UNIVERSIDADE DE BRASÍLIA
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

EFEITOS DA DIVERSIDADE DE PLANTAS E DA PRODUÇÃO PRIMÁRIA NA DECOMPOSIÇÃO EM RIACHOS TROPICAIS

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TESE DE DOUTORADO

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SUMÁRIO

RESUMO

Os riachos tropicais são ecossistemas com uma alta riqueza em suas florestas ripárias, representada em sua maioria por um elevado número de espécies raras. Entretanto, esses ecossistemas têm sofrido uma considerável e rápida perda de diversidade de espécies devido à distúrbios antrópicos. Espécies raras de plantas são mais suscetíveis de serem perdidas, devido ao menor tamanho de suas populações, e por isso geralmente são essas espécies que direcionam tais mudanças de diversidade em riachos tropicais. Contudo, as espécies vegetais dominantes são geralmente as únicas incluídas em experimentos avaliando as consequências da perda de diversidade, e por isso o efeito da perda de espécies vegetais raras sobre esses ecossistemas ainda é pouco conhecido. Importantemente, a presença de espécies vegetais raras em misturas tem o potencial de acelerar a decomposição de detritos vegetais – um processo geralmente lento e essencial para a ciclagem de carbono e nutrientes nos ecossistemas –, pois às suas características funcionais únicas podem conduzir ao aumento da diversidade funcional, o que tipicamente acelera esse processo. Outro fator ainda pouco investigado em riachos tropicais que pode acelerar a decomposição de detritos vegetais é o efeito *priming* – a aceleração da decomposição dos detritos recalcitrantes na presença do carbono lábil de algas. Estudos de efeito *priming* em ecossistemas aquáticos estiveram concentrados em experimentos em laboratório ou mesocosmos, e por isso evidências de efeito *priming* de sistemas naturais são raras. Desse modo, o objetivo desta tese foi avaliar a importância de espécies vegetais raras e do efeito *priming* para a decomposição em riachos tropicais. Para avaliar a importância de espécies vegetais raras para a decomposição em riachos tropicais, realizamos experimentos de campo em um riacho do bioma Cerrado simulando cenários de perda de espécies vegetais raras de diferentes tipos funcionais (i.e., com características funcionais similares ou dissimilares às de espécies dominantes): um experimento levando em consideração à proporção de espécies dos detritos vegetais que entram no rio, e outro experimento onde nós usamos a mesma biomassa para todas as espécies em misturas de detritos. Os experimentos foram elaborados considerando as características de comunidades de baixa diversidade em ecossistema naturais, caracterizadas comumente pela persistência de espécies vegetais dominantes. No **capítulo 1** nós demonstramos que a perda de espécies vegetais raras reduziu a diversidade funcional de misturas de detritos e consequentemente reduziu a decomposição e dois outros importantes processos relacionados – a perda de nitrogênio (N) e a produção de biomassa fúngica –, via efeito de dissimilaridade de recursos. Além disso, nós observamos que quando somente espécies raras similares estiveram presentes em misturas de detritos, os processos foram mais reduzidos do que no cenário com somente espécies raras dissimilares (exceto para perda de N). No **capítulo 2** evidenciamos que a perda de espécies vegetais raras reduziu a biomassa de fungos, considerados os principais agentes da decomposição em riachos tropicais, mas não afetou nenhum outro parâmetro fúngico avaliado (i.e., taxa de esporulação, diversidade e composição taxonômica de hifomicetos aquáticos). Ainda, nós observamos que a redução na biomassa fúngica foi maior em misturas de detritos com proporções de espécies naturais do que em misturas com a mesma biomassa para todas as espécies, indicando que a uniformidade modulou o efeito da perda de espécies raras sobre a biomassa fúngica. Para investigar a importância do efeito *priming* para a decomposição em riachos tropicais, no **capítulo 3** realizamos um experimento de campo usando sombreamento artificial em três riachos do bioma Cerrado. Apesar de termos encontrado somente evidências de redução da decomposição com o aumento do carbono lábil de algas (efeito *priming* negativo), nós demonstramos que o efeito *priming* tem um papel relevante na decomposição de detritos vegetais mediada por microrganimos decompositores e invertebrados fragmentadores nesses ecossistemas. Nossas descobertas destacam a relevância de espécies vegetais raras (especialmente as espécies raras funcionalmente dissimilares), da manutenção de proporções naturais de espécies em florestas ripárias e do efeito *priming* para o funcionamento de riachos tropicais. Diante disso, esta tese tem o potencial de contribuir com questões práticas da restauração de zonas ripárias (principalmente do Cerrado), uma vez que indica que as espécies e os aspectos mencionados acima devem ser levados em consideração em projetos de restauração futuros.

Palavras-chave: biodiversidade, decomposição, ciclagem de nutrientes, hifomicetos aquáticos, efeito *priming*, riachos tropicais.

ABSTRACT

Tropical streams are ecosystems with a high richness in their riparian forests, represented mostly by a high number of rare species. However, these ecosystems have suffered a considerable and rapid loss of species diversity due to anthropic disturbances. Rare plant species are more likely to be lost due to the smaller size of their populations, and so it is often these species that drive such diversity changes in tropical streams. However, the dominant plant species are usually the only ones included in experiments evaluating the consequences of the loss of diversity, and therefore the effect of the loss of rare plant species on these ecosystems is still poorly understood. Importantly, the presence of these species in plant litter mixtures has the potential to accelerate the litter decomposition – a generally slow and essential process for carbon and nutrient cycling in ecosystems –, as their unique functional traits can lead to increased functional diversity, which typically accelerates this process. Another factor that has not yet been investigated in tropical streams that can accelerate the litter decomposition is the priming effect – the acceleration of the recalcitrant litter decomposition in the presence of the labile carbon of algae. Studies of the priming effect in aquatic ecosystems have focused on laboratory experiments or mesocosms, and therefore evidence of a priming effect in natural systems is rare. Thus, the objective of this thesis was to evaluate the importance of rare plant species and the priming effect for the litter decomposition in tropical streams. To investigate the importance of rare plant species for the litter decomposition in tropical streams, we carried out field experiments in a stream of the Cerrado biome simulating scenarios of loss of rare plant species of different functional types (i.e., with functional traits similar or dissimilar to those of dominant species): an experiment taking in consideration the proportion of plant litter species entering the stream, and another experiment where we used the same biomass for all species in litter mixtures. The experiments were designed considering the traits of communities of low diversity in natural ecosystems, commonly characterized by the persistence of dominant plant species. In **chapter 1** we demonstrated that the loss of rare plant species reduced the functional diversity of litter mixtures and consequently reduced decomposition and two other important related processes – nitrogen (N) loss and fungal biomass production –, via the resource dissimilarity effect. Furthermore, we observed that when only similar rare species were present in litter mixtures, the processes were more reduced than in the scenario with only dissimilar rare species (except for N loss). In **chapter 2** we showed that the loss of rare plant species reduced the biomass of fungi, considered the main agents of decomposition in tropical streams, but did not affect other fungal parameters evaluated (i.e., aquatic hyphomycetes sporulation rate, diversity and taxonomic composition). Furthermore, we observed that the reduction in fungal biomass was greater in litter mixtures with proportions of natural species than in mixtures with the same biomass for all species, indicating that evenness modulated the effect of rare species loss on fungal biomass. To investigate the importance of the priming effect for the litter decomposition in tropical streams, in **chapter 3** we carried out a field experiment using artificial shading in three streams of the Cerrado biome. Although we found only evidence of reduced litter decomposition with increasing algal labile carbon (negative priming effect), we demonstrate that the priming effect plays an important role in the litter decomposition mediated by decomposer microorganisms and shredder invertebrates in these ecosystems. Our findings highlight the relevance of rare plant species (especially functionally dissimilar rare species), of maintenance the natural proportion of species in riparian forests, and of the priming effect for the functioning of tropical streams. Therefore, this thesis has the potential to contribute to practical questions of the restoration of riparian zones (mainly the Cerrado), since it indicates that the species and aspects mentioned above should be considered in future restoration projects.

Keywords: biodiversity, decomposition, nutrient cycling, aquatic hyphomycetes, priming effect, tropical streams.

INTRODUÇÃO GERAL

O nosso planeta tem experimentado uma grande perda de diversidade de espécies nas últimas décadas devido à distúrbios antrópicos, e as suas espécies remanescentes permanecem em constante declínio (Díaz et al. 2019; IPBES 2019; Isbell et al. 2022). Estima-se que cerca de um milhão de espécies de animais e plantas da Terra estão atualmente ameaçadas de extinção (IPBES 2019). Caso as tendências atuais continuem, o esperado é que mais espécies tornem-se em risco de extinção, e que muitas espécies ameaçadas hoje sejam perdidas antes do fim do século XXI (Isbell et al. 2022). Os principais fatores que contribuem para os impactos negativos na natureza causados por atividades humanas nas últimas décadas são: as mudanças no uso da terra e do mar, a sobre-exploração de recursos naturais, as mudanças climáticas, a poluição e a invasão de espécies exóticas nos ecossistemas (IPBES 2019).

Os ecossistemas tropicais estão entre os ecossistemas mais ameçados do planeta devido aos distúrbios antrópicos, a despeito do fato de abrigarem a maior parte da biodiversidade da Terra (Barlow et al. 2018). Em particular, os riachos tropicais suportam uma alta diversidade de espécies arbóreas em suas zonas ripárias comparado à riachos de outras regiões (Wantzen et al. 2008). Enquanto em outras regiões do planeta são encontradas, comumente, entre 5 a 10 espécies arbóreas em florestas ripárias (e.g., região temperada; Swan & Palmer 2004), nos trópicos com frequência dezenas de espécies são registradas (e.g., França et al. 2009; Wittmann et al. 2010; Gonçalves et al. 2014). Contudo, essa biodiversidade está criticamente ameaçada, uma vez que as florestas ripárias de riachos situados em regiões tropicais estão sendo frequentemente destruídas ou modificadas para agricultura, pecuária, instalação de assentamentos urbanos, geração de energia hidrelétrica, aquicultura ou irrigação (Ramírez et al. 2008). Entre as consequências do declínio da diversidade de espécies arbóreas em zonas ripárias destaca-se a alteração da decomposição de detritos vegetais nos ecossistemas aquáticos (Gessner et al. 2010). Este processo ecológico é responsável pela principal fonte de energia nos riachos de cabeceira. Isto deve-se à baixa produtividade primária causada pelo sombreamento do dossel sobre os riachos (e.g., Wallace et al. 1997).

Em geral, na literatura é bem conhecido que espécies de detritos vegetais com uma alta concentração de nutrientes, como nitrogênio ou fósforo, e pobres em compostos estruturais e secundários, como lignina e polifenois, respectivamente, tendem a se decompor mais rapidamente, e dessa forma acelerar a ciclagem de nutrientes (Graça 2001). Contudo, a decomposição de detritos vegetais misturados, como ocorre na natureza, pode ser acelerada quando comparada à decomposição de detritos de uma única espécie separadamente, comum em experimentos ou em ambientes impactados por silvicultura (Mori et al. 2020), e ainda temos um entendimento limitado sobre essa questão. Estudos de diversidade têm focado no efeito da perda de espécies vegetais dominantes sobre a decomposição de misturas de detritos e organismos decompositores associados, enquanto as consequências da perda de espécies vegetais raras sobre esse processo e organismos decompositores permanecem ainda desconhecidas.

Outro fator com o potencial de acelerar a decomposição de detritos vegetais ainda pouco estudado é o efeito *priming* em riachos de cabeceira – a aceleração da decomposição dos detritos recalcitrantes na presença do carbono lábil de algas (Guenet et al. 2010). O efeito *priming* pode acelerar a decomposição de detritos nesses ecossistemas por meio de vários mecanismos (explicados abaixo na seção “Efeito *priming* e decomposição em riachos tropicais”).

O conhecimento dos fatores com o potencial de acelerar a decomposição é de suma importância, uma vez que a decomposição de detritos vegetais é um processo essencial para a ciclagem de carbono e nutrientes nos ecossistemas, mas é um processo geralmente lento, que pode levar de semanas até meses (e.g., Gonçalves et al. 2007). Em riachos de cabeceira, onde a irradiação solar é limitada pela floresta ripária e as teias alimentares obtêm a maior parte de sua energia da decomposição de detritos terrestres, o estudo desses fatores se torna ainda mais necessário. O retardo da decomposição de detritos vegetais pode alterar o fluxo de carbono e nutrientes através de vários níveis tróficos nesses ecossistemas (de microorganismos decompositores à predadores; Gessner et al. 2010). Com a aceleração da decomposição de detritos vegetais em riachos de cabeceira, ocorre um aumento da perda de carbono para o ambiente, o que modifica o seu estoque, exportação e disponibilidade para os consumidores (Benstead et al. 2009). Isso também pode alterar *feedbacks* climáticos via aumento da perda respiratória do carbono de detritos como CO_2 (Manning et al. 2018). Ainda, com a decomposição mais acelerada ocorre um *turnover* mais rápido de nitrogênio e outros nutrientes dos detritos vegetais para o ecossistema (Graça et al. 2015).

Os riachos tropicais do bioma Cerrado se destacam por possuírem diversas características intrínsecas que contribuem para o retardamento da decomposição de detritos como uma baixa concentração de nutrientes dissolvidos (Medeiros et al. 2015), e presença de espécies arbóreas com detritos foliares com alta concentração de defensivos químicos (compostos secundários), baixa concentração de nutrientes e fortes defesas físicas (maior dureza) (Tonin et al. 2021). Essas restrições tornam a decomposição bastante lenta nesses ecossistemas devido à limitarem a colonização e a atividade de consumidores sobre os detritos (Gonçalves et al. 2007; Medeiros et al. 2015). Além disso, os riachos tropicais do bioma Cerrado possuem uma baixa densidade de invertebrados fragmentadores, o que retarda a sua decomposição de detritos (Gonçalves et al. 2007), uma vez que esses organimos exercem um importante papel na conversão de matéria orgância particulada grossa em matéria orgânica particulada fina (Graça 2001).

Nesse contexto, o foco desta tese foi explorar como as espécies vegetais raras e o efeito *priming* afetam a decomposição de detritos em riachos tropicais do bioma Cerrado, ambos aspectos ainda pouco estudados e que podem acelerar a decomposição nesses ecossistemas. Nas seções abaixo, a importância de espécies vegetais raras e do efeito *priming* para os riachos tropicais e os mecanismos por meio dos quais esses fatores podem afetar a decomposição de detritos e os organismos decompositores associados nesses ecossistemas são explicados.

PERDA DE ESPÉCIES VEGETAIS RARAS EM RIACHOS TROPICAIS

Espécies vegetais raras e decomposição em riachos tropicais

Espécies podem ser consideradas raras quando apresentam baixa abundância, área geográfica restrita ou tolerâncias estreitas quanto aos seus hábitats (Rabinowitz 1981). Quando combinadas, essas características podem levar a diferentes níveis de risco de extinção (Harnik et al. 2012). Em geral, espécies raras são mais suscetíveis de serem extintas, e por isso comumente são essas espécies que direcionam mudanças de diversidade em comunidades frente à distúrbios antrópicos (e.g., Leps 2004). Porém, as espécies dominantes são geralmente as únicas incluídas em experimentos avaliando o efeito da perda de diversidade sobre o funcionamento dos ecossistemas por representarem uma maior proporção das comunidades (Lyons et al. 2005). Com isso, o efeito da perda de espécies raras sobre os ecossistemas ainda é pouco conhecido, especialmente em ecossistemas tropicais (Clarke et al. 2017). Deste modo, é necessário que essas espécies sejam incluídas mais frequentemente em estudos experimentais para avaliar as consêquencias da extinção de espécies sobre processos ecossistêmicos nesses ambientes (Dee et al. 2019).

O efeito da perda de espécies raras sobre o ecossistema pode ser maior que o esperado baseado na baixa abundância relativa dessas espécies. O fator-chave que determina o efeito da perda dessas espécies sobre o funcionamento do ecossistema é a presença de características funcionais únicas (Lyons et al. 2005; Dee et al. 2019). Características funcionais são definidas como atributos morfológicos, bioquímicos, fisiológicos, estruturais, fenológicos ou comportamentais expressos em fenótipos de organismos individuais considerados relevantes para a resposta de tais organismos ao ambiente e/ou seus efeitos sobre funções do ecossistema (Díaz et al. 2013). Espécies raras podem ser funcionalmente redundantes ou podem possuir características funcionais únicas com relação às outras espécies da comunidade (Violle et al. 2017; Dee et al. 2019). Quando espécies raras são funcionalmente redundantes, a extinção de uma ou mais espécie rara não afeta o ecossistema de uma forma significante, porque as espécies remanescentes podem compensar o impacto provocado (Violle et al. 2017). Por outro lado, a extinção de espécies raras com características funcionais únicas pode gerar fortes impactos sobre o funcionamento do ecossistema, uma vez que o papel funcional único dessas espécies não pode ser compensado pelas outras espécies remanescentes da comunidade (Lyons et al. 2005; Dee et al. 2019).

Estudos experimentais avaliando o efeito da perda de espécies raras com cararcterísticas funcionais únicas indicam que essas espécies, mesmo com uma proporção muito menor do que outras espécies na comunidade, podem exercer um forte impacto no ecossistema aumentando a resistência às invasões (e.g., Lyons & Schwartz 2001), a ciclagem de nutrientes (e.g., Marsh et al. 2000) e a multifuncionalidade do ecossistema (e.g., Soliveres et al. 2016), ou agindo como espécies-chave (e.g., Power et al. 1996). Embora a contribuição de espécies raras para esses processos já tenha sido documentada, as consequências da perda de espécies vegetais raras para a decomposição de detritos permanecem ainda desconhecidas (como mencionado acima, no préambulo da 'Introdução Geral').

Em teoria, a perda de espécies vegetais raras (conceituado aqui como aquelas com menor contribuição em biomassa) pode reduzir a diversidade funcional de misturas de detritos, ou seja, a variação em características relevantes das espécies para as funções do ecossistema (Tilman 2001), e influenciar a concentração de recursos – misturas de detritos com maior concentração de nutrientes, compostos secundários ou dureza (Graça 2001). Esses dois aspectos desempenham um papel crítico na decomposição de detritos (García-Palacios et al. 2017). O esperado é que a perda de espécies vegetais raras tenha um impacto principalmente na diversidade funcional de misturas de detritos, desde que espécies raras têm uma maior probabilidade de possuírem características funcionais únicas do que outras espécies mais comuns (e.g., alta concentração de recursos escassos para a comunidade; Mouillot et al. 2013; Dee et al. 2019). Como espécies raras apresentam uma baixa representatividade em termos de abundância ou biomassa das comunidades (Rabinowitz 1981), o efeito de espécies vegetais raras sobre a concentração de recursos é esperado ser baixo.

Evidências de estudos prévios sugerem que a diversidade funcional de misturas de detritos pode acelerar a decomposição e outros processos relacionados como a ciclagem de nutrientes (Handa et al. 2014; García-Palacios et al. 2017). Entre os principais mecanimos que podem levar à aceleração da decomposição em misturas de detritos com uma maior diversidade funcional se destacam a transferência de nutrientes mediada por fungos ou via lixiviação entre detritos de espécies distintas (e.g., Handa et al. 2014), a complementaridade no uso de recursos – o uso de recursos complementares derivados de diferentes tipos de detritos vegetais por consumidores (e.g., Vos et al. 2013), e a disponibilidade de um ambiente físico mais diverso para os detritívoros (e.g., Sanpera-Calbet et al. 2009). Deste modo, a perda de espécies vegetais raras tem o potencial de reduzir a decomposição nos ecossistemas por meio de seus efeitos sobre a diversidade funcional de misturas de detritos, especialmente em ecossistemas tropicais com uma alta riqueza representada em sua maioria por um elevado número de espécies raras (Slik et al. 2015). Em riachos tropicais, a perda dessas espécies em florestas ripárias pode alterar a decomposição dos detritos vegetais que entram no rio, um processo chave para o funcionamento desses ecossistemas (como explicado acima, no préambulo da 'Introdução Geral').

Espécies vegetais raras e hifomicetos aquáticos em riachos tropicais

Os detritos vegetais em decomposição em riachos se destacam como um importante local de produção de biomassa fúngica (e.g., Mathuriau & Chauvet 2002). Enquanto a colonização bacteriana tem uma maior contribuição principalmente nos estágios iniciais e promove apenas à decomposição de moléculas de fácil assimilação, os fungos tornam-se mais atuantes após poucas semanas do início da decomposição, e possuem a capacidade de metabolizar compostos estruturais de difícil degradação, como celulose e lignina (Pascoal & Cássio 2004; Gonçalves et al. 2006). Entre os fungos que atuam no processamento dos detritos vegetais, os fungos aquáticos – conhecidos também como hifomicetos – são considerados os principais decompositores (Gessner et al. 2007).

As mudanças na diversidade de espécies dos detritos vegetais que entram no rio podem ocasionar alterações nas comunidades de hifomicetos aquáticos que colonizam os detritos (Krauss et al. 2011). Em particular, a perda de espécies vegetais raras pode impactar principalmente a diversidade funcional de misturas de detritos , especialmente em riachos tropicais (como explicado acima na subseção 'Espécies vegetais raras e decomposição em riachos tropicais'), mas pouco se sabe sobre o efeito da diversidade funcional de misturas de detritos sobre os hifomicetos aquáticos. Contudo, evidências de alguns estudos sugerem que as comunidades desses fungos podem ser impactadas por mudanças na diversidade funcional de misturas de detritos. Estudos anteriores encontraram que misturas com detritos vegetais de características funcionais distintas podem favorecer a colonização de hifomicetos com diferentes características funcionais ou necessidades energéticas, e assim permitir que comunidades fúngicas mais diversas existam sobre os detritos (e.g., Jabiol & Chauvet 2012). Além disso, estudos indicam que a presença de recursos complementares em misturas com detritos funcionalmente mais diversos pode proporcionar uma maior oportunidade para os consumidores otimizarem sua aquisição de nutrientes comparado a monoculturas (complementaridade no uso de recursos; Hättenschwiler et al. 2005), o que pode contribuir para o crescimento de comunidades decompositoras (e.g., Chapman et al. 2013; López-Rojo et al. 2020).

EFEITO *PRIMING* E DECOMPOSIÇÃO EM RIACHOS TROPICAIS

A energia química sintetizada na forma de compostos orgânicos e que sustenta os riachos de cabeceira é classificada em dois tipos: autóctone, que é produzida dentro do sistema pelos produtores primários por meio da fotossíntese; e alóctone, que entra no sistema vinda dos ecossistemas terrestres (Allan & Castillo 2007). Desse modo, essas fontes de energia atuam na base da cadeia trófica e disponibilizam energia para os demais níveis tróficos (Neres-Lima et al. 2016) (Figura 1). A matéria orgânica alóctone é composta principalmente de detritos vegetais oriundos de florestas ripárias ao longo do rio (Tonin et al. 2017), enquanto a produção primária autóctone dos riachos de cabeceira é proveniente em sua maior parte de algas perifíticas (Davies et al. 2008).

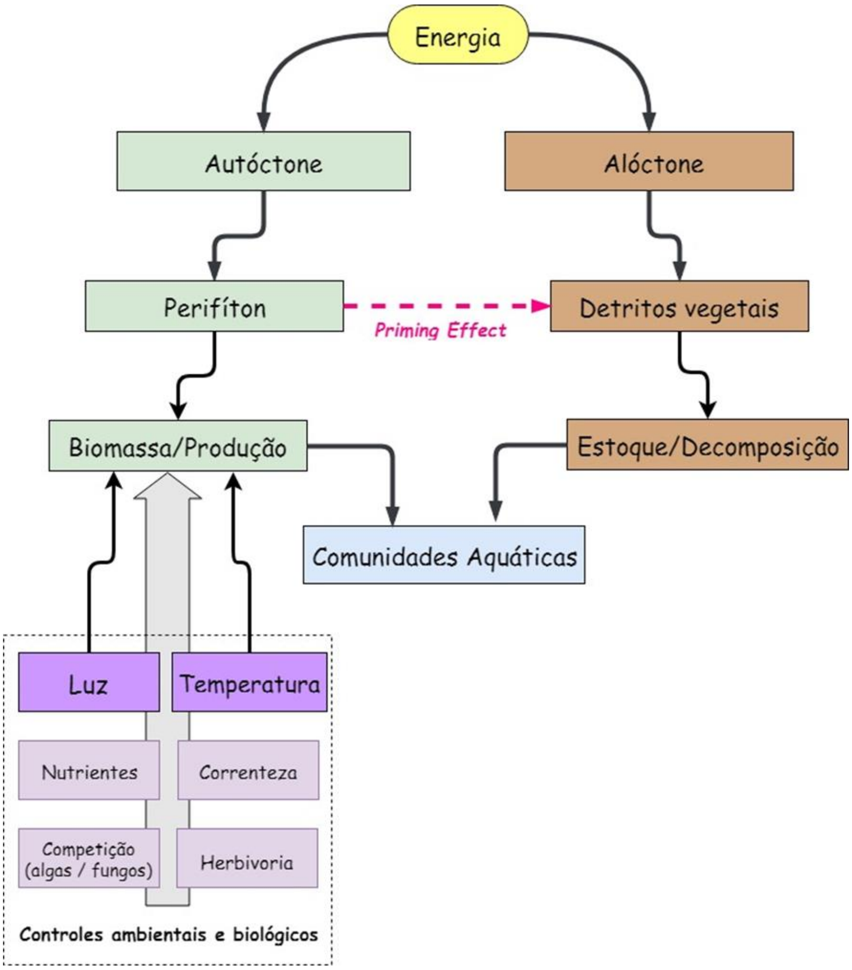

Figura 1. Diagrama conceitual explicitando a conexão da energia autóctone (perifíton) e alóctone (detritos vegetais) com as comunidades aquáticas, e a regulação da biomassa e produção perifítica por fatores abióticos e bióticos em riachos de cabeceira. Além disso, o diagrama destaca que o perifíton pode mediar o efeito *priming*, o qual tem relevância para a decomposição de detritos vegetais.

As algas perifíticas representam de 95 à 99% da comunidade do perifíton, mas este também é composto de diferentes quantidades de bactérias, fungos, detritos inorgânicos e orgânicos (Battin et al. 2016). Os compostos de carbono de algas do perifíton são mais lábeis do que os de detritos vegetais e facilmente assimiláveis pelos organismos (Thorp & Delong 2002). Além disso, o perifíton possui uma alta qualidade nutricional (i.e., baixa proporção C:N e C:P), sendo mais nutritivo do que os detritos vegetais para a fauna aquática (Lau et al. 2009). Em particular, as algas perifíticas são ricas em ácidos graxos comparado aos detritos vegetais (Lau et al. 2009; Guo et al. 2016a). Os ácidos graxos, especificamente os ácidos graxos poliinsaturados, são abundantes somente em alguns tipos de algas e são essenciais para o crescimento e reprodução de invertebrados fragmentadores (Guo et al. 2016a). Esses ácidos são precursores de hormônios de invertebrados fragmentadores e contribuirem para a regulação da fluidez de sua membrana celular (Brett & Muller-Navarra 1997).

O perifíton pode ser afetado por múltiplos fatores e interações – como por exemplo, a luz, concentração de nutrientes, herbivoria e interações de competição com outros microrganismos (Battin et al. 2016). Os fatores abióticos e bióticos que afetam o perifíton podem gerar efeitos de dois tipos: *bottom-up* e *top-down*. Entre os fatores que atuam para gerar o efeito *bottom-up* estão aqueles que estimulam ou limitam o crescimento do perifíton, como a luminosidade, a temperatura, os nutrientes e a velocidade da água (e.g., Izagirre & Elosegi 2005; Hillebrand 2005), enquanto os fatores que atuam para gerar o efeito *top-down* incluem a herbivoria e as interações tróficas (e.g., predação de herbívoros pastadores; Moulton et al. 2004) (Figura 1). Tais fatores podem influenciar sua distribuição, acúmulo e produção de biomassa, dos quais a luminosidade é frequentemente o mais importante devido a sua necessidade para a fotossíntese de algas perifíticas (Davies et al. 2008; Warren et al. 2016).

Em riachos naturais é esperado um aumento marcante da produção primária autóctone apenas com o distanciamento da nascente, onde ocorre a maior abertura do dossel ripário e não há formação de galerias sobre o canal limitando a incidência de luz (Thorp & Delong 2002). Porém, através da abertura de frestas (*sunflecks*) no dossel – seja pela queda de folhas, galhos ou árvores inteiras – a luz do sol pode passar e alcançar o leito do riacho de cabeceira diretamente, e aumentar significativamente a produtividade de algas perifíticas localmente (e.g., Warren et al. 2016). Como resultado, os riachos de cabeceira podem apresentar uma produção primária autóctone significativa mesmo com uma alta cobertura de floresta ripária, especialmente os riachos tropicais (e.g., Neres-Lima et al. 2017).

Enquanto a cadeia de detritivoria de riachos de cabeceira é sustentada principalmente por detritos vegetais alóctones constituídos por frações altamente recalcitrantes (e.g., lignina, celulose, hemicelulose) e de pouca qualidade nutricional, a cadeia de herbivoria é mantida pelos compostos de carbono mais lábeis da produção primária autóctone facilmente assimiláveis pelos organismos (Cebrian 1999; Thorp & Delong 2002). Entretanto, os detritos vegetais alóctones representam uma proporção muito superior da biomassa total do sistema do que o carbono proveniente da produção primária (Neres-Lima et al. 2017). Nesse contexto, há indícios que a colonização desses detritos por algas perifíticas pode aumentar a qualidade deste recurso e acelerar a decomposição dos detritos vegetais recalcitrantes (e.g., Danger et al. 2013; Howard-Parker et al. 2020), o que é denominado de efeito *priming* (Guenet et al. 2010) (Figura 1).

Documentado primeiramente em ecossistemas terrestres, o efeito *priming* descreve a influência estimulatória de frações lábeis de carbono na decomposição e mineralização de matéria orgânica recalcitrante, a qual é mediada por microrganismos (Kuzyakov 2000). Em riachos, o efeito *priming* pode ocorrer pela transferência do carbono lábil de algas para microrganismos decompositores (e.g., Danger et al. 2013; Howard-Parker et al. 2020), ou pelo consumo direto de algas por invertebrados fragmentadores (e.g., Guo et al. 2016b). Os detritos vegetais impõem um grande desafio para esses invertebrados devido a sua baixa qualidade, e invertebrados tendem a preferir detritos previamente colonizados por microrganismos, quando ocorre um aumento da qualidade nutricional e da palatabilidade (Krauss et al. 2011). No entanto, mesmo após a colonização dos detritos por microrganismos, a falta de ácidos graxos essenciais ainda pode limitar a qualidade nutricional dos detritos para os invertebrados fragmentadores (Guo et al. 2016a). Nesse contexto, há evidências de que a colonização dos detritos por algas perifíticas – mais ricas em ácidos graxos –, estimula a qualidade nutricional dos detritos e acelera a decomposição e o consumo pelos invertebrados (Guo et al. 2016b; Vonk et al. 2016; Kühmayer et al. 2020).

Apesar de evidências consistentes apontarem para a existência e generalidade do efeito *priming* em diferentes ecossistemas terrestres (Kuzyakov et al. 2000; Perveen et al. 2019), esse efeito ainda é muito controverso e carece de um embasamento robusto em ecossistemas aquáticos, especialmente em riachos (ver Bengtsson et al. 2018). Até o momento, as evidências sobre o efeito *priming* em ambientes aquáticos estiveram concentradas em experimentos laboratoriais (microcosmos) ou de mesocosmos (canais artificiais; Bengtsson et al. 2018), enquanto evidências de sistemas naturais são essencialmente raras (Elosegi et al. 2018).

Os poucos estudos existentes em sistemas naturais foram majoritariamente executados em ecossistemas de climas temperados (e.g., Elosegi et al. 2018), os quais diferem de ecossistemas tropicais em pontos relevantes para a produção primária devido à menor quantidade de radiação solar recebida, temperatura da água (Lewis Jr. 2008) e maior sazonalidade climática (e.g., Elosegi et al. 2018). Como resultado, a taxa de produção primária autóctone em riachos tropicais pode alcançar até uma ordem de grandeza a mais do que seus equivalentes em riachos temperados (Davies et al. 2008). Nesse contexto, não seria inesperado que riachos tropicais respondessem de modo diferente do que os temperados ao efeito *priming*, tampouco que esse efeito fosse evidenciado sob condições distintas. Desse modo, estudos testando o efeito *priming* em riachos tropicais sob condições de campo são necessários para o entendimento da importância desse efeito nesses ecossistemas, especialmente em sistemas oligotróficos - como é o caso dos riachos do bioma Cerrado – onde o efeito *priming* pode ser exacerbado devido ao estresse nutricional aumentar a produção de exsudatos de carbono por algas (Danger et al. 2013).

OBJETIVO & ESTRUTURA DA TESE

O objetivo principal desta tese foi avaliar a importância de espécies vegetais raras e do efeito *priming* para a decomposição em riachos tropicais (Figura 2). Para investigar a importância de espécies vegetais raras para a decomposição nesses riachos, realizamos um experimento de campo usando misturas de detritos simulando cenários de perda de espécies vegetais raras de diferentes tipos funcionais (i.e., com características funcionais similares ou dissimilares às de espécies dominantes) em um riacho do bioma Cerrado, levando em consideração à proporção de espécies dos detritos vegetais que entram no rio e as características de comunidades de baixa diversidade no ecossistema natural, caracterizadas comumente pela persistência de espécies vegetais dominantes (Hillebrand et al. 2008). No **capítulo 1** exploramos o efeito da perda de espécies vegetais raras sobre a decomposição dos detritos vegetais no ecossistema aquático, considerando a liberação de nitrogênio e a biomassa fúngica, e no **capítulo 2** exploramos as consequências da perda dessas espécies para os fungos – especialmente os hifomicetos aquáticos –, considerados os principais microorganimos decompositores de detritos em ecossistemas de riacho (Krauss et al. 2011). Em adição, neste capítulo também realizamos um experimento de campo similar ao descrito acima, mas usando a mesma biomassa para todas as espécies em misturas de detritos, e exploramos o papel da uniformidade em modular os efeitos da perda de espécies vegetais raras sobre as comunidades de hifomicetos aquáticos.

Para investigar a importância do efeito *priming* para a decomposição de detritos vegetais em riachos tropicais, no **capítulo 3** realizamos um experimento de campo usando sombreamento artificial em três riachos do bioma Cerrado, e exploramos o efeito das mudanças causadas no acúmulo de algas perifíticas sobre a decomposição mediada por microrganimos decompositores e invertebrados fragmentadores.

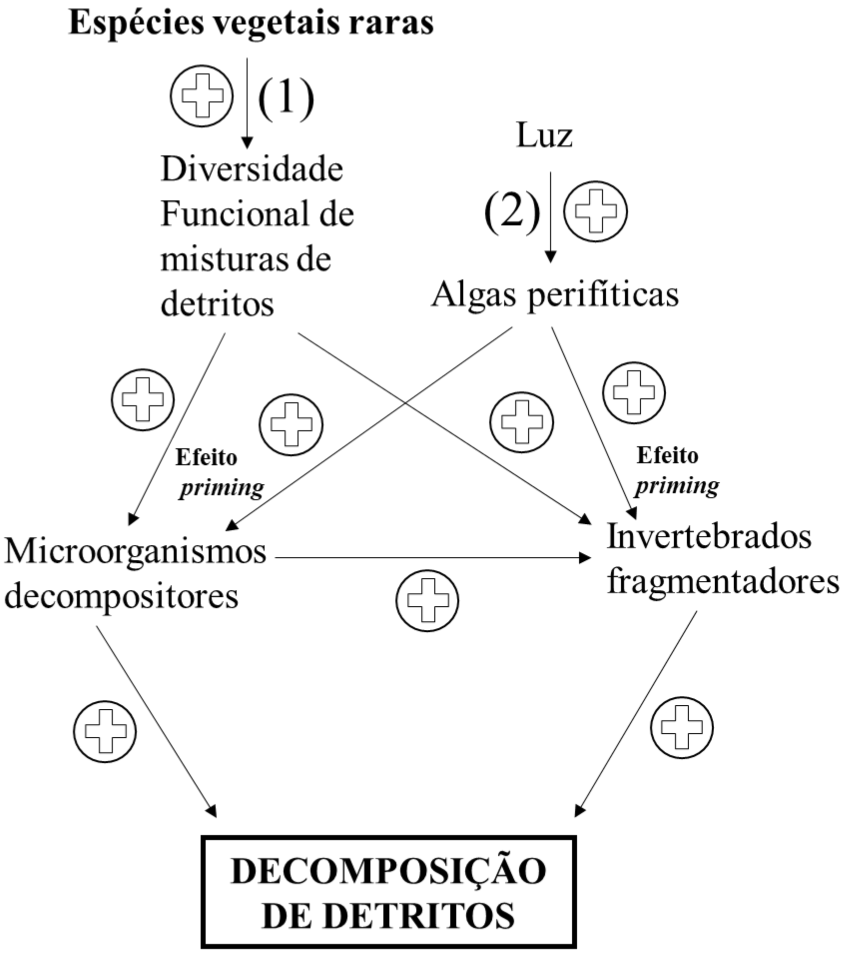

Figura 2. Representação gráfica de como as espécies vegetais raras e o efeito *priming* podem ter potenciais efeitos de estimulação/aceleração (+) sobre a decomposição de detritos em riachos de cabeceira: (1) as espécies vegetais raras podem aumentar a diversidade funcional de misturas de detritos, o que pode acelerar a decomposição mediada por microorganismos decompositores e invertebrados fragmentadores e (2) a disponibilidade de luz pode aumentar o acúmulo de algas perifíticas, e com isso acelerar a decomposição microbina e mediada por invertebrados fragmentadores via efeito *priming*.

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CAPÍTULO 1 – PLANT LITTER FROM RARE SPECIES INCREASES FUNCTIONAL DIVERSITY AND DECOMPOSITION OF SPECIES MIXTURES

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ABSTRACT

Biodiversity loss is altering key ecosystem processes as primary production and decomposition, however, the after-life effects of plant diversity (species mixing effects) on instream organic matter (litter) decomposition is still under debate. Available evidence of litter species-mixing effect (or the lack of) comes from studies using dominant plant species, despite rare species comprising the majority of species in an ecosystem and can contribute to ecosystem functions or in the provisioning of essential elements. Here, we simulated different extinction scenarios of plant from rare species by incubating leaf litter in artificial channels located within a tropical stream. We thus, assessed whether the loss of litter from rare plant species alters functional diversity (resource dissimilarity) and litter quality (resource concentration) of species mixtures and change decomposition, N loss and fungal biomass production. We show that the loss of litter from rare plant species reduced the functional diversity of litter mixtures and consequently, reduced decomposition, N loss and fungal biomass production. Although species lost also changed the nutritional quality of litter mixtures (resource concentration), it did not affect decomposition or N loss but fungal biomass production. Also, when only similar rare species were present, processes were reduced to higher rates than in the scenario with only dissimilar rare species (except for N loss). Our findings reveal the relevance of litter from rare plant species to key ecosystem processes related to carbon and nutrient flow in tropical streams, especially when dissimilar traits are added to litter pools.

Key-words: dominant species; species diversity; biodiversity-ecosystem functioning; nitrogen cycling; fungal biomass production; tropical stream; functional traits.

INTRODUCTION

The alarming loss of biodiversity across multiple trophic levels and ecosystems has motivated thousands of experimental studies primarily focused on the diverse effects of plants on key ecosystem processes such as primary productivity and decomposition (Caliman et al. 2010; Cardinale et al. 2011; Tilman et al. 2012). Mounting evidence has accumulated for how the diversity of living plants is fundamental to support ecosystem functions (Cardinale et al. 2011; Tilman et al. 2012). However, the after-life effects of plant diversity (species-mixing effects) on organic matter (litter) decomposition remains an open question, especially in stream ecosystems where divergent effects were commonly reported. For example, a recent meta-analysis found no litter species-mixing effect on decomposition in streams (Mori et al. 2020), which is contrasting to previous and recent studies supporting the role of litter functional diversity (quantified as functional types or species traits) to decomposition (Handa et al. 2014; Boyero et al. 2021).

Importantly, most studies of litter species-mixing effect on decomposition have used only dominant species from communities, despite the greater probability of rare species (that is, those with limited geographical range and/or low abundance in the local community; Rabinowitz 1981) disappearing from communities (Wardle 2016; Dee et al. 2019). As a result, available evidence of diversity effects (or the lack of) on decomposition are from experiments using unrealistic extinction scenarios or assuming uniform biomass across species (but see Gonçalves & Canhoto 2009; Swan et al. 2009), despite different species contributions to litterfall in forests and riparian ecosystems (Zhang et al. 2014; Tonin et al. 2021). For instance, previous studies ignore that few dominant species produce the largest amount of litterfall, while many rare species contribute with only modest quantities of litter (Tonin et al. 2021), although with potentially dissimilar and complementary traits. It is therefore important to understand whether these issues lead to under- or overestimation of diversity effect on instream decomposition and unravel the mechanisms behind the observed effects.

In theory, we can expect to find species mixtures with higher functional diversity when rare species add dissimilar traits to litter mixtures and lower functional diversity when rare species are mostly similar to overall mixtures. Possible mechanisms underlying the functional diversity effect on decomposition include nutrient transfer from litter of a nutrient-rich to nutrient-poor species (Handa et al. 2014) and divergent use of resources that are complementary in their nutritional composition (that is, litter with different concentration of nutrients and different degrees of toughness) (Vos et al. 2013), which translates into higher consumption by decomposers. However, diversity effects on decomposition may occur not only due to the functional diversity of litter mixtures, but due to differences in average litter quality of mixtures (resource concentration effect, that is, mixtures with higher concentration of nutrients or higher toughness), as appear to operate at higher latitudes (Boyero et al. 2021). Consequently, the integration of both metrics to describe species mixtures (functional diversity and average quality of litter) provide a powerful tool to investigate whether and how rare species influence diversity effects on instream decomposition, which remains unexplored.

Tropical streams have a primer relevance in this context because their riparian forests harbor a high diversity of tree species, most of which are rare species (Slik et al. 2015), are historically understudied ecosystems and are experiencing rapid species loss (Ramírez et al. 2008). Such losses not only reduce species diversity but may change the functional diversity of litter species mixtures (dissimilarity in species traits) that end up in forest soils and streams through alterations in species composition (Gessner et al. 2010). It is often assumed that losing a rare species has weaker effect than losing a dominant one to ecosystem functions given the low representativeness of a rare species in a community (that is, mass ratio hypothesis; Grime 1998). However, previous studies pointed to the disproportionately negative effect of losing rare species to resistance to invasions (for example, Lyons & Schwartz 2001), nutrient cycling (for example, Marsh et al. 2000) and ecosystem multifunctionality (Soliveres et al. 2016), although its effects on litter decomposition remain largely unknown. In contrast to the most previous studies that focused on litter mass loss only, we also investigate diversity effects on instream processes as an indicator of nutrient transformations (immobilization and mineralization) and biological activity of decomposers (fungal biomass production). While nutrient transformations are essential to stream biota (Wallace et al. 1997), especially in detritus-based systems where most energy comes in the form of terrestrial litter, fungi are efficient decomposers and indicators of greater biological activity on plant litter (Krauss et al. 2011).

Here, we assessed how the loss of litter from rare plant species influenced three key processes in stream ecosystems (litter decomposition, litter N loss and fungal biomass production), and how that effect depended on whether the lost rare species were functionally similar or dissimilar to dominant species. We did so through an experiment conducted in 20 artificial channels located within a tropical stream and simulating different extinction scenarios that are described below. We hypothesized that (i) the loss of litter from rare plant species would decrease functional diversity of whole litter mixtures and reduce decomposition, N loss and fungal biomass production, and (ii) the loss of litter from rare plant species that are functionally dissimilar to dominant species would have greater influence on these processes than the loss of litter from rare species that are functionally similar. Although we anticipate differences in average litter quality of the mixtures (that is, resource concentration) with changes in species composition, we expect these differences would be of lower importance than the functional diversity of mixtures due to the major influence of dominant species on resource concentration (Tonin et al. 2021).

METHODS

Study site

The experiment was carried out in artificial channels set up at the Cabeça-de-Veado stream (15°53'22.15" S and 47°50'34.10" W, 1079 m asl), which is located within a preserved area at the Ecological Station of the Botanical Garden of Brasília, Federal District, Brazil. The riparian forest is typical of the Cerrado biome, mostly composed of evergreen species, with dense vegetation cover (Tonin et al. 2020) and high species diversity (ca. 111 plant species; Bambi et al. 2016). The climate is seasonal (savanna climate, Aw, according to the Köppen-Geiger classification), with a dry season

(May to September), a rainy season (October to April) and two transitional seasons: dry–wet (September to October) and wet-dry (April to May; Eiten 1972).

The experimental period (August to October 2016) encompassed the transition between the dry and the rainy season and part of the rainy season; this coincides with the period of highest litterfall and litter storage and fastest litter decomposition in streams of the Cerrado savanna biome (Bambi et al. 2017; Tonin et al. 2017, 2020). During the experimental period, stream water temperature was 21.1 ± 0.1°C, conductivity 5.5 ± 0.2 µS cm^{-1}, pH 6.8 ± 0.1, water flow 0.96 ± 0.06 m^3 s^{-1} and dissolved oxygen 6.6 ± 1.3 mg L^{-1} (Table S1). Stream water was nutrient-poor (28.7 ± 1.7 µg L^{-1} for dissolved inorganic nitrogen (N) and 20.2 ± 1.2 µg L^{-1} for orthophosphate; Tonin et al. 2020).

Dominant and rare plant species

We selected four dominant and four rare species based on the composition of litter mixtures entering the stream. Dominant species considered were those that together contributed with greater than 50% of total litterfall biomass – and thus, are major drivers of litterfall chemistry (Tonin et al. 2021) –, whereas rare species had an individual contribution of less than 1% to total litterfall biomass – and thus, less likely to drive litterfall chemistry (Figure S1). The selected dominant species were *Protium spruceanum* (Benth.) Engl., *Calophyllum brasiliense* Cambess., *Ormosia arborea* (Vell.) Harms and *Hyeronima alchorneoides* Allemão, which have chemical and structural litter traits similar of riparian forests of the Cerrado biome (that is, moderate N and phosphorus [P] concentrations and high toughness) (Tonin et al. 2021). The selected rare species were *Maprounea guianensis* Aubl., *Tapirira guianensis* Aubl., *Hymenaea courbaril* L. and *Copaifera langsdorffii* Desf., which represented two distinct groups: species with similar (*H. courbaril* and *C. langsdorffii*) or dissimilar (*M. guianensis* and *T. guianensis*) chemical and structural traits to dominant species (Fig. 1a). Species selection was based on litter traits that are usually most relevant for litter decomposition worldwide, including the Cerrado biome (that is, N and P concentrations and leaf toughness; Gonçalves et al. 2007; Zhang et al. 2019), using a database of more than 30 plant species from riparian forests of the Cerrado biome.

Litter was collected in the riparian forest of the experimental stream, air-dried at the lab and stored until used. Litter sub-samples were oven-dried (60 ºC, 72 h), ground in a ball mill to powder and analyzed for the initial concentration of carbon (C), N and P. The concentrations of C and N were obtained from total combustion at 950 °C of one sample (100 mg; accuracy of 0.01 mg) per species in an elemental analyzer (Leco Corporation – TruSpec Micro CHN628), which detects C and N in the form of CO2 and N2 by means of infrared cells and thermal conductivity, respectively. The P concentration was obtained from three replicates of each species, using the ascorbic acid method (Flindt & Lillebo 2005). Leaf toughness was estimated from five measurements (in different parts of the leaf mesophyll) using five leaves of each species with a penetrometer, according to the method of Graça & Zimmer (2005).

Litter quality and functional diversity of mixtures

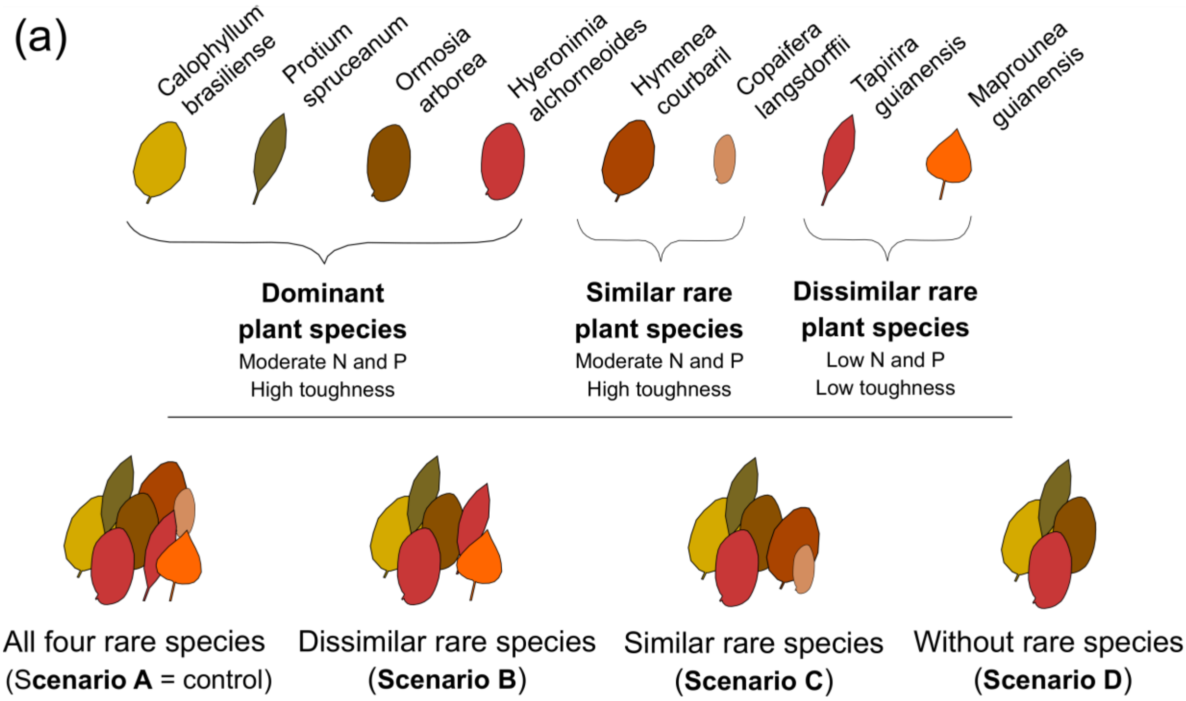
Litter quality and functional diversity of species mixtures were quantified for each replicate (as detailed below) and used as continuous predictors in linear models to indicate resource concentration or resource dissimilarity, respectively.

We used the community-weighted mean (CWM) traits of different species mixtures to quantify the average litter quality of mixtures. CWM is often used to describe the functional composition of communities which varies according to the abundances of species (Ricotta & Moretti 2011), but we rather used species dry mass in the mixture as widely used in decomposition studies (García Palacios et al. 2017). First, we calculated the CWM for each trait individually (N and P concentrations and toughness) using the functcomp function from the FD package. Second, we conducted a principal component analysis (PCA; PCA function, FactoMineR package) with the CWM values of each trait after z-score standardization. Finally, litter quality of species mixtures was expressed as the scores of the first PCA axis (hereafter CWM 1), which summarized 89% of total variance of traits (Figure 2a). CWM 1 was positively correlated with N and P concentration and toughness, indicating that resource concentration increases with CWM 1 scores (Fig. 2a).

Due to the limitations of categorical classifications (for example, lack of variation within categorical groups; Ricotta 2005), we used the Rao's quadratic entropy index to determine the functional diversity of litter mixtures. The index was calculated using the same three litter traits (N and P concentrations and toughness) weighted by the initial dry mass of each species in the sample, incorporating both the functional dissimilarity between species and their relative biomass (Epps et al. 2007). Rao's quadratic entropy index was calculated using all traits simultaneously rather than for each trait individually due to the former approach is more appropriate when there are more species than traits in the dataset (see Laliberte & Legendre 2010), as in our study. It was done using the dbFD function and FD package (Laliberte & Legendre 2010). To explore whether the loss of litter from rare plant species decreases functional diversity of whole litter mixtures we compared 95% confidence intervals for the functional diversity of litter mixtures across the four scenarios (Fig. 2b). Confidence intervals were based on 1,000 resamples using the BCa method with the boot function and package (Davison & Hinkley 1997; Canty & Ripley 2020). All analyses were performed using R v. 4.0.3 (R Core Team 2020).

Experimental design

We set four different scenarios of rare species loss using litter from all selected dominant and rare species (Figure 1a). All scenarios contained the same four dominant species, but differed in the presence of rare species and/or in the proportion of litter from each species: in scenario A (used as a control), there was no loss of rare species (so all four species were present); in scenario B, two rare species were lost (those functionally similar to the dominant ones; that is, *H. courbaril* and *C. langsdorffii*); in scenario C, two rare species were lost (those functionally dissimilar to the dominant ones; that is, *M. guianensis* and *T. guianensis*); and, in scenario D, all rare species were lost (that is, there were only dominant species) (Figure 1a, S2; Table 1).



(b) Artificial channels within the stream

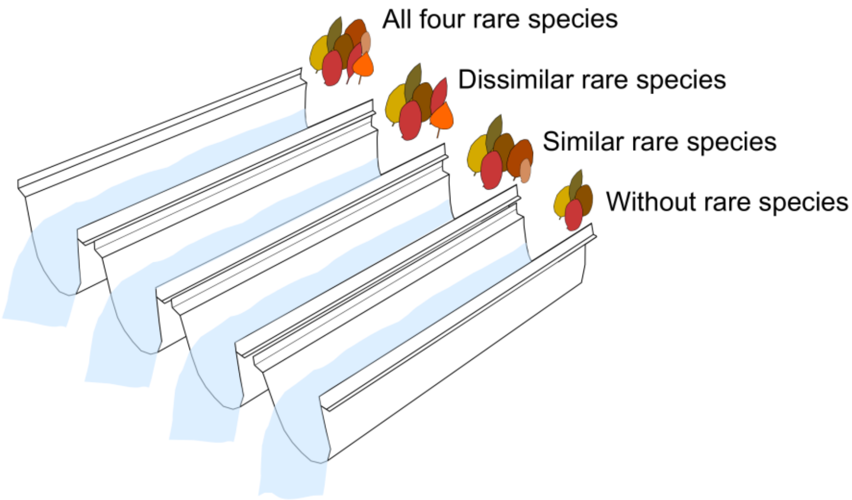


Figure 1. Experimental design representing (a) dominant, similar and dissimilar rare plant species used, their functional traits and the four extinction scenarios of rare species loss (A, B, C and D) and (b) exemplification of how the four scenarios were distributed in one set of artificial channels located within the stream.

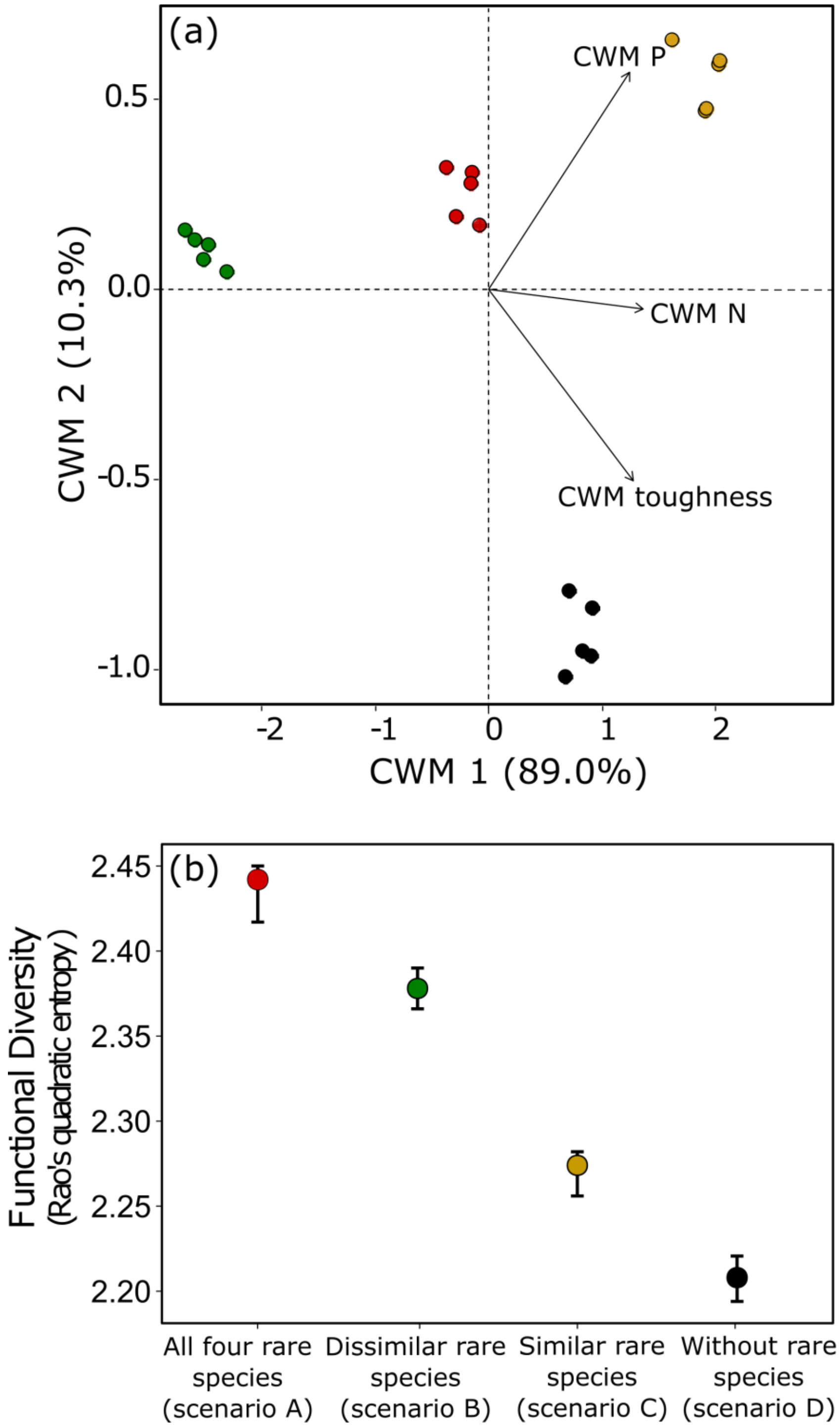


Figure 2. Litter quality (represented by the first axis of a principal component analysis of community-weighted mean [CWM] traits: nitrogen [N] and phosphorus [P] concentrations and toughness, a); and functional diversity (as the confidence intervals of Rao's quadratic entropy index, b) of plant mixtures in the four scenarios of rare species loss.

Table 1. Litter traits of the eight plant species used in the experiment according to functional group category (dominant species or rare species with similar or dissimilar traits in relation to the dominant species). Values are averages ± SE for all litter traits, except C, N and C:N mass ratio that were estimated using one replicate (see methods for details).

Functional group	**Plant species**	**C**	**N**	**P**	**C:N**	**C:P**	**N:P**	**Toughness**
Dominant	*Hyeronima alchorneoides*	48.24	0.83	0.12 ± 0.02	58	428 ± 85	7 ± 1	69 ± 3
Dominant	*Calophyllum brasiliense*	53.05	0.65	0.11 ± 0.01	82	472 ±16	6 ± 1	279 ± 10
Dominant	*Ormosia arborea*	52.83	1.24	0.16 ± 0.01	43	335 ± 6	8 ± 1	238 ± 17
Dominant	*Protium spruceanum*	43.94	0.81	0.11 ± 0.01	54	391 ± 42	7 ± 1	84 ± 11
Rare (similar)	*Hymenaea courbaril*	51.81	0.82	0.26 ± 0.02	63	205 ± 16	3 ± 1	169 ± 13
Rare (similar)	*Copaifera langsdorffii*	53.76	1.07	0.20 ± 0.01	50	271 ± 9	5 ± 1	132 ± 6
Rare (dissimilar)	*Tapirira guianensis*	45.43	0.72	0.06 ± 0.01	63	742 ± 114	12 ± 2	34 ± 3
Rare (dissimilar)	*Maprounea guianensis*	51.40	0.60	0.06 ± 0.01	86	913 ± 43	11 ± 1	61 ± 10

Litter decomposition was evaluated using fine mesh litter bags (250 μm), which allowed colonization and biological degradation of litter only by microorganisms. Although detritivore animals are responsible for a large fraction of litter mass loss in several regions worldwide (and recent evidence pointed to the greatest effect of detritivore diversity loss in tropical streams; Boyero et al. 2021), they are mostly absent or have a minor role to decomposition in Cerrado streams, where most decomposition is mediated by microorganism (for example, Gonçalves et al. 2007; Boyero et al. 2011). The experiment consisted of 20 litter bags (20 x 13 cm), containing 5.0 ± 0.3 g of litter from eight, six or four species (Figure 1a), with a 4:1 biomass ratio between dominant and rare species (except in scenario D where there were only dominant species). The 4:1 biomass ratio between dominant and rare species corresponds approximately to the proportion found in the period of the year when litterfall is greatest (that is, dry season and the transition of dry–wet seasons), which coincided with the experimental period (see above; Figure S3). Thus, litter bags of scenarios A, B and C contained 4 g of litter from dominant species (1 g per species) and 1 g of litter from rare species (0.25 g per species in scenario A and 0.50 g per species in scenarios B and C); and scenario D contained 5 g of litter from dominant species only (1.25 g per species). Only intact leaves (not broken or with any sign of decomposition) were used in the experiment and the number of leaves used to each species was kept constant within replicates and scenarios to homogenize litter mixtures.

Litter bags were deployed inside artificial channels (PVC channels of 0.5 length x 0.15 height x 0.15 width m), which were distributed in five sections along a 150 m stream reach. Each section had a set of four channels, one for each treatment. Each channel received three litter bags, one for each recovery time (30, 60 and 90 days of instream incubation), but for this study, only litter bags recovered after 90 days were used (Figure 1b). The use of artificial channels was intended to homogenize the environmental conditions commonly found in field experiments – for example, type of substrate, depth, current, accumulation of fine sediments, presence of other organic materials – and to impede cross-contamination between scenarios. Channels were placed inside the stream in non-sinuous areas to reduce the accumulation of sediment, with regular water flow and with a depth of no more than 40 cm. Channels were covered at the top and closed upstream with a 0.2 cm screen to prevent the entrance of litter other than the experimental one. The experiment was monitored weekly to ensure the homogeneity of environmental conditions inside the channels (for example, water flow and oxygenation) and to prevent the accumulation of organic material upstream of the channels.

Laboratory procedures

Litter bags were recovered from the field and transported to the laboratory packed in a thermal container with ice. In the laboratory, litter was cleaned with distilled water and two sub-samples were collected: four discs (8 mm in diameter) of each dominant species and 1–2 discs of each rare species present in the mixture, keeping the 4:1 ratio between dominant and rare species. Discs of the first sub-sample were freeze-dried for 48 h and used to estimate fungal biomass, through the concentration of ergosterol of the dominant species and each one of the rare species in the mixture. Ergosterol was quantified by extraction in methanol/KOH solution at 80ºC, filtration with SEP-PACK® columns (Waters, VAC RC tC18 500 mg) using vacuum, isopropanol elution and quantification in a high-performance liquid chromatograph (Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC) (Gessner 2005). The extraction efficiency was monitored using an ergosterol standard (Ergosterol ≥ 95% [HPLC], Sigma®), quantified in parallel. Discs of the second sub-sample were oven-dried (60ºC, 72 h) and weighed (accuracy of 0.01 mg) to estimate their dry mass. They were then ground separately in a ball mill to powder to quantify final C and N concentrations (as above).

Response variables

Three response variables were quantified for the entire mixture (mean values of dominant and rare species present in each sample): litter decomposition, litter N loss and fungal biomass production. Litter decomposition was estimated as litter C loss, that is, the difference between initial and final C concentrations (Ci and Cf, respectively), weighted by litter mass loss, that is, the difference between initial and final DM (Mi and Mf, respectively): C loss (prop.) = [(Mi x Ci) - (Mf x Cf)]/(Mi x Ci). The use of C to estimate the decomposition allowed to correct for potential inorganic contamination of litter during the experiment, similar to the use of ash-free dry mass but with greater precision in the estimates (cf. Handa et al. 2014). Similarly, the N loss was estimated through the difference between initial and final N concentrations (Ni and Nf, respectively), weighted by litter mass loss: N loss (prop.) = [(Mi x Ni) - (Mf x Nf)]/(Mi x Ni). Positive N loss values indicate N decreased during litter incubation, while negative values indicate N increased. Fungal biomass production was estimated as the concentration of ergosterol (in µg) divided by litter discs dry mass (in grams).

Statistical analysis

We used linear models to test whether functional diversity (resource dissimilarity) and litter quality (resource concentration) of species mixtures or their interaction affect litter decomposition, N loss and fungal biomass production. The models were performed using the gls function (generalized least squares) and the restricted maximum likelihood method (REML), both from the nlme package (Pinheiro et al. 2020). The stream section – to which artificial channels were distributed – was treated as a random factor in previous models, but discarded in the final models because both residuals and Akaike information criterion of this model indicated no spatial dependency or need of this component (Table S2). Residuals of each model were explored visually with graphical tools (for example, boxplots and scatterplots), and no violation of the assumptions of linear models was detected (Zuur et al. 2009) after the incorporation of variance components in some models (varIdent; Zuur et al. 2009; Table S2).

To test whether the loss of functionally dissimilar rare species had greater influence on decomposition, N loss and fungal biomass production of litter mixtures than the loss of functionally similar rare species, we quantified the magnitude of such effects. For decomposition and fungal biomass production, the magnitude of effects was calculated through the ratio between the values of each scenario in relation to the control values (that is, scenario A, with all four rare species present), for each replicate; for the N loss, this was calculated by subtracting the values of each scenario from the control values (due to the presence of both positive and negative values), for each replicate. We then calculated non-parametric 95% bootstrapped confidence intervals (as detailed above) for each ratio or difference to test whether these intervals contained a value of either one (for decomposition and fungal biomass production) or zero (N loss) – that is, the null expectation that the decomposition, N loss or fungal biomass production of different scenarios is similar to what would be expected based on the control scenario. The use of this analytical technique eliminates the need to meet assumptions of parametric models (for example, normal distribution and homogeneity of variance), and facilitates the interpretation of the results (Wood 2005). All analyses were performed using R v. 4.0.3 (R Core Team 2020).

RESULTS

Confidence intervals of functional diversity index for each scenario evidenced non-overlapping values for the four scenarios and showed that the loss of litter from rare plant species reduced functional diversity of whole litter mixtures by 3 to 11%, on average (Figure 2b).

After 90 days of incubation of litter mixtures in the stream, the average ± SE values of mixtures were: 0.318 ± 0.015 (proportion) for litter decomposition, 0.108 ± 0.023 (proportion) for N loss and 182.6 ± 22.8 μg ergosterol g^{-1} litter dry mass. Using linear models, we observed that an increase in the functional diversity of litter mixtures resulted in a consistent increase in litter decomposition, N loss and fungal biomass production (Figure 3; Table 2); whereas increased litter quality (that is, resource concentration) reduced fungal biomass production, although did not affect decomposition or N loss (Figure 4; Table 2). We did not find any interaction between functional diversity and litter quality in the models (Table 2). The presence of all four rare species in the litter mixtures (scenario A) increased decomposition (by 19% or 84 ± 53 mg C g^{-1}), N loss (18% or 182 ± 68 mg N g^{-1}) and fungal biomass production (80% or 261 ± 23 μg ergosterol g^{-1} DM) when comparing to the scenario without rare species (scenario D; Figure 5; Table S3). These results support the relevance of functional diversity and of rare species to these processes.

To assess the relative importance of similar versus dissimilar rare species in litter mixtures we compared bootstrapped confidence intervals of these two scenarios and their differences in relation to the control (all rare species present). The presence of only dissimilar rare species (scenario B) did not change decomposition to the rates observed in the presence of all rare species, but (ii) reduced N loss (on average, 13% or 130 ± 77 mg g^{-1}) and fungal biomass production (43% or 144 ± 18 μg g^{-1}) (Figure 5; Table S3). In contrast, all processes were reduced in the presence of only similar rare species (scenario C) to similar rates (N loss by 14% or 62 ± 35 mg g^{-1}) or even higher (decomposition by 11% or 112 ± 49 mg g^{-1} and fungal biomass by 53% or 174 ± 17 μg g^{-1}) than those observed in the presence of only dissimilar rare species (Figure 5; Table S3). These results suggested that the presence of dissimilar rare species (rather than similar ones) in litter mixtures was critical to the maintenance of these process.

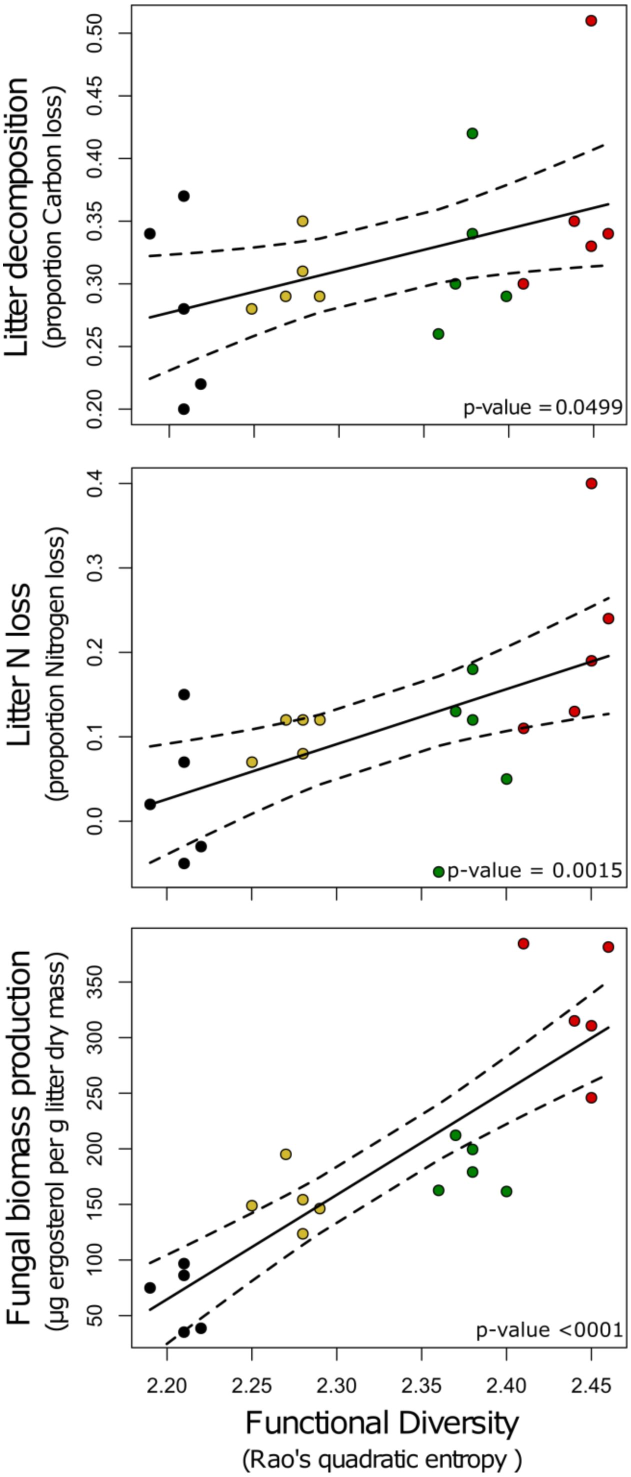


Figure 3. Linear models showing the relationship between functional diversity (Rao's quadratic entropy) of plant litter mixtures and the three processes: litter decomposition, litter nitrogen (N) loss and fungal biomass production. Circles colors represent scenarios that lost rare species: B (green; dissimilar rare species present), C (yellow; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species). Continuous lines are the fit of linear models and dashed lines the 95% confidence intervals.

Table 2. Results of linear models testing for the effect of litter quality (expressed as the first axis of a PCA using the community weighted mean values for litter traits, CWM 1), functional diversity (FD, calculated using Rao's quadratic entropy) and their interaction on decomposition, nitrogen (N) loss and fungal biomass production. Bold values indicate statistically significant differences.

	df	F-value	p-value
Decomposition			
Intercept	1	492.25	
CWM 1	1	0.47	0.5004
FD	1	4.50	**0.0499**
CWM 1 x FD	1	>0.01	0.9749
N loss			
Intercept	1	170.70	
CWM 1	1	0.10	0.7497
FD	1	14.60	**0.0015**
CWM 1 x FD	1	0.16	0.6904
Fungal biomass			
Intercept	1	311.89	
CWM 1	1	7.24	**0.0160**
FD	1	68.52	**<.0001**
CWM 1 x FD	1	0.80	0.3831

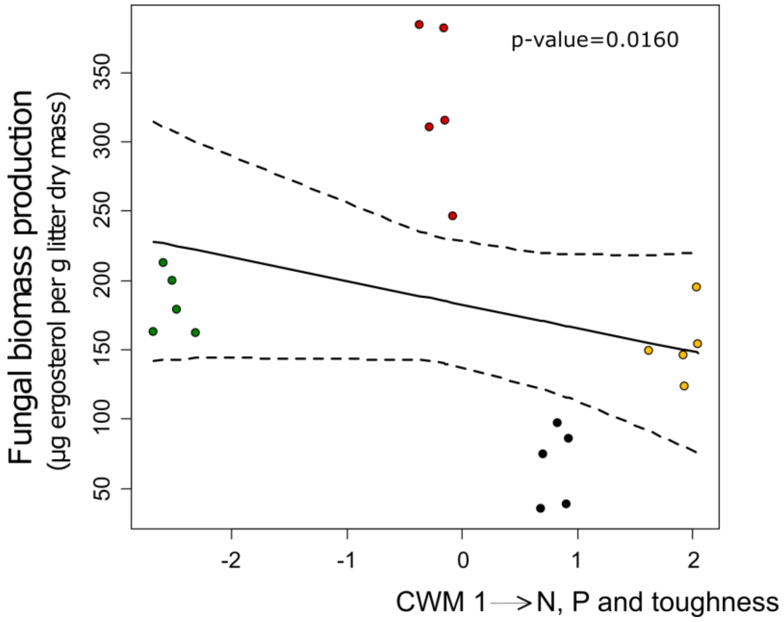


Figure 4. Relationship between litter quality of plant litter mixtures (expressed as the first axis of a PCA using the community weighted mean values for litter traits, CWM 1; Figure 2a) and fungal biomass production. Scores of CWM 1 were positively related to nitrogen (N) and phosphorus (P) concentrations and toughness. *P* value was obtained from a linear model testing for the effect of litter quality, functional diversity and their interactions on the fungal biomass production of litter mixtures (Table 2). Circles colors represent scenarios that lost rare species: B (green; dissimilar rare species present), C (yellow; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species). Continuous line is the fit of the linear model and dashed lines are the 95% confidence intervals.

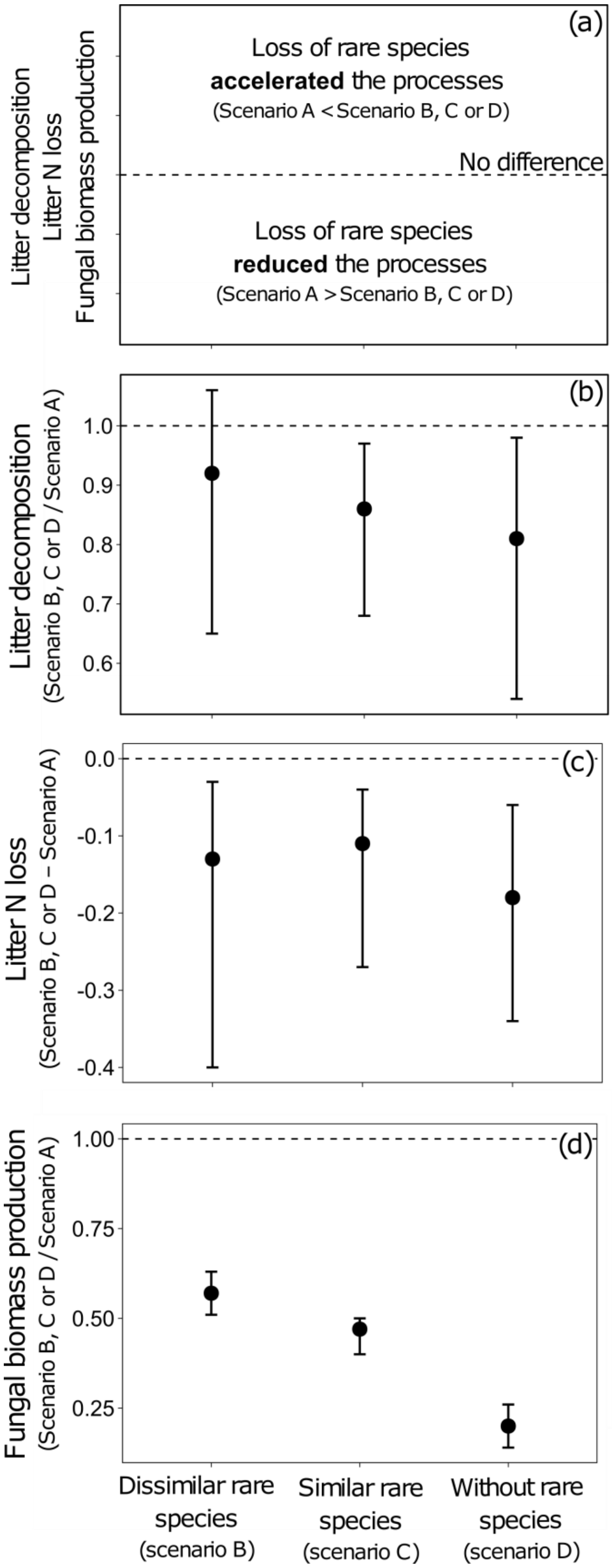


Figure 5. Ratio (for litter decomposition and fungal biomass production) or the relative difference (for litter N loss) between scenarios that lost rare species (B, C or D) and the scenario with all four rare species (A): (a) expected responses of processes to rare species loss; (b) litter decomposition; (c) litter N loss; and (d) fungal biomass production. Circles are means and vertical lines denote upper and lower limits of 95% non-parametric bootstrapped confidence intervals. The dashed line denotes the value of one (for litter decomposition and fungal biomass production) or zero (for litter

N loss), that is, the null expectation that the values of processes in the scenarios that lost rare species are not different from the scenario with all four rare species.

DISCUSSION

Plant species diversity effects on litter decomposition have been demonstrated using a variety of approaches in both observational and experimental studies (see Cardinale et al. 2011; Mori et al. 2020). However, considering that most studies were performed outside tropical areas, that is, in relatively low diverse forests with few numbers of rare plant species (for example, Swan & Palmer 2004), it is unsurprising that the role of these rare species has been seldom explored. Here, using artificial channels located within a tropical stream, we showed that losing litter from rare species reduces functional diversity of litter mixtures and inhibits several key ecosystem processes, namely litter decomposition, litter N loss and fungal biomass production. While the functional diversity of mixtures was important to all three instream processes, the quality of litter mixtures was relevant only to fungal biomass production, which is reduced by resource concentration (that is, higher N, P and toughness). Also, our results evidenced that the presence of litter from functionally dissimilar rare species in litter mixtures maintained or caused less reduction on the above-mentioned processes (except for N loss).

Our finding that rare species influence key ecosystem processes contradicts the mass ratio hypothesis (Grime 1998), which states that species in a community have an effect on the ecosystem that is proportional to their mass. Thus, we could expect no effect of rare species presence or their functional traits on ecosystem processes due to their low representativeness in terms of litter mixture total mass (that is, one quarter of total mass) (Tardif et al. 2014). However, we showed that even a relatively small decrease in the average functional diversity of litter mixtures (RaoQ from 2.44 to 2.21) – for example, through the removal of rare species – was sufficient to considerably reduce the studied processes. Other studies have also found a decrease in the functional diversity (or richness, specialization and originality) with the loss of rare species in tallgrass prairies in the North America (Jain et al. 2014), stream fish in the Brazilian Amazon, rainforest trees in French Guiana and birds in the Australian wet tropics (Leitão et al. 2016). Similarly, others have found a reduction in key ecosystem processes – for example, productivity and nutrient cycling of terrestrial ecosystems – with the loss of rare species (Dee et al. 2019).

Positive effects of functional diversity on instream litter decomposition have been previously reported in field, laboratory and mesocosm experiments (for example, Handa et al. 2014; Oliva et al. 2019; López-Rojo et al. 2018; Boyero et al. 2021), and on N loss in field experiments (Handa et al. 2014). These results contrast with others showing weak or no evidence of litter trait dissimilarity effects on decomposition (for example, Hoorens et al. 2003; Schindler & Gessner 2009; Frainer et al. 2015) and on N loss (Frainer et al. 2015). Inconsistent responses to litter functional diversity could be due to (i) its short-term effect that may disappear with time (Patoine et al. 2017) and/or (ii) the different or arbitrary choice of functional diversity metrics across studies. For example, some studies have used functional diversity based on a single litter trait or on a categorical classification such as functional groups (Hoorens et al. 2003; Schindler & Gessner 2009; Oliva et al. 2019). Although positive effects of functional diversity on decomposition using these approaches have been observed (for example, Oliva et al. 2019), they may not be adequate because they do not consider the complexity of multiple litter traits that potentially influence decomposition (Epps et al. 2007).

Increased decomposition and N loss with greater functional diversity of mixtures may be due to nutrient transfer from nutrient-rich (dominant and similar rare species in our study) to nutrient-poor species (dissimilar rare species), which is promoted by leaching or fungi growing on litter (for example, Handa et al. 2014). In streams, aquatic hyphomycetes dominate fungal communities in terms of biomass (Krauss et al. 2011) and are capable of extending their hyphae to acquire resources from considerable distant litter (Ritz 2006). Laboratory studies using isotopes supported the occurrence of nutrient transfer between chemically distinct litter types (for example, Lummer et al. 2012), which was also observed in a large-scale experimental field study using litter mixtures of N-fixing and rapidly decomposing deciduous plants (Handa et al. 2014). In our study, carbon of high quality (that is, litter of lower toughness) from dissimilar rare species may have favored microbial growth, which in turn increased the demand for nutrients and promoted the transfer of N from neighboring dominant species (and/or rare species functionally similar to the dominant ones) (Lummer et al. 2012). In this context, the increase of fungal biomass production with functional diversity suggests these fungi benefited from the variability of resources via complementarity or facilitation mechanisms — as previously observed (for example, Santoja et al. 2017).

It is important to recognize that our experimental design did not fully allow disentangling species richness from functional diversity in different scenarios of rare species loss. For instance, the scenario with greatest functional diversity has also more species, while lower functional diversity occurred when fewer species were present. This could indicate that the enhanced processes were due to increased species richness of mixtures; however, this is unlikely, as all processes increased consistently and linearly across the functional diversity gradient even when species richness was the same. Also, recent studies support the use of functional diversity rather than species richness as a more important predictor of litter decomposition (Handa et al. 2014; Boyero et al. 2021), as well as of other ecosystem processes (Cadotte et al. 2011).

In line with previous BEF studies (for example, Marsh et al. 2000; Lyons & Schwartz 2001; Soliveres et al. 2016), our study provides support that functionally unique rare species may contribute disproportionately to ecosystem functioning. Despite the rapid biodiversity decline faced by the tropics, BEF research has mostly neglected this region (Clarke et al. 2017). This lack of knowledge in such rapidly growing research topic is surprising, considering that rare species are more vulnerable to disturbances (Lavergne et al. 2005) and predominantly support distinct sets of traits capable to influence the functioning of ecosystems (Mouillot et al. 2013). In consequence, there is a crucial need to a better integration of BEF research into the tropical context, especially to understand the role of rare species in such diverse ecosystems.

We provide novel evidence that litter from rare plant species is capable of accelerating key ecosystem processes related to carbon flow in tropical streams, especially when species provide dissimilar traits to litter pools. Our findings have important management implications and relevance to restoration of riparian forests in the tropics if the aim is to prioritize efforts and resources in the maintenance of species with greater functional role to ecosystems. Although such findings have emerged from studies in tropical streams, they are not limited to these ecosystems considering that the diversity of rare species is often greater than that of dominant species, and litter traits of species greatly differ within ecosystems (Homeier et al. 2021). Also, our results provide empirical support for using trait-based approaches rather than species identity when modeling the effects of environmental changes such as species extinction or invasions on ecosystems.

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SUPPORTING INFORMATION

SI 1 INFORMATION OF THE SAMPLING SITE

Table S1. Physical and chemical parameters (average ± standard error) of stream water recorded during the experiment (August to October of 2016). The parameters were measured *in situ* in three different occasions with portable equipment (except temperature which was continuously recorded using data loggers, HOBO Pendant, Onset): conductivity and pH (Lenway 430 pH / meter, Meter), dissolved oxygen (Lenway 970 Meter DO2) and water flow (estimated through the current velocity [Global Water, FP101] and the stream wetted width and depth).

Parameter	**Average** ± **SE**
Temperature (°C)	21.1 ± 0.1
Conductivity (µS cm^{-1})	5.5 ± 0.2
pH	6.8 ± 0.1
Dissolved oxygen (mg L^{-1})	6.6 ± 1.3
Dissolved oxygen (%)	81 ± 3
Flow (m^3 s^{-1})	0.96 ± 0.06

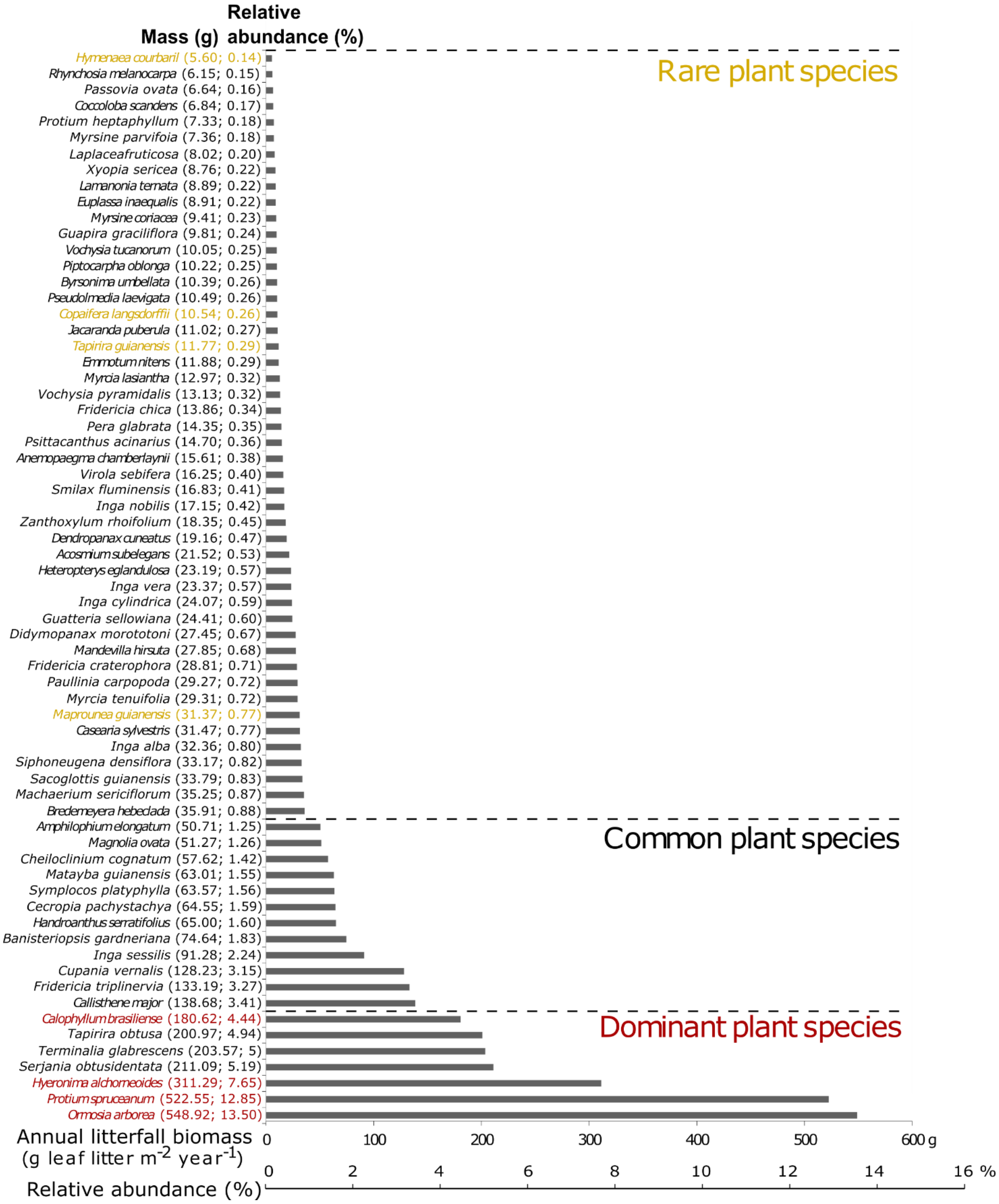

Figure S1. Leaf litter biomass (g m^{-2}) and relative abundance from dominant (those that contributed with more than 50% of the total biomass), rare (those with individual contribution lower than 1% of the total biomass) and common species (those that were not dominant or rare species) in the litterfall of a whole year (September 2010 to August 2011, unpublished data).

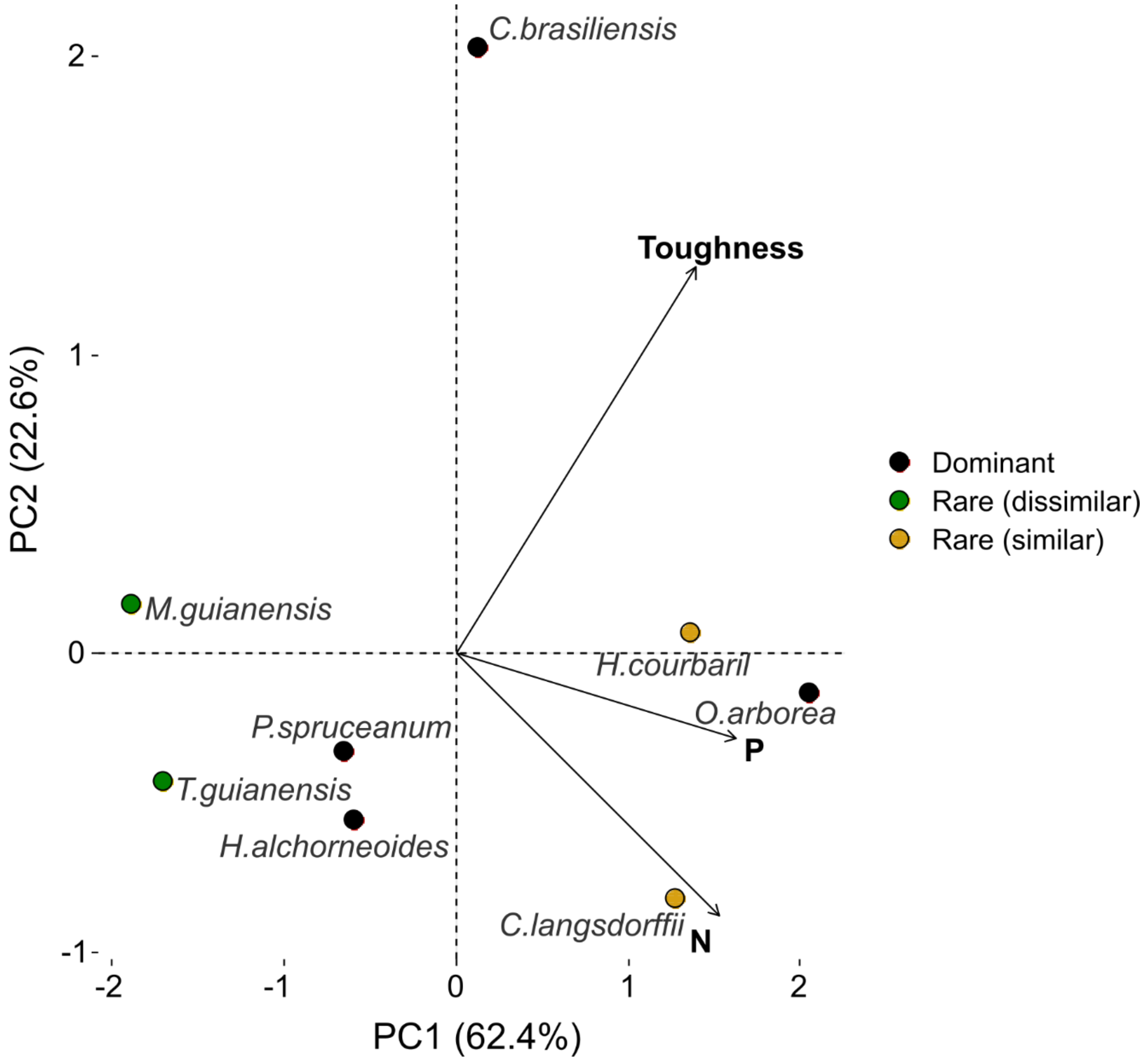

Figure S2. Principal component analysis (PCA; PCA function, FactoMineR package) using chemical and structural traits of species (nitrogen [N] and phosphorus [P] concentrations and leaf toughness) previously standardized with z-scores, used to quantify and visualize the trait differences among our 8 litter species. Circles colors represent species groups. PCA allowed us to visually confirm dominant species traits and the differences in chemical and structural traits between the two distinct groups of rare species: species with similar (*H. courbaril* and *C. langsdorffii*) or dissimilar (*M. guianensis* and *T. guianensis*) traits to dominant species, based on the position of each species (i.e., proximity with traits) in the litter trait space. The points represent the ordering of the species, and the arrows the ordering of the traits.

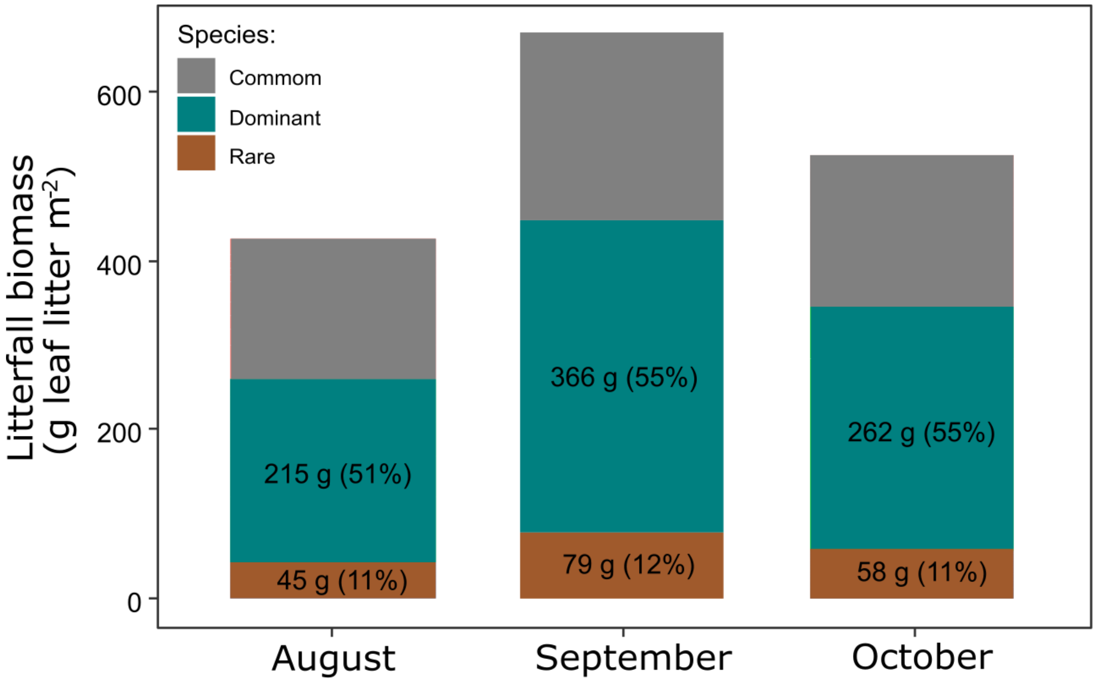

Figure S3. Leaf litter biomass (g m^{-2}) from dominant (those that contributed with more than 50% of the total biomass), rare (those with individual contribution lower than 1% of the total biomass) and common species (those that were not dominant or rare species) in the litterfall of August, September and October 2010 (unpublished data). Biomass ratio between dominant and rare species in the litterfall of approximately 4:1 was found in the three months. (n =10 samples)

Litterfall sampling

Litterfall was estimated using 10 suspended 1-m^2 nets distributed in a 70-m long reach of the riparian forest (near or in the stream bank). Samples were collected once a month for a year (September 2010 to August 2011), oven dried and leaves sorted (additional details of sampling design and processing are found in Tonin and others 2017). Leaves were identified to the species level based on exsiccates and databases [Tropics projects (http://www.tropicos.org), Flora do Brasil (http://floradobrasil.jbrj.gov.br/reflora) and Field Museum (http://www.fieldmuseum.org)] and weighed separately by species.

Table S2. Results of the best-fitting models (M) to test the effect of quality, functional diversity and their interactions on litter decomposition, nitrogen (N) loss and fungal biomass production. Best-fitting models are in bold.

	Random factor (stream sections)	Variance component (varIdent)	M	df	AIC	p-value
Decomposition	**Absent**	**Absent**	**1**	**5**	**-31.71**	
Decomposition	Present	Absent	2	6	-29.71	0.99
Decomposition	Absent	Present	3	8	-30.16	0.11
N loss	Absent	Absent	1	5	-24.47	
N loss	Present	Absent	2	6	-22.47	1
N loss	**Absent**	**Present**	**3**	**8**	**-26.86**	**0.01**
Fungal biomass	**Absent**	**Absent**	**1**	**5**	**178.84**	
Fungal biomass	Present	Absent	2	6	180.02	0.37
Fungal biomass	Absent	Present	3	8	180.63	0.18

Table S3. Averages and upper and lower limits of 95% non-parametric bootstrapped confidence intervals for the ratio of decomposition and fungal biomass, and relative difference in litter N loss between scenarios that lost rare species (B, C or D) and the scenario without rare species loss (A). Confidence intervals that do not contain the value of one (litter decomposition and ergosterol) or zero (litter N loss) indicate statistical differences (in bold).

	Average	95% Intervals
Litter decomposition		
Scenario D vs. Scenario A	0.81	**0.54, 0.98**
Scenario C vs. Scenario A	0.86	**0.68, 0.97**
Scenario B vs. Scenario A	0.92	0.65, 1.06
Litter N loss		
Scenario D vs. Scenario A	-0.18	**-0.34, -0.06**
Scenario C vs. Scenario A	-0.11	**-0.27, -0.04**
Scenario B vs. Scenario A	-0.13	**-0.40, -0.03**
Fungal biomass		
Scenario D vs. Scenario A	0.20	**0.14, 0.26**
Scenario C vs. Scenario A	0.47	**0.40, 0.50**
Scenario B vs. Scenario A	0.57	**0.51, 0.63**

CAPÍTULO 2 – EFFECTS OF THE LOSS OF RARE PLANT SPECIES ON COLONIZATION BY AQUATIC HYPHOMYCETES: RESPONSES TO VARIATION IN EVENNESS

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Em revisão na *Fungal Ecology.*

ABSTRACT

Aquatic fungi are important decomposers of plant litter in tropical streams, which are suffering the rapid loss of species diversity in their riparian forests due to anthropogenic disturbances. While most studies evaluating plant species diversity effects on in-stream fungal decomposers have focused on the quality of dominant species (i.e., resource concentration), there is little evidence on the importance of rare species. Our study simulated different extinction scenarios of plant litter from rare species comprising a decreasing gradient of functional diversity (i.e., resource dissimilarity). We assessed whether the loss of these species altered the fungal biomass and aquatic hyphomycete sporulation, diversity and taxonomic composition in two experiments: even experiment, where we used the same biomass for all species (i.e., even litter mixtures); and natural proportion (NP) experiment, where we kept proportions of plant species as found in stream leaf litter (i.e., natural litter mixtures).We found that the loss of litter from rare plant species reduced fungal biomass and the reduction was greater for natural litter mixtures, indicating that evenness modulated the effect of species loss on fungal biomass (even if evenness alone had no effect), possibly through resource dissimilarity. Our findings reveal the relevance of litter from rare plant species and the maintenance of natural species proportions in tropical riparian forests for the functioning of stream ecosystems.

Keywords: sporulation; species diversity; fungal biomass; riparian forests; tropical streams.

INTRODUCTION

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

In this context, rare plant species may add unique traits to litter mixtures and increase their functional diversity, that is, the variation in species trait values. This may affect both organic matter processing and decomposer organisms (resource dissimilarity effects; Rabelo et al. 2022). Numerous mechanisms have been put forward to explain positive effects of functional diversity related to the organic matter processing such as nutrient transfer among distinct litter species via fungal hyphae or leaching (Handa et al. 2014), and complementary resource use by consumers (i.e., litter with different nutrients concentration or toughness; Chapman et al. 2013; Vos et al. 2013; Handa et al. 2014). Functional diversity in litter mixtures seems to enable a more efficient use of resources by decomposers. Similarly, rare plant species can change the average litter quality of mixtures and affect decomposer organisms (resource concentration effects; Rabelo et al. 2022), even although they have low representativeness in terms of biomass in litter mixtures.

Plant species evenness (i.e., relative abundance of species) in litter mixtures influences the heterogeneity and overall quality of litter resources to consumers (e.g., nutrient contents, phenolics and structural compounds), and thus also can affect decomposer organisms (e.g., Gonçalves & Canhoto 2009; Pereira & Ferreira 2022). Despite that, in most biodiversity-ecosystem function (BEF) experimental studies conducted in the tropics, litter mixtures involve higher species evenness than is normally encountered in nature. In general, these studies equal diversity to species richness (see Hillebrand et al. 2008), without taking evenness into account. However, leaf litter in tropical streams is often dominated by a few species, with the rest being rare species (Tonin et al. 2021). Thus, it is important to investigate how this can influence the effects of species diversity in litter mixtures on decomposer organisms (Swan et al. 2009; Liu et al. 2020).

The aquatic hyphomycetes include a variety of fungal species commonly associated with litter in streams (Krauss et al. 2011), and are main agents of litter breakdown in tropical streams (Gonçalves et al. 2007; Graça et al. 2016). Even though few studies have investigated the effect of changes in the functional diversity in litter mixtures on these fungi, evidence from some studies indicates that it can affect aquatic hyphomycete communities. Previous studies observed more diverse communities of these fungi (Rajashekhar & Kaveriappa 2003; Laitung & Chauvet 2005; Jabiol & Chauvet 2012) and higher fungal biomass (Chapman et al. 2013; López-Rojo et al. 2020; Rabelo et al. 2022) on litter mixtures or riparian vegetation streams more diverse. The aquatic hyphomycetes can benefit from more heterogeneous resources because it can provide substrata for the growth of their different species (Rajashekhar & Kaveriappa 2003), and to enable the complementary resource use (Handa et al. 2014).

The aquatic hyphomycetes have a high degree of functional redundancy (Gessner et al. 2007), but some studies have indicated species preferences for specific litter substrates, which are mainly determined by differences in chemical and structural traits. The litter that is soft (low toughness) and has high concentrations of nutrients is generally preferred by these fungi (e.g., Gomes et al. 2016). The changes in the proportions (evenness) of litter species alter the overall quality of litter mixtures for these traits (resource concentration), which can influence the aquatic hyphomycetes grown, sporulation rate and community structure (Gonçalves & Canhoto 2009; Pereira & Ferreira 2022).

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

METHODS

Study site

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

The riparian forest of the stream presents perennial vegetation, high floristic diversity (ca. 111 plant species; Bambi et al. 2016b) and dense vegetation cover (70 to 95%; Ribeiro & Walter 2008). During the experimental period the stream water presented circumneutral pH (6.79 ± 0.12), low conductivity (5.51 ± 0.18 µS cm^{-1}), good oxygenation (6.62 ± 1.3 mg L^{-1}), stable temperature (21.15 ± 0.01 °C) and low discharge (0.96 ± 0.06 $m^3 s^{-1}$; Rabelo et al. 2022). The concentration of dissolved nutrients was low (28.7 ±1.7 µg L^{-1} and 20.2 ±1.2 µg L^{-1} of dissolved inorganic nitrogen [N] and orthophosphate, respectively; Tonin et al. 2019).

Dominant and rare plant species

Four dominant and four rare species were selected from those present in the riparian forest. The selection was made based on the composition of terrestrial litterfall of the experimental stream throughout one year, studied previosly (September 2010 to August 2011; Rabelo et al. 2022). Species were considered dominant when they together contributed > 50% of total litterfall biomass, and rare when contributed <1% of the total. Dominant species with high toughness and moderate nutrient concentrations (i.e., N and phosphorus [P]) were chosen to represent the chemical and structural traits of dominant species in the Cerrado (Tonin et al. 2021). Rare species were selected to represent two distinct groups: species with either similar or dissimilar chemical and structural traits (i.e., toughness and nutrient concentrations) to dominant species (Fig. S1a; Table S1). The dominant species selected were *Protium spruceanum* (Benth.) Engl. (hereafter *P. spruceanum*), *Calophyllum brasiliense* Cambess. (*C. brasiliense*), *Ormosia arborea* (Vell.) Harms (*O. arborea*) and *Hyeronima alchorneoides* Allemão (*H. alchorneoides*); similar rare species were *Hymenaea courbaril* L. (*H. courbaril*) and *Copaifera langsdorffii* Desf. (*C. langsdorffii*), and dissimilar rare species were *Maprounea guianensis* Aubl. (*M. guianensis*) and *Tapirira guianensis* Aubl. (*T. guianensis*). For more detailed information on dominant and rare species selection and their chemical and structural traits, see Rabelo et al. (2022).

Experimental design

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

Four litter mixtures made of different combinations of dominant and rare species were used in the experiments, in order to simulate different scenarios of rare species loss. All scenarios contained the four dominant species mentioned above, plus different numbers and combinations of rare species: scenario A had four rare species, with no species loss (control); scenario B had two rare species (*M. guianensis* and *T guianensis*) and represented the loss of rare species functionally similar to dominant species; scenario C had two rare species (*H. courbaril* and *C. langsdorffii*) and represented the loss of rare species functionally dissimilar to dominant species; and scenario D had no rare species (Fig. S1a).

Whole leaves of each species were collected from natural litterfall in the study stream, air-dried and stored until used. Then, they were weighed and introduced into fine-mesh litterbags (250 μm), which allowed colonization and biological degradation only by microorganisms. For even experiment, we used 60 litterbags (20 x 13 cm), 15 for each scenario. Each dominant and rare litter species was represented equally by biomass (as mentioned above). That is, each litterbag contained a total of 5.0 ± 0.3 g – 0.63 ± 0.3 g per species in scenario A, 0.83 ± 0.3 g per species in scenarios B and C and 1.25 ± 0.3 g per species in scenario D. Litterbags were distributed in 20 PVC artificial channels (0.5 X 0.15 X 0.15 m), which were placed along a 150 m stretch of the stream in five groups of four channels (sections), with each channel within a group representing a different scenario (Fig. S1b). Each channel received three litterbags, one for each incubation period (20, 40 and 68 days; Fig. S1b). There was thus a total of five litterbags replicated per scenario for each incubation period. We used artificial channels to homogenize the environmental conditions found in field as type of substrate, depth, current, accumulation of fine sediments and presence of other organic materials. The channels were placed in areas non-sinuous, with regular water flow and with a depth of around 40 cm, being covered at the top and closed upstream with a screen (0.2 cm) to prevent the entrance of litter other than the experimental one.

For NP experiment, methods were similar, except for the litter and the incubation periods. Litter proportions followed a 4:1 ratio, with 4 ± 0.3 g in total from dominant species (1 ± 0.3 g per species) and 1 ± 0.3 g in total from rare species (0.25 ± 0.3 g per species in scenario A and 0.50 ± 0.3 g per species in scenarios B and C); the exception was scenario D, where there were only dominant species (1.25 ± 0.3 g per species). Incubation periods in this case were 30, 60 and 90 days (Fig. S1b).

Laboratory procedures

At each retrieval time, litterbags were collected and transported to the laboratory within a thermal container with ice. In the laboratory, the litter was washed carefully with distilled water. Two sub-samples of discs (8 mm in diameter) were taken for the even (2 discs per species in each mixture) and NP (4 discs per dominant species and 1-2 discs per rare species in each mixture, maintaining the 4:1 ratio) experiments, except for the last incubation period (i.e., 68 or 90-days period of even and NP experiment, respectively), when three sub-samples were taken.

The discs of the first sub-sample were used to induce sporulation by shaking (60 rpm) the leaf discs in 50 mL of distilled water for 48 h in a 12:12 h light regime with constant temperature (20º C), conditions like those observed in the stream. After 48 h of agitation, the water was collected and spores fixed with 4% formalin. Aliquots of the conidial suspensions were filtered (Millipore, pore size 5 µm, Billerica, MA, USA), and filters were stained with 0,05% of cotton blue (Sigma-Aldrich, Steinheim, Germany) in 60% lactic acid (Vetec, Rio de Janeiro, Brazil) and observed with a compound microscope at 400 x magnification (Olympus BX 43). Conidial morphology was used to identify aquatic hyphomycete species (Barlocher 2020), using taxonomic keys (Gulis et al. 2005).

The discs of the second sub-sample were oven-dried (60ºC, 72 h) and weighed to determine dry mass (DM; accuracy 0.01 mg). The discs of the third sub-sample (taken only for the last period) were freeze-dried for 48 h and used to estimate fungal biomass, through concentration of ergosterol of the dominant species group and each one of the rare species in the mixture. Ergosterol was quantified by extraction in methanol/KOH solution at 80ºC, filtration with SEP-PACK® columns (Waters, VAC RC tC18 500 mg) using vacuum, isopropanol elution and quantification in high-performance liquid chromatograph (Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC) (Gessner et al. 2020). The remaining litter was oven-dried (60 ºC, 72 h), weighed to determine DM and added to the DM of the discs taken from the sub-samples to determine the final DM of the mixture: final DM = litter DM + (disc DM × 2 or 3).

Litter quality and functional diversity of mixtures

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

Community-weighted mean (CWM) traits of different species mixtures were used to quantify the average litter quality of mixtures. Trait values were based on measurements by Rabelo et al. (2022) from litter collected in the riparian forest of the study stream (Table S1). The traits measured were N and P concentrations and toughness (see Rabelo et al. (2022) for details on measurement procedures). For abundance values, we used species dry mass in the mixture as used in decomposition studies (García-Palacios et al. 2017). First, we calculated the CWM for each trait individually using the functcomp function from the FD package. Then, we conducted a principal components analysis (PCA) using the CWM values of all the traits measured. Before conducting the PCA, we applied standardization using Z-scores. Litter quality of species mixtures was assessed by retaining the first PCA axis (hereafter CWM 1), which explained most of the variance of the mean trait values (84.1%). The CWM 1 was positively loaded by higher concentrations of N, P and toughness (Fig. 1a).

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

We compared 95% confidence intervals for the functional diversity of litter mixtures across the four scenarios to assess whether the loss of litter from rare plant species decreases the functional diversity of whole litter mixtures. Confidence intervals were based on 1,000 resamples using the BCa method with the boot function and package (Davison & Hinkley 1997; Canty & Ripley 2020). Confidence intervals of the functional diversity index for each scenario evidenced non-overlapping values for the four scenarios in NP experiment. Even experiment values were overlapping only for the scenarios with presence of only similar rare species (scenario C) and without rare species (scenario D). Despite that, mixtures in both experiments showed reduced functional diversity with the loss of litter from rare plant species (Fig. 1b). All analyses were performed using R version 4.1.3 (R Development Core Team, 2022).

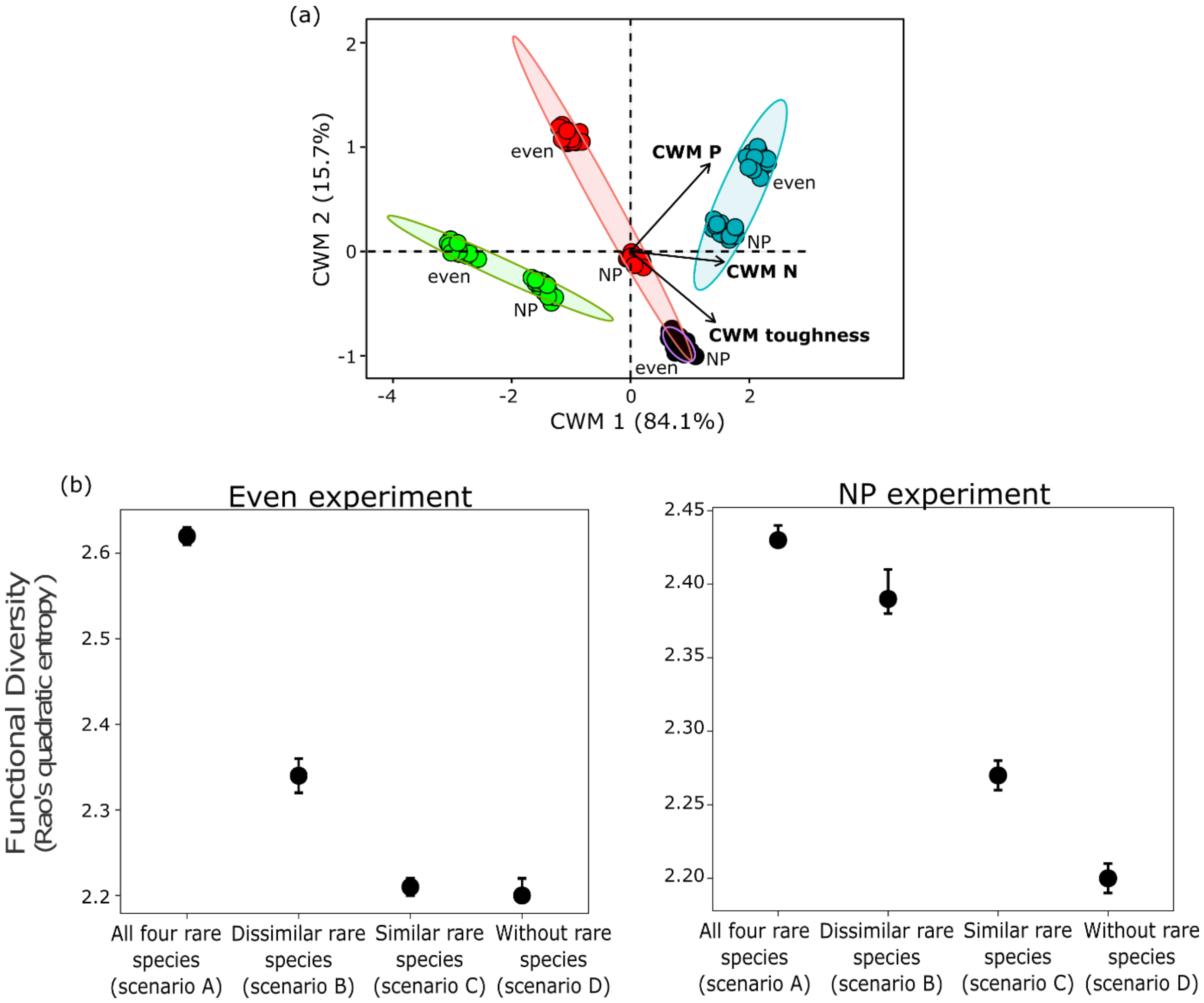

Figure 1. Litter quality (represented by the first axis of principal component analysis of community-weighted mean [CWM] traits: nitrogen [N] and phosphorus [P] concentrations and toughness; a) and functional diversity (as the confidence intervals of Rao's quadratic entropy index; b) of plant mixtures in the four scenarios of rare species loss in even and natural proportion (NP) experiments. Circles colors (a) represent scenarios that lost rare species: B (green; dissimilar rare species present), C (blue; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species).

Calculation of response variables

The percent of litter mass loss from the mixture was calculated using the initial (Mi) and final (Mf) DM of litter: (Mi – Mf) / Mi x 100. Fungal biomass was estimated as the concentration of ergosterol (in µg) divided by litter disc dry mass (in g; Gessner & Chauvet 1993); then, it was quantified for the entire mixture (i.e., mean values of dominant and rare species present in each sample). The sporulation rate from the mixture was expressed as the number of spores per mg of DM per day (conidia day^{-1} mg^{-1} DM) (Barlocher 2020). Aquatic hyphomycete diversity in the mixture was assessed using the Simpson diversity index (Magurran 2010) and calculated using the diversity function of the vegan package (Oksanen et al. 2018).

Statistical analyses

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

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

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

RESULTS

Using linear models, we found that the scenarios of rare plant species loss significantly explained variation in fungal biomass. However, we did not find an effect on aquatic hyphomycete sporulation rate or diversity (Fig. 2; Table 1). The presence of rare plant species, especially the dissimilar ones, tended to increase fungal biomass – being the increase significant for mixtures with the presence of only dissimilar (scenario B; on average by more than two times higher) or only similar rare species (scenario C; 95%) when comparing to mixtures without rare species (scenario D; Fig. 2a). We observed that an increase in the fungal biomass resulted in a significant increase in litter mass loss (Fig. 3).

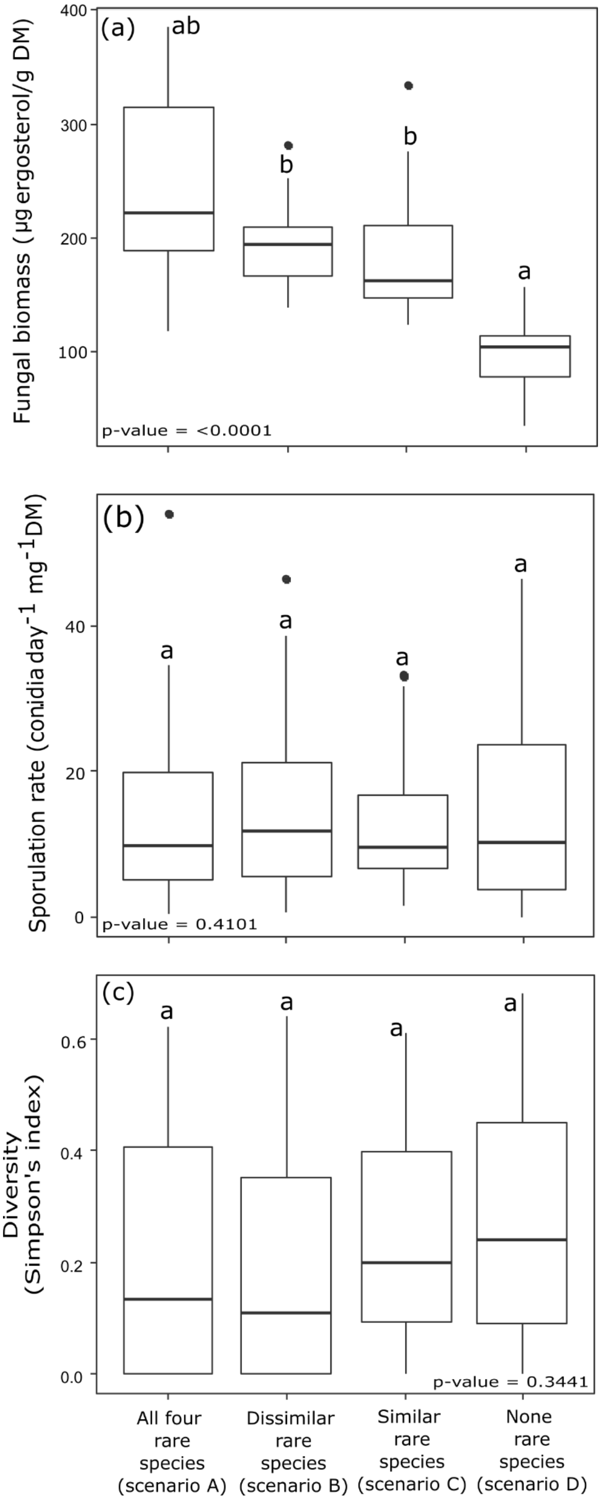

Figure 2. Effect of the scenarios of rare species loss (A, B, C and D) on the (a) fungal biomass and aquatic hyphomycete (b) sporulation rate and (c) diversity (Simpson's index). Different letters

indicate statistically significant differences ($p < 0.05$).

Table 1. Results of linear models testing for the effect of the scenarios of rare species loss: B (dissimilar rare species present), C (similar rare species present) or D (without rare species), and the scenario A (with all four rare species), evenness (even and natural proportion experiment) and their interaction on the fungal biomass and aquatic hyphomycete sporulation rate and diversity (Simpson's index). Bold values indicate statistically significant differences.

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

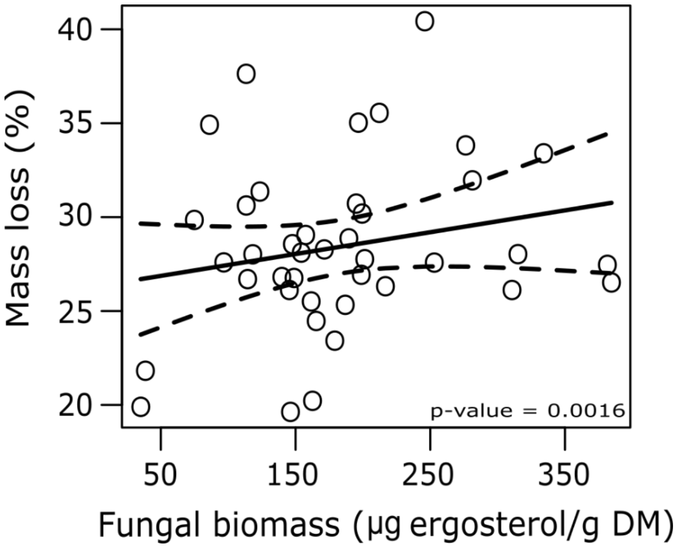

Figure 3. Linear model showing the relationship between fungal biomass and litter mass loss. Continuous line is the fit of the linear model, and dashed lines are the 95% confidence intervals.

We did not find an effect of litter evenness alone on fungal biomass, aquatic hyphomycete sporulation rate or diversity (Table 1 and 2). However, there was a significant interaction between scenario and evenness for fungal biomass (Table 1). While in even experiment the presence of only dissimilar (scenario B) or similar rare species (scenario C) increased fungal biomass by 65 and 78%, respectively, when compared to mixtures without rare species (scenario D), in NP experiment fungal biomass was more than twice higher in scenarios B and C compared to D (Fig. 4).

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

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

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

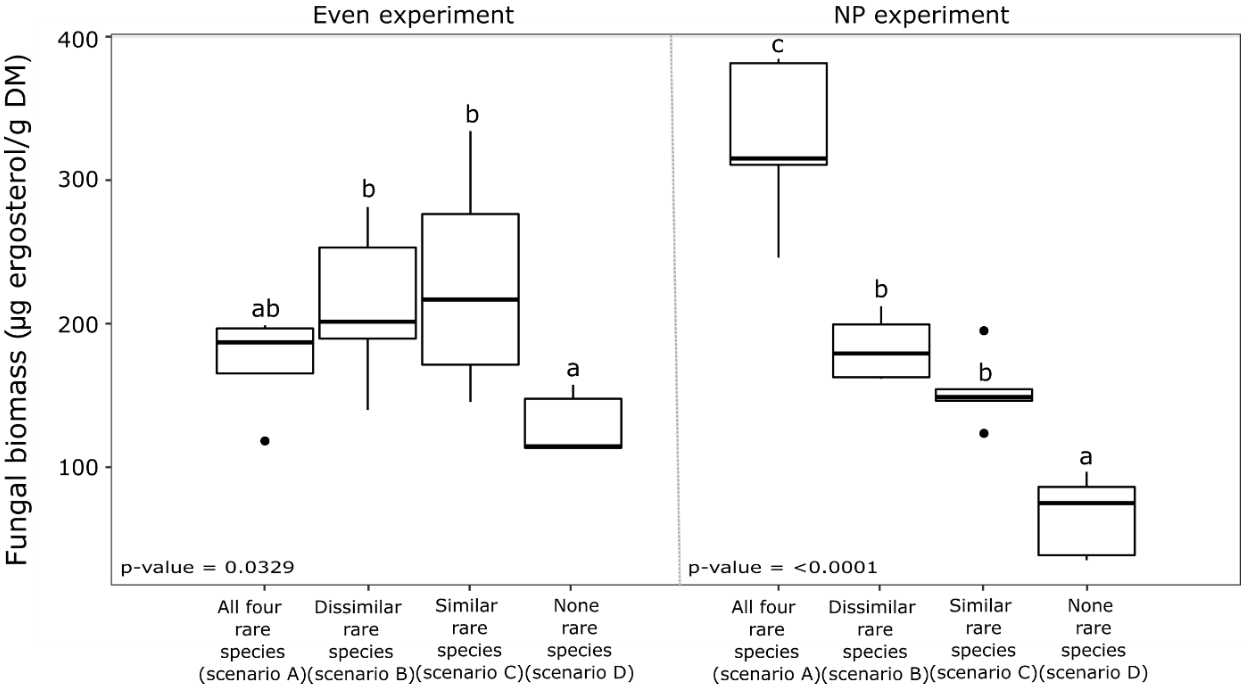

Figure 4. Effect of the scenarios of rare species loss (A, B, C and D) on fungal biomass in even and natural proportion (NP) experiments. Different letters indicate statistically significant differences ($p < 0.05$).

The increase in litter mixture functional diversity (resource dissimilarity) resulted in an increase in fungal biomass but had no effect on other fungal parameters (Fig. S2; Table 2). Similarly, litter quality (resource concentration) influenced only fungal biomass, but its effect was dependent upon evenness of litter mixtures (Table 2). Litter quality reduced fungal biomass in both experiments, but it had a much weaker effect on fungal biomass in NP experiment than in even experiment (r^2 values of 0.02 and 0.32, respectively; Fig. 5a, b). Functional diversity interacted with evenness to affect fungal biomass: the increase in functional diversity resulted in a great increase in fungal biomass in NP experiment ($r^2 = 0.75$), whereas it had no effect on fungal biomass in even experiment (Fig. 5c, d). Functional diversity and litter quality did not interact to affect any fungal parameter (Table 2).

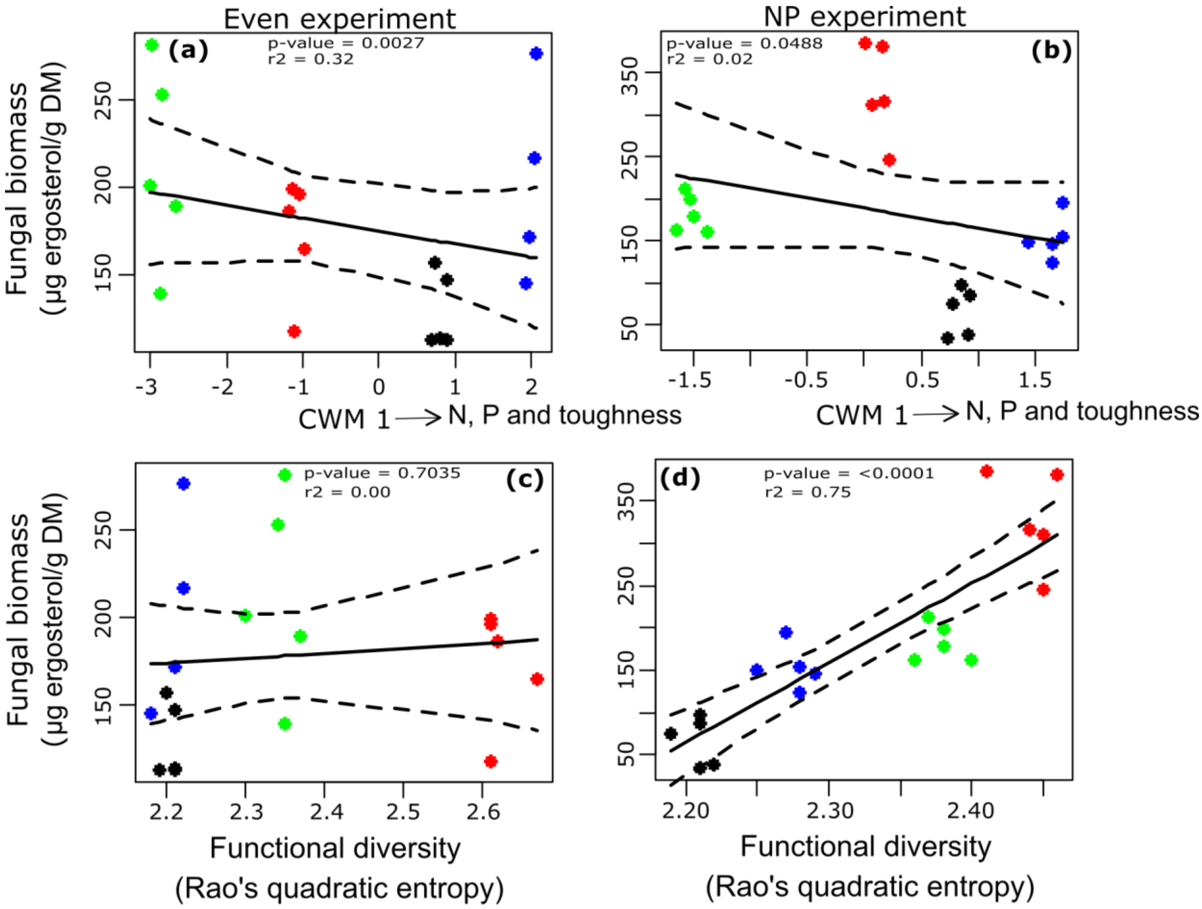

Figure 5. Relationship between litter quality (expressed as the first axis of a PCA using the community weighted mean values for litter traits, CWM 1; Fig. 1a) and functional diversity (calculated using Rao's quadratic entropy index) of plant litter mixtures and fungal biomass in even (a, c) and natural proportion (NP; b, d) experiments. Scores of CWM 1 were positively related to

nitrogen (N) and phosphorus (P) concentrations and toughness. Circles' colors represent scenarios that lost rare species: B (green; dissimilar rare species present), C (blue; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species). Continuous line is the fit of the linear model, and dashed lines are the 95% confidence intervals.

Ten species of aquatic hyphomycetes were identified on decomposing litter: *Alatospora acuminata* Ingold, *Anguillospora filiformis* Greath, *Anguillospora longissima* (Sacc. & P. Syd.) Ingold, *Colispora curvata* Nawawi & Kuthub, *Flagellospora curvula* Ingold, *Heliscus submersus* Hudson, *Lunulospora curvula* Ingold, *Triscelophorus acuminatus* Nawawi, *Triscelophorus monosporus* Ingold and an unidentified species (Fig. S3). Most variation in community composition (93%) was not explained by our predictor variables (Table 3). Evenness significantly explained variation in community composition (although it only explained 5%; Fig. S3a; Table 3), while scenario explained no variation (Fig. S3b; Table 3). There was no effect of the interaction between scenario and evenness (Table 3).

Table 3. Results of PERMANOVA analysis testing for the effect of the scenarios of rare species loss: B (dissimilar rare species present), C (similar rare species present) or D (without rare species), and the scenario A (with all four rare species), evenness (even and natural proportion experiment) and their interaction on aquatic hyphomycetes composition. Bold values indicate statistically significant differences.

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

Abbreviations: SS: mean sum of squares.
[a]Significance values based on 999 permutations.

Litter mixtures in NP experiment were colonized by only five species of aquatic hyphomycetes, while mixtures in even experiment had higher species richness (i.e., nine species;

Fig. S4). Four species were shared by the two experiments – *F. curvula*, *H. submersus*, *L. curvula* and *T. monosporus*. *T. acuminatus* was recorded only in NP experiment, while *A. acuminata*, *A. filiformis*, *A. longissima*, *C. curvata* and an unidentified species were found only in even experiment (Fig. S4). *F. curvula* was the dominant species both in even and NP experiment (mean ± SE of 80.8 ± 3.2 and 80.5 ± 2.8 %, respectively). *H. submersus* was the second most abundant species in even experiment (10.8 ± 1.6 %), whereas *L. curvula* was in NP experiment (9.3 ± 1.9 %; Fig. S4).

DISCUSSION

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

Despite the lower proportion of rare plant species in litter mixtures, their loss can change the average litter quality and functional diversity in these mixtures (see Rabelo et al. 2022), which may explain the effects of the loss of rare plant species on fungal decomposers. Differences in average litter quality in mixtures can affect decomposer organisms via resource concentration effects, while differences in functional diversity can determine the availability of complementary resources and thus affect fungi through resource dissimilarity effects (Handa et al. 2014; García-Palacios et al. 2017). While we expected a greater effect of rare species loss from mixtures in even experiment (i.e., even litter mixtures), due to the higher proportional biomass that these species have in those mixtures, which is know to influence the fungal colonization process (mass ratio hypothesis; Grime 1998), we found the opposite: fungal biomass was more affected (reduced) when rare species were lost from natural litter mixtures. Despite that, litter quality had a weaker effect on fungal biomass in these mixtures, corroborating our hypothesis. On the other hand, functional diversity explained most variation in fungal biomass in natural litter mixtures, whereas it had no effect on fungal biomass in even litter mixtures. Thus, functional diversity was the key factor for the higher reduction in fungal biomass due to rare species loss in natural litter mixtures compared to even litter mixtures. Mixtures reflecting ambient evenness may have a higher occurrence of positive non-additive effects, and thus favor more microbial growth (Swan et al. 2009; Liu et al. 2020), which may explain the results found. For example, fungi can benefit from the higher resource variability via nutrient transfer by fungal hyphae, which can contribute to their growth (Chapman et al. 2013; López-Rojo et al. 2020; Rabelo et al. 2022).

Even though species evenness differs significantly in the litterfall from tropical streams (i.e., few species provide most litterfall and many species provide only small amounts of litter; Tonin et al. 2021), studies considering the effect of evenness on decomposers still are scarce in these ecosystems. Since decomposers show substrate preferences (Gulis 2001), aquatic fungi would be affected by species evenness in decomposing litter (Gonçalves & Canhoto 2009; Pereira & Ferreira 2022). Although effects alone of species evenness were weak or undetectable on fungal parameters in our study, the interaction between diversity and evenness effects on growth in biomass confirms the important effect of evenness on decomposer organisms.

Our results show that the loss of rare plant species may substantially affect decomposers in tropical streams through resource dissimilarity effects. However, this is most likely to be observed when species proportions found naturally in the ecosystem are respected. In contrast, most experimental studies evaluating the impact of plant species diversity on decomposers in tropical streams have simulated overly simplified conditions of this reality (Wardle et al. 2016), despite the high diversity of plant species commonly found on litter in these streams (see Wantzen et al. 2008). Therefore, it is imperative that future studies of diversity effects on decomposer organisms simulate the real conditions observed in ecosystems.

Evenness explained variation in aquatic hyphomycete community composition in our study, as previously observed (Gonçaves & Canhoto 2009), although it had a weak effect. Differences in community composition between even and natural litter mixtures may be due to differences in the overall quality of litter mixtures. By mixing species at different evenness levels, the ratios are altered due to changes in the contents of phenolics, structural compounds, and nutrients in the mixture. Litter quality may act as an environmental filter, benefiting some fungal species (Gulis 2001), potentially leading to their dominance by competitive exclusion (Treton et al. 2004). We found that even litter mixtures had higher aquatic hyphomycete species richness than natural litter mixtures. This may have occurred because mixtures with increasing evenness have greater distribution of traits such as nutrient availability, levels of phenolic compounds, and contrasting structural traits (Hillebrand et al. 2008), which enhance the range of niches available to decomposers.

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

Aquatic fungi exert primary biological control on litter decomposition in tropical streams, since shredder invertebrates are scarce in most of these ecosystems (Gonçalves et al. 2007; Boyero et al. 2011). Therefore, the basis for understanding litter decomposition in tropical streams may lie principally in assessing the importance of different factors on these organisms. Our study showed that the loss of rare plant species from litter mixtures in tropical streams affected the production of fungal biomass (a key fungal parameter; Krauss et al. 2011), but it did not affect other parameters. Moreover, we showed the importance of plant species evenness on fungal biomass through its interaction with diversity. Thus, taking evenness into account seems important to understand the effects of plant species diversity on fungal decomposers in tropical streams. Our study provides support for the importance of rare species for stream ecosystem functioning, an aspect rarely considered (Dee et al. 2019). We thus suggest the necessity of further research on rare plant species to support conservation and management decisions aimed at mitigating environmental impacts on stream ecosystems.

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

SI 1 INFORMATION OF THE SAMPLING SITE

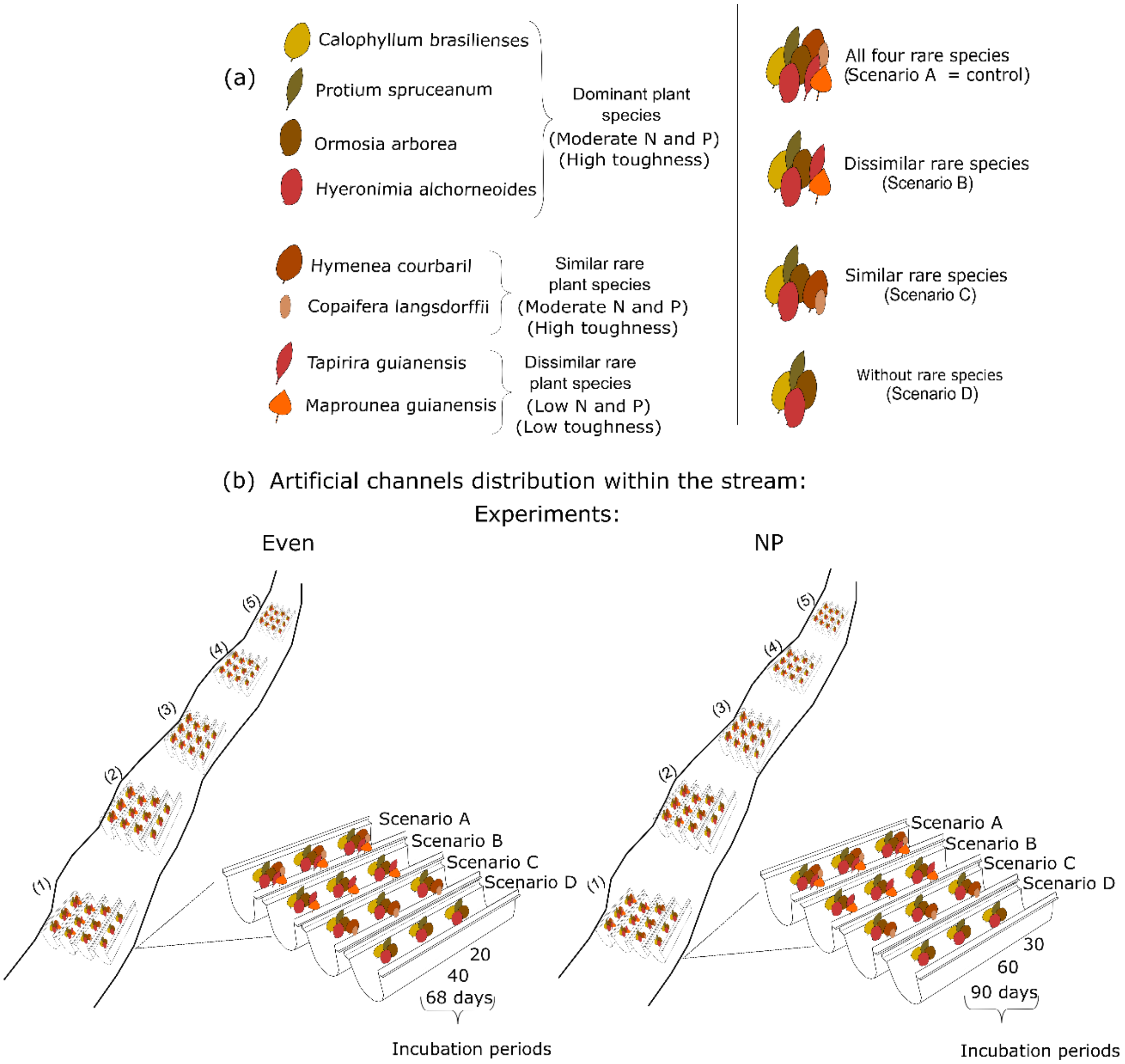

Figure S1. Experimental design representing (a) dominant, similar and dissimilar rare plant species used, their functional traits and the four extinction scenarios of rare species loss (A, B, C and D), and (b) organization of litterbags of each scenario in the artificial channels, even and natural proportion (NP) experiments, incubation periods and distribution of the channels composing for each experiment a total of five sections with four channels along the stream.

Table S1. Litter traits of the eight plant species used in the experiment according to functional group category (dominant species or rare species with similar or dissimilar traits to dominant species). Values are averages ± SE, except for N concentration. The N concentration was estimated using one replicate, whereas the P concentration and toughness were estimated using three and five replicates, respectively.

Functional group	**Plant species**	**N**	**P**	**Toughness**
Dominant	*Hyeronima alchorneoides*	0.83	0.12 ± 0.02	69 ± 3
Dominant	*Calophyllum brasiliense*	0.65	0.11 ± 0.01	279 ± 10
Dominant	*Ormosia arborea*	1.24	0.16 ± 0.01	238 ± 17
Dominant	*Protium spruceanum*	0.81	0.11 ± 0.01	84 ± 11
Rare (similar)	*Hymenaea courbaril*	0.82	0.26 ± 0.02	169 ± 13
Rare (similar)	*Copaifera langsdorffii*	1.07	0.20 ± 0.01	132 ± 6
Rare (dissimilar)	*Tapirira guianensis*	0.72	0.06 ± 0.01	34 ± 3
Rare (dissimilar)	*Maprounea guianensis*	0.60	0.06 ± 0.01	61 ± 10

Note. Data are from Rabelo et al. 2022.

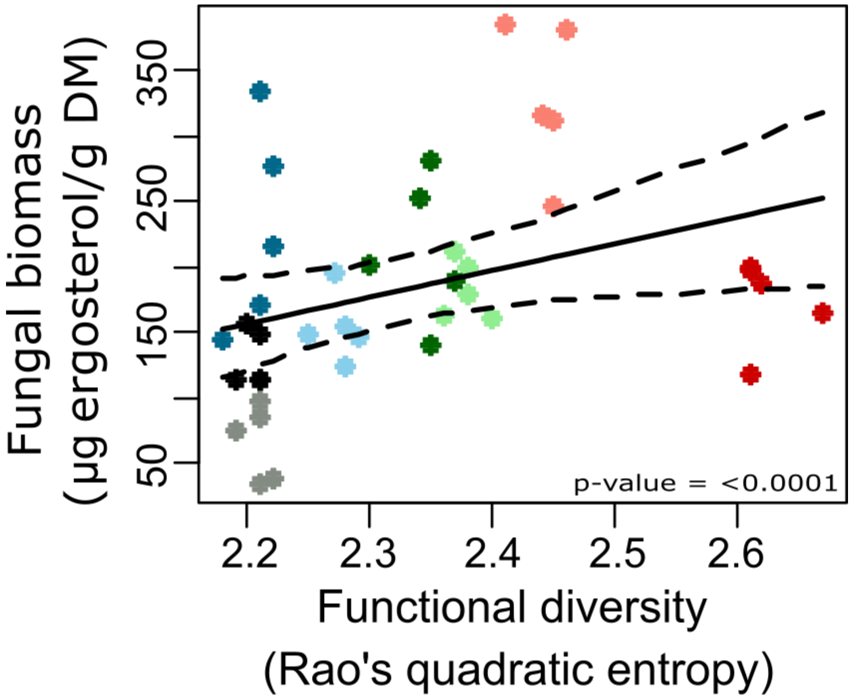

Figure S2. Linear model showing the relationship between functional diversity (Rao’s quadratic entropy) of plant litter mixtures and fungal biomass. Circles' colors represent scenarios that lost rare species: B (green; dissimilar rare species present), C (blue; similar rare species present) or D (black; without rare species), and the scenario A (red; with all four rare species). Darker and lighter colors represent even and natural proportion (NP) experiments, respectively. Continuous lines are the fit of linear models, and dashed lines are the 95% confidence intervals.

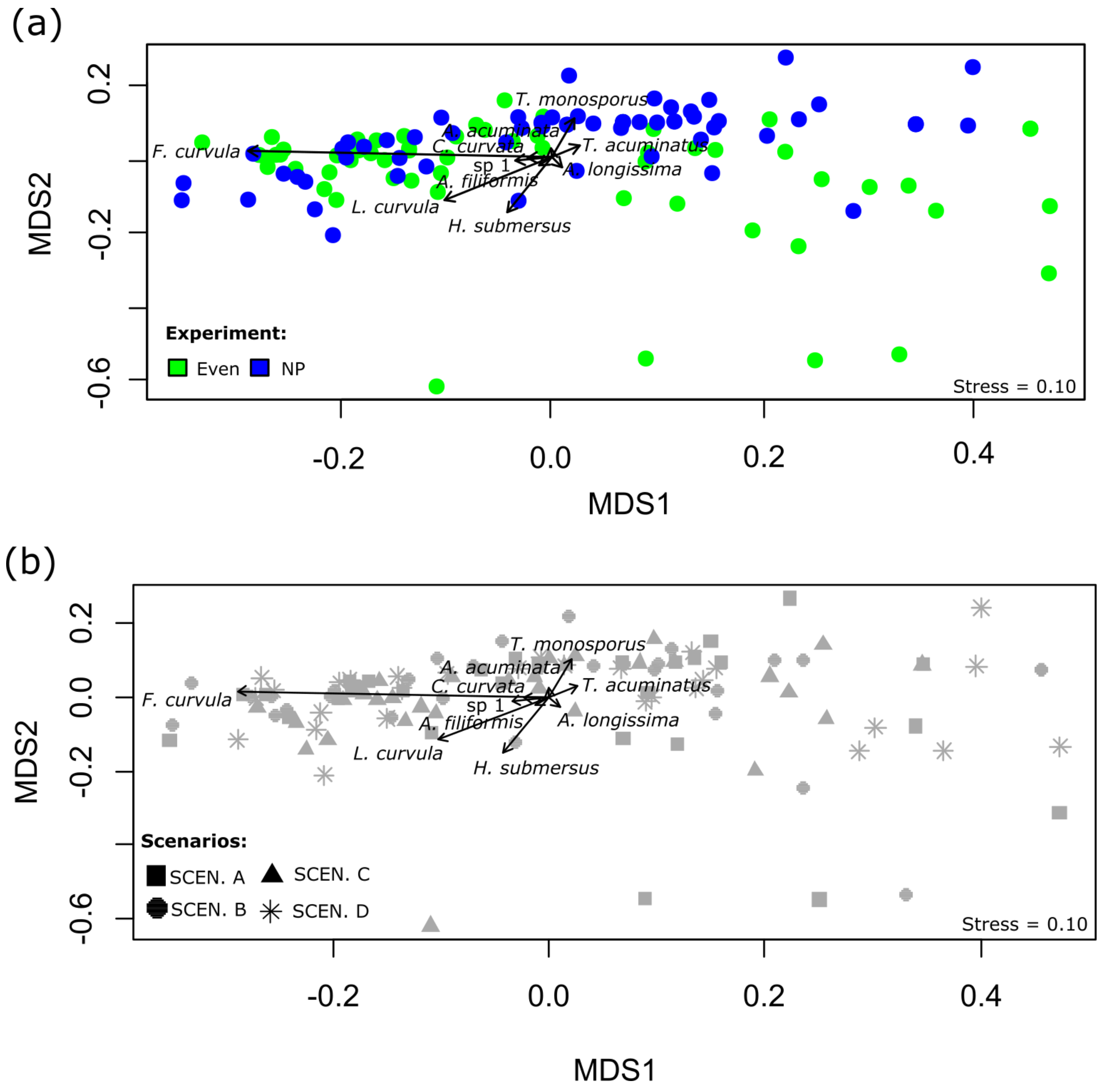

Figure S3. Non-metric Multidimensional Scaling (NMDS) ordination diagrams of distance matrix (Bray-Curtis dissimilarity index) of aquatic hyphomycetes communities composition according to (a) evenness (even and natural proportion experiment) and (b) scenarios (SCEN.) that lost rare species: B (dissimilar rare species present), C (similar rare species present) or D (without rare species), and the scenario A (with all four rare species). Positions of aquatic hyphomycetes species are shown with arrows.

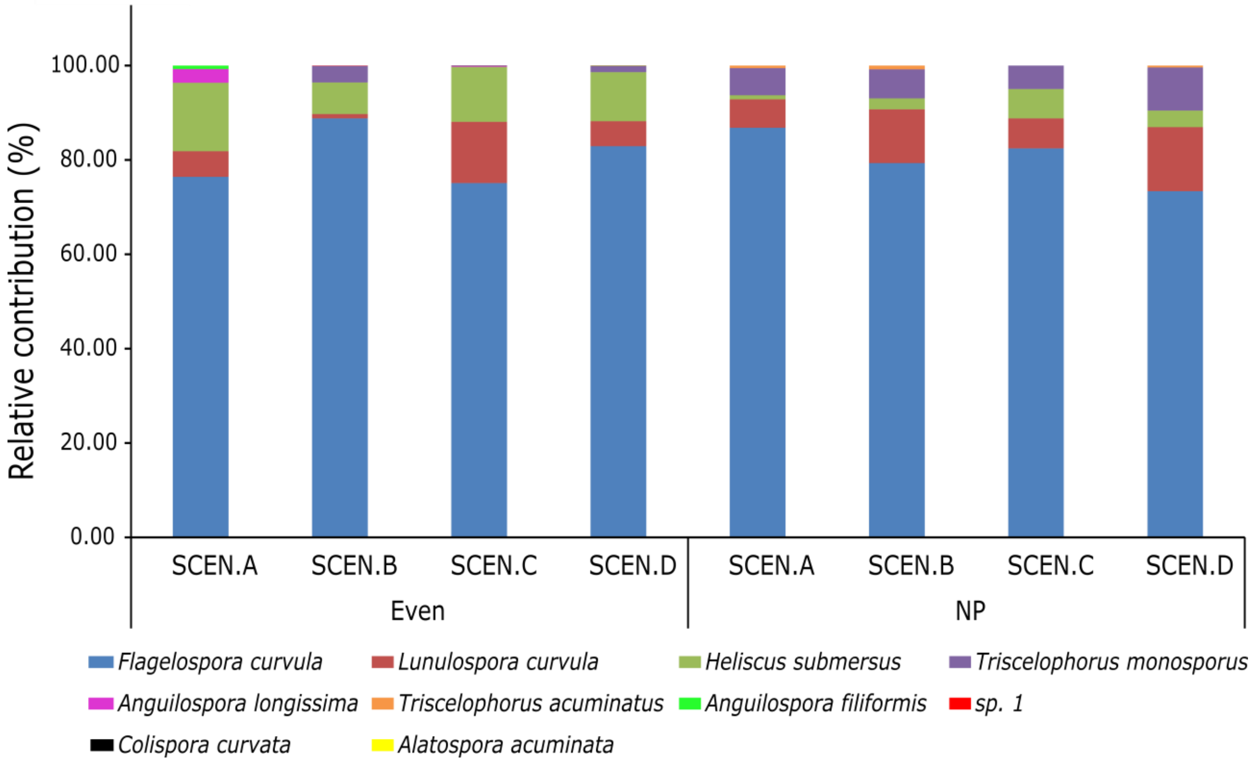

Figure S4. Relative contribution of aquatic hyphomycetes species to the total conidial production of each scenario (SCEN.) of rare species loss: B (dissimilar rare species present), C (similar rare species present) or D (without rare species), and the scenario A (with all four rare species) in even and natural proportion (NP) experiments.

CAPÍTULO 3 – EXPERIMENTAL SHADING CONTROL ON LITTER DECOMPOSITION IN TROPICAL STREAMS VIA THE PRIMING EFFECT

Raiane S. Rabelo, Alan M. Tonin, Monalisa Silva-Araujo, José F. Gonçalves Júnior

ABSTRACT

The canopy cover in headwater streams limits its autotrophic production due to shading effects. However, some studies have been highlighting the importance of periphytic algae to headwater stream food webs, even in streams with high riparian forest cover. Importantly, the presence of periphytic algae may stimulate allochthonous organic matter degradation via the priming effect – the enhancement of decomposition of recalcitrant organic matter by addition of labile carbon (C). Studies investigating the importance of the priming effect for the litter decomposition in streams under field conditions are scarce, especially in tropical regions. This can affect the validity of statements from priming effect studies concerning the ecosystem scale. Here, we aimed to investigate the importance of the algal priming effect for the litter decomposition in natural streams from a tropical region. We did so through a field experiment using artificial shading in three streams of the Cerrado biome. Our hypothesis is that the higher periphytic algae accrual in reaches without artificial cover (non-shaded sections) would led to a higher microbial and invertebrate-mediated litter decomposition due to increased priming effect. The non-shaded sections had periphytic algae quantity (chlorophyll a) 43% higher and quality (total fatty acid content) 26% higher than the artificially shaded sections. We showed that the microbial and invertebrate-mediated litter decomposition was reduced by the increased periphytic algae accrual in non-shaded sections – implying that priming was negative, although this difference has not been consistent among periods. Using linear models, we observed that the increase in the periphytic algae quantity reduced the LML mediated by microbial and invertebrate communities, which supports these results. Despite the fact that we found only evidence of negative priming, our results supports the relevance of algal priming on litter decomposition in natural tropical streams. Our results indicate that through bottom-up stimulation of periphytic algae on consumers, light can reduce litter decomposition in natural tropical streams. This can alter energy and nutrients available to these ecosystems enhancing labile C incorporation and recalcitrant C storage or export in aquatic habitats with high luminosity. Thus, our fidings contribute to the understanding of how primary production can modulate the natural tropical streams functioning via the priming effect.

Keywords: periphytic algae; fatty acids; riparian forest; autochthonous primary production; shredder invertebrates.

INTRODUCTION

The canopy cover in headwater streams promotes significant input of litterfall and limits autotrophic production due to shading effects (Vannote et al. 1980; Tonin et al. 2017). Thus, the allochthonous organic matter is the primary carbon (C) source for consumers in these streams (Wallace et al. 1997). However, some studies have been highlighting the importance of periphytic algae to headwater stream food webs by using stable isotope methods, even in streams with high riparian forest cover (e.g., Füreder et al. 2003; McNeely et al. 2006; Neres-Lima et al. 2016). Importantly, living primary producers may provide high quality resource subsidies to decomposer microbes and/or shredder invertebrates and affect the functioning of these ecosystems through its interaction with the allochthonous organic matter (Guenet et al. 2010; Guo et al. 2016).

The presence of periphytic algae can stimulate allochthonous organic matter degradation by decomposer microbes through the providing labile C exudates (Franken et al. 2005; Danger et al. 2013; Ward et al. 2016; Howard-Parker et al. 2020). In addition, periphytic algae are rich in essential polyunsaturated fatty acids relative to litter, and therefore their presence add these compounds (Lau et al. 2009). This can lead to enhanced litter processing activity by shredder invertebrates (Guo et al. 2016). Such effects of periphytic algae on the transformation of allochthonous organic matter in headwater streams are examples of the ‘‘priming effect’’, i.e, the enhancement of decomposition of recalcitrant organic matter by addition of labile C (Kuzyakov et al. 2000; Guenet et al. 2010).

The priming effect is a broadly accepted process in soils, where labile C additions have consistently increased the decomposition of recalcitrant C due to positive priming (Kuzyakov et al. 2000; Perveen et al. 2019). Despite this, it remains poorly studied in aquatic ecosystems (Bengtsson et al. 2018; Halvorson et al. 2019). The few studies investigating the priming effect on litter decomposition in freshwater ecosystems have divergent results. Some studies have reported positive priming effect (Franken et al. 2005; Danger et al. 2013; Guenet et al. 2014; Ward et al. 2016; Howard-Parker et al. 2020), whereas others did not find a priming effect or have reported negative priming effect – i.e., reduced organic matter decomposition with the increased algal labile C

(Catalán et al. 2015; Elosegi et al. 2018; Halvorson et al. 2018; Brady et al. 2020). In addition, most studies investigating the priming effect in freshwater ecosystems are in laboratory (microcosms) or mesocosms, where is added variable quantity of easily degradable C sources (e.g., glucose, acetate or algal extracts or exudates; see Bengtsson et al. 2018). However, the omission or distortion of key features of these ecosystems in microcosm and mesocosm experiments affects the validity of statements concerning the ecosystem scale (Petersen et al. 1999). The complexity of ecosystems is often overlooked, such as air–water and sediment-water exchanges, size, hydrodynamics and larger organisms' activities (Schindler 1998). Thus, further studies investigating the priming effect in freshwater ecosystems toward to clear results and to better validate conclusions for real ecosystems are required.

In particular, the importance of the priming effect in headwater streams has been tested in situ to our knowledge by a single study in a temperate region via a comparison of closed-versus open-canopy streams, where a weak positive or no priming effect was detected on litter decomposition (Elosegi et al. 2018). Similarly, another recent study testing the priming effect on litter decomposition using artificial streams in a temperate region found no priming effect (Brady et al. 2020). However, it is likely that interactions of physical and chemical variables and biological communities in tropical streams will cause a priming effect in a different range than that observed in temperate streams due to their differences in abiotic factors that control the abundance of periphytic algae such as climate seasonality, light availability and water temperature (Lewis Jr. 2008; Boyero et al. 2009). Among these factors, the light availability can be considered a key factor because algae requires light for photosynthesis (Hornbach et al. 2015). The tropical streams receive greater solar radiation than temperate streams (Lewis Jr. 2008), which contributes for the algal growth (Izagirre & Elosegi 2005; Graça et al. 2018) and therefore may stimulate the occurrences of priming effect (Howard-Parker et al. 2020). In this context, it is therefore crucial to understand the importance of the priming effect for the litter decomposition in tropical streams under field conditions.

We aimed to investigate the importance of the algal priming effect for the litter decomposition in natural streams from a tropical region. We did so through a field experiment using artificial shading in three streams of the Cerrado biome. Our hypothesis is that the higher periphytic algae accrual in reaches without artificial cover would led to a higher microbial and invertebrate-mediated litter decomposition due to increased priming effect.

METHODS

Study area

The study was carried out in three affluents of the Cabeça-de-Veado stream (15° 53' 22.15" S, 47° 50'34.10" W): Abelha, Caesb, and Nascente referred to hereafter as ABE, CAE and NAS, respectively. The three streams are in the Ecological Station of the Botanical Garden of Brasília, Federal District, Brazil. They have a riparian forest typical of the Cerrado biome (savanna), mostly with dense canopy cover (>80%; visual estimation), evergreen species and high species diversity (Bambi et al. 2016). The climate of the region is seasonal (Aw type, according to the Köppen-Geiger classification) with a dry season (May to September), a rainy season (October to April) and dry–wet (September to October) and wet-dry (April to May) transitional seasons (Eiten 1972).

The experimental period was from July to October 2019, which encompassed the driest periods of the year (dry and dry-wet transition seasons), when there is higher litterfall as a response of plants to reduce hydric stress (Tonin et al. 2017), and thus, enhanced sunlight reaching the streambed. The water temperature of the streams was on average ± SE 19.4 ± 1.1 °C (data loggers, HOBO Pendant, Onset), conductivity 5.2 ± 0.8 $\mu S\ cm^{-1}$, pH 5.9 ± 0.1 (Lenway 430 pH/meter, Meter), dissolved oxygen 7.4 ± 0.3 mg L^{-1} or 80 ± 5.4 % (Lenway 970 Meter DO2), turbidity 2.9 ± 1.8 NTU (turbidimeter Q279P, Quimis) and current velocity 0.2 ± 0.1 $m.s^{-1}$ (Global Water, FP10) (Table 1). The water of the streams was nutrient-poor (28.7 ± 1.7 $mg.L^{-1}$ for dissolved inorganic nitrogen (N) and 20.2 ± 1.2 $mg.L^{-1}$ for orthophosphate; Tonin et al. 2020). Water's physical and chemical parameters were generally similar in all streams (Table 1).

Table 1. Luminosity and water physical and chemical parameters (average ± standard error) of the three experimental streams: Abelha (ABE), Caesb (CAE), and Nascente (NAS), and luminosity and water temperature of non-shaded (control) and artificially shaded sections recorded during the experiment (July to October 2019).

	Stream			**Section**	
	ABE	CAE	NAS	Non-shaded	Artificially shaded
Luminosity (lumen/m^2)	616.1 ± 211.3	599.5 ± 180.1	1153 ± 288.6	831.2 ± 146.3	47.4 ± 26.6
Temperature (°C)	17.8 ± 0.3	19 ± 0.2	21.5 ± 0.1	19.6 ± 0.2	19.5 ± 0.2
Dissolved oxygen (mg L^{-1})	7.8 ± 0.1	6.8 ± 0.4	7.7 ± 0.0	—	—
Dissolved oxygen (%)	84.3 ± 0.8	69.3 ± 2.1	86.4 ± 0.6	—	—
pH	5.9 ± 0.1	5.8 ± 0.1	6.1 ± 0.2	—	—
Conductivity (µS cm^{-1})	3.9 ± 0.4	6.5 ± 2.1	5.4 ± 0.9	—	—
Turbidity (NTU)	6.6 ± 1.5	1.5 ± 0.2	0.7 ± 0.2	—	—
Current velocity (m s^{-1})	0.3 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	—	—

Experimental design

We selected three reaches of 20 m with a distance of around 30 m from each other in each stream (ABE, CAE, and NAS; Fig. 1a). Then, we established an artificially shaded section in each of the reaches. For this, we use a double-coated canvas (3 x 3 m) placed above the stream (Fig. 1b). The cover was *c.* 50 cm above the water surface. The control was defined as a section without artificial cover (hereafter non-shaded section) 5 to 10 m upstream of the artificially shaded section (Fig. 1b). Sunlight intensity reaching the water surface and the water temperature were measured continuously using HOBO Pendant (Onset®) data *loggers* both over non-shaded and artificially shaded sections. On average, sunlight intensity reaching the water surface was 17 times higher in the non-shaded than in the artificially shaded section (Table 1). The water temperature was similar in non-shaded and artificially shaded sections (Table 1).

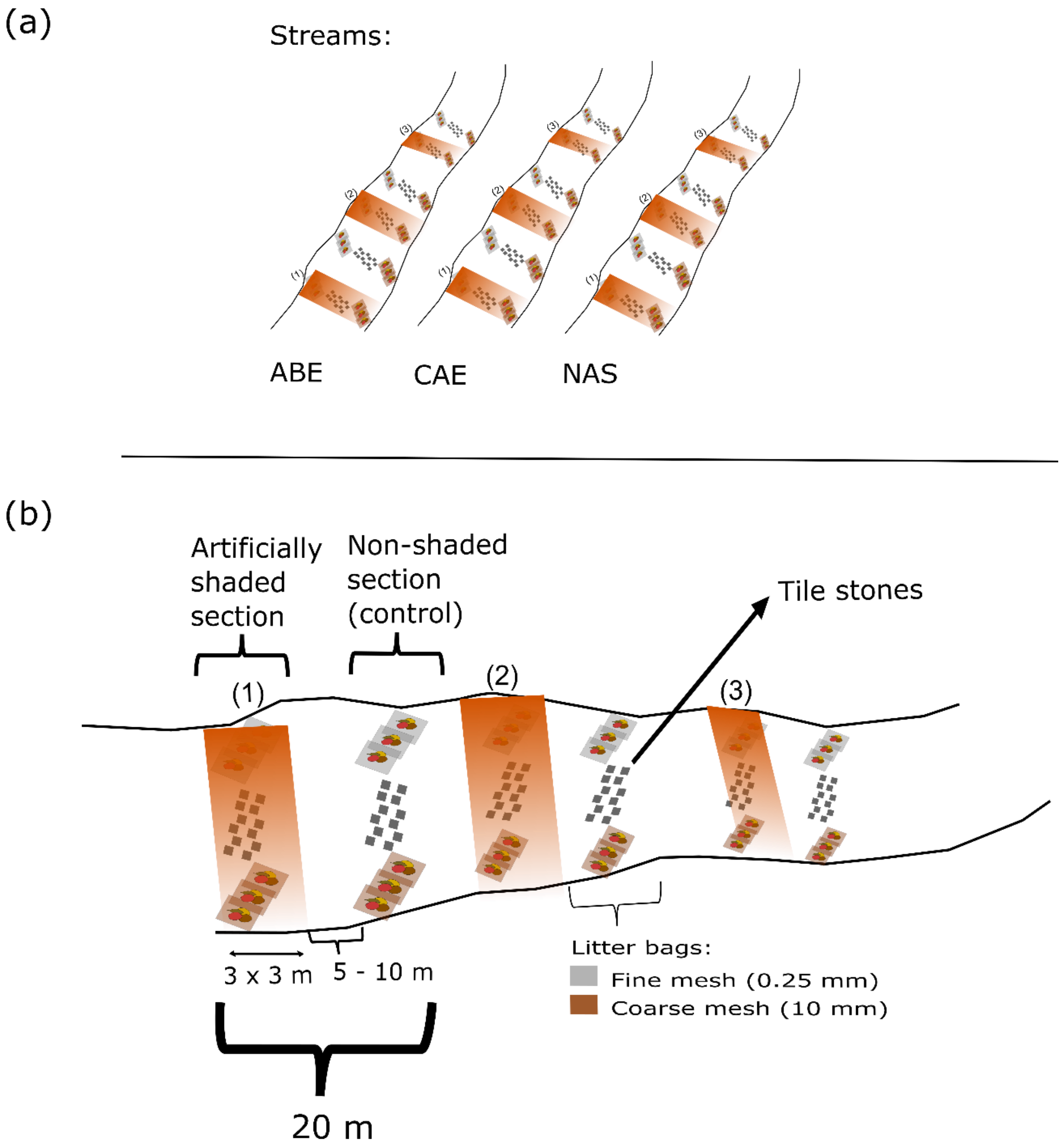

Figure 1. Experimental design representing (a) the three streams where the experiment was carried out – Abelha (ABE), Caesb (CAE) and Nascente (NAS) and (b) the three reaches containing a non-shaded section (control) and an artificially shaded section in the stream and organization of litter bags and tile stones in each section.

Litter decomposition

The litter decomposition was estimated through the incubation of a mixture of senescent leaves of tree species found in riparian zones of the Cerrado biome (*Tapirira guianensis* Aubl., *Protium spruceanum* (Benth.) Engl., *Calophyllum brasiliense* Cambess. and *Ormosia arborea* (Vell.) Harms; Bambi et al. 2017). We use a species mixture instead of just one species to better represent available and decaying resources in the environment (Handa et al. 2014). The tree species native of the Cerrado biome have majority tough leaves, poor in nutrients and which contain large amounts of secondary compounds as a response to high levels of herbivory and hydric stresses (Tonin et al. 2021). Therefore, most of these species (including our tree species mentioned above) have leaves that decompose very slowly after entering streams (Gonçalves et al. 2007). This made us choose to incubate litter for long incubation periods (i.e., 28, 64 and 120 days). We selected three different incubation periods to account for the chemical changes of litter over time (Abelho 2001), and its influence in mediating priming effect (e.g., Steen et al. 2016).

We weighed ca. 1.5 g of each leaf species for each litter bag (totaling 6 g). We had fine mesh litter bags (0.25 mm; impeding consumption by shredder invertebrates) and coarse mesh litter bags (10 mm; allowing consumption by shredder invertebrates). Three litter bags of each mesh type (totaling six litter bags) were incubated in the stream under artificial canopy cover and in non-shaded section (control) of each reach, one for each incubation period (28, 64 and 120 days; Fig. 1b). A total of 108 litter bags were deployed in the streams and used to the experiment.

Litter bags were retrieved from the field and stored in a thermal container with ice for transport to the laboratory. In the laboratory, we washed the litter with a slight run of distilled water to remove sediment and inorganic particles adhered. The litter was oven-dried (60ºC, 72h) and weighed (accuracy of 0.01 mg) to determine its dry mass (DM).

The litter mass loss (LML) was calculated using the initial (Mi) and final (Mf) DM of litter: $\mathrm{LML} = 1 - [(\mathrm{Mi} - \mathrm{Mf}) / \mathrm{Mi}]$. Total and microbe-mediated litter mass loss ($\mathrm{LML}_{\mathrm{TOTAL}}$ and $\mathrm{LML}_{\mathrm{MICROBIAL}}$) were estimated from litter bags of coarse and fine mesh, respectively. We estimated the contribution of invertebrates to litter mass loss ($\mathrm{LML}_{\mathrm{INVERTEBRATE}}$) by the ratio between the LML in coarse and fine mesh litter bags (cf. Tonin et al. 2018).

Periphytic algae

To evaluate periphytic algae accrual we used tile stones. For each sample section (non-shaded and artificially shaded) of the three reaches selected in each stream (ABE, CAE, and NAS), 12 autoclaved tile stones (10 x 10 cm, 7 mm thick, and 0, 01 m^2 of area) with a minimum distance of 10 cm from each other were deployed (Fig. 1b), and retrieved after 64 days of incubation. The material adhered to each tile stone was collected by brushing and washing with 100 ml of filtered stream water (0.45 µm filter), stored in Falcon tubes, and transported to the laboratory cooled in darkness.

In the laboratory, the material collected was divided into two sub-samples (50 ml). The first sub-sample was filtered with a Whatman GF/F 0.45 µm filter (pre-weighed for DM) and used to estimate the periphytic algae biomass (hereafter quantity). We estimated the quantity as chlorophyll a (Chl-a), an estimator of algal biomass growth with more reliable measurement compared to other alternatives used (e.g., dry mass; Morin & Cattaneo 1992). We extracted Chl-a from sample filters in organic solvent ethanol 90% (4°C, 24 h; Lorenzen 1967). Chl-a concentrations were measured with a BEL Engineering S2000 spectrophotometer (467 - 664 nm).

The second sub-sample was used to measure the total of fatty acids, used to estimate the essential polyunsaturated fatty acids content in periphytic algae accrual. The samples were stored (-80 ºC) and, after 48 h, lyophilized (-20 °C, 48 h under vacuum)(Laurens et al. 2012). Lyophilized periphyton was weighed (4 ± 3 mg) and stored in a glass tube (2 ml). Then, methanol-chloroform 1:2 (0.2 ml), C13 TAG internal standard (25µl) and HCl in methanol 5% (0.3 ml) were added. After, it was heated (85ºC) in a dry block and gently agitated for one h. Finally, hexane (1 ml) was added, and after one h at room temperature, the upper organic layer was removed and stored (20ºC). Samples were analyzed on a gas chromatograph (Agilent Technologies 7890-5975) with a 30-m INNOWAX capillary column (Agilent Technologies) and flame ionization detector, efficient methodology for small biomass quantities as mentioned above (Laurens et al. 2012). Fatty acid methyl esters (FAMEs) were quantified using the Agilent ChemStation software and reference curve based on known standard concentration (10 mg/ml of FAME-C13; Laurens et al. 2012). The total fatty acid content was calculated as the sum of all the individual FAMEs of periphyton.

We expressed the Chl-a as the concentration of Chl-a (µg) per surface area of tile stone (cm^-

^{2}) (Steinman et al. 2017). Total fatty acid content (hereafter quality) was expressed as the percentage of FAMEs per mg of DM per surface area of tile stone (in cm^{-2}).

Statistical analysis

We used linear mixed-effects models (LMMs, lme function of the nlme package; Pinheiro et al., 2020) to examine the effects of experimental shading (fixed factor) on periphytic algae quantity (Chl-a) and quality (total fatty acid content) and total, microbial and invertebrate-mediated LML. We used separate models for each incubation period to test the effect of the experimental shading separately for each period, since the influence of the effect of compounds added by periphytic algae attached to litter on consumers can change due to chemical changes of litter over time (Steen et al. 2016). The streams and reaches (nested within stream) were included in the models as random factors (Zuur et al. 2009). We also investigated the effects of the periphytic algae quantity and quality on total, microbial and invertebrate-mediated LML using LMMs with the same random structure mentioned above.

The selection of the best random structure models was based on the Akaike Information Criterion (AIC), and when required was used a variance component (varIdent; Zuur et al. 2009). Model residues were visually explored using graphical tools (boxplots and scatterplots), and no violation of the assumptions of linear models was detected (Zuur et al. 2009; Ieno & Zuur 2015). All analyses were performed using R v. 4.1.3 (R Core Team 2022)**.**

RESULTS

The periphytic algae quantity (Chl-a) in tile stones was on average ± SE 0.11 ± 0.01 and 0.06 ± 0.02 µg cm^{-2} in the non-shaded (control) and artificially shaded sections, respectively, while the quality (total fatty acid content) was 2.21 ± 0.37 and 1.63 ± 0.24 %/DM cm^{-2} (Fig. 2). There were significant statistically differences between non-shaded and artificially shaded sections – the non-shaded sections had periphytic algae quantity 76% higher and quality 35% higher than the artificially shaded sections (Fig. 2; Table 2).

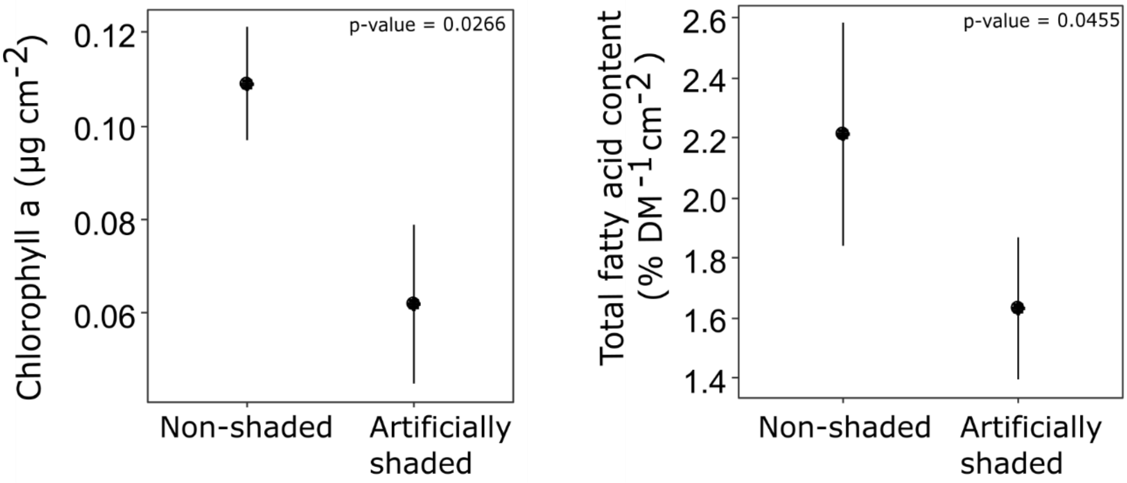

Figure 2. Effect of the experimental shading (non-shaded and artificially shaded section) on the periphytic algae quantity (chlorophyll a) and quality (total fatty acid content) for the incubation period of 64 days. Circles are means, and vertical lines denote upper and lower limits of 95% confidence intervals.

We found significant effects of the experimental shading on the LML mediated by microbial and invertebrate communities (Table 2). Microbial LML was, on average, 11 % higher in artificially shaded than in the non-shaded sections in the 64-day incubation period (Fig. 3b; Table 2). Similarly, the experimental shading increased the invertebrate-mediated LML in the 120-day incubation period (17 %; Fig. 3c; Table 2). No effect of the experimental shading on the microbial and invertebrate-mediated LML was found in other periods (Fig. 3b and c; Table 2). The experimental shading did not influence the total LML in any of the periods (Fig. 3a; Table 2).

Table 2. Results of linear models testing the effect of the experimental shading (exp. shading; non-shaded and artificially shaded section) for each incubation period separately (28, 64, and 120 days) on the total (LML$_{\text{TOTAL}}$), microbial (LML$_{\text{MICROBIAL}}$) and invertebrate-mediated (LML$_{\text{INVERTEBRATE}}$) litter mass loss (LML) and periphytic algae quantity (chlorophyll a) and quality (total fatty acid content). Asterisks (*) indicate statistically significant differences.

	28 days			**64 days**			**120 days**		
	DF	t-value	p-value	DF	t-value	p-value	DF	t-value	p-value
LML$_{\text{TOTAL}}$									
Intercept	8	21.91		8	8.93		8	10.94	
Exp. shading	8	-0.81	0.4403	8	-1.47	0.1793	8	1.02	0.3388
LML$_{\text{MICROBIAL}}$									
Intercept	8	26.45		8	11.00		8	8.17	
Exp. shading	8	0.61	0.5577	8	2.40	0.0429*	8	-1.28	0.2365
LML$_{\text{INVERTEBRATE}}$									
Intercept	8	15.28		8	11.84		8	12.81	
Exp. shading	8	-1.03	0.3322	8	-0.98	0.3562	8	3.46	0.0086*
Chlorophyll a									
Intercept	-	-	-	8	8.83		-	-	-
Exp. shading	-	-	-	8	-2.71	0.0266*	-	-	-
Total fatty acid content									
Intercept	-	-	-	7	5.94		-	-	-
Exp. shading	-	-	-	7	-2.43	0.0455*	-	-	-

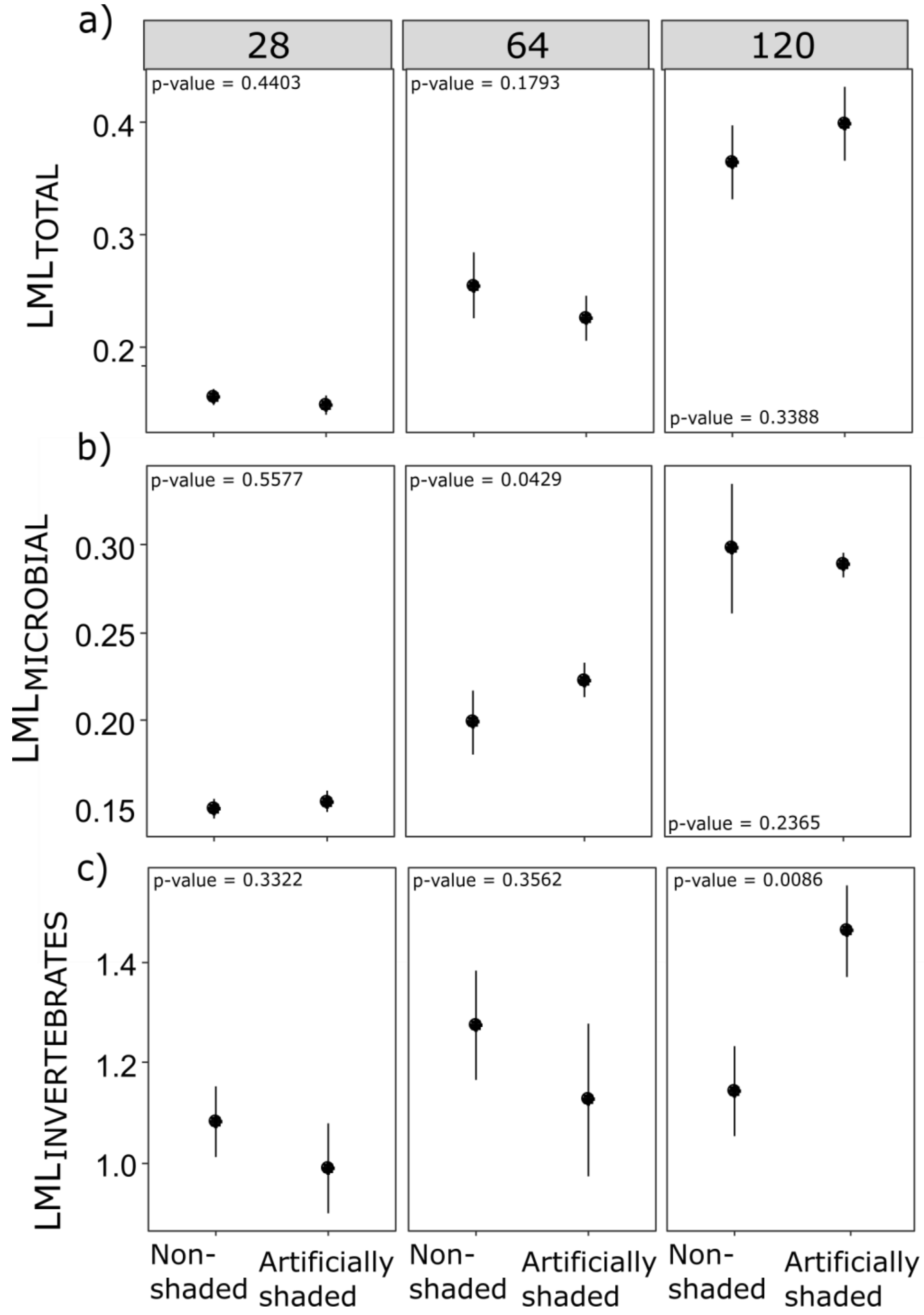

Figure 3. Effect of the experimental shading (non-shaded and artificially shaded section) on the (a) total (LML_{TOTAL}), (b) microbial ($LML_{MICROBIAL}$) and (c) invertebrate-mediated ($LML_{INVERTEBRATE}$) litter mass loss (LML) for the incubation periods of 28, 64 and 120 days. Circles are means, and vertical lines denote upper and lower limits of 95% confidence intervals.

Using linear models, we observed that the increase in the periphytic algae quantity (Chl-a) resulted in a decrease of the LML mediated by microbial and invertebrate communities (Fig. 4c and e), but it did not affect the total LML (Figure 4a; Table 3). In contrast, the increase in the quality (total fatty acid content) increased the total and invertebrate-mediated LML (Fig. 4b and f), but it did not affect the microbial LML (Figure 4d; Table 3).

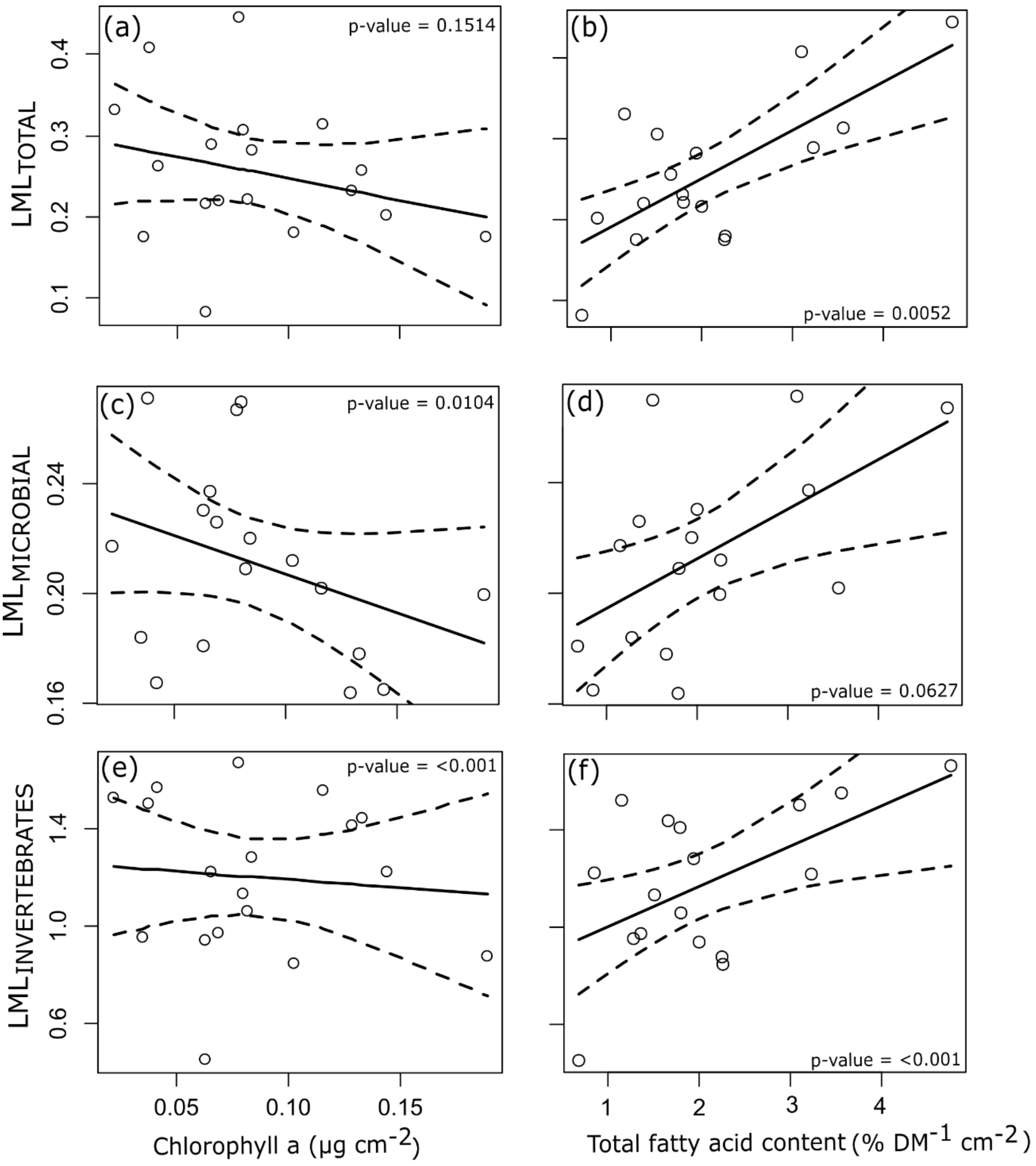

Figure 4. Linear models showing the relationship between periphytic algae quantity (chlorophyll a) and quality (total fatty acid content) and the total ($\mathrm{LML_{TOTAL}}$), microbial ($\mathrm{LML_{MICROBIAL}}$) and invertebrate-mediated ($\mathrm{LML_{INVERTEBRATE}}$) litter mass loss (LML) for the incubation period of 64 days. Continuous lines are the fit of linear models, and dashed lines are the 95% confidence intervals.

Table 3. Results of linear models testing the effect of the periphytic algae quantity (chlorophyll a) and quality (total fatty acid content) on the total (LMLTOTAL), microbial (LMLMICROBIAL) and invertebrate-mediated (LMLINVERTEBRATE) litter mass loss (LML) for the incubation period of 64 days. Asterisks (*) indicate statistically significant differences.

	DF	t-value	p-value
LMLTOTAL			
Intercept	6	3.91	
Chlorophyll a	6	-1.64	0.1514
Total fatty acid content	6	4.27	0.0052*
LMLMICROBIAL			
Intercept	6	12.64	
Chlorophyll a	6	-3.68	0.0104*
Total fatty acid content	6	2.28	0.0627
LMLINVERTEBRATE			
Intercept	6	8.52	
Chlorophyll a	6	-16.70	<0.001*
Total fatty acid content	6	21.91	<0.001*

DISCUSSION

The results presented in this study suggest that priming have a significant effect on litter decomposition in natural tropical streams. However, contrary to our hypothesis we found that the microbial and invertebrate-mediated litter decomposition was reduced by the increased periphytic algae accrual in non-shaded sections – implying that priming was negative (Catalán et al. 2015; Halvorson et al. 2018), although this difference has not been consistent among periods. Using linear models, we observed that the increase in the periphytic algae quantity (Chl-a) reduced the LML mediated by microbial and invertebrate communities, which supports these results. Despite this, the total litter decomposition (mediated by both microbes and shredder invertebrates) was not affected.

Negative or nonsignificant priming effects for the microbe-mediated litter decomposition may have occurred due to microbial consumers have preferred to use the labile C of periphytic algae instead of the recalcitrant C from litter in our study (e.g., Catalán et al. 2015; Halvorson et al. 2018). The use preferential of easily degradable C sources instead of the recalcitrant C may result in a decreased overall consumption of less labile C sources and absent or negative priming (Guenet et al. 2010). In addition, the positive priming effect mediated by microbes in streams involves the stimulation of litter decomposition by periphytic algae due to their presence stimulates degradative enzymes production and activity (e.g., Rier et al. 2007). However, if the labile C of periphytic algae is used for microbial population maintenance (respiration) or growth, priming will not occur, as no labile C will be available for enzymatic anabolism (Guenet et al. 2010). Importantly, if microbial consumers prefer to use labile C from periphytic algae instead of recalcitrant C from litter, this contributes to the incorporation of a higher quality energy to the stream ecosystem (Neres-Lima et al. 2016).

The negative or nonsignificant priming effect for the invertebrate-mediated litter decomposition suggest that invertebrates may have sometimes been unable to discriminate between better and worse quality litter — as previously observed (Franken et al. 2005; Albariño et al. 2008). Moreover, the invertebrates may have been more protected from predation in artificially shaded sections and fed more on litter (Trussel et al. 2006; Hawlena et al. 2012), which may have led to the negative priming effect for the invertebrate-mediated litter decomposition in our study. Also, others sources of natural variation may have overruled the influence of the periphytic algae quality attached to litter such as the heterogeneity in invertebrate community composition or availability of other food sources as litter with differences in fungal colonization or stage of decay (e.g., Vonk et al. 2016).

Importantly, the leaf mass loss in non-shaded sections may have been masking by increase in leaf mass due to microbial biofilms growing on the leaf surface in our study (e.g., Alvim et al. 2015). Thus, it also may have caused the negative or nonsignificant priming effects for the microbial and invertebrate-mediated litter decomposition observed.

The increase of litter fragmentation by the invertebrates due its increase quality (total fatty acid content) by periphytic algae colonization in the 64-day incubation period suggests that invertebrates benefited from the essential polyunsaturated fatty acids of algae attached to litter (Guo et al. 2016; Vonk et al. 2016; Kühmayer et al. 2020). However, it did not lead to a positive priming effect in our study. As the increase in the periphytic algae quantity (Chl-a) had a negative effect on invertebrate-mediated litter decomposition, priming seems to have been equally likely to be positive or negative, leading the annulment of priming effect (on average). The experimental shading did not affect LML mediated by shredder invertebrates in this period, which provides supports for this conclusion.

It is essential to recognize that our study did not allow disentangling polyunsaturated fatty acids from other types of periphytic acids, such as saturated or mono-unsaturated fatty acids, nor the origin of fatty acids found (cf., Guo et al. 2015). This could indicate that the enhanced invertebrate-mediated litter decomposition was due to increased other types of periphytic acids instead of the algal polyunsaturated fatty acids; however, this is unlikely, as several studies support consistently the use of these fatty acids rather than other periphytic acids by the shredder invertebrates (Guo et al. 2016; Kühmayer et al. 2020). Polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6ω-3), are only abundant in certain groups of algae and are required for the shredder invertebrates' growth and reproduction (e.g., Guo et al. 2016). They are precursors of animal hormones and help regulate cell membrane fluidity (Brett & Muller-Navarra 1997).

In theory, priming effect in aquatic ecosystems should be positive and stronger in shorter incubation periods, when the organic matter is less recalcitrant (Abelho 2001), compared to longer incubation periods, when heterotrophs are strongly limited by labile C availability, as has been found for terrestrial soils (Zhang et al. 2013). Despite this, the direction and timing of priming effect seems to vary substantially among aquatic environments. For example, Steen et al. (2016) observed positive priming effect in a microcosm experiment using planktonic estuarine microbial communities at the beginning of the experiment (before 21-24 days), but later no evidence of priming effect was observed. Similarly, other study in Swedish lakes found no evidence of priming after around 30 days (Catalán et al. 2015). On the other hand, we found no evidence of priming effect for the 28-days period in tropical streams, but a negative priming effect was observed on the microbial and invertebrate-mediated litter decomposition after longer incubation periods. This is not surprising since that the priming effect involves complex interactions among disparate decomposer organisms, recalcitrant organic matter and labile organic matter (see Guenet et al. 2010).

The small amounts of labile C exudates release by periphytic algae attached to litter in theory should increase the litter decomposition via priming effect in streams (Guenet et al. 2010). However, Bengtsson et al. (2018) in their meta-analysis found that the priming effect may be negligible or non-existent in aquatic ecosystems. Despite the fact that we found only evidence of negative priming, our results supports the relevance of algal priming on litter decomposition in natural tropical streams. Our findings show that the priming effect may be more critical in tropical streams than in temperate streams (e.g., Elosegi et al. 2018), where abiotic conditions are less likely to promote priming effect – temperate streams receive less solar radiation, have lower water temperatures and higher climate seasonality than tropical streams (Lewis Jr., 2008), which can reduce the algal growth (Izagirre & Elosegi 2005; Elosegi et al. 2018; Graça et al. 2018) and therefore to limit the occurrence of priming effect (e.g., Elosegi et al. 2018; Howard-Parker et al. 2020). Future studies should test the role of algal priming on litter decomposition under field conditions in streams of different climatic regions to understand better when the priming effect is an important driver for the litter decomposition in natural streams.

Our results indicate that through bottom-up stimulation of periphytic algae on consumers, light can reduce litter decomposition in natural tropical streams. This can alter energy and nutrients available to these ecosystems enhancing labile C incorporation and recalcitrant C storage or export in aquatic habitats with high luminosity (e.g., Norman et al. 2017). While there is clearly much more work needed, our fidings contribute to the understanding of how primary production can modulate the natural tropical streams functioning via the priming effect.

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CONSIDERAÇÕES FINAIS

A alarmante perda de biodiversidade atual tem impulsionado a realização de centenas de estudos experimentais com a temática biodiversidade e funcionamento de ecossistemas (BEF) para avaliar as implicações da redução da diversidade de espécies sobre processos ecossistêmicos chave (Mori et al. 2020; Chen et al. 2022). Nesse contexto, esta tese demonstrou pela primeira vez que a perda de espécies vegetais raras, especialmente das espécies com características funcionais dissimilares, pode reduzir a decomposição de detritos e a ciclagem de nutrientes em riachos tropicais. Além disso, encontramos que a perda de espécies vegetais raras reduz a produtividade fúngica, especialmente quando o *pool* de detritos vegetais apresenta as proporções de espécies observadas no ecossistema natural.

Esses resultados contradizem a percepção generalizada de que as espécies raras têm pouco efeito sobre o funcionamento dos ecossistemas devido à baixa abundância que possuem, conforme a conhecida hipótese de razão de massa (Grime 1998). A despeito de muitas espécies raras possuírem características funcionais redundantes com relação as outras espécies, e por isso não afetarem de forma significativa as funções do ecossistema quando extintas (Violle et al. 2017), a perda de espécies raras com características funcionais únicas pode alterar significativamente processos chave do ecossistema como a decomposição e a ciclagem de nutrientes (Dee et al. 2019), como o encontrado em nosso estudo.

Em ecossistemas tropicais altamente diversos, a maior parte da riqueza é representada por um elevado número de espécies raras (e.g., Slik et al. 2015), as quais suportam a maioria das combinações únicas de características funcionais nesses ecossistemas (Mouillot et al. 2013). Em razão disso, o efeito da perda de espécies raras sobre o funcionamento de ecossistemas tropicais deveria ser um tópico já bastante investigado. Contudo, estudos nesse sentido são escassos (Zhang et al. 2022). Dessa forma, estudos de diversidade futuros em regiões tropicais deveriam focar no efeito da perda de espécies raras sobre o ecossistema, uma questão urgente dado as altas taxas de extinção de espécies atuais (Díaz et al. 2019; IPBES 2019), e ainda pouco explorada.

Apesar de nossos resultados terem sido obtidos de experimentos realizados em um riacho tropical, esses resultados não se limitam a esse sistema. Assim como nesses riachos, a diversidade de espécies raras é muito maior do que a de espécies dominantes e as espécies raras têm uma probabilidade muito maior de possuírem características funcionais únicas do que outras espécies em diversos tipos de ecossistemas nos trópicos, como florestas e recifes de corais (Mouillot et al. 2013; Slik et al., 2015; Leitão et al. 2016). Desse modo, essas descobertas podem ser úteis para o entendimento das consequências da perda de espécies vegetais raras para a decomposição de matéria orgânica e a ciclagem de nutrientes em outros ecossistemas tropicais.

Nós demonstramos a importância do efeito *priming* para a decomposição de detritos vegetais em riachos tropicais, o que fornece suporte para a ocorrência do efeito *priming* em ecossistemas aquáticos naturais (Guenet et al. 2010), uma questão ainda pouco explorada e com resultados bastante controversos (Bengtsson et al. 2018). Além disso, esta tese contribui para o entendimento de como esse efeito influência a decomposição de detritos em riachos nos trópicos, uma vez que estudos anteriores em riachos naturais foram realizados até o nosso conhecimento em regiões temperadas (e.g., Elosegi et al. 2018). Este estudo indicou que a decomposição de detritos vegetais mediada por microrganismos decompositores e invertebrados fragmentadores pode ser reduzida com o aumento do carbono lábil de algas em riachos tropicais (efeito *priming* negativo).

Em conjunto, os nossos resultados sugerem que a perda de espécies vegetais raras em riachos tropicais naturais, especialmente de espécies raras dissimilares, pode retardar o processamento de detritos no rio, e que esse retardo pode ser exacerbado devido ao efeito *priming* negativo. Nossas descobertas são muito relevantes dentro de um contexto de mudanças globais, desde que a perda de biodiversidade é uma das principais alterações atuais causadas em ecossistemas devido à distúrbios antrópicos (Díaz et al. 2019; IPBES 2019). Diante disso, esta tese contribui principalmente para o entendimento de duas importantes questões relacionadas ao funcionamento de riachos tropicais: as repercussões da perda de espécies vegetais raras e a importância da produtividade primária para a decomposição de detritos, o que contribui para o auxílio do aumento da eficiência dos programas de restauração.

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