UNIVERSIDADE DE BRASÍLIA
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PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

OS EFEITOS DA HETEROGENEIDADE AMBIENTAL NO FLUXO DE ENERGIA EM RIACHOS DO CERRADO

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TESE DE DOUTORADO

OS EFEITOS DA HETEROGENEIDADE AMBIENTAL NO FLUXO DE ENERGIA EM RIACHOS DO CERRADO

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“Não fui eu que ordenei a você? Seja forte e corajoso! Não se apavore nem desanime, pois o Senhor, o seu Deus, estará com você por onde você andar”. Josué 1:9

SUMÁRIO

RESUMO

A vegetação ripária fornece importantes serviços ecossistêmicos para ambientes aquáticos através da sua importância para a heterogeneidade de habitat e para o fornecimento de carbono e nutrientes para as cadeias alimentares de riachos. No entanto, ainda faltam informações sobre como a vegetação ripária influencia a capacidade de retenção dos riachos considerando as diferentes características dos detritos foliares e das condições hidrológicas dos riachos do cerrado. Além disso, há poucos estudos considerando como as características da bacia hidrográfica, do uso do solo e da heterogeneidade de habitat local influenciam a origem e o suplemento de nitrogênio e carbono nas cadeias alimentares de riachos. Desse modo, conduzimos três estudos em riachos de sistemas tropicais, com o objetivo de entender os padrões e mecanismos das características do habitat local e da bacia hidrográfica nos processos ecossistêmicos de riachos e nas interações tróficas de comunidades aquáticas. Foram avaliados os processos ecossistêmicos nos riachos através da retenção e da decomposição de detritos, e a estrutura trófica da biota aquática e seus recursos alimentares (macroinvertebrados, peixes e recursos basais) através de análises do conteúdo estomacal e de isótopos estáveis de carbono (C) e nitrogênio (N). Em um experimento de campo, observamos conexões estreitas entre a heterogeneidade do habitat, a retenção e decomposição de detritos foliares. Riachos com maior heterogeneidade de habitat potencializa a capacidade de retenção de detritos foliares, especialmente folhas maiores, enquanto que o fluxo da água aumenta a decomposição de detritos foliares, especialmente em folhas macias (Capítulo I). Riachos com menor velocidade da água e menor temperatura (escala local) foram responsáveis por maiores valores de δ^{13}C da teia trófica, ao passo que riachos mais distantes da nascente e com altas cargas de nitrato nos riachos (escala regional) aumentaram os valores de δ^{15}N. Além disso, os resultados isotópicos mostraram que maior parte dos organismos nos riachos são sustentados pela energia autóctone, em vez de alóctones (Capítulo II). Riachos localizados em áreas com mudanças intensas no uso da terra (bacias ocupadas por pastagem e agricultura) apresentaram redução de espécies de predadores (peixes), redes tróficas com menos links envolvidos e menos modulares. Essas mudanças gerais na estrutura das redes tróficas de peixes indicam uma redução de espécies especialistas e aumento da abundância de espécies generalistas (Capítulo III). Nossos resultados sugerem que mudanças locais nas condições hidráulicas, como eventos de seca extrema ou por impactos antrópicos, e mudanças na composição da vegetação que influenciam na qualidade dos detritos foliares, tem o potencial de alterar os fluxos de detritos em riachos, e os ciclos de carbono e nutrientes na interface riacho-floresta. Por último, demonstramos que a organização hierárquica de vários fatores ambientais e escalas espaciais, bem como dos potenciais efeitos da perturbação antrópica sobre o funcionamento desse ecossistema, afetam as teias tróficas dos riachos, por meio das alterações na disponibilidade de recursos alimentares. Assim, restaurar a integridade das paisagens, poderia melhorar o funcionamento dos sistemas aquáticos e de sua biodiversidade.

Palavras-chave: decomposição, retenção, sistemas lóticos, vegetação ripária, isótopos estáveis, dieta, redes tróficas, aninhamento, uso do solo, funcionamento de ecossistemas.

ABSTRACT

Riparian vegetation provides important ecosystem services to aquatic environments through its importance for habitat heterogeneity and the supply of carbon and nutrients to stream food chains. However, information is still lacking on how riparian vegetation influences the retention capacity of streams considering the different characteristics of leaf debris and the hydrological conditions of streams in the cerrado. Furthermore, there are few studies considering how watershed characteristics, land use and local habitat heterogeneity influence the origin and supply of nitrogen and carbon in stream food chains. Thus, we conducted three studies in streams of tropical systems, aiming to understand the patterns and mechanisms of local habitat and watershed characteristics in the ecosystem processes of streams and in the trophic interactions of aquatic communities. Ecosystem processes in streams were evaluated through debris retention and decomposition, and the trophic structure of aquatic biota and its food resources (macroinvertebrates, fish and basal resources) through analysis of stomach contents and stable carbon isotopes (C) and nitrogen (N). In a field experiment, we observed close connections between habitat heterogeneity, retention and decomposition of leaf debris. Streams with greater habitat heterogeneity enhance the retention capacity of leaf debris, especially larger leaves, while water flow increases the decomposition of leaf debris, especially in soft leaves (Chapter I). Streams with lower water velocity and lower temperature (local scale) were responsible for higher values of δ13C of the food web, while streams more distant from the source and with high nitrate loads in the streams (regional scale) increased the values of δ15N. Furthermore, isotopic results showed that most organisms in streams are sustained by autochthonous energy rather than allochthonous (Chapter II). Streams located in areas with intense changes in land use (basins occupied by pasture and agriculture) showed a reduction in predator species (fish), trophic networks with fewer links involved and less modular. These general changes in the structure of trophic fish nets indicate a reduction in specialist species and an increase in the abundance of generalist species (Chapter III). Our results suggest that local changes in hydraulic conditions, such as extreme drought events or anthropogenic impacts, and changes in vegetation composition that influence leaf debris quality, have the potential to alter debris flows in streams, and cycles of debris. carbon and nutrients at the stream-forest interface. Finally, we demonstrate that the hierarchical organization of various environmental factors and spatial scales, as well as the potential effects of human disturbance on the functioning of this ecosystem, affect the trophic webs of streams, through changes in the availability of food resources. Thus, restoring the integrity of landscapes could improve the functioning of aquatic systems and their biodiversity.

Key-words: decomposition, retention, lotic systems, riparian vegetation, stable isotopes, diet, trophic network, nestedness, land use, ecosystem functioning, tropical streams.

INTRODUÇÃO GERAL

Contexto da coleta de dados dos capítulos

Essa tese é fruto de um grande esforço amostral com diversos colaboradores. Os dados de campo do capítulo 1 foi obtido através de coletas de campo da disciplina de Ciência do Ambiente do curso de Ciências Biológicas no ano de 2016. Esse estudo foi conduzido pelo professor José Francisco Gonçalves Júnior e o desenho amostral contou com a participação dos colaboradores: Alan Tonin e Renan Rezende. As coletas de dados foram feitas por esses pesquisadores, juntamente com os colaboradores: Guilherme Sena e Paulino Bambi e os estudantes do curso de Ciências Biológicas. O processamento dos dados foi feito por estes colaboradores e estudantes de graduação no laboratório de Limnologia.

Os dados ambientais e biológicos do capítulo 2 foi obtido a partir de um banco de dados do laboratório que compôs a tese de Gustavo Figueiredo Marques Leite (Leite, 2018). A coleta desses dados foi feio durante o doutorado deste pesquisador entre os anos de 2014 a 2018. Gustavo Leite também realizou todo o processo de coleta, identificação das espécies de plantas, peixes e invertebrados. Além disso, fez todo o processamento das amostras de folhiço, biofilme e de isótopos estáveis no laboratório de Limnologia. Já o levantamento dos dados do capítulo 3 foi feito pela autora desta tese com a colaboração de estagiários do laboratório e dos orientadores (professores Júnior e Murilo).

Efeito da heterogeneidade ambiental na retenção de detritos foliares

Riachos de cabeceira são considerados ecossistemas heterotróficos, decorrente do seu sombreamento levando-os à apresentarem atividade autóctone reduzida, utilizando a matéria orgânica alóctone como sua principal fonte de energia (Journal *et al.*, 2001; Kobayashi & Kagaya, 2008; Pettit *et al.*, 2012). Essa matéria orgânica alóctone é basicamente formada por detritos de origem vegetal ou animal que conectam os ecossistemas aquáticos e terrestres, contribuindo para a produtividade secundária nos riachos através da decomposição deste material (Gonçalves, França & Callisto, 2006; Gonçalves *et al.*, 2014; Gonçalves & Callisto, 2013). No leito dos riachos, essa matéria orgânica pode ser classificada como: a) matéria orgânica particulada grossa (MOPG), que são as partículas maiores de 1mm, sendo composta por troncos, galhos, folhas, flores e frutos; b) matéria orgânica particulada fina (MOPF), que são as partículas com tamanho entre 1mm e 0,45µm, originada pela quebra da MOPG ou fezes de pequenos consumidores; c) matéria orgânica dissolvida (MOD), que são as partículas menores que 0,45µm (Webster *et al.*, 1999). Após a abscisão foliar ou eventos de chuva intensa, esses detritos podem entrar no riacho por meio de diferentes vias, como transportada pelo vento, água, gravidade ou animais (aporte lateral) ou diretamente (aporte vertical). Os materias vegetais derivados da vegetação ripária (folhas, sementes, flores, frutos, galhos e troncos - MOPG) pode representar 90% do total da produção primária vegetal a cada ano (Cebrian, 1999). Esse material vegetal é a principal base energética para as comunidades de riachos florestados (Bambi *et al.*, 2016; Neres-Lima *et al.*, 2017). A quantidade de detritos foliares armazenados no leito do riacho é regulada principalmente por interações entre três fatores: entrada de detritos foliares, capacidade de retenção de riachos e decomposição de detritos foliares. As entradas de detritos foliares aumentam o armazenamento de detritos foliares pela maior eficiência de retenção das entradas. A capacidade de retenção aumentam o armazenamento de detritos foliares, pois reduz o transporte in-situ. A decomposição de detritos foliares, por sua vez, diminui o armazenamento de detritos foliares por meio da transformação de MOPG em MOPF e MOD (Gessner, Chauvet & Dobson, 1999), que é mais facilmente transportado pelo fluxo de água, enterrado nos sedimentos (Webster *et al.*, 1999) ou incorporado na biomassa microbiana e animal.

Após a queda de detritos no riacho, estes podem ser retidos no leito do riacho por estruturas retentivas como rochas, troncos e raízes ou transportada a alguma distância antes de serem retidas em algum obstáculo (Cummins, 1974; Webster *et al.*, 1999) . Essas estruturas retentivas são elementos que proporcionam maior capacidade de retenção de detritos foliares

(Kobayashi & Kagaya, 2008). Em geral, a capacidade retentiva do leito do rio aumenta com o tamanho do substrato - por exemplo, rochas e seixos são mais eficientes na retenção de detritos foliares do que cascalho e areia (Jones, 1997). Troncos grandes podem aumentar muito a retenção, redirecionando o fluxo e retendo fisicamente os detritos foliares diretamente ou em remansos. Ao serem retidas, as folhas podem se acumular na superfície a montante dos obstáculos, criando pacotes de folhas. Pacotes de folhas, sejam eles compostos de algumas ou muitas folhas, representam manchas discretas de recursos abundantes para detritívoros (Hoover, Richardson & Yonemitsu, 2006a; Kobayashi & Kagaya, 2008; Tank *et al.*, 2010). No entanto, o fluxo da água também desempenha um papel importante nesse processo. Os detritos foliares podem ser retidos temporariamente quando a velocidade da água for menor do que a velocidade necessária para mantê-la à deriva na coluna d'água ou ao longo do leito do riacho (Jones & Smock, 1991). Assim, a capacidade retentiva dos riachos é uma função das suas características hidrológicas e da eficiência de retenção das estruturas retentivas (por exemplo, rocha, tronco, raiz, etc., Hoover, Richardson & Yonemitsu, 2006b; Koljonen *et al.*, 2012; Bastias *et al.*, 2019). Portanto, é crucial quantificar os efeitos da heterogeneidade de habitat nos processos em nível de ecossistema, pois ela pode ser um *proxy* para o acúmulo ou transporte de matéria orgânica e importante nas transferências de energia dentro de sistemas lóticos.

Outros aspectos como as características morfológicas dos detritos foliares também são importantes para a retenção em ambientes com alta heterogeneidade de habitat (Kobayashi & Kagaya, 2008). Os detritos foliares diferem em propriedades morfológicas, algumas das quais (por exemplo, tamanho, flexibilidade, massa) podem afetar sua hidrodinâmica e padrão de retenção em riachos. A flexibilidade e o tamanho da folha são características foliares especialmente importantes; as folhas são geralmente grandes e flexíveis, o que permite que uma grande proporção da área de partículas à deriva entre em contato com elementos de rugosidade no leito do riacho (Kobayashi & Kagaya, 2008; Prochazka et al. 1991; Hoover et al. (2010). Além disso, as propriedades morfológicas dos detritos foliares que determinam sua retenção no leito do riacho pode depender de sua localização no canal. Por exemplo, a retenção em locais de corredeira pode ser mais provável para detritos foliares com maior flexibilidade, o que permite uma maior superfície de contato com as estruturas retentivas (Prochazka *et al.*, 1991; Steart *et al.*, 2002; Kobayashi & Kagaya, 2008). Por outro lado, a retenção em remansos pode ser mais comum em detritos foliares com alta probabilidade de deposição, determinada pela sua massa, esperado para detritos foliares menos flexíveis. Isso sugere que a retenção de detritos foliares não depende apenas da probabilidade da folha depositar em uma estrutura retentiva no leito do riacho (Koljonen *et al.*, 2012), mas também em função da capacidade das folhas de permanecerem retidas nas estruturas retentivas (Kobayashi & Kagaya, 2008), o que pode determinar o ciclo da matéria orgânica e o fluxo de energia em ecossistemas aquáticos.

Processamento dos detritos foliares em riachos tropicais

Uma vez retido, o tempo de residência dos detritos foliares em riachos permite a colonização e decomposição por bactérias, fungos hifomicetos e animais detritívoros (Hildrew *et al.*, 1991), além de processos abióticos como a fragmentação física (Gessner *et al.*, 1999). Assim, os detritos foliares estão sujeitos a alguns processos: lixiviação de componentes solúveis, processo no qual há uma rápida perda de compostos solúveis; a colonização e degradação microbiana (condicionamento); e a fragmentação através do consumo por invertebrados e pela abrasão física da água (Cummins, 1974; Webster & Benfield, 1986). O grau de lixiviação dos detritos foliares pode ser determinado pela concentração de componentes solúveis, baixa recalcitrância de carbono e ausência de ceras superficiais de detritos foliares, especialmente em águas mais rápidas e turbulentas (Schreeg et al., 2013; Ferreira *et al.*, 2012; Fonseca *et al.*, 2013). A MOPG é então convertida em MOPF, por meios físicos e biológicos; a ação da água leva à abrasão física, e a ação biológica do metabolismo microbiano e alimentação por trituradores (alimentadores de partículas grossas, Cummins, 1974). Os invertebrados detritívoros são organismos-chave na decomposição da serapilheira em ambientes tropicais e são beneficiados pela colonização microbiana (Boyero *et al.*, 2015, 2021; Rezende *et al.*, 2019). A colonização microbiana dos detritos foliares (ou seja, condicionamento microbiano) aumenta a qualidade nutricional dos detritos foliares e converte o material indigestível em compostos mais lábeis (Bärlocher, 1985; Casotti *et al.*, 2019). Essas fases não ocorrem de forma isolada como um processo temporal, mas são simultâneas e interagem durante toda a decomposição (Gessner *et al.*, 1999).

A fragmentação física é considerada um fator importante que afeta a decomposição dos detritos foliares (Ferreira *et al.*, 2006; Fonseca *et al.*, 2013; Bastias *et al.*, 2019), mas seus efeitos estão associados a outros componentes, como a decomposição mediada por detritívoros (Rader, Mcarthur & Aho, 1994). O fluxo da água aumenta a fragmentação física por meio da abrasão da superfície do detrito foliar com material particulado em suspensão, especialmente pequenas partículas, que são mais propensas a serem transportadas pela corrente (Heard *et al.*, 1999; Fonseca *et al.*, 2013). Entre os fatores que influenciam a fragmentação física, a tenacidade dos detritos foliares é especialmente importante (Fonseca *et al.*, 2013; Tonin *et al.*, 2021). A tenacidade dos detritos foliares se caracteriza por altas concentrações de lignina, celulose e hemicelulose. Folhas com alta tenacidade geralmente são cobertas por uma densa camada de cera e com uma cutícula difícil de remover, o que dificulta a liberação dos compostos solúveis pela fragmentação física (Moretti *et al.*, 2007; Bieras & Sajo, 2009).

Mecanismos locais e regionais na disponibilidade de recursos alimentares

Os riachos de cabeceira são tipicamente caracterizados por alta conectividade com as bacias hidrográficas circundantes e o sombreamento pela vegetação ripária. As características da bacia (geologia e a topografia) 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; Frissell *et al.*, 1986; Allan, 2004; Goldstein & Sponaugle, 2017). O uso e ocupação do solo que atua em escalas regionais também pode causar grandes mudanças nas temperaturas dos riachos, na hidrologia, no transporte de sedimentos e nutrientes, nos regimes de luz, e na quantidade de matéria orgânica terrestre (Jankowsk et al., 2021; Whittier & Stoddard, 2006; Steel *et al.*, 2010; Allan, 2004; Macedo *et al.*, 2014, Figura 1). Por exemplo, a substituição da vegetação nativa por atividades antrópicas (ex. pastagens e sistemas de monocultura) liberam nos rios uma quantidade excessiva de resíduos, agrotóxicos e nutrientes, o que pode refletir diretamente na fonte de nitrogênio para os consumidores (Leitão, 2015; Carvalho et al., 2017). Por outro lado, fatores que atuam em escalas locais como a cobertura do dossel, fluxo e temperatura são regidos por mecanismos em níveis hierárquicos superiores e determinam processo locais como a retenção de detritos foliares e disponibilidade de carbono para a teia trófica em riachos, O'Neill, 1986). Assim, mudanças locais na cobertura do dossel podem gerar um aumento na biomassa da perifíton (pela abertura do dossel e aumento da luz e temperatura) ou um aumento da entrada de folhas (pelo aumento da cobertura do dossel) para os riachos. Em resumo, a origem e o suplemento dos recursos de nitrogênio para a teia alimentar podem ser dependentes de fatores regionais, relacionados à bacia hidrográfica (altitude, declividade) e variáveis de uso do solo (cobertura vegetal da bacia e concentração de nitrato nos riachos, Finlay, 2001). Por outro lado, a origem e o suplemento dos recursos de carbono são determinados por fatores locais, como a variação na entrada de luz (Peterson *et al.*, 2011, Figura 1).

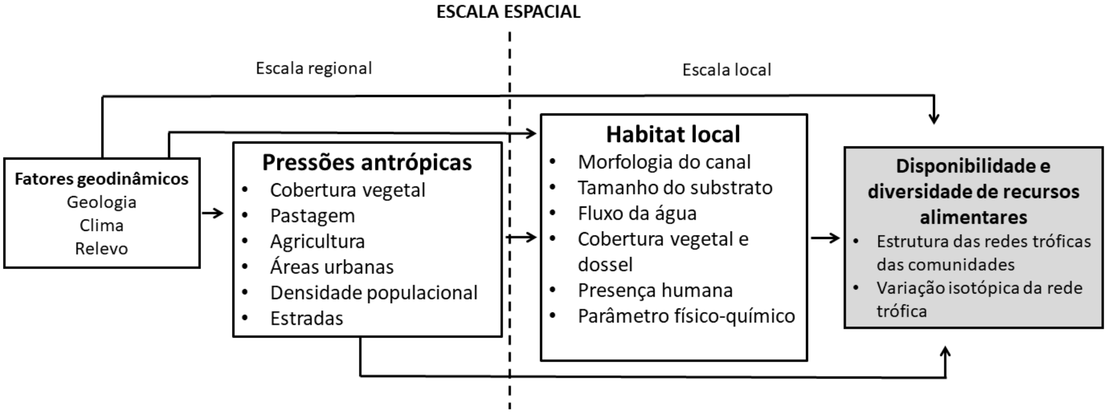

Figura 1. Organização hierárquica e interações de elementos da paisagem. Os processos biológicos das teias alimentares aquáticas podem ser influenciados por fatores que atuam em escala local, ou por fatores de escala regional (da bacia hidrográfica) e fatores antropogênicos. Fonte: Macedo et al. 2014 (adaptado).

Uso de isótopos estáveis em estudos de fluxo de energia

A estrutura trófica de uma comunidade biológica pode ser definida como um sistema adaptativo complexo, no qual diversos componentes (e.g., produtores primários e consumidores) se conectam e interagem por meio das suas relações alimentares (Dunne, Williams & Martinez, 2002; Filho, 2015; Bellmore *et al.*, 2017). O fluxo de energia e as relações tróficas entre os organismos em um ecossistema podem ser avaliadas usando a análise de isótopos estáveis (AIE) de carbono (C) e nitrogênio (N) (Post, 2002; Layman et al., 2011). Os consumidores têm uma composição isotópica semelhante à dieta de suas presas em relação ao $\delta^{13}C$, devido ao seu fator de discriminação, que varia de 0 ‰ a 1 ‰ ao longo da cadeia alimentar. Portanto, o $\delta^{13}C$ permite a obtenção da fonte da dieta assimilada na teia trófica. Diferentemente do método “tradicional” de análise do conteúdo estomacal, o isótopo de carbono de um organismo irá refletir a história alimentar do consumidor em longo prazo (considerando as taxas de *turnover*, ou de reposição os tecidos), uma vez que o carbono é acumulado no tecido animal durante a sua vida. Para o nitrogênio, o fracionamento isotópico do $\delta^{15}N$ (^{15}N e ^{14}N) varia geralmente de 2 a 4 ‰ a cada nível trófico (Zanden & Rasmussen, 2001; Post, 2002; McCutchan *et al.*, 2003). Valores de $\delta^{15}N$ aumentam mais a cada transferência do que os de $\delta^{13}C$ e têm sido usados para identificar a distância de consumidores da base da cadeia.

Fatores regionais e locais podem determinar o fracionamento isotópico do $\delta^{15}N$ e $\delta^{13}C$ da teia alimentar (Castro *et al.*, 2016; Carvalho *et al.*, 2017; Price *et al.*, 2019; Reis, Albrecht & Bunn, 2020). Fatores que atuam em escalas regionais são os principais determinantes da variação das assinaturas isotópicas de $\delta^{15}N$ das teias alimentares (Finlay, 2011). A concentração de nutrientes em riachos de cabeceira é naturalmente baixa (Finlay, 2011), no entanto, a entrada de nutrientes causada pelos fatores regionais pode enriquecer os valores isotópicos de nitrogênio, o que pode ser rastreado pelas assinaturas $\delta^{15}N$ através de níveis tróficos sucessivos nas teias alimentares (Carvalho *et al.*, 2017; Lee *et al.*, 2018; Price *et al.*, 2019). Por outro lado, as assinaturas isotópicas de $\delta^{13}C$ da teia alimentar podem variar em função das mudanças locais nas zonas ripárias e na cobertura do dossel (Neres-Lima *et al.*, 2016, 2017; Carvalho *et al.*, 2017; Castro, Dolédec & Callisto, 2018; Reis *et al.*, 2020). O aumento da taxa de fotossíntese das algas em locais com maior entrada de luz pode fornecer valores de $\delta^{13}C$ mais enriquecidos aos consumidores.

Uso de redes tróficas em estudos de fluxo de energia

Diante das mudanças substanciais na cobertura da terra é importante ampliar a compreensão sobre a maneira como as comunidades biológicas dos riachos reagem a essas alterações ambientais. Dentre os métodos mais recentes destaca-se a análise das redes de interação ecológica (Delmas *et al.*, 2018). Uma das representações das relações tróficas de uma comunidade é por meio de redes de interações (redes tróficas, Figura 2), a qual vem sendo amplamente utilizada em estudos ambientais (Manoel & Uieda, 2017; Kortsch *et al.*, 2018; Felipe-Lucia *et al.*, 2020). Uma das características principais de uma rede é o grau de aninhamento, modularidade e densidade das ligações (Fortuna *et al.*, 2010). Redes aninhadas ocorrem quando espécies especializadas tendem a interagir fortemente com subconjuntos de recursos da maioria espécies generalistas (Bascompte, Jordano & Melia, 2003; Jordano, Bascompte & Olesen, 2006; Ulrich & Almeida-neto, 2012) e com distribuição assimétrica e hierárquica de interações entre espécies, esperado para ambientes instáveis e com algum grau de impacto ambiental (Bascompte *et al.*, 2003). Além disso, uma das explicações para esse padrão de matriz aninhada envolve diferenças na abundância das interações predador-presa (Lewinsohn *et al.*, 2006) e maiores taxas de extinção de espécies especialistas (Ollerton *et al.*, 2003). Por outro lado, as redes modulares são representadas por módulos interconectados e cada módulo inclui um grupo de espécies intimamente ligadas entre si e com poucas interações com o resto das espécies presentes em outros módulos com subconjuntos não-sobrepostos (Olesen *et al.*, 2007; Dormann & Strauss, 2014). Assim, nesse tipo de matriz, as espécies são classificadas pelas afinidades de ligações com outras espécies, enquanto que a matriz de aninhamento é de acordo com o numero de links. Essas estruturas podem sofrer interferências em diferentes ambientes de acordo com a integridade ambiental dos riachos.

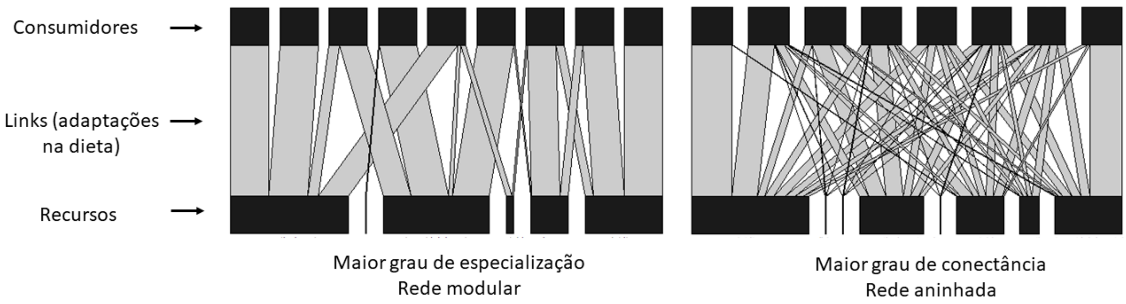

Figura 2. Representação das estruturas de redes aninhadas e modulares.

Dada a importância das redes ecológicas para o funcionamento dos ecossistemas (Schleuning, Fründ & García, 2015), tem havido um interesse crescente em grandes estudos de redes tróficas em função de gradientes ambientais (por exemplo, Sebastián-González *et al.*, 2015; Dalsgaard *et al.*, 2017; Dugger *et al.*, 2018; Galiana, Hawkins & Montoya, 2019; Felipe-Lucia *et al.*, 2020). Comparar redes ao longo de gradientes ambientais tem o potencial de fornecer informações sobre como as condições abióticas determinam a variação nas interações das espécies (Fuller, Doyle & Strayer, 2015; Pellissier *et al.*, 2017), Atualmente, a pesquisa sobre os efeitos dos gradientes de perturbação nas comunidades de peixes tem se concentrado amplamente na riqueza taxonômica e funcional (Brejão, Gerhard & Zuanon, 2013; Teresa, Casatti & Cianciaruso, 2015; Castro *et al.*, 2018). No entanto, ainda existem poucos estudos mostrando como as modificações de habitat no nível da paisagem influenciam as interações tróficas avaliando diretamente as redes tróficas (Schleuning *et al.*, 2015).

Em redes tróficas, os peixes usam diferentes microhabitats preferidos, como remansos ou corredeiras, posições na coluna de água, no meio da coluna ou no fundo da água e em diferentes tipos de substratos durante o forrageamento. Diferentes estratégias de forrageamento de peixes podem depender do grau de integridade ambiental dos ambientes aquáticos, o que afeta o habitat e a disponibilidade de alimentos (Macarthur & Pianka, 1966). As teias alimentares encontradas em ambientas sob influência antrópica são representadas por espécies mais persistentes a esses tipos de ambientes. Além disso, tendem a apresentar dieta generalista e podem influenciar a redundância / conectividade da rede alimentar, aumentando o grau de aninhamento das redes (Manoel & Uieda, 2017). Em áreas preservadas, há maior heterogeneidade de habitat e diversidade de alimento, contribuindo para a complementaridade taxonômica e funcional (Keller *et al.*, 2009; Teresa & Casatti, 2017), aumentando então a complexidade das ligações tróficas (Ceneviva-Bastos *et al.*, 2017), padrão compatível a redes modulares. Assim, o conhecimento da estrutura e organização trófica de um ecossistema nos permite interpretar e prever os efeitos de impactos antrópicos sobre os organismos (Schalk *et al.*, 2017). Torna-se necessário desenvolver abordagens preditivas capazes de identificar as consequências das alterações na cobertura vegetal e uso do solo pelas atividades humanas para a estrutura das assembleias e para o funcionamento dos ecossistemas.

OBJETIVO & ESTRUTURA DA TESE

O objetivo geral desta tese foi avaliar a influência da heterogeneidade de habitat e do fluxo de água no funcionamento dos ecossistemas aquáticos (capítulo I), e como mecanismos locais (mudanças na zona ripária) e regionais (características da paisagem e/ou uso do solo) se relacionam com a estrutura trófica da teia alimentar (capítulos II e III). Para isso, no **capítulo I** exploramos, através de um experimento, como a retenção e a decomposição de detritos foliares em riachos de cabeceira podem ser afetadas pela heterogeneidade de hábitat e pelo fluxo de água. Considerando a diversidade física e morfológica das espécies vegetais na vegetação ripária, utilizamos detritos foliares com diferentes tamanhos para o experimento de retenção, e macios e recalcitrantes para o experimento de decomposição. Esperamos que os resultados desse capítulo contribuam para embasar estratégias de manejo que promovam a retenção e a decomposição de detritos foliares em riachos de cabeceira, tendo em vista a importante contribuição desses processos para o funcionamento de ecossistemas aquáticos.

Considerando que mudanças locais na zona ripária influenciam a entrada de matéria orgânica, e que características da paisagem e uso do solo influenciam o carregamento de sedimentos e nutrientes para os riachos, no **capítulo II** investigamos como esses mecanismos locais e regionais afetam os valores de $\delta^{13}C$ e $\delta^{15}N$ da teia trófica de riachos do cerrado. Nossos resultados destacam que, mesmo em pequenos riachos florestados, as fontes de recursos de algas desempenham um papel importante na cadeia alimentar aquática. Além disso, destacamos a importância de se considerar a estrutura do habitat local e as características da bacia hidrográfica como impulsionadores da disponibilidade de carbono e nitrogênio para os consumidores em riachos de cabeceira.

No **capítulo III**, investigamos como as atividades antrópicas nas bacias de drenagem afetam a estrutura das redes tróficas de peixes em riachos utilizando métricas de redes. Para isso, utilizamos uma compilação de dados de dieta de peixes de todo o Brasil em riachos com diferentes impactos ambientais na bacia (pastagem, agricultura e urbanização) amostrados entre 1982 a 2019. Esperamos que os resultados desse capítulo contribuam para o embasamento de medidas para a preservação de comunidades de peixes em riachos influenciados por atividades antrópicas em suas bacias de drenagem.

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CAPÍTULO I - HABITAT HETEROGENEITY INCREASES LITTER RETENTION AND DECOMPOSITION IN A CERRADO SAVANNA STREAM

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ABSTRACT

Leaf litter fragmentation is one of the main determinants of the availability of food resources for aquatic biota, and this process is strongly influenced by the retention capacity of streams. Retention capacity depends on habitat heterogeneity, as well as on other factors such as leaf litter characteristics and microhabitat diversity. However, the effects of these factors have not yet been clearly understood. In this study, our hypotheses were as follows: (i) habitat heterogeneity increases leaf litter retention, particularly of larger leaves, and (ii) water flow enhances leaf litter fragmentation by promoting physical abrasion of leaf litter, particularly of softer leaves. We tested these hypotheses using (i) the proportion of leaves in transport and the variation of retentive structures such as rock, pebbles, trunks, and roots for habitat heterogeneity in three reaches and (ii) litterbags of two mesh sizes (fine and coarse) incubated in riffle and pool habitats in three reaches of a headwater stream of the Cerrado biome. Our results demonstrated that habitat heterogeneity and leaf litter size increased leaf litter retention rates, and that water flow is an important factor for leaf litter fragmentation in tropical streams. Large leaves interacted with local conditions of streambed heterogeneity (trunks and roots) to reduce transport distances, and hydrology (water flow) accelerated the fragmentation of soft leaves. Our findings suggest that management strategies promoting the accumulation of woody leaf litter in the streambed (trunks and roots), and the diversity of habitats (pool-riffle) can be valuable to increase the retention capacity of streams and processing of allochthonous organic matter.

Keywords: Leaf litter fragmentation; Leaf processing; Pool; Riffle; Retention capacity; Roughness; Trunks

INTRODUCTION

Headwater streams are important connections between terrestrial vegetation and large rivers because they receive and transform large amounts of organic matter (mostly litter) from the riparian forest each year (Rezende et al., 2017; Tonin et al., 2017). They are capable of retaining and incorporating a significant fraction of this leaf litter into their food webs (Entrekin et al., 2020). Consequently, streams contribute to leaf litter processing because of their high retention capacity, which increases leaf litter residence time and variable water flow in space (e.g., pool-riffle configuration) or time (e.g., low-high water flow conditions) (Bastias et al., 2018; Lamberti et al., 2017). Headwater stream food webs rely on the terrestrial sources of leaf litter, given that the canopy cover above the streambed limits the available sunlight and subsequently the in-stream primary production (Neres-Lima et al., 2017). Once in the stream, litter can be (i) retained and later stored in pool areas, (ii) decomposed by microorganisms and detritivores, or (iii) transported downstream until its retention (Bastias et al., 2019; Gonçalves et al., 2017).

Leaf litter retention and transport are contrasting processes that are influenced by stream morphology (e.g., width, depth, sinuosity, and slope of stream channel), water flow variability (e.g., riffle and pool distribution within the stream), and streambed heterogeneity (Bastias et al., 2019; Hoover et al., 2006; Lamberti et al., 2017). Stream heterogeneity is characterized by substrates of different sizes (e.g., pebbles, stones, gravel, and sand) and by structures derived from riparian plants (e.g., living tree roots and dead trunks) where leaf litter may be retained, decreasing the downstream leaf litter transport (Frainer et al., 2018). Retention occurs when leaf litter encounters streambed substrates or other retentive structures, such as living tree roots, large pieces of deadwood, and trunks (see Webster et al., 1994). However, there are many other variables that vary systematically with leaf litter transport distances and are more likely to be directly related to leaf litter retention. For example, larger leaves are more likely to be trapped by substrates (Brouwer et al., 2017), and thus have higher retention rates (Kobayashi & Kagaya, 2008). Although it is evident that leaves must come into contact with the roughness elements before being retained, it is not known how leaf morphology influences the likelihood of contact with retentive structures such as rocks, pebbles, roots, and trunks.

Once retained, the residence time of coarse particulate organic matter in streams is affected by (i) the action of water flow, which dissolves soluble compounds (i.e., leaching), (ii) water abrasion on leaf litter tissues (i.e., physical fragmentation), and (iii) biological action (i.e., microorganisms and detritivores). These processes lead to the conversion of leaf litter into fine particulate organic matter and dissolved organic matter, thereby leading to the flow of energy in the detritus food web (Graça et al., 2005). Invertebrates are important for the processing of organic matter in tropical streams (Boyero et al., 2021; Rezende et al., 2020; Salomão et al., 2019). Nonetheless, the Cerrado savanna streams have low diversity and abundance of shredders (Alvim et al., 2015b; Boyero et al., 2012, 2011; Bruder et al., 2014; Ferreira et al., 2012a; Fonseca et al., 2013; Gonçalves et al., 2017; Graça et al., 2015) because of the low quality of the litter (i.e., lower phosphorus content and higher toughness; Ardón et al., 2009; Ramos et al., 2021). For example, changes in leaf litter availability, mainly during the rainy season, have the potential to affect leaf-shredding (Boyero et al., 2012, 2011), which explains its low density and contribution to leaf litter processing in Cerrado streams (Alvim et al., 2015; Rezende et al., 2019). Therefore, differences in the environmental conditions of streams, such as increased water flow, can play a determining role in the processing of organic matter (Bastias et al., 2019).

Differences in the environmental conditions of streams, such as increased water flow, can play a determining role in the processing of organic matter (Bastias et al., 2019). For example, in two tropical rivers in Australia, Pettit et al. (2012) found that water velocity had a greater impact on leaf mass loss than aquatic macroinvertebrates and leaf-associated microbial communities. Water flow can increase leaf litter fragmentation, thereby stimulating fungal activity (Ferreira and Graça, 2006), and promoting physical abrasion (Ferreira et al., 2012b; Fonseca et al., 2013), particularly in the final stages of the decomposition process (Abril et al., 2021; Bastias et al., 2019), when leaves are more fragile due to microbial-mediated enzymatic maceration. Physical fragmentation can be expected in softer leaves (which are more susceptible to physical abrasion) and in riffles (where physical abrasion is higher). Initially, the chemical quality of the leaf litter can affect leaching by determining the amount of water-soluble compounds (such as certain micro- and macro-nutrients, low-molecular-weight molecules, and certain secondary compounds) and their resistance to dissolution (Schreeg et al., 2013). On the other hand, slower water flow in pools can decrease fungal activity and development through reduced fluxes of dissolved oxygen and nutrients (Bruder et al., 2016) and increased fine sediment deposition (Marjakangas et al., 2019). In pools, the leaf litter patches can act as microhabitats for shredders, leading to higher fragmentation rates by shredders than that in riffles (Gonçalves et al., 2012; Kobayashi and Kagaya, 2002; Moretti et al., 2007; Rezende et al., 2016). Given the tight connections between habitat heterogeneity, leaf litter retention, and fragmentation (Frainer et al., 2018; Muotka and Laasonen, 2002), an integrated assessment of these processes is important to understand the effects of the stream-riparian forest link for the functioning of aquatic ecosystems (e.g., Bastias et al., 2019; Lamberti et al., 2017).

In tropical streams, many studies have considered the effects of characteristics of leaf litter, such as leaf quality, on the functioning of aquatic ecosystems (Navarro and Júnior, 2020; Sena et al., 2020; Tonin et al., 2014). However, there is little evidence on how the diversity in leaf litter morphology and tenacity together affect leaf litter retention and processing. Therefore, our aims were to: (i) quantify leaf litter retention rates by releasing and estimating the proportion of marked leaves retained, (ii) compare leaf litter fragmentation by incubating leaf litter in leaf riffle and pool areas using litterbags, and (iii) assess in-stream leaf litter transport using drift nets. The study premise was that the stream physical characteristics (e.g., habitat heterogeneity and water flow) influenced the two key related processes of leaf litter dynamics of leaf litter retention and fragmentation. We hypothesized that (i) in-stream heterogeneity enhances leaf litter retention (particularly of larger leaves), and that (ii) water flow increases leaf litter fragmentation by water abrasion (particularly of softer leaves).

METHODS

2.1 Study site and experimental design

The study was carried out in a headwater stream (Cabeça-de-Veado) (15°53′11.74″ S; 47°50′33.27″ W), located at the Ecological Station of Botanical Garden of Brasilia in Federal District, Brazil, during the rainy season, from November to December of 2016. The climate is tropical savanna (Aw), with a dry season from May to September (mean temperature of 18°C), and a rainy season from October to April (mean temperature of 29°C). The average annual precipitation is 1500 mm, which ranges from 750 mm to 2000 mm (Silva et al., 2008). During the experimental period, water pH was 6.6 ± 0.1 (mean and standard deviation), temperature 20.0 ± 0.2°C, water flow 0.99 ± 0.10 m s^{-1}, conductivity 6.3 ± 1.6 μS cm^{-1}, nitrate (0.13 mg L^{-1}), and turbidity (1.8 ± 0.2 NTU).

The study consisted of two independent experiments on leaf litter retention and fragmentation, and an estimation of leaf litter transport, which were conducted on three adjacent and 50-m long stream reaches (Figure 1). This extension of the stream reach was important for assessing environmental heterogeneity based on data on productivity, habitat diversity, and organic matter dynamics observed in other studies carried out in the same stream (Bambi et al., 2017; Tonin et al., 2019). We calculated the coefficient of variation (CV) to estimate the spatial heterogeneity between the reaches, considering the sediment data (percentage of silt, clay, sand, gravel, pebbles, and stones), benthic stock of organic matter (% of reach), root density, density of branches (apparent range %), and stream channel morphology (bank height, stream width, and angle of inclination of the curves or sinuosity). The coefficient of variation (CV) is the relationship between the standard deviation and the mean of the environmental variables measured in situ. The CV has been provided as a percentage; a CV close to 100% indicates high heterogeneity of the reach. We selected leaves from four plant species: *Protium spruceanum* (Benth.) Engl., *Richeria grandis* Vahl., *Calophyllum brasiliense* Cambess., and *Maprounea guianensis* Aubl. All plant species are native and commonly found in the Cerrado riparian zone, as well as in our study stream (Bambi et al., 2017). Fresh leaf litter from the four plant species was collected in the study area during the period of greatest leaf litter production, air-dried, and stored until the beginning of the experiments.

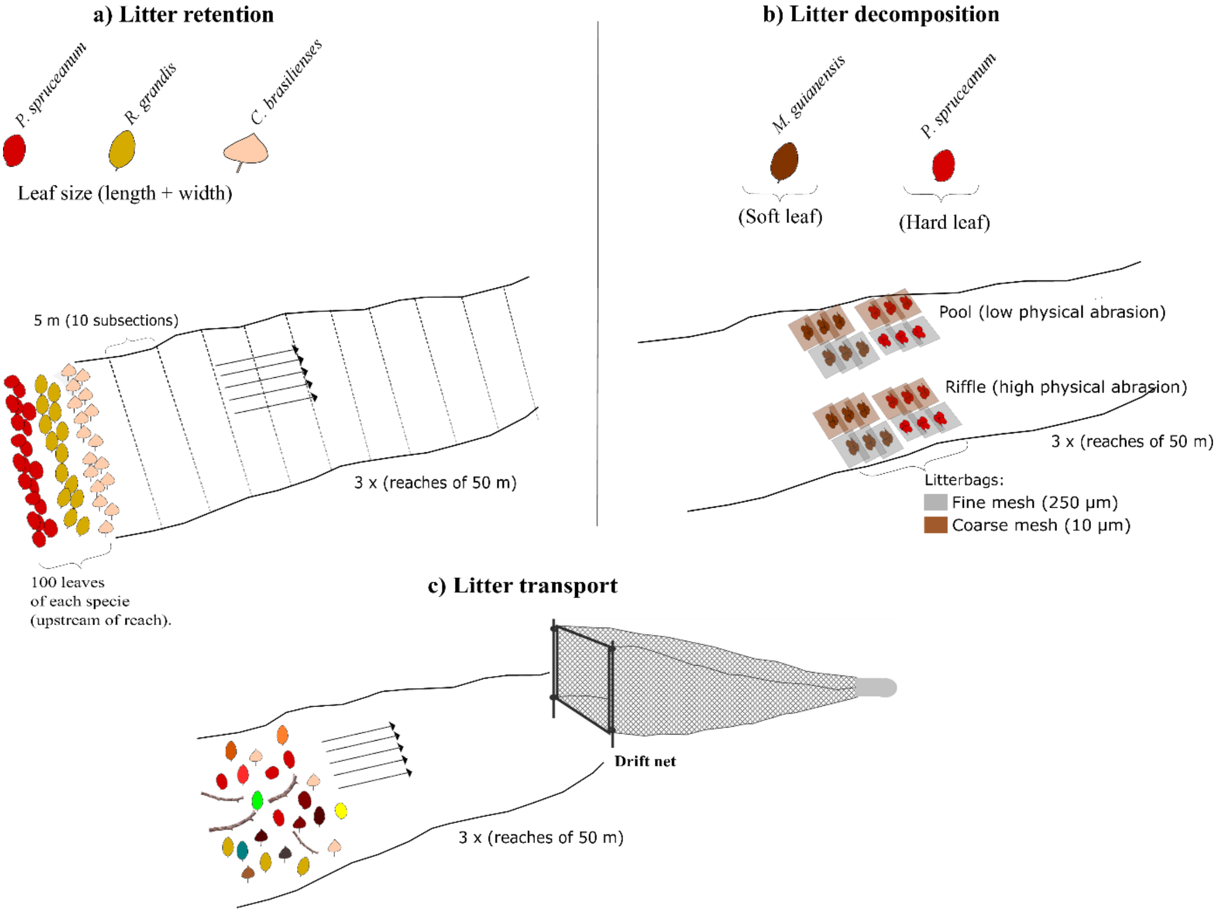

Figure 1. A schematic representation of the study design. (a) Leaf litter retention experiment, in which three types of leaf litter with different sizes and morphology were deployed in the upstream part of each reach; (b) Leaf litter fragmentation experiment, in which different leaf litter species (hard and soft) were deposited in litterbags in two micro-habitats (riffles and

pools); (c) Leaf litter transport experiment, in which drift nets were installed at the end of each section. Species used in the study: *Calophyllum brasiliense, Maprounea guianensis, Protium spruceanum,* and *Richeria grandis*.

2.2 Litter retention experiment

Leaves of *Protium spruceanum*, *Calophyllum brasiliense*, and *Richeria grandis* (n= 100 of each species) were marked with colored ink, soaked in water to confer neutral buoyancy (Speaker, Moore and Gregory, 1984), and released uniformly across the width of the channel upstream of each stream reach. We determined 10 subsections of 5 m for the retention experiment in each of the stream reach. We counted the number of leaves of each species retained after one hour in each 5 m subsection (Figure 1a). We measured the length and width of all retained leaf litter using calipers. The leaf litter average proportional size was calculated by adding its width and length for each species and in each reach (Figure 2). The values (mean ± SE) for *P. spruceanum*, *R. grandis,* and *C. brasiliense* were 14.41 ± 0.30, 17.98 ± 0.42, and 11.49 ± 0.27. The calculation of the area (length × width) was not used, as the lanceolate and spatulate shape of the Cerrado leaves does not allow exact measurements of the area. Thus, when we added the width and length, we estimated the proportional leaf litter size in relation to its length and width. We measured leaf litter size only of the leaves that were retained in the streambed in the retention experiment. Thus, from a total of 300 leaves that were released in each reach, we measured only 139 for reach 1, 259 for reach 2, and 281 for reach 3. At each stream reach, we determined the substrate composition through a visual estimate of the proportion of each substrate type in all 10 subsections of 5 m (Cummins, 1962). For each subsection, we evaluated the visual percentages of clay, sand, gravel, silt, pebbles, stones, and leaf litter bank on the streambed. We also evaluated the proportion (0 to 10) of internal habitat structures, such as roots and trunks in the channel and in-stream reach margins

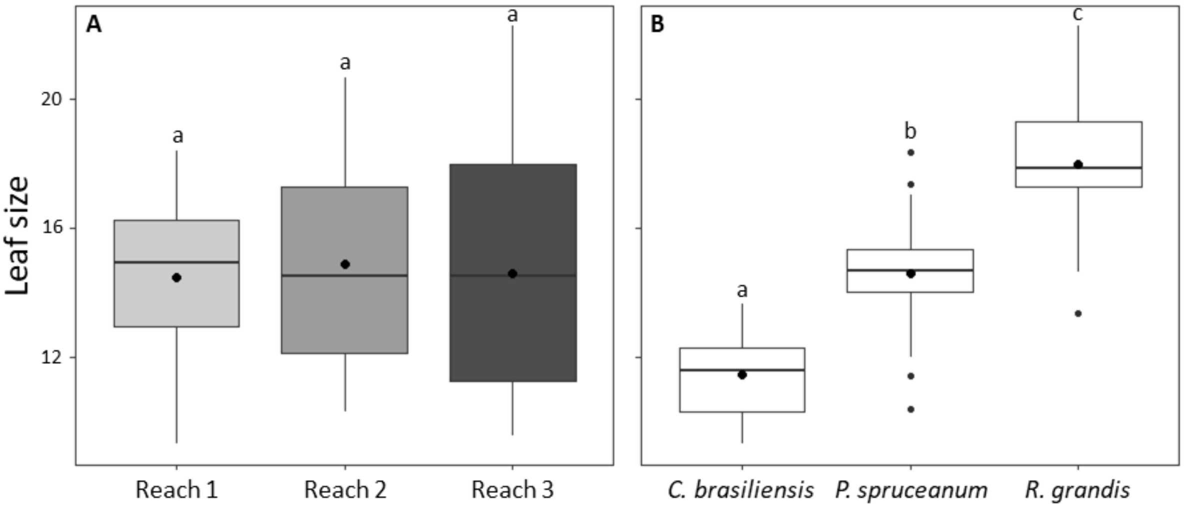

Figure 2. Boxplots representing leaf litter size for the three plant species (*Calophyllum brasiliense*, *Protium spruceanum*, and *Richeria grandis*) retained in each stream reach (A) and for each species (B). Different letters (a, b, and c) indicate significant differences (Tukey's Multiple Comparison test, $p < 0.05$).

We calculated the average leaf litter path distance from the release point in each study reach. Therefore, we plotted the proportion of leaves transported at a given displacement distance (Speaker et al., 1984). Thus, the number of released leaves transported was plotted against the drift distance (subsection). We fitted the retention dynamics to a negative exponential model (Young et al., 1978):

$$L_d = L_0 \, e^{-kd} \quad (1)$$

where, L_0 is the number of leaves (i.e., 100 leaves for each species) released into the reach during each sampling occasion, L_d is the number of leaves transported to a downstream distance d (meters) from the release point, and $-k$ is the retention coefficient (m^{-1}), which is related to the proportion of leaves settling per meter (Larrañaga et al., 2003; Webster et al., 1999). Larger values of k indicate higher retention rates. From the calculations, the average leaf litter drift distance, $1/k$, can be determined. The slope $-k$, is the instantaneous leaf litter retention rate, and $1/k$ is the average distance traveled by a leaf in the stream before its retention (Speaker, Moore and Gregory 1984).

2.3 Leaf litter fragmentation experiment

The plant species used for this experiment were *Maprounea guianensis* and *Protium spruceanum*. *M. guianensis* (considered here as softer leaves), and *P. spruceanum* (harder leaves) (Navarro and Júnior, 2020; Rezende et al., 2019, 2020). Leaf litter fragmentation was evaluated using fine (0.25 mm mesh size, which allows for colonization only by microorganisms) and coarse mesh (10 mm mesh size, which allows for colonization by microorganisms and invertebrates) litterbags. We prepared 48 litterbags of fine and coarse mesh, totaling 72 litterbags. Litterbags were filled with 2 g (± 0.2 g) of *M. guianensis* (n = 18 each in coarse and fine mesh) or *P. spruceanum* (n = 18 each in coarse and fine mesh). Litterbags were incubated in riffles (high physical abrasion) or pools (low physical abrasion) in all three stream reaches. Thus, each stream reach had 24 litterbags, 12 for each microhabitat (riffle and pool) of two leaf litter species (soft or hard) in coarse or fine mesh bags (Figure 1b).

The litterbags were recovered after 45 days, placed in plastic bags, and transported to a laboratory in a thermal container with ice. The leaves from the litterbags were carefully washed with distilled water to remove sediments and invertebrates. From each litterbag, we chose five leaf litter samples and then removed five discs (12 mm; one disc from each leaf litter), totaling five discs for each litterbag. This disc set was used to estimate the dry mass (DM) of the removed discs and to obtain the ash-free dry mass (AFDM). The discs were dried at 60 °C for 72 h, weighed, incinerated at 550 °C for 4 h, and weighed again (with an accuracy of 0.01 mg).

The remaining leaf litter was dried at 60 °C for 72 h, weighed, and added to removed discs DM: Final DM = litter DM + (discs DM). We quantified leaf litter fragmentation in each litterbag as the proportion of litter mass loss (LML) (after 45 days of incubation): LML = [initial AFDM (g) – final AFDM (g)] / initial AFDM (g), with initial AFDM corrected by drying, and ash content (i.e., after multiplying discs AFDM by dry mass; Bärlocher, 2005; Webster et al., 1999). We calculated the contribution of invertebrates to leaf litter fragmentation as the difference in LML between paired coarse- and fine-mesh litterbags for each block (n = 3).

2.4 Leaf litter transport estimation

Litter transport was quantified using a drift net (30 × 30 cm, with mesh size of 0.25 mm) centrally arranged in the stream channel and fixed to the streambed (Figure 1c). The net was installed at the end of each selected stream reach. In each stream reach, the water flow and cross-sectional area intercepted by the drift net were measured upstream. After one hour, plant materials (leaves, branches, and others) in the net were collected and transported in plastic bags to the laboratory.

In the laboratory, the plant materials were dried (50°C, 72 h) and weighed (with an accuracy of 0.01 mg) to determine dry mass (DM). We calculated the AFDM of each plant material by subtracting the ash mass from that of the dry matter and adding all categories in a sample to obtain the total AFDM. To calculate the water filtered volume by each drift net, we multiply the cross-sectional area of water funneled into the net by the average water flow measured immediately both after introducing and before removing the net. To calculate the concentration of coarse particulate organic matter (CPOM) transported from upstream, we divided the AFDM of transport inputs by the filtered water volume (see Graça et al., 2005). The results were expressed in g AFDM m^{-3}.

2.5 Statistical analyses

We tested separate models for overall fragmentation in coarse mesh litterbags, microbial fragmentation in fine-mesh bags, and detritivore-mediated fragmentation. We used linear models with the gls (generalized least squares) function of the ‘nlme’ package (Pinheiro et al., 2018) to test the effect of the microhabitats (pool and riffle) and leaf litter species (soft or hard) (predictor variables) and all interactions on leaf litter fragmentation (response variable).

A principal component analysis (PCA; cmdscale function of the vegan package) was used to encapsulate a set of environmental information of in-stream heterogeneity. We first selected the retentive structures relevant to leaf litter retention (percentage of pebbles, stones, roots, and trunks). We then retained the first axis (hereafter PCA1), which summarized 60% of the total variation in PCA and was mostly related to stones and pebbles ($r = -0.90$), and tree roots ($r = 0.92$). This provided a single variable that represented stream heterogeneity to be used in further analyses. We examined the individual and interactive effects of habitat heterogeneity (PCA1), leaf litter size, and plant species (*P. spruceanum*, *C. brasiliense*, and *R. grandis*) on leaf litter retention (of transported leaves) using linear mixed-effect models. The models were first defined in terms of a random structure, and a model selection procedure was used to identify the interactions between the predictors. The random structure of the fitted models included reach as a random term and a constant variance function structure (VarIdent) to allow different variances among reaches. Individual and interactive effects were explored through seven models, all containing at least one predictor, but varying in the number of interactions. The null model (model 7) assumed no interactions between predictors (i.e., intercept only), two models (models 1 and 2) included interactions between habitat heterogeneity and species type, or habitat heterogeneity and leaf litter size, and one model (model 1) included all interactions, including the three-way interaction. The seven models were compared using an Akaike's information criterion corrected for sample size (AICc)-based model selection approach, with the most plausible models being selected based on delta AICc (Δi; i.e., difference in AICc value relative to the best model) and Akaike weights (*wi*; i.e., the probability that a model is the best among the whole set of models). Residuals from each model were inspected to ensure that the parametric assumptions for the linear models were not violated. Models were constructed and selected using nlme ("gls", "lme" and "VarIdent" functions; Pinheiro et al., 2020) and MuMIn packages ("model. Sel"; Bartoń, 2019). To test whether leaf litter retention depended on features of reaches, species, or both, we used linear models (gls function of the nmle package; Pinheiro et al., 2020). Models were first defined in terms of the best random structure, which were attained with the presence of a constant variance function structure (VarIdent) in relation to species and reach, and a temporal correlation component (corAR1) that considered the dependence of adjacent subsections within each reach (Zuur et al., 2009). When a statistically significant interaction between reach and species was detected, we refitted the model using only reach as a predictor and data for each species separately. Pairwise comparisons among reaches were performed with Tukey tests using the glht function of the multcomp package, with adjusted p-values (Hothorn et al., 2008). The analyses were performed using the software R version 4.0.4 (R Development Core Team, 2020)**.**

RESULTS

3.1 Leaf litter retention

The reaches 2 and 3 presented a higher spatial heterogeneity than reach 1 (Tukey test; $p < 0.001$, Figure 3). We found significant differences in the retention of leaves downstream between the leaf litter species (*P. spruceanum*, *C. brasiliense*, and *R. grandis*) and the type of reach (Tables 1 and 2). The retention rates were higher in reaches 2 and 3 (mean k ± SE, 0.032 ± 0.009; 0.038 ± 0.007, n = 3, respectively) than in reach 1 (0.01 ± 0.002, n = 3; Figure 4, Table 1S). In our study, we observed instantaneous retention rates ranging from 0.006 $k.\mathrm{m}^{-1}$ (*C. brasiliense* in reach 1) to 0.052 $k.\mathrm{m}^{-1}$ (*R. grandis* in reach 3). These retention rates represent ranges that require 166 m to 20 m to retain 31% and 98% of leaves inserted, respectively (Table 1S).

Table 1. Results of linear models testing the effects of the leaf litter species (*P. spruceanum*, *C. brasiliensis* and *R. grandis*) in three different reaches (1, 2 and 3) on the leaf litter retention.

	DF	**F-value**	***P*-value**	**Contrast analysis**
Protium spruceanum				
Intercept	1	65.97	<0.001	
Reach	2	10.27	<0.001*	T3 > T1 = T2
Calophyllum brasiliense				
Intercept	1	132.87	<0.001	
Reach	2	2.32	0.11	
Richeria grandis				
Intercept	1	142.12	<0.001	
Reach	2	0.71	0.49	

*****Statistically significant differences

Table 2. Results of linear models and contrast analyses (AC; P<0.05) testing the differences in leaf litter retention for the three plant species (*Protium spruceanum*, *Calophyllum brasiliense* and *Richeria grandis*) in each stream reach.

	DF	**F-value**	***P*-value**
Intercept	1	25.11	<0.001
Reach	2	18.39	<0.001*
Species	2	67.87	<0.001*
Reach x Species	4	3.90	0.006*

*****Statistically significant differences

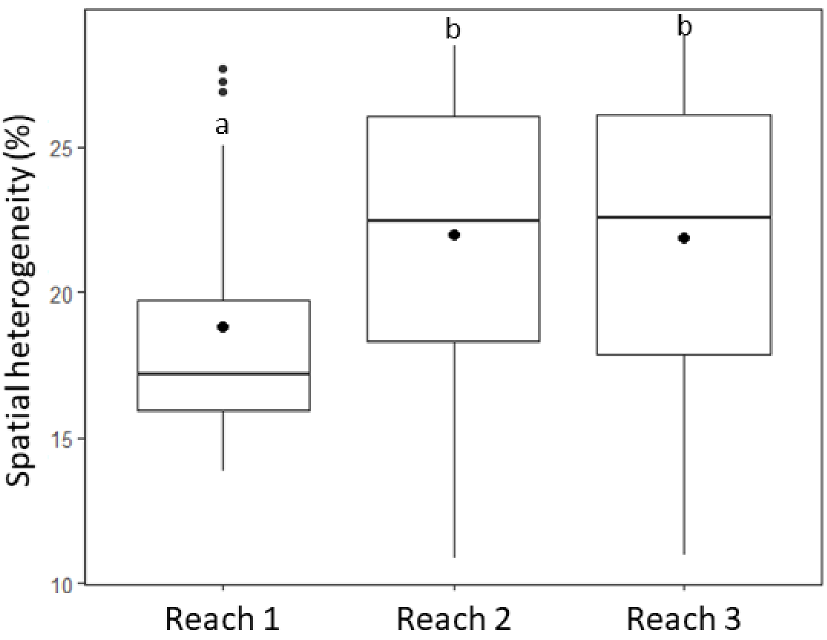

Figure 3. Environmental coefficient of variation (%) by sediment (percent of silt, clay, sand, gravel, pebbles, and stones), benthic stock of organic matter (% in reach), root density, branches density (% apparent in reach), and morphology of the stream channel (height of the margins, stream width, and angle of inclination of curves or sinuosity) in three savanna streams. First (lower line) and third (higher line) quartiles, the median (bold line), and outliers (circles) are shown. Different letters (a and b) indicate significant differences between reaches (Tukey's Multiple Comparison test, $p < 0.05$).

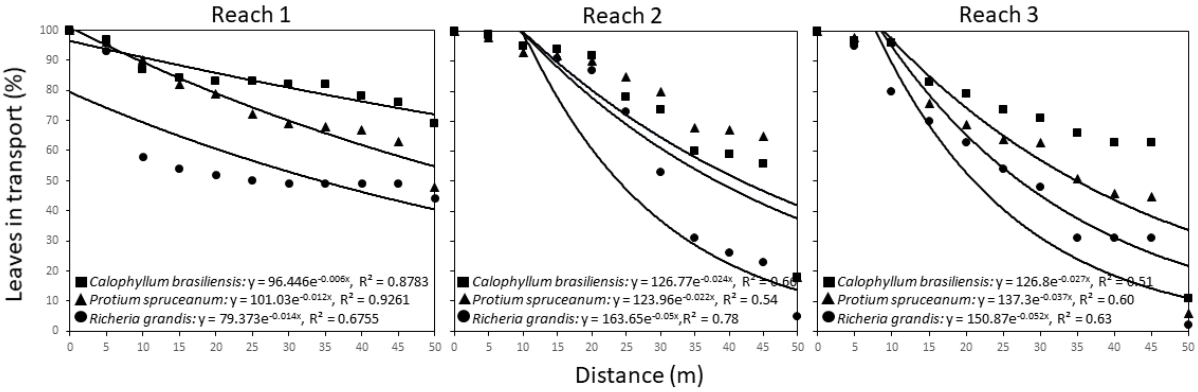

Figure 4 Relationship between leaf litter retention (percentage of leaves in transport) and distance traveled from the release point in each study stream reach. The regression line represents fit to the negative exponential model. Squares represents *Calophyllum brasiliense*; triangles: *Protium spruceanum* and circles: *Richeria grandis.*

Habitat heterogeneity (in terms of pebbles, stones, and roots) was more correlated with axis 1 of the PCA. The percentage pebbles and stones were negatively related to axis 1, whereas the percentage of root structures was positively related to axis 1 (Figure 1S, Table 2S). The percentage of trunk structures was more closely related to PCA axis 2 (Table 2S). The model selection procedure revealed one model that best explained the observed patterns (90% probability based on Akaike weights). This model included habitat heterogeneity and leaf litter size, which are important for leaf litter retention (Table 3). In addition, leaf litter retention depends on the interaction between the type of reach and the leaf litter species. Only *P. spruceanum* showed a difference in retention between reaches, with higher values for reach 3 ($p < 0.001$).

Table 3. Summary of model selection procedure for the set of models used to test the effects of habitat heterogeneity (PC1), leaf litter size (size), leaf litter species (species) on leaf litter retention based on the Akaike weights (*wi* is the probability that a model is the best among the whole set of models). Models are ordered from the best to the poorest fit according to Akaike weights. df, number of estimated parameters for each model; AICc, Akaike information criterion corrected for sample size; Δi (delta AICc), difference in AICc value relative to the best model.

Model	**Intercept**	**PC1**	**size**	**species**	**PC1:size**	**PC1:species**	**df**	**AICc**	Δ_i	w_i
M4	0.069	0.050	0.004				7	-71.6	0.00	0.908
M3	0.066	0.050	0.004	+			9	-65.0	6.56	0.034
M6	0.131	0.057					6	-64.9	6.66	0.032
M2	0.049	0.038	0.005	+		+	11	-64.2	7.40	0.022
M1	0.053	0.014	0.005	+	0.002	+	12	-59.7	11.91	0.002
M5	0.023		0.007				6	-56.8	14.80	0.001
M7	0.132						5	-52.4	19.24	0

*Statistically significant differences

3.2 Leaf litter fragmentation

Soft and hard leaves incubated in pool and riffle habitats lost 18.1% ± 0.1% (mean and standard deviation) of their initial mass at the end of the experiment. We observed consistent responses of leaf litter species in both habitats (pools and riffles) for both mesh sizes, as indicated by the non-significant interactions between leaf litter species and habitats (fine mesh bags: $F_{1,30} = 2.10$, $p = 0.158$; coarse mesh bags: $F_{1,30} = 0.19$, $p = 0.662$). For instance, leaf litter incubated in riffles lost 33–50% more mass than those in pools, irrespective of litterbag mesh size (i.e., coarse or fine). Soft leaves showed 2.5- and 3.0-fold higher fragmentation than hard leaves in coarse and fine-mesh bags, respectively (Figure 5, Table 4). Finally, the model that explained detritivore-mediated leaf litter fragmentation showed that the interactions between leaf litter species and microhabitat were significant (Table 4). We explored the interactions with a second type of model by evaluating the micro-habitat effect (predictive variable) for the detritivore-mediated fragmentation (response variable) for each leaf litter species (soft and hard). These models revealed that the contribution of invertebrates was similar between habitats (riffle vs. pool) for soft leaves ($p = 0.30$), while for hard leaves it was greater in pools than in riffles ($p = 0.01$; Table 3S).

Table 4. Results of linear models testing for the effects of leaf litter species (soft or hard), micro-habitat (pool or riffle) and their interaction on the proportion of leaf litter fragmentation in fine and coarse mesh bags. Denominator degrees of freedom is 31 for the first two models and 12 for the last model.

	DF	F-value	*P*-value	Details
Fine mesh bags				
Leaf litter species	1	239.38	<0.001*	Soft leaves > Hard leaves
Micro-habitat	1	52.14	<0.001*	Riffle > Pool
Leaf litter x micro-habitat	1	2.09	0.15	
Coarse mesh bags				
Leaf litter species	1	175.25	<0.001*	Soft leaves > Hard leaves
Micro-habitat	1	5.52	0.02*	Riffle > Pool
Litter x micro-habitat	1	0.19	0.66	
Detritivore-mediated fragmentation				
Leaf litter species	1	0.18	0.66	
Micro-habitat	1	2.71	0.11	
Leaf litter x micro-habitat	1	6.57	0.01*	

*****Statistically significant differences

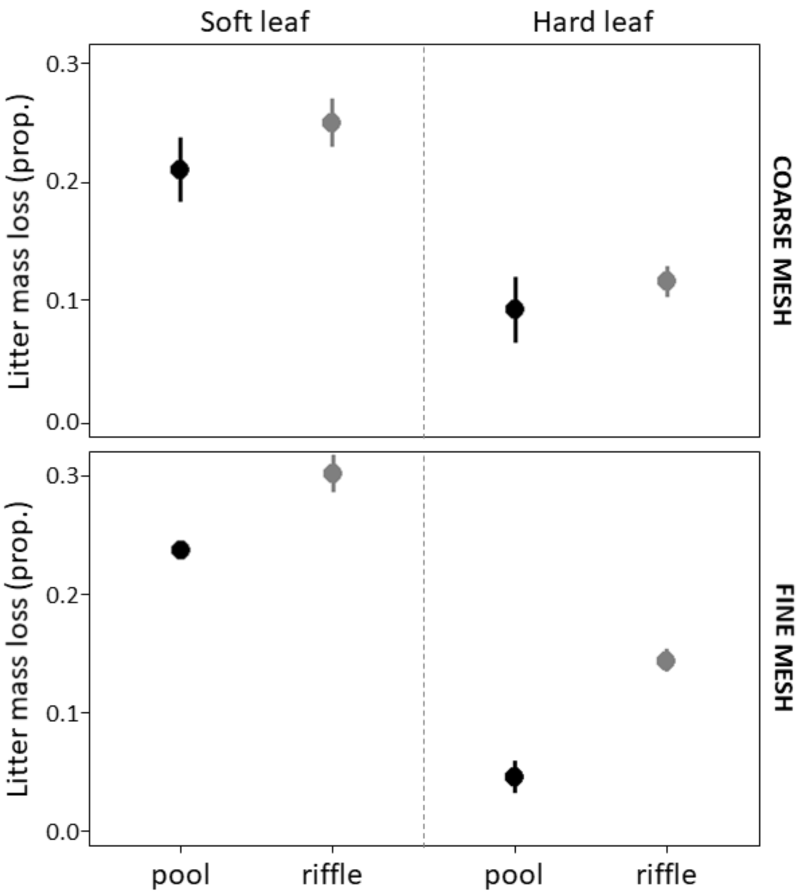

Figure 5. Proportion of litter mass loss for each leaf litter species (soft and hard) incubated in riffle and pool habitats in coarse- and fine-mesh litterbags.

3.3 Leaf litter transport

The reach with less spatial heterogeneity (reach 1) had a higher value of leaf litter transport (0.11 g.m^{-3}), while the reaches with higher spatial heterogeneity (reaches 2 and 3) had lower values of transport (0.07 g.m^{-3} and 0.004 g.m^{-3}, respectively).

DISCUSSION

The findings of our study showed that both habitat heterogeneity and water flow can be important drivers of leaf litter dynamics in streams, as they control retention patterns and can influence leaf litter fragmentation (Bastias et al., 2019; Lamberti et al., 2017). Since leaf litter inputs are temporarily retained in retentive structures on the reach, the leaf litter is susceptible to the action of local hydrological conditions, which through the abrasion of water contribute to the physical fragmentation of leaf litter. Therefore, our results suggest that the interactions between habitat heterogeneity and physical abrasion in the reaches of streams generate certain patterns consistent with the formation of leaf litter patches that provide resources and habitats for aquatic communities. The efficiency of leaf litter retention in streams is ecologically relevant, as it determines the fraction of these inputs that will be available to be processed later, contributing to the water flow of nutrients and secondary production (Bastias et al., 2019; Brouwer et al., 2017). Leaf litter retention in the study sections followed a negative exponential decay model. In addition, the number of drifting leaves decreased with increasing distance downstream from the input point, due to increased heterogeneity. This result corroborates our hypothesis that in-stream heterogeneity increases leaf litter retention capacity (e.g., Bastias et al., 2019; Brouwer et al., 2017; Kobayashi and Kagaya, 2008). However, our results differ from those of previous studies, as we consider both the intrinsic characteristics of leaf litter and the importance of the water flow for the leaf litter retention and fragmentation processes in Cerrado streams.

Riparian vegetation plays an important role in leaf litter retention through the supply of trunks and roots (Brouwer et al., 2017) and contributes to the storage of plant organic matter (Brouwer et al., 2017; Hoover et al., 2010; Koljonen et al., 2012). The high quantity of trunks and rooting by riverside vegetation may stabilize the stream banks and may act as an obstacle for the leaves, creating local retention zones (Lamberti et al., 2017). Previous studies have shown that trunks can be used in stream restoration. The addition of whole logs with branches that fill the water column, often extending to the water surface, leads to increased water flow resistance (and corresponding changes in depth and water flow), which often initiates the formation of debris dams that contribute significantly to the long-term retention capacity of a stream (Elosegi et al., 2016). For example, Flores et al. (2011) observed an up to 70-fold increase in organic matter storage after large trunks and branches were introduced in mountain streams in the Basque country (Spain). In our study, the complexity of trunks and roots may have increased over time, which intensified the blockages of woody materials and increased leaf litter retention. Therefore, any disturbance in the riparian zone (i.e., removal of riparian vegetation) has direct effects on nutrient dynamics in streams, affecting the efficiency of retention and the flow of terrestrial nutrients to streams. Thus, the addition of riparian forest structures such as roots and trunks in the streambed can contribute to the dynamics of organic matter in streams and is important for restoring forested streams (Brouwer et al., 2017; Koljonen et al., 2012).

Leaf litter size was also a key variable for retention rates during its drift downstream (Inoue et al., 2012; Kobayashi and Kagaya, 2008). We found that the retention coefficients were higher in *R. grandis* than in the other three leaf species, which can be explained by leaf litter size (17.98 ± 0.42, Figure 2B). Previous studies have also shown that large leaves may increase the probability of contact with roughness elements (Inoue et al., 2012; Kobayashi and Kagaya, 2008). The leaves are generally flat and flexible, which allows them to be ‘wrapped’ by the water flow force around obstacles, such as protruding stones and woody debris (Kobayashi and Kagaya, 2008). In addition, smaller leaves tend to travel longer distances (Cordova et al., 2008). In our study, leaf litter drift distances were also different in the three study reaches, with shorter drift distances (20-m path) in reaches with greater spatial heterogeneity (reach 3), mainly for larger leaves such as *R. grandis*. In addition, the retention efficiency of larger leaves can be related to the degree of leaf litter flexibility (Kobayashi and Kagaya, 2008; Steart et al., 2002). Leaves with high flexibility may be more easily retained by retentive structures (e.g., trunks and roots) as the flexibility increases the possibility of adhering to or contacting these structures (Steart et al., 2002). We did not measure flexibility, but according to previous studies, the larger leaves in our study, such as those of *R. grandis*, are the most flexible (Navarro and Júnior, 2020; Rezende et al., 2019), which may have contributed to higher retention coefficients. Therefore, changes in leaf litter size in the riparian zone of streams, such as phenological processes that change leaf litter size during periods of drought, can alter leaf litter retention rates and potentially fragmentation, thereby influencing the availability of organic matter and nutrients in headwater streams.

As expected, the increased fragmentation in riffles and the low effect of detritivores suggest that physical abrasion caused by water flow has direct implications for leaf litter fragmentation in tropical ecosystems (Bastias et al., 2019; Colas et al., 2017). However, the higher leaf litter fragmentation in the riffle in the fine mesh for the soft leaves suggests that water flow can stimulate the activity of microbial decomposers (Ferreira and Graça, 2006), as well as the physical fragmentation of leaf litter (Heard et al., 1999). The positive effect of microbial activity on physical fragmentation is in line with the descriptions in previous studies in tropical streams that leaf litter decomposition is a sequential process that begins with leaf litter leaching, followed by microbial colonization and continues with the mechanical effects of physical abrasion and macroinvertebrate activity (Alvim et al., 2015a; Cid et al., 2019; Rezende et al., 2020, 2018; Sena et al., 2020). Furthermore, other studies in the same stream have shown that microorganisms may be more important than shredders for leaf litter fragmentation (Rezende et al., 2014). These results can be expected in tropical systems (Graça and Cressa, 2010), considering the lower leaf litter processing effects by shredders and the high litter tenacity observed in these environments (Gonçalves et al., 2007). Our results differ from the findings of other studies in which the presence of shredders in the riffles increased the rate of litter mass loss (Abril et al., 2021; Ferreira et al., 2006; Ferreira and Graça, 2006). According to a study carried out in the same stream, Leite (et al., 2016) found a low density of shredders throughout the year. Particularly in the Cerrado, shredders have little influence on leaf litter fragmentation (Gonçalves et al., 2012; M. S. Moretti et al., 2007; Rezende et al., 2016), possibly because of the low abundance of these organisms (Boyero et al., 2012; Gonçalves et al., 2007; Moretti et al., 2007; Moulton et al., 2010; Rezende et al., 2016).

In summary, our results suggest that physical abrasion through the effect of water flow is important for leaf litter fragmentation, especially for softer leaves. Harder leaves are more resistant to physical abrasion and fragmentation (Fonseca et al., 2013). During physical fragmentation, many water-soluble compounds, such as proteins, amino acids, carbohydrates, and lipids, are leached. This process is important for the subsequent stages of fragmentation that are affected by microorganisms and invertebrates in aquatic systems (Bastias et al., 2019; Fonseca et al., 2013; Graça et al., 2015). The higher detritivore-mediated fragmentation in pools, especially for hard leaves, suggests that water flow is the main driver of leaf litter fragmentation in riffles. Furthermore, leaf litter patches can be more heterogeneous in pools than in riffles and act as microhabitats for detritivores (Kobayashi and Kagaya, 2002; Mendes et al., 2017). Therefore, we found considerable evidence that human activities that lead to reduced water flow, such as the presence of dams and irrigation, may decrease the transfer of resources for stream aquatic communities (Colas et al., 2017). Furthermore, changes in the vegetation composition that influence leaf litter quality, can be determining factors for the processing of allochthonous organic matter.

CONCLUSIONS

In summary, our results suggest that leaf litter can be retained spatially, depending on the presence of trunks and roots, especially for larger leaves. The interaction between habitat heterogeneity and leaf litter size is necessary to create leaf litter patches that provide resources and habitats for aquatic communities. In addition, since leaf litter inputs are temporarily retained within the stream reach, hydrological conditions on a local scale can influence leaf litter fragmentation rates through physical abrasion. Therefore, an assessment of the retention is necessary to understand the role of leaf litter inputs as subsidies for organic matter in streams. Management policies that increase the capacity of streams to retain leaf litter should be encouraged. In addition, it is important to preserve riparian forests in less retentive reaches and within areas with anthropogenic influence, since riparian forests are primary sources of woody debris such as trunks and roots; structures whose effect on leaf litter retention seems significant. Detailed studies should preferably be carried out throughout the year in a large number of streams and ideally using a diverse pool of species to test the consistency of our findings. Furthermore, studies should be carried out to explore the different effects of seasonal changes in the leaf litter phenology of riparian vegetation and water flow regimes on retention, transport, and fragmentation of leaf litter.

Authorship contributions

A. Tonin, R. Rezende and J. F. Gonçalves Jr. conceived the study. A. Tonin, R. Rezende, J. F. Gonçalves Jr. and P. Bambi performed the experiments. G. Sena performed the limnological analysis. D. Silva, A. Tonin, R. and R. Rezende performed statistical analysis. D. Silva wrote the article, with contributions from R. Rabelo, A. Tonin, R. Rezende, J. F. Gonçalves Jr., P. Bambi and G. Sena.

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SUPPORTING INFORMATION

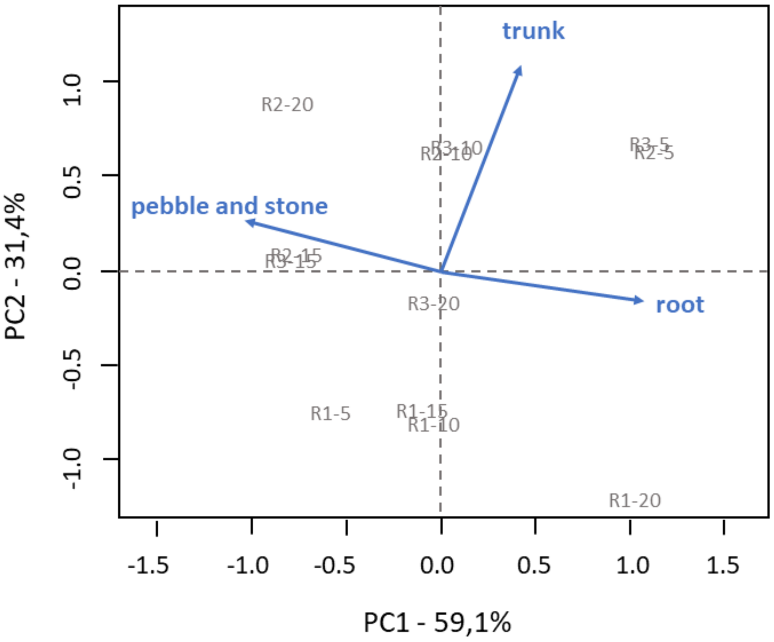


Figure 1S. PCA biplot of the percentage of pebbles, stones, roots and wood found in each subsection within reaches. The numbers in each axis indicate the percentage of variance. Letters and numbers represent the reach number followed by the subsection number, example: R1-5= Reach 1, subsection 5.

Table 1S. Leaf litter retention (percentage of leaves transported), distance travelled from the release point in each study reach (subsection), retention coefficient (*k*) and average leaf litter drift distance (1/*k*) of *C. brasiliensis*, *P. spruceanum* and *R. grandis.*

Reach	Subsection	*P. spruceanum*	*R. grandis*	*C. brasiliensis*
Reach 1	5	96	93	97
	10	90	58	87
	15	82	54	84
	20	79	52	83
	25	72	50	83
	30	69	49	82
	35	68	49	82
	40	67	49	78
	45	63	49	76
	50	48	44	69
Total leaves retained (%)		52	56	31
Retention coefficient (*k*)		0.01	0.01	0.006
Average leaf litter drift		83.33	71.42	166.66

distance (m)				
Reach 2	5	98	98	99
	10	93	95	95
	15	92	91	94
	20	90	87	92
	25	85	73	78
	30	80	53	74
	35	68	31	60
	40	67	26	59
	45	65	23	56
	50	18	5	18
Total leaves retained (%)		82	95	82
Retention coefficient (*k*)		0.02	0.05	0.02
Average leaf litter drift distance (m)		45.45	20	41.66
Reach 3	5	98	95	97
	10	97	80	96
	15	76	70	83
	20	69	63	79
	25	64	54	74
	30	63	48	71
	35	51	31	66
	40	46	31	63
	45	45	31	63
	50	6	2	11
Total leaves retained (%)		94	98	89
Retention coefficient (*k*)		0.03	0.05	0.02
Average leaf litter drift distance (m)		27.02	19.23	37.03

Table 2S. Correlation values of variables with PCA axis (PC1 and PC2). Bold values indicate correlations greater than 80%.

Retentive structures	PC1	PC2
Stone and pebble	**-0.89**	0.23
Roots	**0.91**	-0.13
Trunks	0.35	**0.93**

Table 3S. Results of linear models testing for the effects of leaf litter species (soft or hard) and micro-habitat (riffle or pool) on the detritivore-mediated fragmentation. Denominator degrees of freedom is 12 for the models.

	DF	t-value	*P*-value	Details
Soft leaves				
Micro-habitat	1	-1.07	0.30	
Hard leaves				
Micro-habitat	1	-2.85	0.01*	Pool > Riffle

*****Statistically significant differences

CAPÍTULO II - ASSESSMENT OF STREAM FOOD WEBS UNDER EFFECTS OF THE LOCAL AND REGIONAL SCALES

Dianne M. A. Silva, Andressa da Silva Reis, Murilo Sversut Dias, Raiane Rabelo Serejo, José Francisco Gonçalves Junior

ABSTRACT

Headwater streams are typically characterized by high connectivity to neighboring watersheds and shading by adjacent vegetation, which decreases in-stream primary productivity. The carbon source for the basal resources and consumers may depend on local factors, such as canopy cover, light and temperature in which algae can form the basis of food webs in tropical streams. Moreover, regional mechanisms in the watersheds related to the increase of in-stream nutrients supply may reflect directly on the nitrogen source for consumers. We investigated the influence of local (canopy openness, flow and temperature) and regional (altitude, distance from the source, forest cover in the watershed, total dissolved solids and nitrate concentration) mechanisms in macroconsumers and basal resources using $\delta^{13}C$ and $\delta^{15}N$ isotopic composition in Cerrado streams. We studied 12 headwater streams of the Brazilian cerrado that varied along an environmental gradient of local vegetation cover and watershed, and a gradiet of resource availability and quantity. We compared the isotopic composition of consumers using stable isotope mixing models and tested how the $\delta^{13}C$ and $\delta^{15}N$ isotopic values of basal resources (litter, periphyton and dissolved organic matter), insects (primary and secondary consumers) and fish (tertiary consumers) change in relation to variations in local and regional factors of streams. The main energy source for most aquatic organisms is autochthonous rather than allochthonous. Local conditions such as water temperature accounted for lower periphyton $\delta^{13}C$ values, while the flow negatively influenced the fish $\delta^{13}C$ values. On the other hand, streams more distant from the source and with higher nitrate concentrations increased the isotopic values of $\delta^{15}N$ of basal resources (DOM) and consumers (fish). The results highlight the importance of considering the structure of the local habitat and the characteristics of the watershed as drivers of the availability of carbon and nitrogen for in-stream consumers. Furthermore, we evidence that anthropogenic impacts in watersheds are a strong driver of the increased $\delta^{15}N$ values of the aquatic food web.

Keywords: tropical streams; autochthonous energy; stable isotopes; land use; riparian forest.

INTRODUCTION

The riparian vegetation constitutes the interface between terrestrial and aquatic ecosystems and promotes important ecological functions (Reis et al., 2020). The main functions of riparian vegetation are to regulate the dynamics of organic matter, the transfer of energy between terrestrial and aquatic ecosystems, and the interception of contaminants and nutrients in the watershed (Tonin et al. 2017; Guimarães-Souto et al. 2021). In addition, it also limits primary productivity in headwater streams (Brett et al. 2017). However, these streams can also present naturally less dense riparian vegetation and reduced canopy cover, resulting from physical factors such as topography and soil variation (Peressin & Cetra 2014; Dala-corte & Becker 2016). When the canopy is reduced, the greater incidence of light enables the growth of periphyton, increasing primary production and reflecting on the secondary production of invertebrates and fish (Neres-Lima et al. 2016; Reis et al. 2020). For aquatic consumers, terrestrial leaves are considered a low-quality resource (Allan & Castillo, 2007), as they have a high content of recalcitrant organic matter, such as cellulose, lignin and hemicelluloses (Meyers & Ishiwatari, 1993), and short-chain polyunsaturated fatty acids (PUFA). On the other hand, the algae present in the periphyton are recognized as having higher quality due to the higher content of PUFA, being rich in energy and proteins (Lamberti, 1996; Guo *et al.*, 2016), and assimilated more quickly than terrestrial plants. Thus, the assumption that plant litter is the main energy source that metabolically sustains headwater streams has not always been observed, and autochthonous sources (e.g. algae) may assume this role for consumers (Reis et al. 2020).

Studies with analysis of stable isotopes allow us to estimate changes in the use of resources as energy sources (Peterson & Fry *et al.*, 1987; Fraley *et al.*, 2021; Hayden *et al.*, 2021). Several elements can be used in the isotopic methodology to trace the origin of resources in ecosystems (e.g. C, N, S, H, O; Peterson & Fry, 1987). But, nitrogen and carbon isotopes are still the most used in studies of food webs, mainly due to the better understanding of the fractionation rates of these elements during assimilation and metabolic paths that food travels before being incorporated into some tissue (Albrecht *et al.*, 2021). Consumers have an isotopic composition similar to their prey's diet compared to $\delta^{13}C$, due to its discrimination factor, which varies from 0 ‰ to 1 ‰ along the food web (Zanden & Rasmussen, 2001; Post, 2002). Thus, $\delta^{13}C$ values reflect the isotopic composition of the diet, predicting information about the original source of carbon in the food web (Peterson & Fry *et al.*, 1987). Local factors such as temperature, light, flow and vegetation cover exert powerful control over the $\delta^{13}C$ values of the food web. For example, water velocity can decrease the thickness of the algae's surface layer (Finlay, Power & Cabana, 1999; Finlay, 2004), creating resistance to the diffusion of CO_2 into the cell and strongly influencing the supply of this resource to photosynthesis (Keeley & Sandquist, 1992). As a result, the CO_2 supplement tends to be greater, as the thickness of the surface layer will be smaller, facilitating the diffusion of CO_2 and, with this, the carbon values will be more depleted of $\delta^{13}C$ with the increase in fractionation (Finlay *et al.*, 1999; Finlay, 2004). Other local factors such as light and temperature also stimulate the production of algae and the enrichment of the $\delta^{13}C$ values of the periphyton (Finlay, 2004). The $\delta^{13}C$ of the periphyton tends to be more enriched due to the high photosynthetic rates of algae in streams with higher light and temperature input (Lamberti; Steinman, 1997). Thus, there is an increase in the use of the $CO_{2(aq)}$ reserve available in the surface layer of the algae ('boundary layer') (Keeley; Sandquist, 1992), and there is also a reduction in ^{13}C discrimination (Finlay; Power; Cabana, 1999).

The $\delta^{15}N$ values allow estimating the trophic positions of organisms by gradual enrichment that varies from 3 to 5 ‰ from one trophic level to another (Post, 2002). $\delta^{15}N$ values can be useful in assessing the effects of environmental gradients (whether natural or anthropic impacts) on the community. Regional mechanisms in the watershed related to the anthropic impact into streams may reflect directly the nitrogen source for consumers, as they can be determined by more enriched values of $\delta^{15}N$ in basal resources(Smucker *et al.*, 2018; Price *et al.*, 2019; Carvalho *et al.*, 2020; Oeding *et al.*, 2020). The replacement of native vegetation in the basin by agriculture or pasture can change the type of organic matter that enters the streams, with CPOM predominating in streams with native vegetation, and fine particulate organic matter (FPOM) and dissolved organic matter (DOM) in streams influenced by pasture and agriculture. In addition, the replacement of native vegetation in the basin can increase nutrient input into streams due to the use of organic and/or chemical fertilizers (Dala-Corte *et al.*, 2016). Nitrate and ammonium concentrations are highly influenced by anthropogenic activities (Schlesinger, 2009). In natural surface waters, the concentrations of nitrate and ammonium are low (<1 mg/ L); in contrast, higher concentrations usually indicate the addition of fertilizers used in agriculture, or industrial, human and animal wastes (Xu *et al.*, 2014). Moreover, features related to the catchment area, such as slope and altitude, can also increase the organic material input and nutrients supply due to their influence on the level of particles mobilized in the watersheds and/or transported to streams (Lintern *et al.*, 2018). The variations in sediment distribution and nutrients supply mediated by these regional mechanisms (catchment area and land use/coverage) may determine nutrient assimilation by the food web (Carvalho et al. 2017; Lintern et al. 2018; Price et al. 2019).

Our objectives were to (i) investigate whether the values of $\delta^{13}C$ and $\delta^{15}N$ of basal resources and macro-consumers vary as a function of regional and local factors in cerrado streams; and (ii) identify the main basal carbon sources (autochthonous and allochthonous resources) for macroconsumers using isotopic composition. Our first hypothesis is that the $\delta^{13}C$ isotopic values of periphyton and consumers to be positively related to temperature and less shaded streams, and negatively related to flow. So, we expect regional variables (altitude, distance from the source, forest cover in the watershed, total dissolved solids, and nitrate concentration) indirectly affect basal resources mediated by variations in local variables (canopy openness, flow, and temperature), which is reflected in the $\delta^{13}C$ isotopic values of the food web. Our second hypothesis is that streams further away from their source and at low altitudes tend to have less canopy cover, and thus, they have increased nitrate concentration, especially in areas with anthropic influence, which have a direct effect on enriching the $\delta^{15}N$ isotopic values of the food web. So, we expect that local stream variables indirectly affect the $\delta^{15}N$ isotopic values of the food web, mediated by variations in regional variables (as a consequence of regional variations in additions of urban or agricultural waste). Our third hypothesis is that periphyton is the main basal food source for consumers, even though they are not abundant in headwater streams. We expect consumers to assimilate in the most nutritious resource rather than the most abundant.

METHODS

Study area

We conducted the study in 12 headwater streams (primary to 5th order; Figure 1) sampled during the dry season, between June and August 2015, and June to September 2016. Eight of these streams are located in legally protected areas in the Brazilian Federal District: Environmental Protection Area Gama e Cabeça-de-Veado, Brasília National Park and Águas Emendadas Ecological Station (Rezende et al. 2017), and the other four are headwater streams close to these legally protected areas (Figure 1). One of the streams (stream 5) is located in the Tocantins-Araguaia basin, while the rest is located in the Paraná Basin. Land use and other environmental data for these streams, which represent a spatial gradient of land use and water quality are described in the Table S1. The climate in this region is Tropical Savannah, with two well-defined seasons: wet and hot (from October to April) and dry and cold (from May to September) (Ribeiro et al. 2001). The monthly average temperature is 22 ± 1°C in the rainy season (precipitation 197 ± 60 mm / month, with peaks between January and March) and 20 ±

1°C in the dry season (precipitation of 2 ± 3 mm / month) (Bambi et al. 2016; Tonin et al. 2019).

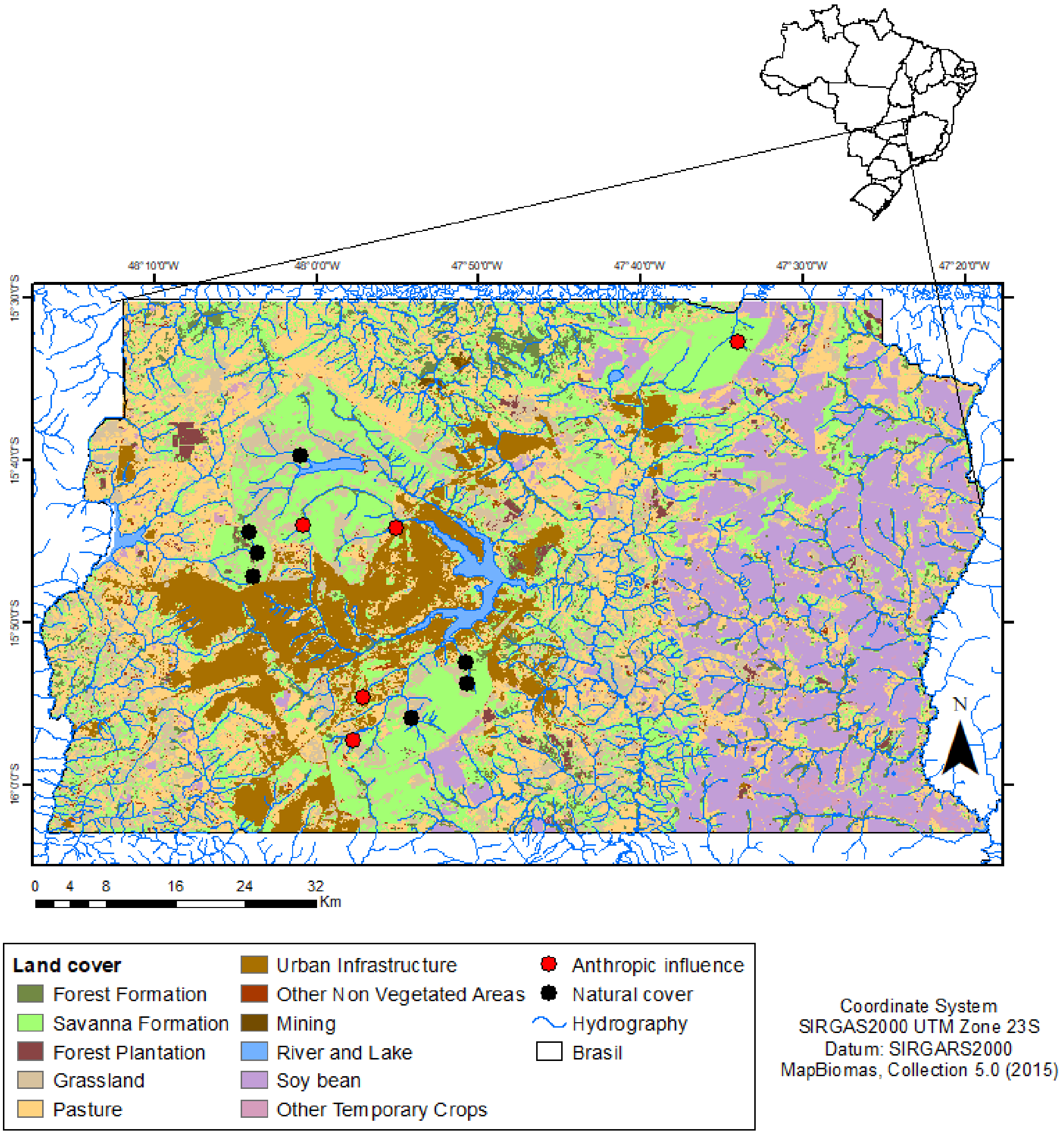


Figure 1. Geographical location of the study areas in Federal District, Brazil. Colors represent land cover classes. Sampling points are represented by dots. Dot colors represent streams with forest cover and with anthropic influence.

Sample collection and processing

The canopy openness (%) of the stream was determined from hemispherical photographs [digital camera (Nikon D5100) with a 10-mm Fisheye lens (Sigma)]. After analysing the images, using Gap Light Analyzer software, canopy opening values were defined. The chlorophyll concentration was estimated from artificial substrates, where three acetate sheets (10 x 15 cm) were incubated in each stream. These substrates remained submerged in streams for 60 days. At the end of the incubation period, the acetate sheets were taken to the laboratory and cut into 5 x 5 cm squares. All squares were individually scraped with a soft toothbrush and filtered through glass fiber filters (0.22 µm aperture), previously calcined and weighed. To estimate the concentration of chlorophyll-a (Chl-a), a filter was subjected to the organic solvent 90% ethanol for 24 h at 4°C to extract the pigments (Lorenzen, 1967). After extraction, the Chl-a concentration was measured using a spectrophotometer. The Chl-a values were then converted to dry mass, corrected by the sampling area and expressed in $mg.m^{-2}$. The dry ash free mass (AFDM) of the scraped periphyton was also obtained in triplicate by drying the filters at 60 ºC for 72 hours, weighing and burning at 550 ºC in an oven for 2 hours. From the Chl-a and AFDM of the periphyton, the autotrophic index (IA) was calculated, which provides the proportion of the algae biomass in relation to the total biomass of the periphyton and is calculated as the quotient between AFDM and Chl-a ($mg.\ m^{-2}$ AFDM/chltotal) (Sierra et al. 2013, Table 1).

In each stream, one sample of the coarse particulate organic matter (CPOM) present in the benthic stock was collected with a Surber sampler (0.1024 m^{-2}; 0.25 mm mesh) in triplicates (Table 1). The CPOM present in the benthic stock were taken to the laboratory and washed in distilled water to eliminate litter and adhered invertebrates. After that, they were dried at 60 ºC for 48 hours. Subsequently, the samples were separated into leaves, branches, flowers or fruits and miscellaneous, and weighed with a precision scale (0.0001g).

The availability of allochthonous resources was estimated from the input of coarse particulate organic matter (CPOM) from the canopy (vertical input; VI). The vertical input was measured with six buckets (sampling area of each bucket 0.043 m^2) arranged on the watercourses during 60 days to capture the organic matter that falls on the stream, totalling a sampling effort of 0.26 m^2 per stream. The sampled CPOM was transported to the laboratory, dried in an oven at 60ºC for 72 hours. Subsequently, the samples were separated into leaves, branches, flowers or fruits and miscellaneous, and weighed with a precision scale (0.0001g). The mass of organic matter collected from the traps was corrected by the exposure time and sampling area and the values for each fraction were expressed in $g.m^{-2}.day$.

Benthic macroinvertebrates and fish were collected in all streams. The macroinvertebrates were sampled with a D-frame kick net (250 μm mesh, 0.3 m aperture). To standardize the sampling effort, each macroinvertebrate sample was collected covering five sections of sediment of one meter each, totaling a sampling area of 1.5 m^2 per stream. In the laboratory, macroinvertebrate samples were washed with a 0.5 mm mesh sieve and the retained individuals were separated and preserved frozen until identification at the family level (Merritt and Cummins 1996; Cummins et al. 2005; Mugnai et al 2010; Hamada et al. 2014).

The fish were sampled using a combination of seines (5 × 1.5 m and 15 mm mesh) and funnel trap, aiming to capture the maximum number of individuals in each sample unit. The two nets remained submerged for 24 hours in each stream. We use bait to lure consumers into the funnel traps. The captured fish and shrimp were placed in plastic bags with water collected in the stream and placed in the refrigerator (4°C) for 30 minutes, which leads to stunning after a certain time. After that period, individuals were sacrificed by spinal cord section or beheading, as recommended by the Federal Council of Veterinary Medicine. After euthanasia, individuals were classified at the lowest possible taxonomic level and frozen (-20°C).

Natural and disturbing features of the landscape

The percentage of forest cover in the watershed was evaluated using geographic information system (GIS) products processed in ArcMap 10.6.1 software. We first modeled the contributing area upstream each sampling point, using the Hydrology toolset (Figure S1). We used the digital elevation surface model from the Alaska Satellite Facility, with 12,5 m pixel-resolution, to derive the water flow model. Then we calculated the percentage area of each land-cover class for the entire drainage network upstream of the sampled site, considering the sampling year reported in this study (2016). We used land use data from the Brazilian Project for the Annual Mapping of Land Use and Coverage (MapBiomas, Collection 5.0, MapBiomas, 2020). This project produces annual land use digital maps with 30m pixel, obtained from the Landsat Data Collection. We consider the forest cover in the area upstream of each stream by the sum of the forest land-cover classes (e.g. forest formation and savanna formation, Table S1). This type of vegetation cover has greater potential to protect streams against nutrient discharge than grassland formation. The 10-land use and land cover classes that occur at the assessed sites have been consolidated into five broader categories of land use and land cover: natural vegetation, pasture, agriculture, urbanization, and others. We represented agriculture by the sum of the classes related to agricultural use (Soybean, Other Temporary Crops), the pasture metric included the pasture class, urbanization included the Urban Infrastructure class, and Others we represented by sum of Grassland and Other non-vegetated areas (Table S1).

The nitrate (NO_{3-}) anions was determined by collecting a water sample from each sample stream. These samples were collected in the water column with plastic bottles, filtered in laboratory through a 0.22 μm GF/C glass fiber filter to remove organic particles and analyzed by ion chromatography (Metrohm 930 Compact Flex IC Flex chromatograph).

Isotopic analyses

The most abundant taxa were selected for the analysis of the isotopic diet. For benthic macroinvertebrates, families were separated into groups of 1 to 20 individuals in centrifugation microtubes. Small animals (e.g., small dipterans of the Chironomidae family) were previously grouped to compose a sample, while larger taxa (e.g. Odonata or Megaloptera) composed a sample with only one individual. The larger individuals of each species of fish and shrimp had their dorsal muscles devoid of scales removed and frozen. All samples for stable isotope analysis were cleaned manually to remove contaminants from the environment, dried in an oven at 60°C for 72 hours and crushed with mortar and Wiley mill.

The acetate was cleaned with running water and then the adhered material was scraped for stable isotopic analysis. The obtained material was then filtered on calcined glass fiber filters. The material on the filter was then inspected under stereomicroscope (40-fold increase) for the presence of non-algae materials. Diatoms were the predominant taxonomic group in all samples. Green leaves from dominant riparian C3 plant species were used as surrogate to isotopic values of CPOM resources. The dominant plant species in CPOM were *Aspidosperma cylindrocarpon* Müll. Arg., *Chrysophyllum marginatum* (Hook. & Arn.) Radlk., *Dicksonia sellowiana* Hook., *Miconia cuspidate* Mart. ex Naudin, Miconia hirtella Cogn., *Piper aduncum* L., *Protium heptaphyllum* (Aubl.) Marchand, *Sorocea bonplandii* (Baill.) W.C. Burger, Lanj. & Wess. Boer, *Vochysia tucanorum* Mart., *Xylopia emarginata* Mart. and *Xylopia sericea* A. St.-Hil.

The isotopic compositions of the samples (carbon and nitrogen) were determined using aliquots of 0.2 to mg of dry and ponded sample and put inside small tin capsules. This analysis was determined using a Flash EA 1112 Series elementary analyzer coupled in line via the Finningan Conflo III interface to a Thermo delta V S mass spectrometer, in the laboratories of the Center for Marine and Environmental Sciences (MARE) of the University of Coimbra, Portugal. The carbon and nitrogen isotopes ratios are expressed in delta (δ) notation, defined as the deviation of parts per thousand (‰) of a standard material (limestone PDB for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$): $\delta^{13}C$ or $\delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000$, where $R = {}^{13}C / {}^{12}C$ or ${}^{15}N / {}^{14}N$. The precision in the overall preparation and analysis was better than 0.1 ‰ for $\delta^{13}C$ and 0.3 ‰ for $\delta^{15}N$.

Classification of trophic levels of the food web

To estimate the potential contribution of allochthonous and autochthonous resources to the isotopic diet of consumers we use Bayesian stable-isotope mixing model analysis (R: SIAR, version 4.1.1; Parnell *et al.*, 2010). The fractionation values for muscles samples were +1.3 ‰ (SD = 1.27) for δ^{13}C and +2.9 ‰ (SD = 1.24) for δ^{15}N, while for samples in which the whole animal was analyzed (smaller macroinvertebrates) the fractionation values were +0.3 ‰ (SD = 1.3) for δ^{13}C and +2.1‰ (SD = 1.60) for δ^{15}N (McCutchan et al., 2003).

To quantify the importance of autochthonous and allochthonous resources for macroconsumers as a whole, the mixing models were built separately for different trophic levels (Primary consumer, secondary consumer or terciary consumer). These groups were used only for the definition of potential food sources for consumers at different trophic levels and were made based on literature for each taxon. For primary consumers, the CPOM and periphytic were the food sources used in the mixing model. As a result, primary consumers were divided into algivorous (algae assimilation > 50%) or herbivores (CPOM consumption > 50%) according to the importance of the resources obtained for each taxon by the model. As secondary consumers are typically predator macroinvertebrates, we chose not to include CPOM and algae in the mixing model for this trophic level. Thus, only algivores and herbivores were used as food sources in the mixing model for secondary consumers. Likewise, secondary consumers were divided into predators of algivorous (consumption of algivores > 50%) or predators of herbivores (consumption of herbivores > 50%). All sources were used in the mixing model for tertiary consumers. However, in this study we used only the most representative taxa of each trophic level (Primary consumer, secondary consumer and terciary consumer).

Among primary consumers, we selected 10 taxa of macroinvertebrates (Chironomidae, Elmidae, Pyralidae, Hydropsychidae, Baetidae, Grypopterigidae, Calamoceratidae, Psephenidae, Leptoceridae and Simulidae, Figure S2). Among secondary consumers, we selected nine macroinvertebrate taxa (Calopterygidae, Corydalidae, Gomphidae, Libellulidae, Megapodagrionidae, Perlidae, Sialidae, Tipulidae and Veliidae, Figure S2). Among the third consumers, we selected six fish species (*Hyphessobrycon balbus*, *Astyanax scabripinnis*, *Moenkausia* sp., *Hasemania crenuchoides*, Moenkausia sp. and *Rhamdia quelen* and one species of shrimp, *Macrobrachium* sp., Figure S2, Table S2). Among tertiary consumers, two groups were identified: generalist fish (*Hyphessobrycon balbus*, *Astyanax scabripinnis* and *Moenkausia* sp.), and specialists fish (with a tendency to consume predators, the shrimp Macrobrachium sp. and the fish *Hasemania crenuchoides* and *Rhamdia quelen*).

Data analysis

We use a redundancy analysis (RDA) to assess how local and regional effects on the isotopic values of the food web (basal resources and consumers together) are correlated. For this, we use two RDAs, one considering the $\delta^{13}C$ data and the other considering $\delta^{15}N$ data as the response variable. In place of the seven local and regional predictors for the RDA, we used site scores associated with the three retained axes of the Principal component analysis (PCA). Eigenvalues were used to determine the number of principal components to retain after PCA (Kaiser 1961). Thus, the principal components that together explain more than 70% of the variation were retained (Figure S3). The most correlated variables (>0.6) in each main component were interpreted as being representative to explain the variability in the data set. To RDA, we run 1,000 permutations to assess the significance of constraints. Analyzes were performed for the model (global test) and for each predicting variables of the three data sets (PC1, PC2 and PC3) to test the significance of these predictors for the variation of $\delta^{13}C$ and $\delta^{15}N$. We used the *rda* function of *vegan* package (Oksanen et al., 2016).

A pRDA was performed to evaluate possible spatial pattern in residuals and to check for possible spatial autocorrelation in each RDA model (values of δ^{13}C and δ^{15}N), which could inflate the type 1 error. For this, the residuals of each RDA model were used as the response variable and included spatial filters taken from principal coordinates of neighbour matrices (PCNM; Borcard and Legendre 2002) as predictors in the pRDA model. The spatial filters were generated using decimal degree geographical coordinates and retaining only the axes that presented Moran's I > 0.5. For this, we used the 'PCNM' function from the PCNM package (Legendre et al. 2013). This analysis showed absence of spatial autocorrelation in the models (δ^{13}C, $R^2 = -0.24$; $p = 0.75$; and δ^{15}N, $R^2 = -1.24$; $p = 0.99$), indicating that effects of local and regional environmental factors on isotope composition were not influenced by any spatial structure.

We performed Structural Equation Modeling (SEM) to assess potential causal pathways and direct and indirect effects of regional and local factors on δ^{13}C and δ^{15}N values of the stream food web. We developed the SEM based on a model concept (Figure S4) synthesized from previous studies (e.g. Maloney and Weller 2011; Chará-Serna and others 2015). Land use and natural landscape factors affect aquatic communities indirectly through effects on riparian condition and in-situ habitat. Land use and natural landscape factors are often correlated. SEM is based upon the calculation of path coefficients (standardized partial regression coefficients), which can be used to calculate direct, indirect and total effects. Direct effects were the path coefficients between two variables connected by a path. Indirect effects are effects mediated through another variable. For example, stream reaches in forested watersheds may have greater riparian coverage, which in turn affects carbon sources for communities, reflecting on communities δ^{13}C values; thus, vegetation cover indirectly affected the δ^{13}C values of the stream biota through its effect on riparian cover. Indirect effects are calculated as the summation of the product of all paths between two variables. Total effects are the summation of indirect and direct effects (standardized path coefficients). We chose SEM because it can simultaneously evaluate these direct, indirect, and total effects of regional and local variables on several stream response variables. We evaluated model fit using the χ^2 statistic, a goodness of fit index (GFI) and root mean square error of approximation (RMSEA). A non-significant χ^2 statistic ($P > 0.05$) indicates that the data set fit the SEM model; GFI values close to 1.0 and RMSEA below ~0.05 indicate a good fit of the model (Byrne, 2010). Significance of effects was estimated using a bootstrap procedure (1000 bootstrap samples). All the coefficients presented in this work were significant. SEM procedures were carried out using the lavaan package in R (R Development Core Team).

To determine the main carbon sources of aquatic organisms, we used the gradient approach (Rasmussen, 2010) to examine the relationships between the $\delta^{13}C$ values of consumers (insects and fish) and basal resources (periphyton and litter) regional land use gradient. Thus, in each stream, mean values of $\delta^{13}C$ were grouped into three trophic levels of aquatic insects that are directly related to periphytic and litter: (1) primary consumer, which include algivores and herbivores insects (terrestrial plant consumer); (2) secondary consumer, which include predator insects of algivores and herbivores; and (3) tertiary consumer, which include fish as top predators. In the gradient approach the slope estimates of the regressions (β_1) were examined. Thus, a slope = 1 suggests that consumers assimilate organic C entirely from the periphyton or litter, whereas a slope > 0 but < 1 would suggest that the periphyton or litter only partially contributes to the organic C and slope = 0 suggests that there is no contribution (Rasmussen, 2010). Slope confidence intervals between 0 and 1 indicate partial dependence on periphyton or litter and those that include both 0 and 1 indicate an unresolved model (Bunn; Leigh; Jardine, 2013; Jardine et al. 2012).

To test whether streams with anthropogenic influence have a direct effect on enriching $\delta^{15}N$ or $\delta^{13}C$ isotopic values of the food web, we use linear models with the gls (generalized least squares) function of the ‘nlme’ R package (version 4.0.4; R Development Core Team, 2020). Then, a Two-way analysis of variance (Two-way ANOVA; anova function of the vegan package) was performed to test the observed differences between streams (impacted and non-impacted) and trophic level (resources, insects and fish), in the mean of values of δ^{15}N. For this, we calculated the mean values of stable nitrogen isotopes (δ^{15}N and δ^{13}C) for all resources (periphyton, litter and dissolved organic matter; DOM), for insects (primary and secondary) and for fish. All analyses were carried out in the R environment (version 4.0.4; R Core Team, 2017).

RESULTS

Structure and productivity of streams

The canopy cover of streams varied from very shaded (7.7% of openness) to exposed (90% of openness), but the majority of sampling streams were shaded (Table 1; 22% ± 21) and characterized by dense riparian vegetation. The biofilm colonization rate varied between 0.003 to 0.014 mg.m^{-2}.day^{-1} among streams, with mean of 0.006 mg.m^{-2} (0.003 SD). The autotrophic index ranged between 135.44 and 3,086.03 among streams (1273.62 ± 762.35). The total allochthonous carbon represented by leaves, twigs and fruits was much more abundant than autochthonous resources within streams, ranging from 1.4 to 101.33 g.m^{-2}. day^{-1} (mean ± SD, 12.88 ± 28.02, Table 1). The forest cover in the drainage network upstream of the streams ranged between 92 and 29% (58.92 ± 23). The distance from the source ranged between 7 and 0.07 km (2.35 ± 0.64) (Table S1).

Table 1. Geographic location, mean (N=3) of productivity of autochthonous and allochthonous resource in the sampled streams.

				Autochthonous resources		Allochthonous resources
Streams	Latitude (S)	Longitude (W)	Canopy openness (%)	Periphyton growth rate (mg	Autotrophic index	Total (mg.m^{-2}. day^{-1}) (leaves, twigs and fruits)

					AFDM.m^{-2}.day^{-1})		
Natural cover	3	15°39'45.05"S	48° 1'1.13"O	14.6	2.01	1714.29	1.4
	6	15°53'46.33"S	47°50'43.62"O	18.56	7.32	1474.06	101.33
	7	15°52'28.59"S	47°50'48.84"O	13.91	7.50	1312.09	6.7
	9	15°55'53.75"S	47°54'9.58"O	18.14	3.27	1171.48	7.41
	10	15°44'25.67"S	48° 4'10.23"O	89.99	7.91	3086.03	3.10
	11	15°45'42.44"S	48° 3'42.52"O	16.78	4.78	1426.21	6.36
	12	15°47'11.71"S	48° 3'54.33"O	15.9	4.39	1150.20	4.45
Anthropic influence	1	15°44'9.64"S	47°55'7.36"O	18.13	2.11	1419.86	11.5
	2	15°44'0.33"S	48° 0'53.16"O	26.28	3.62	882.91	7.4
	4	15°57'17.72"S	47°57'45.97"O	13.8	9.75	135.44	1.4
	5	15°32'43.45"S	47°33'59.28"O	12.57	3.80	1092.64	2.2
	8	15°54'40.78"S	47°57'11.02"O	7.77	13.93	418.23	1.41

Local and regional variables in the food web

The first 3 axes of the PCA explained 76% of the total variation in the environmental data of the streams, of which 40% were explained in axis 1, 23% in axis 2, and 12% in axis 3 (Figure 2). The 1 axis was positively correlated with altitude (0.78), autotrophic index (0.70) and canopy openness (0.69), and negatively correlated with distant from the source (-0.77) and water flow (-0.69). The axis 2 was positively correlated with total dissolved solids (TDS, 0.69) and nitrate (0.68). The axis 3 was negatively correlated with temperature (-0.70).

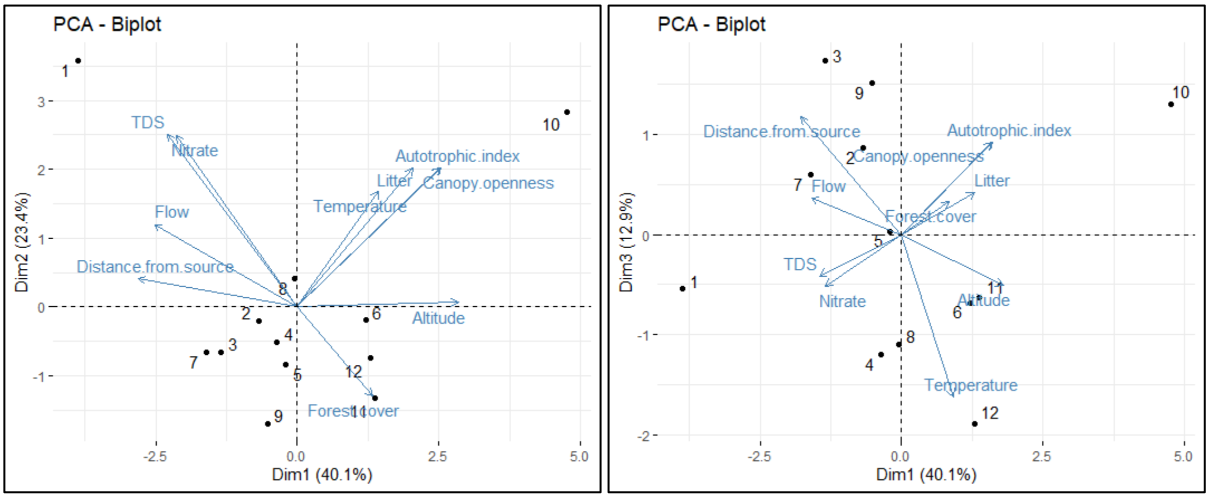


Figure 2. Biplot PCA with local (temperature, flow, canopy openness), regional (altitude, TDS, nitrate, distance from the source, and forest cover in the watershed) variables and productivity in streams (autotrophic index, litter). Numbers in parentheses indicate the percentage variance explained by the respective axis. The numbers represent the streams.

The first and second axes of the RDA considering the δ^{13}C data of consumers and basal resources, explained 16 and 8%, respectively, of the variation in isotopic data (Figure 3A). Axis 1 of the RDA was positively associated with PC3 (Table S3). That is, this axis was negatively correlated with temperature. This axis was positively correlated to the δ^{13}C isotopic values of litter (Figure 3A, Table S4). The environmental variables represented by PC1, PC2 and PC3 were not significantly related to the δ^{13}C isotopic values of the food web (Table 2). These relationships were more evident in streams with less anthropogenic influence (Figure 3A).

The first and second axes of the RDA considering the δ^{15}N data of consumers and basal resources, explained 22 and 5%, respectively, of the variation in isotopic data (Figure 3B). Axis 1 of the RDA was also negatively associated with PC2 (Table S3). That is, this axis was negatively correlated with total dissolved solids and nitrate. This axis was negatively correlated to the δ^{15}N isotopic values of primary, secondary and tertiary consumers (Figure 3B, Table S4). These relationships were more evident in streams with greater anthropogenic influence (Figure 3B).

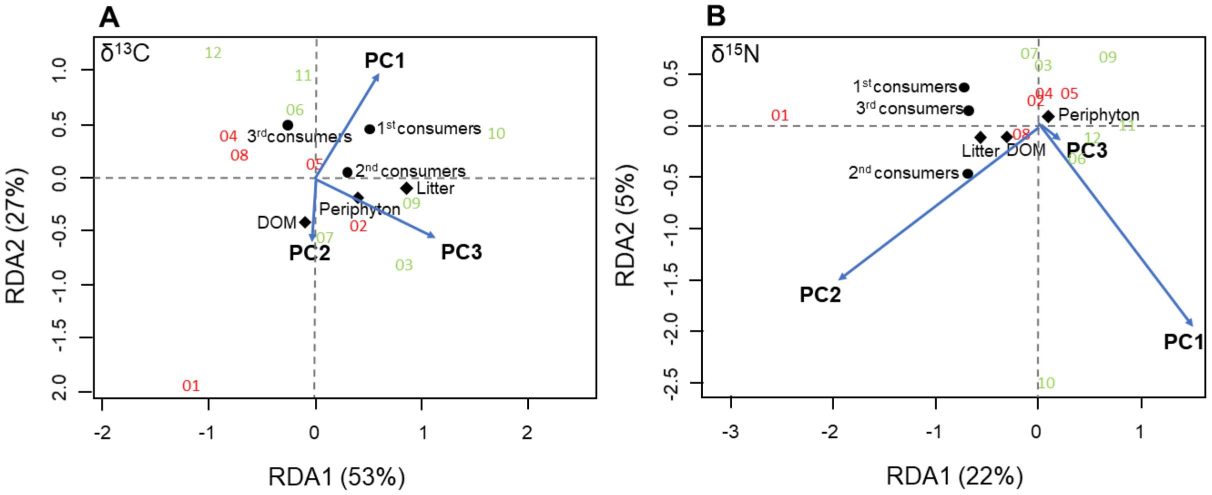


Figure 3. Biplot of the redundancy analysis (RDA) between the δ^{13}C (A) and δ^{15}N (B) isotopic values of the food web and the local and regional variables (axes of the PCA). Circles represent consumers and triangles represent resources**.** Number of sites in red indicates streams with urban and pasture influence, while number of sites in gray indicate preserved streams.

Table 2. Results of permutation test testing the effect of local and regional streams variables (axes of the PCA) on the $\delta^{13}C$ and $\delta^{15}N$ isotopic values of the food web.*Statistically significant differences

δ-value	**Variable**	**Variance**	**F-value**	**P-value**
$\delta^{13}C$	PC1	0.59	1.16	0.36
	PC2	0.39	1.76	0.63
	PC3	0.88	1.72	0.09
	Residual	4.12		
$\delta^{15}N$	PC1	0.69	1.35	0.25
	PC2	0.95	1.88	0.10
	PC3	0.28	0.57	0.73
	Residual	4.06		

The model diagram includes the standardized coefficients for each path, all of which were significant at $p \leq 0.05$. The $\chi 2$ statistics for the four models (SEM) were not significant (Table 3) indicating that both models fit the data well, that is, that the data set fit the SEM model. Models with resources $\delta^{13}C$ values explained 35% of the variation of the temperature (Figure 4 A). While models with consumer $\delta^{13}C$ values explained 33% of the variation of the flow (Figure 4 C). Models with resources $\delta^{15}N$ values explained 41% of the variation of the nitrate and 37% of the distance from the source (Figure 4B). While models with consumer $\delta^{15}N$ values explained 61% of the variation of the distance of the source (Figure 4D).

On a local scale, temperature had a negative effect on the $\delta^{13}C$ values of periphyton (standardized total effect [STE] = -0.65; p = 0.05, Figure 4 A), while the water flow had a negative effect on the $\delta^{13}C$ values of fish (STE = 0.50, p = 0.05). Regional factors such as the distance of the source had an indirect effect on the $\delta^{13}C$ values of periphyton (STE = 0.34), and on the $\delta^{13}C$ values of fish (STE = -0.28).

On a regional scale, nitrate had a positive effect on the $\delta^{15}N$ values of DOM (STE = 0.41, p = 0.04). While the distance from the source had a positive effect on the $\delta^{15}N$ values of fish (STE = 0.50, p = 0.03). Local factors such as temperature had an indirect effect on the $\delta^{15}N$

values of DOM (STE = 0.26).

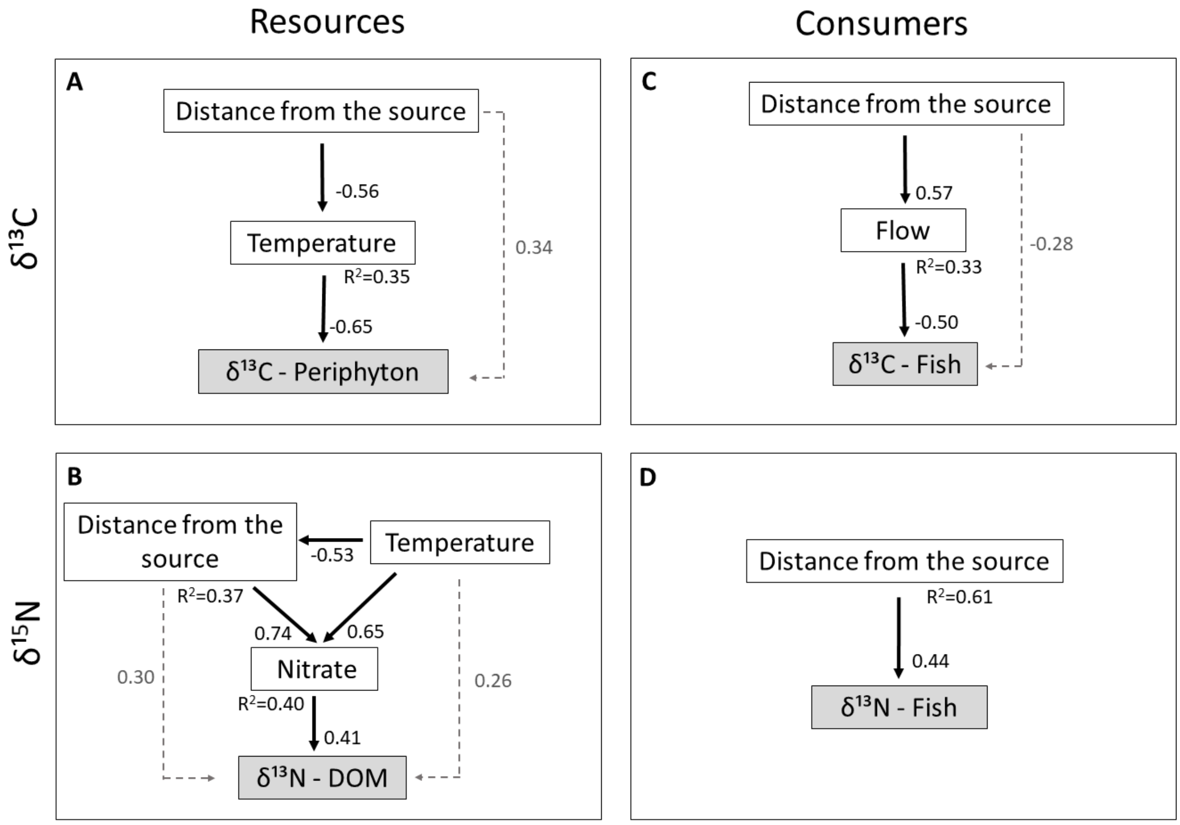


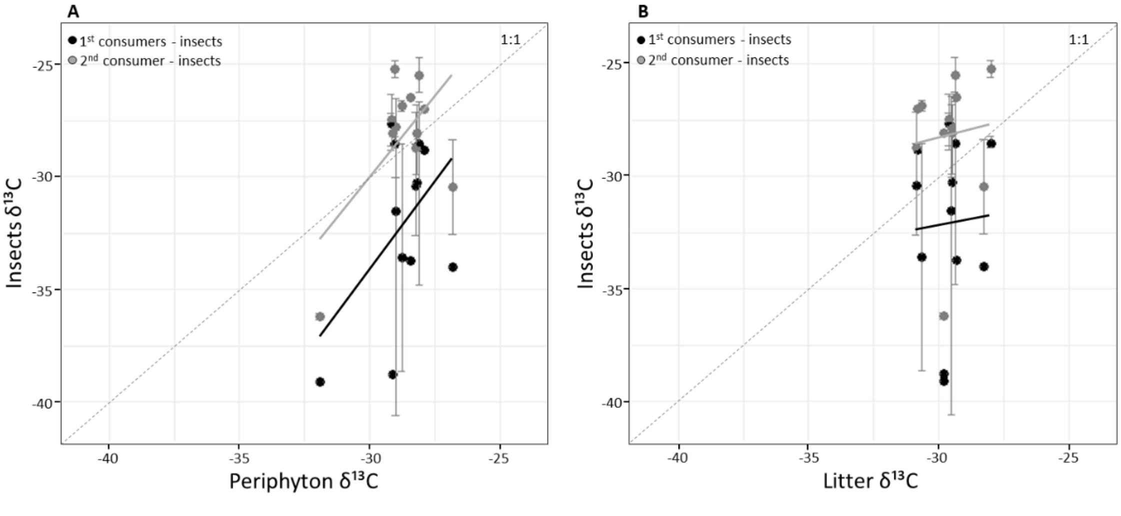
Figure 4. Structural equation model diagrams showing the effects of local and regional variables on the $\delta^{13}C$ (A and C) and $\delta^{15}N$ (B and D) isotopic values of consumers and resources. Direct effects are represented by solid line and indirect effects by dashed line. Numbers next to arrows are standardized path coefficients. Note that the structure of the model assumes direct effects of each independent variable, alongside covariance between all independent variables.

Table 3. Supporting fit statistics for structural equation models showing the effects of local and regional variables on the $\delta^{13}C$ and $\delta^{15}N$ isotopic values of consumers and resources in cerrado streams (RMSEA: Root Mean Square Error Approximation, CFI: Comparative Fit Index. Con= Consumers; Res=Resources).

Model	χ^2	df.	P-value	RMSEA	RMSEA P-value	CFI
$\delta^{15}N$-Con	35.59	11	0.75	0.51	0	0.40
$\delta^{13}C$-Con	27.14	12	0.84	0.52	0	0.29
$\delta^{15}N$-Res	26.29	21	1	0.27	0.02	0.85
$\delta^{13}C$-Res	50.74	23	0.96	0.49	0	0.50

Basal food sources for consumers

We found variation in the values of carbon isotopes of periphyton (δ^{13}C mean ± SD = -28.72 ± 1.20 ‰) and in nitrogen isotope values of periphyton (δ^{15}N average ± SD = 2.68 ± 2.78‰) and litter (-0.63 ± 1.41 ‰) among the streams (Table S5). Much of the observed variation in carbon isotope values of aquatic insects (primary consumers and secondary) was explained by variation in carbon isotope values of periphytic but not of leaf litter (Figure 5, A and B, Table 4). However, spatial variation in the nitrogen isotope values of these aquatic insects was not explained by periphyton (Figure 5 E; Table 4). While for litter, the spatial variation in nitrogen isotope values was more related only to primary consumers and fish (Figure 5, F and H, Table 4). Spatial variation in the δ^{13}C values of generalist fish was more associated with the values of litter than periphyton (Figure 5 C; Table 4). While generalist and specialist fish had their δ^{15}N values more associated with litter δ^{15}N values (Figure 5 H; Table 4).



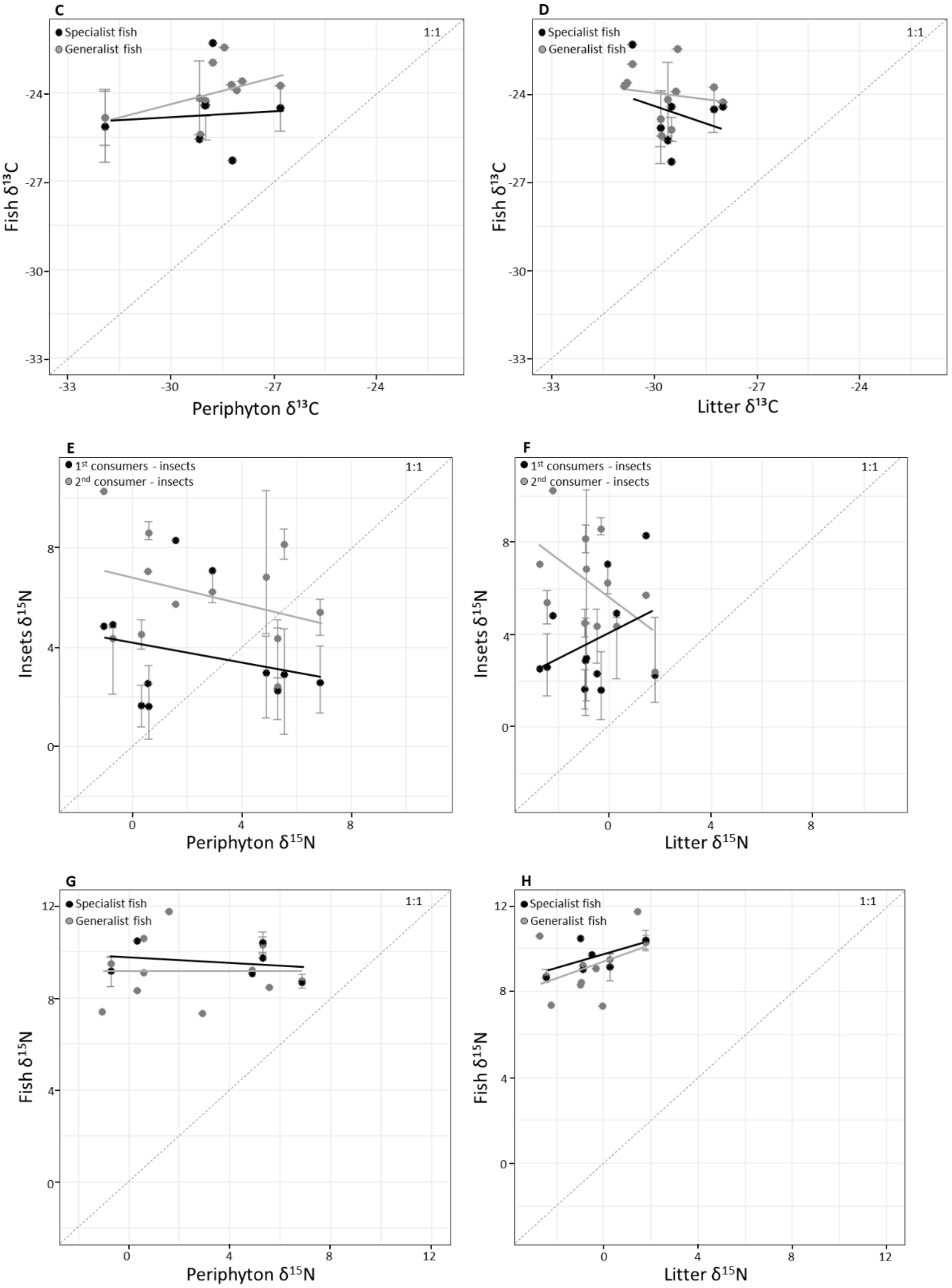


Figure 5. Relationship between δ^{13}C and δ^{15}N isotopic values of consumers (insects and fish) and basal resources (periphyton and litter). Corresponding regression values (R^2 adjusted and slope) are provided in Table 5.

Table 4. Linear regression between δ^{13}C values of basal sources (periphyton, litter) and primary, secondary and tertiary consumers. N = sample number; β_1= slope, slope of the straight

line; 95% CI = confidence intervals. *****Statistically significant differences.

δ-value		**Resources**					
$\delta^{13}C$	Insects		R^2_{adj}	*p*	N	β_1	95% CI
	Primary consumer	Periphyton	0.15	0.11	12	1.53	-0.45 - 3.52
		Litter	-0.09	0.86	12	0.24	-2.82 - 3.30
	Secondary consumer	Periphyton	0.28	0.04*	12	1.42	0.05 - 2.78
		Litter	-0.09	0.78	12	0.28	-1.99 - 2.57
	Fish						
	Terciary consumer - generalist	Periphyton	0.14	0.13	11	0.31	-0.12 - 0.74
		Litter	-0.087	0.66	11	-0.14	-0.85 - 0.57
	Terciary consumer - specialist	Periphyton	-0.18	0.84	7	0.07	-0.86 - 1.01
		Litter	-0.10	0.54	7	-0.38	-1.90 - 1.14
$\delta^{15}N$	Insects						
	Primary consumer	Periphyton	-0.02	0.42	12	-0.19	-0.72 - 0.33
		Litter	0.03	0.25	12	0.54	-0.46 - 1.55
	Secondary consumer	Periphyton	0.02	0.28	12	-0.26	-0.77 - 0.25
		Litter	0.22	0.06	12	-0.83	-1.74 - 0.07
	Fish						
	Terciary consumer - generalist	Periphyton	-0.11	0.96	11	0.007	-0.35 - 0.36
		Litter	0.08	0.19	11	0.38	-0.23 - 1.00
	Terciary consumer - specialist	Periphyton	-0.16	0.61	7	0.11	-0.39 - 0.26
		Litter	0.20	0.20	7	0.31	-0.27 - 0.90

Anthropogenic influence on $\delta^{15}N$ or $\delta^{13}C$ values

Insect $\delta^{15}N$ isotopic values were significantly higher in streams with anthropogenic influence ($F_{1,55} = 4.91$, p = 0.03; Figure 6). Isotopic values of $\delta^{13}C$ consumers and resources were not different between streams with anthropogenic influence and streams with natural cover. When comparing all trophic levels in the food web, the enrichment of $\delta^{13}C$ and $\delta^{15}N$ were higher in predators (fish) in both stream categories (Table 5)

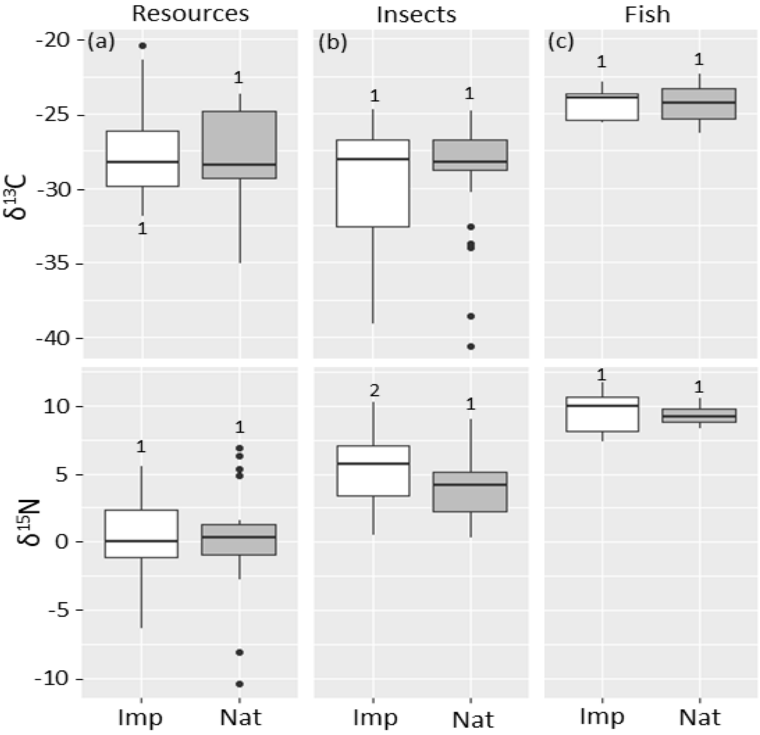


Figure 6. Effect of stream categories (Imp= impacted streams and Nat= streams with natural cover) on $\delta^{13}C$ and $\delta^{15}N$ isotopic values of (a) resources (periphyton and litter), (b) insects (primary and secondary consumers) and (c) fish (tertiary consumers). The box plots illustrate the median (centerline), the interquartile range of the data (box) and the distribution bars (1.5 × interquartile range). Different numbers (1 and 2) indicate significant differences (Tukey's Multiple Comparison test, $p < 0.05$).

Table 5. Results of linear models testing for the effects of stream categories (streams with anthropic influence and natural cover), trophic level (resources, insects and fish) and interactions on $\delta^{15}N$ and $\delta^{13}C$ isotopic values of the food web. *Statistically significant differences.

δ-value		**df**	**F-value**	**P-value**	**Contrast analysis**
$\delta^{13}C$	*Intercept*	1	8484.55	<.0001	
	Stream	1	0.95	0.33	
	Trophic level	2	17.98	<.0001*	Fish > Insects = Resouces
	Stream * Trophic level	2	0.47	0.62	

$\delta^{15}N$	*Intercept*	1	273.63	<.0001	Fish > Insects > Resouces
	Stream	1	1.17	0.28	
	Trophic level	2	82.55	<0.0001*	
	Stream * Trophic level	2	0.52	0.59	

DISCUSSION

The availability of allochthonous organic carbon in streams was higher than the autochthonous organic carbon. This was a consequence of the dense riparian vegetation around preserved headwater streams of the Brazilian Cerrado, which provide constant organic matter (Gonçalves & Callisto, 2013; Bambi *et al.*, 2016). Despite the low autochthonous productivity available in streams, microalgae can be an alternative source of energy, given their high nutritional content and low amount of composite structures that inhibit their digestibility (Neres-Lima *et al.*, 2017; Kühmayer *et al.*, 2020, Guo et al., 2017). Our result suggests that the availability of autochthonous resources was independent of the canopy cover over the streams, which is consistent with the results of other tropical streams (Brito *et al.*, 2006; Lau, Leung & Dudgeon, 2009; Neres-Lima *et al.*, 2016; Reis, Albrecht & Bunn, 2020). We have indicated that, despite the low amount compared to litter, algae is an important resource that regulates the flow of energy in streams (Lau *et al.*, 2009; Brett *et al.*, 2017). Our study was carried out in a dry period, when the opening of the canopy due to the abrupt fall of leaves may have contributed to the growth of benthic algae, due to the entrance of light during this period. According to Graça (*et al.*, 2018) primary production can increase in streams with semi-open canopy compared to streams with full shade.

Although some of the consumers studied (generalist fish and secondary consumers) have assimilated litter, strong spatial relationships between $\delta^{13}C$ values of periphyton and of secondary consumers (slopes similar to 1.0) indicate that the periphyton was the main food source (Rasmussen, 2010; Jardine *et al.*, 2012). This result is expected since the feeding of generalist species is the most diversified and their food sources vary depending on their habitat, which probably results in correspondingly strong variations in the $\delta^{13}C$ of the food sources consumed. Primary consumers that include several functional feeding groups (e.g., collectors, scrapers, shredders) obtain carbon not only directly from periphyton, but also from an important fraction of litter. This result indicates that these functional groups can be considerably generalist, feeding on more than one basal resource type (Cornejo *et al.*, 2021). The positive slopes between fish and primary consumers, and leaf litter nitrogen isotopes also could be explained by the assimilation of nitrogen from algal biofilms growing on the leaf surface (Guo *et al.*, 2016; Alonso *et al.*, 2020). The negative relationship of $\delta^{15}N$ of predatory insects with the $\delta^{15}N$ values of periphyton suggests that the nitrogen isotope may be assimilated from sources other than those that provide their carbon source (Bunn, Leigh & Jardine, 2013). For example, predatory insects assimilate carbon from the periphyton, but nitrogen is assimilated from other primary insects, whose adjustment of carbon values with the periphyton is much higher than the adjustment with the nitrogen values of this resource. However, our study is limited in terms of a more robust understanding of the structure of the food web, as we do not use dietary methods.

The water velocity directly contributed to the less enriched $\delta^{13}C$ of the fish, as expected, which may be related to the decrease in the surface layer around the benthic algae, facilitating the diffusion of CO_2. Carbon values tend to be more depleted of $\delta^{13}C$ with increasing fractionation (Finlay, 2004). The temperature had direct effects on the depleted $\delta^{13}C$ values of the periphyton. This result indicates that warmer waters have had low concentrations of $CO_{2(aq)}$, which may limit this resource for photosynthesis reducing ^{13}C discrimination (Finlay; Power; Cabana, 1999). Distance from the source, a variable that acts on the watershed scale, was indirectly related to the enrichment $\delta^{13}C$ values of the periphyton. In regions further downstream from the stream source there is greater canopy opening, which results in higher rates of periphytic algae photosynthesis and $\delta^{13}C$ enrichment (Carvalho et al., 2017). On the other hand, the distance from the source was responsible for higher values of $\delta^{15}N$ in the fish. This result suggests that streams further downstream from the source are larger, which may favor the presence of top predators. Predators fish are infrequent and abundant in low order streams (Ceneviva-Bastos *et al.*, 2012), as some characteristics that favor the presence of these organisms are necessary, such as greater depth and width of streams and greater abundance of prey.

Although our study was carried out in environmental preservation areas, some of the streams showed urban use, pasture, and agriculture in their drainage area. These streams clearly discriminated axis 2 of the two RDAs, being characterized by the higher influence of regional variables and responsible for the increase of $\delta^{15}N$ in the food web. The $\delta^{15}N$ of resources and consumers were effective indicators associated with increased nitrogen and TDS concentrations in streams further away from the source and with greater flow. This is due to the higher anthropogenic influence which can increase the uptake of suspended solids and nutrients from the watershed in streams located further downstream from its source (Allan, Erickson & Fay, 1997; Xu *et al.*, 2021). These relationships provide context for understanding how differences in nitrogen concentrations and extents of stream watershed development affect stream ecosystems and support their use as response indicators in monitoring and management efforts at regional and watershed scales.

In streams of the cerrado the $\delta^{15}N$ of insects reflected the land use in the watershed possibly due to changes in nitrate concentrations in streams (Carvalho *et al.*, 2020). The basal resource most affected by the enrichment of nitrate in streams was the DOM, which reveals that this resource can be an indicator of environmental impacts. This suggests that changes in land use can alter the composition and availability of dietary resources for consumers, as higher nutrient loads facilitate the growth of different trophic groups, like filter collectors (Allan, 2004; Dülger *et al.*, 2017). In this study, organisms considered as filter collectors of the

Hydropsychidae family (Tomanova, Goitia & Helešic, 2006) for example, were only present in streams with natural cover (Table S2). These organisms may have incorporated DOM nitrogen into their diet, and therefore were responsible for the highest $\delta^{15}N$ values in streams with anthropogenic influence. The effect of land use on isotopic compositions of primary producers could also have a significant impact on those of higher trophic level species (Bunn et al., 1999; Carvalho et al., 2017; Lee et al., 2018; Price et al., 2019; Wang et al., 2021). However, this was not reflected in our results. The $\delta^{15}N$ of basal resources was not further enriched in areas with anthropogenic influence. The concentrations of nitrate in areas with anthropogenic influence were not significantly higher than in forest areas (Figure S). However, even anthropized sites had no significantly higher $\delta^{15}N$ values than woodland sites, but they had higher concentrations of nitrate and TDS in some sites. Our findings suggest that predators (fish) may be assimilating their nitrogen from a greater array of resources, other than insects both in anthropized and forested locations, emphasizing that the influence of land use in these streams was important for the enrichment of $\delta^{15}N$ for insects, however, it was not for the entire food web.

Although the variance in values mainly of $\delta^{15}N$ consumers (insects) in streams with anthropogenic influence may indicate high nitrogen pollution, the identification of nitrogen sources can be complicated. Multiple sources are present with distinct signatures and biogeochemical processes that alter the isotopic composition (Kendall, 1998). For example, the discharge of sewage, chemical fertilizers and animal manure derived from urbanization and pasture generally is enriched in ^{15}N due to preferential use of the lighter isotope by bacteria during denitrification (Nikolenko *et al.*, 2018). This is due to ammonia volatilization during storage, with ^{15}N enrichment in NH_4 + residual (converted to NO_3- enriched with ^{15}N). Consequently, the enrichment of $\delta^{15}N$ in manure and sewage may reflect on consumers (Carvalho *et al.*, 2020). In relation to $\delta^{13}C$ data, the values were less diagnostic of anthropogenic influences, suggesting that the carbon cycle dynamics are more complex compared to nitrogen.

The trophic fractionation of δ^{13}C across multiple trophic levels can be much more variable than that observed with δ^{15}N (Hesslein *et al.*, 1991; Post, 2002).

The higher concentration of total dissolved solids in streams influenced by anthropogenic activities highlights the importance of in-situ vegetation cover and in the watershed in controlling the transport of sediments (Beltrão, Medeiros & Ramos, 2009; Rocha, Casatti & Pereira, 2009). This may explain the direct effect of nitrate on the δ^{15}N values of dissolved organic matter. Forested watersheds are more effective in removing nitrogen from catchment soils compared to pastures and non-vegetated areas (Lintern *et al.*, 2018). However, the intact riparian zones in the streams of this study were not sufficient to dampen the effects of changes in the land use of the catchment. In a recent study in the same study region, Campos, Kennard & Gonçalves (2021) demonstrated that headwater streams are especially vulnerable to relatively small increases in land uses in the riparian zone and upstream watershed. They found a significant increase in diatom assemblages. In our study, the high connectivity of streams with adjacent landscapes confirms the importance of considering regional factors in nutrients uptake by aquatic environments (Taniwaki *et al.*, 2018).

CONCLUSION

Our findings highlight that even in small forested streams, algae resource sources play an important role in the aquatic food web. Thus, local factors influencing algae composition and production are likely to have a significant effect on consumers. However, it was not possible to infer that variations in the canopy cover, are responsible for the availability of autochthonous resources in the stream. Our results seem to indicate that algae availability is independent of canopy cover. Temperature and flow were the parameters that most influenced the variations in the δ^{13}C values of the food web. While nitrate concentrations in streams and distance from the source were the parameters that most influenced the variations in the δ^{15}N values of the food web. We suggest that the origin of anthropogenic nitrogen in the studied watersheds can be traced through the $\delta^{15}N$ signatures of the food web. The $\delta^{15}N$ biota responsiveness to anthropic influences in watersheds supports its use as an indicator in monitoring programs, particularly at larger scale watersheds. Finally, we have highlighted the importance of using isotopic analysis as a useful method for characterizing freshwater food webs, also for understanding the mechanisms and pathways that affect different components of the ecosystem.

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SUPPORTING INFORMATION

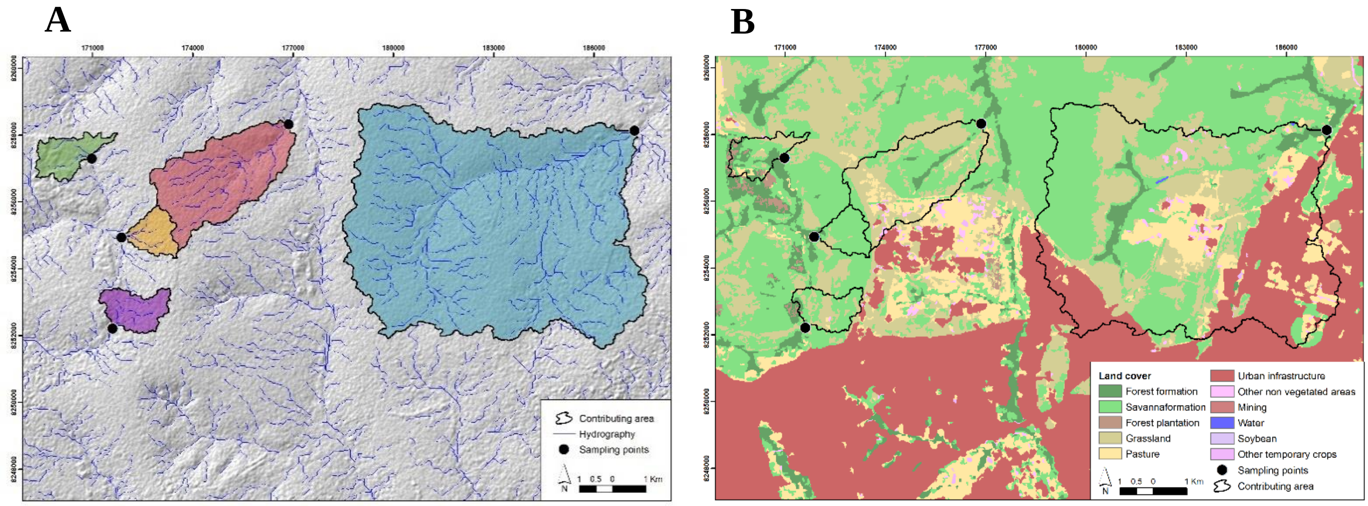

Figure S1. Representation of the catchment scale considering upstream catchment along the entire sample site drainage network. A - representation of the stream contribution area; B - representation of the contribution area of the streams with the land cover classes

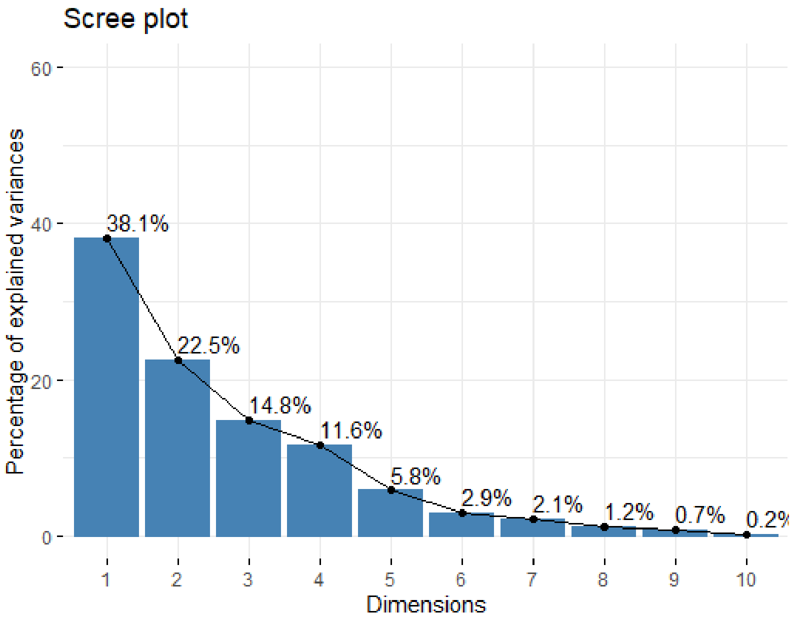

Figure S2. Percentage of explained variance for each PCA axis. Axes 1, 2 and 3 together explained 75.46% of the data variation.

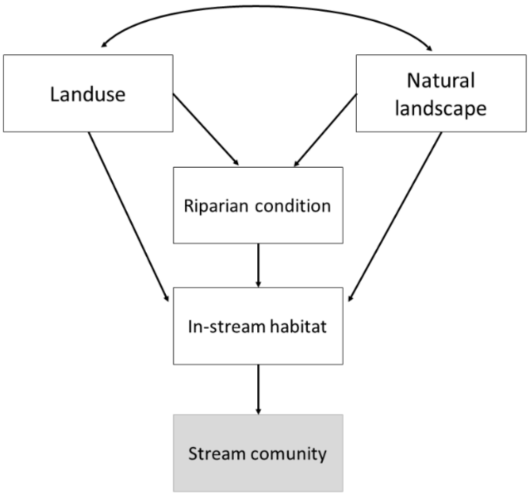

Figure S3. Conceptual model of the possible pathways through which anthropogenic and natural factors affect stream ecosystems. This model was used to build the structural equation model. Double-headed arrows indicate correlation, while single-headed arrows indicate possible governing mechanisms. Model based on previous studies (Maloney & Weller, 2011; Chará-Serna et al., 2015).

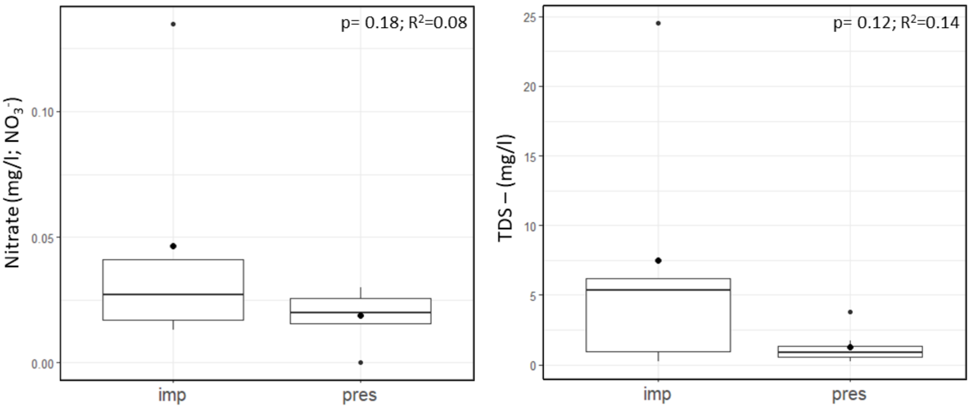

Figure S4. Nitrate and TDS concentrations in forested and anthropogenic-influenced streams. No significant differences were found.

Table S1. Physical characteristics, land use, autochthonous and allochthonous resources calculated for the twelve streams of the cerrado. Order = classification of flow orders according to Strahler; Alt = Altitude; Past= Pasture; Agri=Agriculture; Urb= Urbanization; Others= sum between Grassland and Other non-vegetated areas.

Streams		Characteristics of streams								Land use				
		Alt (m)	Order	Mean depth (m)	Mean width (m)	Flow (m/s)	Nitrate (mg/l; NO$_3^-$)	TDS – Sólidos dissolvidos totais (mg/l)	Distance from source (km)	Natural cover (%)	Past (%)	Agri (%)	Urb (%)	Others (%)
Natural cover	3	1088	5th	0.6	1.2	0.31	0.015	3.8	6.99	38.41	1.81	0	0	59.77
	6	1097	terciary	0.19	0.86	0.11	0.023	0.82	0.84	80.63	0	0	0	19.36
	7	1046	4th	0.6	3.4	3.12	0.03	0.96	3.95	86.16	1.01	0	0.69	12.12
	9	1053	5th	0.5	3	0.69	0	1.72	3.52	85.50	0.45	0.40	0	12.81
	10	1207	secondary	0.15	0.7	0.03	0.016	0.91	0.07	67.57	4.94	0	0	20.06
	11	1195	terciary	0.3	1.5	0.09	0.02	0.31	0.34	92.43	0	0	0	7.56
	12	1218	secondary	0.25	1	0.05	0.028	0.23	0.19	60.52	0.86	0	0	37.97
Anthropic influence	1	1034	5th	0.6	4	2.88	0.135	24.56	5.56	37.21	12.68	0.10	24.62	25.31
	2	1100	terciary	0.4	2	1.35	0.041	0.95	2.69	41.57	11.84	0	0.73	45.84
	4	1090	terciary	0.5	2.4	0.06	0.027	5.37	1.33	42.52	6.39	0	6.05	45.03
	5	1042	4th	0.2	2	0.32	0.017	0.22	1.31	45.77	0.21	42.98	0	11.02
	8	1073	secondary	0.25	1.5	0.46	0.013	6.21	1.45	28.75	22.28	0	42.72	6.22

Table S2. List of taxa that were evaluated in this study

Stream		Primary consumer	Secondary consumer	Terciary consumer
Natural cover	3	Elmidae	Perlidae	*Astyanax scabripinnis*
	6	Calamoceratidae, Chironomidae, Leptoceridae and Pyralidae	Gomphidae, Libellulidae, Megapodagrionidae, Perlidae	*Astyanax scabripinnis, Hyphessobrycon balbus* and *Hasemania crenuchoides*
	7	Elmidae	Calopterygidae, Libellulidae and Veliidae	*Astyanax scabripinnis* and *Rhamdia quelen*
	9	Simulidae	Gomphidae and Libellulidae	*Astyanax scabripinnis, Hyphessobrycon balbus* and *Macrobrachium* sp.
	10	Leptoceridae and Pyralidae	Tipulidae and Perlidae	*Hyphessobrycon balbus*
	11	Pyralidae and Calamoceratidae, Grypopterigidae and Leptoceridae	Tipulidae, Perlidae, Tipulidae, Calopterygidae, Libellulidae and Sialidae	*Astyanax scabripinnis, Hyphessobrycon balbus* and *Hasemania crenuchoides*
	12	Elmidae and Leptoceridae	Gomphidae and Libellulidae	*Astyanax scabripinnis* and *Hasemania crenuchoides*
Anthropic influence	1	Elmidae	Perlidae	*Moenkausia* sp.
	2	Elmidae	Perlidae	*Astyanax scabripinnis*
	4	Baetidae, Hydropsychidae and Simulidae	Corydalidae and Libellulidae	*Astyanax scabripinnis*
	5	Elmidae, Hydropsychidae, Leptoceridae, Psephenidae and Simulidae	Gomphidae, Perlidae, Tipulidae and Veliidae	*Hyphessobrycon balbus*
	8	Elmidae	Libellulidae	*Astyanax scabripinnis, Hyphessobrycon balbus* and *Hasemania crenuchoides*

Table S3. Correlations between environmental variables and RDA scores (loadings) of the 12 Cerrado streams, Brazil. Metrics in bold have a relationship to the axis (either positive or negative).

Isotopic value	Variable	RDA1	RDA2
$\delta^{13}C$	PC1	0.47	**0.78**
	PC2	-0.03	-0.45
	PC3	**0.88**	-0.43
$\delta^{15}N$	PC1	0.60	**-0.79**

	PC2	**-0.79**	-0.61
	PC3	0.07	-0.03

Table S4. Correlations of δ^{13}C and δ^{15}N values of consumers and resources and RDA scores (loadings) of the 12 Cerrado streams, Brazil. Metrics in bold have a relationship to the axis (either positive or negative).

Isotopic value	Food web	RDA1	RDA2
δ^{13}C	primary consumer	0.51	0.47
	secondary consumer	0.32	0.05
	terciary consumer	-0.25	0.51
	Periphyton	0.44	-0.17
	Litter	**0.84**	-0.09
	DOM	-0.04	-0.41
δ^{15}N	primary consumer	**-0.74**	0.38
	secondary consumer	**-0.69**	-0.46
	terciary consumer	**-0.65**	0.17
	Periphyton	0.11	0.09
	Litter	-0.54	-0.08
	DOM	-0.27	-0.09

Table S4. Mean ± S.D. isotopic signatures of resources and consumers sampled in streams.

	δ^{15}N (‰)	δ^{13}C (‰)
Periphyton	2.68 ± 2.78	-28.72 ± 1.20
Litter	-0.63 ± 0.88	-29.62 ± 0.88
DOM	-1.41 ± 4.77	-24.82 ± 3.62
primary consumer (insects)	3.36 ± 2.08	-30.34 ± 4.00
secondary consumer (insects)	6.12 ± 1.50	-27.89 ± 2.81
terciary consumer (fish)	9.37 ± 1.08	-24.35 ± 1.18

Table S5. Isotopic values of carbon and nitrogen from basal resources in cerrado streams

	δ^{13}C			δ^{15}N		
Stream	DOM	Periphyton	Litter	DOM	Periphyton	Litter
1	-20.401	-29.136	-29.801	2.881	1.597	1.426
2	-25.76	-27.925	-30.797	-1.257	-1.045	-2.233
3	-24.838	-28.449	-29.32	-10.363	0.584	-2.727

4	-23.903	-28.24	-30.872	-6.32	2.922	-0.058
5	-21.403	-28.09	-29.373	-2.253	5.559	-0.934
6	-35.082	-29.17	-29.61	-8.075	6.871	-2.452
7	-24.399	-28.2	-29.493	6.264	5.309	-0.497
8	-26.491	-31.911	-29.818	0.015	5.31	1.789
9	-24.078	-26.815	-28.273	1.3	-0.707	0.281
10	-23.685	-29.019	-27.997	1.094	0.61	-0.334
11	-23.807	-28.988	-29.512	1.541	4.894	-0.892
12	-23.994	-28.77	-30.64	-1.845	0.333	-0.966

CAPÍTULO III - LAND USE EFFECTS ON THE STRUCTURE OF TROPHIC NETWORKS FROM STREAM FISH

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ABSTRACT

In studies of fish trophic networks, one of the main gaps is how interactions between species respond to anthropogenic stressors. Considering that areas with landscape integrity have a high supply of food resources through vegetation, the structure of networks can be influenced by environmental characteristics such as the availability of habitats and food resources. To understand the effects of land use on the structure of freshwater fish trophic networks, we compiled a dataset of 49 neotropical fish trophic webs sampled from 1982 to 2019 across Brazil. We hypothesize that increasing land-use intensity will change the structure of trophic networks, reducing modularity and trophic specialization, and increasing nestedness. We calculated nestedness, modularity, trophic specialization, number of links and link density from diet information. We assessed land-use in a 500-meter buffer around each sampling point from MapBiomas information. Our analysis provides a comprehensive overview of the responses of trophic networks to land-use and shows that variation in modularity was greater in pastures, while changes in network complexity were more evident in agriculture. We found a negative relationship between land-use gradient and modularity and links density. The nestedness and trophic specialization degree were not affected by the land-use gradient. These findings highlight the relevance of preserving natural vegetation along watercourses and its key contribution to the functioning of aquatic ecosystems. Our results demonstrate that the interactions between consumer and resource represented by trophic network descriptors should also be considered in future studies on the importance of conservation of riparian forests.

Keywords: Ecological networks, food web, Neotropical stream, modularity, nestedness

INTRODUCTION

Natural communities are composed of different species interacting with each other in different ways, which can be organized as ecological networks (Fortunato & Hric, 2016). A network can be represented by a graph where the species or individuals are nodes and the biological interactions are the links between them (Boccaletti, Latora, Moreno, Chavez & Hwang, 2006). The study of networks provides an efficient way of representing, characterizing and revealing the determinants of the structure of natural interaction systems. This approach has been successfully applied to understand complex interaction systems such as mutualism (Mougi & Kondoh, 2014), trophic interactions (Baumgartner & Robinson, 2016), neural or genetic networks (Roth et al., 2016) and how these structures changes over space and time (Warren, 1989; Pinter-Wollman, 2015). It provides new insights into the ecological and evolutionary processes in structuring and organizing biotic interactions (Tylianakis & Morris, 2017).

Recent research has been focused on the human effect on the dynamic and structure of networks. Authors have found that habitat degradation, for instance, promotes homogenization and a reduction in network complexity and stability (Ollerton, McCollin, Fautin & Allen, 2007; Sebastián-González et al., 2015; Song, 2017; Pellissier et al., 2017; Dáttilo & Vasconcelos, 2019; Felipe-Lucia et al., 2020). This nestedness pattern emerges when specialized species tend to interact with subsets of partners of most generalist species (Bascompte, Jordano, & Melia, 2003). It involves differences in the abundance of predator-prey interactions and higher rates of extinction of specialist species (Pires & Guimarães, 2013). On the other hand, modular networks are formed by subgroups of consumer species interacting more with one resource group than other resources outside its subgroup, hence forming many modules in the network (Olesen, Bascompte, Dupont, & Jordano, 2007; Dormann & Strauss, 2014). In trophic interactions, other network descriptors such as trophic specialization also enable the understanding of the organization of networks and its ecological drivers (Staudacher et al., 2017). This network metric indicates the degree of species consumption on exclusive food items. Investigations of how the properties of networks vary according to anthropic stressors have the potential to provide insights into how the trophic networks may vary under the influences of processes such as environmental filtering, competition or facilitation (Pellissier et al., 2017).

Trophic interactions are an essential component for understanding the dynamics of populations, and consequently the emerging patterns of coexistence and diversity in communities. Despite the increased interest in this field, the knowledge of how and why trophic networks vary along land-use gradients is elusive (Pellissier et al., 2017; Felipe-Lucia et al., 2020). Recently, theoretical studies have shown that the main determinants of network structure differentiation at different sites are variations in ecological and environmental factors (Emer, Venticinque, & Fonseca, 2013; Dugger et al., 2018). In studies of fish trophic interactions, one of the main gaps is how interactions between species respond to anthropogenic impact (Tylianakis et al., 2007). Based on local scale studies, it has been suggested that trophic networks in aquatic ecosystems under strong anthropogenic impact tend to present generalist species with greater connectivity among nodes and a high level of nestedness (Manoel & Uieda, 2017) compared to regions with preserved native vegetation (Thompson & Townsend, 2005). Recently, Felipe-Lucia et al. (2020) showed that increasing land use intensity resulted in homogeneous and less modular networks, while forest formations resulted in more complex networks. The integrity of riparian forest is crucial for the provision of food resources for aquatic communities and ecosystem change are threatening their supply (Zeni & Casatti, 2014; Carvalho et al., 2019). To understand these impacts, it is essential to investigate the trophic relationships between communities (Lobón-Cerviá, Mazzoni, & Rezende, 2016), and how the drivers of global change (ie, intensification of land use) affect these trophic relationships. Thus, understanding the variation of trophic structures at large scale and testing the generality of these predictions is important to build consistent knowledge of the effects of land-use on trophic networks.

Most impacts on freshwater bodies are directly or indirectly related to the conversion of the adjacent vegetation to pasture or cropland (Vorosmarty et al., 2010). Croplands and pastures influence fish communities through multiple paths (Dala-Corte et al., 2016). Croplands cause increased siltation, nutrient input, increase the organic loading through fertilizers and homogenization of the stream substrate (Sutherland, Culp, & Benoy, 2012), and pastures also increase the dissolved organic matter into watercourses (Neill, Deegan, Thomas & Cerri, 2001). At the same time, local reduction in riparian vegetation cover reduce nutrient supply and the input of allochthonous material, increase autochthonous production and completely change the quality and quantity of available food resources (Bambi et al., 2016; Zeni & Casatti, 2014). As a consequence, fish species often change their resource use in response to changes in resource availability (Prejs & Prejs, 1987) and poor environmental conditions (Alonso, Carvalho, Alves, Moreira, & Pompeu, 2019). These modifications can increase niche overlap due to an expansion of generalist/opportunistic feeding strategy and a reduction in specialist species. Consequently, the structure of the trophic network (Pimm, Lawton, & Cohen, 1991) should vary according to the degree of land-use change (Winemiller, 1990).

Here, we aim to assess the anthropogenic effects on the trophic networks structure of Neotropical stream fish. We addressed the following questions: (a) are the structure of fish trophic networks related to land-use changes? and (b) which of the major land-use classes (i.e. pasture or cropland) has the greater effect on the structure of fish trophic networks? The first hypothesis is that basins with greater vegetation cover can increase the supply and diversity of food resources (terrestrial insects, terrestrial allochthonous resources) to streams, resulting in networks more complex (ie large number of links, species and link density) and specialized (ie larger modularity and greater trophic specialization). The second hypothesis is that basins with greater anthropogenic influence may decrease the diversity of food resources, resulting in the predominance of generalist species (ie, more nested and less specialized trophic networks).

METHODS

Dietary data base

We searched for dietary studies with fish assemblages available in the literature, considering the Scopus, Web of Science and Google Scholar databases. Several combinations of keywords were used for the searches, containing (fish*) AND (stream) AND (feeding OR diet) AND (other terms of interest). We only considered studies (articles) with more than five species in studies that represent the local community, because we were interested in describing the local community network structure. The study site samples covered large reaches of rivers (rivers and streams) in multiple habitats located in natural, pasture and agricultural areas. In addition, we focused on the papers expressing the diet of species as the feeding index (IAi) or numeric or volume percentage of the food item in the diet of each individual (Santos et al., 2021; Souza et al., 2020; Peressin et al., 2018; Bonato et al., 2012), to make sure they were fully characterizing feeding habitats. We compiled 49 peer-reviewed articles covering dietary data and study location coordinates (Table S1). We extracted information from the dietary tables of the papers (row food items and column species) that included different food items represented by different families of terrestrial and aquatic insects, algae, plant material, crustaceans and mollusks. The studies diverge in their way to quantify consumed food items and these matrices served as the basis for building the trophic networks detailed below. Thus, we used information on the presence/absence of interactions (i.e., binary matrices) for calculating trophic network descriptors, except for the trophic specialization metric (H2’, considers interaction frequencies).

Network Structure

We built the bipartite networks in which the nodes represent the fish species (consumers) and food items (resources), while the links between them represent the items consumed by each species. We used each interaction matrices to calculate independent trophic network descriptors. There are many network indices, and they differ in sensitivity to detect the property in question, network size robustness and sampling intensity. To understand how land-use intensity modifies food webs, we chose metrics that represent the overall network structure, such as network nestedness, modularity, trophic specialization, and food-web complexity metrics (Number of species, Link density and Number of links). The number of species (nodes), defined by the total number of consumers and resources (May 1973; Tilman 1996). The link density is defined as the number of trophic links (L) divided by the total number of nodes (consumers and resources, S) in a food web (L/S). The average number of links per species informs about how connected species are within the food web (Dunne, Williams, & Martinez, 2002; Bersier, Dixon, & Sugihara, 1994). The number of links is related to the number of trophic interactions in a food web. Number of links has implications for the complexity of the food web, and the number of pathways along which energy can flow (Dunne, Williams, & Martinez, 2002). We quantified nestedness with the nestedness metric based on overlap and decreasing fill (NODF), which is based on the concepts of overlap and decreasing fill of the adjacency matrix (Almeida-Neto, Guimaraes, Guimaraes, Loyola, & Ulrich, 2008). Nestedness describes webs with overlapping interactions, where in a perfectly nested network all interactions must be subsets of generalist interactions (Bascompte, Jordano, & Melia, 2003).

We calculate specialization of trophic networks by quantifying the Modularity and Trophic specialization (H_2') indices. We quantified the modularity with the metric Q that measures the difference between the observed fraction of links connecting species in the same module and the fraction expected by chance using an algorithm modified for two-mode networks (Dormann, & Strauss, 2014). A modular network consists of interconnected modules. Each module is a group of species, which are more closely connected to each other than to species in other modules. (e.g., Olesen, Bascompte, Dupont, & Jordano, 2007) and here it is an indicator of the degree of specificity of trophic interactions, representing sets of species specialized in a resource type or resource subset. Network specialization was quantified by the H_2' index, which is based on the deviation of the number of interactions performed by a species and the expected total number of interactions per species. H_2’ is a two-dimensional index derived from the Shannon index used to compare different networks, and ranges from 0 (no specialization, highly generalist) to 1 (complete specialization) (Blüthgen, Menzel, & Blüthgen, 2006). The metric is calculated by a comparison between observed and expected interaction frequencies, based on the species marginal totals (Blüthgen et al., 2006). In the case of a food web, a species may be feeding only on a particular food item, but if this item presents higher frequency of interactions in the system, it may limit the specialization degree and therefore the species would receive a low H_2’ value. In contrast, a species that feeds on only two rarer food items would have a very high H_2’ value. The higher the level of selectivity of the species, the greater the H_2’.

Null model of trophic network structure

When calculating network descriptors, it is important to control for a possible sampling bias related to network dimensions (i.e. number of species and number of trophic links), which could prevent comparing descriptors among networks. Therefore, we compared all observed index values in individual networks to those calculated under null models (Dáttilo & Vasconcelos, 2018; Kortsch et al., 2018; Quimbayo et al., 2018). We randomized the observed trophic networks over 1,000 matrices for each network descriptor, using a null model that fixes both marginal totals and connectivity (swap.web null model), i.e., maintaining constant the number of interactions (and therefore connectivity), as implemented in the "bipartite" package in R (Dormann, Fründ, Blüthgen, & Gruber, 2009). Then, we used the standardized effect size of each index (z transformations), calculated as (observed - μ) / σ, where observed is the value of the focal index (e.g. Q, NODF, Link density and Number of links), μ is the mean value of focal index over all null matrices and σ is its standard deviation, and used it as the response variables. Empirical values of trophic network descriptors were considered to deviate strongly from the randomized food webs if these were outside the 0.05 to 0.95 quantile range of the null distribution. For each local network, the probabilities of the empirical values under the null model are presented in the Supplementary Material (Table S2). Note that we did not use any standardization in H_2' because a standardization is already performed when this network descriptor is calculated (Blüthgen, Menzel, & Blüthgen, 2006).

Anthropogenic impact in watersheds

We calculated anthropogenic impact surrounding each sampling site to account for the influence of land-use on the structure of fish trophic networks. We created overlapping concentric buffers around each sampling site, with 500 meters radius, and calculated the percentage area of each land-use class (Figure 1) using ArcMap 10.6.1 (ESRI, 2018). We tested several buffer sizes from 500 to 10000 m in 500-m increments (500 – 10000 m) and the 500 m buffer was best suited to our models. In addition, at sampling sites where fish sampling was carried out over more than one stream reach, we calculated the average land use. We used land-use data from the Brazilian Annual Land-use and Land Cover Mapping Project (MapBiomas, Collection 4.1, MapBiomas, 2020). This project produces 30-m pixel resolution digital annual maps of land-use in Brazil based on random forest and machine learning automatic classification processes applied to Landsat Data Collection satellite images (from 1985 until

2018). We retrieved MapBiomas land use information for the respective sampling year reported in each study. The year correspondence was not possible only for a sample obtained in 1982 and another in 2019, for which MapBiomas information was not available then, so we used the closest available information in the time series (1985 and 2018, respectively). The 18 land-use and land-cover classes occurring in the evaluated sites were consolidated into two broader categories of natural and non-natural land-cover. Natural land-cover encompasses forest formation, savanna formation, mangrove, wetland, grassland, salt flat, rocky outcrop, other non-forest natural formation, beach and dune, river, lake and ocean. The overall anthropogenic impact includes forest plantation, pasture, annual and perennial crop, semi-perennial crop (sugarcane), mosaic of cropland and pasture, urban infrastructure, mining and other non-vegetated areas. Using these categories, we were able to create a gradient of natural land-cover loss across sites, ranging from completely natural (100%) to no-remaining natural cover. After the classification of the areas, we represented cropland by the sum of the classes related to agricultural use (annual and perennial crop, and semi-perennial crop – sugarcane –), while pasture metric included the pasture class (Table S3).

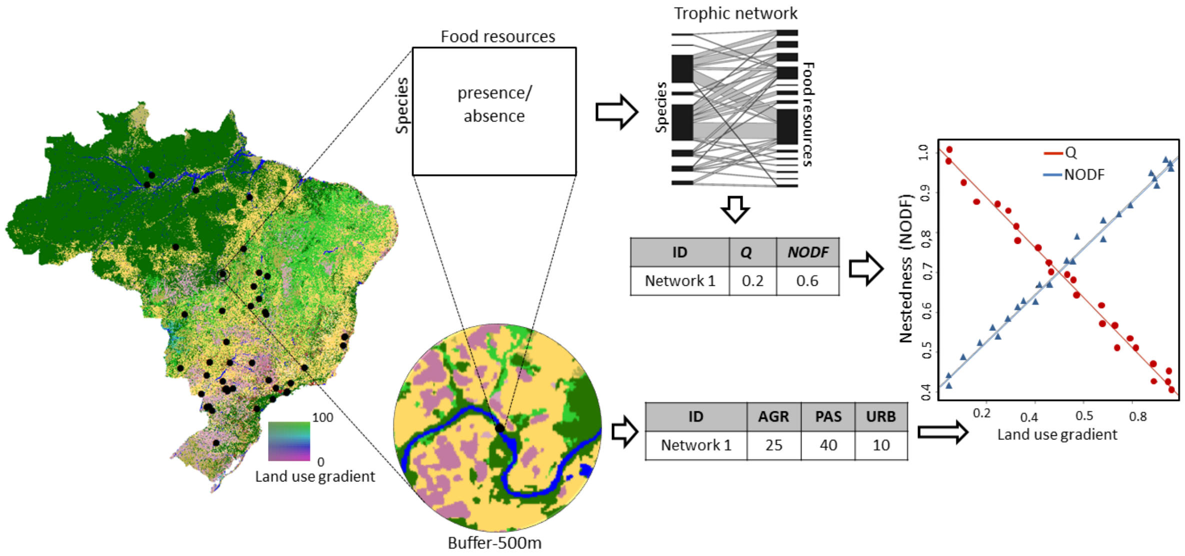

Figure 1. Description of the procedure of sampling the trophic network. We quantified land-use information around a 500-m radius buffer at each sampling point, obtaining data from the

year the study was conducted. Diet data were used to generate food webs within each sub-basin. We calculated the indexes of modularity, nestedness, trophic specialization and food-web complexity metrics (Number of species, Link density and Number of links) for each trophic network.

Linear models

We constructed models in which the response variables were the standardized z value of each index and the predictors were the proportional area of anthropogenic land-use, cropland and pasture. We used simple linear regressions to assess whether the impact in a 500-meter buffer influences the structure (values using Z-scores to NODF, Q, and H_2’, Link species, Number of links and Number of species) of the trophic networks (Figure 1). Based on the geographic coordinates of sampling sites, we tested for spatial autocorrelation in the residuals of our models using global Moran's I and found no evidence of positive spatial autocorrelation in most of them, except for the link density and species number (Table S4). For this, we used spatial models to control these variations and to correct the degrees of freedom of our models, using ‘dbmem’ function from ‘adespatial’ package (Borcard & Legendre, 2011).

We checked for normality of the residuals of the models using the Shapiro test. The assumption of normality was met in all cases, except for the density of link, for which the we calculated the Cook's distance and removed outliers with cook's distance greater than 1 to correct the model (Supporting Information Figure S1). Analyzes were performed in R v. 3.5.3 (R Core Team, 2016), using ‘networklevel’ function (Dormann, Fründ, Blüthgen, & Gruber, 2009), ‘nested’ function and ‘metaComputeModules’ function, all from ‘bipartite’ package (Marquitti et al., 2014).

RESULTS

Overall, we recorded 24,214 trophic interactions involving 608 species of fish and 185 food items (mainly invertebrates, plant material, detritus and algae). Locally, we registered between 5 to 99 species of fish (mean ± SD, 20.93 ± 20.04) and 5 to 58 consumed items (15.55 ± 10.62). In relation to land-use, we found a greater proportion of pasture (19.81% ± 28.72%) and followed by cropland (14.73% ± 27.81%). Most species had an overall invertivore diet (49% aquatic or terrestrial invertebrates), while 18% were detritivores, 10% were omnivorous, 9% were herbivorous, and about 7% consumed mainly fish and 4% consumed algae (Table S5). Networks in general were more nested (0.33 ± 0.13) then modular (0.26 ± 0.11), with high trophic specialization (0.58 ± 0.15) (Table S6). Sites with greater number of species presented greater number of links, lower nestedness and higher modularity (Table S7).

In terms of food-web complexity, the link density of local networks was negatively associated with the land-use gradient (p=0.02; Figure 2), whereas the number of links was positively associated, but not significant (p> 0.05; Figure 2). We did not find land-use effects in the degree of specialization, number of species and nestedness of network (p=0.67; p=0.31; p=0.23, respectively), however, sites located in impacted locations showed low modularity values (p=0.04). When evaluating which of the land uses influence the structure of the trophic network, cropland was positively associated with the food-web complexity metrics (number of species). We found no individual influence of cropland and pasture on nestedness nor on specialization degree of trophic networks (Table 2). The pasture was negatively related to modularity, on the other hand, cropland was negatively related to the number of species (Table 2).

Table 1. Linear regression coefficients between the natural land-use across sites and trophic network descriptors (Nestedness ($wNODF_{zscore}$), Modularity (Q_{zscore}), Specialization (H^{2} '), Link density, Number of links (links por species) and Number of species) of communities of fish. We use Z-scored values for all metrics, except for number of species, in which log was used (x + 1) and Trophic specialization.

Network descriptor	Estimate	SE	t-value	r^2 adj	p-value

NODF	0.006	0.005	1.19	0.009	0.23
H_2'	-0.000	0.000	-0.42	-0.01	0.67
Q	-0.01	0.006	-2.03	0.06	0.04*
Link density	-0.08	0.03	-2.36	0.09	0.01*
Number of links	0.008	0.003	0.26	0.25	0.14
Number of species	0.000	0.00	1.01	0.00	0.31

SE= Standard error. Asterisk (*) denotes statistical significance, *p < .05. **p < .01. ***p < .001

Table 2. Regression coefficients of two land-use variables (cropland and pasture) in explaining the observed variation in nestedness (NODF), modularity (Q), Specialization ($H^{2'}$), Number of links, Link density and Number of species. We use Z-scored values for all metrics, except for number of species, in which log was used (x + 1) and Trophic specialization.

Network descriptor	Predictor variables	Averaged coefficients	SE	t-value	p-value	Moran´s I
NODF						
	Cropland	0.008	0.007	1.04	0.30	-0.02
	Pasture	0.001	0.007	0.17	0.86	-0.02
Q						
	Cropland	-0.009	0.010	-0.88	0.38	-0.04
	Pasture	-0.019	0.010	-1.93	0.05*	-0.03
H_2'						
	Cropland	-0.000	0.000	-0.54	0.58	-0.01
	Pasture	0.000	0.000	0.44	0.65	-0.02
Link density						
	Cropland	-0.06	0.03	-1.65	0.10	-0.09*
	Pasture	-0.05	0.03	-1.72	0.09	-0.11*
Number of links						
	Cropland	0.006	0.005	1.20	0.23	-0.02
	Pasture	0.001	0.005	0.32	0.74	-0.04
Number of species						
	Cropland	-0.006	0.003	-2.01	0.04*	-0.10*
	Pasture	-0.006	0.005	-1.84	0.07	-0.12*

SE: standard error; t-value: test statistic; asterisk (*) denotes statistical significance, *p < .05. **p < .01. ***p < .001.

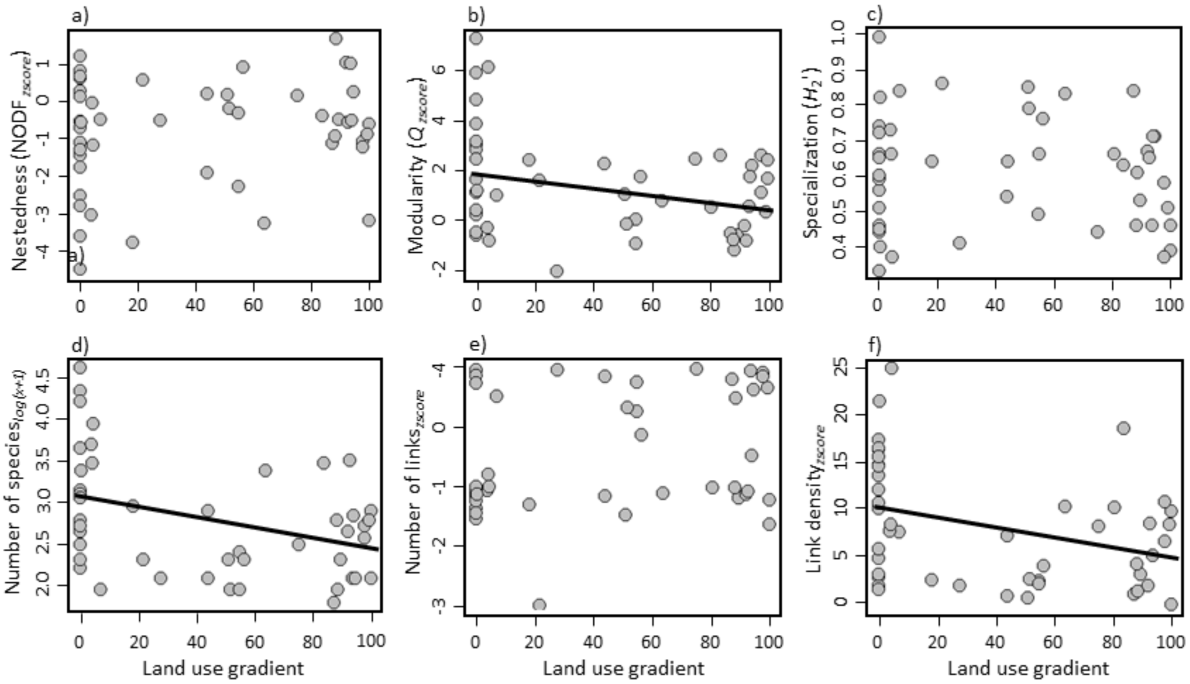

Figure 2. Scatterplots of the relationships between land-use gradient on watersheds and trophic network descriptors and food-web complexity metrics. a) Nestedness (NODF$_{zscore}$), b) Trophic specialization (H_2'), c) Modularity (Q_{zscore}); d) Density of link (zscore), e) Link per species (zscore) and f) Numbers of species (log (x + 1)). Each point represents a sampling site (n = 49 independent fish networks). Linear regression coefficients are shown in table 1.

DISCUSSION

By gathering data from stream fish assemblages, we studied trophic network descriptors and assessed the extent to which the structure of networks was determined by land-use gradient across Brazil. Our study over a large spatial scale generalize previous ones at local scale (Kortsch et al., 2019, Manoel & Uieda, 2017; Peterson, Keppeler, Saenz, Bower, & Winemiller, 2017), and shows evidence of land-use effects on the structure of trophic networks of freshwater fish. Sites located in areas with intense land-use change had less species and less links involved, as well as less modular trophic networks. Decomposing land-use into the two major land-uses in Brazil shows that both the percentage of cropland and pasture dictated the structure of trophic networks, but their effects are mainly on the number of species and modularity, whereas we did not observe effects on nestedness, nor the specialization index.

Disturbance in lotic ecosystems related to land-use change in surrounding terrestrial habitat often reduce the heterogeneity of microhabitats, increase siltation, and modify physico-chemical water conditions (Nessimian et al., 2008; Casatti et al., 2006; Almada et al., 2019). These overall changes in habitat structure contribute to simplifying fish assemblages by excluding habitat specialists, and increasing the abundance of generalist species (Winemiller 1990; Thompson & Townsend, 2005; Dala-Corte, Becker, & Melo, 2017; Arantes et al., 2018). This mechanism is in line with our results as we found that the link density and modularity were negatively related to the land-use gradient, supporting that the structure of trophic networks is simplified under an intense disturbance regime as reported also by other aquatic groups (Pellissier et al., 2017; Lara et al., 2020; Mokross, Ryder, Côrtes, Wolfe & Stouffer, 2014; Sebastián-González et al., 2015). On the other hand, two important metrics, nestedness and the degree of trophic specialization were not affected by the land-use gradient, although the first showed a positive, but not significant (Table 1, Figure 2) effect, and an the second a negative effect, and not significant (Table 2).

Taken together, the lack of effect of land-use on nestedness and specialization, and the greater effect on modularity and link density suggest that the structure of trophic networks could be much more influenced by the loss of specialist species. In general, specialists species are lost as increases the disturbances in altered environments. Previous assessments have addressed the effects of environmental gradients on network ecology (Pellissier et al., 2017; Tylianakis & Morris, 2017), concluding that these are primarily related to changes in species composition and relative abundances. A recent study on spatial patterns in the food web associated with environmental gradients (Kortsch et al., 2019) showed that the spatial variation in the structure of the fish food web is related to turnover in species composition. As a result, the structure of the trophic network became more connected and less modular. However, the turnover in species composition alone should not be the main cause of the lower modularity of the networks. The decrease in modularity can be linked to the reduction in the diversity of trophic groups, generating changes in the composition of the module. This is expected when each trophic group feeds on a wide range of resources (Felipe-Lucia, 2020). Therefore, the decrease in modularity indicates that species trophic interactions are being driven by a small number of less specialized trophic groups. Our models indicate that the modularity and the number of species are determined by cropland and pasture (that is, of the most common classes of land-use change). According to previous studies, the decrease in the number of species with high levels of local farming practices suggests that these activities around the streams simplify aquatic trophic networks (Bonato, Delariva, & Silva, 2012; Zeni & Casatti, 2014; Santos, Ferreira, & Esteves, 2015).

The maintenance of the riparian vegetation cover influences the supply of allochthonous material (fruits, leaves and insects) and increas the physical heterogeneity of the channel, providing different feeding habitats for the species. In fact, in highly impacted environments, the supply of autochthonous and allochthonous resources decreases dramatically due to the absence of riparian vegetation and, consequently, to the availability of terrestrial insects and plant material for fish (Zeni & Casatti, 2014). Previous studies have shown that fish in agricultural landscapes can increase the consumption of low-protein and indigestible foods, such as detritus, sediments and organic waste (Dala-Corte et al., 2016). This is associated with the ability of opportunists species to deal with changes in the availability of food resources, particularly low-quality food items (Dala-Corte, Becker & Melo, 2017; Ferreira et al., 2012).

CONCLUSION

Our study indicates that habitat degradation by land-use change affects the structure of trophic interactions, resulting in large-scale variation in the food web structure throughout an anthropogenic impact. There is a trend towards simplification of aquatic environments influenced by cropland and pasture in neotropical aquatic environments, often resulting in less complex and less modular networks. Moreover, cropland and pasture showed more pronounced changes in food-web complexity metrics than on structural attributes of networks. In sum, our findings highlight the importance of riparian vegetation modulating the trophic structure of fish communities. Since the trophic structure of fish communities responds to the degree of land-use change, restoring the integrity of landscapes, even partially, could improve the functioning of aquatic systems and their biodiversity. Further developments to quantify the land-use at a finer scale, the degree of native vegetation integrity, the time since the land-cover change, the spatial pattern of fragments in the landscape, in addition to local environmental characteristics such as the input of organic matter, and limnological and structural measures at the study sites will help to capture more subtle differences and better understand the influence of riparian forest removal on trophic networks. Our work could be replicated and further expanded to include data from other highly biodiverse tropical regions facing similar pressures from land-use and land-cover change.

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SUPPORTING INFORMATION

Table S1. Study locations. Biomes, richness of consuming species, number of food resources, network size and connectance.

ID	Biome	lat	long	Species richness	Food resources	Network size	Network connectance	Ref.
	Amazon							
		-2.12737	-59.3277	31	19	589	0.28	Silva 2006
12		-3.08246	-59.7675	75	11	825	0.34	Mérona & Rankin-de-Mérona, 2004
32		-9.23588	-56.9429	66	11	726	0.42	Dary *et al.*, 2017
34		-3.56246	-54.8903	21	5	105	0.42	Cardoso & Couceiro, 2017
43		-4.33037	-49.5653	99	7	693	0.49	Mérona *et al.*, 2001
45		-4.73313	-62.1543	28	17	476	0.54	(Duarte and others 2019)
	Cerrado							
01		-15.9329	-47.884	8	34	272	0.47	Leite *et al.*, 2015
02		-15.7186	-48.0138	13	36	468	0.60	Schneider *et al.*, 2011
03		-14.4367	-48.5814	17	7	119	0.45	Mazzoni *et al.*, 2010
04		-11.888	-52.2246	13	8	104	0.17	Carmo 2013
06		-15.58	-52.3067	71	10	710	0.45	Melo *et al.*, 2004
08		-15.934	-56.0369	39	8	312	0.40	Corrêa *et al.*, 2011
		-21.2666	-44.059	32	9	288	0.45	Gandini *et al.*, 2012
21		-20.6853	-53.5437	7	21	147	0.68	Silva *et al.*, 2017
22		-22.0035	-53.8057	7	7	49	0.59	Brandão-Gonçalves *et al.*, 2010
26		-12.1667	-47.75	20	9	180	0.59	Pereira 2010
27		-9.43333	-50.1667	37	9	333	0.61	Pereira 2010
28		-11.7833	-48.6167	50	9	450	0.81	Pereira *et al.*, 2007
31		-21.3003	-56.4355	9	6	54	0.30	Romero 2011
39		-15.1032	-49.4467	6	10	60	0.32	Mello 2019
40		-18.6031	-51.953	6	9	54	0.69	Aloisio 2006
41		-13.1558	-49.1653	28	8	224	0.34	Sales 2015
49		-20.685	-56.7783	9	9	81	0.25	Fuentes 2011
	Atlantic Forest							
06 and 36		-23.3965	-51.8506	9	7	63	0.76	Silva 2013
07		-19.0248	-40.2295	6	13	78	0.27	Machado 2017
09		-18.2261	-40.0756	5	18	90	0.44	Nascimento 2019
19		-25.1621	-53.8295	9	16	144	0.61	Baldasso *et al.*, 2019
13		-23.8382	-54.349	13	5	65	0.57	Lopes *et al.*, 2016
14		-23.256	-46.9613	22	12	264	0.66	Rolla *et al.*, 2009

15	-25.365	-48.8321	15	8	120	0.58	Wolff *et al.*, 2013
16	-25.5461	-53.2977	18	12	216	0.44	Delariva *et al.*, 2013
17	-23.5365	-51.7831	14	32	448	0.47	Silva *et al.*, 2012
18	-25.08	-53.6242	7	20	140	0.43	Neves *et al.*, 2015
19	-22.5984	-52.2459	11	27	297	0.27	Casatti, 2002
23	-22.8022	-45.4489	10	12	120	0.54	Andrade 2004
24	-22.5156	-47.6706	17	17	289	0.53	Rondineli 2007
25	-23.3234	-51.8903	15	21	315	0.46	Bonato *et al.*, 2012
29	-23.3197	-51.1964	7	34	238	0.53	Oliveira & Bennemann, 2005
30	-23.6374	-45.8131	15	10	150	0.55	Esteves *et al.*, 2008
33	-28.7068	-52.8734	11	58	638	0.40	(Bonato and others 2017)
35	-24.4166	-47.25	20	16	320	0.43	Gonçalves *et al.*, 2018
10	-23.3643	-52.0189	6	8	48	0.63	Silva 2013
37	-20.7951	-51.5146	31	27	837	0.26	Luiz *et al.*, 1998
38	-18.1388	-40.0213	7	30	210	0.31	Silva 2019
42	-20.75	-49.3333	12	29	348	0.37	Rocha *et al.*, 2009
44	-23.7333	-45.85	14	12	168	0.58	Esteves & Lobon-Cervia, 2001
46	-23.5334	-52.0185	16	22	352	0.40	Garcia 2019
47	-23.3847	-51.947	15	6	90	0.86	Mise 2012
48	-20.5761	-47.785	9	11	99	0.38	Brambilla *et al.*, 2019

Table S2. Results of null model analyzes performed with the Bascompte algorithm. P-value describes the probability of obtaining a network structure equal to or more extreme than the empirical structure under the null model. If the empirical values were outside the 0.05 to 0.95 quantile range of the null distribution, they were considered to deviate strongly from the randomized trophic networks. Empirical values below or equal to 0.05 are indicated with bold numbers.

ID	**Nestedness ($NODF_{zscore}$)**	**Modularity (Q_{zscore})**	**Number of links**	**Link density**
01	**0.00**	0.91	1.773227	**0.000999**
02	**0.00**	0.64	1.746254	**0.000999**
03	0.20	**0.00**	0.110889	**0.000999**
04	0.32	0.78	0.155844	**0.012987**
05	0.34	0.56	0.398601	**0.006993**
06	**0.01**	0.06	0.120879	**0.000999**
07	**0.05**	0.35	1.814186	**0.000999**
08	**0.00**	0.75	0.124875	**0.000999**
09	0.10	0.54	1.652348	0.452547
10	**0.00**	**0.00**	1.66034	**0.000999**
11	0.15	0.63	1.729271	0.100899

12	**0.00**	**0.00**	0.072927	**0.000999**
13	0.33	0.82	0.180819	0.081918
14	**0.00**	0.25	0.158841	**0.000999**
15	0.96	**0.01**	0.221778	0.202797
16	0.57	**0.02**	0.112887	**0.02997**
17	**0.00**	**0.01**	1.766234	**0.000999**
18	0.09	**0.02**	1.839161	0.615385
19	**0.00**	**0.00**	1.979021	**0.006993**
20	0.06	0.41	0.107892	**0.000999**
21	**0.00**	0.04	1.675325	0.090909
22	**0.01**	0.19	0.413586	0.747253
23	**0.00**	0.97	1.856144	0.02997
24	**0.00**	**0.02**	0.160839	**0.000999**
25	**0.00**	0.71	1.893107	**0.000999**
26	0.87	**0.00**	0.174825	**0.000999**
27	0.12	**0.02**	0.14985	**0.000999**
28	0.70	0.39	0.176823	**0.000999**
29	**0.00**	0.59	1.703297	**0.000999**
30	**0.00**	0.67	0.134865	**0.000999**
31	0.38	0.20	0.146853	0.739261
32	0.25	**0.00**	0.115884	**0.000999**
33	**0.00**	**0.03**	1.802198	**0.000999**
34	**0.04**	0.72	0.156843	**0.000999**
35	**0.00**	**0.00**	0.135864	**0.000999**
36	**0.00**	0.40	1.658342	0.304695
37	**0.00**	**0.01**	**0.032967**	**0.000999**
38	**0.00**	**0.02**	2	**0.000999**
39	**0.01**	0.95	1.835165	**0.025974**
40	**0.03**	0.31	1.523477	0.071928
41	0.66	0.43	0.140859	**0.000999**
42	**0.00**	0.36	1.97003	**0.000999**
43	**0.00**	**0.00**	0.128871	**0.000999**
44	0.06	0.08	0.194805	**0.000999**
45	**0.00**	0.38	0.093906	**0.000999**
46	**0.00**	**0.03**	1.999001	**0.000999**
47	**0.05**	0.37	0.362637	**0.000999**
48	**0.00**	0.07	1.785215	**0.000999**
49	**0.00**	0.15	**0.001998**	**0.000999**

* Metric logarithm did not calculate modules. This can happen on very small networks.

Table S3. Classes of anthropic impact on land use in a 500-meter buffer on each local network. P (pasture); APC (annual and perennial culture); SPC (semi perennial culture); MAP (Mosaic of cropland and pasture); UI (urban infrastructure); ANVA (another non-vegetated area); PPF (percent planted forest); M (mining).

ID	P	APC	SPC	MAP	UI	ANVA	PPF	M
01	0	0	0	0	0	0	0	0
02	0	0	0	0	0	0	0	0
03	43.9483	0	0	0	0	0	0	0
04	0	0	0	0	0	0	0	0
05	0	82.31511	0	7.181136	0	0	0	0
06	80.66667	0	0	0	0	0	0	0
07	0	0	0	7.075472	0	0	0	0
08	3.912543	0	0	0	0	0	0	0
09	66.89655	0	0	20.45977	0	0	0	0
10	4.282407	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0
13	58.60963	8.128342	0	25.5615	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
16	15.20165	0	0	2.895553	0	0	0	0
17	9.195402	65.4023	0	23.21839	0	0	0	0
18	12.73533	9.634551	0	21.70543	0	0	0	0
19	0	0	0	0	0	0	0	0
20	70.72626	0	0	4.022346	0	0	18.10056	0
21	27.73019	0	0	0	0	0	0	0
22	99.5785	0	0	0.421496	0	0	0	0
23	26.44444	0	0	28.11111	0	0	0.222222	0
24	9.237875	1.732102	65.35797	23.67206	0	0	0	0
25	25.73363	60.72235	0	12.86682	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	4.508671	0	0
29	5.995717	6.102784	0	41.64882	39.93576	0	0	0
30	0	0	0	0	0	0	0	0
31	51.08324	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0.913242	67.57991	0	6.621005	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0.222222	0	0	0	0
36	5.989305	52.62032	0	29.94652	0	0	0	0
37	69.95662	0	0	13.88286	0	0	0	0
38	1.194743	78.01673	0	11.23059	0	0	4.181601	0
39	38.8009	3.054299	9.728507	0	0	0	0	0
40	38.27581	5.658085	0	0	0	1.603977	0	0

41	63.90728	0	0	0	0	0	0	0
42	90.90909	0	0	7.134638	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0
45	0.493093	0	0	0	0	0	0	0
46	0.446429	71.79236	0	3.106803	0	0	0	0
47	6.417756	56.34418	2.125261	12.90419	14.67073	0.054549	0.089954	0
48	3.026906	5.381166	47.98206	0	0	0	0	0
49	21.71123	0	0	0	0	0	0	0

Table S4. Test of spatial correlation in the residuals of the linear models that evaluated the influence of Anthropogenic land use on various network descriptors. Shown are the observed and expected Global Moran´s *I* values, and associated *P*-values.

	Specialization (H_2’)	**Nestedness ($NODF_{zscore}$)**	**Modularity (Q_{zscore})**	**Number of links**	**Link density**	**Number of species**
Observed	-0.1004	-0.1004	-0.0105	-0,0792	-0,0556	-0,0520
Expected	-0.0212	-0.0212	-0.0212	-0,0212	-0,0222	-0,0212
P-value	0.1253	0.1253	0.5344	0,0693	0,0961	0,5485

* Significantly negative value indicates negative spatial autocorrelation, in which neighboring values are dissimilar and, therefore, spatial autocorrelation did not impact the analysis.

Table S5. Percentage of the number of species that preferentially feed on terrestrial and aquatic insects, debris, plant material, algae and fish for each local network.

ID	**Omnivores**	**Invertivores**	**Detritivores**	**Herbivores**	**Algivores**	**Piscivores**
01	0.50	0.13	0.25	0.00	0.00	0.13
02	0.23	0.46	0.15	0.08	0.00	0.00
03	0.18	0.53	0.29	0.00	0.00	0.00
04	0.00	0.08	0.15	0.00	0.08	0.54
05	0.00	0.78	0.22	0.00	0.00	0.00
06	0.07	0.45	0.20	0.18	0.04	0.04
07	0.00	1.00	0.00	0.00	0.00	0.00
08	0.10	0.33	0.18	0.08	0.00	0.31
09	0.00	0.20	0.00	0.20	0.60	0.00
10	0.06	0.38	0.19	0.13	0.00	0.25
11	0.00	0.78	0.22	0.00	0.00	0.00
12	0.07	0.33	0.15	0.21	0.07	0.17
13	0.15	0.31	0.38	0.08	0.08	0.00
14	0.36	0.45	0.14	0.05	0.00	0.00

15	0.00	0.53	0.33	0.07	0.00	0.07
16	0.06	0.39	0.06	0.28	0.00	0.22
17	0.07	0.50	0.29	0.14	0.00	0.00
18	0.29	0.57	0.14	0.00	0.00	0.00
19	0.00	1.00	0.00	0.00	0.00	0.00
20	0.19	0.13	0.23	0.35	0.00	0.10
21	0.00	0.57	0.00	0.43	0.00	0.00
22	0.43	0.00	0.29	0.14	0.14	0.00
23	0.00	0.70	0.10	0.10	0.00	0.10
24	0.06	0.82	0.06	0.00	0.00	0.06
25	0.18	0.71	0.06	0.00	0.00	0.06
26	0.00	0.70	0.10	0.10	0.05	0.05
27	0.14	0.68	0.00	0.03	0.14	0.03
28	0.10	0.68	0.16	0.00	0.00	0.06
29	0.57	0.00	0.29	0.00	0.14	0.00
30	0.13	0.47	0.40	0.00	0.00	0.00
31	0.00	0.67	0.22	0.00	0.11	0.00
32	0.09	0.35	0.03	0.23	0.05	0.26
33	0.27	0.45	0.00	0.09	0.00	0.18
34	0.05	0.90	0.00	0.05	0.00	0.00
35	0.05	0.70	0.25	0.00	0.00	0.00
36	0.00	0.33	0.33	0.33	0.00	0.00
37	0.10	0.29	0.35	0.10	0.03	0.13
38	0.00	0.71	0.00	0.00	0.29	0.00
39	0.00	0.00	0.33	0.33	0.00	0.17
40	0.00	0.50	0.00	0.17	0.00	0.33
41	0.00	0.64	0.29	0.07	0.00	0.00
42	0.17	0.58	0.17	0.00	0.00	0.00
43	0.24	0.27	0.21	0.15	0.03	0.25
44	0.14	0.36	0.43	0.00	0.00	0.07
45	0.39	0.39	0.00	0.17	0.00	0.00
46	0.00	0.50	0.37	0.12	0.00	0.00
47	0.00	0.86	0.06	0.06	0.00	0.00
48	0.22	0.33	0.33	0.11	0.11	0.00
49	0.00	0.66	0.22	0.00	0.11	0.00
Mean	**0.11**	**0.49**	**0.17**	**0.09**	**0.04**	**0.07**

Table S6. Network descriptors used in the study. Reference consulted: May 1973, Tilman 1996; Dunne et al., 2002; May 1973, Stouffer & Bascompte 2011; Almeida-Neto, et al., 2008.

ID	Nestedness (*NODF)*	Specialization (H_2')	Modularity (*Q*)	Number of links	Link density	Number of species
01	57.87	0.33	0.20	3.02	5.92	34
02	48.02	0.46	0.16	5.69	6.02	36
03	17.19	0.54	0.36	2.25	5.58	7
04	1.89	0.99	0.65	0.86	2.36	8
05	38.04	0.53	0.17	3.00	3.79	7
06	18.27	0.66	0.18	3.93	11.14	10
07	18.05	0.84	0.55	1.11	3.37	13
08	12.84	0.73	0.23	2.68	7.00	8
09	27.95	0.84	0.40	1.65	2.11	15
10	29.17	0.66	0.30	3.26	4.66	19
11	33.76	0.51	0.14	3.52	4.29	16
12	13.51	0.66	0.27	3.27	11.78	11
13	20.64	0.67	0.27	2.06	3.92	5
14	47.33	0.44	0.20	5.15	7.69	12
15	27.70	0.74	0.20	3.04	3.49	8
16	20.35	0.64	0.23	3.17	4.19	12
17	40.42	0.58	0.18	4.61	4.85	32
18	26.97	0.64	0.30	2.22	3.34	20
19	20.25	0.56	0.40	2.11	4.57	27
20	18.09	0.65	0.20	3.17	6.79	9
21	42.99	0.41	0.13	3.57	4.92	21
22	10.83	0.46	0.24	2.07	3.57	7
23	40.09	0.49	0.19	2.95	4.38	12
24	34.80	0.39	0.17	4.53	6.98	17
25	35.67	0.51	0.21	4.06	5.49	21
26	27.77	0.59	0.17	3.69	5.98	9
27	32.30	0.60	0.16	4.41	8.73	9
28	34.76	0.37	0.11	6.20	16.30	9
29	47.89	0.46	0.25	2.94	4.72	28
30	35.34	0.44	0.21	3.28	5.97	10
31	6.86	0.85	0.49	1.07	2.23	6
32	19.26	0.72	0.24	3.95	9.81	11
33	48.75	0.44	0.22	3.72	6.49	58
34	11.27	0.45	0.22	1.69	8.83	5
35	38.74	0.82	0.42	3.78	7.01	16
36	56.94	0.61	0.19	2.14	2.83	8
37	29.33	0.63	0.25	3.72	6.86	27
38	29.63	0.71	0.37	1.78	2.79	30
39	24.44	0.79	0.54	1.19	2.45	10
40	44.34	0.66	0.20	2.47	2.69	9
41	18.02	0.83	0.29	2.14	5.80	8
42	38.50	0.37	0.24	3.17	7.36	29
43	16.71	0.65	0.22	3.21	17.90	7
44	33.07	0.60	0.20	3.73	4.43	12

45	41.29	0.40	0.16	5.67	9.54	17
46	29.16	0.71	0.22	3.68	3.82	22
47	41.08	0.46	0.17	3.67	5.40	6
48	46.89	0.76	0.29	1.90	2.90	11
49	13.02	0.86	0.52	1.06	2.24	8

Table S7. Pearson’s correlation among the network descriptors

	Nestedness ($NODF_{zscore}$)	**Modularity (Q_{zscore})**	**Specialiation (H_2’)**	**Number of Links**	**Number of species**	**Link density**
Nestedness ($NODF_{zscore}$)	1.0	-0.11	0.03	0.16	-0.40**	-0.21
Modularity (Q_{zscore})		1.0	0.10	-0.25	0.38**	0.08
Specialiation (H_2’)			1.0	-0.23	0.05	-0.27
Number of Links				1.0	-0.50***	-0.09
Number of species					1.0	0.50***
Link density						1.0

*** $P < 0.001$, **$P < 0.01$, * $P < 0.05$

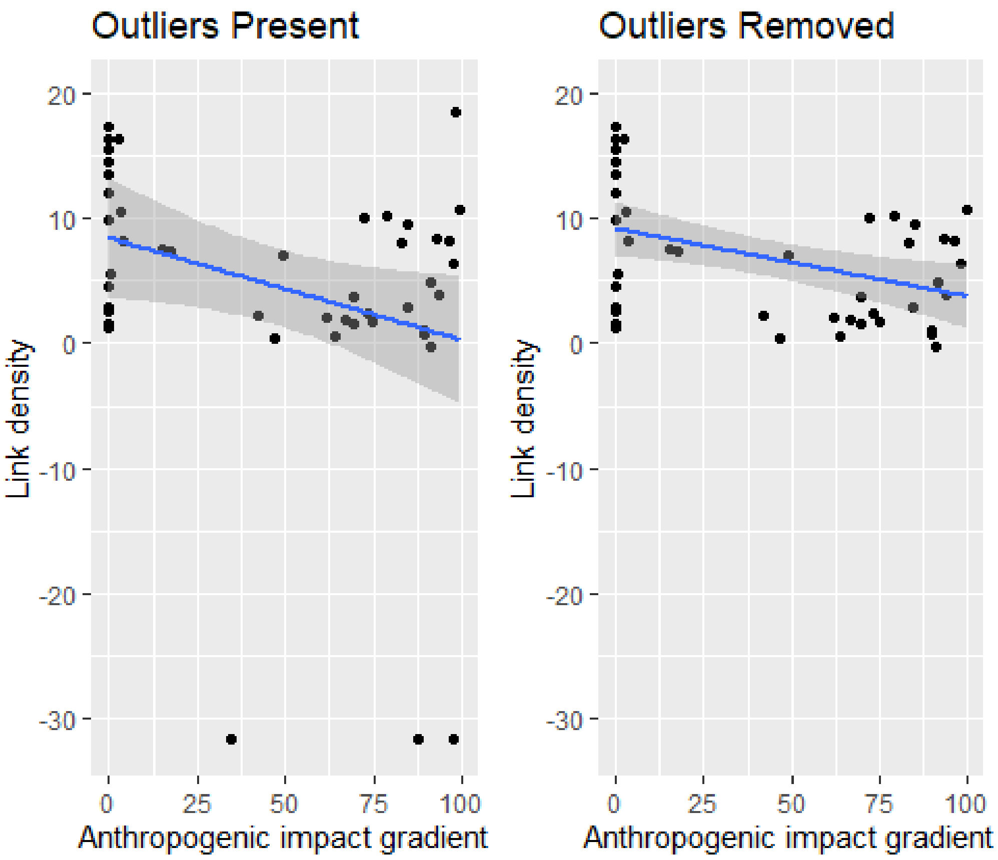

Figure S1. Model correction with removal of outliers with cook's distance greater than 1. Models with outliers present and removed.

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CONSIDERAÇÕES FINAIS

Os resultados desta tese confirmam que riachos do cerrado são ambientes com alta heterogeneidade ambiental, e altamente dependentes da interação riacho e mata ripária. Verificamos que essa interação reflete nos processos ecossistêmicos como a decomposição de detritos foliares e na estrutura das redes tróficas. Os resultados do **capítulo I**, demonstraram que a presença de estruturas vegetais como troncos e galhos, e o aumento do fluxo são importantes para a dinâmica de detritos foliares, regulando a retenção e a decomposição das folhas que caem no leito dos riachos. Reforçamos que a decomposição dos detritos foliares em riachos tropicais tem sua dinâmica diretamente relacionada à velocidade da água. A interação entre a heterogeneidade do habitat e o tamanho da folha pode ser necessária para criar manchas de detritos que fornecem recursos e habitat para as comunidades aquáticas. Sugerimos que medidas para restaurar as funções ecológicas dos riachos devem considerar a heterogeneidade ambiental e a abrasão física do fluxo da água como direcionadores do funcionamento desses ecossistemas.

O **capítulo 2** trouxe um debate sobre a importância de se considerar a estrutura do habitat local e das características da bacia a montante dos riachos como principal direcionador da disponibilidade de carbono e nitrogênio para os riachos, o que interfere na assimilação desses elementos na dieta das comunidades aquáticas. Localmente, as fontes de energia autóctone sustentaram os insetos aquáticos em riachos de cabeceira. Variáveis locais como temperatura e vazão são parâmetros importantes para a variações nos valores de $\delta^{13}C$ da cadeia alimentar. Enquanto as concentrações de nitrato nos riachos e a distância da nascente foram os parâmetros importantes para o $\delta^{15}N$ da cadeia alimentar. Além disso, os impactos antrópicos nas bacias de drenagem dos riachos é um forte impulsionador do comprimento das cadeias alimentares aquáticas. Portanto, nessa tese, os resultados mostraram os efeitos negativos do gradiente de substituição da mata ripária por práticas de agricultura e pecuária sobre as teias tróficas aquáticas em riachos do Cerrado.

O **capítulo 3** apresentou como os estudos de metadados permitem encontrar padrões em larga escala, onde destacamos que os impactos antrópicos nas bacias de drenagem afeta a estrutura das redes tróficas, resultando em redes menos complexas e modulares. Métricas de redes tróficas demonstraram os efeitos dos diferentes usos do solo na estrutura trófica de peixes em riachos tropicais. Os efeitos negativos do uso do solo sobre métricas de redes como a modularidade, destaca a simplificação de módulos da rede, no qual é formado por espécies com dieta altamente especialista. Isso sugere uma redução na diversidade de itens consumidos e um aumento de novos recursos amplamente distribuídos na rede trófica, com predomínio de espécies generalistas. A partir desses resultados, destacamos a importância de compreender os mecanismos pelos quais as atividades antrópicas afetam os ecossistemas de água doce e ajuda a informar as políticas de uso da terra e estratégias de gestão destinadas a mitigar os efeitos do uso da terra sobre a biodiversidade.