Universidade de Brasília
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Ecologia

ORGANIZAÇÃO E DINÂMICA DE COMUNIDADES AQUÁTICAS EM RIACHOS NO CERRADO

Gustavo Figueiredo Marques Leite

Orientadora: Maria João Feio

Brasília – DF
Maio de 2018

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Termo de Aprovação

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CAPES

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Apoio:

MARE

Este trabalho é dedicado a minha
esposa Camila e minha filha Alice.

“Vencer é nunca desistir.”
(Albert Einstein)

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RESUMO GERAL

Riachos representam a maior parte da extensão linear dos cursos d'água em uma bacia hidrográfica e são caracterizados por elevada heterogeneidade de habitats, sobretudo pela sua intensa relação com o meio terrestre. No bioma Cerrado, os riachos são caracterizados por águas levemente ácidas e pobres em nutrientes, mas apresentam elevada diversidade biológica. Apesar disso, esses ecossistemas estão entre os mais ameaçados do mundo devido sua intensa ocupação e fragmentação. Portanto, avaliar os fatores estruturadores das comunidades nesses ambientes, bem como produzir potenciais ferramentas de avaliação ecológica para o Cerrado tornam-se tarefas fundamentais para sua manutenção. Nesse sentido, o objetivo central do presente estudo foi investigar a relação entre variações ambientais e a organização e dinâmica de comunidades aquáticas em 28 riachos de cabeceira no Cerrado e desenvolver um índice sensível a perturbações na qualidade ambiental em riachos baseado na estrutura trófica das comunidades de macroconsumidores aquáticos. Os riachos estudados apresentaram uma elevada heterogeneidade de habitats e uma complexa estrutura biótica. Fatores ambientais locais foram os principais responsáveis pela estrutura e composição das assembleias de macroinvertebrados bentônicos e sua diversidade apresentou-se diretamente relacionada a heterogeneidade de habitats amostrados. Estes resultados evidenciaram a necessidade de se considerar grandes escalas espaciais para o estabelecimento de políticas públicas ambientais uma vez que as características ambientais em larga escala e o uso da terra resultam em diferenças na diversidade da fauna aquática. Ainda, resultados isotópicos mostraram que maior parte dos organismos nos riachos são sustentados pela energia autóctone, mas que diferenças na estrutura e produtividade dos habitats podem afetar significativamente a dieta das espécies, as quais podem inclusive apresentar plasticidade trófica em função da disponibilidade de recursos. A metodologia isotópica mostrou-se ainda útil como ferramenta de avaliação de impactos ambientais sobre as comunidades aquáticas de riachos. Por fim, foi proposto um Índice Isotópico Multimétrico (ISI) como uma ferramenta para a avaliação de distúrbios antrópicos. Os resultados obtidos a partir do ISI em um estudo ao longo do gradiente de perturbação mostrou que este pode ser uma ferramenta mais sensível em detectar mudanças ambientais que metodologias tradicionais de avaliações de impacto ambiental, podendo ser uma alternativa ou complementar em avaliações da qualidade ecológica dos ecossistemas. Esse estudo apresentou resultados novos para o Cerrado, evidenciando que esse bioma ainda tem recebido pouca atenção. Nesse sentido, esse estudo permitiu o aprofundamento acerca da estrutura e composição biótica dos riachos do Cerrado, das relações entre os diferentes componentes da cadeia trófica, bem como dos potenciais efeitos da perturbação antrópica sobre o funcionamento desse ecossistema.

Palavras-chave: riachos de cabeceira, escalas, gradiente ambiental, isótopos estáveis, cadeias tróficas, avaliação ecológica, funcionamento dos ecossistemas, sistemas lóticos

GENERAL ABSTRACT

Streams represent most of the linear extent of watercourses in a river basin and are characterized by high heterogeneity of habitats, especially by their intense relationship with the terrestrial environment. In the Brazilian savannah biome, streams are slightly acidic and nutrient poor waters, but they present high biological diversity. Despite this, these ecosystems are among the most threatened in the world due to their intense human occupation and fragmentation. Therefore, to assess the structuring factors of the biota, as well as producing potential ecological assessment tools for the biome are fundamental tasks for its maintenance. In this sense, the main objectives of the present study was to investigate the relationship between environmental changes and the dynamics of aquatic communities in 28 headwaters in the Brazilian savannah, and to develop a sensitive index to disturbances in streams based on the aquatic food webs. The studied streams showed a high heterogeneity of habitats and a complex biotic structure. Local environmental factors were mainly responsible for the structure and composition of benthic macroinvertebrates assemblages and their diversity was directly related to the heterogeneity of sampled habitats. These results evidenced the need to consider large spatial scales for the establishment of environmental public policies since environmental characteristics in larger scales and land use may result in differences in the diversity of aquatic fauna. Still, isotopic results showed that most of the organisms in the streams are supported by autochthonous energy, but that differences in the structure and productivity of the habitats can significantly affect the diet of the species, which can even present trophic plasticity due to the availability of resources. The isotopic methodology was still useful as a tool to evaluate environmental impacts on the aquatic communities of streams. Finally, an ISotopic multimetric Index (ISI) was proposed as a tool for the evaluation of anthropogenic disturbances. The results obtained along a disturbance gradient showed that ISI may be a more sensitive tool in detecting environmental changes than traditional methodologies of environmental impact assessments, and may be an alternative or complementary evaluation of the ecological quality of the ecosystems. This study presented new results for the Brazilian savannah, evidencing that this biome has received little attention. In this sense, this study allowed the deepening of the structure and biotic composition of the Brazilian savannah streams, the relationships between the different components of the food web, as well as the potential effects of anthropogenic disturbances on the functioning of this ecosystem.

Key words: headwater streams, scales, environmental gradient, stable isotopes, food webs, ecological assessment, ecosystem functioning, lotic ecosystems

INTRODUÇÃO GERAL

As bacias hidrográficas são formadas por diversos corpos hídricos, dentre eles os riachos, que representam cerca de 80% da extensão total dos cursos d'água de uma bacia hidrográfica (Benda et al., 2005). Riachos são ambientes heterogêneos moldados por processo ecológicos e hidrológicos que atuam no tempo e espaço (Legendre et al., 2010), e em diferentes escalas (Ricklefs, 2008; Simberloff, 2004). Esses processos contribuem diretamente para mudanças na morfologia do canal e na formação de um mosaico de habitats (Armitage et al., 1995; Gasith and Resh, 1999). Nesse contexto, habitats podem ser definidos como manchas influenciadas por condições ambientais similares e com uniformidade física (Vannote et al., 1980), ou como um modelo, que é significativo para os padrões de espécies e processos ecológicos para uma dada escala espacial e temporal (Southwood, 1977).

A heterogeneidade dos habitats tem sido frequentemente associada positivamente à diversidade de espécies (Schneider, 2001), pois habitats estruturalmente complexos fornecem mais nichos e formas de explorar os recursos disponíveis (Tews et al., 2004). Esta hipótese também é muitas vezes ampliada para explicar os padrões de diversidade na escala da paisagem, onde paisagens com uma variedade de tipos de habitat devem suportar mais espécies do que aquelas que consistem em um único habitat homogêneo (Būhning-Gaese, 1997; Tews et al., 2004). Nesse sentido, descrever e entender os padrões de diversidade de espécies diante da heterogeneidade de habitats e diferentes escalas tem norteado questões fundamentais no campo da ecologia, biogeografia e evolução e tem sido um objetivo central do estudo da biodiversidade (Al-Shami et al., 2013; Heino et al., 2015; Sutherland et al., 2013).

A importância de se entender os padrões de diversidade tem aumentado ainda mais devido as elevadas taxas de mudança de uso do solo, perda de habitat, invasão de espécies, eutrofização e mudanças climáticas (Callisto et al., 2012). A utilização dos ecossistemas naturais por populações humanas tem sido a principal causa de perda de espécies (Vitousek, 1997). Tais atividades incluem o uso das áreas ripárias, promovendo a alteração de processo físico-químicos e estruturais nos ecossistemas aquáticos, bem como o aumento da demanda de água para usos domésticos e agropecuários (Saunders et al., 2002). Os cursos d'água têm sido ainda submetidos à liberação de poluentes, alterações físicas de hábitat e mudanças em aspectos hidrológicos básicos que exercem uma profunda e quase sempre negativa influência nas comunidades aquáticas. Em riachos, são esperadas que essas alterações sejam ainda mais negativas pois são ecossistemas menores e intimamente ligados às zonas ripárias pelo fluxo de materiais e movimento dos organismos (Baxter et al., 2005).

Riachos são ecossistemas onde a vegetação ripária exerce um papel fundamental no suprimento energético do biota, visto que a copa das árvores encobre o curso d'água e limita a produção de energia autóctone (Vörösmarty et al., 2010; Wallace et al., 1997). Nesses ambientes a vegetação ripária tem papel preponderante na regulação dos processo bioenergéticos sobretudo através de alterações no padrão de sombreamento, manutenção da temperatura da água e suprimento de matéria orgânica alóctone (Hill and Dimick, 2002). Portanto, processos que podem gerar a redução na densidade e/ou extensão da vegetação ripária, sejam esses antropogênicos ou naturais, afetam diretamente os processos bioenergéticos nos riachos e têm efeitos diretos na produção primária e secundária (Franken et al., 2005).

Diversos modelos conceituais tentaram estabelecer como variações ambientais podem afetar o componente biótico dos ecossistemas aquáticos. O primeiro modelo a ligar as características físicas dos cursos d'água a essas mudanças, sobretudo no metabolismo, foi o Conceito do Contínuo Fluvial (RCC; (Vannote et al., 1980). O RCC enfatiza as ligações longitudinais em um rio, onde aspectos abióticos (geomorfologia e hidrologia) são definidos como descritores do compartimento biótico (estrutura, função e relações tróficas das comunidades) no ecossistema. Segundo Vannote et al. (1980), em riachos de cabeceira (cursos d'água de 1^{a}. a 3^{a}. ordem) a produtividade primária é mantida principalmente pela entrada de detritos alóctones, uma vez que a vegetação ripária impede a entrada direta de luminosidade e, consequentemente, o crescimento significativo de algas e macrófitas que são responsáveis pela produtividade autóctone por meio da fotossíntese. Com isso, riachos de cabeceira são considerados sistemas heterotróficos ($P<R$; produtividade primária bruta menor que respiração; (Cummins et al., 1973; Fisher and Likens, 1973; Vannote et al., 1980), onde as cadeias alimentares são baseadas sobretudo no consumo do material alóctone – o detrito vegetal que cai da vegetação circundante (Graça, 2001; Wallace et al., 1997). À medida que as margens dos cursos d'água se alargam (em trechos de 4^{a}. a 6^{a}. ordem), a sombra da vegetação ripária fica restrita às margens e a produção primária autóctone passa a ser a principal fonte de energia (Vannote et al., 1980). Portanto, segundo o RCC, alterações nas propriedades físicas ao longo de um curso d'água são determinantes dos recursos alimentares dominantes que, por sua vez, influenciam a comunidade presente (Vannote et al., 1980).

Anos depois, novos resultados e teorias sobre o metabolismo dos ecossistemas (p. ex., Modelo de Produtividade Fluvial Revisado; RRPM; Thorp et al., 2002) têm contestado o RCC para regiões tropicais, sugerindo que ele subestima o papel da produção primária autóctone local e desconsidera sua importância como fonte alimentar, bem como supervaloriza a natureza recalcitrante da matéria orgânica alóctone nos ecossistemas. Os autores concluem que as cadeias tróficas de metazoários são sustentadas principalmente por fontes autóctones e que apenas micro-organismos (p.ex., bactérias heterotróficas, fungos, ciliados, rotíferos e flagelados heterotróficos) utilizam diretamente a matéria orgânica alóctone como recurso energético principal. Estudos baseados em traçadores químicos (p.ex., isótopos estáveis, ácidos graxos) têm sugerido que o recurso autóctone não é limitante para os macroconsumidores, como sugerido no RCC e, mesmo riachos densamente florestados podem sustentar complexas teias tróficas baseadas no consumo de recursos autóctones. Obviamente, esse fenômeno não é universal e a matéria orgânica alóctone vegetal ainda é o recurso alimentar que sustenta parte dos organismos observados, como algumas larvas de Coleoptera, Ephemeroptera das famílias Leptophlebiidae e Baetidae (March and Pringle, 2003) ou mesmo peixes (Bayley, 1989; March et al., 2001).

De maneira geral, os resultados divergentes entre os estudos sugerem que a complexidade no funcionamento dos ecossistemas aquáticos é superior ao previsto pelos modelos atuais. Essas divergências têm incitado a discussão sobre o paradoxo da heterotrofia para riachos de cabeceira, buscando responder "como a biomassa animal de teias tróficas fluviais pode ser alimentada pela produção autóctone autotrófica se o ecossistema como um todo é heterotrófico?" (Thorp & Delong, 2002). Apesar dos estudos indicarem uma forte associação da biota aquática à matéria orgânica autóctone, existe uma clara interdependência entre riachos e florestas ripárias em termos de fluxo de energia, onde ainda faltam dados quantitativos do movimento da energia na interface terra-água (Callisto et al., 2012). Além disso, a origem dos recursos usados pelos consumidores aquáticos em riachos de cabeceira ainda é pouco documentada (Callisto et al., 2012). A resposta para isso pode estar na maior palatabilidade e alta taxas de crescimento das algas em relação ao material alóctone (como detritos foliares), podendo tornar esse recurso suficiente para sustentar as comunidades aquáticas (Pettit et al., 2012), pelo menos durante certas estações (Brito et al., 2006). Nesse sentido, o conhecimento da importância relativa de diferentes fontes basais de energia é fundamental para a compreensão da dinâmica dos ecossistemas aquáticos (Leberfinger et al., 2011).

A análise de isótopos estáveis têm sido empregada com sucesso ao se investigar os fluxos de energia, as relações tróficas entre as espécies e identificar as principais fontes energéticas para as comunidades aquáticas (Finlay et al., 2010; Peterson and Fry, 1987; Post, 2002). Diversos elementos podem ser utilizados na metodologia isotópica para se traçar a origem dos recursos nos ecossistemas (p.ex. C, N, S, H, O; (Peterson and Fry, 1987). Contudo, os isótopos estáveis do Nitrogênio (^{15}N) e do Carbono (^{13}C) ainda são os mais utilizados em estudos de teias tróficas, devido sobretudo ao melhor entendimento acerca das taxas de fracionamento desses elementos durante a assimilação e caminhos metabólicos que o alimento percorre antes de ser incorporado em algum tecido. As razões entre isótopos estáveis de ^{13}C e ^{12}C (expressos em relação a um padrão e denominados $\delta^{13}C$) e de ^{15}N e ^{14}N (expressos em relação a um padrão e denominados $\delta^{15}N$) fornecem informações que incorporam escalas espaço-temporais e facilitam a análise de alimentos assimilados por consumidores (Post, 2002) e a definição de seus nichos tróficos (Layman et al., 2007; Newsome et al., 2007).

Como o enriquecimento do ^{13}C entre fontes de alimento e consumidores geralmente é baixo (0-1 ‰) (McCutchan et al., 2003) e os valores de $\delta^{13}C$ tipicamente diferem entre fontes basais (por exemplo, material vegetal de plantas C_3 e C_4), o $\delta^{13}C$ é usado como um indicador de fontes C para certos consumidores ao longo das cadeias alimentares (Perkins et al., 2014; Post, 2002). Por outro lado, o fraccionamento trófico de $\delta^{15}N$ geralmente varia de 2 a 4 ‰ em cada nível trófico (McCutchan et al., 2003), facilitando a definição do comprimento total da cadeia alimentar e a posição de um organismo dentro dela (Perkins et al., 2014; Vander-Zanden and Rasmussen, 1999). Dessa forma, as proporções isotópicas de $\delta^{13}C$ e $\delta^{15}N$ em tecidos anuais refletem informações sobre o uso de habitats físicos e características tróficas e atualmente são usadas para determinar a origem da matéria orgânica, relações tróficas e tamanho de nicho e sobreposição (Bearhop et al., 2004; Newsome et al., 2007). Ainda, o uso de isótopos estáveis em ambientes aquáticos tem sido considerado promissor para avaliação dos efeitos de distúrbios antropogênicos, especialmente ao nível de ecossistemas, uma vez que também provaram ser sensíveis às mudanças ambientais, seja para rastrear mudanças na rede alimentar ao longo de um gradiente ambiental natural, ou para distinguir e quantificar as consequências ecológicas de distúrbios induzidos pelo ser humano (Costanzo et al., 2001; Cucherousset et al., 2012; Gratton and Denno, 2006; Hadwen and Arthington, 2007; Hadwen et al., 2010; Schlacher et al., 2005).

Diversos estudos têm utilizado os valores isotópicos obtidos do habitat e de consumidores como medidas funcionais para a avaliação da saúde dos ecossistemas. Os isótopos estáveis têm sido utilizados como indicadores funcionais da degradação da vegetação ripária (Bunn et al., 1999), impactos de esgoto (di Lascio et al., 2013), uso de terras agrícolas, deposição de nitrogênio antropogênico (Holtgrieve et al., 2011), efeitos das águas residuais (Morrissey et al., 2013), enriquecimento de nutrientes (Bergfur et al., 2009), entre outros. Esses estudos tem como premissa que habitats e consumidores em ecossistemas perturbados terão assinaturas isotópicas distintas de seus homólogos em ambientes preservados, podendo fornecer inclusive um aviso prévio de potenciais problemas de perturbação. No entanto, muitos estudos sobre isótopos estáveis demonstraram uma sobreposição elevada entre as fontes de alimento basais e os consumidores, obscurecendo o efeito claro da perturbação nos consumidores e seus habitats (Bergfur et al., 2009). Nesse sentido, muitas vezes a utilização de isótopos estáveis tem sido combinada em métricas (Layman et al., 2007), as quais se propõem a quantificar de maneira simplificada a estrutura da comunidade como um todo. Essas métricas fornecem medidas da posição relativa e do espaçamento de indivíduos / espécies em um plano a partir de valores isotópicos combinados.

Em geral, as métricas isotópicas de toda a comunidade são ferramentas úteis para avaliar os impactos dos distúrbios antropogênicos, que podem ser usados para fornecer informações sobre a estrutura global da rede alimentar em redes alimentares extremamente reticuladas, além das descrições qualitativas da posição em biótopos de isótopos e fornecem um meio para comparações básicas entre redes alimentares (Layman et al., 2012). No entanto, seu uso muitas vezes requer fontes adicionais de dados para interpretar padrões, pois os padrões observados podem ser função da variação basal e não refletem verdadeiras diferenças tróficas entre os consumidores. Além disso, pode levar a padrões enganosos quando as assinaturas do pool de fontes estão sobrepostas (Layman et al., 2012). Nesse sentido, os valores isotópicos muitas vezes tem sido associados a outras ferramentas analíticas (e.g., ácidos graxos) ou métodos estatísticos para se evidenciar melhor as mudanças na estrutura das comunidades ao longo dos gradientes ambientais, sejam esses naturais ou de perturbação. De toda forma, diversos estudos evidenciam que análises de isótopos estáveis são uma ferramenta relevante para o biomonitoramento e sensível para avaliação dos efeitos de mudança no uso do solo na dinâmica e funcionamento de ecossistemas aquáticos (di Lascio et al., 2013; Parreira de Castro et al., 2016).

Objetivos gerais

Investigar a relação entre variações ambientais e a organização e dinâmica de comunidades aquáticas em 28 riachos de cabeceira no Cerrado e desenvolver um índice sensível a perturbações na qualidade ambiental em riachos baseado na estrutura trófica das comunidades de macroconsumidores aquáticos.

Objetivos específicos

I. Avaliar como a diversidade das assembleias de macroinvertebrados bentônicos está associada a diferenças na estrutura dos habitats em diferentes escalas espaciais em riachos de cabeceira do Cerrado;

II. Quantificar a importância dos recursos autóctones e alóctones para macroinvertebrados bentônicos e peixes em riachos de cabeceira do Cerrado;

III. Identificar padrões de dieta de macroinvertebrados e peixes em riachos do Cerrado;

IV. Identificar variações na rede alimentar ao longo dos gradientes da estrutura do habitat físico (cobertura do dossel), produtividade (disponibilidade de recursos) e qualidade dos recursos nos riachos do Cerrado.

V. Desenvolver e testar um índice multimetrico com base em valores de isótopos estáveis e métricas de toda a comunidade para riachos de cabeceira do Cerrado

VI. Testar a adequação das métricas isotópicas e do índice isotópico como ferramentas de biomonitoramento para riachos de cabeceira.

O CERRADO

O Cerrado é o segundo maior bioma da América Latina, com uma área de aproximadamente 204 milhões de hectares, o que equivale a cerca de 25% do território brasileiro (Fig. 1). O Cerrado é considerado um complexo vegetacional constituído por um mosaico de formações florestais, savânicas e campestres subdivididas em diferentes fitofisionomias (Ribeiro and Walter, 2008). Dentre as formações florestais do Cerrado destacam-se as Matas Ciliares e Matas de Galeria, também definidas como a vegetação ripária que acompanha os cursos d’água (Ribeiro and Walter, 2001). A vegetação ripária cobre cerca de 5% da área do Cerrado e contém aproximadamente 32% da biodiversidade de plantas registrada para o bioma (aproximadamente 100 espécies por hectare)(Felfili and Felfili, 2001; Ribeiro and Walter, 2001; Silva-Júnior, 2005).

Figura 1. Área de abrangência do Cerrado brasileiro.

O clima no Cerrado é definido como tropical sazonal ou tropical de savana (Aw - Köppen- Geiger), com estacionalidade do regime de chuvas e duas estações bem definidas: uma quente e chuvosa (de outubro a abril) e outra mais fria e seca (de maio a setembro). A pluviosidade anual no Cerrado fica entre 1.500 e 1.900 mm. As temperaturas máximas alcançam 25 ºC nos períodos chuvosos (precipitação média de 197.5 mm ±60.2 DP; temperatura média 22.1 ºC ±1.1 DP) e mínima de 19ºC nos períodos secos (2.5 mm ±3.5 DP; 20.3 ºC ±0.6 DP). Em setembro inicia-se o período de chuvas que permanece até meados de abril, sendo os maiores índices pluviométricos observados durante os meses de dezembro a março. Os meses de junho e julho são marcados pelo início de um intenso período de déficit hídrico que permeia até o início de setembro. As taxas de precipitação diminuem cerca de 86% entre os meses de maio e junho. A vegetação ripária é considerada perenifólia, quase não ocorrendo caducifolia no período da seca. Contudo, a comunidade arbórea na zona ripária apresenta eventos reprodutivos bem distribuídos ao longo do ano (Gouveia and Felfili, 1998), promovendo a queda de folhas durante todo o ano (média mensal de ~26 $g.m^{-2}$), com aumento no fim da estação seca (Bambi et al., 2016; Tonin et al., 2017).

Além da riqueza de espécies e diversidade genética, a vegetação ripária é especialmente importante para a estabilização das margens dos cursos d'agua, proteção das nascentes, controle da erosão do solo, funcionando como zona tampão, filtro de sedimentos, substâncias químicas e nutrientes (Gouveia and Felfili, 1998; Lowrance et al., 1997; Naiman and Décamps, 1997; Ribeiro and Walter, 2001). Em virtude de suas características e importância, a legislação ambiental Brasileira considera a vegetação ripária como área de preservação permanente. Apesar disso, mudanças extensivas no uso da terra, sobretudo em decorrência da agricultura, pecuária e retirada de madeira têm levado a intensa degradação dessa vegetação (Sano et al., 2008), com efeitos diretos na química da água dos riachos no Cerrado (Silva-Junior and Moulton, 2011). Entre 2002 e 2011 as taxas de desmatamento no Cerrado foram de 1% ao ano, sendo 2,5 vezes maior que na Amazônia.

Em linhas gerais, os solos associados as zonas ripárias são ácidos, apresentando alta saturação de alumínio e teores de nutrientes essenciais se comparados as outras formações do Cerrado (Ribeiro and Walter, 2001, 2008). A maior parte das espécies de plantas tropicais, sobretudo as encontradas no bioma Cerrado, têm folhas com baixas concentrações de nutrientes, cutículas grossas e elevadas concentrações de compostos estruturais e inibitórios (Graça and Cressa, 2010; Marques et al., 2000; Wantzen and Wagner, 2006), sendo considerado um detrito de baixa qualidade nutricional para os consumidores. Contudo, a vegetação ripária apresenta características claramente diferentes daquela observada nos campos e outras fitofisionomias do Cerrado, sobretudo quanto ao porte e composição de espécies da vegetação e qualidade nutricional da matéria orgânica. Na vegetação ripária são observada não apenas espécies do Cerrado mas também de outros biomas, podendo ser considerada um complexo vegetacional de biomas. A vegetação riparia possui grande importância na manutenção, funcionamento e equilíbrio dos sistemas lóticos, principalmente por controlar a ciclagem de nutrientes e biogeoquímica dos sistemas aquáticos, bem como os fluxos de entrada de matéria e energia por meio do material alóctone (Webster and Meyer, 1997).

A região do Cerrado possui grande importância estratégica para a conservação dos recursos hídricos, uma vez que abrange um grande número de nascentes e parte considerável de algumas das principais bacias hidrográficas da América do Sul. Em particular, destacam-se 78% da área da bacia do rio Araguaia-Tocantins, 47% do rio

São Francisco e 48% do rio Paraná/Paraguai. Os cursos d'água preservados do Cerrado são muitas vezes oligotróficos, densamente florestados, levemente ácidos (com valores médios de pH=6) e com baixa condutividade (até 10 μS/cm)(Fonseca and de Mendonça-Galvão, 2014; Fonseca et al., 2014). Segundo Fonseca et al. (2014) a concentração de fósforo e nitrogênio total em riachos do Cerrado é menor do que em outras regiões tropicais, com concentração de fósforo total variando entre 0,006 e 0,0015 mg L^{-1}, nitrato entre 0,004 e 0,04 mg L^{-1} e amônio entre 0,002 e 0,056 mg L^{-1} (Fonseca & Mendonça-Galvão, 2014).

Áreas de estudo

Este estudo foi conduzido em 28 riachos de cabeceira no Cerrado, dos quais 21 desses são cursos d'água localizados em áreas de proteção ambiental no Distrito Federal, e sete em áreas tipicamente afetadas por atividades antrópicas. As áreas protegidas amostradas foram a Área de Proteção Ambiental Gama e Cabeça-de-Veado, Parque Nacional de Brasília e Estação Ecológica Águas Emendadas.

A Área de Proteção Ambiental (APA) Gama e Cabeça-de-Veado (criada pelo Decreto No 9,417, de 21 de abril de 1986) está localizada na porção centro-sul do Distrito Federal. A APA integra uma área de proteção contínua que compreende a i) Fazenda Água Limpa (15°46' S; 47°55' O, altitude de 1.171m), ii) Reserva ecológica do IBGE – RECOR (15°56' S; 47°53' O, altitude de 1.104m) e iii) Estação Ecológica do Jardim Botânico (15°52' S e 47°50' O, altitude de 1.056m). Estas áreas são importantes centros de pesquisa que contém uma rica flora com mais de 2.000 espécies de plantas vasculares, representando 33% do número listado para o bioma Cerrado (UNESCO, 2000). Esta APA abrange uma área de 23,650 ha a ~1100 metros de altitude onde tem-se como maior finalidade garantir a integridade ecológica dos ecossistemas terrestres e aquáticos e proteger os cursos d'água que integram a bacia do Paranoá. Solos espessos do tipo Latossolo predominam na região (principalmente da Depressão do Paranoá). Nas áreas de relevo mais movimentado, aparecem solos mais rasos do tipo Cambissolo. Os solos Litóticos aparecem nas áreas de afloramento rochoso na porção das cabeceiras dos cursos d'água, basicamente à sudeste da APA. Solos Hidromórficos aparecem em áreas planas (vales, onde aparecem Matas de Galeria e Veredas) principalmente no Núcleo rural da Vargem Bonita. Na APA foram amostrados 11 trechos de referência (Fig. 2).

Figura 2. Riachos de referência amostrados na Área de Proteção Ambiental (APA) Gama e Cabeça-de-Veado, Brasília-DF.

(continuação)

Figura 2. Riachos de referência amostrados na Área de Proteção Ambiental (APA) Gama e Cabeça-de-Veado, Brasília-DF.

O Parque Nacional de Brasília (PNB) está situado na porção noroeste do Distrito Federal, a cerca de 10 km do centro de Brasília e corresponde à maior Unidade de Conservação de Proteção Integral do Distrito Federal, compreendendo uma área de 42.389 ha (criado pelo Dec. N° 241 de 29/11/1961, ampliado pela Lei n° 11.285 de 08/03/2006). O PNB abriga as bacias dos córregos formadores da represa Santa Maria, que é responsável pelo fornecimento de 25% da água potável que abastece a Capital Federal. Por se tratar de uma unidade de conservação de acesso restrito, a vegetação ripária que acompanham os cursos d'água dentro do PNB se encontram em ótimas condições de preservação, mantendo os corpos d'água dentro dos padrões de potabilidade (Resolução CONAMA no 20 de 18/06/87). Os principais grupos de solos encontrados no Parque Nacional de Brasília são o Latossolo Vermelho-Escuro (42%), Latossolo Vermelho-Amarelo (23,12%) e Cambissolo (14,13%). Ocorrem também Gleissolo, Plintossolo, Neossolo Litólico, Espodossolo e Neossolo Quartzarênico, sendo esse último o menos frequente, com superfície de 0,05% (FARIAS, 2008). No PNB foram amostrados sete trechos de referência (Fig. 3).

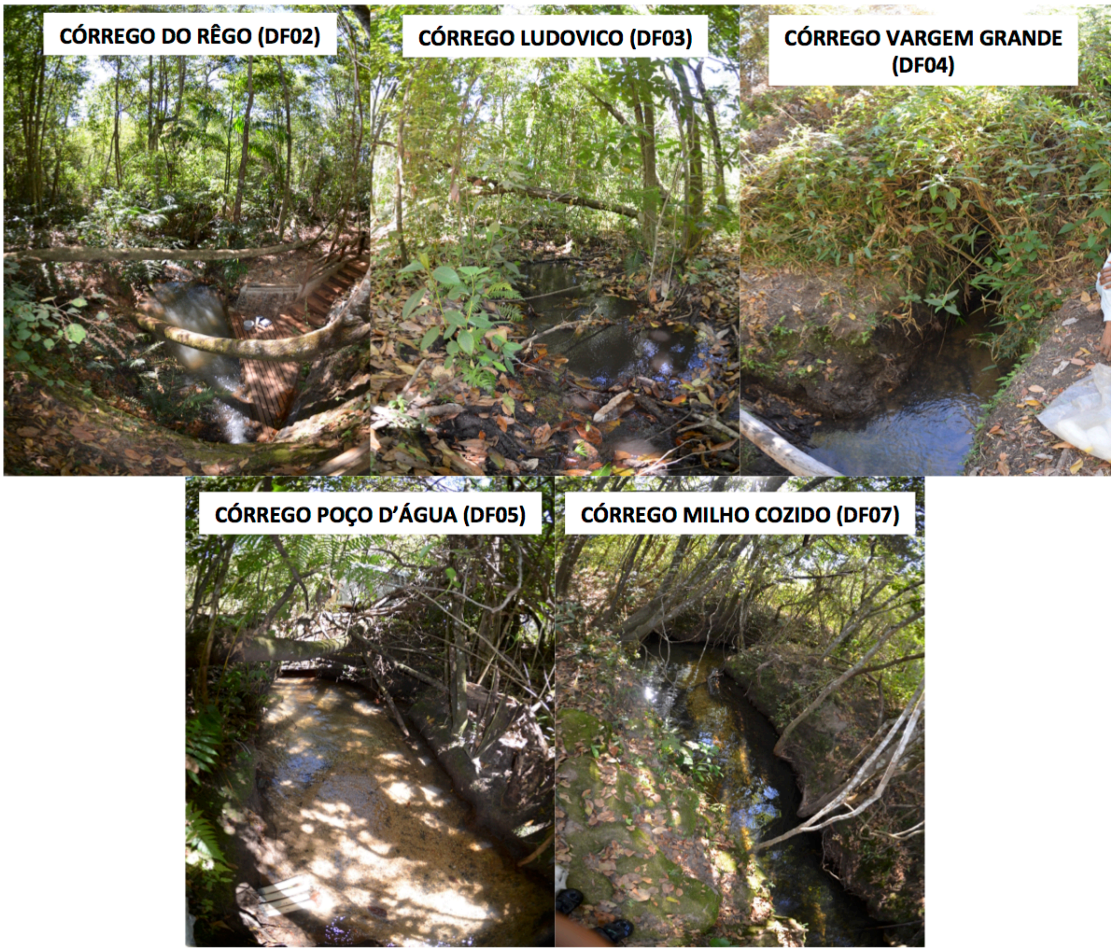

Figura 3. Riachos de referência amostrados no Parque Nacional de Brasília (PNB), Brasília-DF.

A Estação Ecológica de Águas Emendadas (ESECAE; 15°42' a 15°38' S e 47°33' a 47°37' O) é uma reserva de 9.500 ha composta de vegetação típica de Cerrado (criado pelo Dec. Nº 771 de 16/08/1968 e modificado pelo Dec. Nº 11.137 de 20/06/1988). Nessa área ocorre o a união de duas grandes bacias hidrográficas do Brasil – a Araguaia/Tocantins e a do Prata – em uma pequena vereda de aproximadamente 6 km de extensão, topografia plana e completamente hidromorfizada (Couceiro and Padovesi-Fonseca, 2009). A vegetação marginal que acompanha os cursos d'água inseridos na ESECAE apresenta vegetação ripária preservada, enquanto que trechos localizados fora da área de proteção evidenciam uma situação de vegetação ripária degradada (Fernandes, 2007). Os principais grupos de solos encontrados na ESECAE são do tipo Cambissolo Háplico, Neossolo Litólico, Latossolo Vermelho-Amarelo e Latossolo Vermelho (Lacerda & Barbosa 2012). Na ESECAE foram amostrados três trechos de referencia (Fig. 4).

Figura 4. Riachos de referência amostrados na Estação Ecológica Águas Emendadas (ESECAE), Brasília-DF.

Para avaliação da integridade ambiental foram ainda coletados sete riachos em ambientes perturbados, os quais são caracterizados por diferentes tipos e níveis de impacto sobre os ambientes aquáticos. Dentre os riachos perturbados analisados, três são afetados por silvicultura, três por agricultura e pecuária, e um por despejos urbanos (Fig. 5).

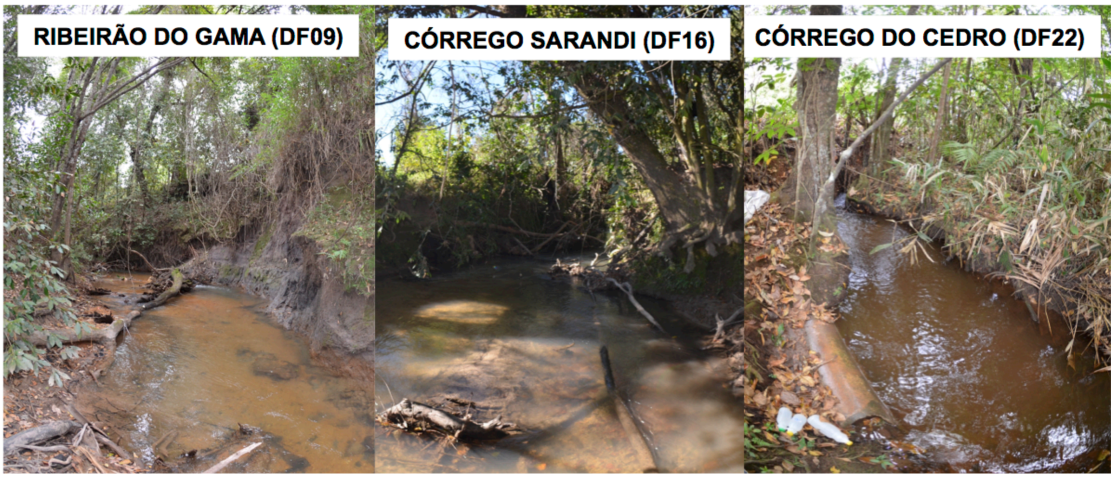

Figura 5. Riachos perturbados amostrados na região do Distrito Federal.

(continuação)

Figura 5. Riachos perturbados amostrados na região do Distrito Federal.

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ESTRUTURA DA TESE

Nesta tese são apresentados os resultados de análises bióticas e abióticas nos compartimentos aquático e terrestre associado a 28 riachos de cabeceira do Cerrado. Esses riachos apresentam diferentes condições ambientais e também representam um gradiente de perturbação. Este documento foi dividido em três capítulos, os quais foram redigidos em formato de artigos. O primeiro, intitulado "**Environmental heterogeneity drives the beta-diversity of aquatic macroinvertebrates in Brazilian savannah headwaters**", é um trabalho onde investigamos os mecanismos que determinam a diversidade de assembleias de macroinvertebrados em 21 riachos preservados de cabeceira no Cerrado em três diferentes escalas: riachos, bacias de drenagem, áreas. Esse estudo parte da premissa que os cursos d'água estão organizados no espaço de maneira hierárquica e, como tal, apresentam elevada heterogeneidade em termos de características ambientais e biológicas. Desta forma, testamos as hipóteses de que a assembleia de macroinvertebrados seria mais fortemente estruturadas por características locais dos riachos, levando a uma maior diversidade β entre os riachos, e que as características da paisagem influenciariam menos as comunidades devido ao efeito amortecedor da vegetação ripária, levando a uma menor diversidade β entre paisagens e bacias de drenagem.

No segundo capítulo, "**Food web and trophic plasticity along environmental gradients in Brazilian savannah headwater streams**", quantificamos a importância dos recursos autóctones e alóctones para macroinvertebrados e peixes em 21 riachos de cabeceira preservados a partir de isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$). Nesse estudo testamos o paradigma atual para os ecossistemas lóticos que afirma que os riachos de cabeceira são sustentados energeticamente pelo carbono alóctone e que mudanças ao longo do gradiente ambiental afetam o fluxo de energia e as relações tróficas em ambientes aquáticos.

O terceiro capítulo, intitulado "**A multimetric index for the functional assessment of Brazilian savannah headwater streams based on aquatic food webs**", utiliza de valores isotópicos de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) para calcular métricas de comunidade em 28 riachos de cabeceira com diferentes graus de perturbação e, então, derivar desses um índice de avaliação de qualidade ambiental para riachos de cabeceira no Cerrado. A partir disso, este estudo buscou testar a eficácia das métricas isotópicas e do índice isotópico multimétrico (ISI) em fornecer uma avaliação de qualidade ecológica de riachos, comparando-as com métricas estruturais mais tradicionais. Desta forma, sugerindo a adequabilidade do uso de métricas isotópicas e do índice como uma ferramenta para avaliação ecológica de riachos.

CAPÍTULO 1

ENVIRONMENTAL HETEROGENEITY DRIVES THE BETA-DIVERSITY OF AQUATIC MACROINVERTEBRATES IN BRAZILIAN SAVANNAH HEADWATERS

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ABSTRACT

Streams are ecosystems organized in a spatial hierarchy that usually show high heterogeneity in terms of biological and environmental characteristics, which difficult the understanding and prediction of their taxonomic composition of biological communities. Based on this perspective, the objective of this study was to identify the mechanisms that drive the beta diversity of benthic macroinvertebrates assemblages in Cerrado low order streams, at three spatial scales (areas, catchments and streams). We sampled 21 streams across seven catchments and three landscape areas. At all sites, standardized protocols were used to assess environmental heterogeneity and sample benthic macroinvertebrates. The streams present a high biotic heterogeneity, when the aquatic invertebrate assemblages are structured by local instream conditions ($R^2 = 0.27$). These assemblages differ from each other better on landscape scale ($\beta 3 = 34.7\%$; $p < 0.001$) than on smaller scales (catchment or stream scales) as result of the greater heterogeneity in local conditions between areas than between streams. This suggests that the land use and regional characteristics have in fact a strong influence over instream macroinvertebrates communities in Cerrado streams, in spite of the buffers created by the dense riparian vegetation. Thus, since the heterogeneity of habitats is represented, the biodiversity conservation measures and monitoring should be planned at this scale, considering the diversity of land uses, under penalty of a mischaracterization of the aquatic biodiversity.

Keywords: beta diversity; spatial scale; metacommunity; headwater streams.

INTRODUCTION

To understand the biodiversity patterns along space and time (Legendre et al. 2010b), and in different scales (Simberloff 2004; Ricklefs 2008), is among the fundamental questions in ecology, biogeography and evolution (Al-Shami et al 2013; Sutherland et al 2013; Heino et al 2015a). In this sense, there has recently been an increasing interest in the patterns of diversity at multiple scales (e.g., regional or local scales), as well as in the identifying and understanding the processes that drive it (Legendre et al 2005). Studies of the beta diversity have been prominent to understand these patterns, and to reveal the processes shaping variation in biotic communities and the efficient design of nature conservation measures (Al-Shami et al 2013).

According to Whittaker (1960) the species diversity can be divided into three components: local species richness (α diversity), regional richness (γ diversity) and differences in species composition between sites (β diversity). In this sense, the beta diversity has been defined as a measure of variation in the community composition across space focusing on variability in species composition among disjoint non-overlapping sites (Legendre et al 2005). While a large number of studies in aquatic ecosystems focused on local or regional units, a smaller part of the recent research focuses on β diversity patterns (Mykrä et al. 2007; Anderson et al. 2011; Grönroos et al. 2013; Ferreira et al. 2017). However, local (α diversity) or regional (γ diversity) approaches are not able to generate predictions that can be extrapolated to other areas or scales of study because patterns measured at small scales do not necessarily hold at larger scales, nor do processes prevailing at regional scales necessarily prevail at large scales (Wiens 1989; Schneider 2001; Wiens 2002). Thus, in spite of the advances and the increasing number of studies, the results achieved are still insufficient to understand how communities are structured, specially in a scenario of changes in biodiversity (Cardinale et al. 2012; Simberloff et al. 2013).

There are three main hypotheses proposed to explain β diversity (Legendre et al. 2005). The first proposes that species composition is stable over large areas, and that biological interactions (e.g., inter- and intra-specific competition) are the drivers of β diversity. The second hypothesis states that species composition varies in a random and autocorrelated way, emphasizing a limited spatial dispersion of organisms. The third hypothesis states that species distribution is driven by environmental conditions, and that landscapes are mosaics in which the local environmental factors control species composition. According to Legendre *et al.* (2005), hypotheses two and three may be associated because often both the spatial patterns in the community can be explained by the environmental conditions of the sites, as the opposite, the environmental conditions are explained by the spatial structure. Testing these hypotheses is important to understand the functioning of ecosystems, for biodiversity conservation and ecosystems management (Legendre et al 2005).

Studies in lotic ecosystems suggest that biodiversity is organized in a spatial hierarchy (Frissell et al. 1986; Wiens 2002; Heino et al. 2015a). The concept of hierarchy in ecology refers to the idea that “scale matters” when the aim is to identify patterns in the compartments of ecosystems. In other words, it refers to hierarchy when a focal level is constrained by large-scale dynamics (Allen and Starr 1982). Also, in streams the community patterns may vary according to the spatial extent of the study and the pattern of species composition is defined by factors that depend on the scale of observation (Bini et al. 2014; Heino et al. 2015a). At local scales, streams present a great heterogeneity of environmental conditions (e.g., heterogeneity in the sediment composition in a stream reach), with direct effect on the structure of the communities (Ligeiro et al. 2009; Heino et al. 2015c). However, community patterns and structures may be different between riffles and pools, but nested within rivers or river basins (Schneider 2001; Wiens 2002).

There are an increasing number of methodologies and theoretical approaches used by ecologists to study the β diversity and to identify the processes related to their generation (Schneider 2001; Legendre et al 2005; Al-Shami et al 2013). Methods using variation partitioning allows determination of the fraction of beta diversity explained solely by environmental or spatial predictors, and by shared effects of both sets of predictors (Legendre 2008; Myers et al. 2013; Legendre and De Cáceres 2013). The most significant progress in identifying biodiversity patterns at different scales is related with the development of the metacommunity theory (Leibold et al. 2004; Logue et al. 2011), which has led to substantial changes in the way that ecologists interpret ecological phenomena at both local and regional scales (Leibold et al. 2004).

In this study, we investigated the effect of spatial scales (streams, catchment and landscape areas) on β diversity of benthic macroinvertebrates in headwater streams of the Brazilian savannah (also called Cerrado biome) and the mechanisms that drive these patterns. Cerrado streams are apparently quite similar to each other (e.g., similar riparian vegetation, geology) and isolated from the surrounding landscape (typical savannah with scattered bushes and small trees adapted to dryness) by dense, large and high riparian galleries, which have a more tropical humid character. However, this biome and their streams are itself highly heterogeneous, ruled by a set of physical factors such as topography and soil characteristics, landscape and rainfall patterns (Budke et al. 2007). Variation in these factors creates an environmental gradient in the streams due to alterations in the supply of organic matter, litter-quality and in the shading pattern (Hill & Dimick, 2002; Schalk et al., 2017), as a result, one can find a variety of habitats even within relatively short distances (hundreds of meters), evidencing the habitat complexity of Brazilian savannah streams, which may define changes in community composition (Ligeiro et al. 2009; Ferreira et al. 2017). Thus, our study hypotheses are that: i) macroinvertebrates assemblages would be more strongly structured by local instream characteristics, leading to a higher β diversity between streams; and ii) the landscape characteristics would influence less the communities due to the buffer effect of the tropical riparian galleries, leading to a lower β diversity between landscapes and catchments. To test this we studied 21 headwater streams belonging to 7 catchments located in the Federal District, Brasil, within the Cerrado biome.

MATERIALS AND METHODS

Study area

This study was conducted in 21 headwater streams (1st to 3rd order) with preserved riparian vegetation, located, in three legally protected areas in the Federal District,: Gama and Cabeça-de-Veado Environmental Protection Area (APA), Brasilia National Park (PNB) and Águas Emendadas Ecological Station (AE). The distance between the studied areas varies between 10 and 35 km, while distance within areas varies from 0.22 to 17 km (Figure 1).

The streams are located within the climatic area of the Cerrado (tropical savannah, Aw; Alvares et al 2013) (Figure 1). The climate in the region is seasonal, with a hot and rainy season (from October to April) and a colder and dry season (from May to September; Ribeiro & Walter, 2001; Ribeiro, Fonseca & Sousa-Silva, 2001). In the rainy season the average air temperature is 22.1 ± 1.1 ° C with precipitation of

197.5 ± 60.2 mm / month and concentrated between January and March. In the dry season the average air temperature is 20.3 ± 0.6 ° C and precipitation of 2.5 ± 3.5 mm / month. The predominant soils near the selected streams are latosol and cambisol. The riparian zone is predominantly composed of natural vegetation with a mean height between 20 and 30 m and 70-95% canopy cover (Ribeiro and Walter 2001; Ribeiro and Walter 2008). The selected streams were separated into seven catchments, which represent important watercourses that cover the preserved areas of the Federal District. Sampling was undertaken during the dry season, between June and August 2015.

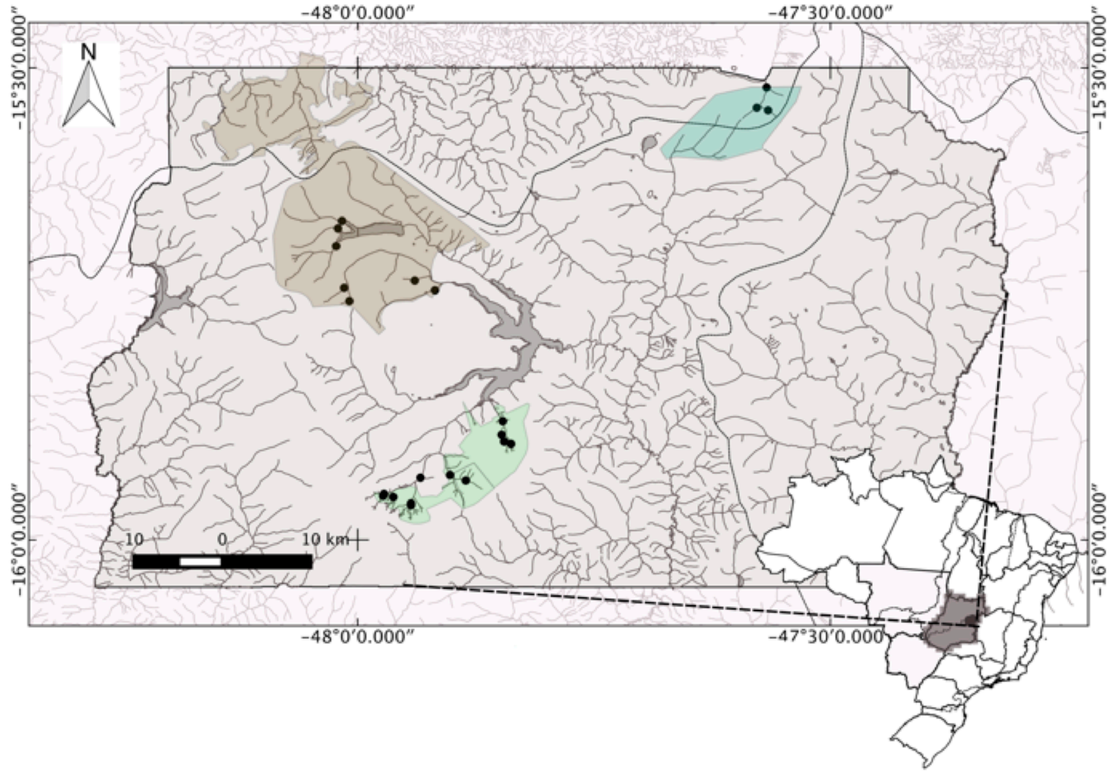

Figure 1 Geographical location of the study areas in Federal District, Brazil.: i) Environmental Protection Area of Gama and Cabeça-de-Veado – light green; ii) National Park of Brasília – brown; and Águas Emendadas Ecological Station – light blue. Sampling points are represented by black dots.

Local environmental variables

In each stream, a water sample was collected for the determination of dissolved cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Br^-, F^-, Cl^-, NO_3^-, $½SO_4^{3-}$). 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). In addition, *in situ* measurements were obtained in triplicates per stream of depth (m), width (m), temperature (°C), pH (pHmeter PHTEK®, Curitiba, PR, BR) electrical conductivity (μS.cm^{-1}; Conductivimeter Quimis®, Diadema, SP, BR), dissolved oxygen (mg.l^{-1}; Jenway 970 Dissolved Oxygen Meter, Staffordshire, OSA, UK) and canopy cover [%; digital camera (Nikon D5100) with a 10-mm Fisheye lens (Sigma)].

A sediment sample was collected in each stream with a quadrat of 0.32 x 0.32 m (total area of 0.1024 m^2) to determine the granulometric composition (Suguio 1973). The collected samples were dried at 60 ºC during 72 hours and separated by gravimetry in sieves to determine the proportions (%) of each type of sediment in the samples: gravel (2.00 mm), very coarse sand (1.00 mm), coarse sand (0, 50 mm), medium sand (0.250 mm), fine sand (0.125 mm), very fine sand (0.063 mm) and silt + clay (less than 0.063 mm).

The vertical input of coarse particulate organic matter (CPOM) in the stream was measured as an estimative of the availability of allochthonous resources in the stream. The vertical input was measured with six buckets (sampling area of each bucket 0.043 m^2), hanged across the streams for 60 days, to capture the organic matter that falls on the stream, totalizing a sample effort of 0.26 m^2 per stream. All the collected samples were taken to the laboratory and dried 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 values for each fraction were expressed in g.m^{-2}.day.

The availability of autochthonous resources was estimated by chlorophyll-*a* in the biofilm. Three acetate leaves (10 x 15 cm) were used as artificial substrate for colonization of the biofilm in each stream. These leaves were submerged in the streams for 60 days in pools with depth between 10 and 50 cm. At the end of the incubation period the acetate leaves were taken to the laboratory and cut into squares pieces of 5 x 5 cm. Squares were individually scraped with a soft toothbrush and filtered through GF/C (0.22 μm aperture) glass fibre filters previously calcined and weighed. For each stream, one of the biofilm filters was dried at 60ºC for 72 hours, weighed, burned at 550ºC in an oven for 2 hour and weighed again to calculate the Ash-Free Dry Mass (AFDM). A second filter was used to determine the chlorophyll-*a* by trichromatic method, extracting the pigments in organic solvents (90% ethanol) and reading the optical density of the solutions containing the pigments in spectrophotometer. The chlorophyll determination was made in triplicates per stream.

In each stream, a sample of the vegetal organic matter present in the benthic stock was collected with a Surber sampler (0.1024 m^{-2}; 0.25 mm mesh size). The leaves collected in the benthic stock were washed in distilled water to eliminate debris and adhered invertebrates, dried at 60 ºC for 48h, milled and sieved (0.5mm mesh). From this material the C/N ratios were obtained by chromatography on CNS elemental analyzer as descriptors of the palatability and nutritional value of the allochthonous vegetal resource.

Regional environmental variables

Drainage area was extracted for each stream, from available information at the National Aeronautics and Space Administration (NASA, *Shuttle Radar Topography Mission* project - SRTM). Landscape analyzes were done using the Geographic Information System (GIS) based on TerraClass images (2013) and multispectral Landsat-8 Thematic Mapper (TM). For land use, the TerraClass Cerrado image was classified as natural environment, annual agriculture, mosaic of occupations, pasture, forestry, urban, or naked soil (Table 1). In addition, Landsat-8 images (OLI) were used to calculate the normalized difference vegetation index (NDVI) for each catchment as an additional tool to detect anthropogenic and natural changes in vegetation (Rouse et al. 1973).

Table 1 Description of the land use features classified from TerraClass images Cerrado (2013) for the study areas.

Variables	**Description**
Natural environment	Forest areas; fields or savannahs whose land cover pattern is compatible with the different physiognomies of the Cerrado; may present include some degree of alteration in relation to the original landscape, due to the presence of non-intensive or subsistence activities, such as natural pastures.
Modified environment	Area in which the land use is constrained by different economic activities and human occupation, responsible for the replacement or eradication of natural cover.
Annual agriculture	Exposed or vegetated soil areas, usually composed of a single species of commercial interest.
Mosaic of occupations	Settlements, villages, sheds and industries.
Pasture	Area generally composed of a single species of grass (*Brachiaria*), with different proportions of regrowth and/or soil exposure, depending on the level of degradation.
Forestry	Vegetated areas usually composed of only one species of commercial interest.
Urban	Buildings, small districts, hamlets, villages with little urban structure.
Naked soil	Anthropogenic areas without vegetation cover, by the analysis of satellite images or history of land use that could be attributed to them; includes also as well as other categories of anthropic use (e.g., roads, rural buildings).

Macroinvertebrates sampling

Benthic macroinvertebrates were collected in all streams with a D-frame kick net (250 μm mesh, 0.3 m aperture) within a 50 m reach. To standardize the sampling effort among streams, five sub-samples of one meter each were distributed to cover the habitats heterogeneity, totalizing a sampling area of 1.5 m^2 per stream. The sampled material was washed on the 0.5 mm mesh sieve and the organisms sorted. Macroinvertebrates were preserved at -20 ºC until their identification to the family level, following appropriate taxonomic keys (Merritt and Cummins 1996; Cummins et al. 2005; Mugnai et al. 2010; Hamada et al. 2014).

Data analysis

The β diversity between spatial scales was estimated by additive partition ($\gamma = \alpha + \beta1 + \beta2 + \beta3$). Alpha (α) is the average species richness in the community found in each stream and gamma (γ) refers to the total species richness observed in the set of all streams. Each component of the beta diversity (β) refers to an analyzed scale: between streams (β1, N = 21), between catchments (β2; N = 7) and between areas (β3; N = 3). These beta components can be interpreted as the difference in mean taxon richness between stream (β1), catchments (β2) or areas (β3). The analyses were performed in Partition 3.0 software (Veech and Crist 2009). The diversity observed for each component was compared to the expected diversity from an individual-based random model, which tests whether the observed partition of diversity could have been explained by a random distribution of individuals (Crist et al. 2003). Contrasting the magnitude of observed beta diversity at each scale against values generated by a null model allows the recognition of the main sources of community variation in the spatial hierarchy (Crist et al., 2003). The individual-based randomization randomly reassigns each individual of the dataset to samples at the lowest level of analysis. The significance of the results was based on the comparison of the percentage of components observed with the expected values of diversity, obtained from 9999 iterations. Therefore, p-values are the proportions of randomized data that obtained diversity values greater than the observed values. Thus, low p-values (e.g., $p < 0.05$) indicate that the observed diversity is significantly higher than the randomly expected from iterations, and high p-values (e.g., $p > 0.95$) indicate that the diversity is significantly lower than expected.

The geographical coordinates (UTM) were used as spatial descriptors to macroinvertebrates communities. Although some studies have suggested that stream distances are more meaningful for aquatic organisms than overland distances (Altermatt 2013), others have found that these two measures of distance are often strongly correlated, providing virtually the same information about the spatial structuring of a community (Thompson and Townsend 2006; Landeiro et al. 2012; Grönroos et al. 2013).

Redundancy analysis (RDA) was performed to divide the specific effects of the local environment variables, regional environmental variables and spatial factors on community composition data (Table 2). The decomposition into fractions [a] to [h] was done from the adjusted R^2 values calculated by RDAs involving 1, 2, and all 3 explanatory data tables. Previously, the local and regional environmental descriptors with low explanatory potential were identified from the Akaike Information Criterion (AIC) model containing only the significant variables. The selection of the variables that best explain the variations in the community was performed by 'double stopping criterion' (forward and backward stepwise selection), which means verifying the inclusion of each variable in the model either by removing or adding a new variable (Blanchet et al 2008). This selection criterion avoids type I errors and the overestimation of the amount of variance explained by the explanatory variables. Composition data were previously transformed by Hellinger method, as recommended by (Borcard et al 2011), and environmental variables were standardized. These analyzes were performed in software R, associated with vegan package for the selection of variables and RDA (Oksanen et al. 2008). To test for spatial relationships among streams, a Mantel test (9999 random permutations) was performed for the macroinvertebrates assemblage composition data (function *mantel*; vegan package). A

Mantel test measures the correlation between two matrices typically containing spatial data of distance for testing for spatial autocorrelations (Legendre & Legendre, 1998).

Table 2 Number of individuals and total biomass (in brackets; mg^{-1}) of benthic macroinvertebrates in the sampled areas: Gama e Cabeça-de-Veado Environmental Protection Area (APA), Águas Emendadas Ecological Station (AE) and National Park of Brasília (PNB).

Taxa	**APA**		**AE**		**PNB**	
Nematoda						
Nematomorpha	5	(2.2)	0	(0)	3	(1.0)
Arthropoda						
Crustacea						
Ostracoda	0	(0)	0	(0)	1	(0.1)
Malacostraca						
Decapoda						
Palaemonidae	2	(552.6)	0	(0)	0	(0)
Amphipoda						
Hyalellidae	0	(0)	0	(0)	4	(1.3)
Insecta						
Ephemeroptera						
Leptophlebiidae	33	(4.4)	6	(0.5)	28	(2.8)
Leptohyphidae	1	(0.1)	0	(0)	3	(0.4)
Baetidae	193	(14.6)	3	(2.4)	2	(0.2)
Euthyplociidae	4	(25.6)	0	(0)	0	(0)
Odonata						
Zygoptera						
Calopterygidae	5	(9.9)	3	(5.8)	0	(0)
Coenagrionidae	6	(6.3)	0	(0)	1	(0.4)
Grypopterygidae	5	(0.7)	2	(0.6)	38	(4.1)
Lestidae	1	(0.6)	0	(0)	0	(0)
Megapodagrionidae	2	(3.8)	0	(0)	0	(0)
Anisoptera						
Libellulidae	16	(185.1)	0	(0)	2	(4.4)
Gomphidae	9	(329.3)	1	(35.6)	2	(3.9)
Cordulidae	0	(0)	0	(0)	1	(0.1)
Aeshnidae	10	(15.7)	0	(0)	0	(0)
Plecoptera						
Perlidae	56	(77.8)	39	(53.1)	22	(17.5)
Hemiptera						
Gerridae	2	(4.3)	0	(0)	1	(1.3)
Veliidae	5	(3.1)	1	(1.0)	9	(5.7)
Megaloptera						
Sialidae	6	(14.6)	0	(0)	1	(9.4)
Corydalidae	2	(1326.6)	0	(0)	0	(0)
Trichoptera						
Calamoceratidae	23	(57.3)	1	(0.6)	1	(0.3)
Hydropsychidae	43	(184.6)	41	(291.5)	27	(63.7)
Glossosomatidae	1	(0.1)	0	(0)	1	(0.1)
Leptoceridae	10	(61.1)	2	(1.6)	13	(2.0)
Odontoceridae	3	(1.7)	0	(0)	2	(0.4)
Hydroptilidae	1	(0.0)	0	(0)	0	(0)
Polycentropodidae	40	(17.9)	1	(2.2)	3	(2.2)
Hydrobiosidae	1	(0.2)	0	(0)	0	(0)
Lepdoptera						
Pyralidae	11	(21.8)	2	(3.2)	0	(0)
Coleoptera						
Unidentified adult	38	(62.2)	20	(22.5)	8	(7.2)
Elmidae (adult)	4	(1.5)	0	(0)	13	(2.7)

Table 2

Elmidae (larvae)	55	(34.8)	38	(28.5)	44	(18.4)
Psephenidae	10	(160.1)	0	(0)	0	(0)
Curculionidae	0	(0)	2	(2.6)	0	(0)
Scirtidae	1	(0.9)	1	(0.2)	1	(0.1)
Dytiscidae (adult)	1	(3.3)	0	(0)	0	(0)
Dytiscidae (larvae)	11	(3.9)	2	(4.7)	0	(0)
Dryopidae	0	(0)	0	(0)	2	(9.8)
Diptera						
Chironomidae	395	(48.2)	23	(9.1)	158	(12.9)
Ceratopogonidae	26	(5.4)	0	(0)	16	(4.6)
Simulidae	293	(25.3)	29	(5.1)	22	(2.0)
Empididae	0	(0)	0	(0)	1	(0.3)
Tipulidae	6	(41.2)	3	(8.9)	3	(3.5)
Psychodidae	0	(0)	1	(0.1)	0	(0)
Unidentified individuals	0	(0)	1	(0.1)	0	(0)

RESULTS

A total of 1991 individuals belonging to 47 families of benthic macroinvertebrates were collected in the 21 streams. The areas presented similar mean taxonomic richness, with AE presenting the highest average richness per stream (13.3 ± 4 families; 22 families in total), followed by APA (12.2 ± 4.4; 39 families) and PNB (10.6 ± 4.6; 31 families). APA presented the highest mean abundance of individuals (121.4 ± 115; N = 1336), followed by AE (74 ± 43.8; N = 222) and PNB (61.8 ± 69.7; N = 433). Only 16 families (34%) were registered in all the three areas (Table 2). The stream richness (α diversity) varied between six and 22 taxa. Only the Chironomidae family occurred in all streams. Six families (Ceratopogonidae, Chironomidae, Elmidae, Hydropsychidae, Leptophlebiidae, Perlidae, Simuliidae) were recorded in at least 12 streams. We also registered that 18 families were found only in one area (10 were in APA, five in PNB and two in AE). The composition of the macroinvertebrates assemblages did not show significant spatial autocorrelation ($r = -0.06$; $p = 0.69$).

The analysis of the hierarchical partition of diversity showed a significant (higher than expected at random) compositional distinction in the assemblages of macroinvertebrates between landscape areas ($\beta 3 = 16.33$; 34.7%; $p < 0.001$) and between catchments (β2 = 8.81; 18.7%; $p < 0.05$) but not between streams (β1 = 10.05; 21.4%; $p = 0.497$) (Figure 2). The α diversity was lower than expected randomly from iterations ($p > 0.999$).

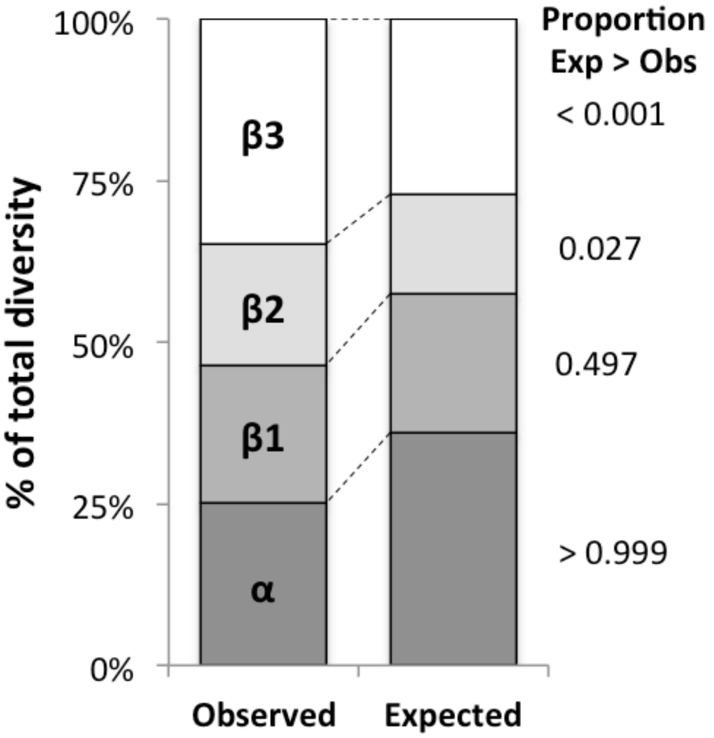

Figure 2. Additive partition of the diversity by taxon of the community of benthic macroinvertebrates observed and expected by null model in headwater streams of Brazilian savannah, Federal District. α = local diversity; β1 = diversity between streams; β2 = diversity among catchments; β3 = diversity between areas.

Among the 33 local environmental descriptors, only five were linearly correlated with communities (AIC criteria, adjusted-R^2 = 0.5). The method didn't select regional variables and thus all eleven variables were considered (adjusted-R^2 = 0.02). Thus, variance partitioning was performed for three data sets: local environmental descriptors (variables: Na^+, K^+, Mg^{2+}, dissolved O_2 and pH), regional environmental descriptors (variables: catchment area, natural area, agriculture, mosaic of occupations, pasture, forestry, urban area, naked soil, percentage of savannah in the catchment, percentage of modified area and NDVI), and spatial descriptors (variable: UTM1 and UTM2). RDAs indicated that the three explanatory data sets explained jointly about 29% of the variability in the abundance data of macroinvertebrates (Figure 3). The local environmental variables explained most of the taxa variation in the community composition (fraction [a]: adjusted $R^2 = 0.27$, $p = 0.026$). On the other hand, no significant portion of variation was explained solely by regional (fraction [b]: adjusted $R^2 = 0.12$, $p = 0.151$), spatial variables (fraction [c]: adjusted $R^2 = 0.09$, $p = 0.188$), or by regional and spatial together. Local, regional and spatial descriptors shared an explanation percentage of 9% (fraction [g]: adjusted $R^2 = 0.09$)(Figure 3).

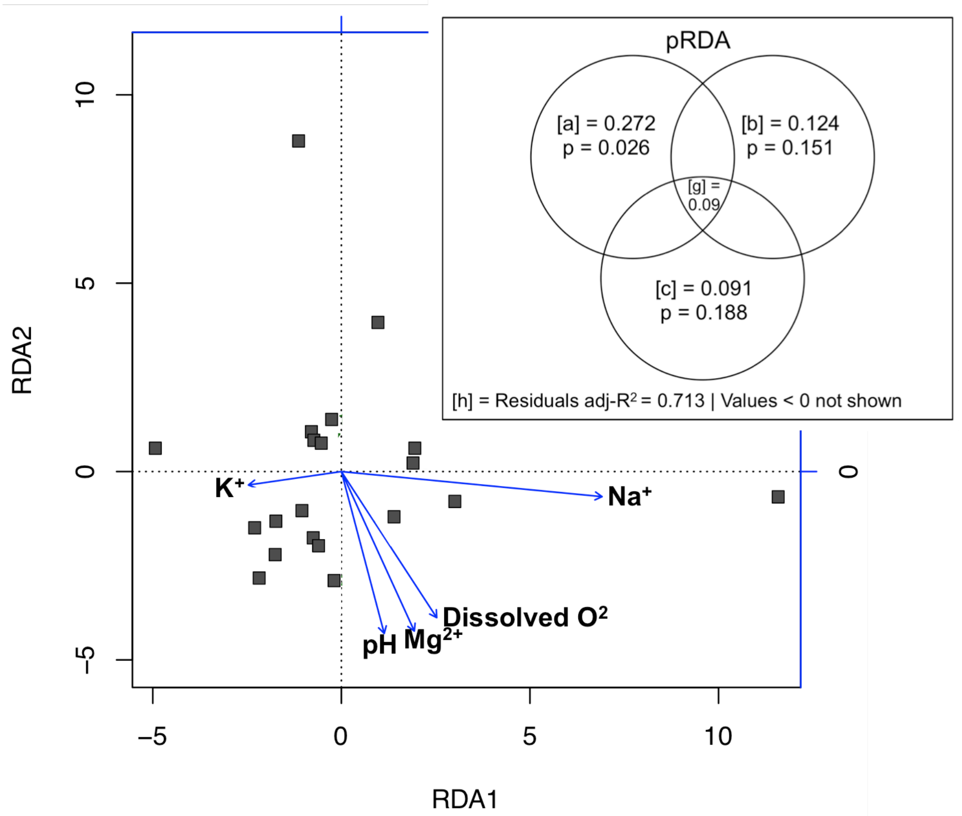

Figure 3. Partial Redundancy Analysis (pRDA) of the composition of benthic macroinvertebrates based on spatial and environmental (local and regional) data. Explanation percentage for each fractional component is shown in the Venn diagram.

DISCUSSION

Beta diversity was high in most of the analysed scales, indicating a highly variable biotic structure in Cerrado streams. This β diversity increased with the scale and was greater at the largest scale (diversity between landscape areas - β3) followed by diversity between streams (β1) and catchments (β2). It was however nonsignificant between streams (β1). This result shows that the diversity is not homogenously distributed in the spatial scales, which confirms the hierarchy ecology statement that "scale matters" to define the diversity patterns in an ecosystem (Allen and Starr 1982). However, these results partially contradict our hypotheses as we expected that most of the total diversity would be due to variations among streams rather than between areas or catchments. Recent studies have indicated higher β diversity at smaller scales (between streams or samples) than at larger scales (catchments or hydrographic units) (Grönroos et al. 2013; Ferreira et al. 2017). We understand that aquatic insects can disperse at all spatial scales (Grönroos et al. 2013; Ferreira et al. 2017) and the limitation of dispersion between areas is not an applausive hypothesis to this pattern in our study. In this sense, an explanation could be that larger areas contain more habitat heterogeneity than smaller areas (Nekola and White 1999; Bini et al. 2014; Heino et al. 2015b), which allows for more species to coexist regionally through niche diversification and differences in resource exploitation (Leibold 1998). Yet, as the species are distributed in an aggregated way, a greater spatial extent could include different pools of species (Heino et al. 2015a). One of the clear examples of taxa aggregation in our study was observed with the mayfly Baetidae, which had about 93% of its total abundance recorded in only one stream in APA.

In addition, despite the broadly similar characteristics in climate, natural vegetation and geology along the preserved Cerrado biome and sampled sites, there was remarkable variation in the habitat characteristics and the riparian corridors among streams. For example, the canopy openness ranged from 7 to 89% and pH from 5.3 to 8.9. This resulted in a high explanatory power of local environmental variables in structuring macroinvertebrates assemblages. This fact associated to the low β diversity among streams could suggests a higher similarity between nearby streams or in the same catchment linked to the proximity and connectivity of sampling sites. The short migrations of individuals or the drift in the watercourses may explain, in part, the homogenization of assemblages within a basin (Robson and Chester 1999; Parsons et al. 2003; Ciesielka and Bailey 2007; Ligeiro et al. 2009). Also studies between distant reaches often found a low similarity in the assemblages (Downes and Hindell 2000; Heino et al. 2004; Heino 2005; Ferreira et al. 2017) showing that both the environmental heterogeneity and dispersion ability of organisms can prevail in determining the organization of assemblages (Heino et al. 2015b). However, the distance decay of community similarity was refuted in our study with the spatial autocorrelation analyses. In this sense, the hypothesis most likely to explain this pattern still is that of greater heterogeneity in local conditions between areas than between streams is responsive to the high macroinvertebrates diversity. In our study about half (49%) of the observed macroinvertebrate families were only found in three streams or less, confirming this indication.

Finally, we identified three possible limitations to our results and associated interpretations. First, we cannot rule out the possibility that unmeasured ecological variables are the main drivers of variation in species composition within each community. Potentially important variables may include fish predation, disturbance regime or ecological relationships among species, which have been shown to be important drivers in single case studies (e.g., Townsend and Douglas 2000). Second, this study was conducted during a single season (dry period). A different pattern may occur during the rainy season due to increased stream flow, which may promote the drift or displacement of organisms, with potential for further homogenization of the distribution of organisms (Townsend and Hildrew 1976). Third, as in previous studies addressing several spatial scales, this study included little replication at the coarsest scale (three landscape areas) and a relatively small distance between areas. Thus, care must be taken in extrapolating our results. In addition, some may argue that the low taxonomic resolution may mischaracterize the patterns of the assemblages. However, although this study was restricted to families, we don't expect that richness will be much higher at lower taxonomic levels because significant proportion of macroinvertebrate families in the Neotropical region are represented by a single species in a catchment (Melo and Froehlich 2001).

Despite the mentioned limitations, this study presented effective evidences that the Brazilian savannah headwater streams are highly heterogeneous habitats where the macroinvertebrate assemblages are structured by local environmental conditions. This also has practical implications as this results show that for monitoring purposes it is important to establish sampling protocols covering the in stream habitat heterogeneity as this is the major influence on the diversity of taxa collected. Finally, this study highlights the need to consider different landscape units for biodiversity conservation and management purposes as large-scale characteristics and land use result in differences in diversity of aquatic fauna.

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CAPÍTULO 2

FOOD WEB AND TROPHIC PLASTICITY ALONG ENVIRONMENTAL GRADIENTS IN BRAZILIAN SAVANNAH HEADWATER STREAMS

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ABSTRACT

The current paradigm for lotic ecosystems states that headwater streams are fueled by allochthonous carbon and changes along the environmental gradient affects the energy flow and trophic relations in aquatic environments. To confirm this, we surveyed in 21 references Brazilian headwater savannah streams that varied along an environmental gradient of canopy cover, availability and quality of resources. We quantified the importance of autochthonous and allochthonous resources to macroinvertebrates and fish with Bayesian mixing models for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes. Patterns across diets of the aquatic community were identified in bipartite networks and variations in food web structure along gradients were revealed with linear regressions. We have gained important information about the community-relevant taxa diet. We found that the major source of energy for the aquatic biota is autochthonous instead of allochthonous. Few taxa are effectively sustained by allochthonous organic matter, which suggests a low trophic specialization and high diet overlap within the community. Our results also show that differences in the availability of resources and canopy cover are drivers of the food web. The diversity of resources exploited by community increases with the input of allochthonous resources. Yet, the availability of algae is independent of canopy cover.

Key-words: fish; freshwater; macroinvertebrates; stable isotopes; tropical streams

INTRODUCTION

Since the publication of The Stream And Its Valley (Hynes 1975) and the development of early conceptual models of lotic ecosystems, such as the River Continuum Concept (RCC) (Vannote et al. 1980), it has been advocated that allochthonous carbon fuelled the food webs in small forest streams. Most studies supporting that model were based on the direct observations of gut content, feeding behaviour of organisms, and corroborated by the substantial influx of leaf litter and reduced photosynthetic radiation on the stream caused by the extensive riparian vegetation, a dearth of visible benthic algae and higher values of stream respiration compared with production (Vannote et al. 1980).

In fact, the riparian vegetation exerts a powerful control on aquatic ecosystems metabolism (Cummins 1974), mainly regulating the dynamics and transport of organic matter and nutrients, the transfer of energy between terrestrial and aquatic ecosystems and intercepting of potential contaminants (Pusey and Arthington 2003, Baxter et al. 2005). In headwater streams, the very high extent of riparian vegetation and the canopy closure over streams also limits *in situ* primary productivity and provides massive input of allochthonous organic material, such as leaves, flowers, seeds, insects and other organic materials that may support stream food webs (Wallace et al. 1997; Paetzold et al. 2005). However, chemical tracer studies (e.g., stable isotope, fatty acids) have questioned the importance of terrestrial carbon in food webs, suggesting that the assumption of allochthonous resources as the basal food source to macro biota is not true for many freshwater food webs, especially in arid (Bunn et al. 2003) and tropical biomes (Brito et al. 2006; Lau et al. 2009a).

Analysis of stable isotopes has proven to be a useful tool to determine the importance of food resources to consumers, architecture and dynamics of food webs in streams (Peterson and Fry 1987, Brito et al. 2006, Parreira de Castro et al. 2016), as long as basal sources have distinct signatures (Bunn and Boon 1993). Whereas gut analysis indicates what a consumer has recently ingested, Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$) stable isotopes are indicative of the origin of sources of carbon, their flow through food webs (Peterson and Fry 1987), and trophic position (Vander-Zanden et al. 1997; Post 2002). In this sense, stable isotope analysis has been an important and advantageous tool in trophic ecology studies to examine resource partitioning (Young et al. 2010), ecosystem fluxes of carbon and nitrogen (Peterson and Fry 1987), to reconstruct diets (Phillips 2012), and to characterize niche properties (Newsome et al. 2007). The $\delta^{13}C$ values typically differ among basal sources (e.g., plant material from C_3 and C_4 plants) and are used as an indicator of C sources for certain consumers along food chains (Post 2002; Perkins et al. 2014). On the other hand, the $\delta^{15}N$ indicates the total length of the food chain and the position of an organism within it (Vander-Zanden and Rasmussen 1999, Perkins et al. 2014). Moreover, a multiple-source mixing model allows the estimation of the proportion of different food sources contributing to consumers (Parnell et al. 2010).

In contrast to suggested with early theoretical models, the advances in studies of aquatic food webs associated to the use of stable isotopes and other chemical tracers have proposed that food quality, and not quantity, is the more important factor regulating the energy flow through stream ecosystems (Lau et al. 2009b; Lau et al. 2009a; Brett et al. 2017). In this sense, studies have refuted the hypothesis that the availability of basal resources implicitly determines the food-web structure of ecosystems, which has been a shift in emphasis to the disproportionate importance of autotrophic producers to animal consumer production in lotic ecosystems (Bunn et al. 2003; Brito et al. 2006; Lau et al. 2009b; Lau et al. 2009a; Jardine et al. 2015; Neres-Lima et al. 2016). These studies have shown that even well shaded tropical streams can have sufficient light input to sustain a reasonable level of primary production and a complex food web (Bunn et al. 1999b; Bunn et al. 1999a). However, relatively little research of this kind has been published for small, forested tropical streams (but see March & Pringle, 2003; Mantel et al., 2004; Brito et al., 2006; Neres-Lima et al., 2016, 2017; Parreira de Castro et al., 2016).

Although the Brazilian savannah streams are typically characterized as oligotrophic and densely forested (Fonseca et al. 2014), this biome is itself highly heterogeneous, ruled by a set of physical factors such as topography and soil characteristics, landscape and rainfall patterns (Budke et al. 2007). Variation in these factors creates an environmental gradient in the streams due to alterations in the supply of organic matter, litter-quality and in the shading pattern (Hill and Dimick 2002, Schalk et al. 2017), which may simplify trophic structure, reduce biological diversity and change bioenergetic processes in the streams (e.g. breakdown, autochthonous production, periphytic biomass, secondary production) (Lorion and Kennedy 2009, Parreira de Castro et al. 2016). For example, alterations in the openness of riparian vegetation, affect indirectly the stream channel morphology, hydrology and water chemistry, temperature, autochthonous *versus* allochthonous-based production, and habitat structure in mediating forest-stream linkage remains difficult to infer from observational studies (Majdi et al. 2015). Therefore, studies addressing the effects of changes in stream and riparian ecosystems on the energy flow and trophic relations in aquatic environments are essential to an understanding of the mechanisms that drive the ecological structure of those environments (Parreira de

Castro et al. 2016). In addition, to determine the mechanisms responsible for community organization and species coexistence is among the fundamental goals in ecology (Chesson 2000, Siepielski and McPeek 2010).

In this study we hypothesized that the importance of algae to consumers is underestimated by their in-stream availability and we expected a switch in the food-web structure with changes in the canopy cover, availability and quality of resources. Therefore, our aims were (i) to quantify the importance of autochthonous and allochthonous resources to macroconsumers (benthic macroinvertebrates and fishes); (ii) to identify diet patterns across the aquatic community; and (iii) to identify variations in food web structure along gradients of physical habitat structure (canopy cover), productivity (availability of resources), and quality of resources.

MATERIALS AND METHODS

Study area

The study was performed in 21 headwater streams (1st to 3rd order; Table 1) with riparian vegetation preserved in three legally protected areas in the Brazilian Federal District: Gama and Cabeça-de-Veado Environmental Protection Area, Brasilia National Park and Águas Emendadas Ecological Station. The climate in this region is Tropical Savannah, with two well-defined seasons: wet and warm (from October to April), and dry and cold (from May to September) (Ribeiro et al. 2001). The average monthly temperature is 22 ± 1°C in the wet season (rainfall of 197 ± 60 mm/month, with peaks between January and March) and 20 ± 1°C in the dry season (rainfall of 2 ± 3 mm/month). The precipitation rate decreases by approximately 86% in May, when the dry season begins (Brazilian National Institute of Meteorology-INMET; Portuguese acronym). The selection of reaches in the studied streams was based on the spatial independence of reaches, similarity in the geological, geomorphological field and morphological characteristics of vegetation (Brazilian savannah); abiotic characteristics (pH, conductivity, dissolved oxygen) during the dry season; and the excellent conservation status of the riparian vegetation. Sampling was restricted to dry season, between June and August 2015, to reduce the 'noise' of seasonal weather fluctuations (Heino 2014).

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

				Autochthonous resources		**Allochthonous resources**		
Reach	Latitude (S)	Longitude (W)	Canopy openness (%)	Biofilm growth rate ($g.m^{-2}.day^{-1}$)	Primary production ($mg.m^{-2}.day^{-1}$)	Total ($g.m^{-2}.day^{-1}$)	Leaves ($g.m^{-2}.month^{-1}$)	C:N ratio
1	15°44'9.64"	47°55'7.36"	18.1	0.003	395.9	11.5	11.6	45.8
2	15°43'32.45"	47°56'24.23"	13.1	0.006	725.3	2.5	9.2	52.9
3	15°41'21.31"	48° 1'23.18"	11.8	*	*	0.5	2.1	42.7
4	15°40'13.75"	48° 1'16.67"	74.9	0.012	255.5	0.6	4.6	65.5
5	15°44'0.33"	48° 0'53.16"	26.3	0.004	202.1	7.4	12.6	56.4
6	15°44'50.97"	48° 0'32.87"	12.0	0.005	65.1	0.9	2.5	45.1
7	15°39'45.05"	48° 1'1.13"	14.6	0.004	179.3	1.4	11.0	44.1
8	15°57'17.72"	47°57'45.97"	13.8	0.010	1453.9	1.4	6.9	50.8
9	15°57'41.60"	47°56'38.18"	16.5	0.006	284.3	6.3	18.4	61.2
10	15°57'48.77"	47°56'38.59"	27.6	0.007	222.0	0.6	3.1	38.3
11	15°57'13.76"	47°58'24.92"	8.4	0.007	*	3.2	24.3	45.5
12	15°57'5.63"	47°58'21.23"	18.6	*	325.2	0.6	3.1	47.8
13	15°32'43.45"	47°33'59.28"	12.6	0.004	309.2	1.9	12.5	53.1
14	15°32'33.46"	47°34'42.07"	11.3	0.003	156.7	2.2	9.1	57.9
15	15°31'16.26"	47°34'4.83"	13.7	0.005	572.3	2.8	5.5	49.2
16	15°53'46.33"	47°50'43.62"	18.6	0.006	658.4	10.3	18.5	43.1
17	15°53'20.39"	47°50'53.48"	12.8	0.015	474.7	22.4	15.7	70.0
18	15°53'55.26"	47°50'17.63"	14.7	0.007	804.6	0.9	11.7	34.1
19	15°52'28.59"	47°50'48.84"	13.9	0.007	512.2	5.2	4.6	60.8
20	15°56'14.77"	47°53'9.85"	19.1	0.005	226.8	3.1	30.5	50.8
21	15°55'53.75"	47°54'9.58"	18.1	0.008	162.9	6.7	8.9	38.9

* Missing data.

Productivity and physical habitat structure

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, totalizing 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.

Periphytic mass and colonization rate in the biofilm were estimated from artificial substrates, following the procedure of Feio et al. (2010). Three acetate sheets (10 x 15 cm) were used as artificial substrate for these estimative in each stream. These substrates were submerged in the streams during 60 days in both pools and riffles, with depth between 10 and 50 cm, in a way that best represented the heterogeneity of habitats in each sampled stream. At the end of the incubation period the acetate leaves were taken to the laboratory and cut into squares pieces of 5 x 5 cm. All the squares were individually scraped with a soft toothbrush and filtered through GF/C (0.22 μm aperture) glass fibre filters previously calcinated and weighed. The chlorophyll was determined in triplicates per stream with the trichromatic method, extracting the pigments in organic solvents (90% ethanol) and reading the optical density of the solutions containing the pigments in spectrophotometer. The chlorophyll-*a* values were then converted into dry mass of chlorophyll-*a*, corrected by sampling area and expressed in mg.m^{-2}. The dry-mass chlorophyll-*a* was then converted to biomass of algae assuming that chlorophyll-*a* correspond to only 1% of algae biomass (Brito et al. 2006; Neres-Lima et al. 2016). The biofilm colonization rate was estimated as an indicator of algae productivity and calculated by the Ash-Free Dry Mass (AFDM) of the biofilm. The AFDM of the scraped biofilm was obtained also in triplicates by drying the filters at 60 ºC for 72 hours, weighting, burning at 550 ºC in oven for 2 hours and weighting again.

In each stream, one sample of the coarse organic matter present in the benthic stock was collected with a Surber sampler. From those, the leaves were washed in distilled water to eliminate debris and adhered invertebrates, dried at 60 ºC for 48h, milled and sieved (0.5mm mesh). From this material the C/N ratios were obtained by chromatography on CNS elemental analyzer as descriptors of the palatability and nutritional values of the allochthonous plant resource. These C/N ratios were analysed in triplicates. The canopy openness of the stream (%) was determined from hemispherical photographs [digital camera (Nikon D5100) with a 10-mm Fisheye lens (Sigma)] also taken in triplicates by stream.

Sample collection and processing

Benthic macroinvertebrates and fish were collected in all streams. The macroinvertebrates were sampled with D-frame kick net (250 µm mesh, 0.3 m aperture). To standardize the sampling effort, each macroinvertebrates sample was collected by covering five sections of the sediment of one meter each, totalizing 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 organisms retained were sorted and preserved frozen until their identification to the family level, following appropriate taxonomic keys (Merritt and Cummins 1996; Cummins et al. 2005; Mugnai et al. 2010; Hamada et al. 2014).

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. Dog food was used as bait to lure consumers into funnel traps. The seines (one upstream, one down-stream) as funnel traps (four per stream) remained submerged for 24 hours at each stream. The fish and shrimp caught were placed in plastic bags with water collected in the stream in the refrigerator (4°C) for 30 minutes, which leads to stunning after a certain time. After this period individuals were euthanized through the section of the spinal cord or decapitation, according to recommendations of the

Brazilian Federal Council of Veterinary Medicine. After euthanasia, the individuals were classified to the lowest possible taxonomic level and frozen (-20°C).

Isotopic analyses

The most abundant taxa were selected to the analysis of the isotopic diet. For benthic macroinvertebrates, families were separated into groups of 1 to 20 individuals in centrifuge 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 largest individuals of each site and species of fish and shrimps had their dorsal muscles removed and frozen. All samples to stable isotope analysis were manually cleaned to remove contaminants from the environment, oven dried at 60 °C for 72 hours and pounded with mortar and Wiley mill.

The acetate was wiped under running water and then scraped the adhered material for the stable isotopic analyses. 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 to all samples. Green leaves from dominant riparian C_3 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 1 mg of dry and ponded sample and put inside small tin capsules. This analysis was determined using a Flash EA 1112 Series elemental analyser coupled on line via Finningan Conflo III interface to a Thermo delta V S mass spectrometer, at Marine and Environmental Sciences Centre (MARE) labs, University of Coimbra, Portugal. The carbon and nitrogen isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (PDB limestone 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 $\delta^{15}N$.

Data analyses

We performed Bayesian stable-isotope mixing model analysis (R: SIAR, version 4.1.1; Parnell & Jackson 2011) to estimate the potential contribution of allochthonous and autochthonous resources to the isotopic diet of consumers. The fractionation values for muscles samples were +1.3 ‰ (SD = 1.27) for $\delta^{13}C$ and +2.9 ‰ (SD = 1.24) for $\delta^{15}N$, while for samples in which the whole animal was analysed (smaller macroinvertebrates) the fractionation values were +0.3 ‰ (SD = 1.3) for $\delta^{13}C$ and +2.1‰ (SD = 1.60) for $\delta^{15}N$ (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 (1st consumers, 2nd consumers or 3rd consumers) but without distinguishing among streams. Likewise, food sources also differed between trophic levels (Eletronic Supplementary Material 1). For 1st consumers, the CPOM and periphytic algae were the food sources used in the mixing model. As a result, 1st consumers were divided into algivores (algae consumption > 50%) or herbivores (CPOM consumption > 50%) according to the importance of the resources obtained for each taxon by the model. As 2nd 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 used as food sources in the mixing model for 2nd consumers. In the same way, 2nd consumers were divided into predators of algivores (consumption of algivores > 50%) or predators of herbivores (consumption of herbivores > 50%). All sources were used in the mixing model for 3rd consumers. 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.

To describe patterns of diet in the community and identify how the food web could be composed of different sub-communities (referred to as modules) we calculated modularity for the consumer-resource interactions networks using the importance of resources for each taxon obtained with the mixing models (Lewinsohn et al. 2006). The network modularity was derived using the QuaBiMo algorithm (*Q*; Barber 2007), based on a hierarchical random graphs approach adapted for weighted bipartite networks (Clauset et al. 2008; Dormann & Strauss 2014). To evaluate if modularity *Q* calculated for our networks deviated from random expectations, we compared them against a reference distribution derived from 100 random matrices where the marginal totals from the original interaction matrices were retained, following (Dormann and Strauss 2014) recommendation. The random matrices were derived from the 'r2da' method, where the number of interactions per taxa is maintained, changing only the way these interactions are distributed. Since Q values are assumed to be normally distributed, results with standardized difference (SDif) above ≈ 2 are considered significantly modular.

To identify changes in the food-web structure among streams and sub-communities, we used community-wide metrics based on isotopic data (Layman et al. 2007). We examined among streams the degree of trophic redundancy (mean nearest neighbour distance, MNND), evenness of trophic niches (standard deviation of NND), average degree of isotopic diversity (centroid distance, CD), magnitude of food-web trophic diversity (convex hull of the total area, TA), maximum vertical structure [$\delta^{15}N$ range (NR)] and basal resource diversity [$\delta^{13}C$ range (CR)] (Layman et al. 2007). We used regression models (function lm, stats package) to explore these relationships between food-web structure and environmental gradients. When necessary, data was previously log transformed to fulfil the assumptions of normality of residuals of the analyses. All statistical analyses were performed in R version 3.1.2 GUI 1.65 Snow Leopard build (R Development Core Team 2014) using the SIAR package (Parnell and Jackson 2013) for stable isotope analyses and bipartite package (Dormann et al. 2009) for network analyses.

RESULTS

Structure and productivity of streams

The canopy cover of streams varied between very shady (8.4% of openness) to exposed (74% of openness), but the majority of sampling streams were shaded (Table 1; 18% ± 13 SD) and characterized by dense riparian vegetation. As expected, allochthonous carbon was much more abundant than autochthonous resources within streams. The mean input of CPOM was 4.3 $g.m^{-2}.day^{-1}$ ± 5 SD but varied greatly among streams, ranging from 0.5 to 22 $g.m^{-2}.day^{-1}$. Leaves represented most of the CPOM (39%), followed by branches (34%), miscellaneous (27%) and reproductive parts (< 1%). The mean periphytic biomass varied from 0.005 to 0.35 $mg.m^{-2}$ among streams, with mean of 0.04 $mg.m^{-2}$ (0.07 SD). The biofilm colonization rate varied between 0.002 and 0.014 $g.m^{-2}.day^{-1}$ among streams. The CPOM resource quality, represented by C:N elementary ratio of leaf litter, varied between 34 to 70 among streams (Table 1).

Trophic structure

Autochthonous and allochthonous resources showed different isotopic signatures, which allowed us to analyse trophic relationships. The $\delta\ ^{13}C$ values from riparian vegetation were relatively uniform, ranging between -35‰ and -31‰ while those of $\delta\ ^{15}N$ varied between -5.7‰ and 3.6. Periphytic microalgae from artificial substrate had more enriched $\delta\ ^{13}C$ values, ranging between -29.8‰ and -26.8‰, and $\delta\ ^{15}N$ between -1‰ and 10‰ (Figure 1A). The 1st consumers also had a higher variation on isotopic values, with $\delta\ ^{13}C$ ranging between -38.8‰ and -25.4‰ and $\delta\ ^{15}N$ values ranging between 0.5‰ and 9.4‰ (Figure 1A). The 2nd consumers vary between -33.9‰ and -24.7‰ for $\delta\ ^{13}C$ and between 0.4‰ and 10.3‰ for $\delta\ ^{15}N$ (Figure 1B). Except for the values of the fish *Hypostomus* sp. ($\delta\ ^{13}C$ = -40.2‰ and $\delta\ ^{15}N$ = 7.3‰), the 3rd consumers showed less variation in the isotopic values, with most of the taxa aligned with autochthonous resources, with $\delta\ ^{13}C$ ranging from -26.5 to -23.9‰ and $\delta\ ^{15}N$ from 8.4 to 11.7‰ (Figure 1C).

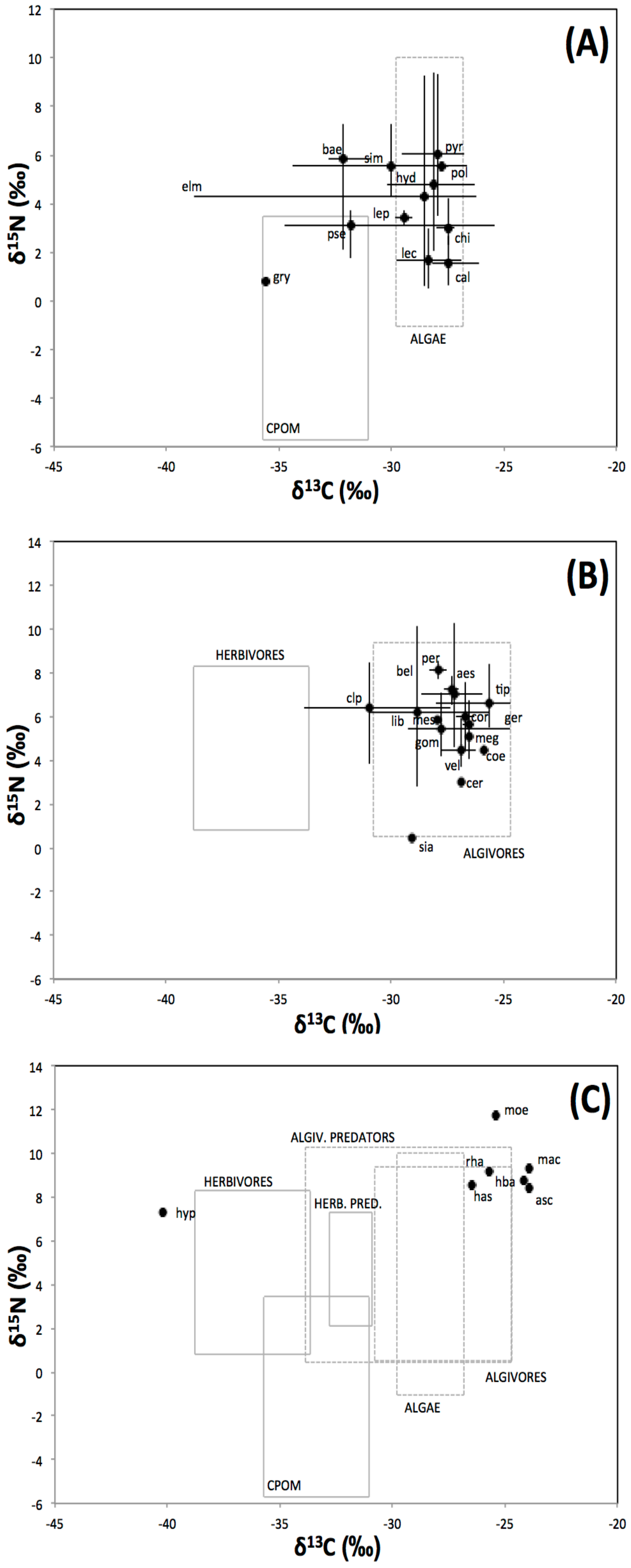

Figure 1. Isotopic values (δ^{13}C and δ^{15}N) from sources (dotted squares) and 1st consumers (A), 2nd consumers (B) and 3rd consumers (C). Taxa code:
elm – Elmidae;
pse – Psephenidae;
cer – Ceratopogonidae;
chi – Chironomidae;
sim – Simulidae;
tip – Tipulidae;
bae – Baetidae;
eut – Euthyplocidae;
lep – Leptophlebiidae;
bel – Belastomatidae;
ger – Gerridae;
mes – Mesoveliidae;
vel – Veliidae;
pyr – Pyralidae;
cor – Corydalidae;
sia – Sialidae;
aes – Aeshnidae;
clp – Calopterygidae;
coe – Coenagrionidae;
gom – Gomphidae;
lib – Libellulidae;
meg – Megapodagrionidae;
per – Perlidae;
cal – Calamoceratidae;
gry – Grypopterigidae;
hyd – Hydropsychidae;
lec – Leptoceridae;
pol – Polycentropodidae;
mac – Macrobrachium sp.;
asc – A. scabripinnis;
has – H. crenuchoides;
hba – H. balbus;
hyp – Hypostomus sp.;
moe – Moenkausia sp.;
rha – R. quelen

The Bayesian mixing model revealed a great variability in the proportions of autochthonous and allochthonous sources in the diet of groups (Figure 2). Most consumers presented carbon isotopic signatures strongly aligned with microalgae, predominating (> 50% of relative importance) in the diet of 80% of primary consumers, 93% of predators, and of all omnivores taxa.

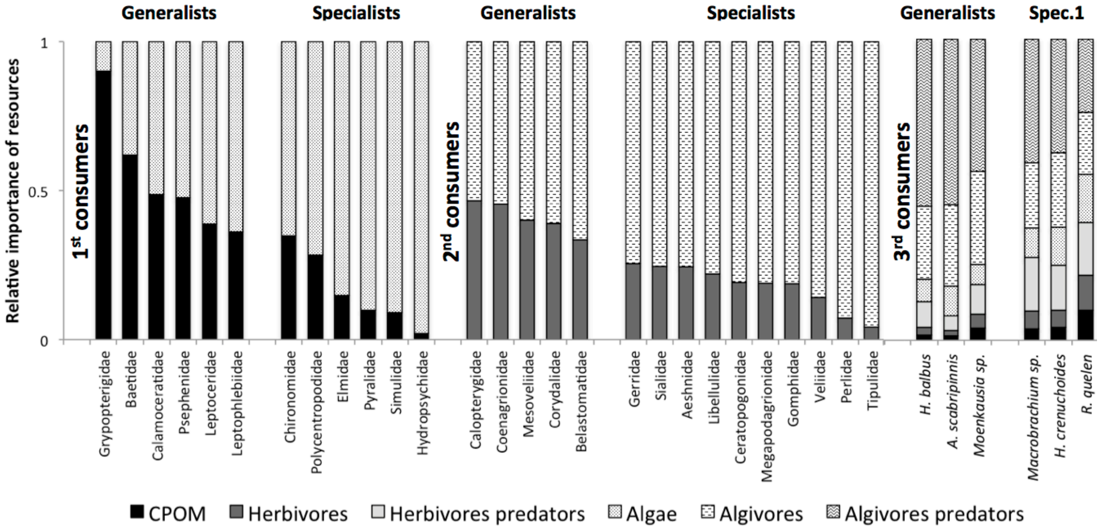

Figure 2. Average importance of allochthonous and autochthonous resources for the isotopic diet of primary consumers, predators and omnivores.

Significant network modularity was identified both for 1st consumers (*z*-score = 0.04; SDif = 22.5), as for 2nd consumers (*z*-score = 0.04; SDif = 10.6) and 3rd consumers (*z*-score = 0.05; SDif = 13.16). These sub-communities based on the relative average importance of resources allowed the identification of specialist and generalist groups at all trophic levels. Specialists were characterized by very high consumption (relative importance above 70%) of single food source, while generalists were characterized by a weighted consumption of all sources. Among 1st consumers, six taxa of macroinvertebrates (Chironomidae, Polycentropodidae, Elmidae, Pyralidae, Simuliidae and Hydropsychidae) were identified as algae specialists and six (Grypopterygidae, Baetidae, Calamoceratidae, Psephenidae, Leptoceridae and

Leptophlebiidae) as generalists. Among 2nd consumers ten macroinvertebrate' taxa (Gerridae, Sialidae, Aeshnidae, Libellulidae, Ceratopogonidae, Megapodagrionidae, Gomphidae, Veliidae, Perlidae and Tipulidae) were grouped as specialists in the consumption of other algivores' organisms and five macroinvertebrate' taxa (Calopterygidae, Coenagrionidae, Mesoveliidae, Corydalidae and Belastomatidae) as generalists, consuming both algivores as herbivores. Among 3rd consumers, three groups were identified: generalist fish (*Hyphessobrycon balbus*, *Astyanax scabripinnis* and *Moenkausia* sp.), a specialist fish with a tendency to consume primary consumers (*Hypostomus* sp.) and specialists with a tendency to consume predators (the shrimp *Macrobrachium* sp. and the fish *Hasemania crenuchoides* and *Rhamdia quelen*).

Interaction networks

The food-web varied along the environmental gradients and across trophic groups (Figure 3). Regarding the generalists, trophic diversity of consumers was significantly related with the canopy openness (CD; adjusted-R^2 = 0.32, $F_{1,8}$ = 5.32, P = 0.04), as well as the trophic redundancy negatively related with biofilm colonization rate (MNND; adjusted-R^2 = 0.48, $F_{1,7}$ = 8.595, P = 0.02). To the specialists, the vertical input was related to diversity of resources used (CR; adjusted-R^2 = 0.17, $F_{1,16}$ = 4.595, P = 0.04) and leaves input related to the diversity of trophic levels (NR; adjusted-R^2 = 0.38, $F_{1,16}$ = 11.83, P = 0.003). As regards to the whole community of streams, the vertical input was significantly related with the diversity of resources used (CR; adjusted-R^2 = 0.27, $F_{1,17}$ = 7.976, P = 0.01) and the isotopic niche (TA; adjusted-R^2 = 0.19, $F_{1,15}$ = 4.874, P = 0.04). The leaves input was significantly related to diversity of resources used (CR; adjusted-R^2 = 0.17, $F_{1,17}$ = 4.92, P = 0.04) and diversity of trophic levels (TA; adjusted-R^2 = 0.16, $F_{1,17}$ = 4.61, P = 0.04). Primary productivity was not significantly related to any of the metrics.

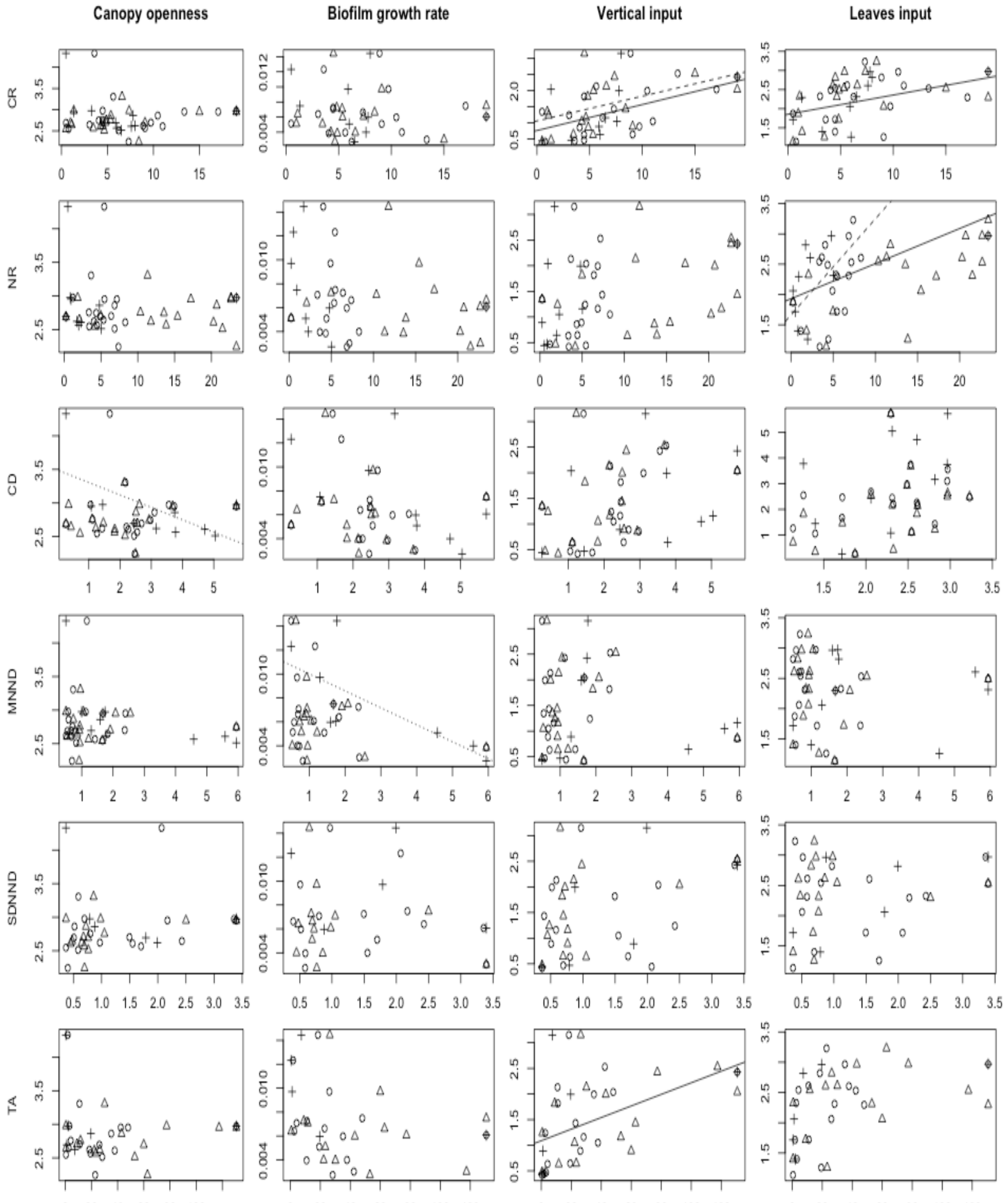

Figure 3. Linear regression between the food web structure and the environmental heterogeneity for specialist consumers (triangle, dashed line), generalists (cross, straight dots) and whole community (circle, solid line). CR - diversity of baseline resources; NR - maximum vertical structure of the trophic web; CD - isotope diversity; MNND - trophic redundancy; SDNND - uniformity of trophic niches; TA - magnitude of trophic diversity.

DISCUSSION

Autochthonous versus allochthonous resources as diet of consumers

The availability of allochthonous organic carbon was overwhelmingly greater than the autochthonous (approximately four times greater), partially corroborating our hypothesis. This is result of the dense riparian vegetation surrounding preserved headwater streams of Brazilian savannah, which supplies massive and constant CPOM (Gonçalves and Callisto 2013; Bambi et al. 2016). It is also consistent with previous studies in tropical headwater streams where the availability of these basal resources was determined (Bunn et al. 1999a; Brito et al. 2006; Neres-Lima et al. 2017). Webster and Meyer (1997) reported 28 similar cases, where the ratio of leaf litter inputs to algal production ranged between 0.6 and 7.8 in temperate streams, with a median of 2.8. In Brazilian tropical rainforest, (Brito et al. 2006) showed that periphytic algae represented less than 1% of the total biomass available to consumers in headwater streams.

However, conversely to availability, algae was the major carbon source in the aquatic food web and allochthonous resources effectively sustained few taxa. This corroborates several studies in tropical streams (March and Pringle 2003, Mantel et al. 2004, Brito et al. 2006, Uieda and Motta 2007, Li and Dudgeon 2008, 2009, Lau et al. 2009a, 2009b, Neres-Lima et al. 2016), suggesting that the importance of algae in the diet of consumers in tropical stream ecosystems is, at least to a certain level, independent of the availability of this food resource (Bunn et al. 1999b; Bunn et al. 1999a; Lau et al. 2009b; Lau et al. 2009a), as hypothesized. This contrasts with the constant and massive entry of organic allochthonous plant material throughout the year in Brazilian savannah streams (monthly average of ~ 26 gm^{-2}; Wantzen et al. 2008; Gonçalves & Callisto 2013; Bambi et al. 2016). The higher nutritive value of algae in relation to litter and fast turnover rates, which allows rapid growth and development of consumers, has been argued to explain this pattern (Salas and Dudgeon 2001). Most species of tropical plants, especially those found in the Brazilian savannah, have thick cuticle leaves and high concentrations of structural and inhibitory compounds (Moretti et al. 2007; Graça & Cressa 2010), making the litter of low nutritional quality and less palatable for consumers.

Only Grypopterygidae showed a strong tendency to be a specialist for assimilation of CPOM in our study. This contrasts with the algae-based diet found for *Gripopteryx* genus in other studies (Brito et al. 2006; Neres-Lima et al. 2016), but can be associated to species-specific diet variations. According to Froehlich (2012), Grypopterygidae larvae are usually scrapers, but some are shredders or collectors, inhabiting streams of clear waters and lotic environments frequently associated to rocky substrate. The importance of allochthonous resources was lower (<50%) than expected even for caddisfly family Calamoceratidae (genus *Phylloicus*), used in several experiments associated with CPOM consumption (Moretti et al. 2009; Rezende et al. 2015). Nevertheless, according to Graça (2001) both autochthonous and allochthonous resources can be ingested by shredders. Apparently algae are still more important for the diet of taxa in Brazilian savannah streams than in other Brazilian biomes, such as Atlantic rainforest (Brito et al. 2006; Neres-Lima et al. 2016), where the allochthonous resources seem to be relevant for a larger number of invertebrate taxa, especially of families Leptophlebiidae, Leptoceridae and Calamoceratidae (Brito et al. 2006; Neres-Lima et al. 2016).

The mean $\delta^{13}C$ values for the siluriform detritivore fish *Hypostomus* sp. also fell close to the CPOM values but $\delta^{15}N$ enriched, equivalent to second consumers. Although few studies point out the consumption of smaller invertebrates in the microscopic diet of *Hypostomus* spp., and others Loricariidae fish in tropical headwater streams, the morphology of the digestive tract of this group is contrary to the expectative of invertivore diet (Lujan et al. 2011). In this sense, we can only conclude that neither periphyton nor CPOM provides the higher direct energy for this species. It is possible that *Hypostomus* sp. is energetically supported in these streams by the consumption of heterotrophs microorganisms (Lujan et al. 2011; Lujan et al. 2012), which could be receiving carbon primarily from heterotrophic organic carbon. In headwater streams, microorganisms (mainly fungal hyphomycetes) are considered the main decomposers of allochthonous organic matter, assimilating the labile molecules and decomposing the structural compounds (Gessner et al. 1999; Mora-Gómez et al. 2016). According to Neres-Lima et al. (2017), most of the allochthonous input in headwater streams is not eaten by primary consumers, but processed by the microbial loop, burial of organic matter or down-stream transported. In this sense, microorganisms have an important role in tropical headwater streams, creating trophic links between aquatic and terrestrial habitats and making available the energy retained in allochthonous organic matter. However, studies addressing the role of microorganisms in aquatic food webs in tropical streams are still scarce and merits future studies.

Finally, CPOM isotopic values observed in our study (ranging between -35‰ and -31‰) were rather different to those referred in the international literature, but consistent with previous studies in the Brazilian savannah. In an extensive review, Finlay (2001) observed $\delta^{13}C$ values close to -28.2 ±0.2‰ in CPOM and -29.3 ±0.5‰ in riparian vegetation. However, this review is based mainly on studies from temperate latitudes, which bias these values. Also, the $\delta^{13}C$ values of CPOM were more depleted than those found for tropical rainforests (Buchmann et al 1997; Bonal et al. 2000; Ometto et al. 2006). On the other hand, previous studies in the Brazilian savannah found more similar values in riparian vegetation, ranging from –33.7‰ to –32.4‰ (Parron et al. 2004). Intrinsic water use efficiency and identified limitations to carbon fixation caused by water stress may be responsible for these differences in Brazilian savannah (Mattos et al. 1997).

Variations in food web structure along gradients of habitat and productivity

Variations along the habitat gradient of aquatic and riparian ecosystems had a significant effect in the food web of headwater streams of Brazilian savannah. This is supported by changes in the habitat structure and availability of resources creating an important environmental gradient that significantly change the trophic structure of the streams at the community level (Mosisch et al. 2001; Schalk et al. 2017). In our study, more shaded streams or with lower biofilm colonization rate were characterized by lower trophic redundancy and high trophic diversity among generalist taxa. Such observation was already mentioned in previous studies and means that consumers may change their diet or simply increase the spectrum of food items consumed in less productive streams due to a shortage of a preferential food and also to avoid competition (Schalk et al. 2017). This is expected especially for generalist species, which are able to exploit various food items and select resources based on their availability (Mihuc and Minshall 1995). Early studies in tropical systems have also indicated that consumers may switch their food habitat with the availability of resources (González-Bergonzoni et al. 2012). However, as previously mentioned, we could not confirm that variations in the habitat structure influenced the availability of algae in the studied streams. Nevertheless, little information exists about spatial patterns in resource utilization in lotic systems except for patterns based on trophic guilds.

Despite the predominance of autochthonous resources in the diet of specialist consumers, these taxa also expanded the diversity of resources exploited in streams of higher input of allochthonous resources. This suggests that allochthonous resources (specially leaves) can also be used as a supplement by specialized biota. Previous studies in temperate streams have reported a trophic switch among aquatic macroinvertebrates towards the assimilation of allochthonous resources in periods of higher productivity of riparian vegetation (e.g., higher leaf fall in autumn or standing crop; Schmid-Araya et al. 2016; Majdi & Traunspurger 2017). This switch was characterized by a reduction of the area of isotopic niches or increase of the food chain length of most consumers in periods of higher availability of allochthonous resources. These results highlight the importance of a donor-controlled linkage, mediated in their study by the riparian vegetation productivity and its assimilation by a stream food web, and emphasize the prominent contribution of allochthonous resources (litter) to a stream invertebrate community (Hall et al. 2000; Whiting et al. 2011). Several studies also reported the importance of leaching, microbial decomposition, and mechanical fragmentation by invertebrate shredders, and the potential of decomposition process to make allochthonous litter palatable to consumers (Graça et al. 2001).

Our study was performed only during the dry season, when the CPOM input in the stream is high. This is a possible limitation to our results, as seasonal variations are expected in the supply of CPOM (Bambi et al. 2016) due to the phenology of tree species in the riparian vegetation, with consequences to the canopy cover, availability, consumptions and assimilation of resources. Further studies should seek to identify the strength of such a relationship. In addition, the autochthonous productivity obtained from the acetates does not accurately reflect the availability of algae.

Previous studies have shown values up to 10 times higher than those observed. In this way, we understand that these differences can be a methodological artefact of the use of an artificial substrate as a sampler of the algae assemblages. Previous studies report that the use of artificial substrates may be good qualitative representations of the periphytic and epiphytic assemblages, but not quantitative (Cattaneo and Amireault 1992). However, algal availability, even if corrected, would be poorly representative in the trophic determination of the species or in the observed patterns for the diet of taxa.

Overall, this study showed that in headwater streams of Brazilian savannah the major source of energy for the aquatic biota is autochthonous instead of allochthonous, in spite of the overwhelming abundance of allochthonous materials in the streams. Although allochthonous energy sources were nonetheless of importance for many consumers, allochthonous resources effectively sustain few taxa, suggesting that despite the high diversity of taxa in tropical headwater streams, this does not necessarily translate into specialization of trophic function. Moreover, we also concluded that differences in the habitat structure and productivity are drivers in food web, both to generalists and specialists. Organisms have expanded the diversity of resources exploited in streams with the highest contribution of allochthonous resources, suggesting that allochthonous resources can also be used as supplementary by biota. From this study it was not possible to infer that variations in the physical habitat, especially changes in the canopy cover, are responsible for the availability of autochthonous resources in the stream. Quite the opposite, our data seem to indicate that the availability of algae is independent of canopy cover. These data cannot be interpreted as an incentive for the reduction of riparian vegetation, since changes in the natural structure of the vegetation generate not measured effects on the aquatic communities and ecosystem.

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CAPÍTULO 3

A MULTIMETRIC INDEX FOR THE FUNCTIONAL ASSESSMENT OF BRAZILIAN SAVANNAH HEADWATER STREAMS BASED ON AQUATIC FOOD WEBS

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ABSTRACT

Multimetric indices (MMIs) have been a valuable tool for recognizing changes in aquatic ecosystems. However, its use is usually dependent on the taxonomic knowledge of the fauna or its conversion into trophic groups, which has often limited its use and generated uncertainties in its application. Our objective was to test an innovative MMI based on isotopic data and community-wide metrics, following the reference condition approach, as a holistic tool for the classification of functional ecological quality of headwater streams. This approach was tested for 28 streams of the tropical Brazilian savannah (Cerrado), covering an anthropogenic disturbance gradient. Isotopic data covered the streams trophic chain and was gathered from the riparian vegetation (leaves), periphyton, invertebrates and fishes. Both the community-wide metrics (centroid distance; carbon range; nitrogen range; mean nearest neighbour distance; standard deviation of the nearest neighbour distance; total area) and the proposed Isotopic multimetric Index (ISI), were effective to discriminate anthropogenic disturbances on aquatic communities (canopy openness, pH, natural area, pasture area, mosaic of occupations, naked soil, percentage of modified area and the general disturbance gradient summarized in the PCA axes). The ISI still revealed a clear disturbance gradient, even among reference sites and was more effective and sensitivity in terms of detecting impacts than traditional diversity descriptors (e.g., Simpson, Shannon-Wiener) or the Biological Monitoring Working Party (BMWP) score system for macroinvertebrates. The use of isotopes provides a relatively quick and cost-effective way to evaluate the anthropogenic effects in aquatic ecosystems, which is even more relevant in the face of scarce taxonomic knowledge for certain freshwater groups in tropical areas, which makes its application feasible for the monitoring of streams. Thus, this approach could constitute a valuable complement to present structural assessments schemes of streams, providing a more holistic overview of the ecosystems and should thus be further tested for wider study areas, for different types of rivers and in other regions and biomes.

Key-words: ecological assessment; stable isotopes; land use; macroinvertebrates;
fish, periphyton

INTRODUCTION

The expansion of agricultural frontiers and the disorderly increase of other anthropogenic activities have generated strong changes in natural systems (Christensen et al., 2006; Crain et al., 2008; Halpern et al., 2007; O'Connor and Crowe, 2005; Vörösmarty et al., 2010). In freshwater ecosystems these pressures lead to alterations in energy and matter flows that comprise a threat to the biotic viability and ecological integrity of rivers and streams (Hughes and Noss, 1992; Karr, 1999, 1998; Macedo et al., 2014). In this scenario, the assessment of ecological integrity of freshwaters has become a growing and fundamental issue in the management of aquatic resources around the world, directing efforts to the development and testing of different methodologies and acquisition of baseline information (Birk et al., 2012; Ferreira et al., 2017; Macedo et al., 2014; Parreira de Castro et al., 2016).

The monitoring of aquatic ecosystems through the use of local biota has been used as a good estimative of the deleterious influences of anthropogenic activities. Among the components of aquatic biodiversity, the benthic macroinvertebrates (Callisto et al., 2002; Gebrehiwot et al., 2017; Rosenberg and Resh, 1993) and fish (Aparicio et al., 2011; Oberdorff et al., 2001) are good indicators of streams and rivers ecological quality, due their sensitivity to environmental conditions, reflecting the physical and chemical conditions of the ecosystem (Hogsden and Harding, 2014; Ligeiro et al., 2013). In fact, land use changes may alter the structural and functional composition of aquatic assemblages, as well as their trophic structure due the replacement, reduction or removal of species (Baerlocher et al., 2010; Jun et al., 2011; Leite et al., 2015; Nessimian et al., 2008). Specifically, disturbances in the riparian vegetation lead to the degradation of physical habitat structure, increased sedimentation rates, hydrological changes, and water temperature oscillations (Ferreira et al., 2012a; Pusey and Arthington, 2003), affecting the input of nutrients, allochthonous resources, autochthonous production (Ferreira et al., 2012a, 2012b), and thus the quantity and quality of available food resources (Pusey and Arthington, 2003). These changes may lead to increases in the frequency of generalists feeding behavior among macroinvertebrates (Parreira de Castro et al., 2016) and fish assemblages (Ferreira et al., 2012b; Leite et al., 2015), increasing the trophic niche overlap (Layman et al., 2007; Mihuc and Minshall, 1995).

Among several methodologies applied to the study of anthropogenic disturbances on aquatic ecosystems, the multimetric indices (MMIs) have been widely used since they integrate the responses of several components of the assemblages (e.g., composition, trophic guilds, dominance), in different scales, in a simple and accurate way (Ferreira et al., 2011; Helson and Williams, 2013; Hering et al., 2006; Hughes et al., 1998; Karr, 1998; Stoddard et al., 2008). When correctly developed, the MMI approach is cost effective easily interpretable and rapidly developed (Hughes and Noss, 1992; Karr, 1998). Metrics relating to habitat guilds, trophic guilds and reproductive guilds have been widely used in MMIs. However, the MMI is a flexible approach that can be used in a wide range of conditions since the list of metrics can change with the target region, country or type of rivers according to purpose of the index and regional peculiarities.

Traditionally, the MMI approach is based on taxonomic community data from single biological elements, such as fish or invertebrates (Aguiar et al., 2011; Helson and Williams, 2013; Hering et al., 2006; Paisley et al., 2014). However and in spite of relevant progresses in this area with macroinvertebrates (Ferreira et al., 2011; Macedo et al., 2014; Reis et al., 2017) these approaches are dependent on the taxonomic knowledge of aquatic fauna, which is still poor for certain groups. Moreover, conversion of taxonomic data into trophic groups (also frequently used as metrics) have also the problem of a lack of validation of taxa actual feeding behavior in these tropical systems which may differ from that of temperate systems, from often the information is derived (e.g., Merritt and Cummins, 1996). In this sense, it is necessary to search for alternatives to the traditional structural evaluations of the communities.

During the last three decades, stable isotopes analysis has been proved a useful tool to food web ecologists (Parnell et al., 2010; Phillips and Gregg, 2003), providing time-integrated measurement of assimilated food (Bearhop et al., 2004; Layman et al., 2007; Newsome et al., 2007; Post, 2002). At the same time, the C and N stable isotopes ($\delta^{13}C$ and $\delta^{15}N$, respectively) have also been proved to be responsive to environmental changes, tracing changes in the food web along a natural environmental gradient (Brito et al., 2006; Bunn et al., 2003; Neres-Lima et al., 2017), or to distinguish and quantify ecological consequences of human-induced disturbances (Cucherousset et al., 2012; di Lascio et al., 2013; Gratton and Denno, 2006; Hadwen and Arthington, 2007; Morrissey et al., 2012; Parreira de Castro et al., 2016; Schlacher et al., 2005; Vander-Zanden and Rasmussen, 1999)

The use of isotopic values for individuals or species in a system has also combined in community-wide measures of trophic structure (Layman et al., 2007). These metrics provide measures of the relative position and spacing of individuals/species in $\delta^{13}C$-$\delta^{15}N$ bi-plot. The most frequently used metrics are measures of the degree of trophic redundancy [mean nearest neighbour distance (MNND)], evenness of trophic niches [standard deviation of the nearest neighbour distance (SDNND)], average degree of isotopic diversity [centroid distance (CD)], magnitude of food-web trophic diversity [convex hull of the total area (TA)], maximum vertical structure [$\delta^{15}N$ range (NR)] and basal resource diversity [$\delta^{13}C$ range (CR)](Layman et al., 2007). Overall, the isotope values and community-wide metrics are potentially useful tools to assess the impacts of anthropogenic disturbances on ecosystem functioning, as they provide insights into overall food web structure and provide ways for comparisons among food webs (Layman et al., 2012). However, their use often requires additional sources of data to interpret patterns, because observed patterns may be a function of baseline variability and not reflect true trophic differences among consumers. In addition, they can lead to misleading patterns when source pool signatures are overlapping (Layman et al., 2012).

In this sense, our primary aim was to test if a multimetric index based on isotopic data and community-wide metrics, following the reference condition approach, could be used as a holistic tool for the classification of functional ecological quality of headwater streams. We tested this approach in streams of Cerrado, the Brazilian savannah, in the Federal District, Brazil, which is simultaneously considered a hotspot of biodiversity and a highly endangered biome. In addition, we aimed to test the efficacy of isotopic metrics and the isotopic index (ISI) in providing a quality evaluation by comparing them with more traditional structural metrics. These isotopic metrics were also never used in the form of an index to provide a classification of streams quality.

MATERIALS AND METHODS

Study area

The Brazilian savannah (also known as Cerrado biome) is a world's hotspot of biodiversity (Myers et al., 2000) due the high level of endemism but is also one of the most threatened biomes in the world, mainly because of the replacement of natural vegetation with pasture and row crop agriculture (Beuchle et al., 2015; Hunke et al., 2015). Currently, it is estimated that about 50% of the native terrestrial vegetation cover of Brazilian savannah has been reduced and fragmented due to the expansion of the agricultural frontiers, thereby degrading aquatic ecosystem and ecological integrity (Macedo et al., 2014). Only 2.2% of the Brazilian savannah biome is located in legally protected areas (Klink and Machado, 2005; Sano et al., 2008). According to Espírito-Santo et al. (2016) extensive agricultural practices, as well as silviculture and the urbanization are the main precursors to land use changes in the Brazilian savannah biome and its degradation has been associated with significant reduction and rapid decline of diversity. Nevertheless, the streams in this region have been suffering from strong anthropogenic pressures, which have changed the structure and composition of the aquatic communities (Beuchle et al., 2015; Leite et al., 2015).

In this scenario, a total of 28 headwater streams from 1st to 3rd order were sampled from June to September 2016. Of this, 21 are preserved streams located in legally protected areas in the Brazilian Federal District and seven are disturbed sites by agriculture, cattle, silviculture or urbanization. The climate in the region is defined as Tropical Savannah (Alvares et al., 2013), with two well-defined seasons: a wet and warm (from October to April), and dry and colder season (from May to September) (Ribeiro et al., 2001). The average temperature is 22 °C (± 1 SD) in the wet season (rainfall of 197 ± 60 mm/month, with peaks between January and March) and 20 °C (± 1 SD) in the dry season (rainfall of 2 ± 3 mm).

Biological data

Benthic macroinvertebrates were collected in all streams using D-frame kick net (250 μm mesh, 0.3 m aperture). To standardize the sampling effort among the streams, the "D" net was passed in the streambed in five sections of one meter each, totalizing a sampling area of 1.5 m^2 per stream. The sampled macroinvertebrates were washed on the 0.5 mm mesh sieve, the organisms were sorted and frozen (-20 °C) and identified to family level, following appropriate taxonomic keys (Cummins et al., 2005; Hamada et al., 2014; Merritt and Cummins, 1996; Mugnai et al., 2010). Sampling was restricted to dry season to reduce the 'noise' of seasonal fluctuations (Heino, 2014).

Fish and shrimp were collected 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. Dog food was used as bait to lure consumers into funnel traps. The seines (one upstream, one down-stream) as funnel traps (four per stream) remained submerged for 24 hours at each stream. The fish and shrimp caught were placed in plastic bags with water collected in the stream and kept in the refrigerator (4°C) for 30 minutes, which leads to stunning after a certain time. After this period individuals were euthanized through the section of the spinal cord or decapitation, according to recommendations of the Brazilian Federal Council of Veterinary Medicine. After euthanasia, the individuals were classified to the lowest possible taxonomic level and frozen (-20°C).

Isotopic analyses

The most abundant taxa were selected the analysis of the isotopic diet. For benthic macroinvertebrates, families were separated into groups of 1 to 20 individuals in centrifuge 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 largest individuals of each site and species of fish and shrimp had their dorsal muscles removed and frozen. All samples were manually cleaned to remove contaminants from the environment, oven dried at 60 °C for 72 hours and pounded with mortar and Wiley mill.

The carbon and nitrogen isotopic compositions of the samples were determined using aliquots of 0.2 to 1 mg of dry and ponded sample and put inside small tin capsules. The analyses were determined using a Flash EA 1112 Series elemental analyser coupled on line via Finningan Conflo III interface to a Thermo delta V S mass spectrometer, at the facilities of Marine and Environmental Sciences Centre (MARE), University of Coimbra, Portugal. The carbon and nitrogen isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (PDB limestone 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 $\delta^{15}N$.

Natural landscape characteristics

Eleven variables were measured as descriptors of natural variations along the environmental gradient. The depth (m), width (m), air and water temperature (°C) were measured *in situ* with multiparameter equipment. The canopy openness (%) of the stream was determined from hemispherical photographs [digital camera (Nikon D5100) with a 10-mm Fisheye lens (Sigma)]. In addition, the hydrologic regime (permanent or ephemeral), valley form (v or u) and morphology of the stream channel (sinuous, naturally contained or artificially contained) were recorded as descriptors of the physical habitats. We also used a geographic information system (GIS) to assess altitude (m), stream order (Strahler system) and distance from the source (km) from Landsat images.

Disturbances characteristics

Fourteen variables were measured as disturbance descriptors of each stream. A water sample was collected for the determination of anions nitrate (NO_3^-), sulfate ($\frac{1}{2}SO_4^{3-}$) and chloride (Cl^-). 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). Electrical conductivity ($\mu S.cm^{-1}$), pH, dissolved oxygen ($mg.L^{-1}$), total of dissolved solids ($mg.L^{-1}$) and runoff ($m.s^{-1}$) were obtained *in situ* and the riparian vegetation width (m) at the reach was measured from Landsat images. In addition, land use at catchment scale was assessed from the Geographic Information System (GIS) based on TerraClass images (2013) to quantify the percentage of natural areas, annual agriculture, mosaic of occupations, pasture, forestry, urban and naked soil (Table 1).

Although the definition of streams as reference or disturbed was made *a priori*, based mainly on catchment modifications, we validated the differences in the level of disturbance between these categories with Wilcoxon tests (function *wilcox.test*; stats package).

Table 1. Description of the land use features classified from TerraClass images Cerrado (2013) for the study areas.

Variables	Description
Natural environment	Forest areas; fields or savannahs whose land cover pattern is compatible with the different physiognomies of the Cerrado; may present include some degree of alteration in relation to the original landscape, due to the presence of non-intensive or subsistence activities, such as natural pastures.
Modified environment	Area in which the land use is constrained by different economic activities and human occupation, responsible for the replacement or eradication of natural cover.
Annual agriculture	Exposed or vegetated soil areas, usually composed of a single species of commercial interest.
Mosaic of occupations	Settlements, villages, sheds and industries.
Pasture	Area generally composed of a single species of grass (*Brachiaria*), with different proportions of regrowth and/or soil exposure, depending on the level of degradation.
Forestry	Vegetated areas usually composed of only one species of commercial interest.
Urban	Buildings, small districts, hamlets, villages with little urban structure.
Naked soil	Anthropogenic areas without vegetation cover, by the analysis of satellite images or history of land use that could be attributed to them; includes also as well as other categories of anthropic use (e.g., roads, rural buildings).

Identification of stream types

To establish adequate reference conditions it is necessary to first check for the existence of different stream types, regarding their natural landscape characteristics, for which different reference values of biological metrics are expected. Thus, a classification analysis was performed (Euclidean distance; Unweighted Pair Group Method with Arithmetic Mean linkage method) and based on the resulting dendrogram, groups of reference streams were established (stream types). The *P*-values for each hierarchical grouping were obtained through a bootstrap multiscale resampling technique (9,999 permutations) generated with the *pvclust* package (Suzuki and Shimodaira, 2006) in R (R Development Core Team, 2014), which is used to evaluate the uncertainty in hierarchical grouping analysis. The *P*-value of a cluster is between 0 and 100 and indicates how strongly the cluster is supported by the data based on two types of *P*-values. The AU (approximately unbiased) *P*-value is calculated by bootstrap resampling and represents an improved unbiased approach to *P*-values in comparison to the BP value. The BP (bootstrap probability) value is calculated by resampling in normal bootstrapping. Only significant hierarchical groupings ($P < 0.05$) were considered as real groups.

To identify the most adequate reference group to which a disturbed stream should be compared (its stream type) we performed Non-metric Multidimensional Scaling (NMDS) based on Euclidean distances individually for each disturbed stream + all reference streams, using only the natural landscape characteristics. Groups were identified by visual inspection of the proximity of each disturbed stream to the reference groups.

Structural and functional descriptors of community

The species richness, Pielou's evenness and diversity index of Shannon and Simpson were calculated for stream (function *diversity*; vegan package). Based on $\delta^{13}C$ and $\delta^{15}N$ values for taxon we calculated the six community-wide metrics as functional descriptors of the communities to each stream (Layman et al., 2007; function *laymanmetrics*; siar package): degree of trophic redundancy [mean nearest neighbour distance (MNND)]; evenness of trophic niches (SDNND); average degree of isotopic diversity [centroid distance (CD)]; magnitude of food-web trophic diversity [convex hull of the total area (TA)]; maximum vertical structure [$\delta^{15}N$ range (NR)]; and basal resource diversity [$\delta^{13}C$ range (CR)].

Selection of metrics

The selection of metrics was based on existing metric classification proposed by Hering et al. (2006). The responses of community-wide metrics to disturbance were assessed through linear regressions (R software; function *lm*; stats package). Only the candidate metrics that presented statistically significance ($P < 0.05$) with one or more disturbance variables were selected to include the index. Outliers revealed using boxplot analysis were removed before the statistical tests and the normality of data was checked with the Shapiro-Wilk test. The efficacy and validity of candidate metrics were then evaluated by looking for significant differences between reference and disturbance sites with t-Tests in R (function *t.test*; stats package). Significantly different metrics were defined as core metrics and included in the index. The data were transformed by log or arcosin when necessary, to fulfil the assumptions of the analyses.

Development of the ISotope multimetric Index (ISI)

The core metrics for each site were standardized by dividing their values by the respective median (reference value) for their river type (given by a group of reference sites). The core metrics were afterwards weighted by the sum of angular coefficients obtained in the regressions with disturbance variables, which provides their relative sensitivity to the measured degradation. Finally, the metrics were transformed to a unitless score, which in practice means to translate each metric into a value between 0 and 1, using the following formula:

$$Value = \frac{Metric\ result - Lower\ limit}{Upper\ limit - Lower\ limit}$$

for metrics decreasing with increasing impairment, and

$$Value = 1 - \frac{Metric\ result - Lower\ limit}{Upper\ limit - Lower\ limit}$$

for metrics increasing with increasing impairment.

The Isotopic multimetric Index (ISI) to each stream was then obtained by the sum of the transformed metrics. The ISI for a given site was finally expressed as an ecological quality ratio (EQR). The EQR represents the relationship between the values of the index for a given body of surface water and the median of the index under the reference conditions applicable to that water body and is expressed as a numerical value between 0 and 1: high ecological status is represented by values close to 1 and bad ecological status by values close to 1 (Hering et al., 2006).

The EQR values were used to derive the class boundaries. The higher quartile was defined as Higher status class for streams, or Reference condition. Three other ordinal categories of water quality were defined below, in bands of equal values: Moderate for low degree of alteration; Poor for an intermediate degree of alteration; and Bad for high degree of alteration.

Model validation

The Biological Monitoring Working Party (BMWP) score system (Hawkes, 1998; Paisley et al., 2014) modified by Monteiro et al. (2008) was calculated as a comparative index to ISI in assesses the effects on the ecosystem along the disturbance gradient. The BMWP is an index based on aquatic macroinvertebrates assemblages and has been widely used by regulatory authorities around the world as the basis of their river status classification system since 1980. Because of its low cost and easily applicability, the BMWP score system have been adapted and extensively used in many South American countries, including Argentina (Capítulo et al., 2001) and Brazil (Junqueira et al., 2000; Reis et al., 2017).

Pearson correlations were performed to identify the relationship between ISI (absolute and EQR values) and the BMWP. Differences in the indices values between reference and disturbance sites were still assessed with t-Tests. Disturbance characteristics were summarized with a Principal Component Analysis (PCA; function *rda*; vegan package). The PCA is a useful approach for selecting metrics wherein patterns of covariation and independent variation are identified to reduce the dimensions of the assemblage space and then the axis with the greatest contribution in explaining the variance was selected to validate the index. We still performed linear regression models (function *lm*, stats package) to complement the validation of the index and quantify individual relationship between EQR values and disturbance characteristics or the scores of the PCA axis. Yet, the structural descriptors of community were compared between reference and disturbance sites with t-tests. When necessary, data were transformed by log or arccosine prior to analyses. We conducted all statistical analyses in R version 3.1.2 GUI 1.65 Snow Leopard build (R Development Core Team, 2014) using the SIAR package for isotopic data analyses (Parnell and Jackson, 2013).

RESULTS

The significant difference between reference and disturbed sites regarding canopy openness (W = 106; $P = 0.04$), pasture (W = 105.5; $P = 0.02$) and forestry areas (W = 94; $P = 0.005$) validated the categorization of sampling sites and evidenced changes in the natural ecosystems. However, we found no significant difference in species richness ($t_{14.6} = 0.91$; p = 0.37), Pielou's evenness (t = -1.94; df = 19.3; p = 0.06), Shannon (t = -1.02; df = 25.6; p = 0.31) or Simpson' diversities (t = -1.37; df = 21.1; p = 0.18) between reference and disturbed sites (Table 2).

The reference streams showed a clear separation into three distinct groups (Figure 1), reflecting similarities among natural landscape characteristics. The values obtained by bootstrap permutations confirmed the separation of the cluster into different reference conditions: smaller streams (AU = 98, BP = 12), larger streams (AU = 98, BP = 19) and one stream uncategorized (DF04) that was then removed from further analysis. This separation suggests greater differences in the characteristics between smaller and larger streams than among the sites within the same group (Supplementary material 1). The differences between small and larger streams were pronounced in stream order (t = 4.67; df = 15.7; p < 0.001), distance from the source (t = 7.17; df = 10.9 p < 0.001) and stream depth (t = 3.48; df = 12.5; p = 0.004) (SM1). The visual inspection of the NMDS based on natural landscape characteristics and containing the reference sites + each disturbed stream indicated that DF22, DF25, DF26, DF27 and DF28 are more similar to larger streams characteristics, while DF09 and DF17 are more similar to smaller streams (Supplementary material 2).

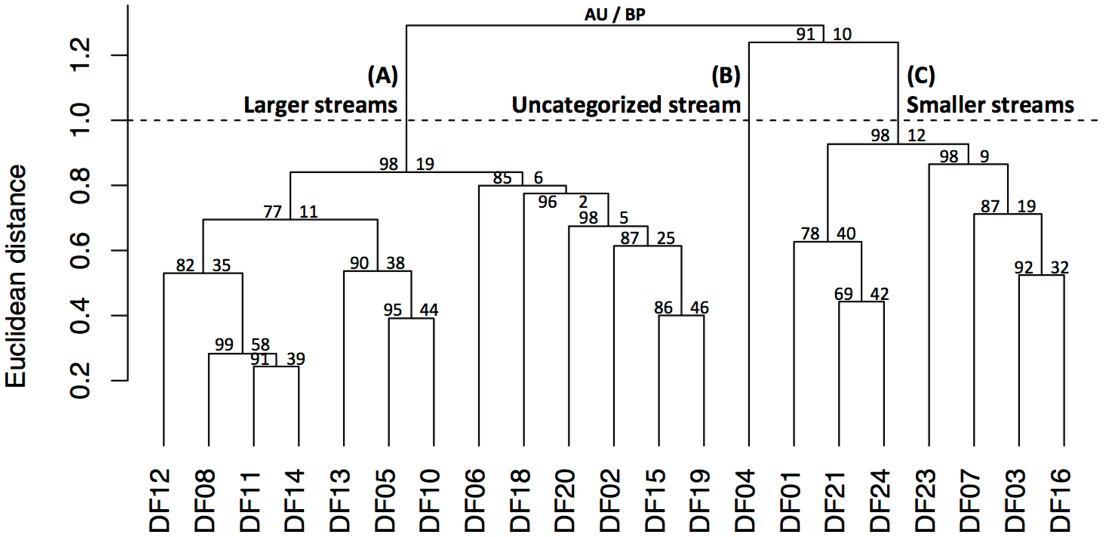

Figure 1 Cluster dendrogram with AU/BP values (%) of the natural landscape characteristics for the reference sites. Significant bootstrap groups (*P* < 0.05) are indicated with dashed line. AU *P*-value: Approximately unbiased bootstrap; BP *P*-value: Bootstrap probability.

The metrics varied widely across the sites, but only four [centroid distance (CD), carbon range (CR), nitrogen range (NR) and total area (TA)] were statistically significant with one or more disturbance characteristics and selected as candidates for the index (Fig 2A). Of these, only CD (t = 2.92; df = 15.6; *P* = 0.01), NR (t = 2.26; df = 8.1; *P* = 0.05) and CR (t = 2.83; df = 12.7; *P* = 0.01) differed between reference and disturbance sites and were finally included in the index (Figure 2). The weighting factors for each core metric obtained from the sum of the angular coefficients of the significant relations of the variables with the disturbance characteristics were -0.12, 0.11 and -0.87 to CD, CR and NR, respectively.

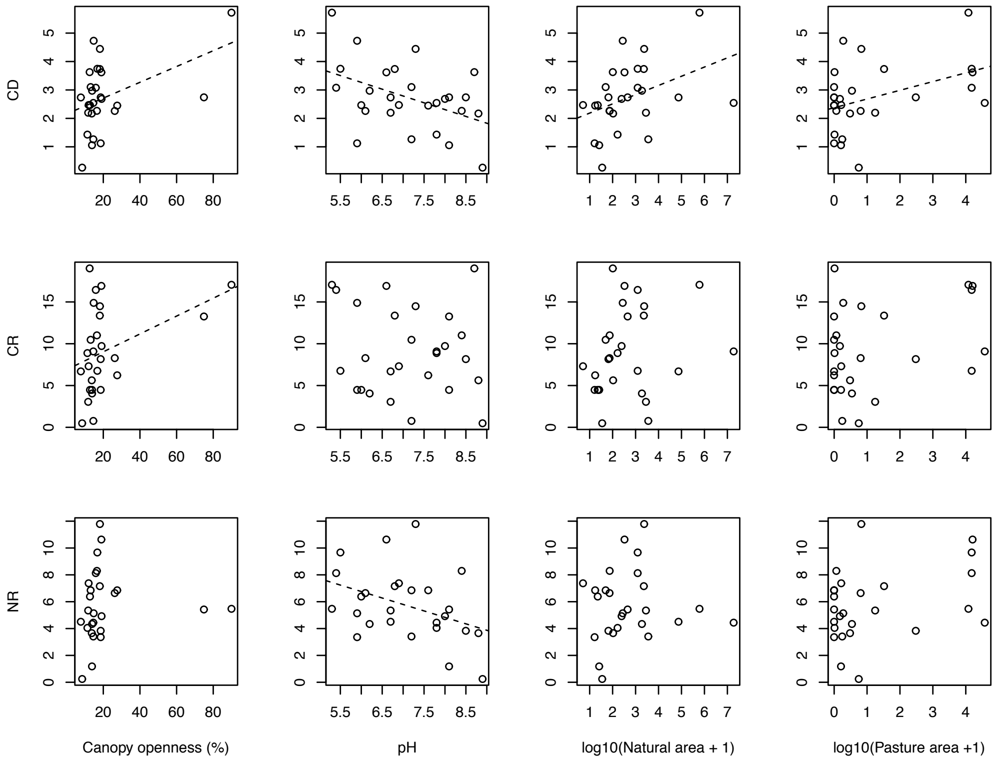

Figure 2 Significant metrics of the food-web structure in relation to the environmental disturbance gradients. Significant relationships between food-web structure and an environmental variable are depicted by the presence of a dashed line. CD: centroid distance; CR: carbon range; NR: nitrogen range.

The calculated stable isotope index (ISI) ranged widely along the disturbance gradient, from 0.01 to 3, with values significantly higher in reference (2 ± 0.56 SD) than in disturbance sites (1.46 ± 0.34 SD) (t = -2.8; df = 16.7; *P* = 0.01). The Ecological Quality Ratio (EQR) presents the same pattern, with values ranging from 0.01 to 1.47. The EQR values for reference sites (0.98 ± 0.28 SD) were also significantly higher than for disturbed sites (0.71 ± 0.17 SD) (t = -2.8, df = 16.7, *P* = 0.01). Among the reference sites, similar values were observed between small and larger streams, with ISI absolute mean values close to 2 and 0.95 to ISI EQR.

From the generated ISI scores we established the boundaries that delimit four quality classes with equal ranges to each reference condition: Reference, Good, Moderate and Poor (Table 3). Among reference streams, 16 (80%) were classified as Reference based on ISI classes. The remaining was classified as Moderate (2 streams; 10%) or Poor (2 streams; 10%). Significant differences were observed in classes between reference and disturbance streams (t = 2.81; df = 10; *P* = 0.01). Pearson correlations tests did not show significant relationships between ISI EQR and BMWP (r = - 0.19; *P* = 0.33) or ISI absolute values and BMWP (r = 0.27; *P* = 0.16).

Table 2. Structural and functional descriptors of community, reference condition and bioassessment scores generated to each reference and disturbance sites. BMWP: Biological Monitoring Working Party score; ISI: ISotopic multimetric Index; EQR: Ecological Quality Ratio; CD: centroid distance; CR: carbon range; NR: nitrogen range; MNND: mean nearest neighbour distance; SDNND: standard deviation of the nearest neighbour distance; TA: total area.

	Prior classification	**Reference condition**	**Species richness**	**Simpson's index**	**Shannon's index**	**Pielou's evenness**	**BMWP score**	**ISI score**	**EQR**	**ISI classification**	**CD**	**CR**	**NR**	**MNND**	**SDNND**	**TA**
DF01	ref	Smaller	10	0.87	2.17	0.94	49	1.23	1	Moderate	3.74	13.36	7.15	2.40	3.40	42.38
DF03	ref	Smaller	9	0.87	2.12	0.96	43	2.64	0.79	Ref	1.26	0.77	3.41	1.67	0.37	0.52
DF07	ref	Smaller	8	0.85	1.98	0.95	45	2.08	1	Ref	2.97	4.05	4.35	5.94	1.86	25.48
DF16	ref	Smaller	10	0.88	2.21	0.96	60	3.00	0.05	Ref	0.27	0.48	0.24	0.54	1.86	25.48
DF21	ref	Smaller	18	0.94	2.83	0.98	116	2.04	1	Ref	2.47	4.48	6.39	2.37	1.50	11.06
DF23	ref	Smaller	8	0.85	1.99	0.96	47	1.95	0.96	Ref	2.74	6.70	4.50	2.43	1.86	25.48
DF24	ref	Smaller	5	0.78	1.55	0.96	23	0.73	1	Poor	5.72	17.05	5.47	1.68	2.17	47.96
DF02	ref	Larger	11	0.90	2.32	0.97	71	2.27	1	Ref	2.20	3.05	5.35	1.83	2.43	2.63
DF05	ref	Larger	7	0.83	1.86	0.96	30	2.27	0.68	Ref	2.17	5.63	3.66	0.72	0.59	10.83
DF06	ref	Larger	20	0.94	2.92	0.97	119	2.04	0.79	Ref	2.55	9.09	4.44	1.42	1.70	19.28
DF08	ref	Larger	12	0.90	2.39	0.96	70	1.96	0.87	Ref	2.69	9.73	4.92	0.66	0.51	26.05
DF10	ref	Larger	13	0.91	2.50	0.98	78	1.77	1	Moderate	3.10	10.47	6.85	0.58	0.52	35.12
DF11	ref	Larger	4	0.69	1.26	0.91	19	2.03	1	Ref	2.26	8.28	6.64	0.86	1.18	28.26
DF12	ref	Larger	13	0.91	2.49	0.97	80	1.99	1	Ref	2.47	7.31	7.37	0.69	0.40	22.84
DF13	ref	Larger	12	0.90	2.40	0.97	73	2.66	0.2	Ref	1.06	4.47	1.18	0.57	0.70	3.14
DF14	ref	Larger	17	0.93	2.76	0.97	98	1.87	1	Ref	2.27	11.00	8.29	0.67	1.55	37.78
DF15	ref	Larger	10	0.88	2.22	0.96	64	2.05	1	Ref	2.45	6.23	6.85	0.82	0.58	28.23
DF18	ref	Larger	14	0.92	2.57	0.97	88	0.65	1	Poor	3.63	19.02	23.38	1.15	3.40	130.66
DF19	ref	Larger	17	0.93	2.77	0.98	97	2.31	0.66	Ref	1.43	8.88	4.05	0.49	0.97	18.42
DF20	ref	Larger	12	0.90	2.37	0.95	71	2.55	0.58	Ref	1.12	4.47	3.36	0.70	0.80	4.18
DF04	ref	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
DF09	imp	Smaller	11	0.89	2.30	0.96	61	0.79	0	Poor	4.44	14.50	11.78	1.89	2.23	32.69
DF17	imp	Smaller	12	0.91	2.43	0.98	63	1.90	0.7	Ref	2.74	8.16	3.83	1.08	0.44	11.06
DF22	imp	Larger	13	0.91	2.50	0.97	67	1.33	0.8	Moderate	4.73	14.89	5.13	1.00	1.23	32.50
DF25	imp	Larger	9	0.87	2.11	0.96	47	1.62	0.58	Moderate	3.75	6.76	9.67	0.72	0.47	16.20
DF26	imp	Larger	19	0.94	2.85	0.97	117	1.52	0.69	Moderate	3.08	16.43	8.13	1.03	2.08	41.51
DF27	imp	Larger	12	0.90	2.41	0.97	64	1.28	0.57	Poor	3.62	16.91	10.63	1.26	2.13	75.46
DF28	imp	Larger	12	0.90	2.41	0.97	69	1.82	0.81	Moderate	2.74	13.27	5.42	1.30	1.61	43.11

Table 3. Ordinal quality classes for smaller and larger streams and their respective class boundaries derived from the Ecological Quality Ratio (EQR) values.

	Upper boundary	Classes	Lower boundary
Smaller streams		Reference	≥ 0.78
	0.78 <	Moderate	≥ 0.52
	0.52 <	Poor	≥ 0.26
	0.26 <	Bad	≥ 0.00
Larger streams		Reference	≥ 0.96
	0.96 <	Moderate	≥ 0.64
	0.64 <	Poor	≥ 0.32
	0.32 <	Bad	≥ 0.00

The PCA1 was the axis with greatest contribution of explanation (23% of the variance) for the disturbances characteristics among sites and, therefore, used to validate the index. The index revealed an anthropogenic pressure gradient across the sites (Fig 3). Pasture area (R^2 = 0.3; *P* = 0.003), naked soil (R^2 = 0.16; *P* = 0.04), mosaic of occupations (R^2 = 0.18; *P* = 0.03) and the percentage of modified area (R^2 = 0.17; *P* = 0.03) were significant related to the index, with higher values related to highly catchment-disturbed sites (Figure 3). The index was also significantly related with the disturbances characteristics summarized in the PCA1 (R^2 = 0.33; *P* = 0.002). On the other hand, BMWP values do not were significantly related to any disturbance characteristic (*P* > 0.05).

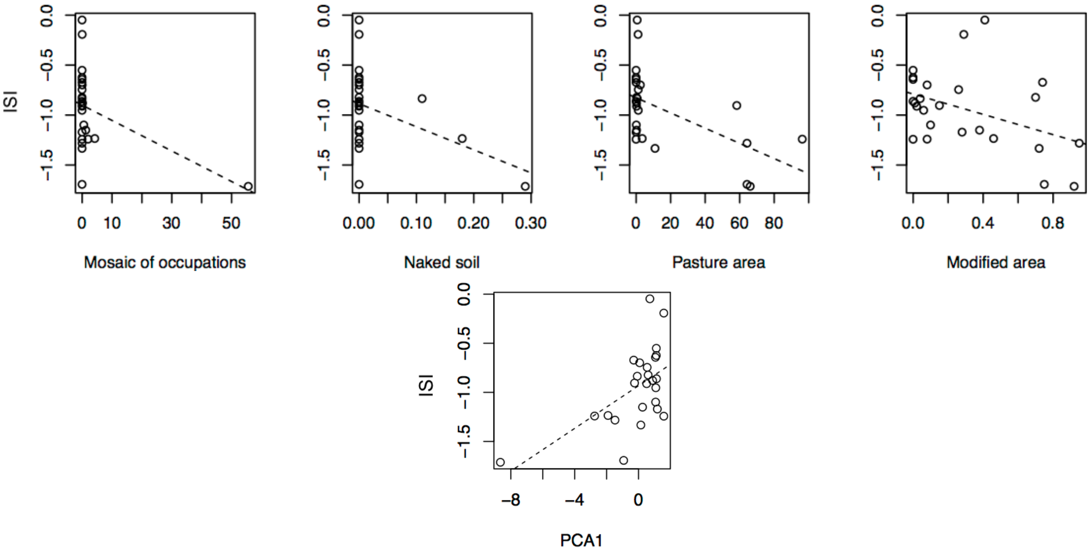

Figure 3. Significant environmental disturbance variables in relation to the Isotopic multimetric Index (ISI).

DISCUSSION

The stable isotopic metrics were effective in discriminating reference from disturbed sites. In particular, the centroid distance, carbon range and nitrogen range were those that effectively discriminate impacts along the disturbance gradient. Changes in these properties are expected in stressed food webs, due to the loss of sensitive species or entire trophic levels (McCann, 2000; Odum, 1985) and have been reported in previous studies associated to food webs of disturbed streams (Parreira de Castro et al., 2016). According to Layman et al. (2007), land use changes can be negatively reflected in all metrics, mainly when associated to the changes in species composition (e.g., loss of a specialized filter feeder), which leads to a decrease in overall trophic diversity in the food web. The decreasing in the NR corroborates this hypothesis since that means a simplification of the aquatic assemblages in terms of trophic levels along the disturbance gradient. Previous studies have observed similar results due the loss of specialist taxa associated with changes in the relative importance of the basal food resources (Benstead and Pringle, 2004; Parreira de Castro et al., 2016). Increases in CR and CD also can also be related to the replacement of species in the community in disturbance sites and the establishment of new basal resources with a relatively enriched $\delta^{13}C$ signature (e.g., C_4 plant species), or even trophic plasticity according to changes in the availability of resources. Parreira de Castro et al. (2016) in a comparison between preserved sites and other disturbed by pastures or sugar cane plantations, in the Brazilian savannah, reported that pasture sites had greater ranges of resources exploited, indicating a higher trophic diversity than sites with natural cover or sugar cane plantation. Recently, a global evaluation of the consequences of non-native species on the isotopic structure of freshwater fish communities demonstrates that communities in lotic ecosystems containing non-native species had a larger total isotopic niche than communities without non-native species (Sagouis et al., 2015).

Our aim was also to test the suitability of an isotopic index as biomonitoring tool by comparing it with more traditional structural metrics. In terms of detecting impacts, the isotopic index provided a more sensitive and complete tool than the BMWP score or traditional structural index. While the BMWP, species richness and Pielou’s evenness, as well as the Shannon’ and Simpson’ diversity have failed in identifying significant effects of disturbances, the index was sensitive in revealing changes along the human-induced disturbance gradient. From the index we could observe that land-use changes can drive the food-web structure in headwater streams.

The index was even more sensitive to disturbances than the metrics themselves, presenting significant associations with a greater number of disturbance variables and with the disturbance characteristics summarized in the PCA axis. In fact, previous freshwater studies have shown traditional species diversity indices to perform poorly in discriminating anthropogenic influences on biodiversity, as relevant information for the description of communities such as the presence of sensitive species, proportion of functional groups or trophic aspects are reduced to presence/absence and number of species (Heino et al., 2007; Magurran and Dornelas, 2010). It has been suggested that biodiversity measures should go beyond indices that only portray variability in species richness and relative abundances, taking into account the evolutionary relatedness of species and/or functional aspects of assemblages (Harper and Hawksworth, 1994). In agreement, Hogsden and Harding (2014), assessing the effects of mine pollution on stream food webs, suggested that stable isotopes provide a more advanced tool for assessing the disturbance effects than any combination of structural and functional metrics, gave new insights into how food webs were disrupted and are likely to be more useful for guiding stream management and rehabilitation strategies.

As reported in previous studies, we understand that interpreting isotopic metrics can be more challenging than for other metrics, which generate “scores” or “values” that are assigned to different levels of pollution (e.g., BMWP biotic score; Wright et al., 1988), because ‘good’ isotopic metric values may differ among ecosystems or regions. However, with the creation of an index we have been able to summarize the sensitivity and effectiveness of the isotopic metrics in a classification of easy understanding. In addition, the index was not only more effective and sensitive to disturbances than traditional measures, as also the sample collection and processing for stable isotope analysis is easy to complete and replicate, and the isotopic metrics are relatively simple to calculate using $\delta^{13}C$ and $\delta^{15}N$ data. This highlight that while the equipment used to run analyses (e.g., mass spectrometer, elemental analyser) is expensive and requires specialized skills to use, the isotopes provide a relatively quick and cost-effective way of constructing food webs and potentially provide more information compared to traditional approaches (Fry, 1991).

Four of the stream a priori selected as reference sites, were classified below Reference by the ISI. These results can be explained by the potential anthropic interference in these specific sites. Although the streams are in conservation units, both the PNB as APA, and have good physical and chemical conditions, they are surrounded by major cities of the Federal District, such as Plano Piloto and Estrutural.. In addition, both conservation units have areas with a high recreational and touristic value for the local population and are thus not pristine. The poorer classifications of these sites reinforce the high sensitivity of ISI to changes in functional properties of the stream, even under a small disturbance level, which was not detected by traditional taxonomic-based metrics.

Overall, our results show that a multimetric index based on isotope values could be a relevant tool for biomonitoring and evaluating the effects of land use changes on the dynamics and functioning of headwater streams. The isotopic analysis contributed also to improve our understanding of how stream consumers and their trophic interactions were affected by land use changes. Thus, we believe that such an isotopic index could be a particularly useful tool when applied to stream management or rehabilitation efforts, supporting and broadening or even substituting the assessment made by taxonomic-based structural methods. This is particularly relevant in regions where the expertise and knowledge and taxonomy of fauna and flora is not so well-developed, leading to poorer ability of methods to detect changes in freshwater ecosystems quality. Further developments of this approach should consist on widening the study area, covering other river types, regions and biomes to allow for generalization of our conclusions.

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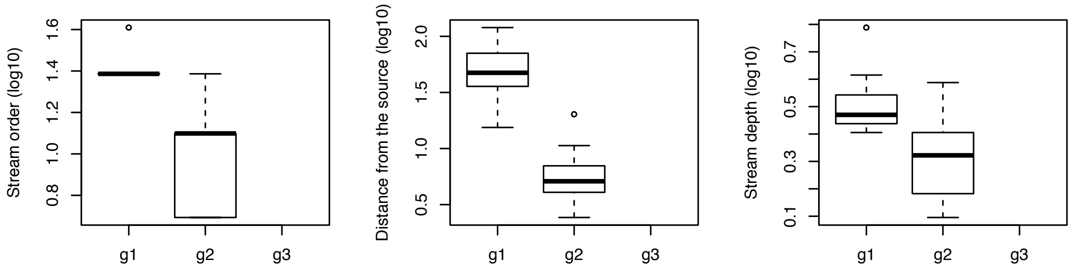
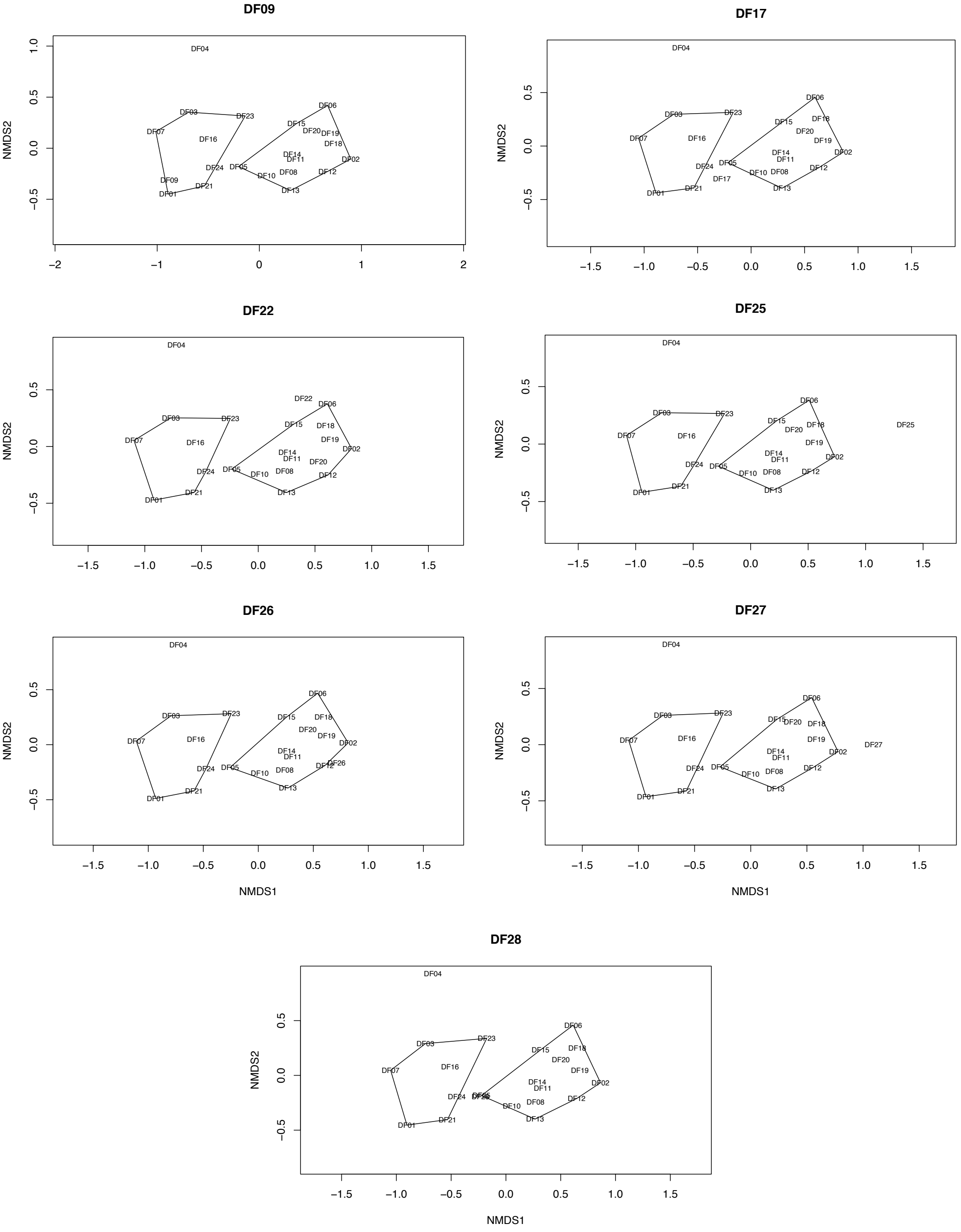
SUPPLEMENTARY MATERIAL 1



SM1. Natural landscape characteristics statistically significant among the stream types observed in cluster.

SUPPLEMENTARY MATERIAL 2



SM2. Non-metric Multidimensional Scaling (NMDS; Euclidean distances) based on natural landscape characteristics for each disturbed stream + all reference streams.

CONCLUSÕES GERAIS

Esta tese utilizou ferramentas internacionalmente validadas (por exemplo, isótopos estáveis e índices multimétrico) com o intuito de investigar a estrutura e dinâmica de comunidades aquáticas em riachos de cabeceira, questões ainda não esclarecidas para bacias tropicais e para o bioma do Cerrado. Os dados biológicos foram obtidos a partir de protocolos padronizados e esforço amostral significativo para os objetivos propostos. Ainda, utilizando de características funcionais das teias tróficas foi proposto um índice sensível a perturbações na qualidade ambiental para riachos de cabeceira.

Os resultados da tese confirmaram que riachos de cabeceira no Cerrado são ambientes de grande heterogeneidade de habitats, o que se reflete em uma complexa estrutura biótica. Fatores ambientais locais foram os principais responsáveis pela estrutura e composição das assembleias de macroinvertebrados bentônicos nos ambientes estudados. Porém, a escala amostral apresentou-se como um fator importante na determinação da diversidade nesses ecossistemas. Esta avaliação ambiental integrada em várias escalas espaciais, além de possibilitar a comparação temporal, no futuro, poderá potencialmente oferecer subsídios para a gestão de bacias hidrográficas.

Apesar da diversidade das assembleias ter respondido significativamente a complexidade dos ecossistemas, o mesmo não foi observado na dinâmica trófica desses ambientes. Fontes de energia autóctone sustentaram maior parte de organismos em riachos de cabeceira no Distrito Federal, sugerindo baixo nível de especialização na dieta dos taxa. Esse resultado apontam que a disponibilidade de recursos autóctones é independente da cobertura do dossel ou vegetação ripária sobre os riachos. Contudo, a dieta das espécies foi afetada significativamente pela estrutura e produtividade dos habitats e os organismos, tanto generalistas quanto especialistas, podem apresentar plasticidade trófica em função de variações no ecossistema.

A metodologia isotópica mostrou-se útil não apenas no estudos de teias tróficas e dos efeitos do gradiente ambiental sobre as comunidades aquáticas, mas também na composição de um índice multimétrico. O proposto Índice Isotópico Multimétrico (ISI) foi eficiente para a avaliação de distúrbios antropogênicos, sendo mais sensível em detectar mudanças ambientais que ferramentas tradicionais de avaliações de impacto ambiental como descritores de diversidade ou mesmo o indicador biológico BMWP (Biological Monitoring Working Party). Desta forma, o ISI poderia ser considerado uma alternativa ou complementar em avaliações da qualidade ecológica dos ecossistemas.

PERSPECTIVAS FUTURAS

Em pesquisas futuras, alguns tópicos podem ser considerados para serem explorados em outros projetos:

- Aumento do número de pontos amostrais em maiores escalas com o intuito de obter um esforço amostral balanceado para avaliação da diversidade.
- A possibilidade de incorporação de dados adquiridos em outras bacias hidrográficas com metodologia semelhante, o que permitirá avançar no entendimento de como outras escalas espaciais influenciam as assembleias biológicas na região neotropical, bem como permitindo comparação e validação da metodologia utilizada nesta tese.
- Avaliação dos fluxos e estoques de carbono na comunidade aquática com a finalidade de aumentar o entendimento da importância relativa de fontes alimentares autóctones e alóctones em riachos tropicais.
- Estudo das influências de variações temporais sobre a estrutura e dinâmica das comunidades, o que permitiria um refinamento do modelo multimétrico, bem como dos fatores determinantes da diversidade.