P]), and mass ratios of N:P (D), C:N (E) and C:P (F); specific leaf area (G), toughness (H) and phenol concentration (I) of litter from Amazon (dark green circles), Atlantic Forest (blue circles), and Cerrado Neotropical savanna (orange circles). The circles are the means of different species and the vertical lines denote the upper and lower limits of 95% confidence intervals. Different letters indicate statistically significant differences between ecoregions.
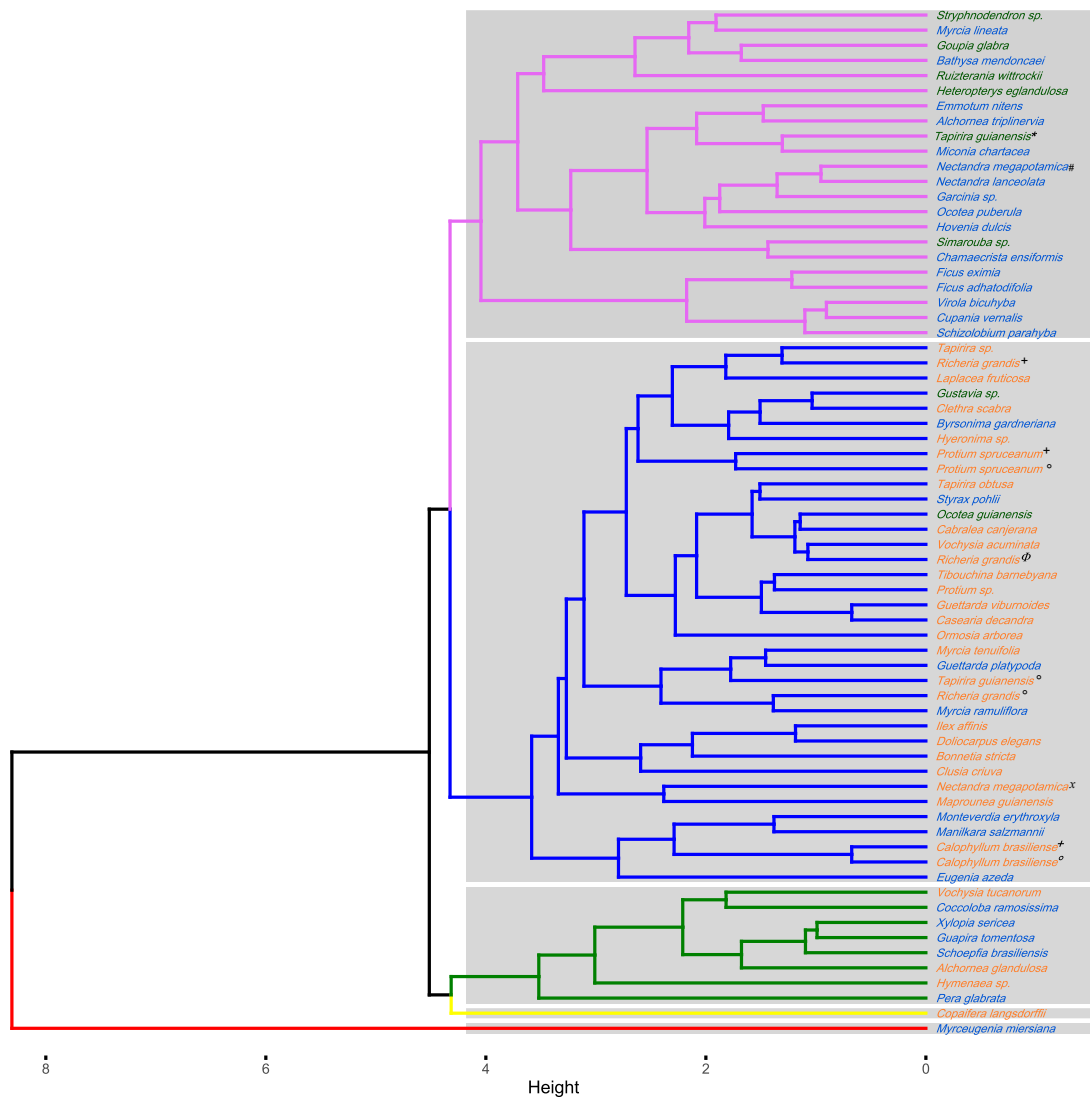
Figure 4. Hierarchical agglomerative clustering of litter traits of tree species from Amazon Forest (dark green), Atlantic Forest (blue) and Cerrado (orange) biomes. Branches with different colors indicate more similar clusters in relation to the physical and chemical traits of litter. Cophenetic correlation coefficient of 0.75. Different symbols denote species from Cantá (*), Palmas (+), Brasília (°), Patrocínio (*x*), Erechim (#) and Mucugê (Φ).
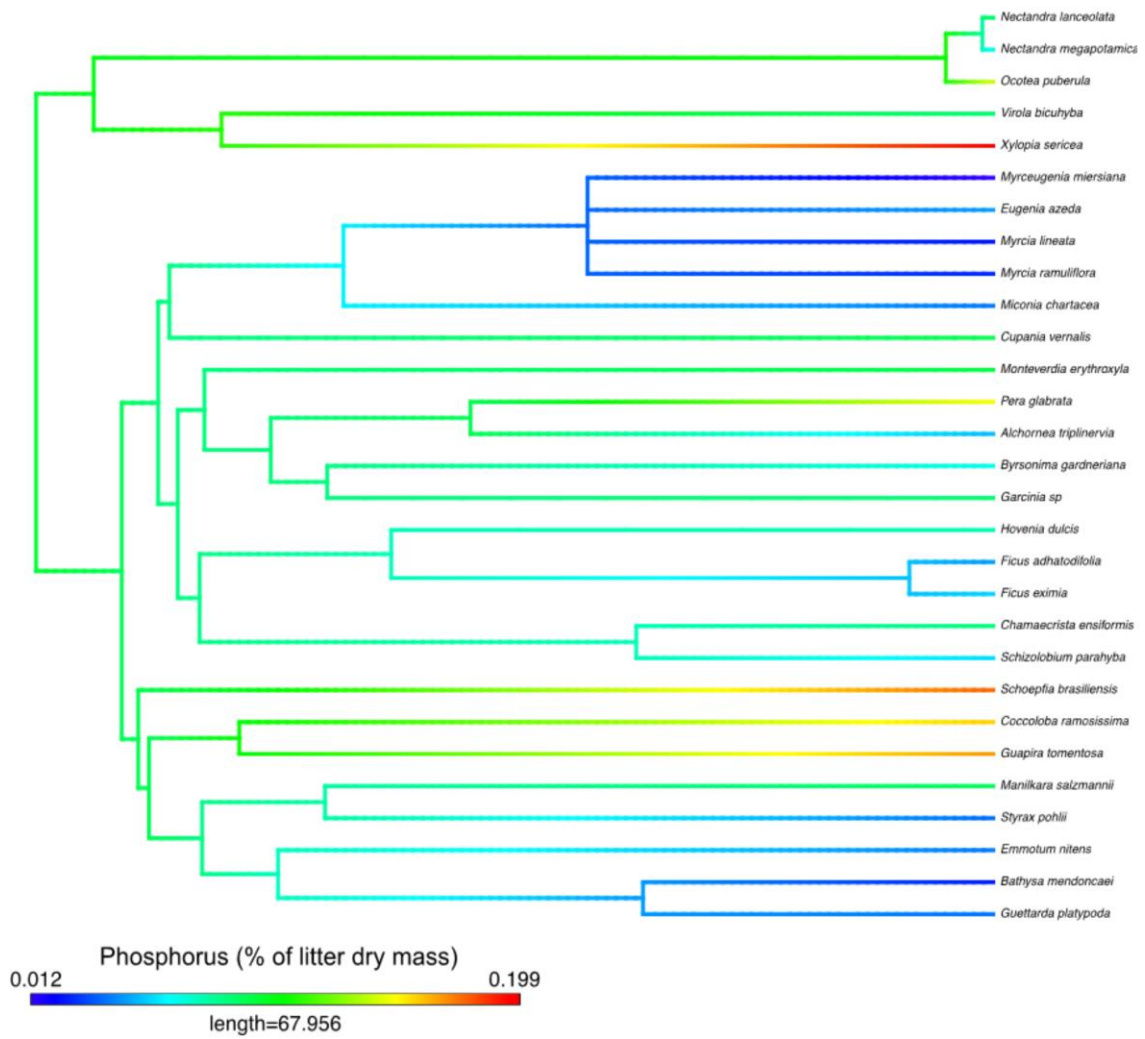
Figure 5. Observed phosphorus (P) content on litter of 29 plant species from Atlantic Forest and the reconstructed estimation of the ancestral P content. The colored scale indicates the content of P, which varies from low (blue) to high (red). The length of the legend gives a scale for the branch lengths in Atlantic Forest phylogenetic tree.

Table 1. Phylogenetic signal (Pagel's λ) and its statistical significance (*P*-value at 0.05) for each litter trait using phylogenetic reconstructed trees for all three biomes and for each biome separately. Note that only phosphorus showed a phylogenetic signal in the Atlantic Forest biome.

Litter Trait	Phylogenetic tree	Pagel's λ	*P*-value
Carbon	All biomes	0.137	0.925
	Atlantic Forest	< 0.001	1.000
	Amazon Forest	< 0.001	1.000
	Cerrado	1.040	0.074
Nitrogen	All biomes	0.168	0.315
	Atlantic Forest	0.203	0.479
	Amazon Forest	< 0.001	1.000
	Cerrado	< 0.001	1.000
Phosphorus	All biomes	0.591	0.087
	Atlantic Forest	0.709	**0.045**
	Amazon Forest	< 0.001	1.000
	Cerrado	< 0.001	1.000
N:P	All biomes	< 0.001	1.000
	Atlantic Forest	0.013	0.971
	Amazon Forest	< 0.001	1.000
	Cerrado	< 0.001	1.000
C:N	All biomes	0.183	0.597
	Atlantic Forest	< 0.001	1.000
	Amazon Forest	< 0.001	1.000
	Cerrado	< 0.001	1.000
C:P	All biomes	0.144	0.464
	Atlantic Forest	0.423	0.196
	Amazon Forest	< 0.001	1.000
	Cerrado	< 0.001	1.000
Phenols	All biomes	< 0.001	1.000
	Atlantic Forest	< 0.001	1.000
	Amazon Forest	< 0.001	1.000
	Cerrado	< 0.001	1.000
Toughness	All biomes	0.198	0.558
	Atlantic Forest	0.448	0.606
	Amazon Forest	< 0.001	1.000
	Cerrado	< 0.001	1.000
SLA	All biomes	< 0.001	1.000
	Atlantic Forest	< 0.001	1.000
	Amazon Forest	< 0.001	1.000
	Cerrado	< 0.001	1.000

DISCUSSION

This study revealed striking similarities in the physical and chemical traits of litter from the Amazon and Atlantic Forest biomes. Although these biomes are spatially isolated, there is evidence of past connections between them during the expansion periods of rainforests (Fouquet et al., 2012; Ledo and Colli, 2017). This finding corroborates the connection of floristic composition and functional traits among neotropical forests (Fouquet et al., 2012). Despite the importance of phylogenetic relatedness of species to determine species traits, we found no phylogenetic signals for the studied traits across biomes; however, within the Atlantic Forest biome, we found a strong phylogenetic signal for P (see below). Consequently, litter traits seem to be driven by environmental differences among biomes, such as temperature, rainfall, edaphic conditions, and topography.

We found higher N contents in litter from the Amazon and the Atlantic Forest than in litter from the Cerrado. However, there were similar P contents in litter across all three biomes, partially corroborating our first hypothesis that litter from the Amazon and Atlantic Forest biomes would be richer in nutrients than that from the Cerrado biome. According to Reich & Oleksyn (2004), N:P mass ratios < 14 would indicate N limitation. Thus, the higher N contents of litter from the Amazon and the Atlantic Forest led to higher N:P mass ratios, indicating that litter from the most humid biomes (that is, Amazon and Atlantic Forest) is more P-limited than that from the Cerrado. Considering that the P contents in litter were similar among the biomes, the observed difference in the N:P mass ratio suggests that P resorption rates are similar and that the variation in the N:P ratio was due to differences in N resorption rates before leaf senescence (Haridasan, 2008; Grau et al., 2017). These different nutritional limitations of the litter may influence the nutrient availability for decomposers (Güsewell, 2004; Sena et al., 2021).

Riparian forests of the Cerrado produced litter with higher phenolic content and higher C:N mass ratio than litter from the Amazon and the Atlantic Forest, but the content of C, P, C:P mass ratio, and toughness values were similar among all the biomes, partially corroborating our second hypothesis that litter from the Cerrado would be more refractory than litter from the Amazon and the Atlantic Forest. The highest phenolic content was observed for litter from the Cerrado, supporting previous evidence that tree species from this biome produce more chemically defended litter (Haridasan, 2008; Miatto, Wright & Batalha, 2016). The low N content for litter from the Cerrado – and thus the high C:N ratio – is likely a strategy used by plants to conserve the limited and essential available N in the soils. Consequently, N-poorer litter may decompose at slower rates in both forest soils and in streambeds, as shown elsewhere (García-Palacios et al., 2015; Zhang et al., 2019). Although SLA is considered an opposite proxy for litter toughness (Boyero et al., 2017), these two litter traits did not differ among the three biomes, indicating that riparian species from these biomes produce tough and malleable litter. Although multiple traits were similar across the biomes, we showed the existence of relevant differences in terms of the C:N mass ratio and phenolic content between drier (Cerrado) and humid biomes (Amazon and Atlantic Forest), which possibly constrain litter decomposition in the Cerrado, reducing the turnover and availability of nutrients to aquatic and terrestrial food webs as well as for plant growth (Ordoñez et al., 2009).

Although no phylogenetic signal was observed for the studied traits using a tree for all biomes, we found a strong phylogenetic signal for P from the Atlantic Forest. This result indicates that closely related species have similar P contents in the Atlantic Forest and suggests that phylogeny is essential for determining P content within the Atlantic Forest biome. This finding contrasts with no phylogenetic signal observed for the Amazon and Cerrado biomes in the present study and with findings of a global survey

(Boyero et al., 2017) but agrees with regional studies using green leaves, which reported a significant signal for P (Kraft & Ackerly 2010, Silva & Batalha, 2010). However, differences among studies should be interpreted cautiously, as the phylogenetic signal can depend on the study scale, sample size, model, index, and phylogenetic tree used (Münkemüller et al. 2012).

Several tree species were found across sites, which allowed us to examine differences between phenotypic plasticity and environmental regulation. On the one hand, litter specimens of *Protium spruceanum* (Benth.) Engl. and *Calophyllum brasiliensis* Cambess. were found in different areas of the Cerrado and clustered closely, suggesting that individuals of these two species produce litter with low phenotypic plasticity (Sultan, 2000; Kroon et al., 2005). On the other hand, litter specimens of *Richeria grandis* Vahl were sampled at different sites of the Cerrado and did not cluster closely, suggesting some phenotypic plasticity (Sultan, 2000; Kroon et al., 2005). A similar pattern occurred with the litter of *Nectandra megapotamica* (Spreng.) Mez, the only species sampled in more than one biome. *Nectandra megapotamica* litter showed greater trait similarity with litter specimens of other tree species from the same biome sampled, suggesting that functional responses of individual traits are induced by environmental conditions (Sultan, 2000; Boyero et al., 2017). These plastic responses of riparian tree species may have important consequences for litter stoichiometry and influence nutrient availability in detritus-based ecosystems such as forest streams (Danger, 2020).

Here, we demonstrated that litter traits for tree species from the Amazon and Atlantic Forest biomes of South America are remarkably similar, mainly in terms of C, P, C:P mass ratio, SLA, and toughness. However, litter from the Cerrado was nutrient-poor and highly chemically defended (although similar in terms of toughness of litter tissues), traits which usually retard litter decomposition and biological activity. We

showed that species phylogeny seems to have lower importance than environment in the determination of litter traits, at least at the biome scale. Finally, we demonstrate how the litter of riparian tree species from different South American biomes are physically and chemically characterized, providing evidence for their possible repercussions to carbon and nutrient dynamics.

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CAPÍTULO 2 - SHREDDERS BIOMASS IS LIMITED BY C:N RATIO OF LITTER, BUT NOT BY LITTER QUANTITY IN AN AMAZONIAN STREAM

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ABSTRACT

The co-limation of more than one independent resource can take various forms depending on the resource category and it has been recognized for a long time in studies in Ecology. The idea that quantity and quality of food resources co-limit metabolism and nutrient restriction is well understood in autotrophic systems, but little explored in detritus food webs. Forested headwater streams are ecosystems dependent on plant litter – mostly dead leaves – from riparian forests, supporting stream detritus-based food webs. We filled this gap by assessing the effects of the limitation by litter quantity and quality on detritivores biomass over time in an Amazonian stream. We found that the biomass variation of shredding detritivores in an Amazonian streams may not be co-limited by the quantity and quality of litter storage mass over time, but instead by the variation in C:N mass ratio variation of litter storage mass. Our results did not show a pattern in the temporal dynamics of litter storage mass. Our study provides evidence for possible repercussion to the food web and nutrient dynamics in Amazonian streams due to N availability limitation affecting shredders' metabolism. Thus, changes in plants' composition in the Amazon riparian forest might affect not only the processing of organic matter in streams but also the nutrient pathway driven by shredding detritivores.

Keywords: Organic matter; Decomposition; Aquatic shredder; Stream; Amazon

INTRODUCTION

The notion of simultaneous constraints by multiple resources has been recognized for a long time in Ecology (Tilman, Kilham and Kilham, 1982; Saito, Goepfert and Ritt, 2008). Co-limitation of two or more independent resources can take various forms depending on the resource category (Tilman, Kilham and Kilham, 1982; Saito, Goepfert and Ritt, 2008). The quantity and quality (physical and chemical characteristics) of food resources can limit the consumers' metabolism by restricting the acquisition of Carbon (C), Nitrogen (N), and Phosphorous (P); (Sperfeld, Raubenheimer and Wacker, 2016). This phenom is well-understood as a bottom-up control in autotrophic systems, but is poorly understood in detritus food web (Cross et al., 2006; Halvorson, Sperfeld and Evans-White, 2017). The functioning of the detritus food web in forested headwater streams is an example of an ecosystem that may be subject to bottom-up control driven by the quantity and quality of food resources (Halvorson, Sperfeld and Evans-White, 2017), mainly in megadiverse sites as tropical riparian forests.

Tropical riparian forests have shown higher diversity and productivity of plant species during drought season, influencing the direct input of leaf litter into headwater streams (Bambi et al., 2017; Tonin et al., 2017). This litter is processed and available in the food chain by the activity of microorganisms and invertebrate decomposers (Boyero et al., 2021; Tonin et al., 2021). The leaf litter decomposition is controlled by environmental conditions and litter characteristics (Zhang et al., 2019; Sena et al., 2020). The litter chemistry may vary in space and time due to the physiology and phylogeny of riparian plant species and environmental controls acting on leaf senescence (Reich, Ellsworth and Uhl, 1995; Tonin et al., 2021; Sena et al., 2022). As well as riparian plant species composition and the ratio between abundant and rare species play an important role in the litter chemistry (Bambi et al., 2017; Rabelo et al.; 2022). Such as litter quantity

into tropical streams depends on the timing of senescence of multiple plant species present in riparian zones, changing also the elemental ratios (Gonçalves and Callisto, 2013; Bambi et al., 2017).

Litter elemental ratios, one of the litter chemical characteristics, have been proposed as an important factor in regulating leaf litter decomposition worldwide (Zhang et al., 2019). Besides, they can be used to predict nutrient limitation in aquatic ecosystems (Sterner and Hessen, 1994). For example, the excess of C in the litter can directly limit the availability of other elements, thereby changing the balance of the elemental composition of food resources (Cross et al., 2003; Scott et al., 2013). This limitation can result in less nutritional availability (Danger et al., 2021) affecting the decomposers' metabolism (Elser and Bennett, 2011; Zhang et al., 2019). Although detritivores naturally have a diet that is rich in C but deficient in nutrients (Sterner and Elser, 2002). Also, the variation of litter elemental ratios can influence the assimilation of nutrients, growth, and biomass of these organisms (Halvorson, Sperfeld and Evans-White, 2017).

Previous studies have focused on the quality of leaf litter to explain the detritivores activity using the litterbag approach on both on a regional and global scale (e.g., Whiles and Wallace, 1997; Gonçalves and Callisto, 2013; Zhang et al., 2019). Recently, studies have begun to address the influence of both litter quantity and quality in laboratory experiments (e.g. Halvorson, Sperfeld and Evans-White, 2017; Arias-Real et al., 2018; Sena et al., 2020). These studies indicate that detritivores growth may be explained by elevated litter quantity and quality (Halvorson, Sperfeld and Evans-White, 2017; but see Cararo et al., 2023). Additionally, detritivores may selectively feed on higher quality litter even when the availability of this resource is lower compared to lower quality litter (Arias-Real et al., 2018; Sena et al., 2020). However, few studies investigated the influence of the natural temporal dynamics of litter deposited in the

streambed on the metabolism of detritivores. Moreover, the co-limitation among detritivores remains understudied, even though it may be a good way to investigate continuous responses to the quality and quantity of food resources and to fill existing gaps in the study of ecological stoichiometry and ecosystem ecology (Sperfeld, Raubenheimer and Wacker, 2016).

We conducted an observational study to assess the effects of the limitation by litter quantity (in terms of litter mass) and litter quality (as C:N, C:P and N:P mass ratios) on detritivores shredders over different seasonal periods in an Amazonian stream. Therefore, the premises of our study are that (i) shredders growth is co-limited by litter quantity and quality (Halvorson, Sperfeld and Evans-White, 2017); and (ii) detritivores tends to potentialize their consumption of resources that provide better energy for metabolism maintenance (Kaspari et al., 2012; Arias-Real et al., 2018).Thus, we hypothesized that (i) Shredders biomass would increase with higher litter quantity and quality (lower litter C:N and C:P mass ratios),

METHODS

Study site

The experiment was performed in the Barro Branco stream, located in the Adolpho Ducke Reserve; (03°01'S, 59°53' and 59°59'W), Manaus, Amazonas, Brazil (Figure 1). The reserve covers an area of 10,000 ha with vegetation composed of a Dense Ombrophiles Forest of Terra Firme (Hopkins, 2005). The climate is classified as "*Af*", constantly humid (Kottek et al., 2006). Annual rainfall is approximately 2,200 mm, concentrated in the months of December to April, with an average annual temperature of 26°C (Inmet, 2022; Station Code: A101; https://bdmep.inmet.gov.br/; Figure 2). The Barro Branco stream is surrounded by "terra firme" vegetation with yellow latosol-type

soils and sandy bottom with abundant plant litter (Spironello et al., 2004). Stream water

is acid (pH= 4.6 ± 0.1), well oxygenated (6.6 ± 0.1 mg/L) and low in dissolved ions, and

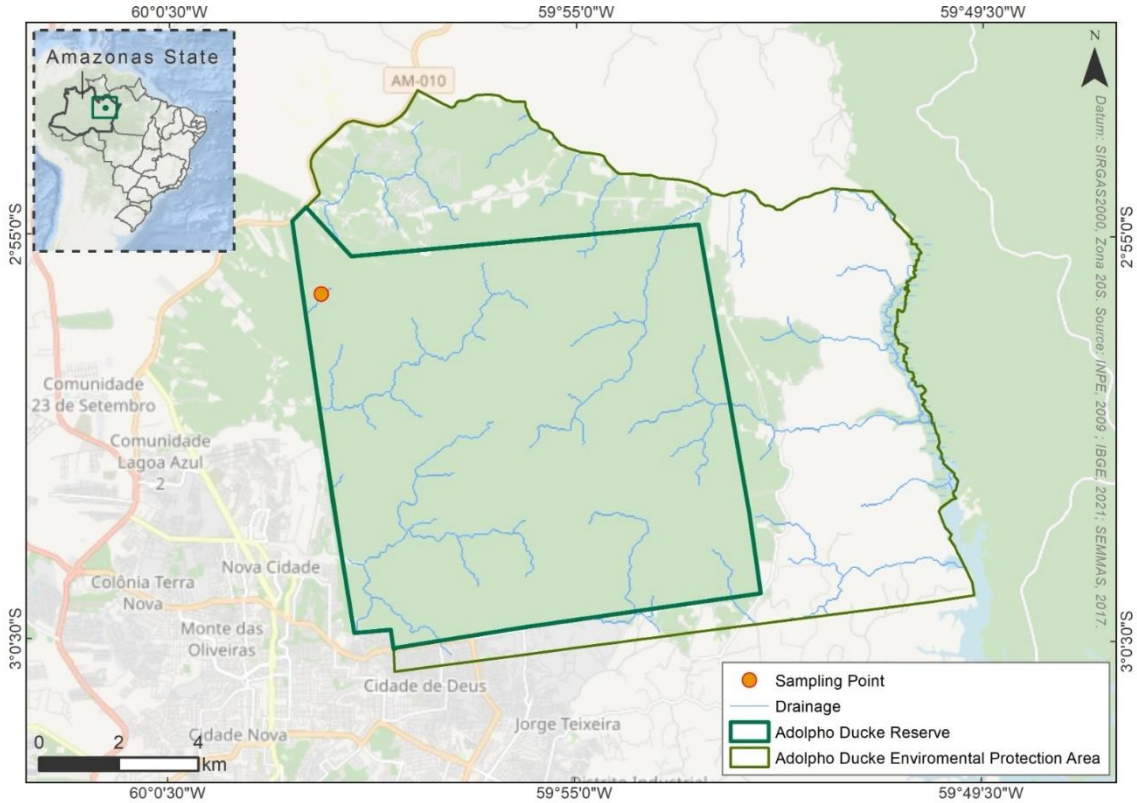electrical conductivity (10.7 ± 0.4 µS/cm) (Martins et al., 2014).

Figure 1. Location of experiment site at Barro Branco stream, located in the Adolpho Ducke Reserve (green area).
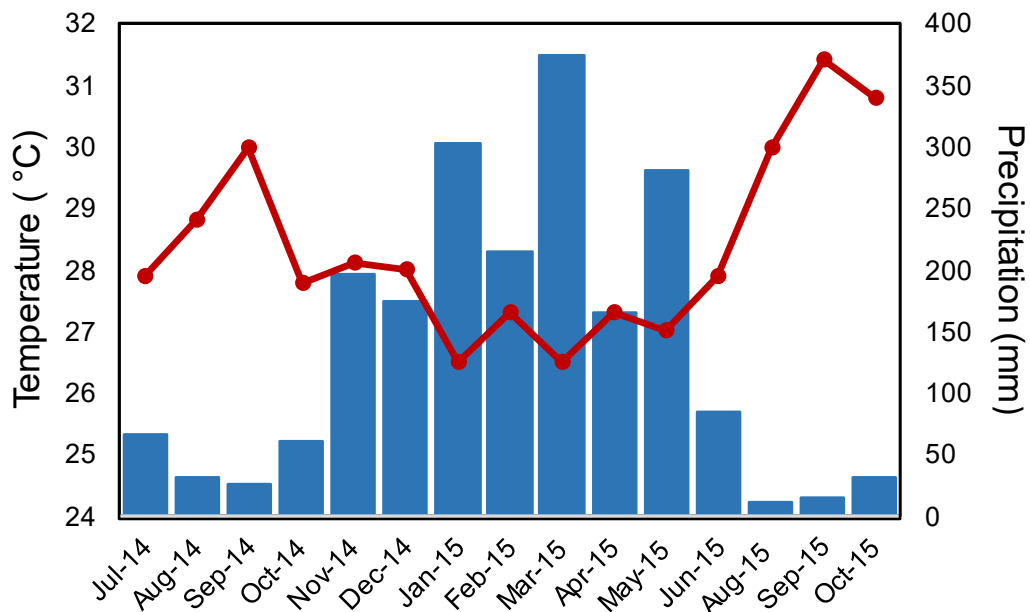
Figure 2. Climatogram of the Amazon using records of precipitation (blue bars) and temperature (red points and lines) from July, 2014 to Octuber, 2015. Data from National Institute of Meteorology (2014-2015) (INMET) for Station Code: A101.

Field sampling

Litter storage was collected monthly (30 ± 3 d) between July 2014 and October 2015, totaling 16 sampling events. Nine samples of benthic litter were collected from three locations within a 120 m stream reach using a Surber® sampler (0.09 m^2 and 250-µm mesh). To ensure a representative sample of the Barro Branco stream conditions, samples were taken haphazardly from pool and riffle areas. Litter was sieved through a 1-mm mesh, enclosed in individual plastic bags, and transported to the laboratory. In the lab, litter was cleaned with distilled water in a sieve (250 µm mesh) to retain any associated detritivores, which were preserved in 70% ethanol, dried (60ºC, 72h), and weighted to the nearest (accuracy 0.001g).

Chemical analysis of litter

Dried litter samples were ground in a vibratory ball mill (Fritsch Pulverisette, Model 0, Idar-Oberstein, Germany) before chemical analysis. Total C and N concentrations (% of dry mass) were analyzed by total combustion (950 °C) in an elemental analyzer (Leco Instruments Ltda, Model Truspec CHN628, San José, Michigan, USA). Total P concentration was quantified spectrophotometrically (measuring absorbance at 882 nm) using the ascorbic acid method after combustion (550°C for 4 h) and digestion (with hydrochloric acid) of litter samples (Flindt et al., 2020). All chemical analyses were performed in triplicate of 100-mg portions of litter powder from each sample. The masses of litter chemical constituents were estimated by multiplying the proportion of each litter constituent by the litter storage mass. The dry mass of litter chemical elements (in grams) was then multiplied by three times the area of Surber® sampler (0.45 m^2 x 3 sample points). Litter mass ratios of C:N, C:P, and N:P where then calculated for each month.

Identification of invertebrates

The detritivores were identified to genus or family level using identification keys of Pes et al. (2005), Domínguez and Fernández (2009) and Hamada, et al. (2019). These organisms were classified into functional feeding groups (FFGs): shredders, collectors, scrapers, and predators (Cummins, Merritt and Andrade, 2005). In present study, we focused on shredders, which directly feed on litter (Cummins, Merritt and Andrade, 2005). Chironomids larvae were not included in the FFG classification due to their generalistfeeding habits. The total biomass of shredders was calculated by summing the biomass of all identified invertebrate shredders.

Data analysis

We examined the temporal dynamics of litter storage and the effects of environmental factors, with additive models (GAM) using the "mgcv" package (Wood, 2011). Additive models allow for non-linear relationships between the response variable and multiple explanatory variables, in contrast to linear models (Hastie and Tibshirani, 1990). We performed linear mixed effects models (LMM) to analyze the relationship between shredders biomass, litter quantity, and litter mass ratios (C:N, C:P and N:P) over time. Initial multicollinearity tests revealed high correlation between C:P and N:P mass ratios ($r = 0.97$; Figure 3); therefore, we used only C:N and N:P mass ratios for the LMM models. Initial exploration of the data revealed the presence of two outliers, which were identified and removed in further analyses (Zuur et al., 2010). We used the "lme4" package (Bates et al., 2014) and the AICctab function from the "bbmle" package (Bolker and Bolker, 2017) to select the best random effect component for the model Season was included in the models as a random term to account for the influence of seasonality. As fixed effects, we used litter mass (as an indicative of the quantity) and mass ratios of C:N

and N:P (quality), and sampling time to analyze the variation of quantity and quality through time (without interaction term) into the model. Visual inspection of residual plots with "RVAideMemoire" package (Hervé, 2022) did not reveal any deviations from homoscedasticity or normality. P-values of models were obtained with "lmerTest" package (Kuznetsova, Brockhoff and Christensen, 2017) using Satterthwaite's degrees of freedom method. All analyses were performed using R software v. 4.1.3 (R Core Team, 2022).

RESULTS

Quantity and quality of litter temporal dynamic

Litter storage mass and N:P mass ratio varied over time (Table 1 and Figure 3). However, neither precipitation nor temperature influenced litter storage mass (Table 1 and Figure 3). But, litter C:N mass ratio did not show any consistent temporal pattern or significant association with climatic variables (Table 1 and Figure 3). Litter C, N and P masses showed variation over time, although they were not influenced by precipitation (Table 1 and Figure 4).

Variation of detritivores biomass and quantity and of litter quality over time

The detritivores biomass was influenced exclusively and negatively by the C:N ratio of litter (Table 2 and Figure 5). However, we did not find any temporal or seasonal pattern or influence of climatic (temperature and precipitation) or environmental factors (litter mass or litter chemical traits) on detritivore biomass (Table 2 and Figure 5).
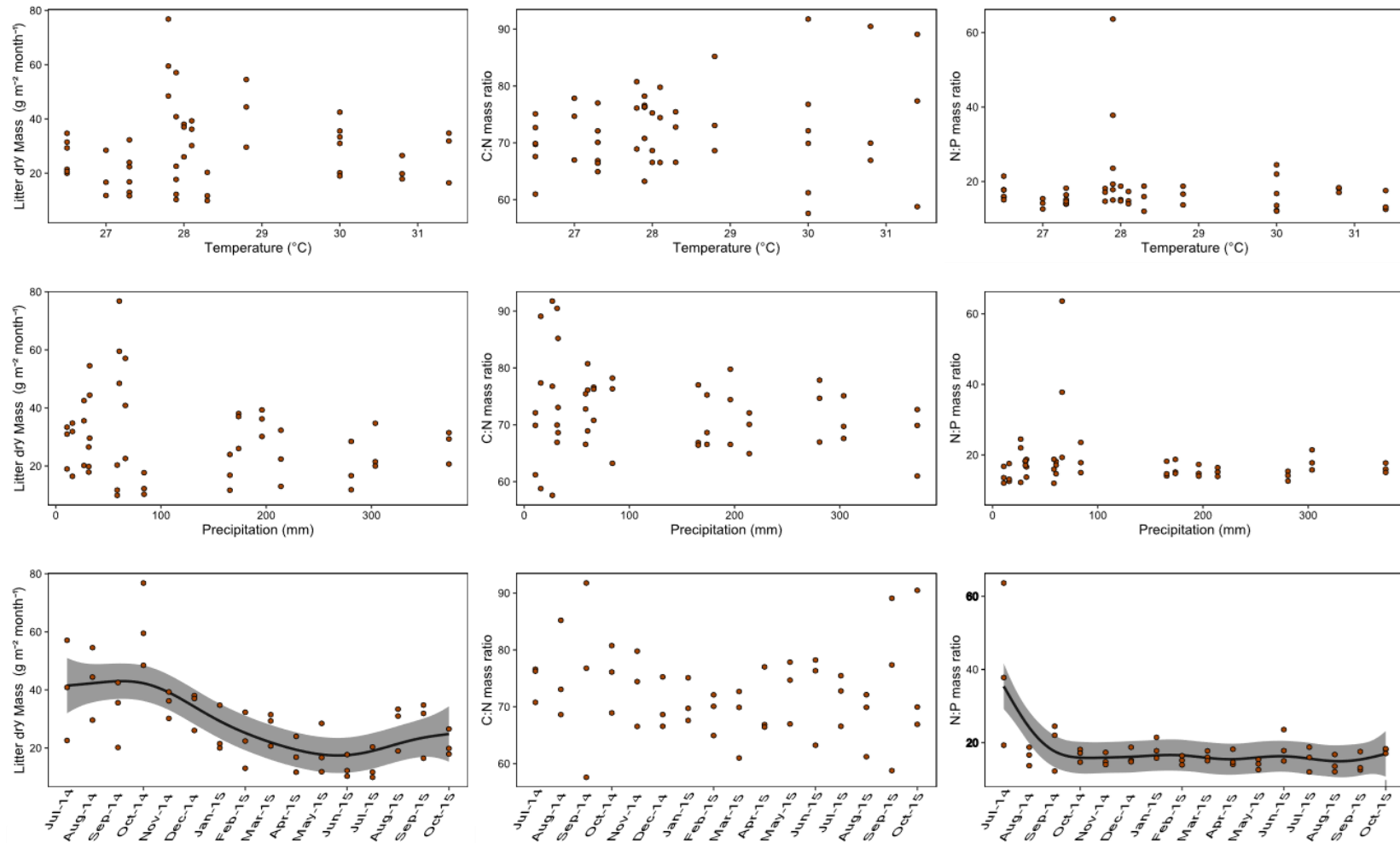
Figure 3. Relationship of litter storage dry mass, C:N and N:P mass ratios with variation of temperature and precipitation over 16 months in an Amazonian stream. C = Carbon; N = Nitrogen; P = Phosphorous.
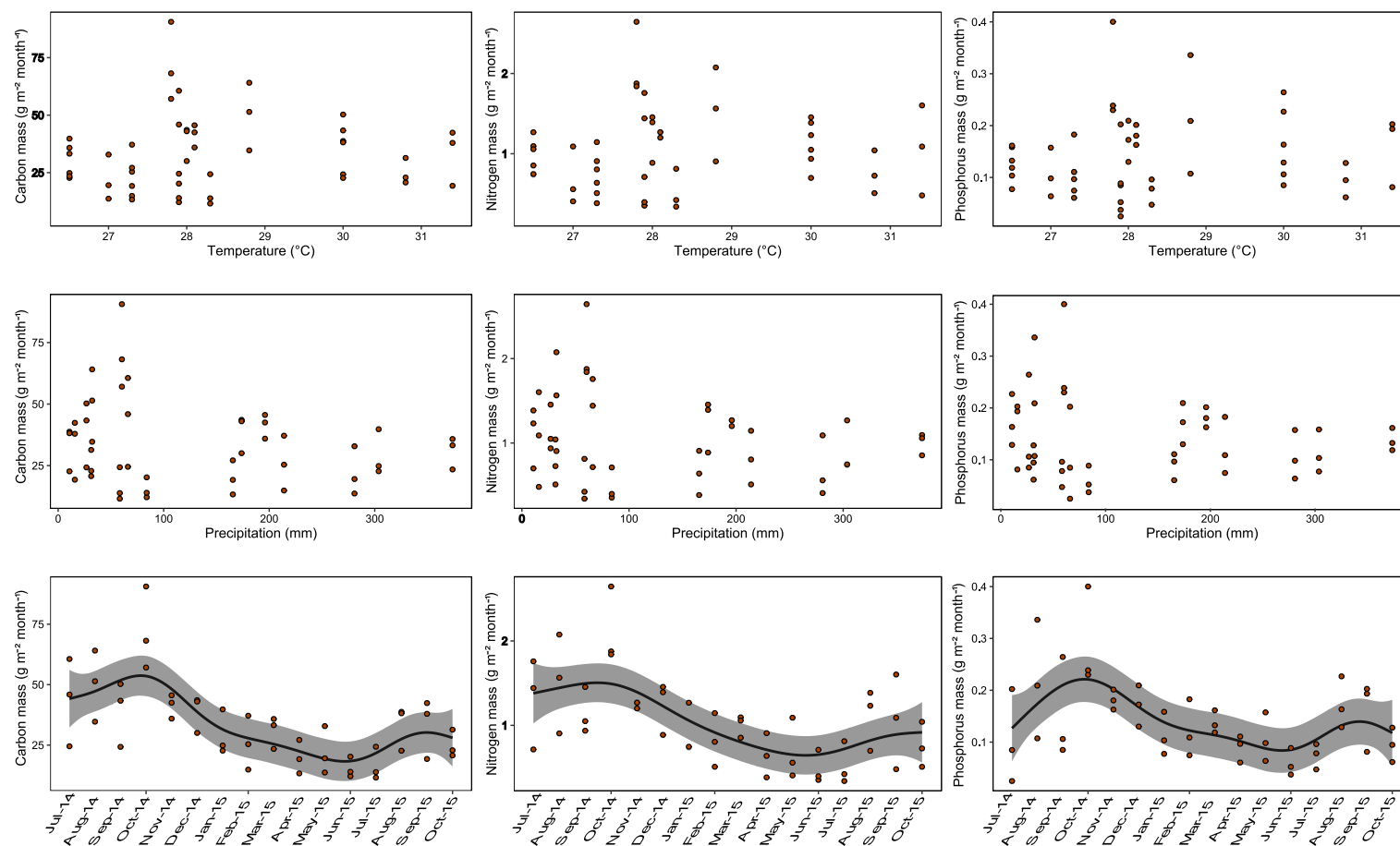
Figure 4. Relationship of Carbon; Nitrogen; Phosphorous in terms of mass (g m^{-2} month^{-1}) with variation of temperature and precipitation over 16 months in an Amazonian stream.

Table 1. Results of generalized additive models (GAMs) exploring non-linear temporal patterns of litter traits in terms of mass (g m^{-2} month^{-1}) or

C:N, C:P, and N:P ratios over over 16 months in an Amazonian stream. *C = Carbon; N = Nitrogen; P = Phosphorous*.

	Intercept	edf	F-value	p-value	R^2
Litter Storage					
Temperature	28.89	7.42	2.01	0.070	0.20
Precipitation	28.89	1.00	1.71	0.196	0.01
Time	28.89	4.01	8.27	**<0.001**	0.45
C:N Mass Ratio					
Temperature	72.39	1.00	1.42	0.238	0.01
Precipitation	72.39	1.00	1.92	0.172	0.02
Time	72.39	1.67	0.90	0.403	0.02
N:P Mass Ratio					
Temperature	17.79	1.00	0.16	0.686	0.03
Precipitation	17.79	3.34	1.25	0.305	0.06
Time	17.79	5.91	4.76	**<0.001**	0.40
Carbon Mass					
Temperature	33.51	7.38	1.95	0.080	0.19
Precipitation	33.51	1.00	2.09	0.155	0.02
Time	33.51	5.93	6.84	**<0.001**	0.49
Nitrogen Mass					
Temperature	1.03	7.20	1.65	0.141	0.25
Precipitation	1.03	1.00	1.62	0.20	0.01
Time	1.03	4.12	6.14	**<0.001**	0.17
Phosphorus Mass					
Temperature	0.14	1.06	0.38	0.524	0.01
Precipitation	0.14	1	0.99	0.324	0.01
Time	0.14	5.60	3.18	**<0.001**	0.29

Table 2. Results of linear mixed effects exploring temporal patterns detritivores biomass with litter storage, ratios of C:N and N:P and variation of

temperature and precipitation in an Amazonian stream. *C = Carbon; N = Nitrogen; P = Phosphorous*.

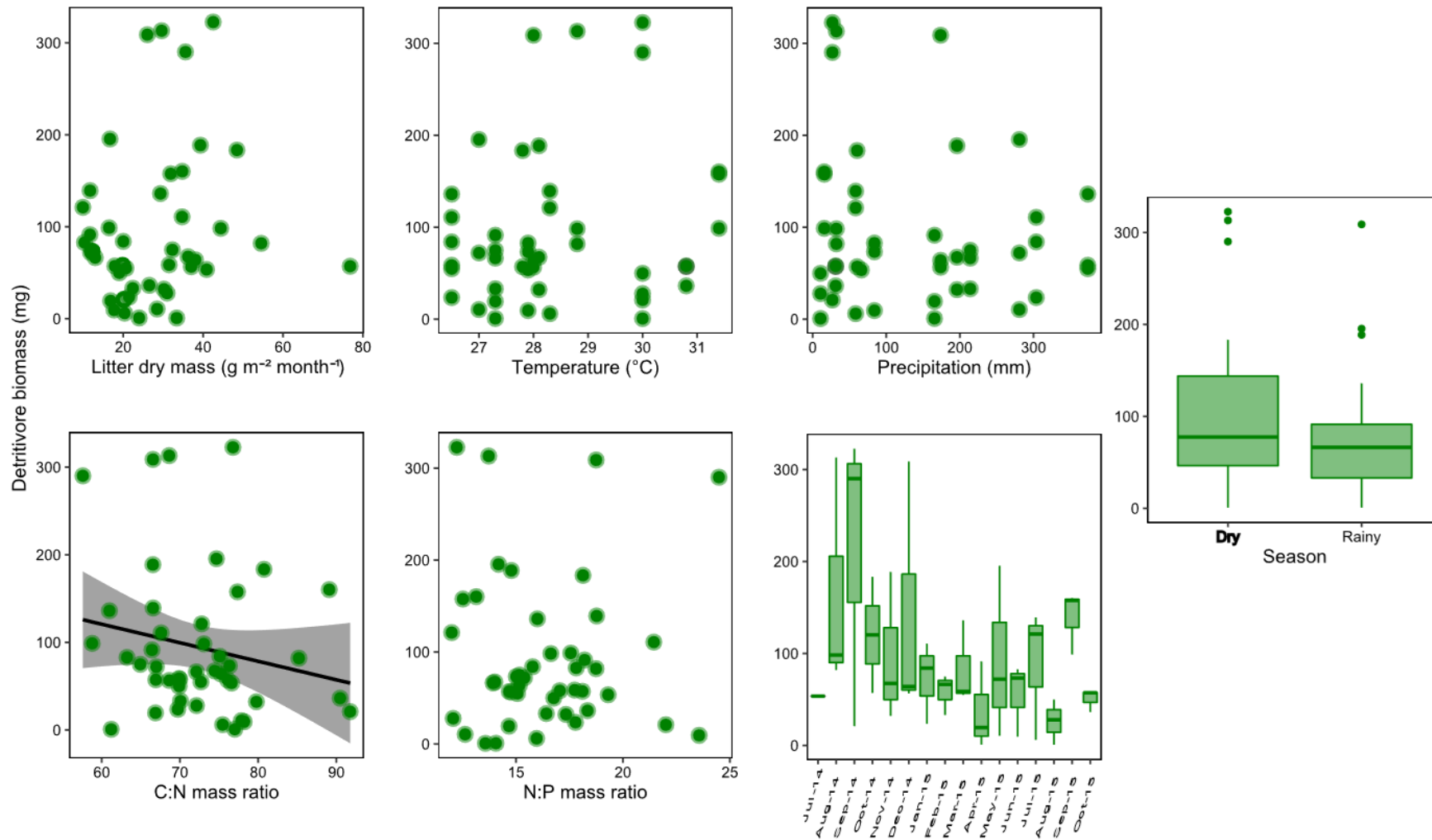| Source of variation	DF	Sum Sq	Mean Sq	F-value	p-value
Litter Storage	1	5056	5056	0.871	0.359
C:N Mass Ratio	1	37028	37028	6.378	**0.017**
N:P Mass Ratio	1	9672	9672	1.666	0.208
Precipitation	1	7273	7273	1.252	0.273
Season	1	7101	7101	1.223	0.549
Temperature	1	6520	6520	1.123	0.298
Time	12	121498	10125	1.744	0.114

Figure 5. Relationship of detritivores biomass with litter storage, C:N and N:P mass ratios, variation of temperature and precipitation, over 16 months and two seasons in an Amazonian stream. C = Carbon; N = Nitrogen; P = Phosphorous.

DISCUSSION

We showed no co-limitation of litter storage quantity and quality, indicating a limitation of detritivores biomass just by chemical litter storage quality. Although, the quantity and quality of food resources have been claimed as controlling factors for energy flow in the ecosystem, as well as co-limiting factors for metabolism and nutrient acquisition by consumers (Cross et al., 2006; Sperfeld el at., 2016; Halvorson, Sperfeld and Evans-White, 2017). Therefore, our findings suggest that litter quality is the preponderant factor controlling litter fragmentation, which is consistent with previous studies in tropical streams (Martins et al., 2015; Gonçalves et al., 2017; Sena et al., 2020).

We did not find a pattern in the temporal dynamics of litter storage mass. Litter storage can be influenced by variation of litterfall, lateral entrances, and downstream transport (Rezende et al., 2016; Tonin et al., 2017). In addition, litter storage can be determined by water flow conditions, precipitation, the stream retention capacity, and litter decomposition rate (Tonin et al., 2017; Elosegi and Pozo, 2020; Nuven et al., 2022). Although we expected a high variance of litter storage in tropical streams, our results have indicated less predictable temporal dynamics for this Amazonian stream than our model for Amazonia biome (Tonin et al., 2017). Surprisingly, the lack of influence of precipitation on litter storage, which contradicts the idea that increased precipitation and, consequently, the rise of stream flow should lead to higher downstream litter transport and decreased deposition on the streambed (Marshal, Iskin and Wohl; 2021; Nuven et al., 2022). These unexpected findings may be related to (i) the intrinsic characteristics of the stream; (ii) the noises in the local precipitation that diminish the prediction of the model and/or the effects of climate change; (iii) the study duration was enough to establish the relationship among variables. These hypotheses need to be tested in future studies.

We did not find variation of litter storage C:N and C:P mass over time. The absence of the temporal or climatic influence on these mass ratios variation may be influenced by variations of some factors such as leaf size and weight, physiology, phylogeny and composition of riparian plant species (Tonin et al., 2021; Nuven et al., 2022; Sena et al., 2022). Nevertheless, we observed variations in the mass of litter C, N and P over time. Litter chemical characteristic is a response to the trade-offs between plant metabolism, resources availability, and plant-environment relationship (Weiher et al. 1999, Craine et al. 2001). These variations in chemistry of litter storage may be due to (i) the assimilation of N and P, and C uptake in response to the environment, mainly precipitation, which can alter litter chemical characteristics, depending on the riparian zone plant species (Tonin et al., 2021); (ii) changes in litter retention capacity, resulting from different sizes and weights, as a function of increasing precipitation and, consequently, the flow of the stream (Nuven et al., 2022); (iii) inter and intraspecific chemical characteristics differences in litter (Danger 2020). Thus, it indicates that the chemical characteristics of litter may be influenced by environment variations, suggesting that dominant species of tropical riparian zones play an important role in the availability of resources for decomposers.

Contrary to our hypothesis, we did not find influence of litter storage mass on shredding detritivores biomass. However, it is well established that litter quantity variation can affect several processes such as colonization, consumption, and litter breakdown by shredders, in both temperate and tropical streams (Tiegs et al., 2008 Ferreira, Encalada and Graça, 2012; Martins et al., 2015; Arias-Real et al., 2018). Although shredding detritivores biomass is not linked to changes in litter availability, it may be related to variation in the quantity of better-quality litter available, as shredder tend to prefer less recalcitrant and more nutrient-rich litter (Arias-Real et al., 2018; Sena

et al., 2020). Thus, changes in plants' composition in the Amazon riparian forest may affect not only the processing of organic matter in streams but also the nutrient pathway driven by shredding detritivores (Bambi et al., 2022; Oester et al., 2022).

We partially confirmed our hypothesis that an increase in the litter C:N mass ratio negatively influence the biomass variation of shredding detritivores. This result could indicate a recalcitrant effect of detritus, whereby higher biomass values seems to be linked with low C:N mass ratio of higher-quality litter. The complexity of interaction between shredders and litter storage is high and depend on the plant species that compose the litter, rather than quantity. We observed limitation based on the expression of plant species that can change the quality of the litter pool, expressing a low value of the C:N mass ratio (Gessner, Chauvet and Dobson, 1999; Jabiol et al., 2019; Zhang et al., 2019). Furthermore, our findings might show that a dystrophic effect of C (an increase in C:N mass ratio) leading a direct limitation of N (an essential element to form proteins, nucleic acids and other cell components; Gruber and Galloway, 2008) in Amazonian shredding detritivores (Sena et al., 2020; Navarro and Carneiro, 2022). This bottom-up control in the food web, driven by litter processing (Martins et al., 2015; Gonçalves et al., 2017; Rezende et al., 2018; Sena et al., 2020) needs to be interpreted with caution because the study was performed in a single stream in the Amazon basin for only one and a half year. In addition, it is necessary to highlight some other possibilities that could influence the increment of biomass by the shredders, such as the influence of the biofilm on the litter, the tested organisms not being exclusive shredders, dense-dependent and competition effect (Rezende et al., 2019; Allen et al., 2020).

We demonstrated that the biomass of shredding detritivores in an Amazonian stream may not be co-limited by the quantity and quality of litter storage mass over time, but instead may be limited by the variation in C:N mass ratio of litter storage mass. Our

study provides evidence for possible repercussion to the food web and nutrient dynamics in Amazonian streams due to N availability limitation affecting shredders' metabolism. There results deepen our understanding of the functioning Amazonian streams and can drive policies for conservation and restoration from riparian zones in the Amazonia River Basin.

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CAPÍTULO 3 - LITTER TRAITS AND STREAM FLOW DRIVE LEAF BREAKDOWN IN TROPICAL STREAMS

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ABSTRACT

Plants of riparian forests provide abundant plant litter – mostly dead leaves for terrestrial and freshwater stream ecosystems, which support detritus-based food webs. The increased replacement from natural riparian forests to *Eucalyptus* plantations, as an exotic species, has been seen as a significant stressor of key ecosystem processes such as leaf breakdown. We evaluate the influence of native and exotic leaf species with distinct physical and chemical characteristics (traits) on the breakdown in two different tropical reference condition streams. We tested the hypothesis that regardless of the leaves' origin, leaf litter with higher nutrients and less recalcitrant compounds has higher decay rates. *Eucalyptus camaldulensis* leaf breakdown was faster than *Miconia chartacea,* the native species from tropical riparian forests. At the end of the experiment *M. chartacea* had 24% and 12% more mass remaining in both streams, respectively. Physical and chemical characteristics of *E. camaldulensis* may have led to more preference for consumption by decomposers. The higher leaf breakdown of *E. camaldulensis* was evidenced in the stream with the most increased water flow, further accelerating the leaf breakdown. However, leaf species did not influence the density of the macro detritivore feeding groups. Our findings suggest that *Eucalyptus* plantations may influence leaf breakdown, accelerating the organic matter and energy flows in tropical streams.

Keywords: Leaf decomposition; Alien species, Plant detritus; Neotropical; Nutrient cycling; Ecosystem functioning.

INTRODUCTION

In several regions of the planet there is a considerable increase in monoculture plantations due to the demand for wood products and/or the establishment of the carbon sequestration market (Zomer et al., 2008). At the same time, the total natural forests area has decreased worldwide, while the area of forest monoculture plantations has increased (Payn et al., 2015). The most planted forest genus in the world is *Eucalyptus* with ca 25 million ha planted (Chen et al., 1996; Martins et al., 2022). Thus, one of the largest *Eucalyptus* producers in the world, out of Australia, is Brazil with about 7.6 million ha planted (IBGE, 2019). Meanwhile, the transformation of natural riparian forests into exotics monoculture plantations may result in changes on freshwater ecosystem processes (Gonçalves et al., 2014; Fernández et al., 2016).

Eucalyptus trees have intrinsic characteristics that may affect both the terrestrial and aquatic ecosystems (Fernández et al., 2006; Lara et al, 2009). Eucalyptus can make the soil hydrophobic due to oils released from its leaves, hampering rainwater to penetrate and replenish underground water (Fernández et al., 2006; Lara et al, 2009). Besides, its high-water demand may lead to decreases in runoff and water flow in streams (Fernández et al., 2006; Amazonas et al., 2018). Furthermore, the presence of *Eucalyptus* in the riparian zone can influence the flow of matter and energy between terrestrial and aquatic ecosystems (Ferreira et al., 2015; 2019). Thus, changes in leaf phenology and litter quantity and quality into streams due to differences of intrinsic characteristics of native riparian trees (Molinero and Pozo 2004, 2006). This replacement of native riparian forests by *Eucalyptus* plantations may strongly affect the litter breakdown, a fundamental ecosystem process for the flow of energy and matter and the maintenance of biodiversity in forested headwater streams (Graça et al., 2015; Gomes et al., 2016; Ferreira et al., 2019).

Forested streams are generally small order rivers located in high altitude regions, with marginal vegetation that often shades the bed, limiting autochthonous primary production (Vannote et al, 1980, Neres-Lima et al., 2017). The contribution of allochthonous organic matter, mainly leaves, is the main source of energy for the metabolism of these streams (Esteves and Gonçalves, 2011; Bambi et al., 2017). Therefore, leaf breakdown is the main energy source available to forested streams (Graça, 2001; 2015). Leaf processing in freshwater ecosystems has three main phases: leaching of water-soluble compounds that occurs by the action of the physical abrasion of water flow, which can have a great influence on the litter mass loss (Nuven et al., 2022); conditioning by aquatic hyphomycetes and bacteria; and fragmentation by physical abrasion and shredders (Graça et al., 2015). Although often analyzed separately, these leaf breakdown phases are simultaneous and interdependent (Gessner, Chauvet and Dobson, 1999).

Previous studies carried out in temperate regions point to a delay in colonization and a reduction in the consumption of *Eucalyptus* leaves by shredders and lower leaf breakdown rates (Gonçalves and Canhoto, 2009; Ferreira et al., 2015; 2019). A recent global assessment found an overall significant inhibition of total litter *Eucalyptus* breakdown (Ferreira et al., 2019). Over time, studies evaluating the influences of *Eucalyptus* litter breakdown as alien litter on tropical freshwater ecosystems (out of Australia) have increased. Some studies indicate an increase in the colonization of shredders and, consequently, an acceleration of *Eucalyptus* litter breakdown (Gonçalves et al., 2012; 2014; Ferreira et al., 2019). Others found a decrease in the diversity of the microbial community comparing to litter from naturally occurring riparian tree species (Gomes et al., 2016; 2018). Such distinct responses in the decomposition of *Eucalyptus*

litter may occur because its higher nutritional quality than other tropical native plant species (Gomes et al., 2018).

Litter traits refer to a set of intrinsic physical, chemical and morphological attributes of plant leaves, quantified in terms of palatability, toxicity, and nutritional value for terrestrial and aquatic decomposers (Gessner, Chauvet, & Dobson, 1999; Graça et al., 2015). Litter physical characteristics such as toughness (Berg and McClaugherty, 2003; Zhang et al., 2019), concentrations of recalcitrant compounds as lignin and polyphenols (Moretti et al., 2009; Jabiol et al., 2019), nutritional concentration of nitrogen (N) and phosphorus (P) (García-Palacios et al., 2016) are important moderators of leaf breakdown. The set of attributes is commonly referred to as 'litter quality', whereas litter of higher quality includes soft leaves, with high nutrient concentrations and low structural and secondary 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; García-Palacios et al., 2015). Therefore, leaves of high nutritional quality decompose faster than low quality leaves (Zhang et al., 2019; Sena et al., 2020).

Despite the worldwide importance of *Eucalyptus* all repercussions of its litter breakdown as alien litter on tropical streams have not yet been fully fulfilled. We filled this gap by conducting a field experiment in two neotropical savanna headwater streams. We aimed to assess the influence of native and exotic leaf species with distinct physical and chemical characteristics on leaf breakdown in Neotropical savanna streams. We assume the premises that leaf breakdown (i) is the result of the activity and influence micro and macro decomposers, (ii) litter physical and chemical characteristics (traits) are constraints for organisms, (iii) water flow influence stream physical and chemical processes. We hypothesized that (i) Regardless of the leaves' origin, leaf litter with higher

nutrients and less recalcitrant compounds has higher litter mass decay; (ii) Increased water flow from streams accelerates litter fragmentation.

METHODS

Study sites

The experiment was performed in two reference condition streams. The Pedras stream is located at the Serra do Cipó National Park (19°12'S, 19°34' and 43°27'W, 43°38'), and the Taboões stream is located in The Serra do Rola Moça State Park (20°02'S and 44°0'W), Minas Gerais state, southeastern Brazil (Figure 1).
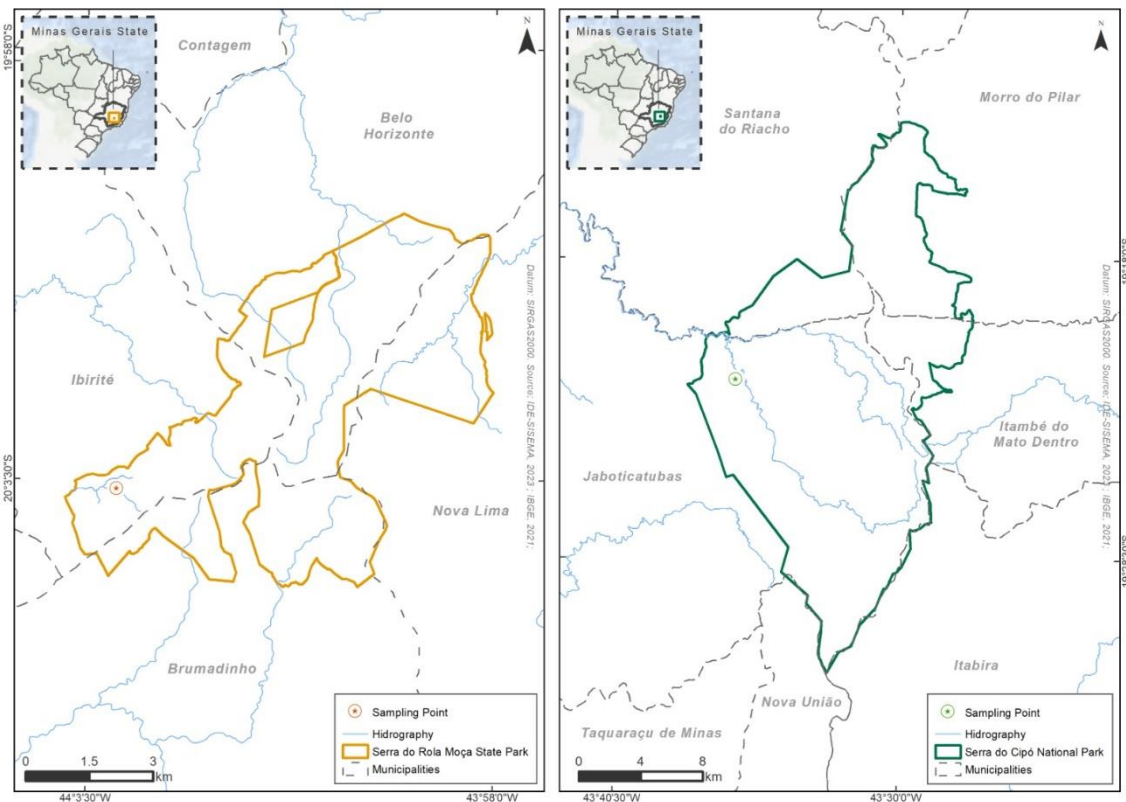

Figure 1. Location of experiment sites at the Taboões stream located in the Serra do Rola Moça State Park (orange area) and the Pedras stream located in the Serra do Cipó National Park (green area).

The climate of the Serra do Cipó National Park is of high altitude tropical type, with cool Cwb summers and a pronounced dry season, according to Köppen and Geiger (1930). It fits into the type of bioclimate called moderately humid sub-tropical, which due to the marked dry season, presents an annual water deficit that can reach 60 mm. Average annual temperatures range between 17º and 18.5ºC and average rainfall between 1,450 and 1,800 mm, causing a potential annual evapotranspiration of 700 to 850 mm (ICMBio, 2023). At least three major plant formations can be defined due to geographic positioning, the varied morphology of the rugged soil and the climate variation at different altitudes, which are Campos de Cerrados, Campos Rupestres and Matas de Galerias (Gonçalves et al., 2006).

The Serra do Rola Moça State Park is in a transition zone from the Cerrado (Neotropical savanna) to the Atlantic Forest biomes. The climate of the park is tropical at altitude, the annual average temperature is 15.3°C and 21.4°C (IEF, 2023). The average altitude of 1,300 meters. It has rugged relief contributing to the various ecological conditions. The relief of the park is extremely rugged, including some mountains such as Serra do Rola-Moça, Serra do Jatobá, among others (IEF, 2023). The rainfall regime well represents the characteristics of the southeastern region of Brazil, with rainy summers and dry winters (Peixoto, 2004). Although the Park is open to public visitation, the spring areas cannot be used by visitors, which guarantees the maintenance of water quality.

Field sampling

The experiment was carried out from April to September 2005 in the Pedras stream (3rd order, around 960 m altitude, PARNA Cipó) and in the Taboões stream (2nd order, around 1180 m altitude in the PE da Serra do Rola-Moça). To collect leaves, plastic nets were tied to riparian forest trees. In this way, the leaves that naturally fell from the trees were retained in the nets and protected from contact with the soil and its

microorganisms, preventing a premature start of the decomposition (Tonin et al., 2017). The leaves retained in the nets were taken to the laboratory, separated, identified and air-dried. Leaves from *Miconia chartacea* Triana was chosen as native litter because of large occurrence in tropical riparian vegetation zones in Brazil and *Eucalyptus camaldulensis* Dehnh. as a non-native litter. As described by Gomes et al. (2016) and Sena et al. (2022), *Miconia chartacea* has higher concentrations of lignin (mean 25 % dry mass, DM), cellulose (21 % DM), lignin:N ratio (16) and lower N (1.5 % DM) and P (0.2 % DM) than *Eucalyptus camaldulensis* (17 % DM, 17 % DM, 11, 1.6 % DM and 0.5% DM, respectively).

Leaves from each species were placed separately in litter bags with 10 mm mesh size. The total litter placed in each litter bag was 3 ± 0.1 g air-dry weight. The litter bags removal periods were at time intervals of 3, 7, 15, 30, 60, 90, 120 and 150 days or until the litter had lost more than 70% of its initial mass (visually estimated value). Four litter bags of 10 mm mesh of each species were removed in each collection period. After each removal period, the litterbags were collected, placed individually in plastic bags and taken to the laboratory in ice boxes. The leaves were cleaned with distilled water in a sieve (250 µm mesh) to retain the invertebrates associated, preserved in 70% ethanol, dried (60ºC, 72h) and weighed to the nearest 0.001g. The invertebrates were identified according to Pes et al. (2005), Domínguez and Fernández (2009) and Hamada et al. (2019). We estimated the total biomass of detritivores for each removal period for both litter species, in both streams. Thus, collected detritivores were classified into different functional feeding groups (FFGs), according to Cummins et al. (2005). FFGs were divided into 5 groups: shredders are those that feed on coarse organic matter (MOPG). Other organisms feed on fine particulate organic matter (FPOM), filtering it in the water column were classified as filtering-collectors, and the ones that collect FPOM directly on the substrate

by gathering-collectors. Predators are those that obtain their nutrients from the tissues of other animals. Scrapers feed on the biofilm that grows on submerged surfaces such as rocks, logs and leaves. The density of each FFG was the relationship between the abundance and the remaining mass of leaf litter in each period (individuals/ g leaf dry mass). Chironomidae larvae were not included in the FFG classification due to their generalist-feeding habits (Pes et al., 2005; Domínguez and Fernández, 2009; Hamada et al., 2019).

In the field, the water's physical and chemical characteristics were evaluated at each collection: temperature, pH, electrical conductivity and turbidity, which were measured in the stream with portable field equipment (DIGIMED brand). Dissolved oxygen (Winkler method) was measured in the laboratory following the methodology proposed in the Standard Methods for the Examination of Water and Wastewater (American Public Health Agency, 2001). A flowmeter (model SWOFFER 2100 series) performed the water flow measurement in each stream per sampling.

Table 1. Water characterization with mean values and standard deviations for each variable analyzed in the studied stretches of Pedras and Taboões streams. Different letters indicate statistically significant differences between streams.

	Mean ± Standard Deviation
Pedras Stream	
Dissolved oxygen (mg L^{-1})	9.67 ± 1.62^a
Water Temperature (ºC)	20.4 ± 2.04^a
pH	6.51 ± 0.09^a
Conductivity ($\mu S/cm^2$)	4.28 ± 0.18^a
Water Flow (m/s)	0.084 ± 0.023^a
Taboões Stream	
Dissolved oxygen (mg L^{-1})	10.7 ± 1.55^b
Water Temperature (ºC)	21.47 ± 1.39^b

pH	7.21 ± 0.33^{b}
Conductivity (μS/cm^2)	11.64 ± 0.21^{b}
Water Flow (m/s)	1.09 ± 0.22^{b}

Data analyses

To verify the similarity of the physical and chemical characteristics of the streams, Student's t test was performed using the t.test function from *stats* package (R Core Team, 2022). Initial multicollinearity tests revealed that pH and conductivity are highly correlated ($r = 0.85$) and later high correlation between pH and water flow ($r = 0.83$); we thus used detritivores biomass, water dissolved concentration, water flow, water temperature, litter species and streams to run the models. We performed Analysis of Variance (Anova) to analyze the relationship between litter mass loss, detritivores biomass, water dissolved oxygen concentration, water flow, water temperature, pH, conductivity, litter species and streams over time. The selection of the best model was performed using AICctab function from *bbmle* package (Bolker, 2022). The multiple linear regression model without random effects had best performance. Visual inspection of residual plots with *RVAideMemoire* package (Hervé, 2022) did not reveal any deviations from homoscedasticity or normality. We find difference of litter mass loss between streams, thus, the models were tested again for each stream separately. To test the influence of the two leaf species on the density of different FFG, we performed linear mixed effects models (LMM) with *lme4* package (Bates, Maechler and Bolker, 2012) and the AICctab function from the *bbmle* package (Bolker, 2022) to select the best random effect component for the model. Time was included in the models as a random term to account for the influence of time during the leaf breakdown. As fixed effects, we used

leaf species, streams, and sampling time (without interaction term) into the model. For LMM, P-values of the models were obtained with *lmerTest* package (Kuznetsova, Brockhoff and Christensen, 2017) via using Satterthwaite's degrees of freedom method. Visual inspection of residual plots with *RVAideMemoire* package (Hervé, 2022) did not reveal any deviations from homoscedasticity or normality. All analyses were performed using R software v. 4.1.3 (R Core Team, 2022).

RESULTS

Litter mass loss at streams

E. camaldulensis had less remaining mass than *M. chartacea* at the end of the experiments, with much of the initial mass lost within 120 days, while *M. chartacea* lasted up to 150 days with 24% more remaining litter mass in the Pedras stream (Table 2; Figure 3A; Figure 2A). As well the increase in O_2 concentration and water flow is related to the decrease in litter remaining mass over time (Table 2; Figure 2A). Nonetheless, the associated detritivores biomass and water temperature did not show significant relationship with litter mass loss (Table 3; Figure 2A).

We also observed the same pattern where *E. camaldulensis* had less remaining mass than *M. chartacea,* but much of the initial mass of *E. camaldulensis* was lost within 30 days, while *M. chartacea* lasted up to 120 days with 12% more remaining litter mass in the Taboões stream (Table 2; Figure 3B; Figure 2B). As well the increase of water flow is related to the decrease in the litter remaining mass (Table 2; Figure 2B). Unlike the Pedras stream, the increase of associated detritivores biomass is also related to the decrease in the litter remaining (Table 2; Figure 3B).

Densities of all FFGs were not influenced by leaves of both species (Table 3; Figure 4). Shredders had higher density in the Taboões stream (Table 3; Figure 4C). The

gathering-collectors and predators had higher densities in the Pedras stream, with increasing of density through the litter breakdown (Table 3; Figure 4B; 4D). The scrapers, on the other hand, showed a density increase through the litter breakdown (Table 3; Figure 4E). The density of filtering-collectors was not related with leaves species, streams or litter breakdown (Table 3; Figure 4A).
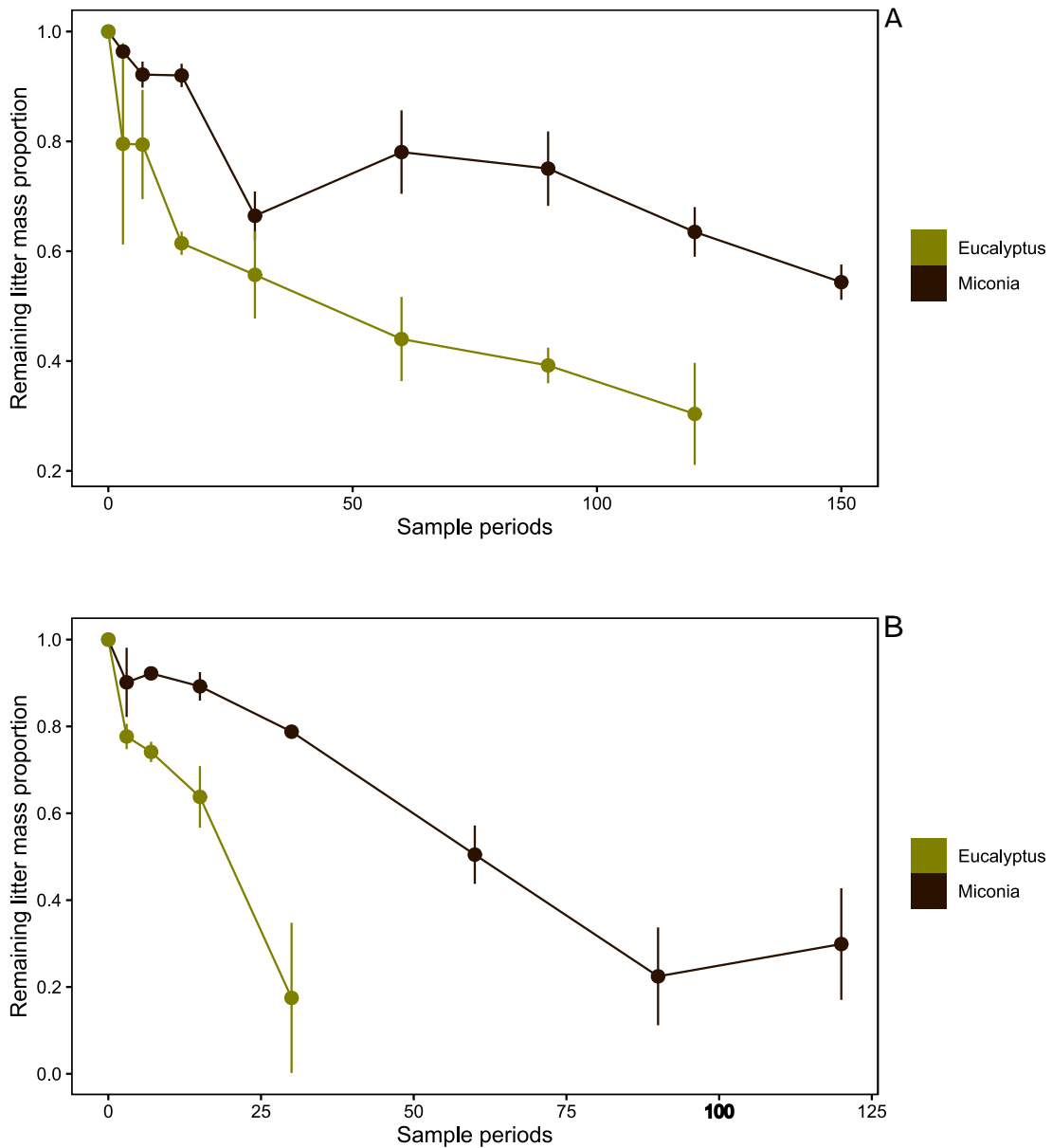

Figure 2. Remaining litter mass proportion of *Eucalyptus camaldulensis* and (olive lines) and *Myconia chartacea* (brown lines) leaves until the end of the experiments in Pedras (A) and Taboões (B) streams.

Table 2. Results of linear model exploring temporal patterns of remaining litter mass proportion with detritivores biomass, O_2 concentration, water flow, water temperature and *Eucalyptus camaldulensis* and *Myconia chartacea* leaves; and sample periods.

Source of variation	DF	Sum Sq	Mean Sq	F-value	p-value
Pedras Stream					
Detritivores Biomass	1	0.062	0.062	2.438	0.123
O_2 Concentration	1	0.483	0.483	18.925	**<0.001**
Water Flow	1	0.166	0.166	6.514	**0.013**
Water Temperature	1	0.031	0.031	1.248	0.268
Leaf Species	1	0.863	0.863	33.833	**<0.001**
Sample Periods	1	1.548	1.548	60.645	**<0.001**
Residuals	63	1.608	0.025		
Taboões Stream					
Detritivores Biomass	1	0.804	0.804	25.278	**<0.001**
O_2 Concentration	1	0.079	0.079	2.489	0.120
Water Flow	1	0.432	0.432	13.489	**<0.001**
Water Temperature	1	0.015	0.015	0.479	0.268
Leaf Species	1	0.300	0.300	9.385	**0.003**
Sample Periods	1	3.036	3.036	94.797	**<0.001**
Residuals	54	1.729	0.032		

Table 3. Results of linear mixed effects exploring temporal patterns of density of Filtering-collectors, Gatherering-collectors, Shredders, Predators and Scrapers detritivores with *Eucalyptus camaldulensis* and *Myconia chartacea* leaves; Pedras and Taboões streams and sample periods.

|	DF	Sum Sq	Mean Sq	F-value	p-value
Filtering-Collectors					
Leaf Species	1	24.09	24.09	0.06	0.796
Local	1	463.89	463.89	1.29	0.258
Time	1	1219.04	1219.04	3.39	0.099
Gatherering-Collectors					
Leaf Species	1	10353	10353	0.85	0.357
Local	1	13965	13965	11.51	**<0.001**
Time	1	98366	98366	8.10	**0.020**
Shredders					
Leaf Species	1	4.65	4.65	0.07	0.791
Local	1	2938.76	2938.76	44.39	**<0.001**
Time	1	29.53	29.53	0.44	0.521
Predators					
Leaf Species	1	8.60	8.60	0.01	0.899
Local	1	8729.60	8729.60	16.07	**<0.001**
Time	1	9809.40	9809.40	18.06	**<0.001**
Scrapers					
Leaf Species	1	69.32	69.32	3.60	0.059
Local	1	64.08	64.08	3.33	0.070
Time	1	207.14	207.14	10.78	**0.025**
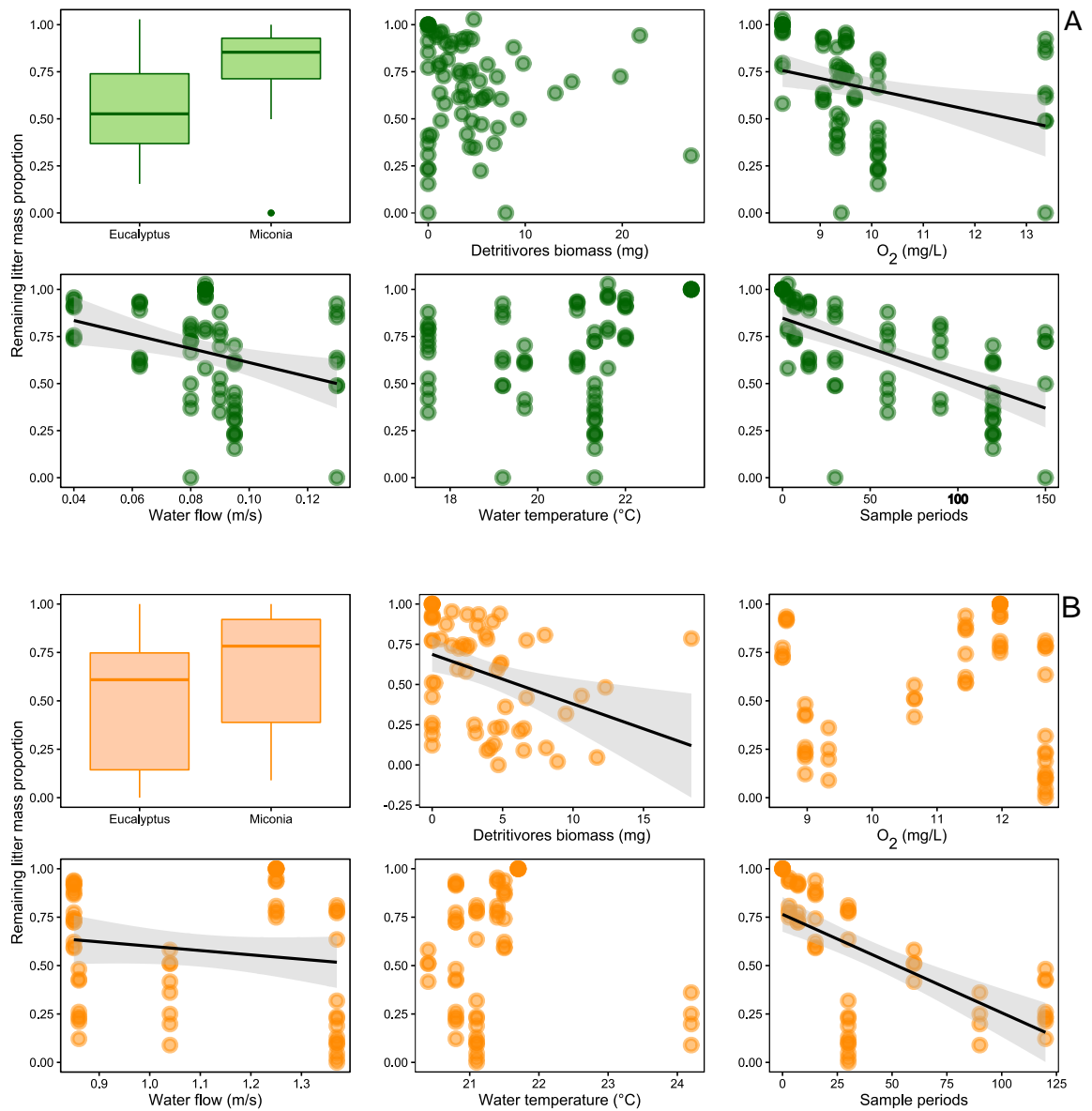
Figure 3. Relation of remaining mass proportion with *Eucalyptus camaldulensis* and *Myconia chartacea* leaves, detritivores biomass, O₂ concentration, water flow, water temperature and sample period. (**A**) Pedras stream (green); (**B**) Taboões stream (orange).
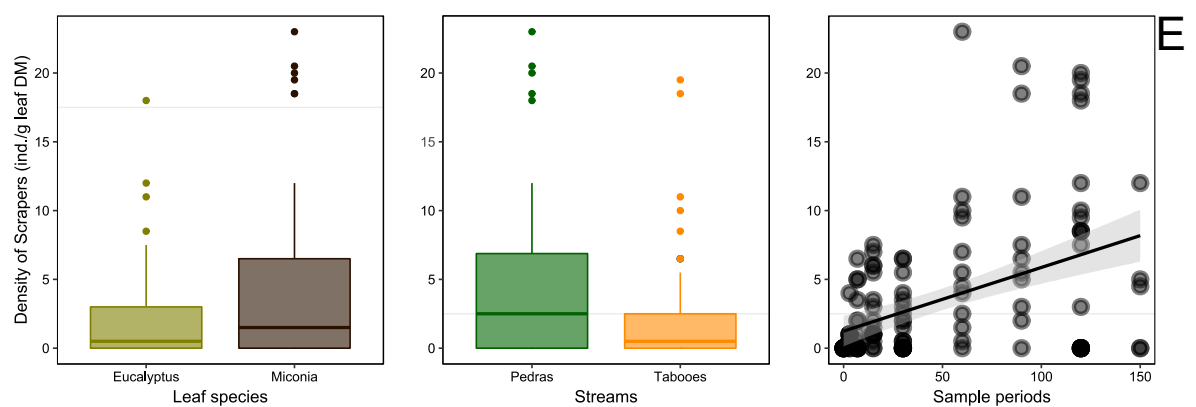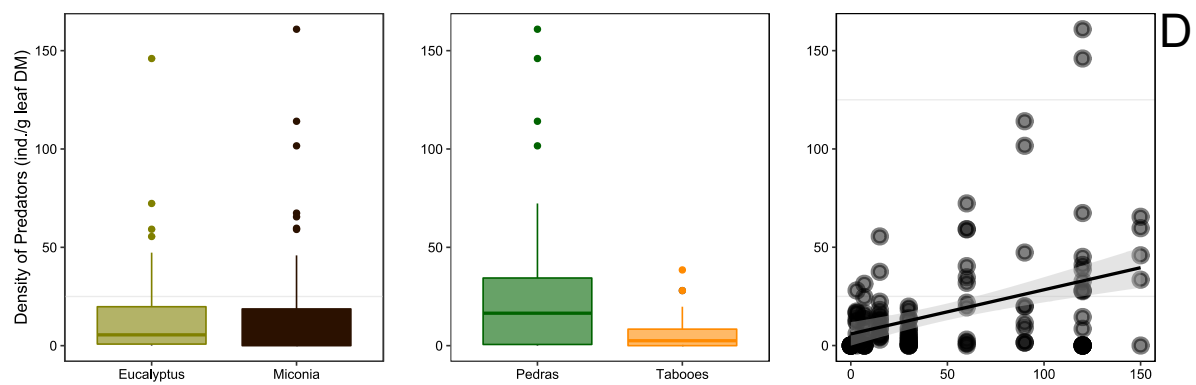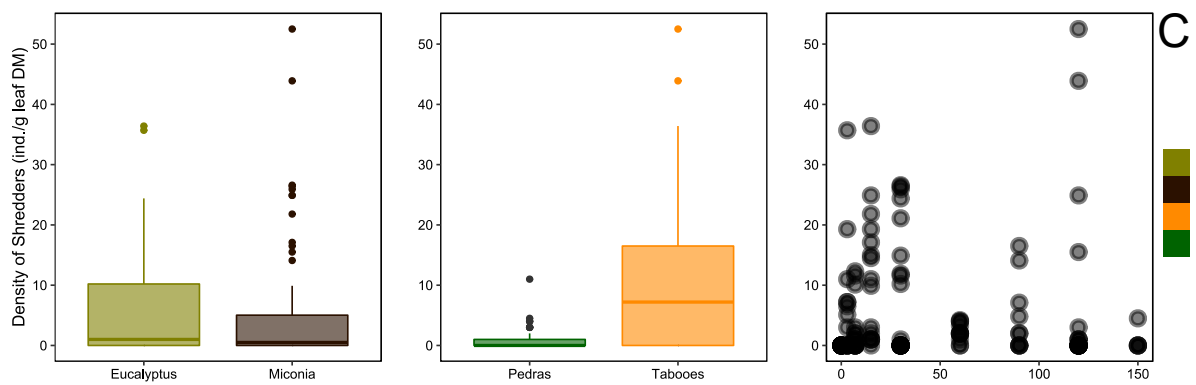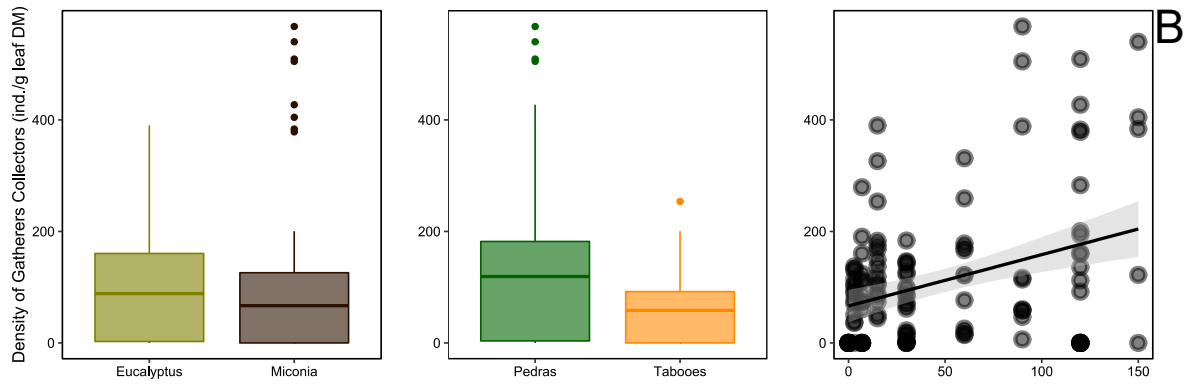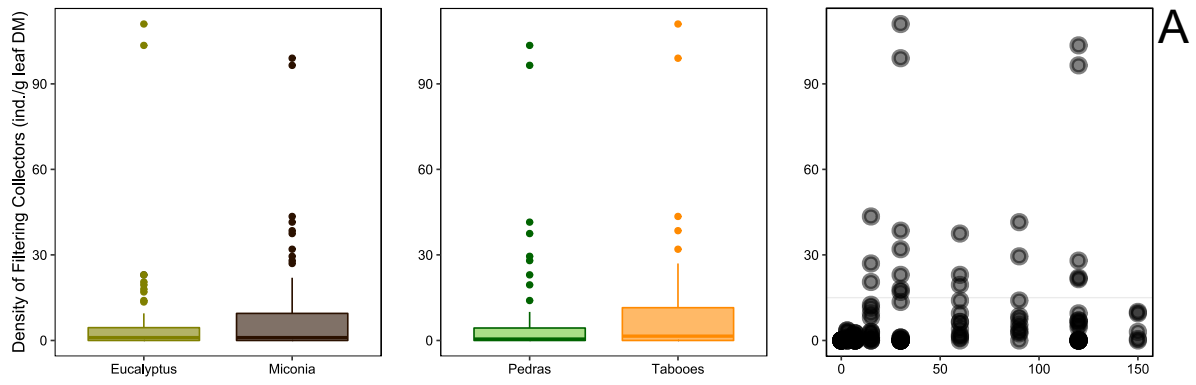
Figure 4. Relation of densities of **(A)** Filtering-collectors, **(B)** Gatherering-collectors**, (C)** Shredders, **(D)** Predators and **(E)** Scrapers with *Eucalyptus camaldulensis* (olive) and *Myconia chartacea* (brown) leaves; Pedras (green) and Taboões (orange) streams.

DISCUSSION

Our data showed that in the two different tropical streams, alien leaf litter tended decaying more rapidly the litter mass than the native leaf litter. In both streams, *E. camaldulensis* had higher litter mass decay than *M. chartacea*. Futhermore, litter mass decay may be also driven by increased water flow into the streams, becoming more evident in the Taboões stream, with higher water flow and litter mass decay. These findings reinforce that litter physical and chemical characteristic (but see Rezende et al., 2023) and the water flow as controlling factors for litter breakdown in tropical streams (Gonçalves et al., 2017; Sena et al., 2020; Nuven et al., 2022), supporting our hypotheses. Therefore, the replacement of natural plant species of riparian zones for alien plant species as *Eucalyptus* may lead to modifications in organic matter dynamics and per instance litter mass decay, affecting the functioning of tropical streams.

This lower litter mass decay of *M. chartacea* observed at both streams must occur due mainly the recalcitrant compounds as lignin and cellulose. High concentration of recalcitrant compounds as lignin and cellulose has a negative influence on litter colonization of decomposing organisms in tropical streams (Medeiros et al., 2015; Rezende et al., 2018). Litter with high refractive compounds and less nutrient concentrations tend to hind microbial colonization (Sales et al., 2015), and hence affecting invertebrate consumption (Graça et al., 2015). *Eucalyptus camaldulensis* physical and chemical characteristics may make it a preferred litter for consumption by decomposers (Gonçalves et al., 2014; Gomes et al., 2018), mainly due to the higher feeding selectivity of shredders on higher quality litter in tropical streams (Gonçalves et al., 2017; Sena et

al., 2020). This directly implies on litter breakdown, accelerating it and transforming the structure of the decomposer community (Gonçalves et al., 2012; Graça et al., 2015; Gomes et al., 2018).

In addition to variations in the inherent litter characteristics, aquatic decomposers and, consequently, the litter breakdown is sensitive to environmental conditions (Boyero et al., 2016; Yue et al., 2022). Water flow influenced litter mass decay in both streams, reinforcing that physical abrasion of the water is one of the main drivers to litter decay (Nuven et al., 2022; Ferreira, Graça & Elosegi, 2023). Besides, we also must consider in this process the composition of the invertebrate assemblage present in litter breakdown, abiotic characteristics, the ability of invertebrates to resist water flow (Smith et al, 2003; Barquin and Death, 2004) and colonization by microorganisms (Abelho, 2001; Graça et al., 2015; Gomes et al., 2018). The increase in invertebrate densities is also directly associated with the increase in microbial biomass along with the progress of decomposition (Gonçalves et al, 2004; Abelho, 2001; Gomes et al., 2016). The litter mass decay was more accelerated in the Taboões stream because the influences of invertebrate biomass, mainly, by the effect of the higher density of shredders, and higher water flow observed between the streams. This reinforces that although in general, shredders detritivores have been found in low abundance in tropical streams (Boyero et al., 2011; Prather, 2003), due to the high biomass can be predominant for the flow of organic matter and energy in tropical aquatic ecosystems (Rezende et al., 2014; Tonin et al., 2014; Martins et al., 2015).

There are generalizations that tropical plant litter is tougher, higher chemically defended and more P-limited (García-Palacios et al. 2016; Boyero et al. 2017) due mainly to environmental influences. However, we should be careful in general patterns because there has been coming distinct patterns of litter characteristics in tropical biomes (Sena et

al., 2022). For example, litter from more humid tropical biomes was higher in nitrogen, although more P-limited, than litter produced in tropical biomes with drier climates (Sena et al., 2022). Furthermore, there is a wide diversity of species in tropical riparian zones (Bambi et al., 2017) that support higher functional diversity, which also influence litter breakdown (Rabelo et al., 2022). Thus, the results found in this study should be taken with care, as only one natural tropical plant litter was used vs. *E. camaldulensis*, not reflecting the diversity found in tropical riparian forests.

We reinforce the evidence that regardless the litter origin, litter characteristics preponderant for litter breakdown in tropical streams. We also demonstrate that different tropical streams may have different decomposition rates, due to the specificity of the natural environmental conditions of these streams, in particular the water flow. Our findings provide empirical support for the influence of replacement of native tropical riparian plants by *Eucalyptus* plantations on decomposition and its possible repercussions on the aquatic community, on the flow of matter and energy in tropical streams. We believe that understanding of the repercussions that human activities may cause on the standards that determine the processing of organic matter in tropical aquatic environments can be useful in face of current global social and economic dynamics. Moreover, for the maintenance of riparian forests (Brazilian Forestry Code; Brasil, 2012), more forest rehabilitation projects are needed with seedlings of native plant species, not the non-native ones, to guarantee the integrity of riparian forests, input of leaf litter and ecological processes such as leaf decomposition.

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CONSIDERAÇÕES FINAIS

A decomposição foliar em riachos tropicais é um importante processo ecológico que influencia a ciclagem de nutrientes e o fluxo de energia dentro desses ecossistemas aquáticos (Gonçalves et al., 2006; 2012). Entretanto, inicialmente, os estudos sobre a decomposição foliar em riachos tinham grande foco principalmente nos ecossistemas aquáticos em regiões temperadas (Meyer, 1980; Webster e Benfield, 1986; Hieber & Gessner, 2002). No Brasil, os estudos sobre decomposição foliar em riachos iniciaram-se com mais ênfase na década de 2000 (Gonçalves et al., 2006; 2007; Moretti et al., 2007; Hepp et al., 2009). Além disso, a alta diversidade de ambientes, a ampla variação climática e elevada riqueza de espécies vegetais das florestas ripárias de riachos tropicais (Rezende et al., 2014; Bambi et al., 2017; Tonin et al., 2017; Nuven et al 2022; Sena et al., 2022), tem exigido um grande esforço na busca do aumento do entendimento do processo de decomposição foliar em riachos tropicais.

Os riachos florestados são peças chave na conexão entre os ambientes terrestres e aquáticos, sendo importantes para grande parte no fluxo de água, de matéria, de energia e manutenção da biodiversidade (Cummins, 1977; Vannote et al., 1980; Bren, 1993). Nesse contexto, esta tese demonstrou avanço nos seguintes pontos deste processo:

1. Pela primeira ver foi identificado que características físicas e químicas de detritos foliares de espécies de árvores oriundas de florestas ripárias tropicais não são totalmente uniformes. Estas características dos detritos são influenciadas por fatores ambientais e filogenéticos.

2. Além disso, também foi sugerido que a biomassa de fragmentadores de riachos tropicais não é colimitada pela quantidade e qualidade dos detritos foliares. Na realidade, há uma limitação pela disponibilidade de N nestes detritos.

3. Reforçou-se que independentemente da origem dos detritos, aqueles com menos compostos recalcitrantes tendem a se decompor em riachos tropicais de forma acelerada.

4. O fluxo da água/vazão intensifica a maior taxa de decomposição de detritos com menos compostos recalcitrantes.

Apesar de nossos resultados terem sido obtidos de banco de dados e de experimentos distintos realizados em diferentes riachos tropicais, esses resultados elucidam padrões e processos ainda pouco estudados. A intersecção floresta ripária-riachos tropicais comporta uma elevada diversidade de espécies, funcional, filogenética e morfológica (Bambi et al., 2017; Sena et al., 2022; Rabelo et al., 2023). Isso faz com que a decomposição foliar em riachos tropicais seja um processo complexo e o seu entendimento completo dependa da investigação de uma série de fatores distintos que variam em função da localização e do tempo (Graça et al., 2015). Desse modo, as descobertas encontradas nesta Tese podem ser úteis para o melhor entendimento da dinâmica de matéria orgânica, fluxo de energia e matéria e a ciclagem de nutrientes em ecossistemas aquáticos tropicais.

Estes resultados reforçam a importância das características físicas e químicas dos detritos foliares para a decomposição foliar em riachos tropicais, além da manutenção da composição florística natural das floretas ripárias. Nossas descobertas são pertinentes no cenário atual de mudanças climáticas e ambientais globais, pois a supressão ou substituição de espécies da floresta ripária pode ser um dos fatores que alteram a decomposição (Bambi et al., 2017; 2022).

Neste sentido, deve-se atentar à influência que o ambiente e a filogenia possam ter nas características fitoquímicas de diversas espécies de plantas das florestas ripárias em diferentes biomas; a repercussão de possíveis limitantes para o metabolismo de

organismos decompositores aquáticos; e como a inserção de diferentes espécies exóticas interferem na composição da comunidade decompositora e no processo de decomposição. Estes são interessantes caminhos a serem perseguidos visando o preenchimento de lacunas no conhecimento da dinâmica e decomposição de matéria orgânica, assim como, a manutenção da biodiversidade da floresta ripária e de riachos tropicais.

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