

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

**CONTROLES MULTIESCALARES BIÓTICOS E ABIÓTICOS
DA DINÂMICA E DECOMPOSIÇÃO
DE DETRITOS FOLIARES EM RIACHOS**

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TESE DE DOUTORADO

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DINÂMICA E DECOMPOSIÇÃO DE DETRITOS FOLIARES EM RIACHOS**

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RESUMO

Riachos e florestas ripárias são funcionalmente conectados pela ciclagem de carbono e nutrientes, especialmente considerando (i) a relativamente baixa produção primária em riachos como consequência da cobertura ripária, (ii) elevadas quantidades de detritos foliares de origem terrestre que entram nos riachos e (iii) a importância desses detritos foliares como fonte de carbono e nutrientes para as cadeias alimentares de riachos, que por fim irão decompor esse material. Contudo, ainda faltam informações sobre processos básicos e suas conexões por trás da dinâmica de detritos, particularmente em riachos tropicais, o que impede um entendimento abrangente do funcionamento de riachos e previsões em cenários prováveis de mudanças ambientais. Essa deficiência é ainda mais crítica considerando as taxas atuais de perda de biodiversidade na maioria dos ecossistemas em todo mundo, que tem o potencial de alterar a disponibilidade de recursos e a interação de espécies dentro de riachos, com sérias consequências para processos ecossistêmicos chave como a decomposição de detritos.

Desse modo, nessa tese utilizamos diferentes abordagens observacionais (Capítulo I & II) e experimentais (Capítulo III & IV) a fim de explorar os padrões e mecanismos da dinâmica de detritos e como eles são afetados pela perda de biodiversidade, em ecossistemas de riachos de diferentes regiões e em várias escalas espaciais e temporais. Em um estudo de campo ambicioso ao longo diversos biomas tropicais, observamos padrões temporais distintos dos aportes e estoque de detritos (de não sazonais à altamente sazonais) dentro de um ciclo anual em riachos na Amazônia, Mata Atlântica e Cerrado, e um papel dominante da precipitação na regulação desses padrões sazonais (Capítulo I). Similarmente, observamos que o transporte de detritos – o qual depende do fluxo de água do riacho e com isso, responde aos regimes de precipitação – é um mecanismo chave na disponibilidade de detritos para os consumidores em climas sazonais

tropicais, apesar do papel predominante da decomposição na remoção de detritos na escala de trecho de riacho com base anual (Capítulo II). Em microcosmos experimentais, inicialmente demonstramos que a perda de diversidade de recursos (detritos foliares) não afetou os detritívoros (como sua sobrevivência, crescimento ou razão C:N), mas reduziu a decomposição mediada por microrganismos e por detritívoros em 7 e 15%, respectivamente, principalmente por meio de efeitos de complementariedade (Capítulo III). Adicionalmente, evidenciamos que a perda de diversidade de detritívoros reduziu a decomposição, mas sobretudo quando espécies grandes de detritívoros foram perdidas de comunidades com espécies pequenas, o que foi explicado pela facilitação dos organismos pequenos pelos grandes (Capítulo IV).

Nossos resultados sugerem que mudanças no regime de precipitação – no qual é previsto aumento na duração de períodos secos em vários biomas, incluindo o Cerrado e algumas partes da Amazônia – tem o potencial de alterar drasticamente os fluxos de detritos em riachos, e finalmente os ciclos de carbono e nutrientes na interface riacho-floresta. Por último, demonstramos que a perda de biodiversidade, tanto na vegetação ripária quanto nas comunidades de detritívoros em riachos, tem efeitos negativos nas interações da cadeia alimentar e em processos ecossistêmicos essenciais.

Palavras-chave: detritos foliares, aporte de detritos, decomposição, funcionamento de ecossistemas, matéria orgânica, escala temporal, escala espacial, detritívoros, partição de recursos, diversidade funcional, biodiversidade, floresta ripária.

ABSTRACT

Streams and riparian forests are functionally linked by carbon and nutrient cycling, especially considering (i) the relatively low in-stream primary production as a consequence of riparian shading, (ii) the high amounts of terrestrial plant litter inputs to the stream, and (iii) the importance of this plant litter as a source of carbon for stream food webs, where it is ultimately decomposed. However, there still is a lack of knowledge of basic processes and their connections behind litter dynamics, particularly in tropical streams, which precludes a comprehensive understanding of stream ecosystem functioning and predictions of likely scenarios of environmental change. This deficiency is even more critical given the current rate of biodiversity loss in most ecosystems worldwide, which has the potential to alter resource availability and species interactions within streams, with serious consequence to key ecosystem processes such as litter decomposition.

Therefore, in this thesis we used different observational (Chapter I & II) and experimental (Chapter III & IV) approaches to explore patterns and mechanisms of plant litter dynamics and how they are affected by biodiversity loss, in stream ecosystems from different regions and over a range of spatial and temporal scales. In an ambitious field study across several tropical biomes, we found distinct temporal patterns of litter inputs and storage (from aseasonal to highly seasonal) within a year cycle across streams in Amazon, Atlantic forest and Cerrado, and a major role of precipitation in driving these seasonal patterns (Chapter I). Similarly, we observed that litter transport – which is a function of stream discharge and thus respond to precipitation regimes – is a key mechanism of in-stream litter availability to consumers in seasonal tropical climates, despite the overall major role of decomposition in removing litter at the reach-scale on an annual basis (Chapter II). In experimental stream microcosms, we first

showed that diversity loss of resources (leaf litter) did not affect detritivores (such as survival, growth or C:N ratios) but reduced microbial and detritivore-mediated decomposition by 7 and 15%, respectively, mostly through complementary effects (Chapter III). Secondly, we observed that detritivore diversity loss reduced decomposition, but mainly when large detritivore species were lost from communities of small-sized species, which was explained by facilitation of small detritivores by larger ones (Chapter IV).

Our findings suggest that changes in precipitation regime – which is expected to enhance the length of drier periods in several biomes, including the Cerrado and some parts of Amazon forest – have the potential to drastically alter plant litter fluxes in streams, and ultimately the carbon and nutrient cycles in the stream-forest interface. Finally, we demonstrate that biodiversity loss, both in the riparian vegetation and in stream detritivore communities, has negative effects on stream food web interactions and key ecosystem processes.

Key-words: leaf litter, litterfall, decomposition, ecosystem functioning, organic matter, spatial scale, temporal scale, detritivores, resource partitioning, functional diversity, biodiversity, riparian forest.

INTRODUÇÃO GERAL

Ecossistemas aquáticos continentais (*i.e.*, banhados, estuários, lagos, rios e riachos) compreendem apenas 0,01% da água do mundo e cobrem aproximadamente 0,8% da superfície da Terra (Gleick 1996). Apesar da minúscula fração mundial, esses sistemas suportam uma riqueza de espécies de plantas e animais desproporcional a sua área de abrangência (revisado por Dudgeon et al. 2006) e contribuem significativamente para o ciclo do carbono, tanto em escala regional quanto global (Cole et al. 2007, Raymond et al. 2013, Hotchkiss et al. 2015). Entre os sistemas aquáticos continentais, os riachos (1^a - 3^a ordem) representam mais que 75% da área da rede de drenagem fluvial (Raymond et al. 2013) e, devido as grandes quantidades de matéria orgânica de origem terrestre que recebem, sua baixa produção primária, elevada capacidade de retenção e decompositores eficientes, são *hotspots* de processamento de matéria orgânica (Battin et al. 2008).

Riachos de cabeceira (daqui em diante ‘riachos’) são sistemas frequentemente heterotróficos – *i.e.*, a respiração total do sistema é superior à produção primária. Devido a limitada produção primária pela cobertura arbórea, a produção secundária é sustentada pelo carbono de origem terrestre. Isso significa que as cadeias alimentares nesses riachos dependem da entrada de energia basal de fontes externas devido à baixa produtividade interna do sistema. Conseqüentemente, a decomposição de detritos foliares de origem terrestre – a qual é influenciada por inúmeros fatores bióticos e abióticos – é um processo central nesses riachos heterotróficos visto que a maior parte da produção primária vegetal torna-se detritos que sustentam as cadeias alimentares em riachos (Cebrian 1999).

Apesar da importância da decomposição e dos fluxos de carbono terrestre em riachos heterotróficos, as taxas atuais de extinção local de espécies de plantas, fungos e animais têm o

potencial de alterar a disponibilidade de recursos, interação entre espécies e com isso, processos ecossistêmicos essenciais como a decomposição de detritos (Cardinale et al. 2012). A perda de biodiversidade é um dos maiores problemas em inúmeros ecossistemas em todo mundo (Dudgeon et al. 2006) e pode afetar a decomposição por meio de sua influência entre diversos níveis tróficos (Gessner et al. 2010). Por exemplo, a perda de biodiversidade reduz a diversidade de detritos foliares disponíveis para consumidores ou a eficiência na captação de recursos pelos consumidores, caso sejam perdidas interações importantes entre as espécies (Cardinale et al. 2002). Nas próximas seções enfocamos nesses aspectos importantes do funcionamento de ecossistemas e seus potenciais controles; inicialmente, introduzimos os processos ecossistêmicos básicos relacionados à disponibilidade de detritos em riachos – como os aportes, transporte e retenção de detritos – e suas conexões com a decomposição de detritos, e então enfocamos nas repercussões da perda de biodiversidade para o processo fundamental da decomposição.

PARTE 1. FLUXO E DECOMPOSIÇÃO DE DETRITOS VEGETAIS

A importância da conexão riacho-floresta ripária

Ecossistemas ripários – conceituado aqui como zonas semi-terrestres de transição influenciadas por ecossistemas aquáticos continentais (Naiman et al. 2005) – são áreas associadas com quase todos os ecossistemas aquáticos continentais e mediam interações entre ecossistemas aquáticos e terrestres. Ecossistemas ripários são caracterizados por uma considerável heterogeneidade de habitats, fluxo constante de energia e materiais entre água e terra, e uma diversidade de processos ecológicos e de espécies (Naiman & Décamps 1997). Por exemplo, ecossistemas ripários formam redes dentro da área de drenagem, as quais contribuem

com água e materiais para riachos e cursos de rios que conectam-se com o oceano (Schlesinger & Melack 1981).

Ecossistemas ripários proporcionam muitos benefícios de natureza estética, cultural e oportunidades recreativas, e produzem valiosos bens como madeira, recursos medicinais e alimentícios (*e.g.*, sementes, frutas e peixes) (Daily 1997). Além disso, esses ambientes desempenham funções ecossistêmicas essenciais como controle de inundações por desacelerar o fluxo de água, retenção de sedimentos (reduzindo a sedimentação), interceptação e retenção do escoamento superficial (incluindo fontes de poluição), prevenção da erosão das margens dos riachos, além de servirem como habitat ou corredores ecológicos para a dispersão de muitas espécies (Postel & Carpenter 1997). Ainda, a vegetação ripária reduz a incidência de radiação solar no leito do riacho por meio do sombreamento, atenuando aumentos da temperatura da água durante os períodos mais quentes do ano e fornece elevadas quantidades de detritos vegetais – aproximadamente 90% do total da produção primária vegetal a cada ano (Cebrian 1999) – para riachos e solos da zona ripária. A decomposição destes detritos é a base para processos fundamentais nos ecossistemas como a ciclagem de nutrientes, fluxo de carbono e, produção primária e secundária (Cebrian 1999, Wardle et al. 2004). Contudo, até o momento, temos um entendimento limitado inclusive de questões básicas relacionadas à dinâmica de matéria orgânica em riachos (*e.g.*, período e magnitude dos aportes de detritos para os riachos, e controles biofísicos da decomposição), especialmente em áreas historicamente pouco estudadas como os trópicos.

Fluxo de detritos em riachos

A matéria orgânica que chega aos riachos geralmente é subdividida em diferentes frações de acordo com seu tamanho: matéria orgânica particulada grossa, MOPG (> 1 mm); matéria orgânica particulada fina, MOPF (< 1 mm mas $> 0,45$ μm); e, matéria orgânica dissolvida, MOD ($< 0,45$ μm) (Allan & Castillo 2007). Essas frações de matéria orgânica podem entrar nos riachos por meio de diferentes vias (*e.g.*, via aporte vertical, também conhecido como *litterfall* ou via aporte lateral a partir dos solos) e seus fluxos provavelmente diferem sazonalmente e em magnitude (*e.g.*, Johnson et al. 2006). Aqui, nosso foco é na matéria orgânica particulada grossa (referida aqui como ‘detritos vegetais’ ou ‘detritos’), a qual é a principal base energética para as comunidades de riachos florestados (Hall et al. 2000, Neres-Lima et al. 2017) e é composta por várias partes vegetais mortas como detritos foliares, galhos (ou ramos), sementes, flores, frutos, cascas e troncos (> 2 cm de diâmetro) (Gonçalves et al. 2014b, Bambi et al. 2017). Em geral, excluindo as entradas ou saídas esporádicas de troncos, os detritos foliares dominam o fluxo de detritos em riachos ($> 60\%$ do total dos fluxos segundo nossas estimativas nos Capítulos I e II). Assim, nessa tese o enfoque será nos detritos foliares de origem terrestre, uma vez que estes constituem a fração de carbono terrestre mais ativa biologicamente em riachos florestados e é renovado anualmente (Wallace et al. 1997, Neres-Lima et al. 2017).

Quando os detritos caem das árvores, eles podem cair no solo da zona ripária ou diretamente no riacho – processo denominado ‘**aporte vertical**’. Contudo, obviamente a maior parte do aporte vertical cai sob os solos da zona ripária devido a sua maior extensão, e uma porção destes detritos eventualmente é transportada pelo vento, água, gravidade ou animais até o riacho – processo denominado ‘**aporte lateral**’. Apesar de negligenciado em inúmeros estudos de dinâmica de detritos, os aportes laterais podem representar uma proporção considerável do

aporte total de detritos para o riacho (como evidenciado no Capítulo I). Também, o transporte lateral de detritos pode representar um recurso diferente para as cadeias alimentares de riachos uma vez que sofre degradação física e biológica durante seu tempo de residência no solo (e.g., Selva et al. 2007, García-Palacios et al. 2016). Após a entrada dos detritos no riacho, vertical ou lateralmente, os detritos podem ser imediatamente retidos por estruturas presentes no riacho (e.g., rochas, raízes ou troncos) ou transportados à jusante até que sejam retidos. A retenção é a força oposta ao transporte e é essencial para aumentar o tempo de residência dos detritos nos riachos para a utilização pelas comunidades aquáticas (Hildrew et al. 1991). Isto é, os detritos geralmente necessitam permanecer retidos por algum tempo para possibilitar sua colonização e degradação por detritívoros e decompositores. Em geral, os detritos não são transportados longe de seu local de entrada até que a decomposição biológica seja iniciada (Webster et al. 1999), porém, podem ser periodicamente transportados à jusante pelo fluxo de água. Apesar da natureza transitória dos detritos nos riachos, uma porção desses detritos são estocados relativamente por longos períodos em áreas de remanso ou em obstáculos com alta capacidade retentiva (e.g., troncos, grandes pedras ou represas naturais) do riacho (Smock et al. 1989), mas também podem ser enterrados no sedimento (e.g., na zona hiporéica - interface entre águas superficiais e subterrâneas; Boulton et al. 1998).

O **estoque** de detritos na zona bêntica (tratado aqui como ‘estoque de detritos’ ou ‘estoque’) usualmente é um componente ativo e importante do fluxo de detritos em riachos, por ser uma fonte fundamental de energia para os consumidores, sujeita à degradação física e potencial transporte à jusante (Jones 1997 e referências citadas). Os detritos acumulam-se no leito dos riachos quando os aportes – vertical, lateral ou à montante – são superiores do que a exportação – pelo **transporte à jusante** e a **decomposição**. Considerando que regiões tropicais

são caracterizadas por maiores volumes de precipitação e/ou maior sazonalidade (Feng et al. 2013), podemos esperar um papel importante de regimes hidrológicos no fluxo e decomposição de detritos nesses ambientes, apesar desse tópico ainda ser pouco explorado (e.g., Johnson et al. 2006, Rueda-Delgado et al. 2006). Entre os fluxos de detritos, o mais complexo é a decomposição ou degradação (utilizados aqui como sinônimos), devido à suas relações multi-tróficas (i.e., entre recursos, consumidores e predadores; Jabiol et al. 2013b) e interações entre controles bióticos e abióticos.

Fluxo de detritos em uma perspectiva hierárquica

Mais de 20 anos depois do artigo seminal de Levin (1992) sobre o significado dos padrões escalares em ecologia, tem havido um crescente reconhecimento de que a identificação da escala na qual os processos ecológicos ocorrem é determinante para a produção de modelos preditivos mais gerais (Chave 2013). Apesar dos avanços nos experimentos de ecologia de riachos ao longo das últimas décadas, a maior parte do conhecimento sobre fluxos e decomposição de detritos é baseada em estudos nas escalas de micro e mesohabitats (veja revisão de Tank et al. 2010 e referências citadas), o que dificulta generalizações nas escalas de bacia hidrográfica ou regionais. Enquanto alguns modelos conceituais (e.g., Royer & Minshall 2003, Graça et al. 2015) proporcionaram um avanço significativo na descrição de fontes potenciais de variabilidade da decomposição em riachos em múltiplas escalas espaciais (e.g., de micro-habitats até biomas), poucos estudos empíricos investigaram essas questões (e.g., Tiegs et al. 2009, Rezende et al. 2014, Tonin et al. 2017b). Além disso, a maioria dos experimentos de larga escala espacial têm ignorado a heterogeneidade local (p.ex., análises baseadas em poucas amostras ou sub-amostras, como uma compensação pelo aumento considerável na escala espacial do estudo)

ou variações sazonais e anuais, o que é geralmente uma importante fonte de variação em ecossistemas naturais (e.g., Bambi et al. 2017, Tonin et al. 2017b).

Mecanismos locais e regionais do fluxo de detritos em riachos

Apesar da importância do fluxo de detritos para o funcionamento dos ecossistemas de riachos, e de sua relevância para a ciclagem global de carbono e nutrientes, as informações existentes sobre esses fluxos são escassas – especialmente em ecossistemas tropicais – e pouco se sabe sobre suas conexões com o processo de decomposição, ainda que este seja muito mais estudado (mas veja Fisher & Likens 1972, Fisher & Likens 1973, Pozo et al. 1997a, Webster & Meyer 1997). Isso é um problema uma vez que impede uma visão mais realista da dinâmica de detritos em riachos, tanto em escalas temporais mais longas quanto em diferentes condições ambientais ou regimes climáticos.

Nesta tese nós superamos essa limitação propondo um novo modelo conceitual conectando os aportes, estoque e decomposição de detritos. Utilizamos uma perspectiva hierárquica para prever o papel de múltiplos fatores em diferentes escalas espaciais sobre os processos estudados, similarmente à modelos prévios de decomposição (Royer & Minshall 2003, Graça et al. 2015) (Figura 1). Esses modelos teóricos buscam estabelecer conexões entre os fatores que atuam em diferentes escalas espaciais e/ou temporais, e isso tem proporcionado uma estrutura básica para o entendimento de processos ecológicos (cf. O'Neill 1986, Wiens 1989) – como a decomposição de detritos. Por exemplo, o clima, a geologia e a biogeografia são fatores que atuam em escalas regionais, e por isso estão no topo da hierarquia e influenciam fatores em níveis hierárquicos mais baixos como a vegetação ripária (O'Neill 1986). Por outro lado, fatores

em níveis locais são regidos por forças em níveis hierárquicos superiores e determinam a magnitude dos processos locais (como os aportes, estoque e decomposição de detritos).

No entanto, o maior desafio ainda permanece se o interesse for entender a dinâmica de detritos e seu papel no funcionamento de ecossistemas de riachos, uma vez que os fatores podem interagir dentro e entre escalas espaciais, produzindo resultados imprevisíveis baseados apenas em simulações teóricas ou no conhecimento empírico de uma escala espacial em particular. Nas seções seguintes descrevemos os diferentes componentes do modelo e suas relações com os diferentes capítulos desta tese (Figura 1).

1- Aporte de detritos

O aporte de detritos consiste em três componentes: aporte vertical, aporte lateral e aporte à montante (*i.e.*, detritos que já estão no riacho, mas são transportados de trechos à montante). Esses aportes são influenciados por uma variedade de fatores. Inicialmente, a **produção de detritos** é um fator chave que medeia os aportes de detritos nos riachos, pois determina a magnitude do aporte vertical, bem como o total de detritos disponível nos solos da zona ripária que podem ser transportados para o riacho (e.g., Gonçalves et al. 2006, França et al. 2009). A produção de detritos depende da fisionomia e composição de espécies da comunidade vegetal, os quais são determinados por fatores climáticos (temperatura e precipitação; Prentice et al. 1992, Woodward et al. 2004) e também pela biogeografia, que resulta em mudanças na distribuição das espécies vegetais ao longo de tempos geológicos (e.g., refúgios glaciais e rotas da expansão pós-glacial; Comès & Kadereit 1998). Em resumo, é esperado elevados aportes de detritos em florestas muito produtivas; e, elevada produtividade em florestas em solos férteis e em ambientes quentes e úmidos (e.g., florestas ombrófilas ou pluviais), enquanto é esperado baixa

produtividade em florestas presentes em solos pouco férteis e em ambientes limitados por água, por baixas temperaturas ou ambos (*e.g.*, florestas em áreas secas e/ou frias).

Adicionalmente, a **morfologia da margem** dos riachos (*e.g.*, heterogeneidade e inclinação) regula o transporte lateral de detritos para o riacho por meio da capacidade de retenção, em relação à topografia, hidrologia e relações com a vegetação ripária (Leopold et al. 1992). A heterogeneidade da margem dos riachos é caracterizada pela presença de obstáculos os quais impedem o transporte de detritos para os riachos, como troncos vivos ou mortos, raízes, pedras, plantas rasteiras ou no sub-bosque e muitos outros. A influência da inclinação das margens está fortemente associada à forças físicas do transporte dos detritos para o riacho (France 1995b). Por exemplo, margens mais declivosas facilitam o movimento dos detritos pela força da gravidade e/ou do vento, e aumentam o escoamento superficial (por meio da precipitação) (Horton 1945). Em resumo, elevados aportes laterais de detritos são esperados em florestas ripárias altamente produtivas, e em margens mais homogêneas (*i.e.*, com poucos obstáculos) e mais declivosas.

Por último, a **morfologia do riacho** e o **fluxo de água** afetam os aportes à montante por meio de sua influência sobre a capacidade de retenção (Quinn et al. 2007). No entanto, os aportes à montante possuem um aspecto diferencial em relação aos aportes vertical ou lateral, pois referem-se a uma fonte de detritos que encontra-se dentro do riacho. Isso significa que os aportes à montante são controlados pelos mesmos fatores que o transporte de detritos dentro do riacho, o qual é discutido na próxima seção (*‘Estoque de detritos’*).

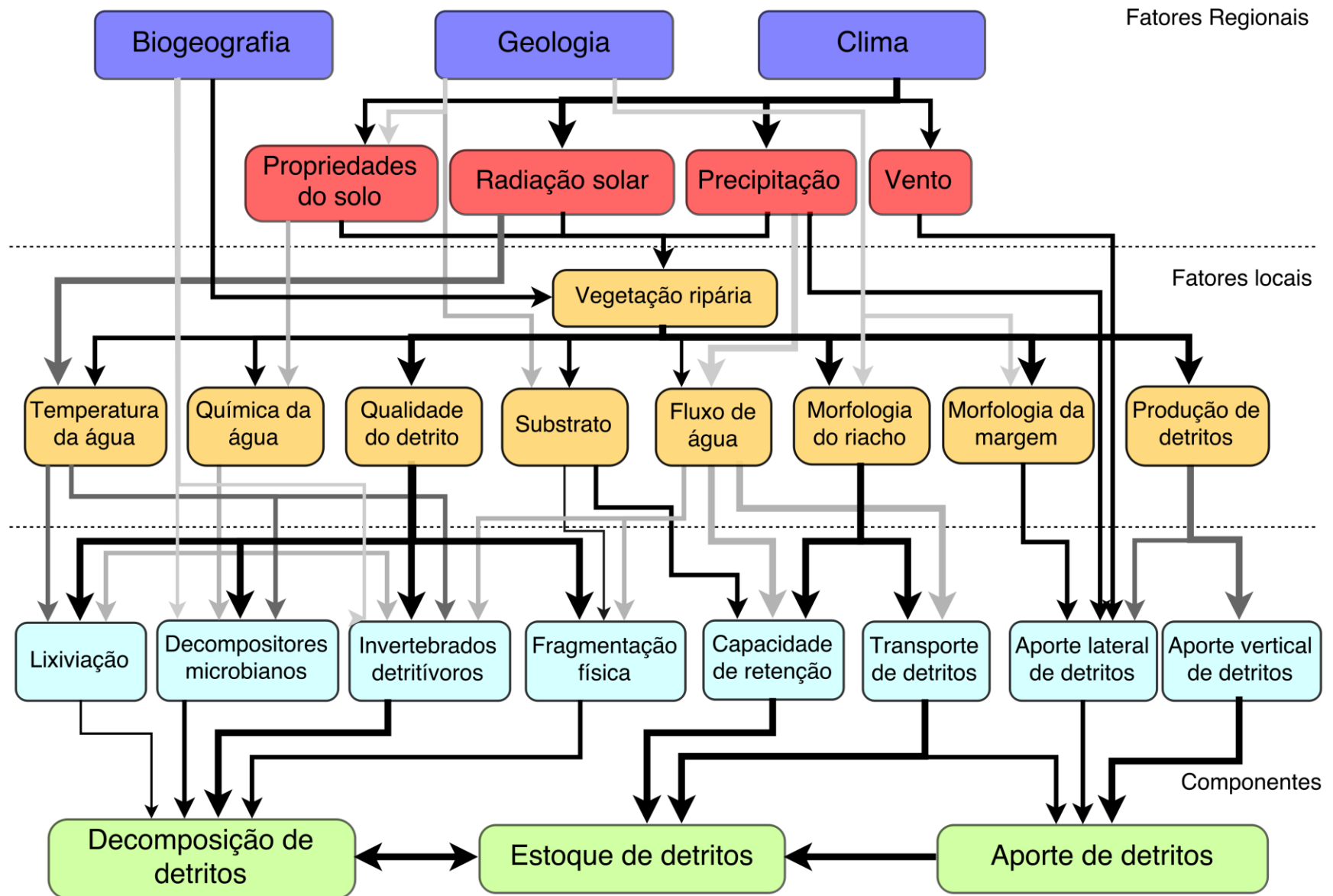


Figura 1. Modelo conceitual da dinâmica de detritos foliares em riachos florestados. Os fatores reguladores dos três processos chave para a dinâmica de detritos foliares (aporte, estoque e decomposição) são apresentados em uma perspectiva hierárquica, em que fatores de escalas regionais modulam o efeito de fatores em escalas locais. A largura das setas é uma tentativa de indicar a contribuição relativa de cada fator.

2- Estoque de detritos

A quantidade de detritos estocada no leito dos riachos é regulada pela interação entre três fatores principais: aporte de detritos, decomposição de detritos e capacidade de retenção dos riachos. Primeiramente, o **aporte de detritos** aumenta linearmente o estoque desse material, caso a retenção ocorra em taxas similares. Contudo, na prática isso raramente ocorre devido à elevada heterogeneidade intrínseca aos riachos (Pringle et al. 1988) e interações múltiplas com processos físicos e biológicos como explicado abaixo. Por outro lado, a **decomposição** diminui o estoque de detritos por meio da transformação de partículas grossas em finas e dissolvidas (Gessner et al. 1999), as quais são mais facilmente transportadas pelo fluxo da água ou enterradas no sedimento (Webster et al. 1999). Os agentes reguladores da decomposição são explorados na próxima seção.

Adicionalmente, a **capacidade de retenção** é uma força chave por trás do estoque de detritos por reduzir o transporte dentro do riacho. A capacidade de retenção de um riacho varia em função de sua morfologia (*e.g.*, largura, profundidade e inclinação), fluxo de água, substratos no leito do riacho (materiais orgânicos e inorgânicos, incluindo tipo, tamanho e quantidade das estruturas de retenção) e suas complexas interações (Quinn et al. 2007). A morfologia do riacho é principalmente um resultado da geomorfologia (por meio de seus efeitos históricos sobre a topografia), mas também é modelada pela hidrologia (por meio da erosão) e da vegetação ripária (de diversas formas, *e.g.*, reduzindo a velocidade do fluxo de água; aumentando a integridade das margens por meio das raízes; ou fornecendo grandes troncos que podem alterar o curso da água) (Hupp et al. 2016). Nesse contexto, a morfologia e a precipitação regulam o fluxo de água (por meio de alterações na vazão e turbulência), enquanto os substratos no leito do riacho são determinados pela geomorfologia, hidrologia e vegetação ripária (*e.g.*, por meio de sua influência

na rocha matriz, processos de intemperismo ou fornecendo diversos tipos de substratos orgânicos) (Leopold et al. 1992). Em geral, a capacidade de retenção dos substratos aumenta com seu tamanho. Por exemplo, seixos e rochas são mais eficientes em reter detritos do que cascalho e areia (Jones 1997). Ainda, grandes pedaços de madeira ou troncos aumentam drasticamente a capacidade de retenção dos riachos por serem eficientes obstáculos e são estruturas de longo prazo (devido a sua lenta decomposição e difícil mobilidade) no leito dos riachos (Wallace et al. 1995, Díez et al. 2000). Consequentemente, é esperada elevada retenção – e então, elevado estoque de detritos – em riachos estreitos, profundos, sinuosos e com pouco declive; em condições de baixo fluxo de água; e em riachos com substratos grandes e abundantes. Além disso, é esperado maior estoque de detritos em riachos com maior aporte, mas com baixas taxas de decomposição dos detritos.

3- Decomposições de detritos foliares

A decomposição é um processo complexo que foi tradicionalmente separado em uma série de sub-processos que ocorrem ao longo do tempo, com o propósito de simplificar seu estudo (e.g., lixiviação, condicionamento microbiano e fragmentação; Gessner et al. 1999). Como a grande maioria dos estudos de decomposição são baseados em detritos foliares nosso foco nesta seção é neste tipo de detrito vegetal. Além disso, os detritos foliares compreendem a maior parte do material vegetal que entra nos córregos (mais de 60% da biomassa total de detritos) e são renovados anualmente – pois, respondem a mecanismos sazonais das plantas e sua degradação é mais acelerada do que a de troncos ou galhos (e.g., Reich 1995, Webster et al. 1999). Isso caracteriza os detritos foliares como uma fonte de carbono e nutrientes essencial para detritívoros e decompositores.

3.1 Lixiviação

A lixiviação é a dissolução inicial de compostos solúveis em água presentes nos detritos foliares (*e.g.*, açúcares e compostos de baixo peso molecular) e pode ser responsável por até 40% da perda inicial de massa em apenas uma semana, porém as maiores perdas ocorrem dentro das primeiras 48h após a imersão (Taylor & Bärlocher 1996, Gomes 2015). A lixiviação dos detritos foliares é o resultado da interação entre quatro fatores principais: qualidade dos detritos foliares, química da água e, temperatura e fluxo da água (*i.e.*, turbulência e velocidade).

A **qualidade dos detritos foliares** é expressa por diversas características físicas e químicas intrínsecas aos detritos como a concentração de nutrientes (principalmente nitrogênio e fósforo), recalcitrância do carbono (*e.g.*, moléculas complexas de difícil degradação como lignina, celulose e hemicelulose) e metabólitos secundários (*e.g.*, substâncias tóxicas ou repelentes utilizadas para proteção das folhas verdes contra herbivoria, mas que ainda permanecem nos detritos foliares, como fenóis). Inicialmente, a qualidade química dos detritos pode afetar a lixiviação por determinar a quantidade de compostos solúveis em água (como alguns micro e macro-nutrientes, moléculas de baixo peso molecular e alguns compostos secundários) e sua resistência à dissolução (Kuiters & Sarink 1986, Schreeg et al. 2013). Deste modo, a lixiviação aumenta com a quantidade de compostos solúveis em água e diminui com a recalcitrância do carbono. A qualidade dos detritos foliares é regulada principalmente pela fisionomia da vegetação – isto é, características estruturais das comunidades vegetais como forma de vida (árvores, lianas, arbustos, ervas), altura dos indivíduos, tamanho das folhas e fenologia (sempre-verdes, semi-decíduas, decíduas) – e composição, a qual varia em função do clima, geologia e biogeografia (como discutido acima, na seção ‘Aporte de detritos’). Ainda

assim, comunidades vegetais com fisionomia e composição de espécies similares podem diferir na qualidade de seus detritos como resultado das características do solo (por meio de diferentes eficiências na reabsorção de nutrientes antes da senescência; Vergutz et al. 2012) ou interações locais entre espécies (p.ex., competição por nutrientes; Casper & Jackson 1997).

Adicionalmente, a **química da água** afeta a lixiviação por meio do pH, dureza e níveis de minerais na água (isto é, devido ao efeito da polaridade, em que compostos do soluto irão se dissolver melhor em solventes com estrutura química similar a eles; Essington 2005). A lixiviação aumenta em pH básicos (> 7). Contudo, a dureza da água (que refere-se a concentração dissolvida de íons de cálcio e magnésio) e os níveis de minerais podem afetar os compostos químicos das folhas de distintas maneiras (p.ex., os polifenóis ligam-se aos minerais de águas mais duras; Gebely 2016). A química da água é regulada pela geologia (*i.e.*, composição elementar da rocha matriz), propriedades do solo (incluindo sua idade e processos de intemperismo) e vegetação ripária (por meio de sua influência sobre moléculas orgânicas e inorgânicas dissolvidas).

A **temperatura da água** influencia a lixiviação (e.g., Chergui & Pattee 1988) pelo seu efeito na solubilidade das moléculas da água (*i.e.*, um aumento da temperatura intensifica a energia cinética das moléculas de água que efetivamente mantém separadas as moléculas do soluto). A temperatura da água é primariamente controlada pelo clima (por meio da radiação solar), mas a densidade do dossel ao longo do curso do riacho também é importante, pois regula a incidência de radiação. Desse modo, podemos esperar uma lixiviação mais rápida em riachos tropicais do que em temperados (devido à maior temperatura da água), o que pode repercutir na qualidade nutricional dos detritos foliares para os consumidores, uma vez que os efeitos inibitórios de metabólitos secundários podem ser reduzidos em riachos tropicais e subtropicais

(Ardón & Pringle 2008, Tonin et al. 2014b). Por último, o **fluxo de água** pode afetar a lixiviação por meio da turbulência e da velocidade da corrente (Fonseca et al. 2013, Gebely 2016), os quais regulam a velocidade de dissolução dos compostos solúveis em água. No entanto, a importância da lixiviação dos detritos foliares para a decomposição e para a liberação de nutrientes nos riachos é sem dúvida o componente menos estudado da decomposição e seus mecanismos ainda carecem de suporte empírico mais consistente. De modo geral, é esperado maior lixiviação em detritos foliares com elevadas concentrações de compostos solúveis em água e com baixa recalcitrância, e em águas mais alcalinas, quentes, rápidas e turbulentas.

3.2 Decomposição microbiana

Existem dois principais grupos de decompositores microbianos que colonizam os detritos foliares em riachos: fungos e bactérias. Apesar da importância de ambos e de suas funções complementares na decomposição (e.g., os fungos podem facilitar a penetração de bactérias no tecido foliar; Schneider et al. 2010), os fungos representam a maior proporção da biomassa microbiana associada aos detritos foliares (Findlay & Arsuffi 1989, Findlay et al. 2002). Dentre os fungos decompositores, os hifomicetos aquáticos têm um papel predominante na decomposição em riachos de climas temperados (Suberkropp & Klug 1974). Contudo, a participação dos hifomicetos aquáticos na decomposição em riachos tropicais ainda é controversa, uma vez que tanto valores elevados quanto baixos de biomassa e diversidade de hifomicetos aquáticos foram observados (e.g., Mathuriau & Chauvet 2002, Gonçalves et al. 2007). Apesar disso, há mais indícios de que os hifomicetos aquáticos em sistemas tropicais e subtropicais sejam menos diversos e abundantes do que em riachos em ambientes temperados (veja revisão de Graça et al. 2016 e referências citadas).

A contribuição dos microrganismos para a decomposição é regulada por quatro fatores principais: biogeografia, temperatura da água, química da água e qualidade dos detritos foliares. A **biogeografia** pode ser responsável pela composição da comunidade de fungos e bactérias (e com isso, eficiências distintas na degradação do carbono dos detritos), apesar de ainda haver controvérsias sobre a importância relativa de condições históricas *versus* condições ambientais contemporâneas na determinação dessas comunidades (Martiny et al. 2006, O'Malley 2007). Do mesmo modo, a **temperatura da água** influencia os microrganismos por meio de seu papel na distribuição destes organismos – selecionando algumas espécies, e em consequência regulando a composição da comunidade e a diversidade de espécies (Dang et al. 2009) –, mas também em sua biomassa e taxas de esporulação (Ferreira & Chauvet 2011). Assim, um aumento na temperatura eleva a atividade e biomassa microbiana (i.e., por meio da regulação das taxas metabólicas dos organismos, de acordo com a Teoria Metabólica da Ecologia; Brown et al. 2004). Desse modo, poderíamos esperar que riachos tropicais apresentassem maior decomposição microbiana do que riachos temperados (e.g., Boyero et al. 2011b). No entanto, muitas vezes isso não é observado, possivelmente devido à limitação dos microrganismos por outros fatores históricos (como discutido anteriormente) ou ambientais como menor disponibilidade de nutrientes na água e nos detritos em ambientes tropicais (e.g., Gonçalves et al. 2007, Ferreira et al. 2012). Entretanto, essas questões ainda carecem de suporte empírico mais consistente, principalmente envolvendo metodologias padronizadas e amplos gradientes ambientais e latitudinais (e.g., Jabiol et al. 2013a, Heffernan et al. 2014b).

Adicionalmente, os microrganismos respondem à **química da água** potencializando sua atividade e aumentando sua biomassa juntamente com a concentração de nutrientes dissolvidos (N e P) (por meio da maximização da ingestão de carbono; Suberkropp & Chauvet 1995) e, pH e

alcalinidade (pelo aumento da atividade de diferentes tipos de enzimas associadas ao amolecimento e maceração dos tecidos foliares; Chamier 1987, Jenkins & Suberkropp 1995). Além disso, a qualidade dos detritos foliares afeta os decompositores microbianos, os quais atuam melhor em detritos mais macios (pois são mais susceptíveis à degradação enzimática), com menos defesas químicas (pois há menos prejuízo em seu desenvolvimento) e mais ricos em nutrientes (pois há um menor desequilíbrio estequiométrico entre seus tecidos e os recursos) (Gessner et al. 2007). Ainda, a atividade alimentar seletiva dos detritívoros (*i.e.*, preferencialmente consumindo detritos colonizados por microrganismos) pode também afetar as comunidades microbianas (*e.g.*, diversidade de espécies e biomassa) por meio do consumo de determinadas espécies de fungos e rejeição de outras (*e.g.*, Arsuffi & Suberkropp 1989, Barlocher 2005).

3.3 Fragmentação por invertebrados detritívoros

Invertebrados detritívoros são organismos fundamentais na decomposição de detritos, geralmente responsáveis por uma elevada proporção do total da decomposição (*e.g.*, 51-64% da perda de massa foliar de acordo com Hieber & Gessner 2002), apesar de que esta proporção é geralmente inferior em riachos tropicais (Boyero et al. 2011b). Além disso, a atividade dos detritívoros produz grandes quantidades de partículas finas (por meio de sua alimentação e excreção; Graça 2001) as quais são usadas por outros invertebrados (Cummins & Klug 1979). A importância relativa dos detritívoros para decomposição é afetada por seis fatores principais: biogeografia, temperatura da água, química da água, qualidade dos detritos, fluxo da água e substrato. Fatores regionais como biogeografia e clima (por meio da temperatura da água) determinam a distribuição das espécies de detritívoros. Por exemplo, alguns táxons de

detritívoros são mais abundantes e diversos em domínios biogeográficos particulares (Boyero et al. 2011a) – tais como a elevada abundância e diversidade de tricópteros no domínio Australiano; o de besouros nos Neotrópicos; e, de plecópteros e anfípodos no domínio Paleártico. Ainda, uma maior densidade e diversidade de detritívoros ocorrem em águas mais frias (i.e., um gradiente latitudinal inverso; Boyero et al. 2011a, Boyero et al. 2012c). Conseqüentemente, a contribuição dos detritívoros para a decomposição tende a aumentar com a abundância (ou densidade por área ou biomassa de recurso), biomassa e diversidade de detritívoros (e.g., Jonsson & Malmqvist 2000a, Tonin et al. 2014a, Tonello et al. 2016) sendo estas superiores em climas mais frios (Boyero et al. 2011b). A composição da comunidade de detritívoros também pode afetar a decomposição, principalmente por meio da presença ou dominância de consumidores eficientes (como é o caso de alguns tricópteros, plecópteros e anfípodos). Além disso, macroconsumidores como peixes, camarões e caranguejos podem ser responsáveis por uma fração considerável da decomposição em riachos tropicais ou subtropicais (e.g., Landeiro et al. 2008, Moulton et al. 2010, Cogo & Santos 2013).

A **química da água** também tem o potencial de influenciar as comunidades de detritívoros (e.g., Herrmann et al. 1993), e assim, a contribuição total dos detritívoros na decomposição. Por exemplo, algumas espécies de tricópteros e anfípodos são mais sensíveis à águas ácidas (e.g., Herrmann et al. 1993, Dangles et al. 2004), enquanto plecópteros estão geralmente associados à águas neutras ou ácidas (e.g., Dangles & Guérold 1999). A **qualidade dos detritos** foliares influencia o consumo dos detritívoros e suas razões corporais de C:N:P, crescimento e sobrevivência (e.g., Graça et al. 2001, Hladyz et al. 2009). Eles geralmente preferem e aumentam a degradação de detritos macios, ricos em nutrientes e pobres em compostos secundários (isto é, detritos foliares de alta qualidade nutricional; Graça 2001,

Martins et al. 2015). O **fluxo da água** e o **substrato** também podem regular a distribuição dos detritívoros, mas na escala de micro-habitats, uma vez que diferentes táxons ocorrem em diferentes tipos de substratos (e.g., substratos minerais como pedras versus substratos orgânicos como detritos foliares; Cheshire et al. 2005), tais como os detritívoros que usualmente formam agregações em áreas com elevado acúmulo de detritos – as quais geralmente ocorrem em remansos ou águas mais calmas (Heino et al. 2004). Deste modo, o estoque de detritos (*i.e.*, sua disponibilidade) e sua distribuição espacial dentro de riachos geralmente determinam a contribuição dos detritívoros para a decomposição (e.g., Tonin et al. 2017b). Finalmente, os detritívoros usualmente se beneficiam da colonização microbiana nos detritos foliares (*i.e.*, condicionamento microbiano), devido aos microrganismos aumentarem a qualidade nutricional dos detritos e converterem compostos de difícil digestão em moléculas mais lábeis (Bärlocher 1985). Em resumo, é esperado uma contribuição superior dos detritívoros em água frias, em detritos foliares com alta qualidade nutricional e condicionados, e em micro-habitats com elevada disponibilidade de detritos foliares.

3.4 Fragmentação física

A fragmentação física é um componente importante da decomposição de detritos em riachos – geralmente responsável pela degradação de quantidades consideráveis do detrito por meio da quebra física dos tecidos vegetais e liberação de partículas finas para a coluna de água (Fonseca et al. 2013). Contudo, na maioria dos casos é um desafio separar sua contribuição dos outros componentes concomitantes, particularmente da fragmentação mediada por detritívoros (principalmente em estudos de campo, mas veja Rader et al. 1994). A fragmentação física depende da qualidade do detrito foliar, do fluxo de água e da interação entre fluxo e substrato. A

recalcitrância do detrito é o fator chave por trás do efeito da **qualidade do detrito**, uma vez que materiais mais duros são mais resistentes à degradação do que os macios (Fonseca et al. 2013). Geralmente, quanto maior a concentração de lignina do detrito, maior sua resistência, porém a celulose e a hemicelulose também são compostos estruturais importantes que retardam a degradação.

O **fluxo da água** afeta a fragmentação física por meio da abrasão da superfície do detrito foliar (Fonseca et al. 2013), contudo, seu efeito pode depender da presença e do tipo de substrato do leito do riacho (e.g., substratos de pequena granulometria, como areia fina e argila, os quais são mais facilmente transportados pelo fluxo de água e, então, podem desgastar a superfície do detrito foliar; Heard et al. 1999, Ferreira et al. 2006). Ainda, a turbulência pode intensificar o atrito e, com isso, a degradação do detrito (por meio do fluxo em diferentes direções). Apesar da existência de alguns estudos que exploraram este tópico, estes não são conclusivos ou foram delineados para situações muito específicas o que limita generalizações sobre o papel da fragmentação física em diferentes sistemas e condições. Consequentemente, podemos esperar maior fragmentação física em detritos menos recalcitrantes, em condições de fluxo de água mais intenso e turbulento e, em riachos com substratos mais finos.

PARTE 2. BIODIVERSIDADE E DECOMPOSIÇÃO

Os ecossistemas aquáticos continentais estão sofrendo perdas de biodiversidade muito superiores aos ecossistemas terrestres mais ameaçados (Sala et al. 2000). As razões principais para essa vulnerabilidade às ações humanas e mudanças ambientais variam da elevada e desproporcional diversidade de plantas, animais, protistas e fungos que estes ambientes suportam (revisado por Dudgeon et al. 2006) até o mais essencial recurso natural que proporcionam: a

água (Vörösmarty et al. 2010). As maiores ameaças à biodiversidade dos ecossistemas aquáticos continentais incluem super-exploração (principalmente sobre vertebrados como peixes, répteis e anfíbios), poluição da água, modificação do fluxo de água, destruição e degradação de habitat, e invasão por espécies exóticas, os quais resultam em declínios populacionais, e extinções locais, regionais ou até globais de espécies (Dudgeon et al. 2006).

A biodiversidade aquática proporciona uma ampla gama de bens e serviços valiosos para os humanos e sustenta inúmeras funções ecossistêmicas que controlam os fluxos de energia, de nutrientes e de matéria orgânica (Postel & Carpenter 1997). Adicionalmente, há evidências irrefutáveis de que a perda de biodiversidade altera processos ecossistêmicos essenciais como a decomposição e a ciclagem de nutrientes (e.g., Balvanera et al. 2006, Srivastava et al. 2009, Cardinale et al. 2011). Apesar dos progressos substanciais nas últimas décadas no entendimento dos efeitos da perda de biodiversidade no funcionamento de ecossistemas, ainda há um número razoável de questões fundamentais para serem respondidas e lacunas no conhecimento para serem preenchidas (Loreau et al. 2001, Cardinale et al. 2012), especialmente considerando que muito menos é conhecido sobre esses ecossistemas aquáticos do que sobre os terrestres (Hooper et al. 2005).

Perda de biodiversidade e repercussões para a decomposição de detritos

A decomposição engloba relações multi-tróficas dentro e entre pelo menos três níveis tróficos em cadeias alimentares de detritos em riachos florestados: recursos basais (e.g., detritos foliares), decompositores microbianos e detritívoros (e.g., invertebrados detritívoros) (Gessner et al. 2010). Conseqüentemente, alterações na diversidade de qualquer um desses níveis tróficos têm o potencial de alterar a decomposição de detritos. Contudo, como a maioria dos fungos são

capazes de degradar uma ampla variedade de polímeros vegetais, há uma probabilidade maior de a redundância funcional limitar os efeitos da diversidade microbiana na decomposição (Gessner et al. 2010). Nesse contexto, nosso foco nesta tese é nas repercussões da perda de diversidade de detritos foliares e de detritívoros para a decomposição. Enfocamos em dois aspectos importantes e complementares da diversidade: a diversidade taxonômica (em particular, a riqueza de espécies ou o número de espécies) e a diversidade funcional (*i.e.*, o número de tipos funcionais ou grupos de espécies que compartilham características particulares). No **Capítulo III** lidamos com a diversidade de espécies de plantas, que influenciam a diversidade de detritos foliares que entram nos riachos; e no **Capítulo IV** enfocamos na diversidade de detritívoros.

Efeitos da diversidade de detritos foliares na decomposição

Espécies vegetais produzem detritos foliares que variam amplamente quanto a suas características físicas e químicas, como resultado de estratégias adaptativas das plantas contra herbivoria e eficiência na obtenção de recursos essenciais (Mattson 1980, Agrawal 2007). Em consequência, detritos foliares com características variadas entram no riacho e formam misturas que são sujeitas à decomposição. É bem reconhecido que a maioria dos microrganismos e dos detritívoros preferencialmente alimentam-se de detritos lábeis e ricos em nutrientes para maximizar sua ingestão de energia e intensificar seu crescimento (e.g., Güsewell & Gessner 2009, Ohta et al. 2016). Contudo, a presença de detritos com características distintas pode acelerar a decomposição por meio de vários mecanismos. Por exemplo, microrganismos e detritívoros podem captar recursos essenciais de diferentes tipos de detritos dependendo de onde forem mais abundantes ou facilmente disponíveis (complementariedade de recursos; e.g., Vos et al. 2013). A decomposição de detritos pobres em nutrientes pode ser intensificada pela presença

de detritos ricos em nutrientes, como resultado da transferência ativa de nutrientes entre os tipos de detritos, a qual é mediada por fungos (facilitação; Gessner et al. 2010, Handa et al. 2014). A diversidade de detritos pode aumentar a heterogeneidade de habitat e, com isso favorecer uma maior abundância de detritívoros (Sanpera-Calbet et al. 2009). A maior diversidade pode também retardar a decomposição, como por exemplo, se a lixiviação de metabólitos secundários de um detrito de pior qualidade reduzir a palatabilidade de um detrito de melhor qualidade (e.g., Horner et al. 1988 em ambientes terrestres). Considerando que tanto efeitos positivos quanto negativos da diversidade de detritos foram descritos (Srivastava et al. 2009), e que há pouco suporte para os mecanismos que regulam essas relações, parece ser crucial o desenvolvimento de estudos futuros para examinar os efeitos dos diferentes tipos de diversidade de detritos (e.g., taxonômica *versus* funcional) na decomposição e explorar os mecanismos biológicos subjacentes à esses efeitos.

Efeitos da diversidade de detritívoros na decomposição

Efeitos *top-down* da diversidade de detritívoros na decomposição parecem ser mais fortes do que efeitos *bottom-up* da diversidade de detritos foliares, como demonstrado por uma compreensiva síntese (Srivastava et al. 2009). Isso é consistente com inúmeros estudos experimentais e meta-análises (Balvanera et al. 2006, Cardinale et al. 2006 e referências citadas), os quais observaram efeitos positivos da diversidade de detritívoros na decomposição. Contudo, os mecanismos biológicos por trás desses efeitos da diversidade são ainda pouco compreendidos e permanecem inexplorados. Enquanto efeitos positivos da diversidade são geralmente associados à partição de recursos (*i.e.*, uso de diferentes tipos de recursos no espaço ou no tempo) ou facilitação (*i.e.*, uma espécie aumenta o desempenho da outra), esses dois mecanismos

de complementariedade raramente são distinguidos experimentalmente (mas veja Cardinale et al. 2002), o que impede generalizações entre organismos e sistemas.

Neste contexto, há evidência de que os efeitos de complementariedade são superiores quando espécies de detritívoros funcionalmente distintas estão presentes na comunidade, isto é, quanto a diversidade funcional é maior (e.g., Heemsbergen et al. 2004, Ohta et al. 2016). Deste modo, espécies com as características mais divergentes relevantes para o processo estudado (e.g., modo de alimentação, uso do habitat, mobilidade ou comportamento) têm uma probabilidade maior de diferir no uso do recurso, e então, competir menos e/ou beneficiar-se mutuamente de sua atividade (Petchey & Gaston 2006). Em consequência, um desafio é derivar predições e variáveis facilmente mensuráveis que adequadamente descrevem os efeitos da diversidade e da interação de espécies (e.g., Berlow et al. 2009, Séguin et al. 2014). Nesta tese exploramos o potencial do tamanho corporal (ou biomassa corporal, utilizados aqui como sinônimos) como uma característica chave por trás dos efeitos da diversidade na decomposição. O tamanho corporal engloba inúmeras características das espécies que são relevantes para um contexto populacional (e.g., taxas de ingestão e taxas de metabolismo relativas à massa), de comunidades (e.g., níveis tróficos e interações entre as espécies como predação e competição) e de ecossistemas (e.g., produção secundária e decomposição) (Woodward et al. 2005). Ainda, o tamanho do corpo pode informar sobre o risco potencial de extinção das espécies, uma vez que organismos maiores tendem a sofrer um risco de extinção superior (Duffy 2003).

OBJETIVO & ESTRUTURA DA TESE

Nesta tese exploramos os padrões e mecanismos da dinâmica de detritos vegetais (aportes, estoque e decomposição) em ecossistemas de riachos florestados tanto com

experimentos de campo (*Capítulos I & II*) e de microcosmos (*Capítulos III & IV*), quanto em escalas temporais curtas (semanas) à longas (anos). Deste modo, asseguramos diferentes níveis de realidade e de manipulação que facilitam, respectivamente, a generalização dos resultados e a determinação das relações causais.

No *Capítulo I* exploramos os padrões dos aportes e estoque de detritos em riachos, ao longo de um ano, entre três biomas tropicais no Brasil utilizando múltiplos locais de coleta e uma rede de colaboradores (AquaRipária). Como a vazão é uma variável chave para muitos processos em riachos, no *Capítulo II* investigamos o papel relativo do transporte e da decomposição na mediação do fluxo de detritos (aportes e exportação), e conseqüentemente, na disponibilidade de detritos para as cadeias alimentares de riachos, com base em uma escala de trecho durante dois anos em riachos do Cerrado brasileiro.

Uma vez que a decomposição é severamente afetada pela perda de diversidade tanto de detritos foliares como de detritívoros, no *Capítulo III* simulamos experimentalmente inúmeros cenários de perda de diversidade de detritos – tanto na riqueza de espécies quanto de tipos funcionais (*e.g.*, estratégias de aquisição de N, isto é, espécies fixadoras *versus* não-fixadoras de N) – e testamos suas repercussões na decomposição microbiana e por detritívoros, e se as respostas dependem do contexto ambiental (*e.g.*, concentração de nitrogênio dissolvido na água). No *Capítulo IV* exploramos experimentalmente o papel do tamanho corporal dos detritívoros e de interações interespecíficas na mediação dos efeitos da diversidade na decomposição. Finalmente, sintetizamos nossos achados mais importantes e suas implicações e, pontuamos perspectivas e desafios para estudos futuros.

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CAPÍTULO I

Plant litter dynamics in the forest-stream interface: precipitation is a major control across tropical biomes

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ABSTRACT

Riparian plant litter is a major energy source for forested streams across the world and its decomposition has repercussions on nutrient cycling, food webs and ecosystem functioning. However, we know little about plant litter dynamics in tropical streams, even if the tropics occupy 40% of the Earth's land surface. Here we investigated spatial and temporal (along a year cycle) patterns of litter inputs and storage in multiple streams of three tropical biomes in Brazil (Atlantic forest, Amazon forest and Cerrado savanna), predicting major differences among biomes in relation to temperature and precipitation regimes. Precipitation explained most of litter inputs and storage, which were generally higher in more humid biomes (litterfall: 384, 422 and 308 g m⁻² y⁻¹, storage: 55, 113 and 38 g m⁻², on average in Atlantic forest, Amazon and Cerrado, respectively). Temporal dynamics varied across biomes in relation to precipitation and temperature, with uniform litter inputs but seasonal storage in Atlantic forest streams, seasonal inputs in Amazon and Cerrado streams, and aseasonal storage in Amazon streams. Our findings suggest that litter dynamics vary greatly within the tropics, but point to the major role of precipitation, which contrasts with the main influence of temperature in temperate areas.

Key-words: litterfall, particulate organic matter, benthic storage, leaf litter, ecosystem functioning, riparian forest, Cerrado, Atlantic forest, Amazon, litter decomposition.

INTRODUCTION

Freshwater ecosystems are widely spread across terrestrial landscapes and receive large amounts of litter from riparian vegetation (Fisher & Likens 1973). In particular, rivers and streams receive, transport and store approximately 2.1 Pg of terrestrial organic carbon each year, which represents a considerable fraction of the overall net ecosystem production of terrestrial ecosystems (Raymond et al. 2013). Despite their small spatial extent, headwater streams significantly contribute to organic matter processing due to their high retentive capacity, constant water flow and high nutrient availability (Wipfli et al. 2007, Battin et al. 2008). Organic material – mostly leaf litter – enters streams through two routes (Webster & Meyer), directly by vertical litterfall (hereafter litterfall), or laterally from the forest soil (hereafter lateral inputs), and can be transported downstream by water flow or retained in depositional habitats or structures such as boulders or logs. The retained litter represents an important energy source for stream food webs (Wallace et al. 1997, Neres-Lima et al. 2017), and its subsequent decomposition contributes significantly to the global carbon cycle (Battin et al. 2009). Thus, quantifying the magnitude and timing of litter inputs and storage in headwater streams seems a major step towards understanding the functioning of ecosystems and the cycling of organic matter globally.

Organic matter inputs and storage in temperate and boreal forest streams have been studied for decades, especially in Europe and North America (Fisher & Likens 1973, Fisher 1977, Benfield 1997, Pozo et al. 1997b), where the timing and the magnitude of these processes are well known. In contrast, comparable studies in tropical streams are scarce, so most basic questions about natural variation of litter inputs and storage within the tropics remain unknown. For example, are there similarities in the timing of litter inputs to the stream within and across tropical biomes? In which periods of the year most litter enters and is accumulated in streams?

The few existing assessments of organic matter inputs and storage in tropical streams have mostly been restricted to single streams (Benson & Pearson 1993, Gonçalves et al. 2006, França et al. 2009) or a single region (Colón-Gaud et al. 2008, Bambi et al. 2016), which limits the identification of spatial and temporal patterns of variation and their main controls at larger scales (Heffernan et al. 2014a). Also, ignoring the natural variation of litter inputs and storage in the tropics may limit the understanding of key ecosystem processes such as litter decomposition and secondary production (Neres-Lima et al. 2017), challenging the development of an integrated view of tropical stream ecosystems.

Litterfall has been widely used by terrestrial ecologists as a good estimator of plant productivity (i.e., annual net primary productivity), and it is generally positively influenced by temperature, precipitation and soil fertility (Chapin III et al. 2011, Wright et al. 2011, Zhang et al. 2014). However, in tropical forests, litterfall annual variability seems to depend mainly on precipitation and solar radiation, with litterfall peaks corresponding to the dry season, which contrasts with most temperate forests, where litter peaks occur in autumn and are predicted by temperature and solar radiation (Zhang et al. 2014). Lateral litter inputs tend to be less predictable than litterfall, as they depend on multiple factors such as litter accumulation in forest soils, the slope of stream banks, litter humidity – (as dry litter is more vulnerable to be transported by the wind; e.g., Shibata et al. 2001) – and physical processes such as overland flow and wind that may enhance litter transport into the stream (Orndorff & Lang 1981, France 1995a). Litter storage in the stream depends on both litterfall and lateral inputs, and is mainly determined by water flow conditions (that is, low-flow streams have lower shearing stress; e.g., Hoover et al. 2006, Quinn et al. 2007), the stream retention capacity (shallow streams have more

retentive structures), which together determine the downstream transport (Pozo & Elosegí 2005) and, the rate at which litter is decomposed that acts as a longer-term control (Pozo 2005).

The complexity of biological and environmental interactions involved in litter dynamics and the lack of basic information have precluded robust tests of which factors control litter inputs and storage in tropical streams. Here we addressed this issue in a multi-site field study across three biomes in Brazil (Atlantic forest, Amazon forest and Cerrado savanna) encompassing 30° of latitude (28°S - 2°N). We aimed to explore the patterns of litter inputs (divided into two routes: litterfall and lateral inputs) and storage in streams across multiple spatial scales (from within stream to among biomes), as well as temporal dynamics within an annual cycle, and to identify which environmental and biological factors are the main influences on these processes. For that purpose we tested the following hypotheses (Fig. 1): (i) spatial patterns of litterfall would mainly depend on plant productivity (which in turn depends on climatic and soil factors), while its temporal dynamics would mainly depend on plant phenology (in turn related to climate) (Fig. 2); (ii) spatial patterns and temporal dynamics in lateral litter inputs would result from the combined effect of multiple environmental factors (including climatic and other factors) and of litterfall (Fig. 2); (iii) litter storage would vary spatially depending on litter inputs and stream channel characteristics (e.g., retention structures) while its temporal dynamics would be greatly influenced by precipitation (Fig. 2); and (iv) the greatest spatial variance of all these processes would occur among biomes, in relation to climatic and geologic variation, with less variance at smaller scales.

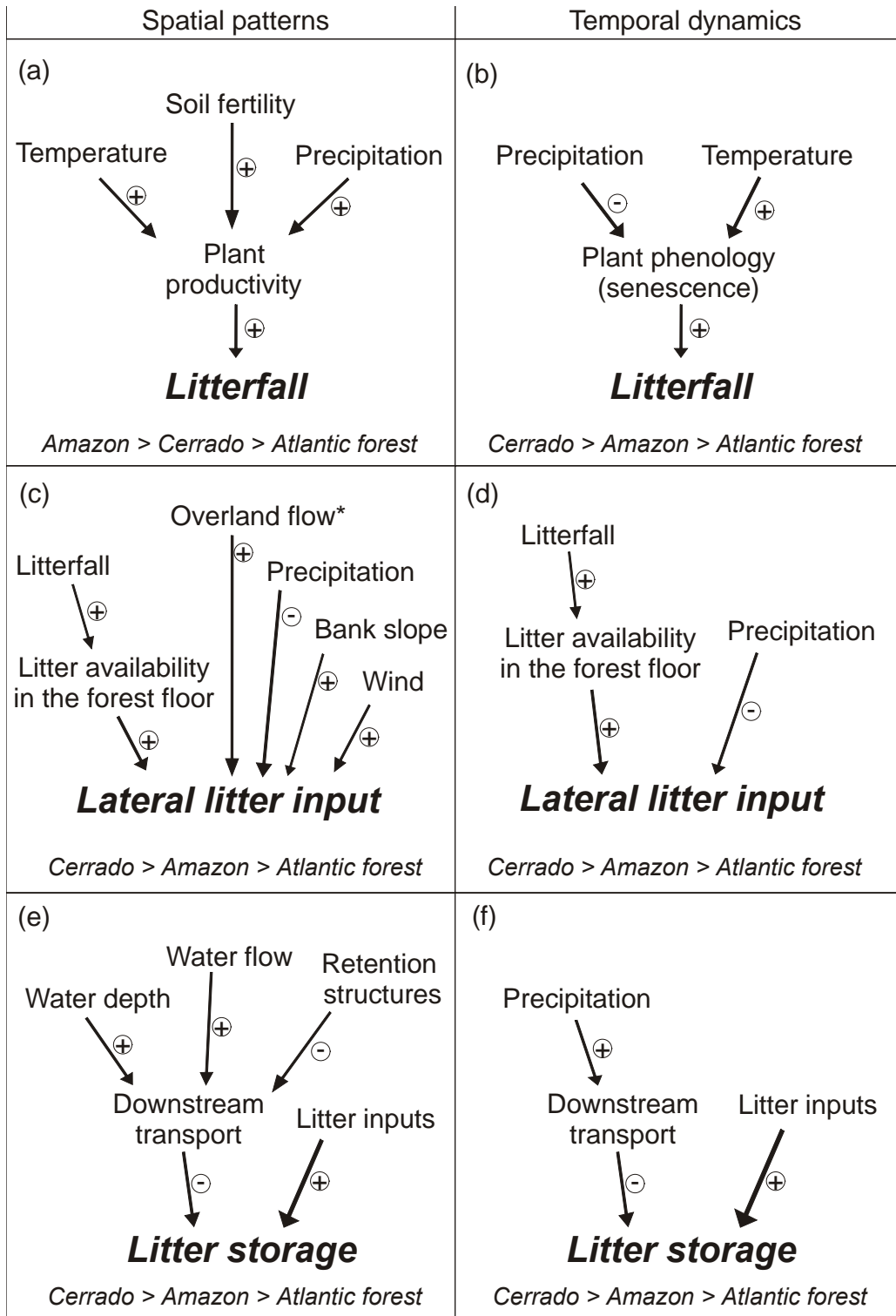


Figure 1. Expected predictors of spatial patterns (a, c, e) and temporal dynamics (b, d, f) of litterfall (a, b), lateral inputs (c, d) and benthic storage (e, f). Plus and minus signs near arrows indicate the direction of effects (positive or negative, respectively). The expectation for the spatial patterns and temporal dynamics of each process is indicated below each process.

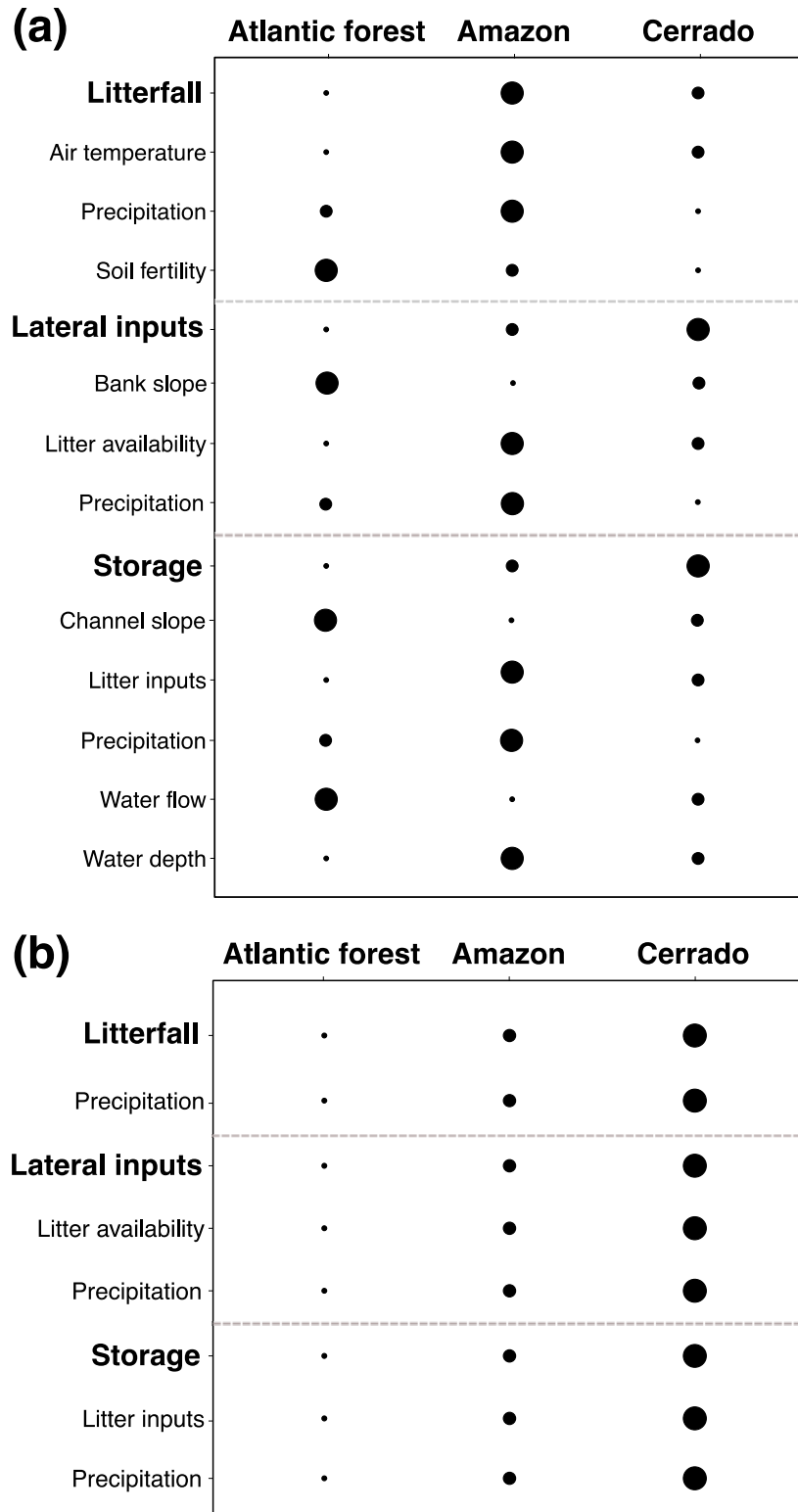


Figure 2. Expected predictors of litterfall, lateral inputs and storage in Atlantic forest, Amazon forest and Cerrado savanna biomes. Circles of different size indicate effects of different magnitude (small, medium and large) for the spatial patterns (a) and temporal dynamics (b) of each process.

METHODS

Study sites

Our study was conducted in 13 streams located in 3 biomes in Brazil: the subtropical Atlantic forest (3 streams), the Amazon tropical forest (3) and the Cerrado tropical savanna (7). Study sites were located at latitudes ranging from 2°N to 28°S (Fig. 3, Table S1). We selected 1st–3rd order streams < 5 m wide and < 50 cm deep (estimated at low flow conditions), with dense riparian canopy (> 70%), in watersheds with no apparent anthropogenic impacts. The riparian forests in all three biomes were highly species diverse, containing deciduous, semi-deciduous and evergreen species (> 50 – 122 species in Atlantic forest, > 50 – 62 in Amazon and 29 – 112 in Cerrado; Table S2). Atlantic forest streams were located in the interior (2 streams) and coast (1) areas of Brazil; the climate is subtropical with frequent precipitation and no dry season; vegetation is mainly composed of Araucaria rainforest and semi-deciduous forest. Cerrado savanna streams drain through dense corridors of evergreen forest known as gallery forest (Mirmanto et al. 1999) and experience a tropical seasonal climate with a dry season from May through September that coincides with the coldest months of the year. The Amazon biome encompasses the largest tropical rainforest in the world; our streams drained non-flooded (*terra firme*) forests located in the central (2 streams) and northern Amazon (1); the climate is tropical humid, with central Amazon sites characterized by a rainy season from December through May and a modest dry season from June through November, and northern Amazon sites with a rainy season from April to September and a pronounced dry season from October to March.

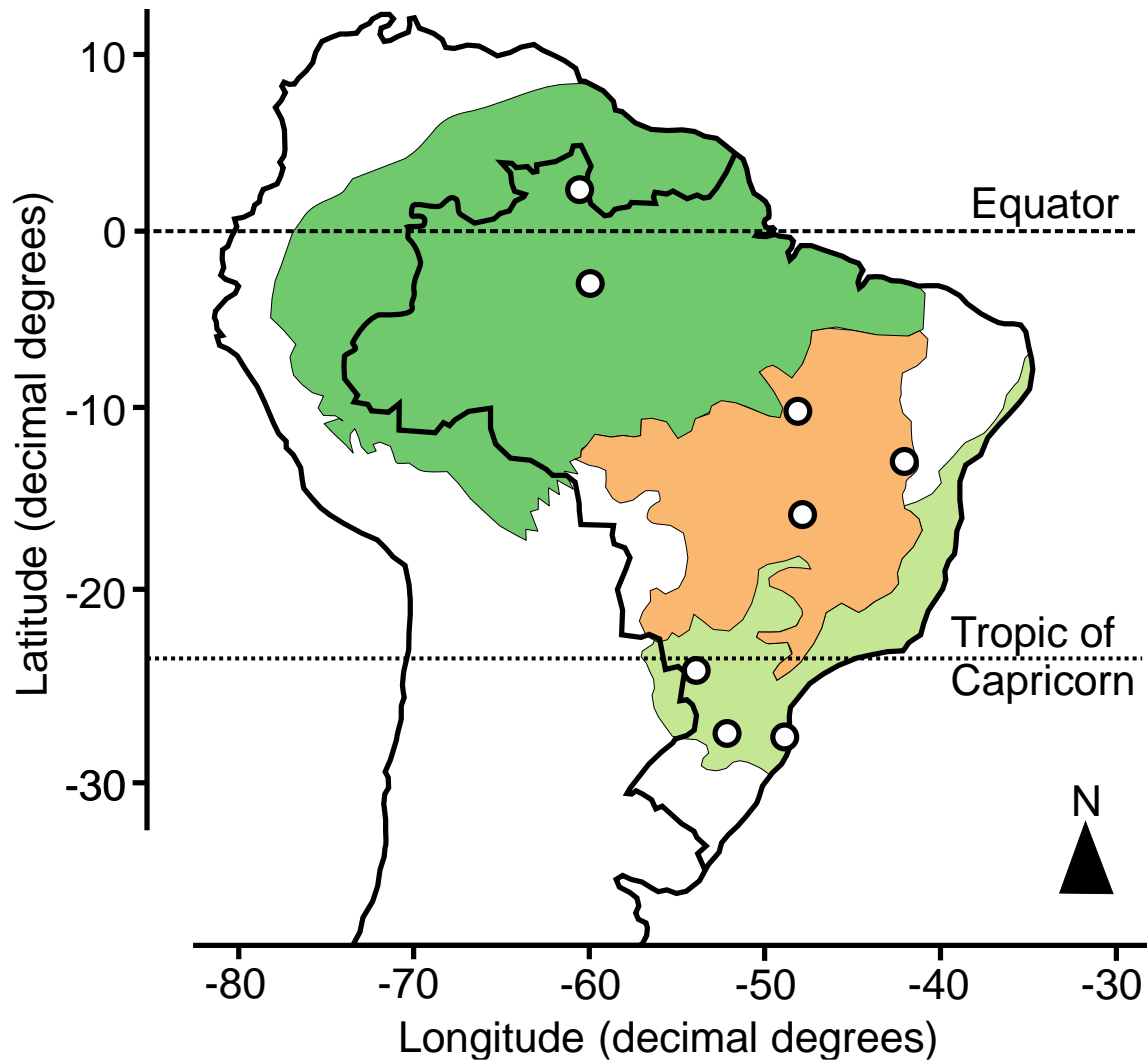


Figure 3. Location of study sites in Atlantic Forest (light green area), Cerrado savanna (orange area) and Amazon forest (dark green area) biomes. This figure was generated using ‘ggmap’ package (<http://journal.r-project.org/archive/2013-1/kahle-wickhampdf>) in R (version 3.2.2; <https://www.R-project.org/>).

Experimental design and procedure

In each stream, we conducted the experiment at 5 equally distanced sampling sites within a 50–100 m long reach. Litterfall and lateral litter inputs were estimated using suspended and lateral traps, respectively. Suspended traps consisted of 90 plastic buckets (18 per site) placed 2 m above the streambed, with a 26-cm diameter and small holes on the bottom to allow water to drain; their total sampling area was 4.75 m². Lateral collectors consisted of 20 traps (4 per site)

of 50x25x50 cm and made of 1-mm mesh; they were distributed along the stream bank and fixed to the soil. Additionally, we estimated litterfall to the riparian forest floor with 10 suspended nets (2 per site) of 1-m² area and 1-mm mesh. Benthic litter storage was estimated with 15 Surber samples (3 per site taken randomly, including pool and riffle areas) of 0.10 m² and 250- μ m mesh that were further sieved through a 1-mm mesh.

Samples were collected once a month for a year (Fig. S1). They were transported to the laboratory, oven dried and sorted into four categories: leaf litter, twigs, reproductive parts (fruits, flowers and seeds) and unidentified parts. However, we mostly focused on leaf litter (henceforth “litter”) in further analyses because it represented the majority of total particulate organic inputs (>60% of dry mass [DM]; SI 2), while the other fractions were absent in many sites and showed large variance across replicates and over time. Monthly litterfall and lateral inputs were estimated as litter DM per m² per year at each sampling site. Storage was estimated as litter DM per m² on each occasion.

At each site we estimated a set of variables related to spatial patterns of litterfall, lateral inputs and storage: stream and bank slope (with a clinometer), and water depth and width (cross sections with 5 depth measures each). We calculated the coefficient of variation (CV) of the width/depth ratio of each site as a measure of channel heterogeneity (as an indicator of stream retentiveness). For each of these variables, we used the 5 values from the different sites to calculate a mean value per stream. Additionally, we extracted temperature and precipitation data for each stream from the WorldClim database v.1.3 (Hijmans et al. 2005) at the highest resolution (2.5 min of arc) using DIVA-GIS software, 7.5.0.0 (<http://www.diva-gis.org>), and wind frequency from the National Institute of Meteorology of Brazil (Automatic Stations from <http://www.inmet.gov.br>). We used the average of minimum and maximum temperatures for

each month to calculate monthly mean temperature, which was used for temporal analyses. For spatial analyses, we used the following climatic predictors: mean annual precipitation (MAP), mean annual temperature (MAT), precipitation of the driest month (PDM, as an indicator of the presence of dry periods) and wind frequency.

Data analysis

Spatial Models

We explored the relationships between litterfall, lateral inputs, storage and their environmental predictors with linear models, after averaging monthly measurements and site data within a stream. Litterfall predictors included MAP and MAT; lateral input predictors were litterfall to the forest (as a surrogate of fresh litter availability in forest soils), wind frequency, PDM and bank slope; and storage predictors were MAP, litter inputs (sum of litterfall and lateral inputs), stream slope, water depth and channel heterogeneity. We first used the variance inflation factor and a cut-off value of 3 to remove collinear explanatory variables³². Next, we selected the best models by removing any non-significant variables and assessing model improvements based on the Akaike Information Criterion (AIC) (Table S2). Models were fitted using the ‘stats’ package and plots were drawn with the ‘ggplot2’ package (Wickham 2016) (and in association with ‘ggmap’ package in the case of Fig 6) in R (R Core Team 2015); version 3.2.2.

Temporal Models

We examined temporal dynamics of litterfall, lateral inputs and storage, as well as the effects of environmental factors, with additive mixed models (GAMM) using a normal distribution and the identity-link function (Wood 2006, Zuur et al. 2009). We used this type of model instead of a

linear model because scatterplots of litter inputs and storage (on the y-axis) for each biome, with the covariates (time, precipitation, temperature and litterfall to the forest) on the x-axis, showed clear non-linear patterns (Zuur et al. 2009, Ieno & Zuur 2015). Importantly, additive models (also called smoothing models) allow for non-linear relationships between the response variable and multiple explanatory variables, in contrast to linear models (Hastie & Tibshirani 1990). Also, the amount of smoothing in an additive model is expressed as effective degrees of freedom (edf) for a smoother. Thus, the higher the edf, the lower the linearity of a curve (Zuur et al. 2009). Initial data exploration using Cleveland dot- and boxplots revealed outliers in the storage data, which required square-root transformation prior to analysis. Examination of multi-panel scatterplots indicated contrasting patterns of litterfall within the Amazon biome, so this biome was separated into central and northern Amazon, but only for litterfall comparisons. All models were fitted using the ‘mgcv’ (Wood 2011) and ‘nlme’ (Pinheiro et al. 2015) packages in R.

We firstly fitted a model to describe temporal patterns for each response variable (litterfall, lateral inputs and storage) that excluded the environmental factors. The explanatory variables in this model were biome (Atlantic forest, Amazon or Cerrado), time (number of the month within a year; continuous variable) and the interaction between biome (categorical) and time (fitted as a smoother). Secondly, we fitted a model that included the environmental covariates. For litterfall, the explanatory variables were precipitation (as a surrogate for flow; smoother), temperature (continuous variable) and the interaction between precipitation and biome. The lateral input model was first fitted using an additive mixed model, with precipitation and litterfall to the forest as smoothers. However, effective degrees of freedom for these smoothers were 1, indicating a linear effect, so a linear mixed model was more appropriate. Explanatory variables for lateral inputs were precipitation (continuous variable), litterfall to

forest (continuous variable) and their interaction with biome. For litter storage, the explanatory variables were precipitation, litterfall to the stream and their interaction with biome (see full models in SI 2). The interactions in additive mixed models were fitted using the ‘by’ command in the ‘mgcv’ package in R. Cross-validation was used to estimate the optimal amount of smoothing (Wood 2006).

We extracted variance components and standard deviations of litterfall, lateral inputs and storage for each hierarchical scale: biomes, streams nested within biomes (hereafter ‘across streams’) and sites nested within streams (hereafter ‘within streams’) using the ‘VarCorr’ function in linear mixed effects models. Biome was treated as a random factor purely to allow comparison with other components (Logan 2011).

RESULTS

Litterfall

Litterfall was 20% higher in Atlantic forest and 40% higher in Amazon than in Cerrado, but similar between Atlantic forest and Amazon (mean \pm SE in Amazon, Atlantic forest and Cerrado, respectively: 384 ± 43 , 422 ± 20 and 308 ± 22 g leaf dry mass m^{-2} year $^{-1}$; Table S3; Fig. S3). Litterfall accounted for $72 \pm 13\%$ in Atlantic forest, $72 \pm 1\%$ in Amazon and $59 \pm 7\%$ of total litter inputs in Cerrado. Although spatial patterns of litterfall were not significantly related to mean annual temperature (MAT) or mean annual precipitation (MAP), litterfall weakly increased with MAP ($F_{1,13} = 3.03$, $P = 0.109$; Fig. 4a), which explained 22% of its variance. A similar but stronger relationship between MAP and all plant components of litterfall (i.e. sum of leaves, twigs and reproductive parts; $F_{1,13} = 5.36$, $P = 0.041$) explained 33% of the variance (Fig. 4b).

Litterfall variance was highest among biomes (30% of total variance), followed by across streams (23%), and lastly, within streams (11%; Table S4).

Temporal patterns of litterfall were consistently different among biomes, with lower variability over a year in Atlantic forest, intermediate in Amazon and higher in Cerrado (i.e., the higher degree of freedom of additive mixed model, the higher seasonality; Fig. 5): litterfall was constant throughout the year in Atlantic forest; peaked in June, July and August in central Amazon; between October to January in northern Amazon; and in July, August and September in Cerrado. Precipitation and temperature were important predictors of litterfall temporal dynamics, although effects were distinct among biomes: there was no relationship for Atlantic forest, a negative linear relationship between precipitation and litterfall for Amazon (both central and northern areas analyzed together) and a negative exponential relationship for Cerrado (Fig. 6a, b). In contrast, there was no relationship between temperature and litterfall for Atlantic forest, but a positive linear relationship for Amazon and a positive non-linear relationship for Cerrado (Fig. 6a, b).

Lateral inputs

Lateral inputs were similar among Atlantic forest, Amazon and Cerrado (131 ± 25 , 165 ± 7 and 213 ± 27 g leaf dry mass $\text{m}^{-2} \text{year}^{-1}$; Table S3; Fig. S3). The contribution of lateral inputs to total litter inputs was $28 \pm 13\%$ for Atlantic forest, $28 \pm 1\%$ for Amazon and $41 \pm 7\%$ for Cerrado. Lateral inputs decreased as a function of precipitation in the driest month, and increased with the amount of total litterfall in the forest ($F_{2,6} = 8.70$; $P = 0.017$; ; Fig. 4c, d). These two predictors of spatial patterns of lateral inputs explained 66% of its variance. Lateral input variance was higher

across streams (9%) than within streams (5%) or among biomes (<0.001%), although residual variance had the largest contribution (86%; Table S4).

Lateral inputs were more constant over a year in Atlantic forest, and more variable in Amazon and Cerrado (Fig. 4): increased from April (autumn) to December (late spring and early summer) in Atlantic forest; showed a bimodal trend with similar peaks in June and October–November in Amazon; and showed a bimodal trend in Cerrado but with a smaller peak in March (rainy season) and a larger one in October (beginning of rainy season and after litterfall peaks; Fig. 5). Precipitation and litterfall to the forest predicted lateral inputs temporal dynamics, but significant interactions between precipitation and biome, and litterfall to forest and biome indicated significant positive relationships only for Cerrado (Fig. 6c, d).

Storage

Litter storage was, on average, two times higher in Amazon than in Atlantic forest and three times higher than in Cerrado, but was similar between Atlantic forest and Cerrado (113 ± 1 , 55 ± 5 and 38 ± 12 g leaf dry mass m^{-2} ; Table S3; Fig. S3). Storage increased as a function of MAP and stream depth, which explained 52% of its spatial pattern ($F_{2,8} = 6.50$; $P = 0.021$; Fig. 4e, f). Storage variance was higher among biomes than across or within streams (6% and <0.001%), but residual variance had the largest contribution (56%; Table S4).

Temporal dynamics of storage over the year was consistently distinct among biomes, with higher variability over a year in Atlantic forest and Cerrado and lower in Amazon (Fig. 5): storage showed a bimodal trend for Atlantic forest streams, with peaks in summer (beginning of the year) and winter (July to September); a peak from July to December in Amazon; and an evident peak from July to September (which correspond to the dry season) in Cerrado (Fig.5).

Precipitation and litter inputs were important predictors of temporal dynamics of storage, although effects were distinct among biomes: there was a negative linear relationship between precipitation and storage only for Cerrado streams, and positive relationships between litter input and storage for Atlantic forest (linear) and Cerrado (non-linear; Fig. 6e, f).

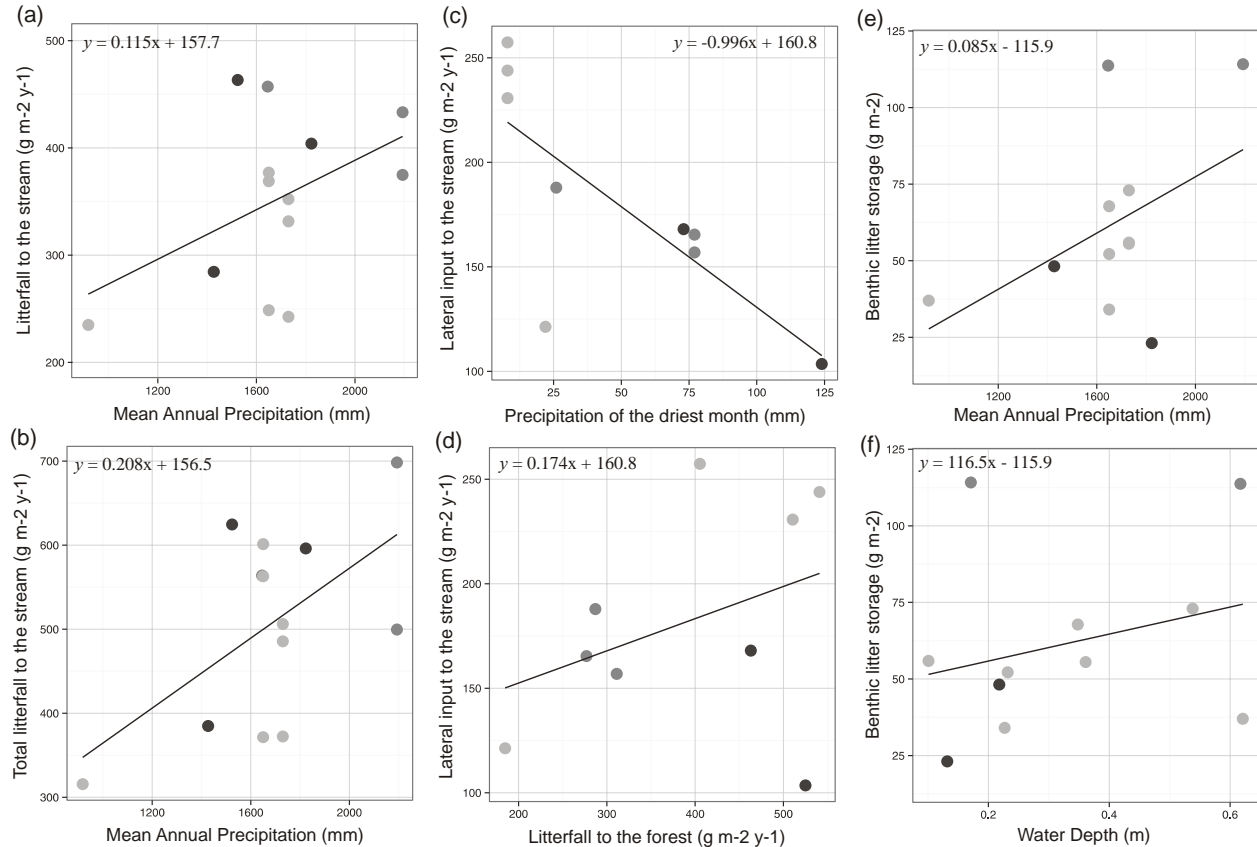


Figure 4. Relationships between litter inputs, benthic storage and their predictors in Atlantic forest (black circles), Amazon (dark grey circles) and Cerrado streams (light grey circles): (a) litterfall vs. mean annual precipitation (MAP); (b) total litterfall vs. MAP; (c) lateral inputs vs. precipitation of the driest month (PDM); (d) lateral inputs vs. litterfall to the forest; (e) storage vs. MAP; and (f) storage vs. water depth. Litter inputs are in g per m² per year and storage in g per m².

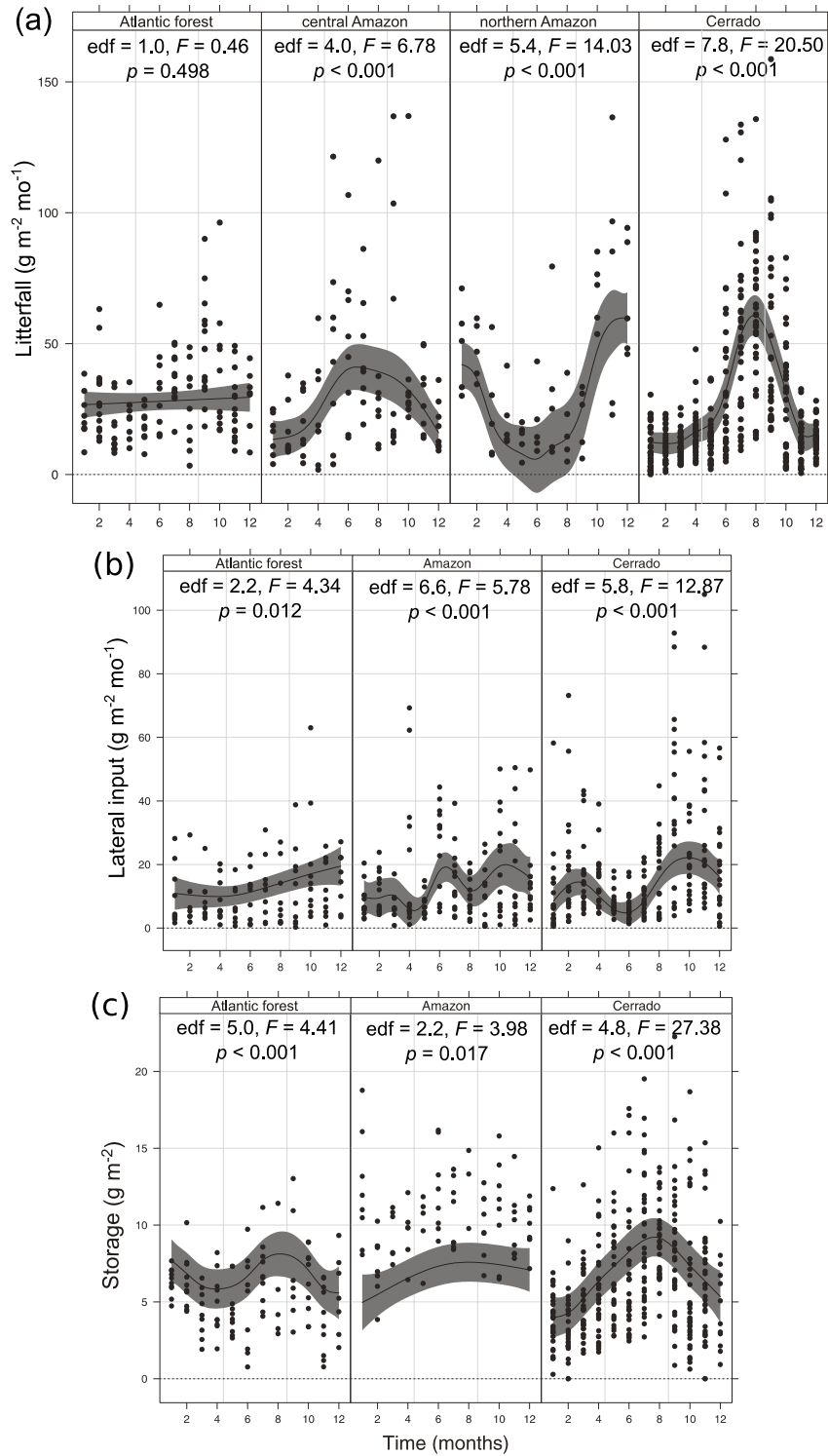


Figure 5. Temporal dynamics of litterfall (a), lateral inputs (b) and benthic storage (c, square-root transformed) over a year in each biome (Atlantic forest, Amazon and Cerrado). Black lines represent the smoothers of litterfall, lateral inputs and storage, and grey areas the 95% confidence intervals from models M_{ILf} , M_{ILi} and M_{ISb} , respectively (Supplementary Information 2). Litter inputs are in g per m² per month and storage in g per m².

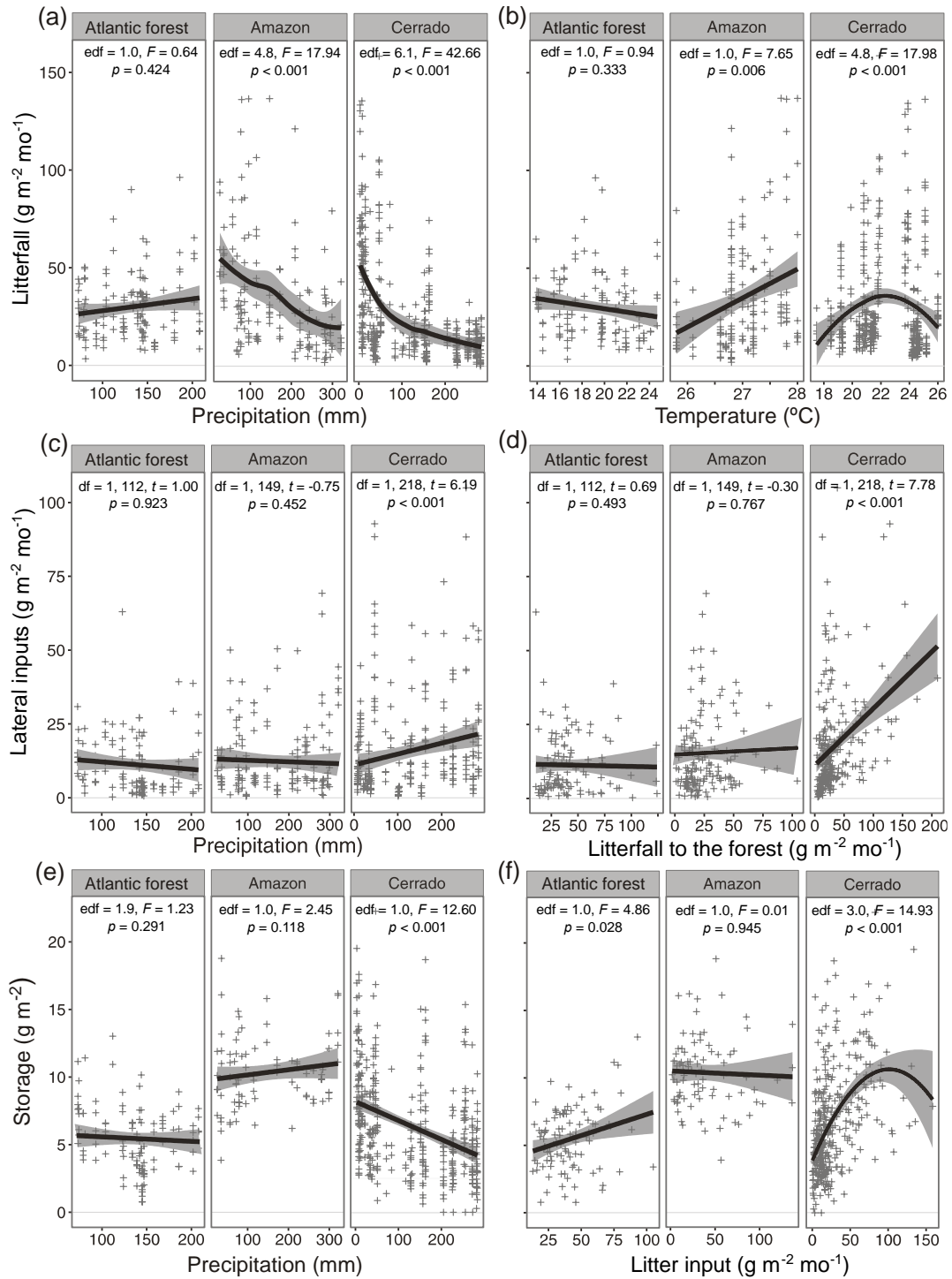


Figure 6. Relationship between litter inputs (g per m² per month), storage (g per m²) and their temporal predictors in Atlantic forest, Amazon and Cerrado streams: (a) litterfall vs. precipitation; (b) litterfall vs. temperature; (c) lateral inputs vs. precipitation; (d) lateral inputs vs. litterfall to the forest; (e) storage vs. precipitation; and (f) storage vs. litter inputs. Black lines represent the smoothers of litterfall, lateral inputs and storage, and grey areas the 95% confidence intervals from models M_{2Lf} , M_{2Li} and M_{2St} , respectively.

DISCUSSION

Higher litterfall at Atlantic forest and Amazon as a result of higher precipitation

Allochthonous sources dominate energy flows in many tropical forested stream food webs (Neres-Lima et al. 2017) as it occurs in streams of temperate zones (Wallace et al. 1997). Most of these allochthonous sources are represented by particulate organic matter in the form of leaf litter, which are of fundamental importance for stream food webs and ecosystem functioning (Wallace et al. 1997). However, to date there was no comprehensive study addressing how litter dynamics varies within the tropics or determining which are its environmental controls. Our study show how litter inputs and storage in tropical streams vary at multiple spatial scales within the tropics and which factors influence such variability, using a large-scale study involving streams across three tropical biomes.

We found that litterfall was higher in Amazon and Atlantic forest than in Cerrado and was positively related to precipitation, but not to temperature, partially supporting our prediction (Figs. 1, 2). These results contrast with those of another study (Chave et al. 2010), which found no relationship between precipitation and annual litterfall in 81 South American tropical sites; however, 77 of those sites were in Amazon or Panamanian rainforests and none in Cerrado savanna, which occupies a large region in the center of South America (Cardoso Da Silva & Bates 2002). It is thus likely that the spatial extent of our study (3 biomes and 30° of latitude) comprised a larger climatic gradient and also more varied forest types. Also, our findings indicated some similarities between tropical and temperate climates: temperate streams flowing through drier forests and with more seasonal precipitation regime (e.g., the Mediterranean biome) showed lower litter inputs than streams in Atlantic temperate forests, which have a more humid climate and more constant precipitation through the year (Sabater et al. 2008). The lack of

a relationship between temperature and litterfall was unexpected, given the strong control that this climatic factor exerts on plant productivity globally (Vitousek 1984). Conversely, a pan-tropical analysis of net primary productivity – which is correlated with litterfall – found that temperature was the most important factor driving differences among tropical forest types (Cleveland et al. 2011). The lack of a temperature effect in our study could be related to the distinct characteristics of the riparian forest compared to other types of forest. It is possible that riparian soil fertility played an important role in determining litterfall, as shown elsewhere (Mirmanto et al. 1999, Adamek et al. 2009, Wright et al. 2011), causing the differences observed among biomes. For example, the lowest litterfall production that we recorded, in riparian forests of Cerrado, may have been the result of its nutrient-poorer soils (Eiten 1972, Paiva et al. 2015)

Precipitation and temperature influence temporal dynamics of litterfall in Amazon and Cerrado

The negative relationship between litterfall and precipitation for Amazon and Cerrado indicate that precipitation is a limiting factor for litterfall regulation, supporting our prediction (Fig. 1, 2) and suggesting that litterfall helps plants reduce water stress during the driest periods (Reich & Borchert 1984, Reich 1995). Higher litterfall in the driest months has been previously reported for riparian forests of Cerrado (Gonçalves et al. 2006, Rezende et al. 2016), in the Mediterranean climate (Gasith & Resh 1999), and for tropical forests worldwide (Zhang et al. 2014), which contrast to the higher litterfall in autumn in temperate deciduous forests (Abelho 2001). However, our study provides further evidence that this occurs in riparian forests of different tropical biomes and extends our understanding in important ways. Firstly, we found consistent evidence of litterfall seasonality in Amazon and Cerrado, and uniform litterfall rates over the year in Atlantic forest. These findings contradict the widespread perception of aseasonal litterfall

in tropical riparian forests (mostly when climate is relatively constant year around; Wantzen et al. 2008) and evidence for different timing of litter inputs in different tropical riparian forests. Secondly, stronger litterfall seasonality in Cerrado and moderate in Amazon (both in central and northern areas) suggest important repercussions for litter decomposition and nutrient recycling in streams and riparian forests, as well as for aquatic and terrestrial food webs. This is due to the fact that leaf litter will not be supplied at same rates over the year, leading to probable reductions in litter quantity and changes in litter quality (i.e., chemical composition of stored litter in pools or soils due to biological or physical processes).

Also importantly, the uniform litterfall rates over the year observed in Atlantic forest may be the result of a mixture of subtropical Atlantic forest types (e.g., rain forests, Araucaria forest and semi-deciduous forest), which represents a mosaic of evergreen, semi-deciduous and deciduous trees (Oliveira-Filho et al. 2013) that may sustained ‘constant’ litterfall rates over the year. Additionally, as the Atlantic forest biome is comprised by heterogeneous forest vegetation subtypes (e.g., rain, cloud, moist and dry forests in the coast and the interior areas) and our Atlantic forest sites were restricted to the southern portions of the Atlantic forest domain (mainly moist forests both in the coast and continental areas) our results for this biome should be interpreted with caution, mostly for different forest subtypes. The positive relationship between litterfall and temperature for Cerrado and Amazon indicates that temperature may also play an important role on litterfall, as shown in other studies (Williams et al. 1997, Parsons et al. 2014). Temperature increases evapotranspiration rates, which may lead to temporary water deficits that accelerate the abscission of senescent leaves (Reich & Borchert 1984). Previous studies also suggested that light availability (e.g. solar radiation and day length) determines seasonal patterns in litterfall in tropical wet forests (Wright 1996, Angulo-Sandoval & Aide 2000), because falling

of mature leaves coincides with the appearance of new leaves during periods of higher radiation (Zalamea & González 2008). However, it is unlikely that light availability explains our seasonal pattern of litterfall in Cerrado, because periods of greatest day length occurred in different months or seasons at each site (INMET 2014); or the aseasonal pattern in Atlantic forest, where there was higher light availability during the summer (cf. Morellato et al. 2000, INMET 2014).

Higher lateral inputs in more productive and drier riparian forests

In contrast to direct litterfall, litter coming from riparian soils may have undergone some degree of decomposition by physical or biological processes (depending on the time since litterfall) and may thus provide a different resource for stream food webs, because of leaching of labile compounds and microbial conditioning (Bruder et al. 2011). Thus, understanding the timing and magnitude of litter inputs from riparian soils represents an important step for future experimental or manipulative studies aiming to address their influence on stream ecosystem processes (e.g., litter decomposition, ecosystem metabolism and secondary production).

We found similar lateral inputs among Atlantic forest, Amazon and Cerrado streams, which did not support our prediction (Figs. 1, 2). However, as expected, we observed a positive relationship of lateral inputs with litterfall to the forest and a negative relationship with precipitation of the driest month. These findings suggest that higher lateral inputs occur in more productive riparian forests, because a higher amount of litter is available in riparian soils and is susceptible of reaching streams; and where drought periods are more intense and/or frequent, because dry litter is more easily transported (Shibata et al. 2001, Hart et al. 2013, Lisboa et al. 2015), although we found no relationship with wind frequency and bank slope. These discrepancies might be the result of interactions between wind, riparian density, ground

complexity (i.e. plants, roots, dead trunks, rocks, etc) and litter characteristics, understanding of which may require specific experimental studies. Moreover, as many environmental factors can affect lateral litter transport, it is not surprising that a range of lateral litter contributions have been reported, from negligible amounts to even surpassing litterfall contributions [e.g., in mixed-hardwood forest Fisher (1977), in tropical rainforests Benson & Pearson (1993); in tropical savanna Gonçalves et al. (2006); and in broadleaf forests Kochi et al. (2010)]. These findings are supported by the higher variability of lateral litter inputs observed at smaller scales (86% of total at sampling sites or samplers), which suggest that local factors (e.g., riparian density, ground complexity, stream bank slope and litter characteristics) are more important than regional ones in driving its dynamics. Also, our results provide evidence that ignoring lateral inputs would result in an considerable underestimation of total litter inputs to the stream, which according our data would be of 19–51% of total litter inputs to the stream.

Temporal dynamics of lateral inputs depend on precipitation and soil litter accumulation in Cerrado

Lateral inputs and litterfall to the forest were positively related throughout the year only in Cerrado, indicating that lateral inputs were intensified in the most productive periods in this biome. Interestingly, lateral inputs increased with precipitation in Cerrado, contrary to our prediction, evidencing the higher lateral litter inputs mainly in the beginning of the rainy season. This is likely to occur through the mobilization of litter in the riparian floor by the wind during intense storms, which although sporadic are more common to occur in the dry-rainy transition. In contrast, there was no temporal relationship between lateral inputs and litterfall to the forest or precipitation in Amazon or Atlantic forest, suggesting that litter transport in these biomes is not

intensified by litter accumulation in riparian soils or overland flow, which is expected to be of minor importance on the well drained soils of riparian zones studied. The lack of relationship between lateral inputs and litterfall to the forest is striking and might indicate the lower movement of litter in riparian soils of Amazon and Atlantic forest, probably slowed down by the high humidity in most periods of the year. Previous studies have reported either a positive or no relationship between precipitation and lateral litter transport (Scarsbrook et al. 2001, Selva et al. 2007, Lisboa et al. 2015), reflecting regional patterns and suggesting that direct field measures (e.g., overland flow and wind intensity on the floor base) of putative predictors should provide a better representation of a highly local variable processes such as litter transport in riparian soils.

Litter storage increases with annual precipitation and stream depth

Benthic litter storage is a major energy source for secondary production in forest stream food webs (Wallace et al. 1997, Neres-Lima et al. 2017), influencing nutrient cycles and the export of particulate and dissolved organic carbon (Cross et al. 2005). Benthic litter also helps with channel stability (through reducing bank erosion), increases stream retentiveness (Keller & Swanson 1979) and it is habitat for microorganisms, invertebrates and fishes (Covich et al. 1999). Thus, spatial and temporal dynamics of litter storage potentially have important consequences for all the above processes and organisms.

Our results showed storage to increase with annual precipitation and water depth. Similarly, Jones (1997) found that litter storage was directly related to annual precipitation, suggesting that storage increased as a result of enhanced litter production with precipitation. The positive relationship between storage and water depth was contrary to our predictions but might be related to the higher litter accumulation in pools, which are deeper and in consequence low-

flow habitats that are able to storage large amounts of materials than riffle habitats. The lack of a relationship with litter inputs suggests that annual storage in these streams is primarily driven by their low retention capacity (5 to 19% of litter inputs) and high downstream litter export in relation to litter inputs. This result contrasts with Jones (1997), who demonstrated an increase of litter storage with inputs in North American streams, but is in accordance to another study in Neotropical streams where low storage ($\sim 10\%$ of total inputs; $13 - 153 \text{ g leaf dry mass m}^{-2}$) was also reported despite high litter inputs ($590 - 918 \text{ g leaf dry mass m}^{-2} \text{ y}^{-1}$) (Colón-Gaud et al. 2008). In our study, storage was up to 3 times higher in Amazon than in other biomes, which is surprising because Amazon streams had sand substrates, which generally show lower retention than cobble-dominated streams (Jones 1997). Also, the high variance (ca. 40%) of litter storage among biomes and its relation with annual precipitation suggest that a considerable proportion of storage dynamics was resulted by regional processes that could directly influence litter retention and export (e.g. precipitation regime and hydrology). Taken together, these results suggest that spatial pattern in litter storage is partly due to biome type, despite large unexplained variance.

Temporal dynamics of litter storage are driven by precipitation and litter inputs

We observed distinct temporal patterns of litter storage among biomes, which were driven by precipitation and litter inputs in Cerrado and inputs in Atlantic forest, supporting our prediction. This indicates that temporal patterns of in-stream storage in Cerrado are more predictable, given that higher inputs coincide with base-flow conditions (during the dry season). Also, temporal storage patterns of Cerrado demonstrated a massive accumulation of benthic litter until the rainy season starts, when the beginning of rainy season flushed out the system most of benthic litter to downstream, banks or hyporheic zone. Notably, most of the removed litter might be in the initial

stages of decomposition, given the low decomposition rates reported for Cerrado streams [$\sim 20 - 50\%$ mass loss in 75-120 days; cf. Gonçalves et al. (2007), Moretti et al. (2007)]. It is possible that storage in Atlantic forest is only predicted by litter inputs due to well distributed precipitation throughout the year, which can limit litter accumulation in streams through the occurrence of spates which scoured benthic litter (which were not reflected in monthly precipitation). This empirical evidence supports theoretical predictions of the role of hydrological regimes in litter availability in streams (Graça et al. 2015) and suggests that retained litter is transported downstream before it is processed by biological communities.

In contrast to Atlantic forest and Cerrado, Amazon streams were characterized by high litter storage throughout the year (Fig.4), and a lack of a relationship with precipitation. For instance, the annual range of litter storage in Amazon streams ($43 - 210$ g leaf dry mass m^{-2}) was higher than those of Atlantic forest and Cerrado streams ($4 - 144$ and $5 - 172$ g leaf dry mass m^{-2} , respectively), which were similar or even higher than those observed for temperate deciduous forest streams [e.g., $0 - 78$, $0 - 20$, $5 - 40$ g leaf dry mass m^{-2} from Petersen et al. (1989), Richardson (1992), González & Pozo (1996), respectively]. These results suggest that Amazon streams did not experience large or periodic litter export to downstream reaches over the year, unlike Cerrado and Atlantic forest streams, respectively. This can be the result of topographic and hydrological characteristics of Amazon streams draining *terra firme* forests, where the altitudinal gradient is low (60–100 m asl) and high precipitation events usually do not disturb the streambed (McClain & Richey 1996, Landeiro et al. 2008). This finding indicates that most benthic litter in Amazon streams might have enough time to be colonized by microbial and invertebrate communities, and possibly its decomposition is driven by different agents and routes than in Atlantic forest streams.

CONCLUSIONS

Our study provides comprehensive evidence of the spatial patterns and temporal dynamics of litter inputs and storage, and the major factors influencing them, in tropical streams across several biomes. Firstly, higher litter inputs occurred in the most humid biomes (Atlantic forest and Amazon forest) because of a positive effect of precipitation on plant production. Secondly, higher litter storage was observed in Amazon forest than in Atlantic forest or Cerrado savanna streams, as a consequence of higher annual precipitation and/or higher water stream depth. Thirdly, there were distinct temporal patterns of litter inputs and storage according to the type of biome: uniform litter inputs but rather seasonal storage in Atlantic forest, and seasonal inputs in both Amazon forest and Cerrado savanna, but aseasonal litter storage in Amazon forest. Fourthly, temporal patterns of inputs were mostly driven by precipitation (although temperature and litter availability were also important), while storage was determined by litter inputs and precipitation. In conclusion, these results evidence that major differences in plant litter dynamics in streams across tropical biomes are mostly influenced by precipitation. However, we still know remarkably little about how this variability might affect litter decomposition, energy flow and complex food webs in streams ecosystems at regional or at broad scales [e.g. Parton et al. (2007), Boyero et al. (2011b), Boyero et al. (2016)]. This information is crucial to predict changes in stream ecosystem functioning and potential effects on the global carbon cycle as a result of future changes in temperature and precipitation regimes (Pachauri et al. 2014).

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SUPPORTING INFORMATION

SI 1 INFORMATION OF SITES AND SAMPLING PERIOD

Table S1. Location of study streams per biome (AF, Atlantic forest; CE, Cerrado savanna; AM, Amazon forest), code of streams, latitude (Lat) and longitude (Long; in degrees), altitude (Alt; m asl), MAT (mean annual precipitation; °C), TS (temperature seasonality; standard deviation of monthly mean temperature × 100), MAP (mean annual precipitation; mm), PS (precipitation seasonality; coefficient of variation of monthly mean precipitation), PDM (precipitation of the driest month; mm), dominant substrate type, stream depth (m) and wetted width (m), canopy cover of streambed (%), and slope of bank and channel (in degrees). Stream depth and wetted width refer to the base-flow conditions. Depth, width, canopy cover, bank slope and channel slope are means of five sites per stream (see methods for additional details).

Biome	Code	Lat	Long	Alt	MAT	TS	MAP	PS	PDM	Substrate	Depth	Width	Canopy cover	Bank slope	Channel slope
AF	CGRANDE	-27.7	-48.5	79	19.6	287	1427	37	73	boulder	0.22	4.6	80	28	26
AF	GAUR	-27.6	-52.1	574	18	312	1823	15	124	cobble	0.13	3.0	69	19	10
AF	QUATI	-24.3	-53.9	295	20.9	317	1524	26	74	silt	0.40	2.5	84	4	2
CE	CAPET	-16.0	-47.9	1090	20.7	112	1650	80	8	gravel	0.23	2.9	84	23	5
CE	CVEADO	-15.9	-47.8	1079	20.7	112	1650	80	8	cobble	0.23	2.8	87	5	3
CE	RONCAD	-15.9	-47.9	1069	20.7	112	1650	80	8	silt	0.35	3.0	92	2	2
CE	BOIAD	-13.0	-41.3	984	19.9	130	918	59	22	sand	0.62	1.8	75	5	1
CE	BURIT	-10.3	-48.1	629	24.6	61	1730	80	3	sand	0.36	1.9	86	39	1
CE	BVISTA	-10.3	-48.2	643	24.6	61	1730	80	3	gravel	0.10	1.5	93	33	2
CE	SBENTO	-10.3	-48.1	544	24.6	61	1730	80	3	sand	0.54	1.7	93	26	1
AM	ACARA	-3.0	-60.0	82	27.1	49	2193	42	77	sand	0.30	2	86	3	5
AM	BBRANCO	-2.9	-59.9	98	27.1	49	2193	42	77	sand	0.62	1.8	85	3	2
AM	ASERRA	2.4	-60.6	100	26.8	63	1646	84	26	sand	0.17	4.2	79	5	4

Table S2. Plant diversity (number of species, including trees and lianas) in the riparian forest of study streams per biome (AF, Atlantic forest; CE, Cerrado savanna; AM, Amazon forest). Local surveys of plant diversity were performed using 10 plots (10×10m) along the watercourse (see more details in Bambi et al. 2017). Local estimations were performed through visual estimates of plant diversity by botanists.

Biome	Code	Plant diversity	Source
AF	CGRANDE	122	Lisboa et al. (2015)
AF	GAUR	80	Capellesso (2016)
AF	QUATI	> 50	Local estimation
CE	CAPET	70	Bambi et al. (2017)
CE	CVEADO	112	Bambi et al. (2017)
CE	RONCAD	29	Bambi et al. (2017)
CE	BOIAD	51	Local survey
CE	BURIT	87	Local survey
CE	BVISTA	83	Local survey
CE	SBENTO	> 80	Local estimation
AM	ACARA	58	Local survey
AM	BBRANCO	62	Local survey
AM	ASERRA	> 50	Local estimation

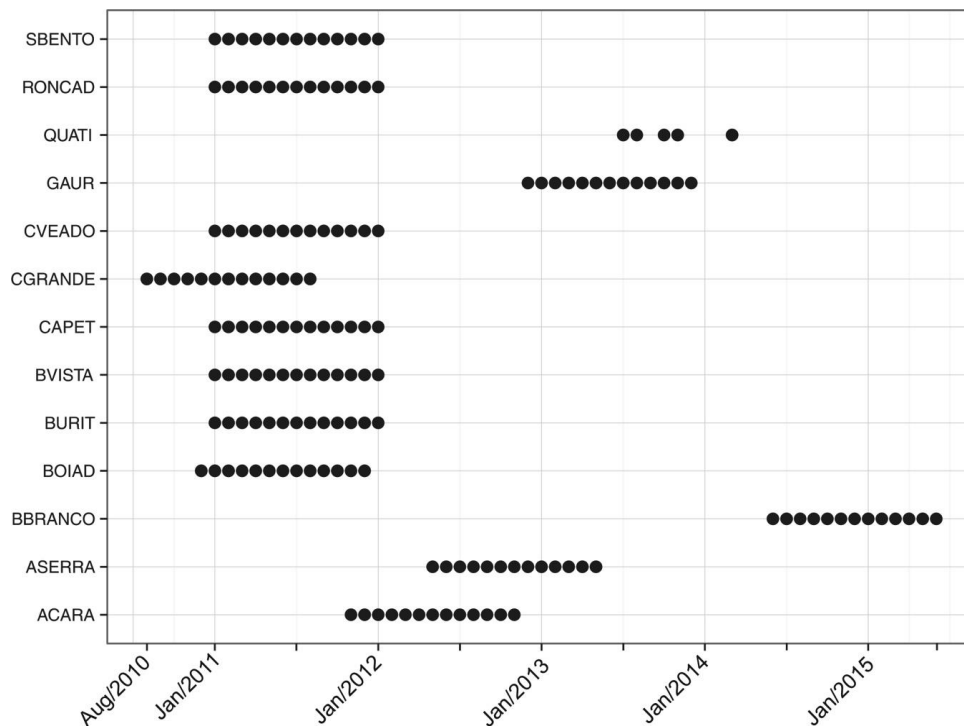


Figure S1. Interval of sampling at each stream (codes are presented in Table 1). The first circle of each stream represent when the samplers were installed in the field.

SI 2 TEMPORAL MODELS

Model M_1 describe temporal patterns for each response variable (litterfall, M_{1Lf} ; lateral inputs, M_{1Li} ; and storage, M_{1St}), which excluded the environmental factors. The explanatory variables in this model were biome (Atlantic forest, Amazon or Cerrado; categorical variable), time (number of the month within a year; continuous variable) and the interaction between biome and time (fitted as a smoother). Model M_2 included the environmental covariates: precipitation (PREC), temperature (TEMP), litterfall to forest (LF; continuous variable) and litterfall to the stream (LS), with respect to each response variable.

$$M_{1Lf}, M_{1Li}, M_{1St}: \text{Litter inputs or storage}_{ijk} = \alpha + f(\text{time}_i) \times \text{biome}_{ijk} + a_k + a_{j|k} + \varepsilon_{ijk}$$

$$M_{2Lf}: \text{Litterfall}_{ijk} = \alpha + f(\text{PREC}_i) \times \text{biome}_{ijk} + f(\text{TEMP}_i) \times \text{biome}_{ijk} + a_k + a_{j|k} + \varepsilon_{ijk}$$

$$M_{2Li}: \text{Lateral Inputs}_{ijk} = \alpha + \text{PREC}_{ijk} \times \text{biome}_{ijk} + \text{LF}_{ijk} \times \text{biome}_{ijk} + a_k + a_{j|k} + \varepsilon_{ijk}$$

$$M_{2St}: \text{Storage}_{ijk} = \alpha + f(\text{PREC}_i) \times \text{biome}_{ijk} + f(\text{LS}_i): \text{biome}_{ijk} + a_k + a_{j|k} + \varepsilon_{ijk},$$

where α is an intercept; f is the smoothing function; a_k and $a_{j|k}$ are random intercepts allowing for variation between the streams and between samples within the same stream, respectively; and ε is independently, normally distributed error with mean zero and variance σ^2 .

Temporal autocorrelation between subsequent samplings was examined using the autocorrelation function of the ‘nlme’ package with respect to month. Temporal autocorrelation was detected in litterfall data and therefore we used an auto-regressive model of order 1. Spatial autocorrelation was detected for litter inputs and storage data with variograms of normalized residuals of each model. To incorporate spatial dependency of data into models, sampling sites nested within streams were considered as random components. Visual inspection of residuals

plots and initial data exploration indicated violation of homogeneity in most cases, requiring the use of a variance structure that allows for different residual spread within biomes over time (i.e., 'VarIdent' function; Zuur et al. 2009). The optimal random structure was defined selecting models with the lowest AIC. Once the optimal random structure was found, we selected the best model in terms of fixed structure by removing any non-significant variables or interactions.

SI 3 SUPPLEMENTARY RESULTS

Table S3. Summary of backward model selection based on Akaike information criterion (AIC) for litterfall, total litterfall (sum of all litter categories), lateral inputs and storage in streams. The *p*-value refers to the comparison between 1st and 2nd, 2nd and 3rd model, and so on; and non-significant *p*-values indicate that both models are similar (at 5% level). MAT, mean annual temperature; MAP, mean annual precipitation; PS, precipitation seasonality; WF, wind frequency; PDM, precipitation of the driest month; SLOPE, bank and stream slope for lateral input and storage models, respectively; LI, litter inputs; DEPTH, stream depth; HCM, heterogeneity of channel morphology.

	Model	DF	AIC	<i>p</i>
	Litterfall			
1	MAT + MAP + PS	5	154.7	
2	MAP + PS	4	153.2	0.514
3	MAP	3	152.4	0.267
	Total litterfall			
1	MAT + MAP + PS	5	163.4	
2	MAP + PS	4	161.4	0.803
3	MAP	3	160.3	0.342
	Lateral inputs			
1	LF + WF + PDM + SLOPE	6	94.6	
2	LF + WF + PDM	5	93.0	0.395
3	LF + PDM	4	91.8	0.795
	Storage			
1	LI + MAP + SLOPE + DEPTH + HCM	7	104.2	
2	MAP + SLOPE + DEPTH + HCM	6	102.2	0.847
3	MAP + DEPTH + HCM	5	102.0	0.181
2	MAP + DEPTH	4	102.1	0.138

Fractions of litter inputs: Litterfall was, on average \pm SE, $70 \pm 2\%$ of leaves, $13 \pm 2\%$ of twigs, $9 \pm 2\%$ of reproductive parts and $8 \pm 1\%$ of other. Lateral litter inputs were $57 \pm 5\%$ of leaves, $19 \pm 4\%$ of twigs, $9 \pm 3\%$ of reproductive parts, and $15 \pm 4\%$ of other litter types. Benthic storage was $47 \pm 5\%$ of leaves, $24 \pm 5\%$ of twigs, $15 \pm 7\%$ of reproductive parts and $14 \pm 3\%$ of others (Fig. S3).

Table S4. Summary of linear mixed effects models testing for differences in monthly litterfall, lateral inputs and storage among Atlantic forest (AF), Amazon forest (AM) and Cerrado savanna (CE) biomes. AF was used as a baseline (intercept) for comparisons with AM and CE, and AM vs. CE comparison was obtained reordering the dataset.

	Value	SE	df	<i>t</i>	<i>P</i>
<i>Litterfall</i>					
Intercept	27.22	4.10	631	6.64	< 0.001
AM vs AF	0.69	6.36	10	0.11	0.916
CE vs AF	-16.95	4.80	10	-3.53	0.005
AM vs CE		5.47	10	3.23	0.009
<i>Lateral Inputs</i>					
Intercept	10.05	2.48	458	4.05	< 0.001
AM vs AF	-3.00	3.17	6	-0.95	0.381
CE vs AF	-1.34	3.02	6	-0.44	0.673
AM vs CE		2.62	6	-0.63	0.551
<i>Storage</i>					
Intercept	24.4	7.0	517	3.5	< 0.001
AM vs AF	74.4	11.3	8	6.6	< 0.001
CE vs AF	7.8	8.3	8	0.9	0.375
AM vs CE		9.9	8	6.7	< 0.001

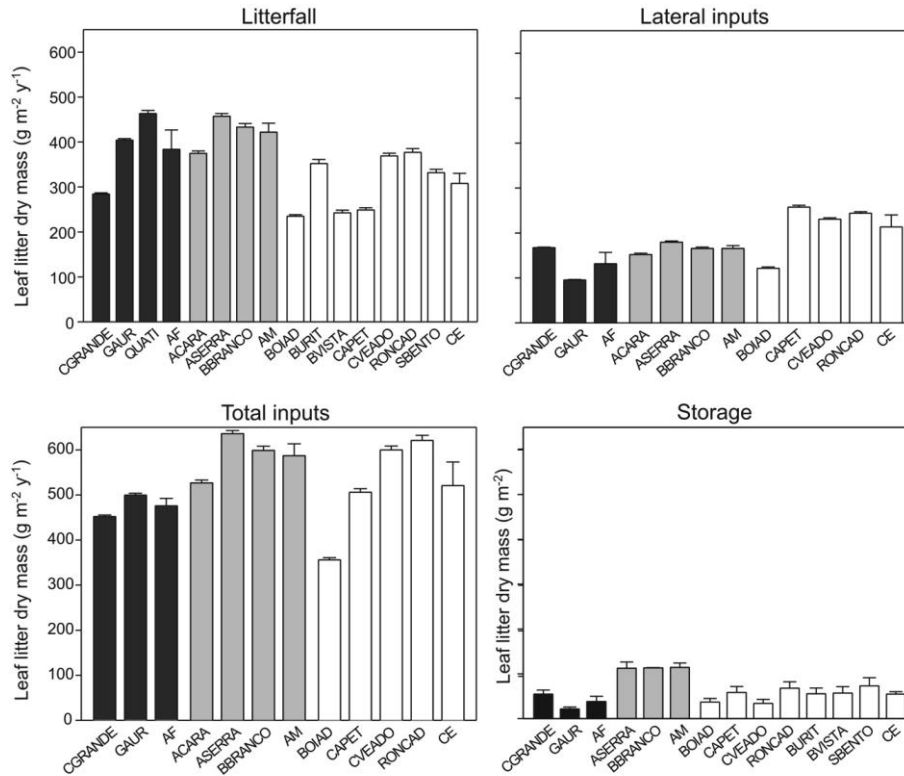


Figure S2. Annual estimates (mean ± SE) of litterfall, lateral inputs and storage at Atlantic Forest (AF; black bars), Amazon (AM; grey bars) and Cerrado (CE; white bars) biomes.

Table S5. Estimated variance, standard deviation (SD) and percent of total variance of litterfall, lateral inputs and benthic storage partitioned in spatial scales (among biomes, across streams and within streams) from the linear mixed effects model.

Terms	Variance	SD	% total variance
<i>Litterfall</i>			
Biome	61.4	7.8	30
Across streams	46.5	6.8	23
Within streams	22.1	4.7	11
Residuals	72.4	8.5	36
<i>Lateral Inputs</i>			
Biome	< 0.001	< 0.01	< 0.001
Across streams	8.68	2.95	9
Within streams	5.46	2.34	5
Residuals	89.27	9.45	86
<i>Storage</i>			
Biome	6.43	2.53	38
Across streams	0.96	0.98	6
Within streams	< 0.001	< 0.001	< 0.001
Residuals	9.31	3.05	56

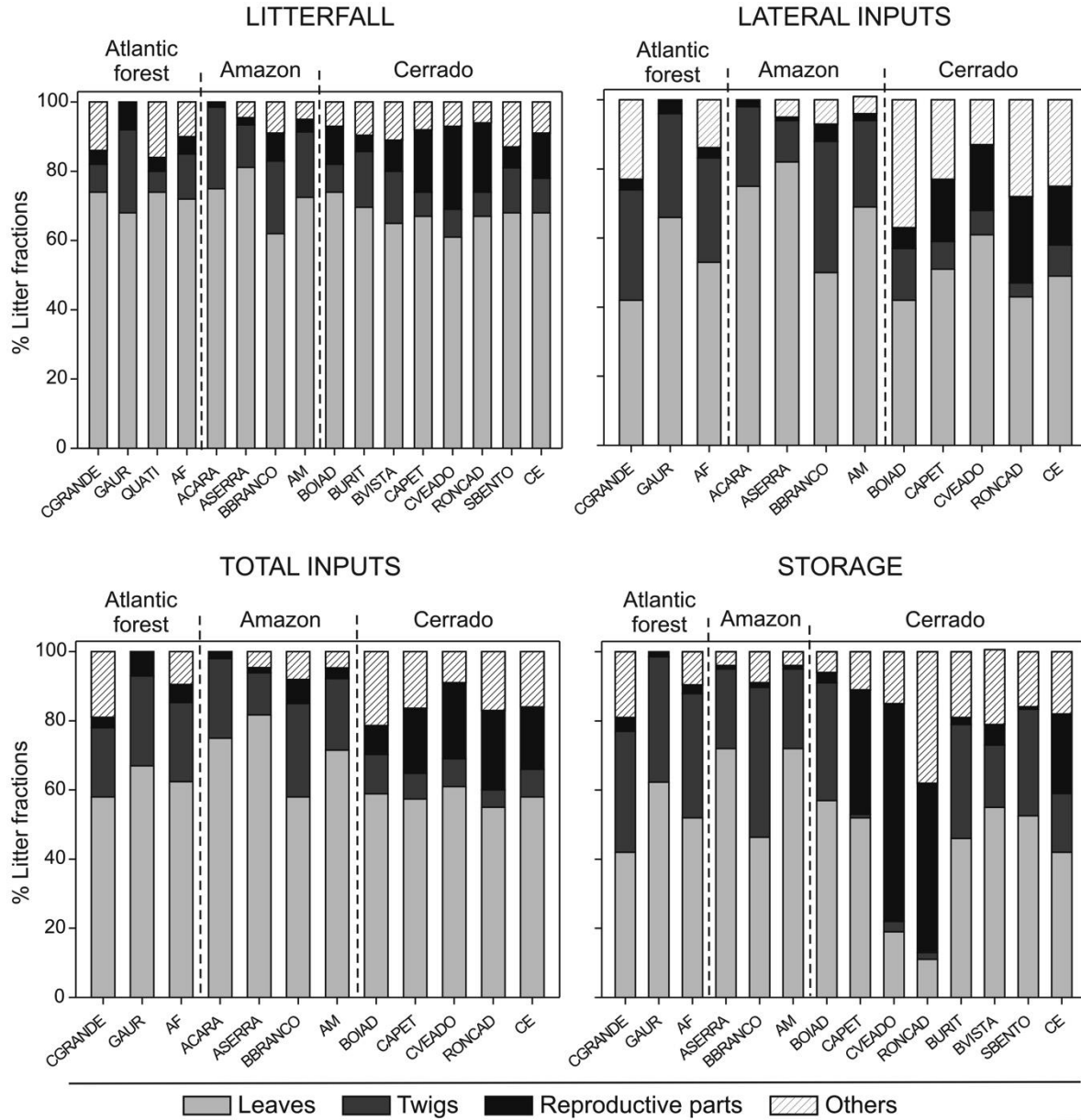


Figure S3. Proportion (%) of leaves, twigs, reproductive parts (flowers, fruits and seeds) and other unidentifiable litter parts of litterfall, lateral and total inputs (sum of litterfall and lateral inputs) to the stream, and storage in Atlantic forest, Cerrado and Amazon biomes.

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CAPÍTULO II

Plant litter fluxes in the forest-stream interface: breakdown and transport play a key role in seasonal tropical streams

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Em revisão na *Ecological Monographs*

ABSTRACT

The availability of terrestrial plant litter, which fuels heterotrophic forest streams, depends on a balance between inputs (litterfall and lateral pathways) and outputs (litter breakdown and downstream transport). However, we know little about how these litter fluxes vary within and among tempo-spatial scales, particularly in the tropics, even if this is critical to predict potential alterations in ecosystem functioning due to anthropogenic stressors. Here we quantified several processes related to litter dynamics (i.e., litterfall, lateral inputs, storage, downstream transport and breakdown) by sampling litter at multiple sites in three streams of the Brazilian Cerrado biome - which is tropical and strongly seasonal - for two years, and assessing the relative contribution of different spatial (among and within streams) and temporal scales (inter-annual, inter- and intra-seasonal) to total variability. Overall, spatial variability of litter fluxes and storage was two-fold higher (65%) than temporal variability (33%), except for litterfall, which varied less spatially (24%) than temporally (76%). We found consistent evidence across streams of the major role of litter transport as determinant of in-stream litter budgets through different seasons: litter inputs and transport were higher in the wet than the dry season (1.45 vs. 0.92 and 1.43 vs. 0.06 g litter m⁻² d⁻¹, respectively), while outputs by breakdown were similar between seasons (0.88 vs. 0.94 g litter m⁻² d⁻¹, respectively). Our results show how litter fluxes and storage in streams may be variable within a relatively small spatial scale (i.e., within stream reaches), suggesting that high within stream replication might be necessary for long-term, large-scale predictions. Further, we demonstrate that seasonal variation in litter storage (hence its availability to consumers) is mostly mediated by downstream transport losses in tropical seasonal streams, despite the largest removal of litter by breakdown on a year- and reach-scale basis. Our

findings entail important repercussions for stream functioning in a scenario of predicted shifts in rainfall seasonality in the tropics.

Key words: organic matter, leaf litter, detritus, decomposition, fungal biomass, spatial scale, temporal scale, tropical, riparian forest.

INTRODUCTION

Streams link multiple components of the landscape including terrestrial vegetation and soils with groundwater and oceans and have been recently identified as essential for regional and global carbon (C) budgets (Raymond et al. 2013). Given their retentive capacity of materials and nutrients, constant water flow, nutrient supply and tightly interface with terrestrial ecosystems, streams have a crucial role in the transformation and storage of terrestrial coarse particulate organic matter (CPOM, mainly litter; Battin et al. 2008), which is an essential C source for stream functioning (Wallace et al. 1997, Neres-Lima et al. 2017). Streams draining forested landscapes receive large amounts of litter (mostly leaves), which is retained by in-stream structures, accumulated in the streambed, and undergoes physical and biological transformation by microbes, detritivores and water flow (see Tank et al. 2010 and Graça et al. 2015 for reviews). Also, litter entering or accumulated in streams is transported to downstream reaches, mainly during high discharge periods, buried in sediments, or broken down. Thus, litter fluxes (i.e., inputs and outputs) and storage can be useful to indicate several processes related to stream functioning as retention capacity of streams, variation in the energetic basis for communities, litter turnover, residence time and organic-matter budgets.

Although litter fluxes and storage provide a means to quantify functional processes of streams, these processes have been assessed mostly in non-tropical regions of the globe (e.g., Tank et al. 2010 and references therein). One of the first studies addressing litter fluxes in forest headwater streams was conducted in the 70's (Fisher & Likens 1973) and later on there was a profusion of similar studies, mainly in North America and Europe (Webster & Meyer 1997), with few examples from the tropics (e.g., Johnson et al. 2006; Bass et al. 2011). Considering that tropical regions cover 40% of the Earth's land surface and show fundamental differences in

climate than most studied temperate regions (i.e., high rainfall intensity, high solar radiation and evapotranspiration in the tropics; Galvin et al. 2015), it is evident that litter fluxes in tropical streams are virtually unknown. The two studies cited above, conducted in tropical forest streams, provided evidence about seasonal variation of dissolved and particulate C, indicating the dominance of litterfall C inputs (over throughfall dissolved C; Johnson et al. 2006) and a substantial mobilization of C forms in the rainy season (Johnson et al. 2006; Bass et al. 2011). Other studies have quantified one or more litter fluxes in tropical streams over a year or at specific periods of the year (mainly litterfall inputs or decomposition; e.g., Rueda-Delgado, Wantzen & Tolosa 2006; Rezende et al. 2016). However, these studies lack comprehensive and integrated data of inputs and outputs especially regarding lateral pathways, storage and breakdown, which are essential components of litter fluxes and budgets. Also, tropical studies generally have comprised temporal scales of months to one year, which has precluded a robust assessment of seasonal variation patterns.

Litter fluxes and storage are processes that occur over different time scales. For example, litter inputs such as litterfall strongly depend on phenology of plant communities, and thus it is expected to vary seasonally (Reich 1995). Litter transport generally responds to short-term disturbances in flow which it is controlled by stream discharge, thus being susceptible to substantial changes at scales from hours to months (Bilby & Likens 1979; Webster et al. 1987). Finally, litter breakdown is a relatively long process controlled by biophysical agents and can vary from weeks to months mostly in relation to factors such as temperature, nutrients and water flow, which modulate the metabolism of organisms or physical abrasion (Ferreira et al. 2014; Graça et al. 2015; Follstad Shah et al. 2017). Similarly to time scales, litter fluxes and storage are also regulated within space by several environmental features acting on larger (e.g., continental

or regional scales, which can vary in climate, geology, hydrology) or smaller scales (e.g., stream segments or micro-habitat scales, which can vary in discharge, substratum type, nutrients, depth, width). However, while it is widely accepted that temporal and spatial scales are critical to understand the sources of variation in multiple ecosystem processes (Levin 1992), we are not aware of any study that quantifies the variability of tempo-spatial scales in litter fluxes and storage. Also, identifying whether and how much certain tempo-spatial scales are an important source of variability in a process can provide support for future research questions aiming at investigating the drivers of variability, and to more efficient sampling or experimental designs which could reduce unexplained variability.

Here we explore the spatial and temporal variability of litter fluxes in forest tropical streams of the Brazilian Cerrado biome. We quantified several processes related to litter dynamics (litterfall, lateral inputs, storage, transport and breakdown) by sampling litter at multiple sites within three streams for two years. We predicted that temporal scales (i.e., inter-annual, inter-seasonal and intra-seasonal) would be responsible for higher variability in litter fluxes and storage than spatial scales (i.e., among and within streams) (hypothesis 1) because (i) our experimental set-up comprised study streams and sites within streams that are close in space (spatial extent < 15 km) and drain adjacent watersheds, which imply relatively similar environmental regulatory factors according to the spatial scaling theory (Wiens 1989); while (ii) temporal variation within streams of Cerrado biome is evidenced by contrasting rainfall periods (i.e., dry and wet seasons) and temperature variation (Alvares et al. 2013), which can modulate directly or indirectly litter dynamics (Bambi et al. 2017). We also predicted that the relative importance of litter losses by breakdown and transport in a reach-scale would change seasonally, that is, reduced losses by breakdown and transport would result in litter accumulation in the dry

season, while the opposite trend would result in litter exportation in the dry season (hypothesis 2). Lower losses by breakdown in the dry season were expected due to the lower temperature and discharge, which reduce the overall biological and physical breakdown (Fonseca et al. 2013; Follstad Shah et al. 2017), respectively, while lower transport would be due to reduced hydrological effect of discharge (Johnson et al. 2006).

METHODS

Study area

We sampled three streams (Capetinga, Cabeça-de-Veado, and Roncador hereafter CAP, CVE and RON, respectively) draining adjacent microbasins within the Cerrado biome. CAP flows through a natural area belonging to the University of Brasilia, used for scientific research (Água Limpa Farm); CVE is located within the Ecological Station of Botanical Garden of Brasilia (EE-JBB); and RON flows through the Ecological Reserve of the Brazilian Institute of Geography and Statistics (RECOR-IBGE; Table 1). These three watersheds are part of the Protected Area of Gama Cabeça-de-Veado (23,650 ha), which includes urban, rural and preserved areas in the Federal District of Brazil and represent sites of the Brazilian Long Term Ecological Research Program. All three catchments are preserved areas with natural vegetation as the dominant land use and similar characteristics in terms of area, slope and normalized difference vegetation index (NDVI; generally used in remote sensing analysis and which indicates the natural vegetation condition) (Table 1). The vegetation type is typical of the Brazilian Cerrado, with dense evergreen riparian forests (i.e., gallery forests) with 70-95% of vegetation cover along the course of streams and adjacent areas of savannah (i.e., cerrado *stricto sensu*; Ribeiro & Walter 2008). The riparian forest at the CVE study stream reach had 71 tree species with a density of 2036

individuals ha⁻¹, while the CAP study reach had 68 species with 2071 ind ha⁻¹, and the RON study reach had 25 species with 4786 ind ha⁻¹. The most common riparian species were *Protium spruceanum*, *Matayba guianensis* and *Cyathea villosa* at CVE; *Protium spruceanum*, *Pseudomenia laevigata* and *Tapirira obtusa* at CAP; and *Xilopia emarginata*, *Richeria grandis* and *Clusia Criuva* at RON (Bambi et al. 2017).

Table 1. Spatial information (latitude, longitude and altitude) and environmental characteristics of drainage area (area, slope and NDVI) and each stream segment (channel width and depth, water temperature, conductivity, pH, dissolved oxygen, turbidity, discharge, dissolved N and P). Values of drainage area slope and NDVI are means \pm SE of all drainage area or upstream riparian forest, respectively. Stream variables are means \pm SE over two years ($n = 24$ in each stream) of *in situ* measurements (except DIN and SRP; Dissolved Inorganic Nitrogen and Soluble Reactive Phosphorus, respectively). DD, decimal degrees; NDVI, Normalized Difference Vegetation Index obtained using Landsat 8 satellite image and ArcGIS software; DIN SRP obtained using filtered stream water (0.45 μ m) and analyzed in a ionic chromatography for inorganic nitrogen fractions (sum of NO₂, NO₃ and NH₄) and orthophosphate (PO₄), respectively.

		CAP	CVE	RON
Stream name	-	Capetinga	Cabeça-de-Veado	Roncador
Latitude	DD	-15.960775	-15.937294	-15.889661
Longitude	DD	-47.943578	-47.886386	-47.842828
Altitude	m asl	1090	1069	1079
Drainage area	km ²	5.8	12.3	16.3
Drainage area slope	°	6.0 \pm 3.4	2.6 \pm 1.3	2.0 \pm 1.1
NDVI ¹	-	0.28 \pm 0.10	0.37 \pm 0.08	0.35 \pm 0.06
Channel width	cm	301 \pm 8	265 \pm 9	193 \pm 9
Channel depth	cm	20 \pm 2	33 \pm 1	62 \pm 4
Water temperature	°C	18.5 \pm 0.4	20.0 \pm 0.2	19.3 \pm 0.2
Water conductivity	μ S cm ⁻¹	4.9 \pm 0.9	6.3 \pm 1.6	7.3 \pm 0.8
pH	-	6.5 \pm 0.2	6.6 \pm 0.1	6.2 \pm 0.1
Dissolved oxygen	mg L ⁻¹	7.9 \pm 0.5	7.0 \pm 0.6	5.8 \pm 0.6
Turbidity	NTU	2.9 \pm 0.5	1.8 \pm 0.2	2.7 \pm 0.5
Discharge	L s ⁻¹	0.27 \pm 0.07	0.77 \pm 0.08	0.57 \pm 0.10
DIN ²	μ g L ⁻¹	20.7 \pm 1.5	28.7 \pm 1.7	29.4 \pm 2.3
SRP ²	μ g L ⁻¹	15.3 \pm 1.2	20.2 \pm 1.2	20.9 \pm 1.3

The Cerrado biome has a seasonal climate with a dry season from May to September and a rainy season from October to April. However, two transition seasons are clearly defined: a dry

to wet season which comprises September – October (hereafter dry-wet) and a wet to dry season between April – May (hereafter wet-dry) (Fig. 1a). The monthly average \pm SE rainfall (and temperature) during the experiment in the dry and wet season was 1 ± 2 and 215 ± 74 mm (20.2 ± 0.2 and 21.2 ± 0.1), respectively; and, 114 ± 133 and 56 ± 41 mm (22.5 ± 0.3 and 20.8 ± 0.3) in the dry-wet and wet-dry transitions, respectively (Fig. 1b; INMET 2014).

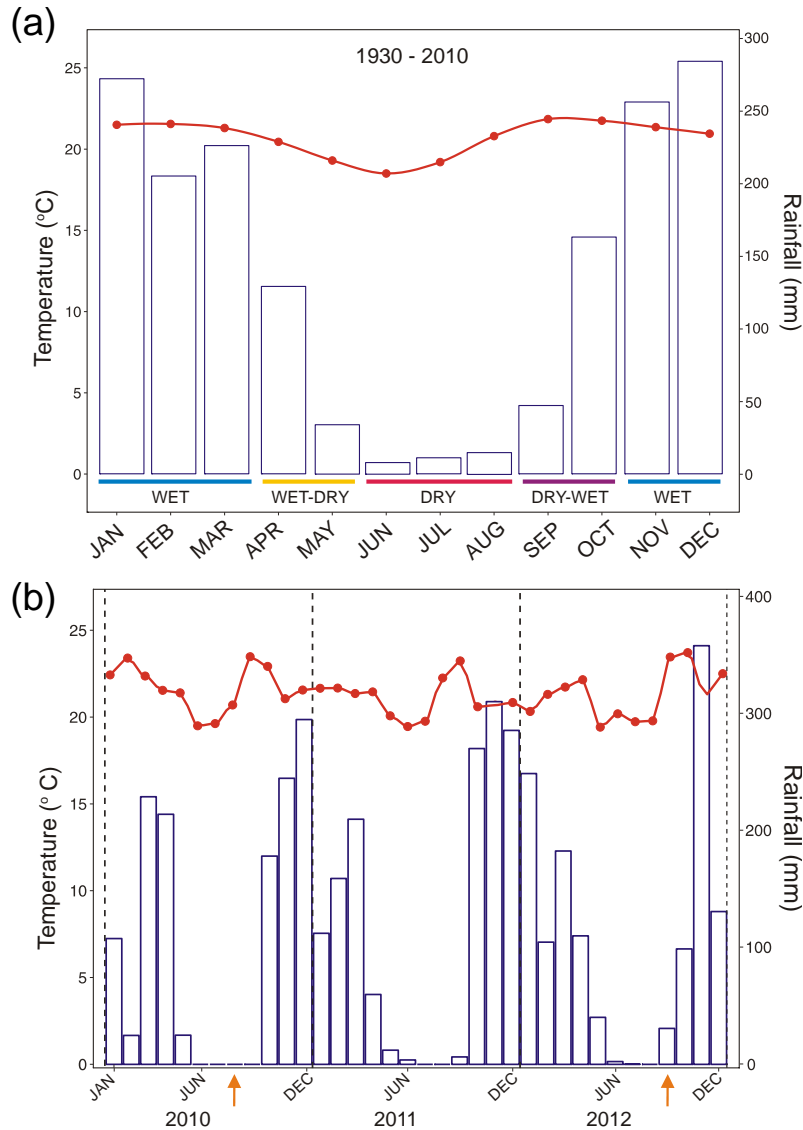


Fig.1. Climatograms of a typical Cerrado savanna climate (i.e., Brasília city) using (a) records from the 1950 - 2000 period and (b) from the 2010 - 2012 period, when the experiment was performed (orange arrows indicate the start and end of the experiment). Red points and lines represent the temperature, while blue bars the rainfall.

Experimental design and procedure

In each stream, we conducted the experiment at five equally distanced sampling sites within a 50–100 m long reach. Litterfall and lateral litter inputs were estimated using suspended and lateral traps, respectively. Suspended traps consisted of 90 plastic buckets (18 per site) placed 2 m above the streambed, with a 26-cm diameter and small holes on the bottom to allow water to drain. Lateral collectors consisted of 20 traps (4 per site) of 0.5 m long x 0.25 high x 0.5 deep and made of 1-mm mesh; they were distributed along the stream bank and fixed to the soil. Benthic litter storage was estimated with 15 Surber samples (3 per site taken randomly, including pool and riffle areas) of 0.10 m² and 250- μ m mesh that were further sieved through a 1-mm mesh. Samples were collected once a month for two years (from September 2010 to September 2012). They were transported to the laboratory, oven dried and sorted into three categories: leaf litter, twigs (< 2 cm diameter) and others (fruits, flowers, seeds and unidentified parts). However, our further analyses were focused on leaf litter because it represented the majority of total particulate litter inputs (> 50% of dry mass [DM]; Appendix S1, Fig S1), is the most biologically active pool of terrestrial litter in forest streams and is renewed annually (Webster et al. 1999).

Leaf litter collected once a month in the suspended traps was mixed and weighed in portions of 2.00 ± 0.05 g (mean \pm SE), which were enclosed in 15 coarse-mesh litterbags (10 mm). Litterbags were incubated at the five sampling sites (i.e., three litterbags per site) and recovered after ~30 days of incubation to estimate breakdown rates. The use of ‘natural’ leaf litter mixtures, rather than leaves from selected species, ensured realistic conditions. Ten leaf discs (10 mm in diameter) were cut from the remaining leaf material to estimate fungal biomass (using five randomly discs through ergosterol content according Gessner, 2005; see below) and DM (using the remaining five discs). The remaining leaf material was oven dried (60°C, 72 h)

and weighed to determine leaf DM, which was summed to the DM of the 10 discs (DM of five discs multiplied by two) to determine the final DM. Ergosterol content on leaf discs was extracted at 80 °C for 30 minutes in a methanol/KOH solution and purified with solid-phase extraction cartridges (Sep-Pak[®], Waters, Milford, MA, USA; Vac RC, tC18, 500 mg) by applying a gentle vacuum. Extraction efficiency was monitored by running standards (Ergosterol $\geq 95\%$ [HPLC], Sigma[®]) in parallel. Ergosterol was eluted in isopropanol and quantified by high-performance liquid chromatography (detection wavelength: 282 nm, flow rate: 1.5 mL s⁻¹, column temperature: 33 °C, injection volume: 20 μ L). Fungal biomass (FB) on litter was expressed as μ g ergosterol content per gram of litter DM.

Estimation of litter fluxes and storage

We estimated two types of litter fluxes (inputs and outputs) and benthic storage (total and variation; hereafter storage) at each site and sampling occasion following Elosegi & Pozo (2005) and Pozo (2005). Litter inputs were litterfall (LF), lateral inputs (LA) and total inputs (TI). We estimated LF (g DM m⁻² d⁻¹) by dividing the total amount of litter collected by the area of the traps and by the elapsed time in days (i.e., g litter); LA (g DM m⁻² d⁻¹) by dividing the total amount of litter by the length of traps in meters and the elapsed time in days; and TI (g DM m⁻² d⁻¹) as the sum of LF and LA. Storage (S; g DM m⁻²) was the total amount of litter divided by sampling area on each occasion, and storage variation (Δ S; g DM m⁻² d⁻¹) was the difference between storage at time zero (S₀) and at time *t* (S_{*t*}) divided by the elapsed time in days. Litter outputs were those by breakdown (O_B) and by downstream transport (O_T). We estimated breakdown rate (*k*; d⁻¹) as the difference between the natural logarithm of final and initial DM divided by incubation time in days, and O_B (DM m⁻² d⁻¹) by multiplying litter storage by litter

breakdown We estimated O_T using the general mass balance equation $O_T = \Delta S + TI - O_B$, where positive values of O_T mean lower downstream outputs than upstream inputs, while negative values mean the opposite. The litter budget resulting from subtracting outputs by inputs is the same as ΔS , where negative values mean litter accumulation (inputs > outputs) in the stream and positive values indicate litter export (inputs < outputs).

Data analysis

To test our first hypothesis (i.e., that litter fluxes and storage vary temporally more than spatially within the Cerrado) we partitioned the total variance of each response variable in a set of tempo-spatial nested scales (three temporal and two spatial scales): intra-annual (which accounted for variation between the first and second years of sampling), inter-seasonal (between the four seasons - dry, dry-wet, wet, and wet-dry), intra-seasonal (within each season), among-stream (among the three streams) and within-stream (among the five sites within each stream). The variance associated with each scale was estimated with the *VarCorr* function fitting a linear mixed model with the intercept-only and all nested scales considered as random factors (*lm* function, both of the *nlme* package of R; Pinheiro et al. 2016; R Core Team 2016).

To test whether our second hypothesis (i.e., whether dry periods store more litter than wet periods due to higher inputs and lower export by transport and breakdown in the former) we calculated ordinary non-parametric bootstrapped 95% confidence intervals (BCa method using the *boot* function and package, and based on 1,000 bootstrap replicates; Davison & Hinkley 1997; Canty & Ripley 2016) for ΔS , TI , O_T , O_B , k , FB response variables separately for dry and wet seasons as for both transition seasons. We tested if bootstrapped 95% confidence intervals (CI) for each response variable differ between dry and wet seasons, and between dry-wet and

wet-dry transition seasons. We also tested if 95% CI for OT and ΔS differ from zero (i.e., the null expectation that there is not CPOM transport or storage variation).

RESULTS

Tempo-spatial variability of litter fluxes, storage and budget

Spatial variability of organic matter fluxes and storage was, on average, almost two-fold higher than temporal variability (65% among and within streams vs. 33% inter-annual, inter- and intra-seasonal; Fig. 2, 3, 4). O_T , ΔS , O_B and LA showed even higher spatial variability, averaging 99, 92, 76 and 75%, respectively, which were more than five-fold higher than temporal variability (85% vs. 15% as a whole; Fig. 2). LF and consequently TI were the only two fluxes with higher temporal than spatial variability (76 vs. 24% and 66 vs. 33%, respectively; Fig. 3a, c). The partitioning of variance into tempo-spatial scales evidenced that most of spatial variability was associated to the within-stream rather than the among-stream scale (62 vs. 12%, which represents a five-fold difference), while most of temporal variability was inter-seasonal (26%) rather than intra-seasonal (7%) or intra-annual (0.05%) (Fig. 2, 3, 4). Overall, most variability of litter fluxes and storage occurred at the within-stream (54%), inter-seasonal (26%), among-stream (11%), intra-seasonal (7%) and, lastly, inter-annual (< 0.1%) scales. Residual variability was generally lower (0.03 – 8%) than all tempo-spatial scales except for the inter-annual scale, in which variability was minimal (< 0.01 – 0.44%).

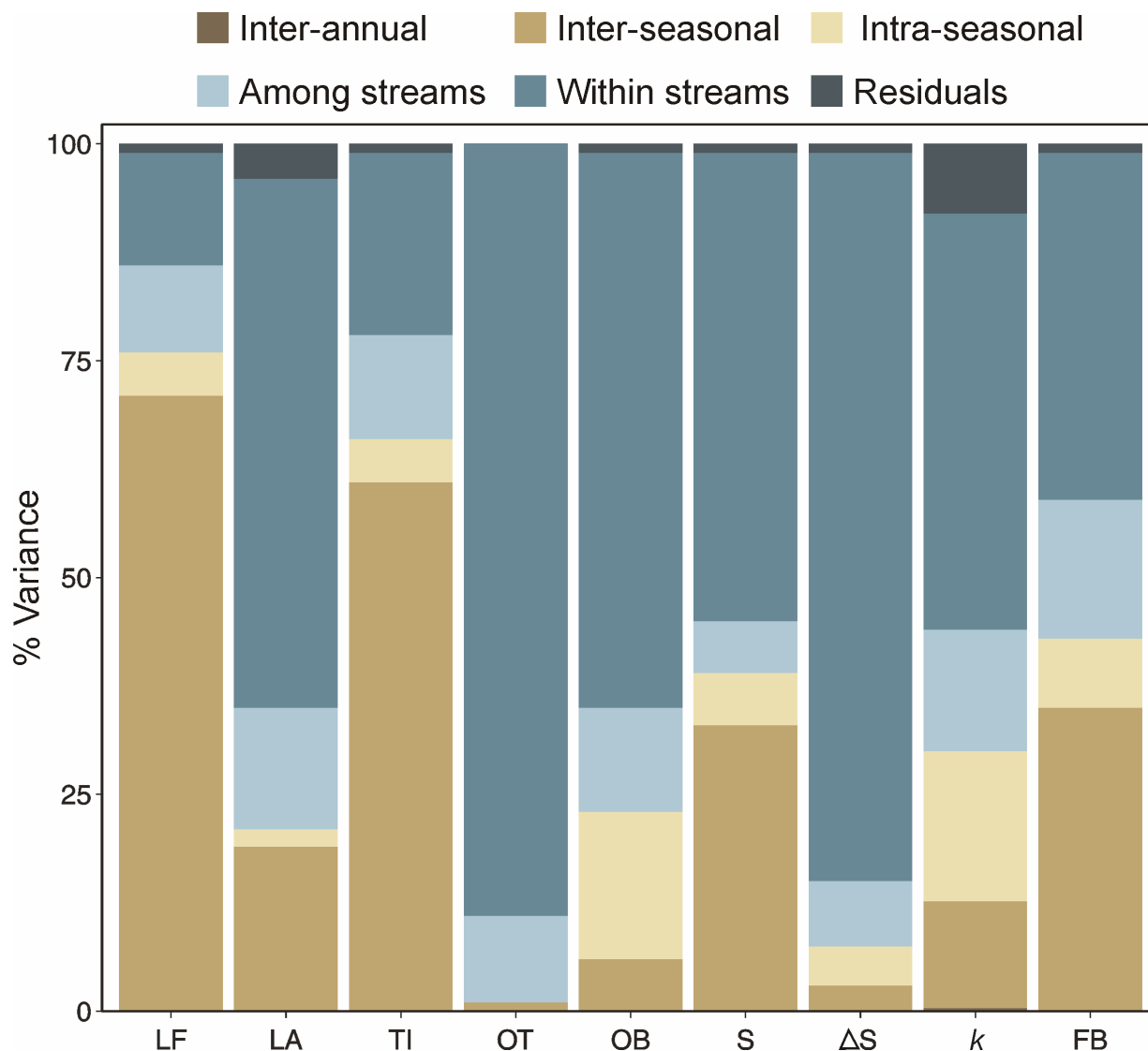


Fig.2. Variance of litter fluxes (LF, litterfall; LA, lateral input; TI, total inputs; O_T , output by transport; O_B , output by breakdown; ΔS , storage variation; and k , breakdown), storage (S) and fungal biomass (FB) partitioned into a set of nested tempo-spatial scales (inter-annual, inter-seasonal, intra-seasonal, among streams and within streams) and remaining residual variation.

Seasonality of litter inputs and outputs

The bootstrapped confidence intervals revealed that the dry season stored on average $0.38 \text{ g litter m}^{-2} \text{ d}^{-1}$ (ΔS ; 95% CI, 0.105 – 0.786), which corresponds to a total storage of $34.11 \text{ g litter m}^{-2}$ by the end of dry season (after 90 days of accumulation). The wet season exported on average $0.86 \text{ g litter m}^{-2} \text{ d}^{-1}$ (ΔS ; 95% CI, 0.41 – 1.44), which was more than two-fold higher than average

storage in the dry season and represent a total output of 129.75 g litter m⁻² by the end of the wet season (i.e., after 150 days). These contrasting litter dynamics between seasons were mostly driven by O_T, but not by TI or O_B: there were lower TI and non-different from zero O_T in the dry season, and higher TI and positive O_T in the wet season (Fig. 5a). Overall, the observed S in the stream was similar between dry and wet season (Fig. 5b). O_B was different from zero but similar between seasons [0.94 (95% CI, 0.75 – 1.19) and 0.88 g m⁻² d⁻¹ (95% CI, 0.68 – 1.87), in the dry and wet seasons, respectively]. Although O_B was similar between seasons, *k* was 40% higher in the wet than the dry season (0.0172 vs. 0.0123 d⁻¹); and FB on decomposing litter was more than two-fold higher in the wet than the dry season (333.63 vs. 158.18 μg ergosterol g⁻¹ leaf DM) (Fig. 5b).

Dry-wet and wet-dry transition seasons showed different litter dynamics than dry or wet seasons, but similar between them: there was no litter accumulation or export (i.e., ΔS was not different from zero; Fig. 5a). However, inputs and O_B differed in their magnitudes: TI and O_B were on average more than three- and five-fold higher in the dry-wet than the wet-dry season, respectively (3.21 vs. 0.93 g m⁻² d⁻¹ and 1.63 vs. 0.31 g m⁻² d⁻¹). Although the dry-wet season showed an overall ΔS non-different from zero, most of the time there was litter accumulation in the streambed as indicated by 74% of bootstrapped values. O_T was similar between transition seasons, but different from zero and positive only in the wet-dry season (Fig. 5a). Observed S in the streambed was five-fold higher in dry-wet than wet-dry season (Fig. 5b). In contrast, *k* was similar between both transition seasons, but FB was 44% higher in the wet-dry than the dry-wet season (Fig. 5b).

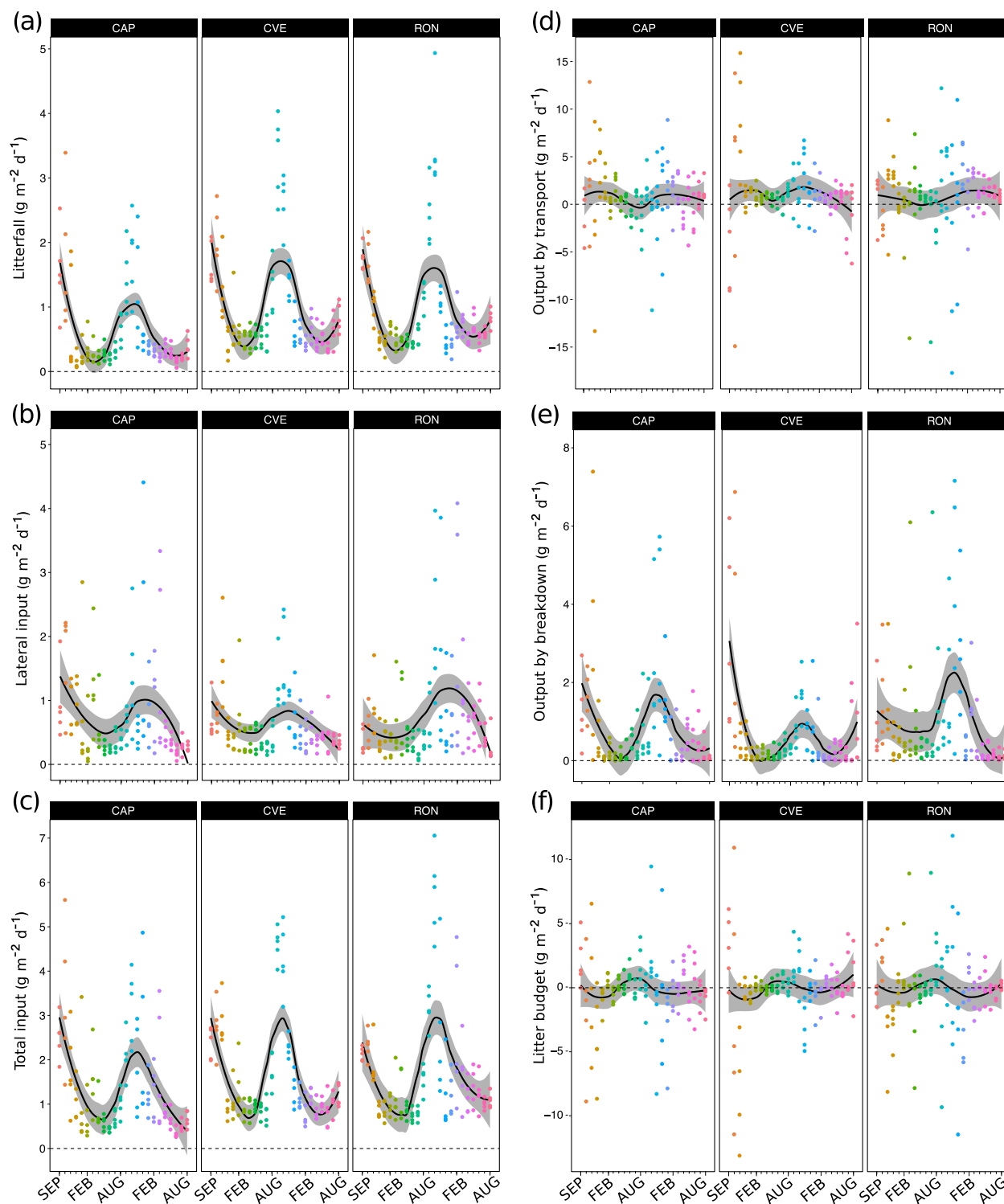


Fig.3. Temporal patterns of litter fluxes (a, litterfall; b, lateral input; c, total input; d, output by breakdown; e, output by transport; and, f, litter budget) over two years in each stream (CAP, CVE, RON). Points within each month represent each sampling site within a stream ($n = 5$). Black lines represent the non-linear temporal trend of each flux and grey areas the 95% confidence intervals. Note the different y-axis among panels.

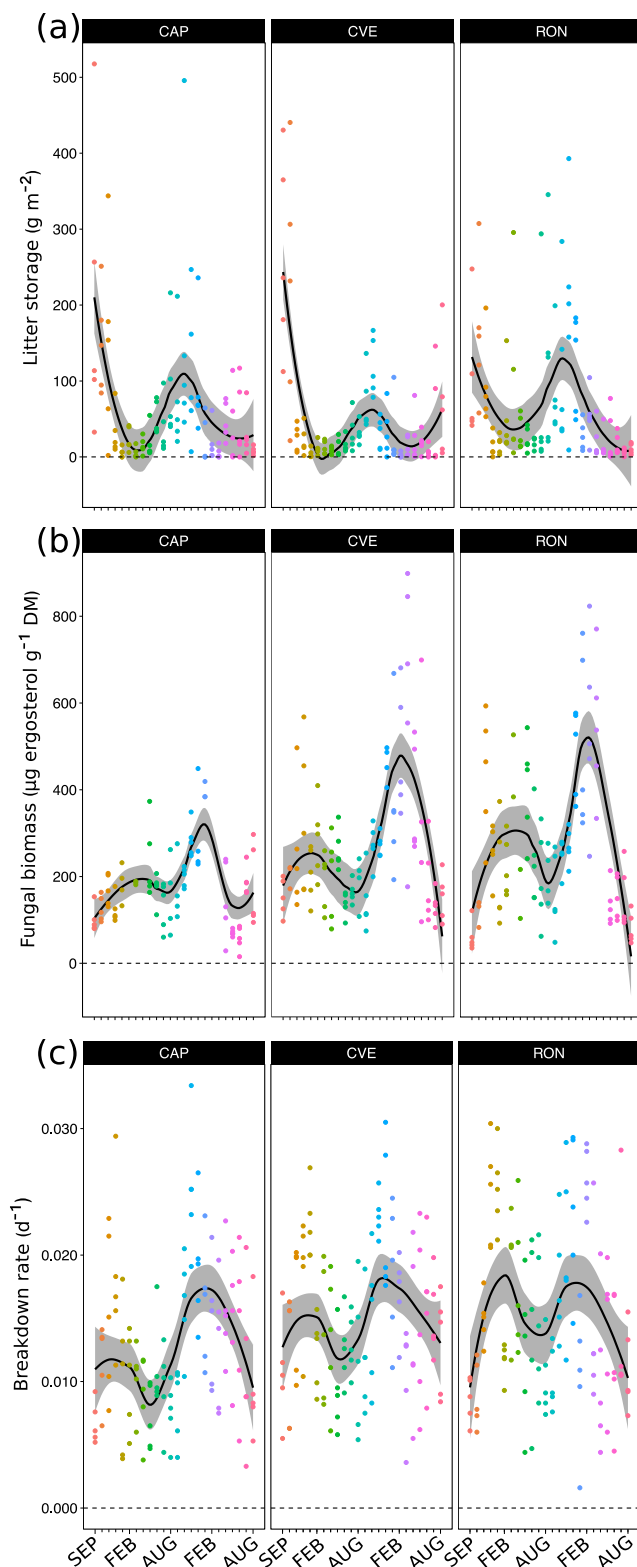


Fig.4. Temporal patterns of (a) litter storage, (b) fungal biomass and (c) litter breakdown over two years in each stream (CAP, CVE, RON). Points within each month represent each sampling site within a stream ($n = 5$). Black lines represent the non-linear temporal trend of each flux and grey areas the 95% confidence intervals.

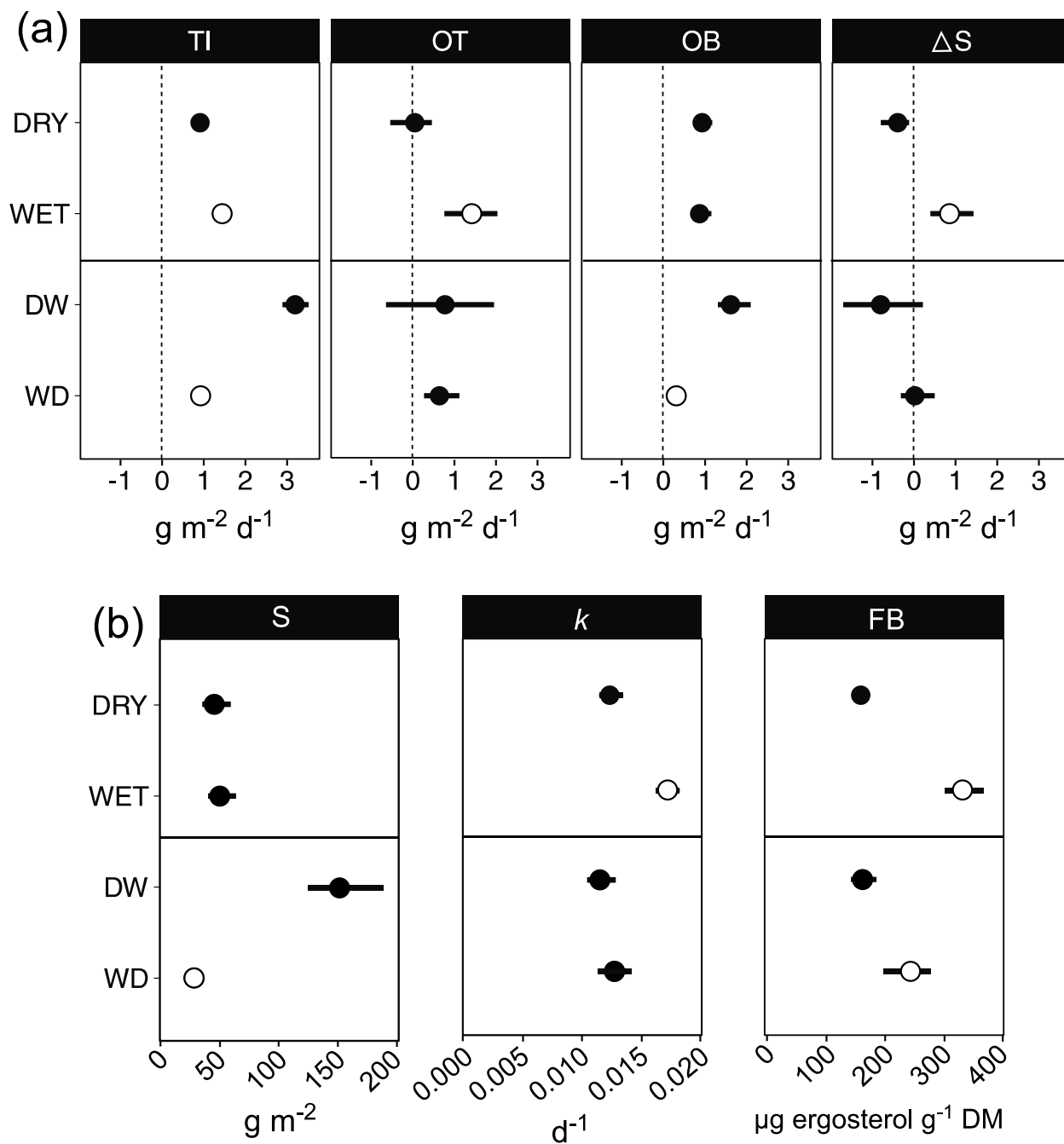


Fig.5. Litter fluxes (TI, total inputs; O_T, output by transport; O_B, output by breakdown; and ΔS, storage variation) (panel a) and, litter storage (S), litter breakdown (k) and fungal biomass (FB) (panel B) in dry (DRY), wet (WET), dry-wet (DW) and wet-dry (WD) seasons. Circles are means and vertical lines denote upper and lower limits of 95% non-parametric bootstrapped confidence intervals (CI); open and closed circles denote whether there is difference (i.e., no overlap between CI) between dry and wet season or dry-wet and wet-dry season; the dashed lines denote the value of zero (which is meaningful only for OT and ΔS, that is, the null expectation that there was no litter transport or storage variation).

DISCUSSION

Longitudinal variability within streams may influence litter dynamics more than seasonality

Our experiment showed how different tempo-spatial scales, which are generally used to investigate ecological patterns over time and space in a variety of ecosystems (Stommel 1963; Delcourt et al. 1982; Palmer & Poff 1997), may produce different outcomes of litter fluxes and storage in stream ecosystems. This implies that information from one scale often cannot be transferred to others without an a priori knowledge of potential sources of variation in a given process, as this extrapolation may result in inconsistent or contrasting conclusions. Although the importance of scale in ecology was highlighted decades ago (Levin 1992), experimental evidence explicitly demonstrating this for stream processes is scarce.

We observed that all litter fluxes and storage, except litterfall and total inputs, were more variable over space than over time scales tested, contrary to our prediction. This suggests that local spatial heterogeneity has a greater effect on these variables than temporal environmental oscillations. The spatial heterogeneity of streams and of its interface with terrestrial environments (i.e., the riparian zone) is an intrinsic characteristic of these ecosystems that has been explored and evidenced elsewhere (e.g., Pringle et al. 1988; Poff & Ward 1990). The higher variability of outputs by transport and storage at the within stream scale may reflect the large influence of stream geomorphology (e.g., width, depth, slope and pool/rifle configuration), which is in turn determined by large-scale, long-term factors such as climate and lithology (Schumm & Lichty 1965). For instance, channel geomorphology determines the capacity to retain litter, with narrow, rough-bottom or debris dams areas being most retentive. The patchy distribution of litter storage was commonly reported elsewhere (Lisboa et al. 2015), while transport was reported to vary mainly due to hydrological regime, with higher transport in high-

flow periods (note that discharge-transport relationship may be not linear, but sigmoid; Richardson et al. 2009). Here we found evidence of higher transport variability at within stream scale, suggesting a major role of channel morphology than hydrological regime. Additionally, the heterogeneity of the aquatic-terrestrial interface also produced the greatest variability of lateral litter inputs, which may be enhanced by stream bank slope and the amount of available litter to be transported or restricted by density of obstacles (e.g., roots, rocks or dead trunks).

Higher within-stream variability was also evidenced for outputs by breakdown, breakdown rate and fungal biomass; however, these variables also varied considerably temporally (20 - 40% of total variability in inter- and/or intra-seasonal scale). The highest variability of breakdown rates and fungal biomass at the within-stream scale agrees with the findings of Tonin et al. (2017), supporting the idea that biological breakdown agents (which includes fungi and invertebrates) are mainly influenced by microhabitat conditions. For instance, there is evidence of the aggregate distribution of shredder invertebrates in microhabitats (Heino, Louhi & Muotka 2004; Schmera et al. 2007). The tempo-spatial variability of outputs by breakdown was somehow similar to that of breakdown rate and storage, as both variables were used to estimate this flux. Seasonal differences of fungal biomass (i.e., 40% of total variability) may be associated to water temperature and nutrient inputs, which are important regulators of fungal activity in streams (Suberkropp 1995; Suberkropp & Chauvet 1995) and both vary seasonally in Cerrado streams (Silva et al. 2011). In turn, inter- and intra-seasonal variation of breakdown rates may be mediated by oscillations in shredder and fungal activity, which are generally stimulated by increases in temperature and nutrients in the water. Also, physical breakdown is a potential mechanism contributing to the observed variability, controlled by water flow (which depended of rainfall; Singh 1997), and that is responsible for a representative litter

mass loss (Fonseca 2013). Previous studies have reported seasonal or monthly variations in litter breakdown (Ferreira et al. 2013; Rezende et al. 2016) and have shown its association with temperature and nutrient increases, especially in highly oligotrophic streams (as our study streams), which are nutrient limited and therefore sensitive to even small increases in nutrient availability (Gulis et al. 2006).

In contrast to other fluxes, most of litterfall variability (70% of total) was associated to the inter-seasonal scale, indicating a large-scale environmental control of litterfall. This is likely due to the influence of climatic factors, such as rainfall and temperature, which drive the phenology of leaf senescence (Reich 1995). Moreover, total litter inputs presented the same pattern of litterfall due to the largest contribution of litterfall to inputs (> 54%). Previous studies have shown a clear seasonal pattern of litterfall in the Cerrado biome (Gonçalves et al. 2006; França et al. 2009) or other biomes experiencing seasonality (Sabater et al. 2008; Gonçalves & Callisto 2013). Considering the strong large-scale climatic control on litterfall, it is not unexpected that litterfall patterns have been consistently identified across a wide range of biomes worldwide (Chave et al. 2010; Zhang et al. 2014), while other important fluxes such as litter breakdown or lateral inputs show inconsistent patterns.

Output by transport drives litter availability in Cerrado streams

Litter is an important food source for forest stream food webs and is often assumed to accumulate in low-flow periods and to be exported in high-flow periods. However, there is little empirical evidence on the importance of these two processes – litter breakdown and transport –, which are responsible for litter availability. While we showed the importance of litter accumulation and exportation in the dry and wet seasons, respectively, this pattern was mostly

mediated by litter transport and not by litter inputs and breakdown, as predicted. For example, we had expected litter accumulations in low-flow conditions (characteristic of the dry season) due to higher litter inputs and lower outputs (i.e., transport and breakdown), and the opposite in the wet season (i.e., lower inputs and higher outputs, leading to litter exportation). We observed, however, that litter availability in the streambed was determined by the amount of litter removed by transport, as outputs by breakdown were similar between seasons and inputs were 57% higher in the wet season. Results from other year-round litter experiments (Colón-Gaud et al. 2008; Gonçalves & Callisto 2013; Lisboa et al. 2015) suggest that temporal dynamics of litter inputs correlate poorly with litter storage. Our results also suggest a considerable role of litter breakdown on litter loss, as this process was 15 times greater than transport by water flow in the dry season, while transport was only 1.6 times higher than breakdown in the wet season. Considering both seasons together, breakdown was responsible for 22% more litter exportation than transport. Yet, it is important to note that litter breakdown results in the production of dissolved and fine organic particles, which can be transported downstream or retained and metabolized (Battin et al. 2008).

Several other studies have investigated the relative importance of litter transport and decomposition in streams. For example, a synthesis of studies from mountain deciduous forest streams found that the transport rate of sticks, leaves and fine particles exceeded their breakdown rate, suggesting a substantial role of transport in litter dynamics (Webster et al. 1999). However, estimates of turnover length indicated that sticks and leaves travel short distances until they are retained again. Using a modeling framework, Richardson et al. (2009) showed that transport of particulate organic matter (POM) was an important component of reach-scale loss, but POM breakdown was a major source of loss (from about 65 to 98% of the inputs) in boreal conifer

forest streams. Additionally, they suggested the biological breakdown of POM is the major source of reach-scale loss during low-flow periods, while POM transport gains importance during high-discharge events. In contrast, other studies have suggested the predominance of litter export by local flushing (e.g., Richardson 1992). Thus, our results are in accordance with others showing that litter export by transport affects seasonal availability of litter in streams. However, our study is one of the first to show the relative importance of breakdown and transport in mediating litter storage variation in the tropics (and possibly the first conducted at tropical savannah streams), thus significantly contributing to a general understanding of these processes.

Our data showed that, despite similar losses by breakdown in the dry and wet seasons, breakdown rates were higher in the wet season (40%, on average). This suggests that losses by breakdown were more related to the amount of accumulated organic material in the streambed than to breakdown rate. However, we found twice more fungal biomass on litter in the wet season, indicating higher fungal conversion of litter to inorganic compounds and incorporation of litter C into mycelial biomass. A possible explanation for the higher fungal biomass in the wet season is the higher input of nutrients from terrestrial ecosystems into streams after the leaching of riparian soils during rainfall periods (Silva et al. 2011); however, further work is needed to investigate this relationship. Also, seasonal differences in fungal biomass are unlikely to be explained by seasonal differences in temperature – as would be expected based on metabolism regulation (Gillooly et al. 2001) – as we found similar fungal biomass in the hottest (dry-wet transition) and coldest (dry season) periods of the year.

Both transition seasons showed similar losses by transport, which probably explained the steady state of litter accumulation and exportation. However, litter inputs and losses by breakdown were much higher in dry-wet than wet-dry transition, even surpassing those of the

dry and wet seasons. Moreover, losses by breakdown did not exceed inputs as much as to prevent large litter accumulations in the streambed (median of 104 g m^{-2} vs. $14 - 20 \text{ g m}^{-2}$ at all other periods). Taken together, these results suggest that seasonality over the year has fundamental repercussions in stream litter budgets, and evidences the particular influence of transitional seasons, especially the dry-wet transition, on litter dynamics.

An important limitation of our study is that we only measured CPOM ($> 1 \text{ mm}$; treated here as litter), thus excluding fine particulate organic matter (FPOM, $0.45 \text{ }\mu\text{m} - 1 \text{ mm}$) and dissolved organic matter (DOM, $< 0.45 \text{ }\mu\text{m}$), which are other important sources of terrestrial C in streams (Fisher and Likens 1973). FPOM is generally the major product of breakdown, with a percentage of refractory FPOM coming from erosion of soil organic matter (Hedges et al. 1986), while DOM may come from in-stream decomposition, groundwater and the terrestrial ecosystem (mostly from organic-rich riparian soils; Bass et al. 2011; Fasching et al. 2016). While FPOM and DOM are important components of organic matter export (37% and 59% of the total export, respectively, reported by Johnson et al. 2006 in the seasonally dry Amazon), litter inputs are a fundamental flux to headwaters (e.g., 43 times greater than DOM in throughfall; Johnson et al. 2006).

CONCLUSIONS

We provide some of the first experimental evidence demonstrating how litter fluxes and storage in streams may be variable within a relatively small spatial scale (i.e., within stream reaches) and how this variation may surpass temporal variation across seasons. Our findings suggest that future studies should investigate drivers of litter dynamics at different spatial scales to help understand how and when extrapolations from small to large scales are valid. Also, our study

indicates the need for higher within-site or within-stream replication in order to reduce the unexplained variability of measurements in regional or larger-scale studies, since streams and the aquatic-terrestrial interface are highly heterogeneous ecosystems.

Further, we conclude that seasonal variation in litter storage (hence its availability to consumers) is mostly mediated by transport losses in a dry-wet or seasonal rainfall climate, which has serious repercussions in a scenario of predicted shifts in rainfall seasonality in the tropics (e.g., Feng et al. 2013). This implies that we may expect higher litter accumulation in low-flow periods and higher litter exportation in high-flow periods, to certain extent independently of inputs and losses by breakdown. Still, even if transport mediates litter dynamics, our data suggest that litter breakdown is responsible for the largest removal of litter on a year- and reach-scale basis. Interestingly, we also show that the contribution of fungal decomposers varies with season in terms of biomass, which suggests that decomposition is higher in wet periods. Our results are likely applicable to other streams and their aquatic-terrestrial interface with respect to spatial variation, and mainly to streams in dry-wet and seasonal rainfall climates with respect to temporal litter dynamics. Our study has implications to conservation, restoration and management of forest-stream interface. For instance, our data could help managers establish a minimum level and seasonality of litter flux to maintain litter availability in restored or disturbed streams. Studies addressing FPOM and DOC, although methodologically more challenging, are the next step in understanding C fluxes in streams and, ideally, would need to be run at multiple sites in stream networks to enable consistent predictions and generalizations.

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SUPPORTING INFORMATION

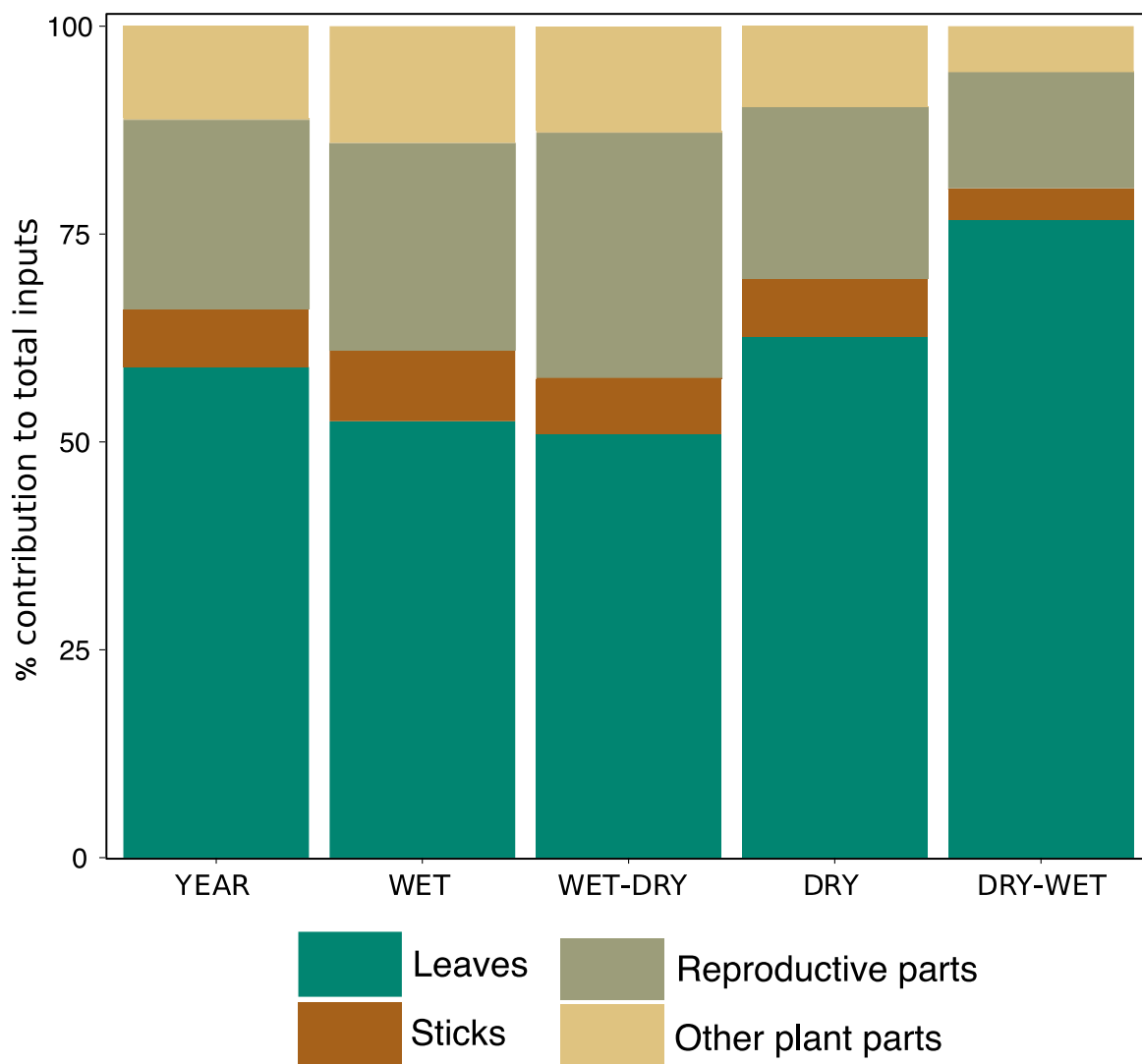


Fig. S1. Contribution of each litter type (mean percentage of dry mass of leaves, wood, reproductive parts and others) to total litter inputs to streams on a year basis (YEAR) and in the dry, dry-wet, wet and wet-dry seasons. Values are means through sampling times and streams. Plant reproductive parts were composed of flowers (sepals and petals), seeds and fruits.

CAPÍTULO III

Stream nitrogen concentration, but not plant N-fixing capacity, modulates litter diversity effects on decomposition

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ABSTRACT

1. We are facing major biodiversity loss and there is evidence that such loss can alter ecosystem functioning. However, the effects of plant diversity on decomposition – a key component of the global carbon cycle – are still unclear. A recent study suggested that a plant trait – their nitrogen (N)-fixing capacity – could mediate effects of litter diversity on decomposition by means of a microbial transfer of N from N-fixers to non-fixers.

2. We explored this possibility in a microcosm experiment in which we manipulated litter species richness (1, 2 or 4 species), N-fixing capacity (N-fixer or non-fixer species), the presence of detritivores (*Sericostoma pyrenaicum* larvae present or absent), and water N concentration [natural stream water (0.366 mgL⁻¹ of NO₃-N) or elevated N concentration (5 times the natural concentration: 1.835 mgL⁻¹)].

3. We show that litter diversity accelerated decomposition by microorganisms and detritivores (by 7 and 15%, respectively), mostly through complementarity effects. However, enhanced decomposition did not result in higher detritivore growth, possibly because all litter combinations provided sufficient resources for their maximum growth.

4. The plant N-fixing capacity had no effect on decomposition, which varied among species most likely because of differences in a combination of litter traits. Detritivores maximized the consumption of their preferred resource in litter mixtures, but also exploited less preferred resources, and their C:N ratios increased during the experiment regardless of litter type or water N concentration.

5. Microbial decomposition of litter with low N content was enhanced at elevated water N concentration, suggesting that microorganisms used nutrients from the water when those

nutrients were limiting in leaf litter. In contrast, detritivore growth was impaired at elevated water N concentration, possibly because a stoichiometric imbalance entails metabolic costs.

6. Our findings suggest that loss of plant diversity in riparian forests would mostly affect decomposition in streams of high nutrient status, where effects on microbial decomposition would be more evident and detritivore populations may be reduced.

Key-words: decomposition rate, detritivores, functional traits, litter breakdown, nitrogen-fixing plants, species richness.

INTRODUCTION

The current major rate of biodiversity loss (Barnosky et al. 2011), and its potential consequences for ecosystem functioning, goods and services (Cardinale et al. 2012), have motivated hundreds of experimental studies testing how changes in species richness might alter rates of primary production and plant litter decomposition (Schmid et al. 2009, Cardinale et al. 2011). Relevant studies on primary production have typically demonstrated that reduction in species richness decreases the efficiency with which biological communities capture resources and convert them into new plant biomass, the mechanisms for which are well understood (Hector et al. 2009). In contrast, our understanding of how species loss affects plant litter decomposition is still in its infancy (Cardinale et al. 2011), despite the importance of this process. Plant litter decomposition is a key component of the global carbon (C) cycle, as 90% of terrestrial plant biomass produced each year dies and is stored or decomposed in soils and fresh waters, with major consequences for nutrient cycling and carbon dioxide emission rates (Gessner et al. 2010, Raymond et al. 2013).

Experimental studies have failed to show a clear effect of plant species richness on decomposition rates. Two meta-analyses, including 90 and 84 observations, respectively, found either no effects of richness on decomposition rates (Srivastava et al. 2009), or a significant but small effect (litter mixtures lost 5% more mass than the average monoculture) (Cardinale et al. 2011). Subsequent studies have similarly found a lack of clear effects, and demonstrated that species identity in litter mixtures, rather than species richness per se, is the major influence on decomposition rates (Ferreira et al. 2012, Boyero et al. 2014, Bruder et al. 2014).

The lack of a clear, unidirectional effect of plant species richness on decomposition rate could be related to the wide variety of functional traits contained in different litter mixtures. A

recent study showed that mixing litter with different key traits (acquisition strategies for C and N, and litter recalcitrance) resulted in accelerated C and N loss compared to monocultures, and the pattern was consistent across biomes and ecosystem types (Handa et al. 2014). Specifically, litter diversity effects on C and N loss were largely explained by the interaction between N-fixing plants [which have symbiotic bacteria that fix atmospheric N and make it available to the plant (Franche et al. 2008)] and non-N-fixing plants, which were deciduous and rapid decomposers. These results suggested that N could be transferred from litter of N-fixers to that of non-fixers, possibly through fungal decomposers, which may use the N reservoir of litter from N-fixers and boost the use of high-quality C from litter of non-fixers.

Here we explore the effects of mixing litter from N-fixer and non-fixer plants (hereafter N-fixer and non-fixer litter, respectively) on decomposition rates in a laboratory experiment. We mixed litter from different species of these two functional types and compared their decomposition rates with those of their monocultures in the presence and absence of detritivores. We also manipulated the concentration of inorganic N in the water to investigate whether it affected any interaction between N-fixer and non-fixer litter. We predicted that (1) an increase in litter species richness would promote decomposition due to positive complementarity effects (Boyer et al. 2000), and would enhance detritivore growth through the use of a greater variety of litter types by detritivores (i.e., a balanced diet effect; DeMott 1998); (2) decomposition of N-fixer litter would be faster than that of non-fixer litter, because the higher N content of N-fixer litter promotes the activity of microbial decomposers and detritivores; because of this, detritivore growth would be higher on N-fixer than non-fixer litter; (3) decomposition and detritivore growth would be enhanced in litter mixtures containing both N-fixers and non-fixers, compared to mixtures of a single functional type or to monocultures, because the high N content of N-fixer

litter would boost the use of C from non-fixer litter, resulting in a more efficient use of both resources (as suggested in Handa et al. 2014); and (4) any effects of litter type on decomposition would only occur when N is limiting in the water; when N is not limiting, microbial decomposers would be able to use it (Cheever et al. 2013), and the N contained in N-fixer litter would be superfluous.

METHODS

Plant species and functional types

In Europe, N-fixing plants include several common riparian tree species such as the black alder *Alnus glutinosa* [L.] Gaertn. (Betulaceae) and the exotic black locust *Robinia pseudoacacia* L. (Fabaceae) (hereafter *Alnus* and *Robinia*). Both species are known to greatly increase the N content of soils (Von Holle et al. 2005), and their leaves generally show higher N content than other common riparian species (Alonso et al. 2010, Casas et al. 2013). We used these two species in our experiment, together with two other common riparian species that are not associated with N-fixing bacteria: the black poplar *Populus nigra* L. (Salicaceae) and the grey willow *Salix atrocinerea* Brot. (Salicaceae) (hereafter *Populus* and *Salix*). Litter of these two species generally has low N content (Casas & Gessner 1999), but is similar to the other selected species in terms of C allocation strategies (i.e., they are all deciduous) and recalcitrance [i.e., they all have relatively fast decomposition rates, although *Alnus* decomposes at a faster rate than the other three species (Casas & Gessner 1999, Alonso et al. 2010, Pozo et al. 2011) and has lower lignin content (ca. 12% dry mass for *Alnus*, 15% for *Robinia*, 18% for *Salix* and 23% for *Populus*) (Chauvet 1987, Gallardo & Merino 1992, Alonso et al. 2010)]. The four species selected were among the most common riparian species in the study area.

Freshly fallen leaves were collected from the ground at various locations from the Biscay province, northern Spain (43.22°N 3.27°W; 43.33°N 2.97°W; 43.29°N 2.99°W), in November 2014. In the laboratory, discs of 12-mm diameter were cut from the leaves using a cork borer. As we could not avoid the central nerve when cutting the disks in *Robinia* leaflets (which are < 3 cm wide), we included the nerves in disks of all species, but avoided the widest part next to the petiole. Discs were air-dried and weighed in groups of 10, 20 or 40, to be used in the different experimental treatments.

Leaf quality

We determined the initial leaf quality of each plant species (N and P contents, C:N and N:P ratios, and ash content) to examine its possible influence on our results. Five replicates of 20 discs per species were air dried and ground into powder (1-mm screen) and their initial nutrient contents determined. C and N contents (% of total DM) were determined using a Perkin Elmer series II CHNS/O elemental analyzer (Perkin Elmer, Norwalk, Connecticut), and P content (%) was measured spectrophotometrically after autoclave-assisted extraction (APHA 1998). Five discs per species were oven dried (60°C, 72 h) to determine their DM and then incinerated (550°C, 4 h) to determine their AFDM and calculate ash content (%). We explored differences in leaf quality (N and P content, C:N and N:P ratios, and ash content) with linear models followed by multiple comparisons.

Experimental set up

In May-June 2015 we conducted an experiment in 220 microcosms (8 cm-diameter glass cups) within a controlled-temperature room set at 10°C, which was lower than the average temperature

of streams when detritivores were collected (approx. 13°C) but which significantly reduced evaporation. Each microcosm contained 40 leaf discs that belonged to 1 species (monocultures) or to 2 or 4 species (litter mixtures of all possible species combinations, containing 20 or 10 discs per species, respectively; Fig. 1). Leaf discs of the same species were marked and kept together in 10-disc groups using labelled safety pins, so they could be easily identified at the end of the experiment. For each plant treatment, 10 replicate microcosms included detritivores and 10 did not. Each replicate with detritivores contained three larvae of the caddisfly *Sericostoma pyrenaicum* Pictet, 1865 (Sericostomatidae), which is a common detritivore in the study area. Detritivore biomass per microcosm was on average 28.07 mg (\pm 5.48 SD; Table S3) [i.e., the average individual biomass was approximately 9.4 mg, which corresponds to the last (7th) larval instar in this species (Basaguren et al. 2002)] and did not differ between plant species richness, plant functional type or water N concentration treatments ($p > 0.27$ in all cases; Table S4). Larvae were collected from leaf litter in streams of the Agüera watershed and starved for 48 h prior to the experiment. For each plant/detritivore combination, half of the microcosms contained 250 mL of filtered (100 μ m) stream water (mean \pm standard error of NO₃-N concentration = 0.366 ± 0.010 mgL⁻¹) and the other half contained 250 mL of filtered stream water with added potassium nitrate to elevate N concentration to 5 times the natural concentration (i.e., to 1.835 ± 0.031 mgL⁻¹), which is similar to the highest concentration found in the study area (Barba et al. 2010). Concentration of soluble reactive phosphorus was 9.5 μ g L⁻¹. We added fine sand and pebbles (previously incinerated at 550 °C for 4h and washed to remove ash) to each microcosm to provide environmental heterogeneity and material for caddisfly case construction.

The experiment was run for 24 days. Initially, only the leaf discs were added to the microcosms to allow initial conditioning and leaching of soluble compounds. On day 3 we

replaced the water and added the detritivores. Water was again replaced on days 11 and 18, and the experiment was terminated on day 24. Microcosms were monitored every two days to ensure there was leaf material of every species available during the experimental period. At the end of the experiment, all leaf material was collected (fragments were identified based on colour and morphology), oven dried (60°C, 72 h) to determine dry mass (DM), and then incinerated (550°C, 4 h) to determine ash-free dry mass (AFDM). DM and AFDM showed a very strong relationship ($r^2 = 0.99$, $F_{1,219} = 20055.2$, $p < 0.001$), so only AFDM was used in the analyses. We used 5 additional sets of 40 leaf discs per species to calculate a DM/AFDM correction factor, which was used to estimate initial AFDM of each microcosm. Leaf mass loss due to leaching was not measured during the experiment, but we measured it a posteriori (several months later) on 5 additional sets of 40 leaf discs per species, which were submerged in filtered stream water for 3 days, oven-dried and weighed.

Detritivores were oven dried (60°C, 72 h) to determine their final DM; initial DM was estimated from a case length (CL)/DM relationship, calculated using 26 additional individuals of similar case length to those used in the experiment ($DM = 0.17 \times CL^2 - 2.87 \times CL + 14.15$; $r^2 = 0.96$). Detritivores were ground and analysed in a Perkin Elmer series II CHNS/O elemental analyzer (Perkin Elmer, Norwalk, Connecticut) to determine their C:N ratio; the initial C:N ratio was determined using 5 replicates of 3 individuals from the pool of 26 additional individuals used to estimate initial DM.

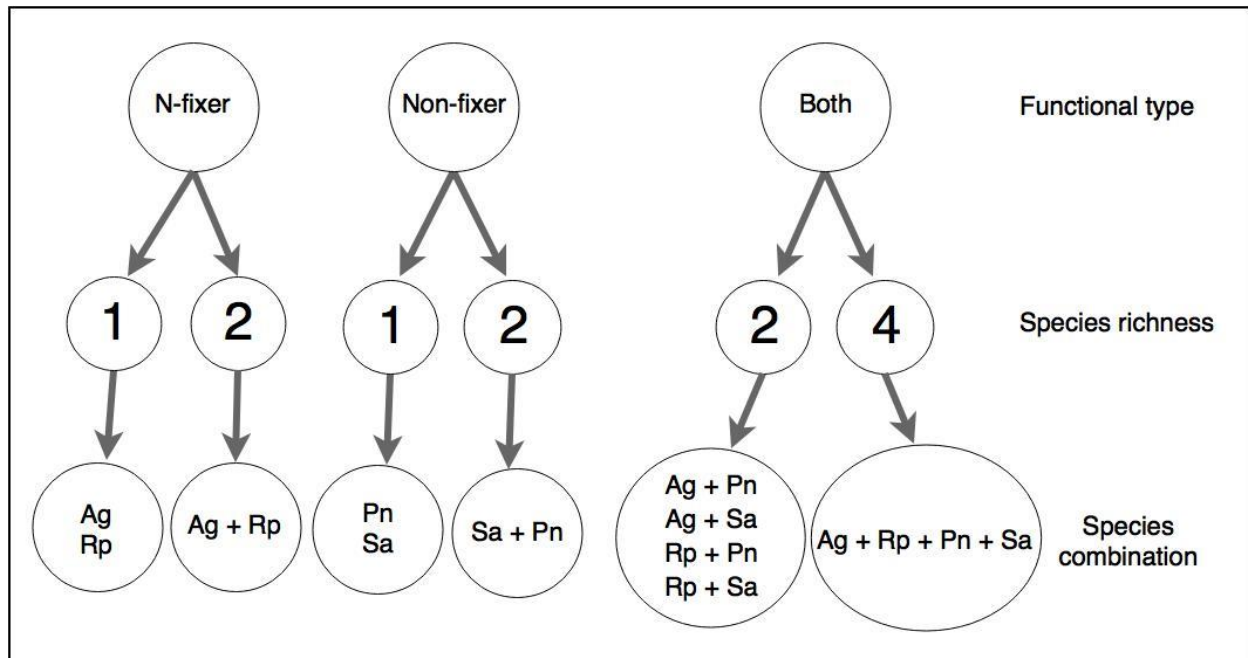


Fig 1. Experimental design with different litter functional types (N-fixer, non-fixer or both), species richness levels (1, 2 or 4 species) and species combinations (Ag, *Alnus glutinosa*; Rp, *Robinia pseudoacacia*; Pn, *Populus nigra*; Sa, *Salix atrocinerea*).

Response variables

Our experiment allowed us to examine the influence of plant species richness, plant functional type (in terms of N-fixing capacity), detritivores (presence and biomass) and water N concentration on litter decomposition rate and detritivore growth. Decomposition rate was estimated through the relative litter mass loss (LML) during the experiment: $LML = (\text{initial AFDM} - \text{final AFDM}) / \text{initial AFDM}$. We calculated LML separately for each plant species in a microcosm, and total LML of all component species in a microcosm. Because the leaf material used in the leaching trial had been stored in the laboratory for several months, apparently increasing leaching (Fig. S1), we did not use LML resulting from this leaching trial to correct initial leaf mass in the experiment, but used the leaching data for comparative purposes among

species. We quantified detritivore growth (DG) as the relative growth during the experiment: $DG = (\text{final DM} - \text{initial DM}) / \text{initial DM}$.

As discs of different plant species were weighed separately, we could also explore the potential mechanisms responsible for any effect of species mixtures on decomposition. We used the additive partitioning method (Loreau & Hector 2001) to measure the *Net Effect* of diversity on decomposition, as well as the relative contribution of a *Complementarity Effect*, which can occur through resource partitioning or from synergistic or antagonistic interactions, and a *Selection Effect*, which arises when the presence of a particular species with high (or low) decomposition rate dominates the rate of decomposition of a mixture (Loreau & Hector 2001, Handa et al. 2014). The net effect was calculated as the difference between the observed LML of a mixture and its expected LML, which was based on LML in the monocultures ($\Delta LML = LML_O - LML_E$). The complementarity effect was calculated as the average deviation from expected LML of species in a mixture multiplied by the mean LML of species in monoculture and the number of species in the mixture ($\text{mean } \Delta LML \times \text{mean } LML \times N$). The selection effect was calculated as the covariance between LML of species in monoculture and their ΔLML multiplied by the number of species [$\text{cov}(\Delta LML, LML) \times N$].

Data analyses

We used linear models to explore variation in leaf mass loss (LML), detritivore growth (DG), and net diversity, complementarity and selection effects in relation to plant species richness (1, 2 and 4 for LML and DG; 2 and 4 for the other variables, as diversity effects are calculated by comparing species mixtures with the monocultures), plant functional type (N-fixer, non-fixer or both), detritivore presence, water treatment (natural or N addition), and the interactions among

these factors. Initial data exploration using Cleveland dot- and boxplots revealed no outliers in the data, so there was no need for transformations (Zuur & Ieno 2015). However, data exploration showed clear differences in the variance of each response variable between detritivore treatments (Fig. S2). For this reason, and to avoid very complex models with many interactions, we examined each of these treatments separately and used a separate model to explore variation in each variable (except DG) between detritivore treatments.

Multi-panel boxplots for each response variable versus species richness and functional type showed that the homogeneity of variances assumption for linear models was violated, requiring the use of a variance structure that takes these differences into account [VarIdent function of ‘nlme’ R package (Pinheiro et al. 2013) in R software (version 3.2.2; R Core Team 2015)]. Detritivore biomass (final DM) was included in the model for microcosms with detritivores, to account for the higher mass loss most likely caused by larger detritivores (Boyero et al. 2014). All variables were treated as categorical except detritivore biomass, which was continuous.

The models were fitted using the gls function (generalized least squares) and restricted maximum likelihood (REML) method in the ‘nlme’ R package. The optimal variance structure was defined by comparing models with different variance structure (using VarIdent), and evaluated using the Akaike information criterion (AIC) using a backward selection procedure. The optimal models allowed residual spread to vary in relation to each species combination (LML and DG), each species and water treatment combination (net diversity, complementarity and selection effects), each species and detritivore presence combination (LML comparing detritivore treatments), or detritivore presence (net diversity, complementarity and selection effects comparing detritivore treatments). Visual exploration of residuals indicated no violation

of the homogeneity assumption. Pairwise multiple comparisons were addressed with Tukey tests using the `glht` function of the ‘`multcomp`’ R package (Hothorn et al. 2008).

We further explored whether results for LML depended on plant species identity in a mixture, using LML data for each plant species. We followed the same steps as above to define the optimal random and fixed structure of models. For these models, we also tested the autocorrelation between species in the same replicate (ID variable), because their LMLs were not independent of each other. Autocorrelation was evaluated with the `acf` function in R, and comparing model improvement with AIC (Zuur et al. 2009). Autocorrelation occurred only when detritivores were present, and was removed by adding a correlation structure to the model (`corCompSymm` function also in the ‘`nlme`’ R package).

RESULTS

Leaf quality

Leaf quality differed among plant species (Table 1): N content was highest for *Alnus* and lowest for *Populus*; P content was highest for *Alnus* and lowest for *Robinia*; the C:N ratio was highest for *Populus* and lowest for *Alnus*; the N:P ratio was highest for *Robinia* and lowest for *Populus*; and ash content was highest for *Populus* and lowest for *Alnus*.

Table 1. Mean (\pm standard error) of nitrogen (N) and phosphorus (P) content (% dry mass), C:N and N:P ratios, and ash content (% dry mass), for each leaf species based on measurements of five replicates. Different letters indicate significant differences on the basis of a linear model followed by pairwise multiple comparisons (significant values $p < 0.05$).

Species	N	P	C:N	N:P	Ash
<i>Alnus glutinosa</i>	2.9 \pm 0.1 ^a	0.10 \pm 0.001 ^a	19.8 \pm 0.2 ^c	62.7 \pm 1.3 ^b	4.59 \pm 1.29 ^d
<i>Robinia pseudoacacia</i>	1.5 \pm 0.03 ^b	0.04 \pm 0.002 ^c	35.2 \pm 0.5 ^b	90.8 \pm 6.6 ^a	13.33 \pm 2.73 ^b
<i>Populus nigra</i>	0.7 \pm 0.03 ^c	0.08 \pm 0.001 ^b	67.4 \pm 2.2 ^a	20.2 \pm 1.0 ^d	15.49 \pm 1.31 ^a
<i>Salix atrocinerea</i>	1.6 \pm 0.1 ^b	0.08 \pm 0.001 ^b	37.7 \pm 2.0 ^b	44.8 \pm 2.9 ^c	7.73 \pm 1.61 ^c

Leaf mass loss

Microcosms had leaf litter present throughout the experiment, except that at the end of the experiment *Alnus* litter was absent from 5% of the microcosms containing this species (Fig. S3). Leaf mass loss (LML) was, on average, more than twice as high when detritivores were present (54%) than when they were absent (25%) ($F_{1,218} = 529.4$, $p < 0.001$). On average, the contribution of detritivores to LML was 68% (± 0.02 SD) and varied from 31% to 89% (Table S5).

When detritivores were present, LML was affected by plant species richness, plant functional type and water N concentration (Tables 2, S1): LML was greater in microcosms having 2 vs. 1, 4 vs. 1 and 4 vs. 2 species (Fig. 2a); it was higher for N-fixers or for both functional types together than for non-fixers (Fig. 2c); and it was higher in microcosms with elevated N concentration (Fig. 2e). As there was a suggestion of weak interaction between species richness and water N concentration ($p = 0.053$; Table 2), we examined the difference between species richness levels separately for natural and elevated N concentrations: at natural N concentration, results were similar to those of total effects; at elevated N concentration, higher LML was only observed for 4 vs. 1 species.

Table 2. Results of linear models testing for effects of plant species richness (1, 2 or 4 species), functional type (N-fixer, non-fixer or both types), water N concentration (natural or elevated), and interactions on relative litter mass loss (LML) in microcosms with and without detritivores (numDF = numerator degrees of freedom; total degrees of freedom: 110).

Term	numDF	F	p
With detritivores			
Intercept	1	6518.7	< 0.0001
Species richness (I)	2	13.6	< 0.0001
Functional type (II)	2	65.0	< 0.0001
Water N concentration (III)	1	15.4	0.0002
I × III	2	3.0	0.0534
Without detritivores			
Intercept	1	8886.7	< 0.0001
Species richness	2	19.6	< 0.0001
Functional type	2	47.7	< 0.0001
Water N concentration	1	15.9	< 0.0001

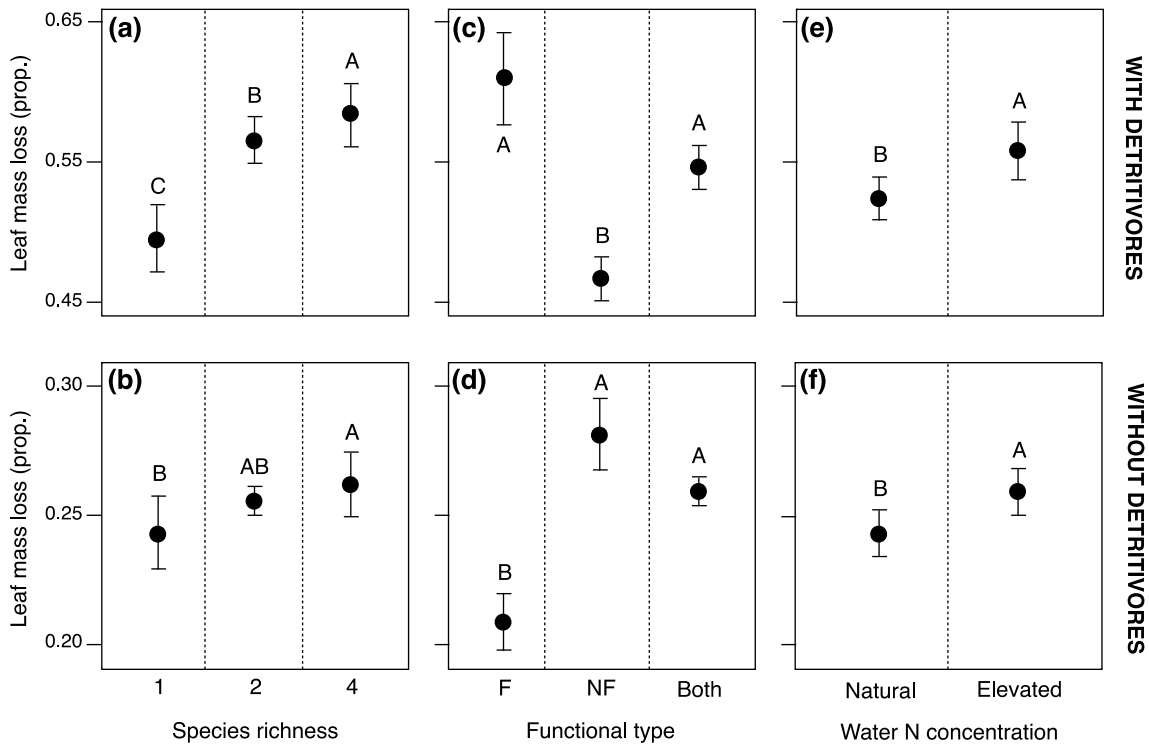


Fig. 2. Relative litter mass loss (LML; mean \pm standard error) in relation to (a, b) species richness (1, 2 or 4 species); (c, d) functional type (F = N-fixer, NF = non-fixer or both); and (e, f) water N concentration (natural or elevated), in the presence (a, c, e) or absence (b, d, f) of detritivores. Different capital letters indicate significant differences between treatments.

When we examined the effect of species identity on LML we found significant differences ($F_{3,203} = 1701.1$, $p < 0.001$): LML was highest for *Alnus* (on average, 84%), intermediate for *Salix* (48%) and *Populus* (47%) and lowest for *Robinia*, (39%) (Fig. 3a). Moreover, there was a significant species identity \times species richness interaction ($F_{6,203} = 6.8$, $p < 0.001$) showing that LML increased with species richness only for *Alnus* (2 vs. 1, 4 vs. 1 and 4 vs. 2 species) and *Salix* (4 vs. 1 species), and a significant species identity \times N concentration interaction ($F_{3,203} = 6.4$, $p < 0.001$), indicating that only *Populus* decomposed faster with elevated N concentration.

When detritivores were absent, LML was also affected by plant species richness, plant functional group and water N concentration (Tables 2, S1): LML increased with 2 vs. 1 and 4 vs. 1 species (Fig. 2b); was higher for non-fixers and for both functional types together than for N-fixers (Fig. 2d); and was higher at elevated N concentration (Fig. 2f). Species identity also affected LML ($F_{3,197} = 239.3$, $p < 0.001$); LML was highest for *Populus* (on average, 37%), intermediate for *Alnus* (24%) and *Salix* (23.0%), and lowest for *Robinia* (16%) (Fig. 3b). The leaching trial performed after the experiment showed that LML due to leaching was highest for *Populus* (on average, 29%), intermediate for *Alnus* (21%) and *Robinia* (21%), and lowest for *Salix* (16%) ($F_{3,16} = 33.4$, $p < 0.001$; Table S2).

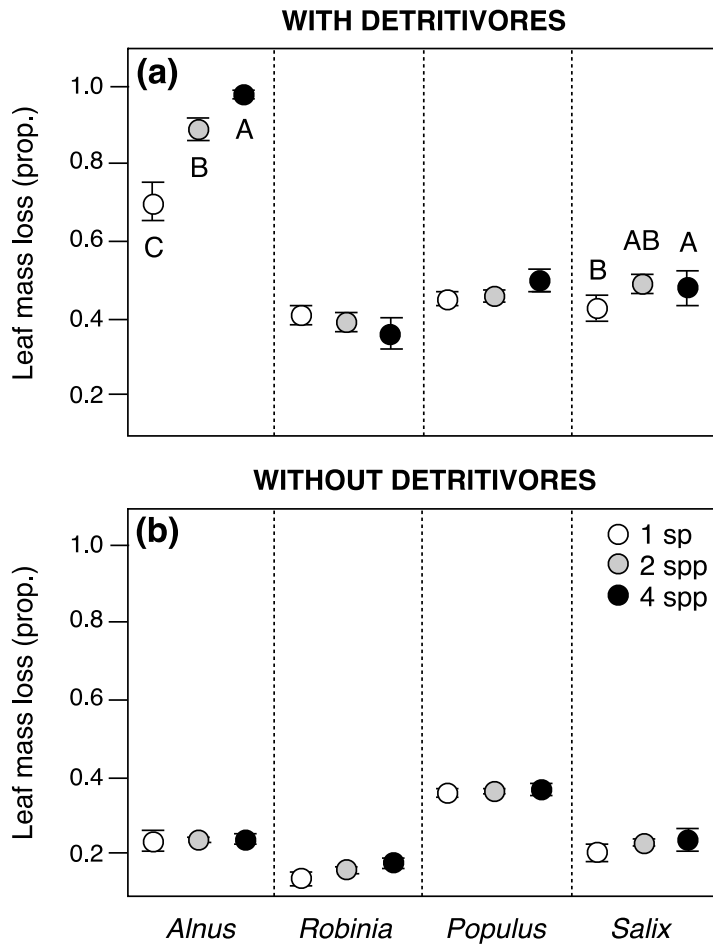


Fig. 3. Relative litter mass loss (LML; mean \pm standard error) of each plant species (*Alnus glutinosa*, *Robinia pseudoacacia*, *Populus nigra* and *Salix atrocinerea*) at different levels of species richness (1, 2 or 4 species) in the presence (a) and absence (b) of detritivores. Different capital letters indicate significant differences between treatments.

Contribution of complementarity and selection to litter mixing effects

Net diversity effects averaged 2.93 (\pm 0.43 standard error), with the additive partitioning showing that complementarity effects (2.41 \pm 0.39) were almost 5-fold higher than selection effects (0.51 \pm 0.08). All effects were higher when detritivores were present than when they were absent ($p < 0.001$ in all cases); on average, net diversity effects were 11 times higher when

detritivores were present (5.32 ± 0.76 vs. 0.47 ± 0.12), complementarity was 10 times higher (4.34 ± 0.69 vs. 0.43 ± 0.12), and selection was 29 times higher (0.98 ± 0.14 vs. 0.03 ± 0.02).

When detritivores were present, increased species richness (from 2 to 4) resulted in higher net diversity (Fig. 4a), complementarity (Fig. 4b) and selection effects (Fig. 4c). Plant functional type also had positive net diversity effects for N-fixers vs. non-fixers and both types together (Fig. 4d); positive complementarity effects for N-fixers vs. both types together (Fig. 4e); and selection effects, dependent on N concentration (Fig. 4f): at natural N concentration, selection was positive and higher for N-fixers than for non-fixers and both types together; at elevated N concentration, selection was higher for N-fixers and for both types together (both positive) than for non-fixers (negative) (Tables 3, S1).

When detritivores were absent, net diversity effects depended on water N concentration: at natural concentration, the effect increased but became negative in 4-species mixtures; at elevated concentration, the effect was positive in all cases and increased from 2- to 4-species mixtures (Fig. 4g). Complementarity effects showed the same trend as net diversity effects (Fig. 4h), and selection effects increased with species richness but were very close to zero (Fig. 4i). Plant functional type affected net diversity effects, which were positive in all cases, being higher for non-fixers than for N-fixers (effect close to zero) and intermediate when both types were present (Fig. 4j). Complementarity effects showed a similar trend but there were no significant differences among functional types (Fig. 4k). Selection effects again depended on N concentration (Fig. 4i): at natural concentration the effect was higher (but negative) for N-fixers than for both types together (close to zero), and intermediate (positive) for non-fixers; at elevated concentration, the effect was higher (positive) for both types together than for N-fixers (close to zero) and non-fixers (negative) (Tables 3, S1).

Table 3. Results of linear models testing for effects of plant species richness (1, 2 or 4 species), functional type (N-fixer, non-fixer or both types), water N concentration (natural or elevated), and interactions on net diversity, complementarity and selection effects in microcosms with and without detritivores (numDF = numerator degrees of freedom; total degrees of freedom of model with detritivores = 71; total degrees of freedom of model without detritivores = 69).

Term	numDF	F	p
With detritivores			
Net diversity			
Intercept	1	75.8	< 0.0001
Species richness	1	12.9	< 0.0001
Functional type	2	26.7	< 0.0001
Complementarity			
Intercept	1	39.2	< 0.0001
Species richness	1	6.7	0.0119
Functional type	2	9.9	0.0002
Selection			
Intercept	1	81.4	< 0.0001
Species richness (I)	1	29.6	< 0.0001
Functional type (II)	2	44.6	< 0.0001
Water N concentration (III)	1	10.1	0.0023
II × III	2	11.5	0.0001
Without detritivores			
Net diversity			
Intercept	1	19.0	< 0.0001
Species richness (I)	1	0.1	0.7941
Functional type (II)	2	4.3	0.0170
Water N concentration (III)	1	17.9	0.0001
I × III	1	5.0	0.0294
Complementarity			
Intercept	1	18.3	0.0001
Species richness (I)	1	0.1	0.7973
Water N concentration (II)	1	10.6	0.0017
I × II	1	4.6	0.0353
Selection			
Intercept	1	71.9	< 0.0001
Species richness (I)	1	19.5	< 0.0001
Functional type (II)	2	68.1	< 0.0001
Water N concentration (III)	1	48.7	< 0.0001
II × III	2	5.0	0.0101

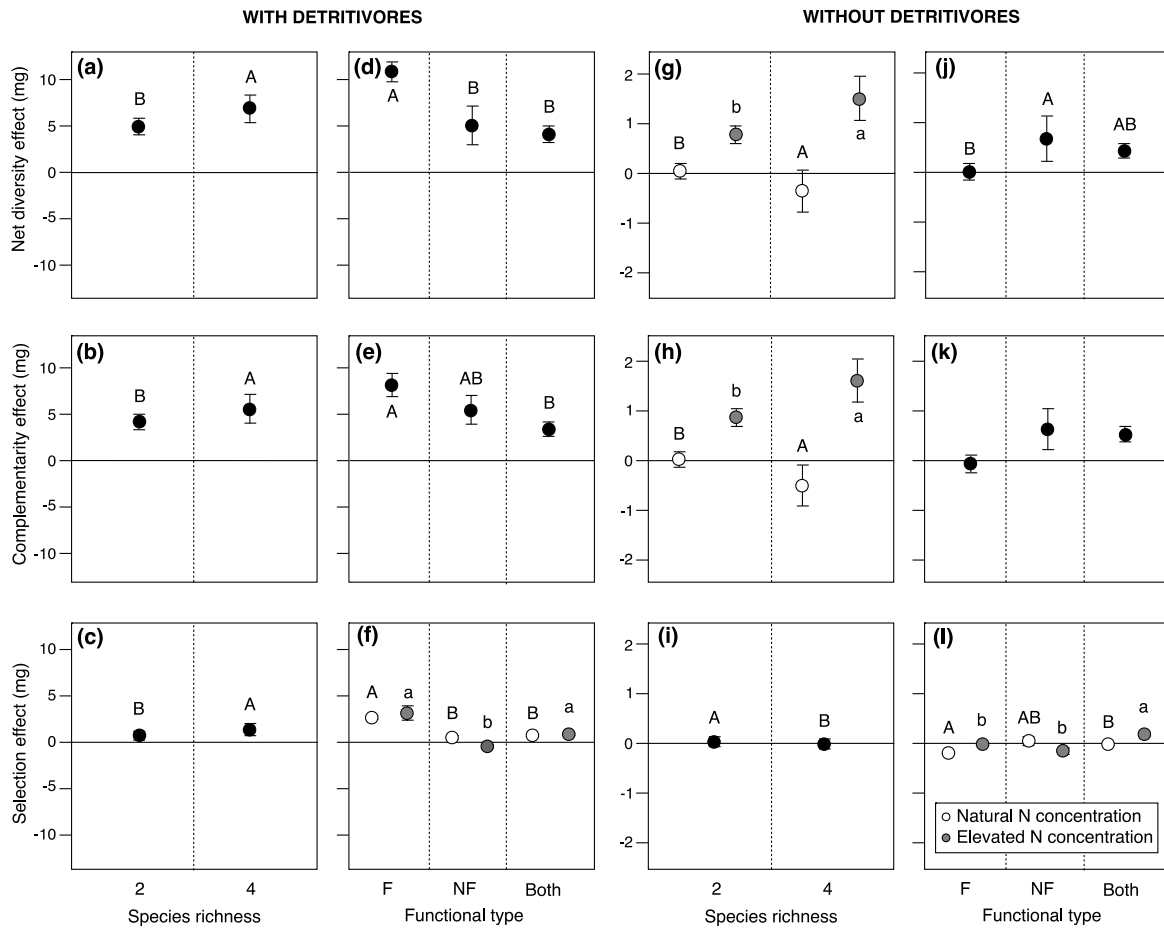


Fig 4. Net diversity effects (top panels), complementarity effects (middle panels) and selection effects (bottom panels) of plant litter mixtures (mean \pm standard error) on LML for different levels of species richness (a,b,c,g,h,i), functional type (d,e,f,j,k,l) and water N concentration (different coloured dots in panels f,g,h,l). Explanation of treatments as in Fig. 1. Different capital letters indicate significant differences between treatments; when the species richness (or functional type) \times water N concentration interaction was significant, capital and non-capital letters were used to denote significant differences within each water N concentration.

Detritivore growth and C:N ratios

Detritivore growth was not affected by plant species richness or functional type, but decreased at elevated N concentration ($F_{1,110} = 5.3$, $p = 0.0234$; Table S1). Detritivore C:N ratios were ~ 1.2 times lower before than after the experiment (5.54 vs. 6.59; $t = -2.71$, $p = 0.0078$), but they were

not affected by plant species richness, functional type or water N concentration (the final model only retained the factor ‘water N concentration’, which was not significant: $F_{1,110} = 1.6$, $P = 0.2022$; Tables S1, S3).

DISCUSSION

1. Plant litter diversity enhances decomposition through complementarity effects

Our results showed that decomposition was faster for litter mixtures than for monocultures, supporting our first hypothesis that litter species richness would promote decomposition. This occurred whether detritivores were present or absent, indicating that microbial decomposers (and possibly detritivores) increased their activity at higher levels of litter diversity. This result contrasts with some previous reports that litter mixing influences detritivores but not microbial decomposers (Swan & Palmer 2004, Sanpera-Calbet et al. 2009). A possible mechanism behind litter mixture effects on microbial decomposition is the active microbial transfer of nutrients among litter types (Gessner et al. 2010), including transfer from litter of N-fixing plants to that of non-fixing, rapidly decomposing plants (Handa et al. 2014). Although we were unable to explore the mechanisms behind litter mixing effects on microbial decomposition, we showed that these effects could vary depending on nutrient concentration in the water, as explained below.

Nevertheless, detritivores played an important role in mediating diversity effects, which were more than 10 times stronger in the presence of detritivores than in their absence. Moreover, when detritivores were present, diversity effects were always stronger at higher levels of diversity (i.e., in 4-species litter mixtures compared to 2-species mixtures), and were mostly due to positive complementarity effects. Positive complementarity can occur through resource partitioning or synergistic interactions (facilitation), although it is difficult to distinguish between these mechanisms (Loreau & Hector 2001). Our results demonstrate, however, that increased

rates of decomposition in litter mixtures were not linked to enhanced detritivore growth, thus not supporting our hypothesis of a balanced diet effect. It is possible that all litter combinations provided sufficient resources for maximum detritivore growth in all cases (Boersma & Elser 2006), or the low concentration of phosphorus may have prevented growth (Frost et al. 2006).

2. Plant N-fixing capacity does not drive differences in decomposition

Our results only partly supported our second hypothesis, which predicted that litter of N-fixers would decompose faster than that of non-fixers and that detritivore growth would be higher on N-fixers. Detritivore growth was similar between functional types, and decomposition was higher on N-fixers only when detritivores were present, mostly because detritivores preferentially fed on *Alnus*, which had the highest quality leaves (greatest N and P content and lowest ash content). *Alnus* is known to decompose faster than many other riparian species, with and without detritivores (Hladyz et al. 2010, Bruder et al. 2014), and the presence of *Alnus* causes litter mixtures to decompose faster than expected (Leroy & Marks 2006, Taylor et al. 2007, Ferreira et al. 2012). In contrast, when detritivores were absent, decomposition was faster on non-fixers, mainly because *Populus* decomposed faster than the other species. *Populus* had the highest C:N ratio and the lowest N:P ratio, suggesting that microorganisms use these leaves to select P over N and thus overcome possible stoichiometric imbalances (Gessner et al. 2010). We note that the higher decomposition of *Populus* could have been partly due to higher leaching, as indicated by the leaching trial conducted a posteriori. However, *Populus* lost on average 51% more mass than other species in the leaching trial, and 80% more mass than other species in experimental microcosms without detritivores; this difference suggests that microbial decomposition was in fact higher for *Populus* than for the other species.

Robinia decomposed more slowly than other species. *Robinia* is a North American N-fixing species that has been introduced to many countries (Contu 2012) and is commonly found in riparian forests in the Iberian peninsula (Castro-Díez et al. 2011). It is unlikely that the exotic nature of *Robinia* unduly influenced the results, as microbial decomposers and detritivorous caddisflies are typically able to process leaves of mixed provenance (Hladyz et al. 2009, Boyero et al. 2012a, Makkonen et al. 2012). Moreover, its lignin content is generally lower than that of *Salix* and *Populus* (see above). It is possible, however, that *Robinia* litter had higher content of condensed tannins (Horigome et al. 1988) that could suppress microbial assimilation and deter detritivores from feeding (Gessner et al. 2010). Moreover, *Robinia* had the lowest P content, and its N content was lower than that of *Alnus* and more similar to that of *Salix*. Although we would have expected *Salix* to have lower N concentration than the N-fixing species, others have reported values similar to ours (Escudero et al. 1992).

These results suggest that decomposition varied among species because of differences in a combination of litter traits, rather than to their N-fixing capacity alone. Mixtures of litter of N-fixers and non-fixers did not increase decomposition rates or detritivore growth, contrasting with findings of Handa *et al.* (2014), the basis of our third hypothesis. However, we have shown that the presence of more refractory (or less preferred) species in litter mixtures can enhance the decomposition of faster decomposing species, possibly because of a greater concentration of decomposers or detritivores on their preferred resource, as suggested by Sanpera-Calbet, Lecerf & Chauvet (2009). Decomposition of the preferred resource (here *Alnus* and, to a lesser extent, *Salix*) may have been enhanced in litter mixtures compared to monocultures (and in 4-species compared to 2-species mixtures) because detritivores processed smaller fragments or even minor leaf nerves of the preferred resource as it became scarce, in preference to the mesophyll of the

more recalcitrant leaves. However, the decomposition of less preferred resources (*Robinia* and *Populus*) when detritivores were present was not reduced in litter mixtures compared to monocultures, suggesting that detritivores also exploited these resources. This contrasts with evidence of slower decomposition of recalcitrant species in litter mixtures (Swan & Palmer 2006). It is likely that functional evenness of litter mixtures (i.e., the relative abundance of different litter types) is at least as important as the number of litter types or species in mediating leaf diversity effects (Sanpera-Calbet et al. 2009).

3. Water N concentration modulates plant litter effects on microbial decomposition

Although our results did not support our fourth hypothesis, they showed that litter diversity effects on microbial decomposition were modulated by water N concentration: net diversity and complementarity effects were positive only at elevated N concentration and became negative in 4-species mixtures at natural N concentration. This suggests that microbial nutrient transfer that causes litter diversity effects (Gessner et al. 2010, Handa et al. 2014) is enhanced when N is readily available in the water.

Faster decomposition at elevated N concentration demonstrated that microorganisms were able to use N from the water, although the effect was only evident for *Populus*. The fact that *Populus* litter had the lowest N content and N:P ratio in our study, and its decomposition was enhanced at elevated water N concentration, suggests that microorganisms were able to use N from the water and P from *Populus* litter (P is more easily leached from litter than N; Gessner 1991) and thus overcome nutrient imbalances and maximize decomposition. In any case, we note that as our experiment lacked microbial inoculation, the only source of fungal spores was the stream water, so microorganisms were probably underrepresented compared to other laboratory

experiments (e.g., Ferreira & Chauvet 2011, Gonçalves et al. 2014a). This might explain the lack of enhanced decomposition at elevated N concentration for most plant species.

Faster decomposition at elevated N concentration was not accompanied by enhanced detritivore growth, which was actually impaired, possibly because nutrient excess (and thus stoichiometric imbalance) can cause metabolic costs through increased excretion rates, slowing down growth even when nutrient availability is higher (Boersma & Elser 2006). C:N ratios did not differ across treatments, but were higher at the end of the experiment, indicating that detritivores had lower N content than initially. This could occur if detritivores initially had higher quality conditioned leaf material from the stream in their guts than the leaf discs offered during the experiment. However, all C:N ratios fell within the range reported for various detritivores (Hladyz et al. 2009).

CONCLUSIONS

Overall, our results provide evidence that litter diversity enhances decomposition through complementarity effects, which are mediated by both microbes and detritivores. Although litter mixing effects on decomposition have been shown previously, our results further suggest that (1) microbes are important in mediating diversity effects on decomposition, although detritivore-mediated effects are stronger; (2) detritivores enhance the decomposition of their preferred resource in litter mixtures but also process less-preferred resources at rates similar to those in monocultures; (3) the plant N-fixing capacity does not drive differences in decomposition, which rather depends on a combination of litter traits; and (4) water N concentration modulates plant litter diversity effects on decomposition through microbial activity.

Our findings suggest that plant diversity loss in riparian forests would have different consequences for in-stream litter decomposition depending on the stream nutrient status as well as the nutritional quality of the remaining litter. It is possible, however, that nutrient enrichment of streams causes the homogenization of nutrient contents of different types of litter, with litter C:N and C:P ratios tending to be generally lower and more similar (Manning et al. 2016). How these changes in litter nutritional quality would affect plant diversity effects on microbial and detritivore-mediated decomposition remains unexplored. We have shown that plant diversity effects on decomposition mediated by detritivores are stronger than those mediated by microorganisms, but microbial processes could become important in streams of high nutrient status, where detritivore populations might be impaired (Woodward et al. 2012). Laboratory experiments like ours are indicative of likely scenarios, but are limited by the selection of species and treatments. Comparable in-stream experiments are the next step in understanding real world scenarios and, ideally, would need to be run at multiple sites globally to enable broad generalisations about the results (Boyero et al. 2011b).

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DATA ACCESSIBILITY

Data are available in the electronic supplementary material and the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.1k7tr> (Tonin et al, 2017).

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SUPPORTING INFORMATION

Table S1. Summary of backward model selection based on the Akaike information criterion (AIC) for leaf mass loss, net diversity effects, complementarity effects and selection effects in microcosms with and without detritivores, and detritivore growth and C:N ratios in microcosms with detritivores. The *p*-value refers to the comparison between 1st and 2nd, 2nd and 3rd model, and so on; non-significant *p*-values (*p* > 0.05) indicate that both models are similar. SR, plant species richness (1, 2 or 4); FT, functional type (N-fixer, non-fixer or both types); WN, Water N concentration (natural or elevated); DM, detritivore biomass (dry mass at the end of the experiment, in mg).

	Model	DF	AIC	<i>p</i>
	Leaf mass loss – With detritivores			
1	SR + FT + WN + DM + SR:WN + FT:WN	22	-173.4	
2	SR + FT + WN + DM + SR:WN	20	-175.8	0.469
3	SR + FT + WN + SR:WN	19	-176.9	0.341
	Leaf mass loss – Without Detritivores			
1	SR + FT + WN + SR:WN + FT:WN	21	-357.5	
2	SR + FT + WN + SR:WN	19	-361.3	0.877
3	SR + FT + WN	17	-361.6	0.162
	Net Diversity – With detritivores			
1	SR + FT + WN + SR:WN + FT:WN	15	459.9	
2	SR + FT + WN + SR:WN	13	457.2	0.530
3	SR + FT + WN	12	455.4	0.652
4	SR + FT	11	453.9	0.453
	Net Diversity – Without detritivores			
1	SR + FT + WN + SR:WN + FT:WN	15	201.6	
2	SR + FT + WN + SR:WN	13	201.7	0.126
	Complementarity – With Detritivores			
1	SR + FT + WN + SR:WN + FT:WN	15	457.0	
2	SR + FT + WN + SR:WN	13	454.2	0.568
3	SR + FT + WN	12	452.5	0.563
4	SR + FT	11	451.2	0.397
	Complementarity – Without detritivores			
1	SR + FT + WN + SR:WN + FT:WN	15	202.7	
2	SR + FT + WN + SR:WN	13	202.9	0.119
3	SR + WN + SR:WN	11	202.4	0.172
	Selection – With detritivores			
1	SR + FT + WN + SR:WN + FT:WN	15	160.5	
2	SR + FT + WN + FT:WN	13	159.9	0.234

	Model	DF	AIC	p
Selection – Without detritivores				
1	SR + FT + WN + SR:WN + FT:WN	15	-113.3	
2	SR + FT + WN + FT:WN	13	-115.2	0.815
Detritivore growth				
1	SR + FT + WN + SR:WN + FT:WN	21	-7.8	
2	SR + FT + WN + FT:WN	19	-9.4	0.302
3	SR + FT + WN	17	-11.1	0.315
4	SR + WN	15	-13.6	0.473
5	WN	13	-15.2	0.301
Detritivore C:N ratios				
1	SR + FT + WN + SR:WN + FT:WN	11	192.1	
2	SR + FT + WN + SR:WN	9	189.1	0.621
3	SR + FT + WN	7	187.4	0.305
4	SR + WN	5	183.7	0.888
5	WN	3	182.3	0.268

Table S2. Mean (\pm standard error) relative leaf mass loss due to leaching for each plant species. Different letters indicate significant differences ($p < 0.05$) examined with a linear model followed by pairwise multiple comparisons.

Species	Leaf mass loss
<i>Alnus glutinosa</i>	0.21 \pm 0.09 ^b
<i>Robinia pseudoacacia</i>	0.21 \pm 0.10 ^b
<i>Populus nigra</i>	0.29 \pm 0.13 ^a
<i>Salix atrocinerea</i>	0.16 \pm 0.07 ^c

Table S3. Mean (\pm standard error) detritivore case length (mm), initial biomass (mg; sum of the three individuals in each microcosm), carbon:nitrogen ratio (C:N) and growth rate (proportion) for each plant species combination (Ag, *Alnus glutinosa*; Rp, *Robinia pseudoacacia*; Pn, *Populus nigra*; Sa, *Salix atrocinerea*), plant species richness level (1, 2 or 4 species), plant functional type (N-fixer, non-fixer or both types) and water N concentration (natural or elevated). Biomass was estimated from a case length – body dry mass relationship (see Methods for additional details); the initial C:N ratio was measured on 15 additional individuals.

Treatment	Case length	Biomass	C:N	Growth rate
Initial			5.54 \pm 0.38	
Plant species combination				
Ag	14.39 \pm 0.25	25.94 \pm 1.70	6.72 \pm 0.11	0.29 \pm 0.08
Ag + Pn	14.70 \pm 0.20	27.86 \pm 1.27	6.55 \pm 0.13	0.13 \pm 0.05
Ag + Rp	14.43 \pm 0.32	26.51 \pm 2.14	6.52 \pm 0.16	0.19 \pm 0.07
Ag + Sa	14.48 \pm 0.26	27.57 \pm 1.66	6.87 \pm 0.17	0.15 \pm 0.09
Ag + Sa + Pn + Rp	14.79 \pm 0.23	28.36 \pm 1.43	6.56 \pm 0.16	0.09 \pm 0.06
Pn	14.62 \pm 0.34	28.29 \pm 2.13	6.45 \pm 0.21	-0.07 \pm 0.04
Rp	14.60 \pm 0.30	27.40 \pm 1.87	6.07 \pm 0.15	-0.06 \pm 0.05
Rp + Pn	15.13 \pm 0.13	31.78 \pm 0.96	6.87 \pm 0.16	-0.11 \pm 0.05
Rp + Sa	14.70 \pm 0.30	27.96 \pm 1.99	6.38 \pm 0.18	0.02 \pm 0.06
Sa	14.70 \pm 0.35	28.75 \pm 2.27	6.72 \pm 0.15	0.09 \pm 0.04
Sa + Pn	14.53 \pm 0.22	28.11 \pm 1.46	6.78 \pm 0.18	0.04 \pm 0.06
Plant species richness				
1	14.58 \pm 0.15	27.64 \pm 0.98	6.48 \pm 0.09	0.06 \pm 0.04
2	14.66 \pm 0.10	28.30 \pm 0.67	6.66 \pm 0.07	0.07 \pm 0.03
4	14.79 \pm 0.23	28.36 \pm 1.43	6.56 \pm 0.16	0.09 \pm 0.06
Plant functional type				
N-fixer	14.45 \pm 0.26	26.29 \pm 1.06	6.61 \pm 0.10	0.13 \pm 0.05
Non-fixer	14.64 \pm 0.15	28.28 \pm 0.96	6.50 \pm 0.10	0.02 \pm 0.03
Both	14.80 \pm 0.11	29.13 \pm 0.70	6.64 \pm 0.07	0.06 \pm 0.03
Water N concentration				
Natural	14.58 \pm 0.12	27.99 \pm 0.76	6.65 \pm 0.08	0.10 \pm 0.03
Elevated	14.72 \pm 0.11	28.15 \pm 0.73	6.52 \pm 0.07	0.03 \pm 0.03
Both	14.80 \pm 0.11	29.13 \pm 0.70	6.64 \pm 0.07	0.06 \pm 0.03

Table S4. Summary of linear model testing for differences in initial biomass of detritivores subjected to different treatments of plant species richness (1, 2 or 4 species), plant functional type (N-fixer, non-fixer or both) and water N concentration (natural vs. elevated); numDF = numerator degrees of freedom; total degrees of freedom: 104.

Species	numDF	F	p
Intercept	1	3309.8	< 0.001
Plant species richness	2	0.747	0.476
Plant functional type	2	1.323	0.271
Water N concentration	1	0.106	0.746

Table S5. Mean (\pm standard error) contribution of detritivores to leaf mass loss (prop.) of different plant species combinations (Ag, *Alnus glutinosa*; Rp, *Robinia pseudoacacia*; Pn, *Populus nigra*; Sa, *Salix atrocinerea*). Leaf mass loss was calculated as described in Methods, where initial mass resulted from multiplying the initial mass of each microcosm with detritivores by the mean leaf mass loss (prop.) of microcosms without detritivores within each treatment.

Species combination	Leaf mass loss
Ag	83.5 \pm 3.0
Ag + Pn	74.3 \pm 3.9
Ag + Rp	89.3 \pm 1.3
Ag + Sa	75.3 \pm 3.3
Ag + Sa + Pn + Rp	74.2 \pm 2.3
Pn	31.1 \pm 3.8
Rp	74.8 \pm 2.5
Rp + Pn	48.3 \pm 2.6
Rp + Sa	70.1 \pm 4.2
Sa	62.5 \pm 5.5
Sa + Pn	61.9 \pm 3.7

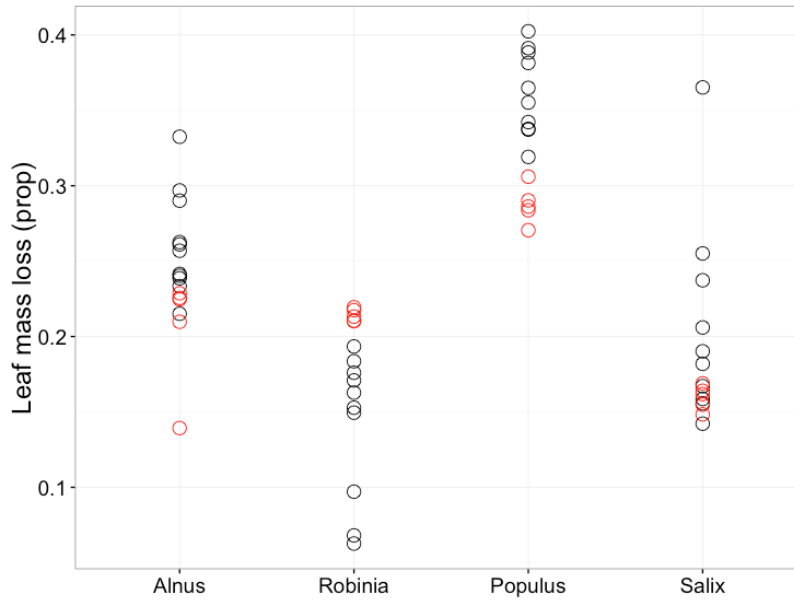


Fig. S1. Leaf mass loss in experimental microcosms with plant monocultures (black circles) and in replicates of the leaching trial conducted several months after the experiment (red circles). Note that, for Robinia, leaf mass loss was higher in the leaching trial (which lasted 3 days) than in the main experiment (which lasted 24 days), which suggested that the leaf material that had been stored for months had suffered physical and/or chemical changes that accelerated the leaching of soluble compounds. For this reason, we did not use the leaching data to correct initial leaf mass in experimental microcosms, but rather used them for comparative purposes among species.

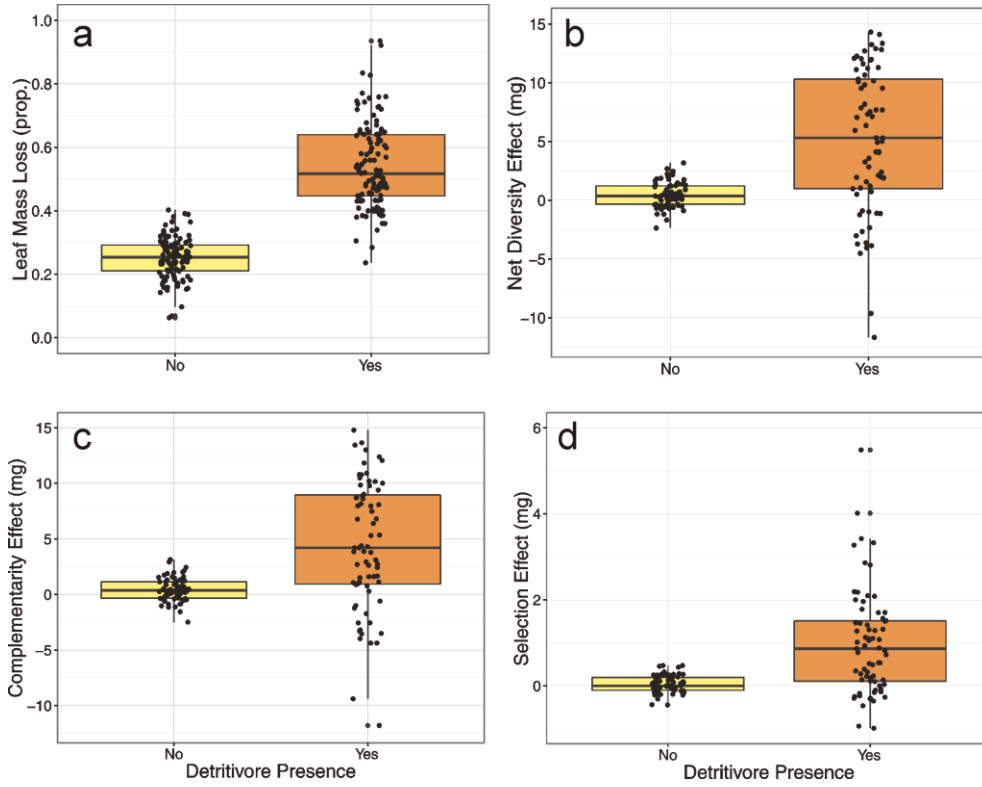


Fig.S2. Boxplots of relative leaf mass loss (a), net diversity (b), complementarity (c) and selection effects (d) in relation to detritivore presence. Note the different variance of the two treatments.



Fig. S3. Relative leaf mass loss in different microcosms. Each dot represents leaf mass loss of a particular species in each microcosm (Ag, *Alnus glutinosa*; Rp, *Robinia pseudoacacia*; Pn, *Populus nigra*; Sa, *Salix atrocinerea*).

CAPÍTULO IV

Interactions between large and small detritivores influence how biodiversity impacts litter decomposition

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ABSTRACT

1. Understanding how biodiversity loss influences litter decomposition is crucial to predict changes in ecosystem functioning, because 90% of plant biomass production enters the detrital pool and is ultimately decomposed. The relationship between detritivore diversity and decomposition is particularly uncertain, as experimental studies have found contrasting results.

2. We predicted that differences in detritivore body size would determine interspecific interactions and thus would be key for predicting effects of detritivore diversity on decomposition. We expected that larger species would facilitate smaller species through the production of smaller litter fragments, resulting in faster decomposition and greater growth of smaller species in polycultures containing species of different body size.

3. We examined these hypotheses in a microcosm experiment where we manipulated detritivore diversity and body size simultaneously using two small (*Leuctra geniculata* and *Lepidostoma hirtum*) and two large detritivore species (*Sericostoma pyrenaicum* and *Echinogammarus berilloni*) in all possible 1-, 2- and 4-species combinations, and litter discs of *Alnus glutinosa*. We explored how decomposition was affected by different interspecific interactions and the role of body size using a set of ‘diversity-interaction’ models, and quantified the magnitude of such effect through ratios of decomposition rates and detritivore growth between polycultures and monocultures.

4. We found a clear positive effect of detritivore diversity on decomposition, which was mainly explained by facilitation of small animals by larger ones (which enhanced decomposition by 12% compared to monocultures) and niche partitioning between large species (19% increase). Facilitation was evidenced by the higher growth of small species in polycultures containing large species with the former feeding on fine particulate organic matter produced by larger animals. In

contrast, large detritivores fed on different parts of litter discs (only one species being able to eat less palatable parts), which resulted in faster decomposition in polycultures with no changes in growth.

5. We conclude that body size is a key animal trait that should be taken into account in diversity-decomposition studies. These should also consider differences in species' vulnerability to extinction depending on body size and how this might affect ecosystem functioning in different scenarios of detritivore diversity and more complex food webs.

Key-words: body size, detritivore assemblages, ecosystem functioning, facilitation, resource partitioning, species richness, streams.

INTRODUCTION

Rapid loss of biodiversity is of major global concern, partly because of its potential consequences for ecosystem processes and the services they provide to humans (Cardinale et al. 2012). Motivated by this concern, hundreds of experimental studies have been conducted across a wide variety of organisms and systems and have confirmed that changes in species richness can alter key ecosystem process rates (Balvanera et al. 2006, Cardinale et al. 2006, Cardinale et al. 2011). However, evidence differs for different ecosystem processes: while it is well established that plant diversity boosts primary production, the relationship between diversity loss and plant litter decomposition is unclear, as shown by the variable results of different studies (Cardinale et al. 2011). Understanding this relationship is a crucial research goal if we are to predict the consequences of diversity loss on global carbon and nutrient cycles, as 90% of the plant biomass produced annually becomes dead plant litter and most of it is ultimately decomposed (Gessner et al. 2010).

Decomposition is a process that involves multi-trophic biological interactions (Scherer-Lorenzen 2008) and thus can be affected by the diversity of plants, microbes and detritivores (Gessner et al. 2010). While there is evidence that detritivore diversity has stronger effects on decomposition than plant diversity (Srivastava et al. 2009), the underlying biological mechanisms are better known for plant diversity (e.g., Handa et al. 2014). This is partly because of the existence of a statistical technique ('additive partitioning') which allows partitioning plant diversity effects into complementarity and selection effects (Loreau & Hector 2001). This technique cannot be applied to investigate effects of detritivore diversity because the contribution of different species to decomposition in an assemblage cannot be separated (Kirwan et al. 2009).

It is thus critical to develop new methods that identify the most plausible mechanisms underlying detritivore diversity effects on decomposition.

Within a detritivore assemblage, the observed net diversity effect on decomposition will depend on a balance between positive and negative interactions between species. The former may include resource partitioning (which can arise if different species exploit litter differently in space or time; Schoener 1974, Fynke & Snyder 2008), facilitation (if a species enhances the performance of another species or both enhance each other's performances; Bruno et al. 2003) and a positive selection effect (if a species with large effects on decomposition dominates the assemblage; Fox 2005), while negative effects are often associated with competition (mainly when one species is a dominant competitor or shows aggressive behaviour; Creed et al. 2009) and a negative selection effect (if a competitively dominant species does not contribute significantly to decomposition; Jiang et al. 2008). Within this context, body size is a relevant animal trait because it is related to (1) ingestion rates and mass-specific metabolic rates (Brown et al. 2004), (2) foraging behaviour (Petchey et al. 2008) and (3) interspecific interactions including trophic relationships, competition and facilitation (Woodward et al. 2005). Remarkably, interspecific differences in body size have not been taken into account when exploring detritivore diversity effects on decomposition.

We explored how detritivore diversity loss affected litter decomposition in stream microcosms, and investigated the potential biological mechanisms underlying such effects, with a suite of methods used novelly in this context. By manipulating detritivore species body size, and using a set of statistical models ('diversity-interactions models') that explicitly take into account the role of species interactions and differences in body size (Kirwan et al. 2009), we tested the hypotheses that diversity enhances decomposition when species differ in body size

because litter processing by larger detritivores facilitates processing by smaller species through the production of smaller litter fragments (hypothesis 1), while diversity has no effect on decomposition when different species in the assemblage are of similar size because they are functionally similar (hypothesis 2). Unlike the additive partitioning method, this approach does not require measuring the contribution of each species in a polyculture, but identifies the most parsimonious description of diversity effects. Further, we examined the magnitude of diversity effects on decomposition using the ratio of decomposition rates in polycultures:monocultures (an analogue of response ratios), and repeated the procedure with growth rates, as we expected that they would be enhanced in smaller detritivores when facilitation by larger detritivores occurred (hypothesis 3). Lastly, we investigated the nature of detritivore interactions by observing the feeding modes and foraging behaviours of large and small species, and behavioural differences between monocultures and polycultures that might indicate the existence of facilitation.

METHODS

Detritivore species

We selected four common detritivore species in our study area (the Agüera catchment in northern Spain, 43°N 3°W) to represent ‘small’ and ‘large’ organisms. Small detritivores were the stonefly *Leuctra geniculata* Stephens, 1835 (Leuctridae) and the caddisfly *Lepidostoma hirtum* Fabricius, 1775 (Lepidostomatidae) (hereafter *Leuctra* and *Lepidostoma*); large detritivores were the caddisfly *Sericostoma pyrenaicum* Pictet, 1865 (Sericostomatidae) and the amphipod *Echinogammarus berilloni* Catta, 1878 (Gammaridae) (hereafter *Sericostoma* and *Echinogammarus*) (Riaño 1998, Basaguren et al. 2002, Larrañaga et al. 2014). Average body dry mass \pm SE was 0.7 ± 0.1 mg for *Leuctra*, 2.3 ± 0.1 mg for *Lepidostoma*, 7.5 ± 0.2 mg for

Sericostoma and 6.1 ± 0.1 mg for *Echinogammarus*. Detritivores were collected in June 2015 from leaf litter in streams. They were transported in aerated containers within a cooler and kept in a controlled-temperature room set at 10°C, which was lower than the average temperature of streams when detritivores were collected (approx. 13°C) but which significantly reduced evaporation during the experiment. Detritivores were starved for 48 h prior to the experiment.

Experimental set-up

Our experiment included all possible 1, 2 and 4 species combinations, which resulted in 11 treatments (i.e., 4 monocultures; six 2-species polycultures, 2 with 1 and 4 with 2 body-size categories; and the single 4-species polyculture), plus a control with no detritivores (Fig. 1). All microcosms (except controls) had 8 detritivore individuals in total (i.e., 2- and 4-species polycultures had 4 and 2 individuals per species, respectively). Each treatment (including controls) was replicated 10 times, resulting in 120 microcosms.

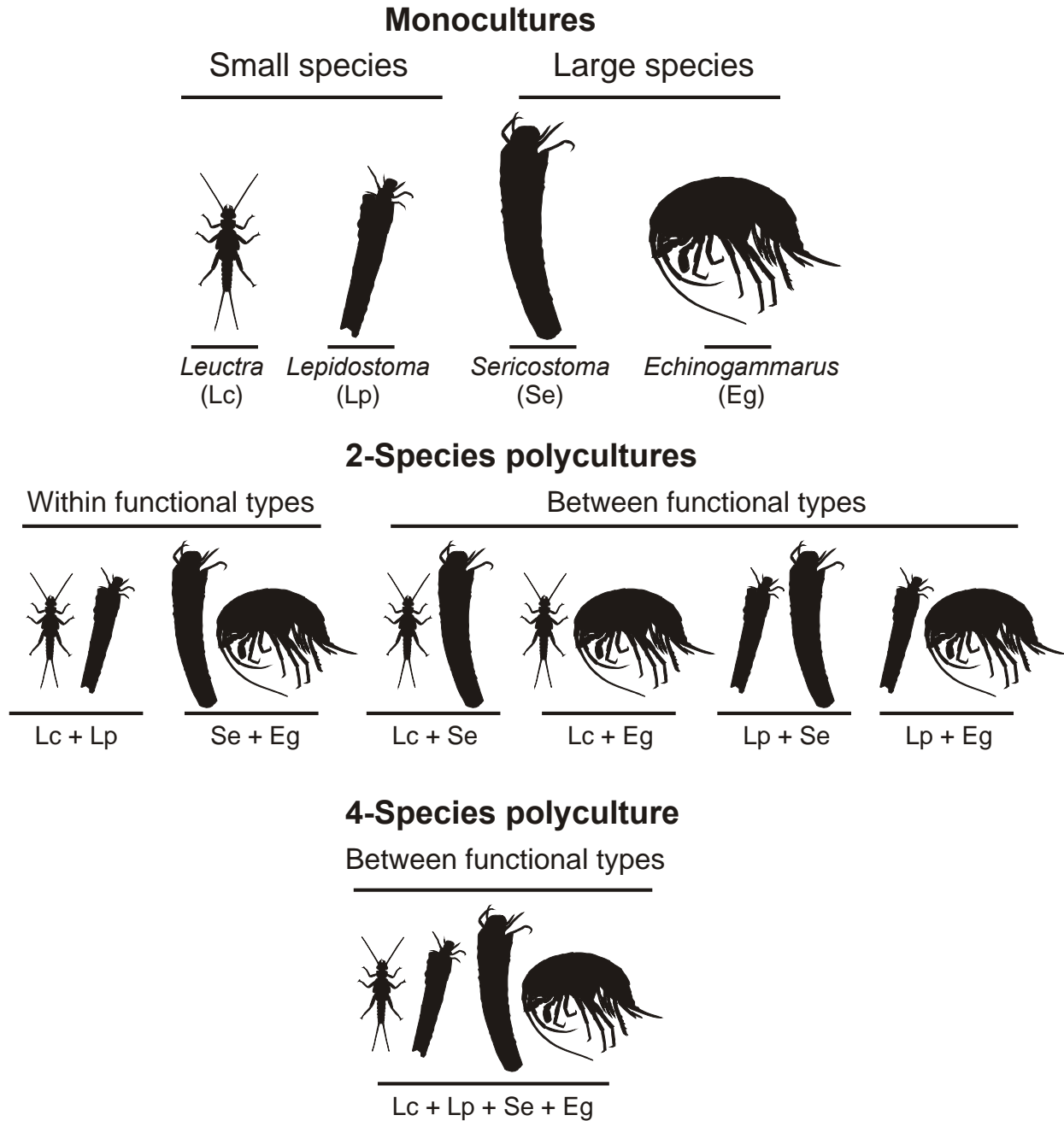


Fig. 1. Experimental design with four detritivore species belonging to two functional types (i.e., large and small body-sized species) in monocultures, 2-species polycultures (six species combinations of the same or different functional type) and the 4-species polyculture.

Plastic cups (13 cm wide, 5 cm deep) were used as microcosms, each containing leaf litter, substrate, 500 mL of stream water, and aeration. Litter was provided in the form of 40

discs of black alder, *Alnus glutinosa* [L.] Gaertn. (Betulaceae). Leaves were collected just after abscission from the forest floor in the Agüera catchment in November 2014; discs were cut with a 12-mm diameter cork borer, air dried and kept in the laboratory; just before the experiment they were weighed to the nearest 0.0001 g. Substrate was provided in the form of fine sand and pebbles collected from streams, which facilitated detritivore movement and served as refuge and material for caddisfly case construction; substrate was incinerated at 550°C for 4h and washed to remove ash before it was introduced in the microcosms. Water was taken from the stream the day before the experiment started, filtered through a 100- μ m mesh, and added to each microcosm. Microcosms were aerated through pipette tips connected to an air injection system.

Litter discs were introduced in the microcosms 6 d before the addition of detritivores to allow leaching of soluble compounds and microbial conditioning. After this period, the water was replaced and detritivores were added. Water was again replaced on days 7 and 14, using newly collected and filtered stream water, and the experiment was terminated on day 21, except for *Sericostoma* monocultures, which were terminated on day 18 because most of the litter material ($90.57\% \pm 0.03$ SE) had been consumed. Microcosms were monitored every 2 d to ensure that detritivores were alive (visual inspection without manipulation) and that there was litter remaining. We video-recorded 4-5 randomly selected microcosms with different species combinations daily for 1 h each day; in total, 3-4 different microcosms of each species combination were video-recorded. At the end of the experiment, litter material was oven dried (60°C, 72 h), weighed to determine dry mass (DM), incinerated (550°C, 4 h) and re-weighed to determine ash-free dry mass (AFDM). We estimated initial AFDM using 10 additional sets of 40 litter discs.

Initial detritivore body mass for each species in each microcosm was estimated from a case length (CL) – body mass (BM) relationship for *Sericostoma* ($BM = 0.170 \times CL^2 - 2.872 \times CL + 14.154$, $r^2 = 0.96$, $n = 26$) and *Lepidostoma* ($BM = 0.099 \times CL^2 - 1.091 \times CL + 3.464$, $r^2 = 0.84$, $n = 41$), and from a body length (BL) – BM relationship for *Leuctra* ($BM = -0.026 \times BL^2 - 0.515 \times BL - 1.502$, $r^2 = 0.70$, $n = 42$) and *Echinogammarus* ($BM = 0.127 \times BL^2 - 1.654 \times BL + 9.383$, $r^2 = 0.82$, $n = 28$) (Fig. S1), using additional individuals of a similar range of body mass or case length to those used in the experiment. At the end of the experiment, detritivores were oven dried (60°C, 72 h) and weighed (grouping individuals of each species from each microcosm) to determine their final body mass. Videos of detritivores were observed to describe animal behavioural patterns that might indicate niche partitioning or facilitation; we noted whether individuals fed on different parts of litter discs or on smaller fragments potentially produced by other species, and whether feeding or foraging behaviour differed between monocultures and polycultures, and calculated the proportion of videos where a given species showed a particular behaviour.

Data analysis

We quantified the decomposition rate mediated by detritivores as the relative daily litter mass loss = $[(LM_i - LM_f) / LM_i] / t$, where LM_i and LM_f were the initial and final litter AFDM in a microcosm, respectively, and t was the duration of the experiment in days. Initial AFDM was previously multiplied by the average proportion of remaining mass in control microcosms (= 0.716) to correct for leaching and microbial losses. Detritivore growth was calculated for each species as: detritivore growth = $(DM_f - DM_i) / DM_i$, where DM_i and DM_f were the initial and final dry mass of a species in a microcosm, respectively. When there were missing individuals,

their mass was estimated as the average body mass of the remaining individuals for that species in the same microcosm.

We explored hypotheses 1 and 2 using a modelling framework that explicitly quantifies the contributions of individual species and species interactions to the diversity effect (Kirwan et al. 2009). This framework included the following models (Fig. 2): (1) null model (i.e., intercept only), which assumes that species perform identically and do not interact with each other; (2) species identity model, where different species have different effects on decomposition, but without interactions among species, so the performance of a polyculture can be predicted from the additive performance of each species; (3) pairwise interaction model, which augments model 2 with interactions between pairs of species, resulting in diversity effects (i.e., a difference between the performance of a polyculture and the additive expectation from the constituent monocultures); (4) species-specific model, in which interspecific interactions are due to the presence of a particular species; (5) functional-type model, which assumes that interactions between species of different functional types (i.e., large or small species) are stronger than interactions between species within a functional type; and (6) functional similarity model, where the contributions of some species to decomposition are similar (used only when model 5 showed no species interactions within a particular functional type). Model 6 was based in Kirwan's (2009) functional redundancy model, but did not assume functional redundancy (i.e., a 100% compensation of a species' function by another), but rather similar effects on decomposition.

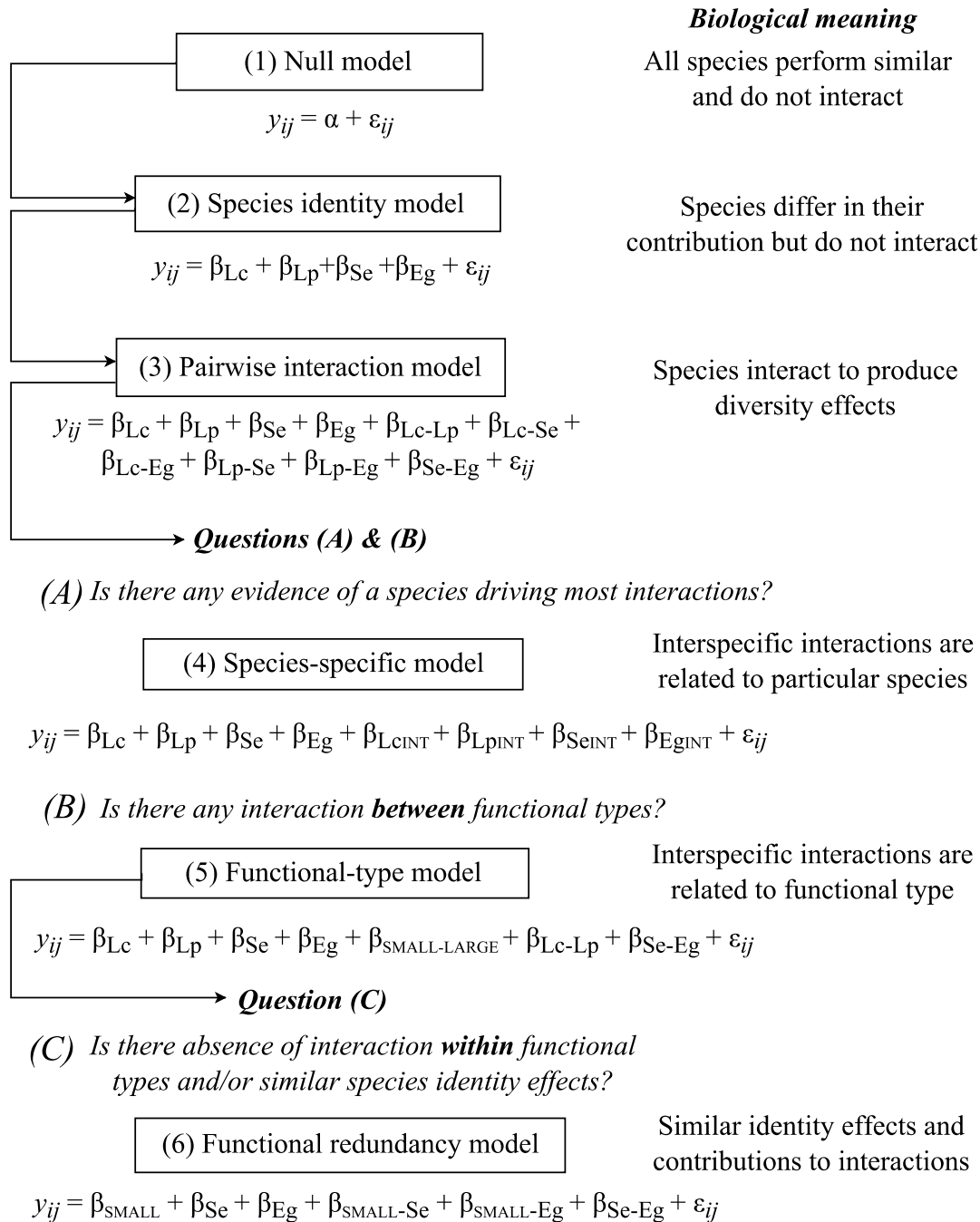


Fig. 2. Diversity-interaction models used to test for diversity effects on decomposition. The biological meaning of each model and model terms are described next to each box; y , response variable; α , intercept; β , estimated parameter of the contribution of each species; ε_{ij} , model residuals, which were allowed to vary with respect to each detritivore combination (see methods). Arrows linking different boxes represent an increase in model complexity. Detritivore species: Lc, *Leuctra geniculata*; Lp, *Lepidostoma hirtum*; Se, *Sericostoma pyrenaicum*; Eg, *Echinogammarus berilloni*; 2-species polyculture interactions: Lc-Lp, Lc-Se, Lc-Eg, Lp-Se, Lp-Eg, Se-Eg; diversity-interaction terms for each species: LC_{INT}, LP_{INT}, SE_{INT}, EG_{INT}; diversity-interaction terms for functional types: SMALL, LARGE.

The models were fitted using the ‘glms’ function and maximum likelihood method in the nlme R package, and they were compared through a model selection procedure based on the Akaike information criterion corrected for sample size (Zuur et al. 2009). Prior to running the models we used Cleveland dot- and boxplots for each response variable and species combination to detect outliers (Zuur & Ieno 2015); a single outlier was revealed (for decomposition) and was removed for subsequent analyses. As boxplots also revealed different variances depending on detritivore species combinations for both response variables (i.e., a violation of the homogeneity assumption for parametric models), we used the *VarIdent* function of the nlme R package (Pinheiro et al. 2016) in R v.3.3.1 (R Core Team 2016) in the models described below to produce an appropriate variance structure (Zuur et al. 2009).

We further examined whether species of similar body size were functionally similar (hypothesis 2) by estimating the performance of each species monoculture, which standardizes for differences in body mass and takes into account the metabolic capacity of species (Jabiol et al. 2013b). Detritivore performance was estimated as litter decomposition rate relative to the detritivore metabolic capacity, which correlates allometrically with body mass and metabolic rate (Brown et al. 2004). Metabolic capacity was estimated for each species as $(DM)^{0.75}$, where DM was the mean value between initial and final dry mass (mg) of a species in a microcosm, and the exponent 0.75 described a general relationship between body mass and metabolism (Brown et al. 2004). We examined whether the expected decomposition rate based on each species’ metabolic capacity matched the observed decomposition rate using linear regression, with the null expectation of equal predicted and observed rates.

When significant effects of species interactions or functional types on decomposition were demonstrated, we quantified the magnitude of such effects by calculating the ratio of

decomposition rate between the value of a polyculture (observed value) and the average value of the corresponding monocultures (expected value). We further examined whether detritivore growth differed from the additive expectation (hypothesis 3), by subtracting the relative growth of a species in a polyculture from the relative growth of the same species in a monoculture. We calculated ordinary non-parametric bootstrapped 95% confidence intervals (BCa method using the 'boot' function and package, and based on 1,000 bootstrap replicates; Davison & Hinkley 1997, Canty & Ripley 2016) to test whether these intervals contained the value of one (for decomposition rate) or zero (for detritivore growth) – that is, the null expectation that the response of the polyculture was not different from the mean responses of the monocultures of species present in the polyculture.

RESULTS

Survival of all detritivore species was high during the experiment (mean \pm SE: $74 \pm 5\%$ for *Leuctra*, $88 \pm 2\%$ for *Lepidostoma*, $94 \pm 2\%$ for *Sericostoma* and $92 \pm 2\%$ for *Echinogammarus*). Decomposition rates were lowest in the *Leuctra* monoculture (mean \pm SE: 0.69 ± 0.10 mg d⁻¹) and highest in the *Sericostoma* monoculture (16.93 ± 0.41 mg d⁻¹) (Fig. S2a; Table S1). Growth rates in monocultures were positive for *Sericostoma*, which increased by 42% their initial body mass, while *Lepidostoma* and *Echinogammarus* growth rates did not differ from zero, and body mass of *Leuctra* was reduced by 18% (Fig. S2b).

The model selection procedure showed that species interacted and produced diversity effects on decomposition rates. Two models were plausible descriptions of species interactions ($\Delta_i < 2$; Table 1): the functional-type model and the species-specific model. The functional-type model had a better fit than the species-specific model, indicating that interspecific interactions

were mostly related to detritivore body size, with some influence of species identity. The bootstrap procedure showed that interactions between functional types (i.e., small and large species) produced a 12% increase in decomposition rates of the average rate of those species in monoculture (Fig. 3a). The decomposition rate of the two large species together (i.e., *Sericostoma* and *Echinogammarus*) was 19% higher than the average of their monocultures (Fig. 3a). In contrast, the interaction between the two small species did not exceed the average contribution of their monocultures (Fig. 3a), which led us to test for functional similarity within this functional type. However, the poor fit of the functional similarity model and the very different performances of *Leuctra* and *Lepidostoma* (see below) indicated that small organisms did not have similar effects on decomposition. The species-specific model and 95% confidence intervals showed that results were not driven by the presence of a single species in a polyculture, because the effect was always higher than the additive expectation (from 9% higher in interactions with *Lepidostoma* to 20% higher in interactions with *Sericostoma*; Fig. 3b).

Detritivore performance in monocultures indicated that *Lepidostoma* and *Sericostoma* were the most efficient species [mean (95% CI): 0.80 (0.72 – 0.88) and 0.70 (0.65 – 0.76), respectively], while *Leuctra* and *Echinogammarus* were less efficient [0.20 (0.14 – 0.25) and 0.18 (0.15 – 0.21), respectively]. There was a positive relationship between metabolic capacity (i.e., decomposition rates predicted from detritivore body mass) and the observed decomposition rates ($t = 13.45$, $df = 2, 110$, $P < 0.0001$).

The differences between observed and expected growth (polyculture minus monocultures) showed (i) higher growth of *Lepidostoma* and *Leuctra* when combined (Fig. 3c); (ii) similar growth of *Sericostoma* and *Echinogammarus* when combined (Fig. 3c); (iii) higher growth of small organisms, but similar growth of large organisms, when both small and large

organisms were combined (Fig. 3c); and (iv) higher overall growth of *Leuctra* and *Lepidostoma* and similar overall growth of *Sericostoma* and *Echinogammarus* (Fig. 3d).

The video observations evidenced differences in feeding behaviour between monocultures and polycultures only for *Leuctra*, who was observed feeding on fine particulate organic matter (FPOM) produced by other species in polycultures; the two caddisflies were observed shredding on litter discs, but *Lepidostoma* ate only the margins, while *Sericostoma* ate the whole discs including the less palatable parts; *Echinogammarus* was a very active swimmer and was observed shredding the margins and scraping the surface of litter discs (Table S2).

Table 1. Summary of model selection for the set of diversity-interaction models used to test for diversity effects on litter decomposition rate (mg d^{-1}), based on the Akaike Information Criterion corrected for sample size (AIC_c). Models are ordered from the best to the poorest fit according to Akaike weights (w_i). The biological meaning of each model is described in the methods and Fig.2. K, number of estimated parameters for each model; Δ_i (delta AIC_c), difference in AIC_c value relative to the best model; w_i , probability that a model is the best among the whole set of models. Detritivore species: Lc, *Leuctra geniculata*; Lp, *Lepidostoma hirtum*; Se, *Sericostoma pyrenaicum*; Eg, *Echinogammarus berilloni*; 2-species polyculture interactions: Lc-Lp, Lc-Se, Lc-Eg, Lp-Se, Lp-Eg, Se-Eg; diversity-interaction terms for each species: LC_{INT} , LP_{INT} , SE_{INT} , EG_{INT} ; diversity-interaction terms for functional types: SMALL, LARGE.

Model	K	Δ_i	w_i
(5) Functional type Lc + Lp + Se + Eg + SMALL-LARGE + Lc-Lp + Se-Eg	18	0.00	0.51
(4) Species-specific Lc + Lp + Se + Eg + LC_{INT} + LP_{INT} + SE_{INT} + EG_{INT}	19	0.39	0.42
(2) Species identity Lc + Lp + Se + Eg	15	4.78	0.05
(3) Pairwise interaction Lc + Lp + Se + Eg + Lc-Lp + Lc-Se + Lc-Eg + Lp-Se + Lp-Eg + Se-Eg	21	5.82	0.03
(6) Functional redundancy SMALL + Se + Eg + SMALL-Se + SMALL-Eg + Se-Eg	17	91.89	0.00
(1) Null Intercept only	12	225.28	0.00

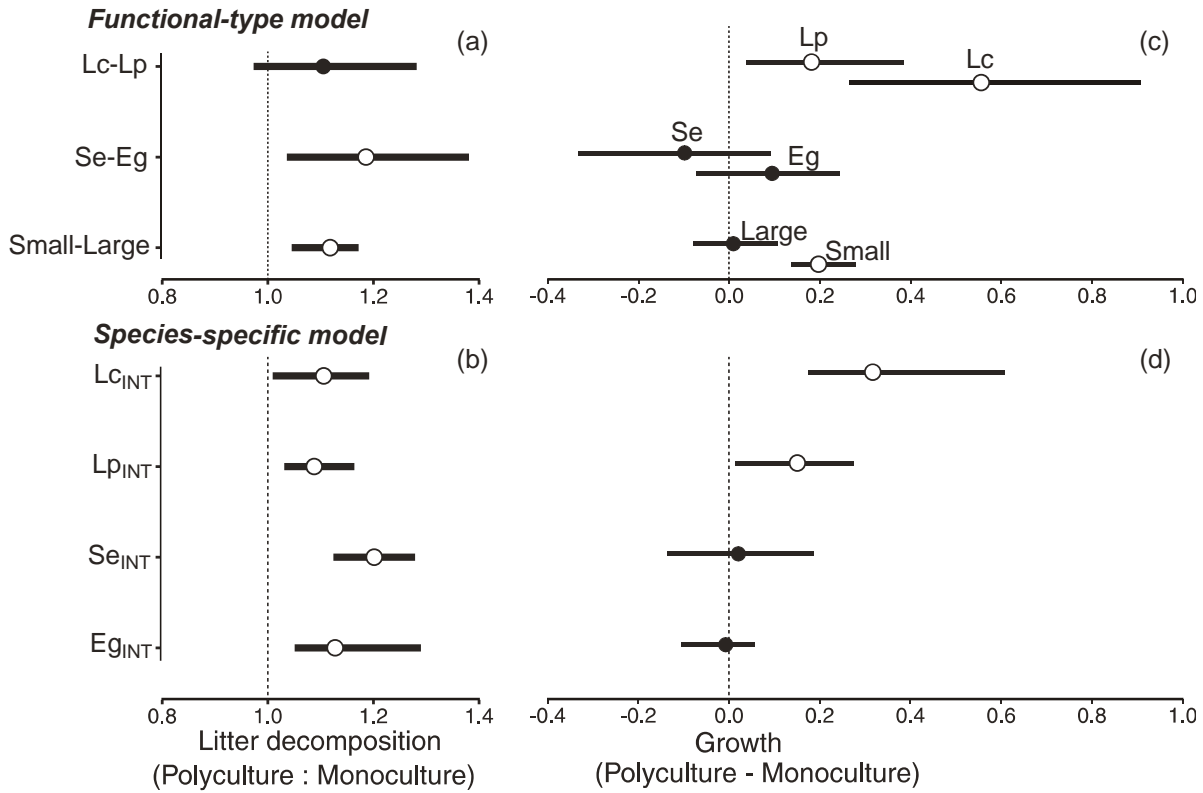


Fig. 3. Ratio of decomposition rates between polycultures and monocultures (a, b) and difference in detritivore growth between polycultures and monocultures (c, d) for the interaction of species of similar (Lc-Lp, Se-Eg) or different body size (small-large) or for the average interaction of each species (see Fig.2 legend). The dashed line denotes the value of one (for decomposition) or zero (for growth), that is, the null expectation that the polyculture value is not different from the mean value of constituent monocultures. Circles are means and vertical lines denote upper and lower limits of 95% non-parametric bootstrapped confidence intervals; closed circles represent intervals that do not reject the null hypothesis (i.e., do not contain the value of one or zero) and open circles represent intervals that do reject the null hypothesis.

DISCUSSION

Our study is the first to manipulate detritivore diversity and interspecific variation in body size simultaneously, and to demonstrate that both factors have an effect on litter decomposition. We show clear positive effects of detritivore diversity on decomposition rates, which are mediated by facilitative interactions between species of different size and niche partitioning between species of similar size. Positive effects of detritivore diversity on decomposition have been shown in

other experimental studies (Jonsson & Malmqvist 2000b, Dangles et al. 2002, Boyero et al. 2007, Constantini & Rossi 2010) and some syntheses (Cardinale et al. 2006, Srivastava et al. 2009), but not in others, which have found either negative or no diversity effects (Bastian et al. 2008, Creed et al. 2009, McKie et al. 2009, Reiss et al. 2011). This lack of consistency across studies has been attributed to differences in assemblage composition, which can lead to the existence of different interspecific interactions (McKie et al. 2008). However, while such interactions are often mediated by body size (Woodward et al. 2005), this animal trait has been rarely taken into account in diversity-decomposition experiments. An exception is Reiss *et al.* (2011), who found that within-species variation in body size had a large effect on decomposition; however, this study showed no effect of diversity on decomposition, and thus the role of body size in diversity-decomposition relationships had remained unexplored.

We showed that diversity effects on decomposition were most evident when species of different body size were combined, which supported our first hypothesis. Leaf litter decomposed faster in polycultures containing large and small detritivores than was expected from their monocultures, indicating that interspecific interactions caused greater effects on decomposition than simple addition. Such effects could arise from mechanisms such as resource partitioning or facilitation, but few experimental studies have distinguished between these mechanisms (exceptions include Cardinale et al. 2002, Jonsson & Malmqvist 2003). The patterns we observed suggested that facilitation was an important mechanism underlying diversity-decomposition effects, as shown by the higher growth of smaller detritivores in the presence of larger species (in support of our third hypothesis). The enhanced growth and the video observations suggested that smaller detritivores could benefit from the feeding activity of larger detritivores, which would produce large amounts of smaller litter fragments and FPOM that could be used by the small

species. *Leuctra* species are known to act as both litter-shredding detritivores and collectors (López-Rodríguez et al. 2012), and are often found in FPOM deposits in streams (Callisto & Graça 2013). The relatively small mouthparts of *Lepidostoma* compared to larger detritivores might be more efficient at handling the smaller litter fragments, although more evidence would be required to support this statement.

In contrast to the enhanced growth of small detritivores in polycultures containing species of different body size, larger detritivores showed similar growth in polycultures and monocultures, indicating that larger species did not benefit from the presence of smaller species. This could indicate that faster decomposition in polycultures was due exclusively to enhanced feeding of small species; however, this is unlikely, as the polyculture containing just the two large species also showed faster decomposition than was expected from monocultures. The absence of enhanced growth in this case, however, suggests that there was no facilitation between the large species. A plausible alternative mechanism underlying diversity effects on decomposition in this case would be resource partitioning, which is common among species belonging to distantly related taxa (Petchey & Gaston 2002), as is the case for *Sericostoma* and *Echinogammarus*, which belong to different subphyla. Gammarids are able to shred leaf litter, but can also scrape on surfaces, as observed in our videos and shown elsewhere (Mayer et al. 2012); in contrast, caddisflies such as *Sericostoma* have mouthparts that are highly specialized for fragmenting leaf material, including the tougher parts (Friberg & Jacobsen 1994). These detritivores also differed in their use of habitat: *Echinogammarus* was a highly mobile swimmer that actively searched for food, as do other gammarids (Friberg & Jacobsen 1994), while *Sericostoma* crawled on the substrate and was more sedentary, as are other sericostomatids (Jackson et al. 1999). Less mobile detritivores are often able to process low quality food, as they

have more limited capacity for finding higher quality food. Thus, *Sericostoma* was able to eat the less palatable parts of leaf discs (minor nerves), as observed in our videos and elsewhere (Tonin et al. 2017a). In contrast, *Echinogammarus* seemed to feed only on the more palatable parts (which would better satisfy their higher energy requirements), resulting in higher consumption overall, but similar growth rates in polycultures.

When the small species were together, decomposition was similar to that of the average monoculture, but growth of both species was enhanced. This suggests that facilitation occurred also between these two species, possibly through the mechanism described above: the feeding activity of *Lepidostoma* released high amounts of FPOM that were most likely used by *Leuctra*; it is also possible that *Lepidostoma* roughened the leaf surface, making it easier for *Leuctra* to eat it, as shown for other detritivores (Iwai et al. 2009). It is unclear, however, how *Lepidostoma* could benefit from the presence of *Leuctra*; it is possible that the presence of *Leuctra* somehow enhances litter quality by increasing microbial conditioning, but this would need to be confirmed experimentally. Importantly, the positive diversity effect on decomposition found in polycultures containing large species, the distinct performance of small species in monocultures, and the poor fit of the functional similarity model indicated that these species were not functionally similar, thus not supporting our second hypothesis. It is also noteworthy that our results were not driven by the presence of particular species with dominant effects, unlike findings elsewhere (Dangles & Malmqvist 2004).

Our study confirms that body size is an important animal trait mediating diversity effects on decomposition (Reiss et al. 2011, Boyero et al. 2014), as it influenced the type of interactions that occurred between species. However, body size did not determine detritivore performance, as would have been expected based on the metabolic theory of ecology: for a given biomass, a

higher number of smaller individuals should result in higher consumption than a lower number of larger individuals, because the former have higher mass-specific metabolic rates than the latter (Brown et al. 2004). This possibly occurred because caddisflies were much more efficient detritivores than non-caddisflies, as reported elsewhere (e.g., Boyero et al. 2012b). The lower efficiency of *Echinogammarus* could be related to their higher energy expenditure as a result of active swimming, as shown in our videos and reported for other gammarids (MacNeil et al. 1999), while the lower efficiency of *Leuctra* merits further examination.

We conclude that body size is a key animal trait to take into account when exploring diversity effects on litter decomposition and related processes, as body size has the potential to mediate such effects through its influence on interspecific interactions. We show how different mechanisms of complementarity (i.e., facilitation and resource partitioning) can mediate interactions between detritivore species of different or similar size, and de-emphasize the existence of functional similarity between similar-sized species. Although microcosm experiments are inherently simple compared to natural systems, these experiments are often crucial to understand complex ecological relationships (Fraser & Keddy 1999, Benton et al. 2007), and our results are supported by empirical evidence that body size is a key driver of many ecological processes (Peters 1986, Woodward et al. 2005). Our study suggests that, if we are to anticipate the consequences of diversity loss for decomposition in stream ecosystems, it is crucial to take into account not only the identity and biomass of detritivore assemblages but also their body-size structure. Ideally, future studies should also address the potential influence of different species' vulnerability to extinction depending on body size (Petchey et al. 1999, Raffaelli 2004), and how this might affect ecosystem functioning on different scenarios of detritivore diversity (Boyero et al. 2012c) and in more complex food webs (Thébault & Loreau 2003).

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SUPPORTING INFORMATION

Table S1. Mean (\pm SE) litter decomposition rate (mg d^{-1}) in each of the 11 detritivore species combinations (Lc, *Leuctra*; Lp, *Lepidostoma*; Se, *Sericostoma*; Eg, *Echinogammarus*), and results (t statistic and p -value) of linear models testing whether decomposition rate differed from zero (i.e., the null expectation that there was no decomposition). Linear models had zero intercept and species combination as the predictor; degrees of freedom: 110, total; 99, residual.

	Decomposition rate	t	p
Lc	0.69 ± 0.10	7.00	< 0.001
Lp	6.58 ± 0.39	16.90	< 0.001
Se	16.93 ± 0.41	41.03	< 0.001
Eg	3.06 ± 0.22	14.16	< 0.001
Lc-Lp	3.95 ± 0.24	16.16	< 0.001
Lc-Se	10.15 ± 1.02	9.90	< 0.001
Lc-Eg	2.45 ± 0.16	15.11	< 0.001
Lp-Se	12.43 ± 0.73	16.99	< 0.001
Lp-Eg	4.93 ± 0.21	23.92	< 0.001
Se-Eg	11.75 ± 0.78	15.01	< 0.001
Lc-Lp-Se-Eg	8.08 ± 0.69	11.74	< 0.001

Table S2. Type of feeding and foraging behaviour of the studied species, as observed in the videos. % Obs: percentage of videos where a given pattern was observed (e.g., the 70% value for *Lepidostoma* making a hole in the mesophyll means that, in 70% of videos containing *Lepidostoma*, this species showed that particular feeding behaviour).

	Food source/feeding mode	% Obs.	Foraging behaviour	% Obs.
Small detritivores				
<i>Leuctra</i>	Monocultures: litter discs	80	Most time spent under litter discs or pebbles	100
	Polycultures: FPOM	20		
<i>Lepidostoma</i>	Litter discs (shredding margins)	80	Crawled around moderately in search for food	50
	Litter discs (making hole in mesophyll, avoiding nerves)	70		
	Produced large amounts of FPOM	100		
Large detritivores				
<i>Sericostoma</i>	Litter discs (mesophyll)	100	Crawled around moderately in search for food	60
	Litter discs (less palatable parts – minor nerves)	65		
	Produced large amounts of FPOM	100		
<i>Echinogammarus</i>	Litter discs (shredding margins)	55	Highly active swimmer	85
	Litter discs (scraping the surface)	20		
	Produced moderate amounts of FPOM	65		

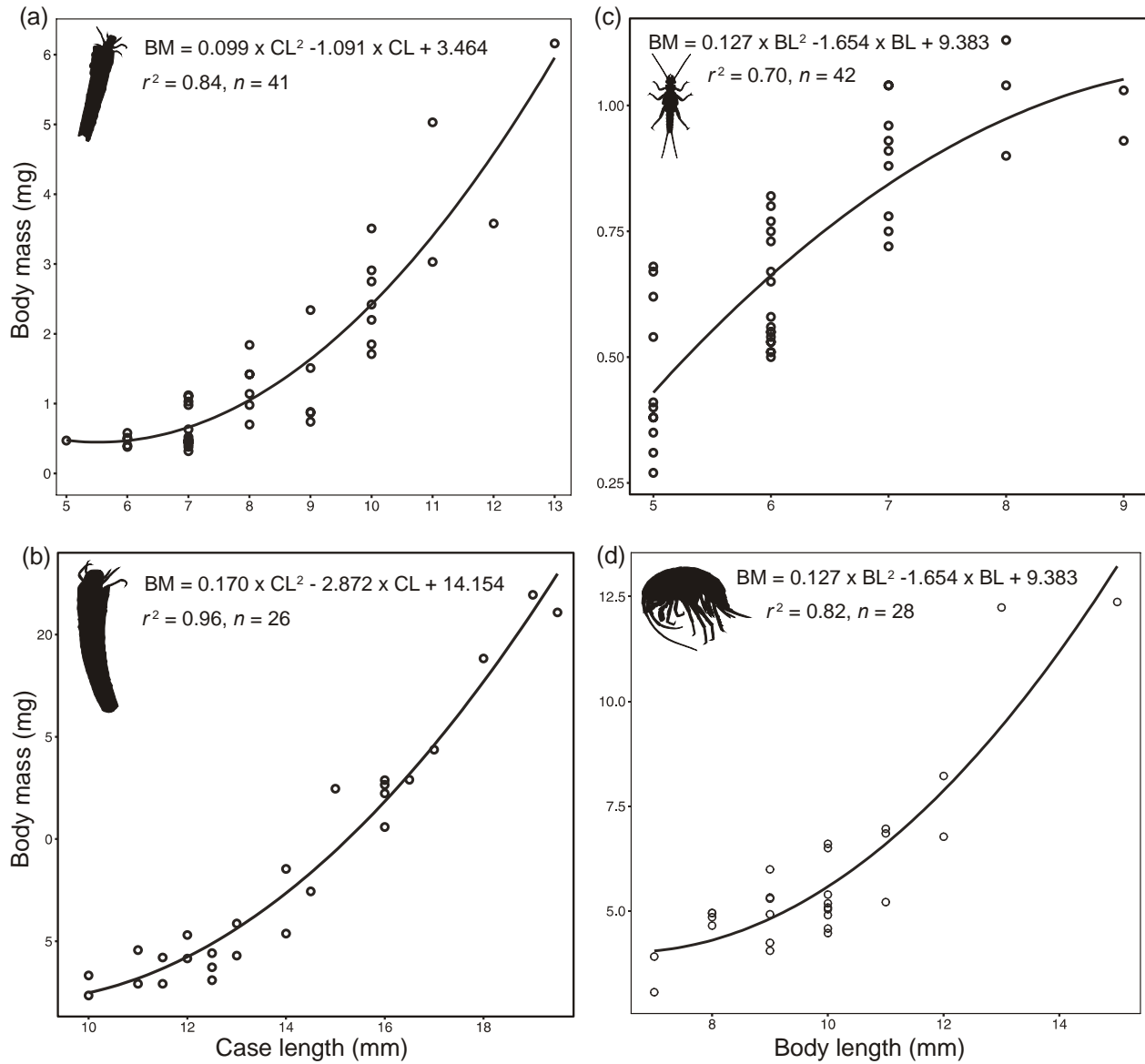


Fig. S1. Allometric relationships between case length (CL) and body mass (BM) for *Lepidostoma* (a) and *Sericostoma* (b), and between body length (BL) and BM for *Leuctra* (c) and *Echinogammarus* (d), used to estimate initial detritivore biomass in experimental microcosms.

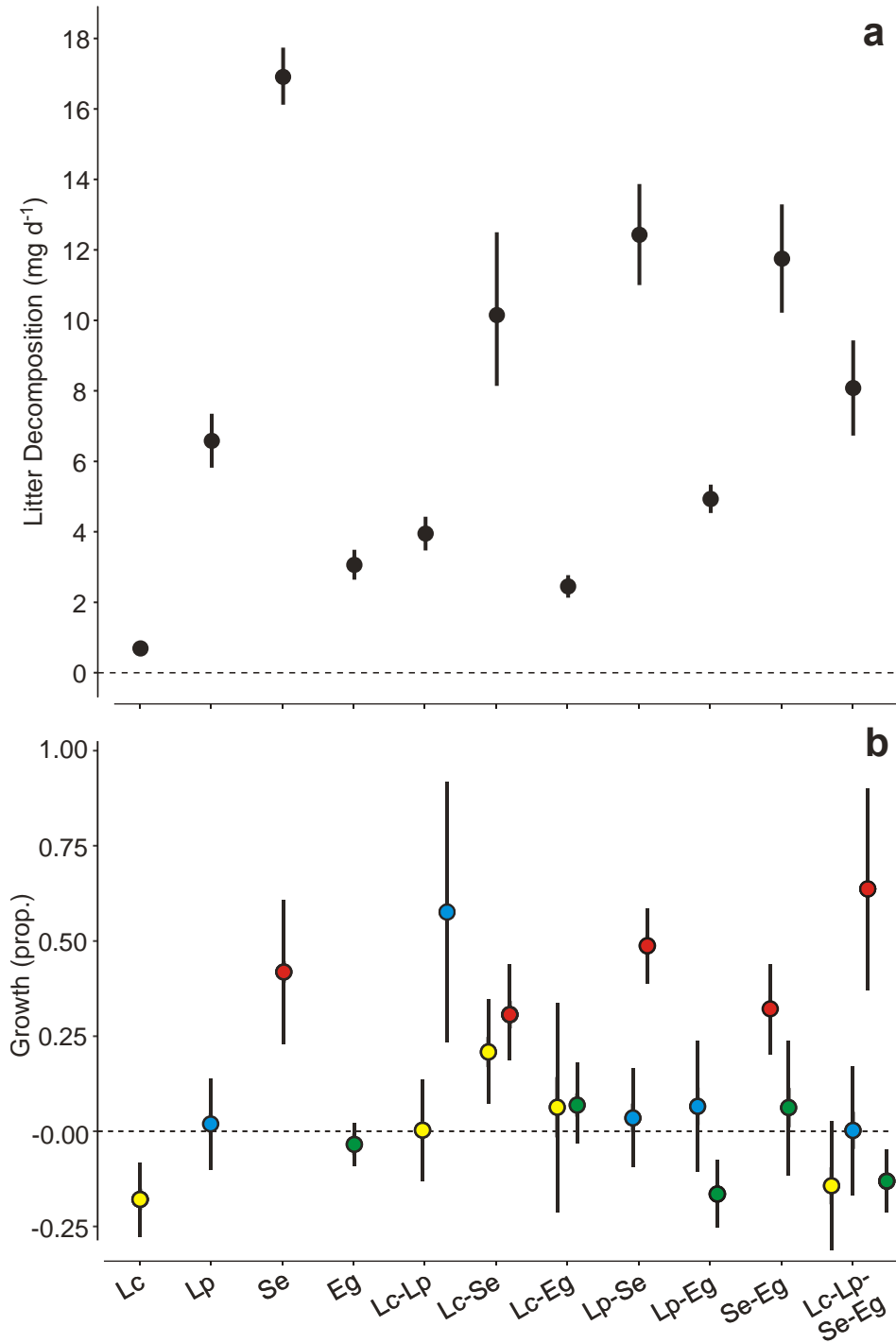


Fig. S2. Litter decomposition rate (a) and relative detritivore growth (b) in monocultures (Lc, *Leuctra*; Lp, *Lepidostoma*; Se, *Sericostoma*; Eg, *Echinogammarus*), 2-species polycultures (Lc-Lp, Lc-Se, Lc-Eg, Lp-Se, Lp-Eg, Se-Eg) and the 4-species polyculture (Lc-Lp-Se-Eg). Circles are means and vertical lines denote upper and lower limits of 95% confidence intervals. The dashed line denotes the value of zero (i.e., the null expectation that observed values are not different from zero).

CONSIDERAÇÕES FINAIS

Nesta tese apresentamos uma avaliação empírica abrangente dos padrões e mecanismos que influenciam a dinâmica de detritos foliares em ecossistemas de riachos ao longo de diferentes escalas temporais e espaciais, e usando diferentes abordagens observacionais e experimentais. Uma das maiores motivações desta tese foi a falta de um conhecimento amplo sobre os padrões e mecanismos da dinâmica de detritos nos trópicos, que compreendem 40% da área superficial global mas são historicamente pouco estudados. Isso contrasta com os padrões bem conhecidos de riachos em florestas decíduas temperadas, que recebem um aporte massivo de detritos durante o outono (Abelho 2001) – principalmente quando o fotoperíodo e a temperatura diminuem (Gill et al. 2015) – e subsequente acúmulo de detritos, que é precedido pelo aumento da decomposição. Considerando que estes processos são essenciais para entender o funcionamento de ecossistemas de riachos e para prever as consequências potenciais de alterações antrópicas, foi realmente necessário um estudo exaustivo destes processos nos trópicos. Desse modo, na primeira parte da tese (**Fluxo de detritos vegetais e Decomposição**) exploramos os padrões espaciais (entre trechos de riachos, riachos e/ou biomas) e temporais (mensais, sazonais e/ou anuais) dos aportes, transporte, estoque e decomposição de detritos, as conexões entre esses processos e seus controles ambientais, em vários grandes biomas tropicais: Amazônia, Mata Atlântica e Cerrado (Capítulo I & II).

Os padrões temporais de aportes e estoque de detritos – ao longo de um ano – diferiram entre os biomas tropicais, com o aporte sazonal de detritos, mas estoque não sazonal na Amazônia; aporte de detritos não sazonal mas estoque sazonal na Mata Atlântica; e, uma sazonalidade marcada tanto do aporte quando do estoque de detritos no Cerrado (**Capítulo I**). No entanto, apesar da evidente diferença temporal na dinâmica de detritos nos trópicos (e possíveis

mecanismos distintos dentro e entre biomas), observamos que a precipitação tem um papel consistente e robusto na regulação dos padrões temporais dos aportes e estoque de detritos (**Capítulo I**). Esses resultados contradizem a percepção generalizada de que os aportes de detritos não são sazonais em climas tropicais pouco sazonais (como em algumas áreas da Amazônia), o que sugere que relativamente pequenas alterações nos regimes de precipitação podem alterar o período e a magnitude dos aporte de detritos, e com isso, sua disponibilidade para as cadeias alimentares de riachos. Ainda, os regimes de precipitação parecem regular a maior parte da dinâmica de detritos em climas pluviais sazonais, uma vez que as exportações de detritos pelo transporte da água aumentam seriamente em períodos chuvosos e diminuem em períodos secos (**Capítulo II**). Adicionalmente, como são previstos aumentos futuros na sazonalidade da precipitação inclusive nos trópicos (e.g., um aumento da duração de períodos mais secos, especialmente no Cerrado e em partes da Amazônia; Feng et al. 2013), podemos esperar que essas mudanças nos regimes de precipitação afetem populações e comunidades (e.g., por meio da regulação da disponibilidade de detritos para microrganismos e detritívoros) e estendam-se para consequências no nível ecossistêmico (e.g., por meio da regulação da quantidade e do tempo de retenção dos detritos até a decomposição ou exportação, o que por fim pode alterar a ciclagem de carbono e nutrientes). Essas repercussões são ainda mais críticas considerando que a decomposição pode ser responsável pela maior remoção de detritos dos riachos (**Capítulo II**; mesmo em um ambiente com detritos foliares de baixa qualidade nutricional; Gonçalves et al. 2007) e é reduzida em períodos mais secos (**Capítulo II**), o que implica em reduções gerais na geração de partículas finas e liberação de CO₂ (pelos organismos atuantes na decomposição).

Outro componente principal das mudanças globais com importantes repercussões para processos ecossistêmicos (como a decomposição) é a perda de biodiversidade, resultado da super-exploração, modificação de habitat, poluição por nutrientes ou invasão de espécies. A perda de biodiversidade é atualmente um dos principais problemas na maioria dos ecossistemas e regiões em todo mundo e tem um grande potencial para impactar a disponibilidade de recursos, a interação de espécies e, finalmente os processos ecossistêmicos. Diante disso, na segunda parte da tese (**Biodiversidade e Decomposição**) exploramos as consequências da perda de biodiversidade de recursos e consumidores em riachos – detritos foliares e espécies de invertebrados detritívoros, respectivamente – ao nível populacional (i.e., sobrevivência, crescimento e razão C:N dos detritívoros) e ecossistêmico (i.e., decomposição) em microcosmos (**Capítulo III e IV**). Embora a diversidade de detritos foliares não tenha afetado a sobrevivência, o crescimento ou a razão C:N dos detritívoros, ela reduziu a decomposição mediada pelos microrganismos e pelos detritívoros (em 7 e 15%, respectivamente), principalmente por meio de efeitos de complementariedade (**Capítulo III**). Ainda, encontramos evidências de efeitos interativos da diversidade de detritos foliares e a concentração de nitrogênio na água, o que sugere que a perda de diversidade de recursos afeta a decomposição principalmente em riachos com elevado estado trófico (**Capítulo III**). Similarmente, a perda de diversidade de espécies de detritívoros resultou na redução da decomposição, mas principalmente quando espécies de tamanhos corporais diferentes foram extintas (**Capítulo IV**). Espécies de detritívoros com tamanho corporal grande tendem a facilitar a atividade alimentar de espécies menores nos detritos foliares, aumentando a decomposição total (em 12%; **Capítulo IV**). Esses resultados têm repercussões importantes do ponto de vista de conservação, uma vez que organismos maiores geralmente apresentam taxas de extinção superiores à de organismos menores (Duffy 2003).

De modo geral, nossos resultados apontam para a importância do entendimento dos efeitos múltiplos e interativos de fatores bióticos (e.g., interações entre espécies, perda de diversidade) e abióticos (e.g., variáveis climáticas como temperatura e precipitação) nos aportes, estoque e decomposição de detritos em riachos, especialmente se estivermos interessados em manter um elevado número de funções ecossistêmicas e de antecipar consequências futuras das alterações ambientais. Com isso, uma das maiores implicações desta tese é de que precisamos modelos mais abrangentes que integrem os aportes, estoque e decomposição de matéria orgânica em riachos, mas particularmente estendendo estes modelos à interface riacho-floresta ripária, uma vez que os riachos e a floresta ripária são funcionalmente conectados pela ciclagem de carbono e nutrientes (Wallace et al. 1997, Bernhardt et al. 2003). Dois caminhos complementares para atingir essa meta são (i) sumarizar informações de distintos estudos utilizando meta-análise e (ii) conduzir estudos experimentais adicionais baseados em protocolos que implementem metodologia padronizada, tanto em riachos quanto em florestas ripárias, a fim de possibilitar generalizações consistentes. Estudos futuros devem idealmente aumentar a realidade dos experimentos alterando de situações de microcosmos para mesocosmos ou campo – e incluir uma variedade de biomas –, proporcionando deste modo informações sobre o alcance no qual resultados de estudos laboratoriais são mantidos em ‘ecossistemas reais’ e em escalas temporais mais longas.

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