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Tiago Borges Kisaka

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Tese apresentada ao Programa de Pós-Graduação em Ecologia da Universidade de Brasília, para a obtenção do Título de Doutor em Ecologia.

Orientadora: Profa. Dra. Gabriela Bielefeld Nardoto

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“*A natureza é perfeita na sua imperfeição, e bela com todos os seus contrastes*”

O Jardim do Rei

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Resumo

Escopo e objetivos

Córregos de cabeceira fornecem importantes serviços ecossistêmicos para ambientes aquáticos a jusante, com intermédio da comunidade de macroinvertebrados bentônicos para decomposição e a disponibilização de matéria orgânica e nutrientes na coluna d'água, bem como, na transferência de energia aos níveis superiores da cadeia trófica. A comunidade de invertebrados é moldada pela reduzida disponibilidade de luz e pelo regime hidrológico sensível aos eventos de precipitação que caracterizam os córregos de cabeceira florestais. Para entender como a estrutura e as interações tróficas da comunidade de macroinvertebrados são influenciadas pelas características do habitat, conduzimos três estudos de caso em córregos de cabeceira florestais no Brasil Central. No primeiro estudo, o monitoramento realizado em quatro córregos na escala de microhabitats permitiu identificar atributos funcionais (traits) da comunidade de macroinvetebrados que foram sensíveis e tolerantes às mudanças sazonais no regime hidrológico (estação chuvosa e seca). No segundo estudo de caso, verificamos o efeito da sazonalidade na transferência de energia dos recursos basais (biofilme, seston e serapilheira) para os invertebrados, utilizando a metodologia com isótopos estáveis de carbono (C) e nitrogênio (N). O monitoramento em campo foi realizado no mesmo arranjo utilizado para o primeiro estudo, permitindo a comparação dos nichos isotópicos dos macroinvertebrados entre as estações chuvosa e seca. No terceiro estudo, avaliamos como os fatores *top-down* (pastejo) e *bottom-up* (luz e nutriente) atuam no desenvolvimento do biofilme e se a atividade fotossintética determina o fracionamento isotópico do carbono no biofilme. Para tanto, a densidade de macroinvertebrados pastejadores foi controlada com pulsos elétricos em quatro córregos, dois oligotróficos e dois enriquecidos em nutrientes. Estruturas eletrificadas (exclusão de roçadores) e não eletrificadas (com raspadores) foram implementadas em áreas abertas e sombreadas em cada córrego. Assim como os invertebrados coletados nas estruturas não eletrificadas, o biofilme extraído em ambas as estruturas (eletrificadas e não eletrificadas) foi submetido à análise do isótopo estável carbono. Matéria seca livre de cinzas (AFDM) e concentração de clorofila-a do biofilme também foram analisadas. A presente tese foi desenvolvida sob a autorização do Sistema de Autorização e Informação em Biodiversidade (SISBIO) nº 56144 e do Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN) nº de cadastro A018ECD.

Conclusões

Sazonalidade e gradiente de luz são forças seletivas para a comunidade de macroinvertebrados bentônicos nos córregos de cabeceira florestais. A alteração hidrológica sazonal demonstrou a dependência dos macroinvertebrados quanto à conformação dos microhabitats, favorecendo traits tolerantes ao estresse hidráulico na estação chuvosa. A redução na disponibilidade de alimentos na estação chuvosa refletiu em redução na diversidade de recursos alimentares assimilados pelos macroinvertebrados, além do aumento na redundância funcional da comunidade. Na estação seca, traits sensíveis apresentaram condições adequadas de desenvolvimento, preditas principalmente pelo acúmulo de serapilheira. Com isso, o estresse hidráulico mostrou-se importante para o entendimento da composição da comunidade de invertebrados, principalmente em função da dependência da serapilheira para fornecimento de alimento, habitat e refúgio. Com relação ao gradiente de luz, invertebrados pastejadores foram indiretamente favorecidos pelas condições ambientais que resultaram em aumento da produtividade primária e mudanças no fracionamento isotópico do carbono no biofilme. É importante destacar que esses córregos são naturalmente sombreados e oligotróficos e as mudanças na cobertura florestal ripária podem promover fluxos de energia a partir de recursos autóctones, afetando as características e o funcionamento da teia alimentar e a capacidade desses em fornecer serviços ecossistêmicos associados à manutenção da qualidade da água.

Palavras-chave: habitat templet, sistemas prístinos, sazonalidade, traits funcionais, relação trófica, nicho isotópico, isótopos estáveis, ecologia experimental, biofilme.

Abstract

Background and goals

Headwater streams provide important ecosystem services for downstream aquatic environments, mediated by the benthic macroinvertebrates community that favors the decomposition and availability of organic matter and nutrients in the water column. The structure and functioning of the invertebrate community are shaped by the reduced availability of light and by the hydrological regime sensitive to precipitation events that characterize the forest headwater streams. To understand how the structure and trophic interactions of the macroinvertebrate community are influenced by habitat features, we conducted three studies case in forest headwater streams in Central Brazil. In the first study, the monitoring of four microhabitat streams allowed the identification of functional traits of benthic macroinvetebrates that were sensitive and tolerant to seasonal changes in the hydrological regime (rainy and dry seasons). In the second case study, we verified the effect of seasonality on the transfer of energy from the basal resources (biofilm, seston and litter) to invertebrates, through the methodology using stable isotopes of carbon (C) and nitrogen (N). Field monitoring was performed in the same arrangement used for the first study, allowing the comparison of the isotopic niches of the macroinvertebrates between the rainy and dry seasons. In the third study, we evaluated how the top-down (grazing) and bottom-up factors (light and nutrient) act in the development of the biofilm and whether the photosynthetic activity of the biofilm determines the isotopic fractionation of carbon. For that, the density of grazers macroinvertebrates was controlled with electric pulses in four streams, two oligotrophic and two enriched in nutrients. Electrified structures (scraper exclusion) and non-electrified structures (with scrapers) were implemented in open and shaded areas in each stream. Like the invertebrates collected in the non-electrified structures, the biofilm extracted in both structures (electrified and non-electrified) was subjected to stable isotope carbon analysis.

Ash-free dry matter (AFDM) and chlorophyll-a concentration of the biofilm were also analyzed.

Conclusions

Seasonality and light gradient are selective forces for the community of benthic macroinvertebrates in forest headwater streams. The seasonal hydrological alteration demonstrated the dependence of the macroinvertebrates on the conformation of the microhabitats, favoring traits tolerant to the hydraulic stress in the rainy season. The reduction in food availability in the rainy season reflected a reduction in the diversity of food resources assimilated by the macroinvertebrates, in addition to the increase in the functional redundancy of the community. In the dry season, sensitive traits presented adequate conditions of development, predicted mainly by the accumulation of litter. Thus, hydraulic stress was important for the understanding of the composition of the invertebrate community, mainly due to the dependence of the litter for food supply, habitat and refuge. Regarding the light gradient, grazer invertebrates were indirectly favored by environmental conditions that resulted in increased primary productivity and changes in carbon isotopic fractionation in the biofilm. It is important to note that these streams are naturally shaded and oligotrophic and changes in riparian forest cover can promote energy flows from autochthonous resources, affecting the characteristics and functioning of the food web and their ability to provide ecosystem services associated with maintenance of the water quality.

Keywords: habitat templet; pristine systems, seasonality, functional traits, trophic relationship; isotope niche, stable isotopes, experimental ecology, biofilm.

Sumário

1 Introdução Geral

Os córregos de cabeceira florestais são caracterizados pelo predomínio da produção primária alóctone e regime hidrológico sensível aos eventos de precipitação (Meyer et al., 2007; Townsend and Hildrew, 1994; Vannote et al., 1980). Suas drenagens são de primeira a terceira ordem, sombreados pela vegetação ripária e que fornecem importantes serviços ecossistêmicos, incluindo água, nutrientes, matéria orgânica e invertebrados (alimento potencial para peixes e outros vertebrados) para ambientes aquáticos a jusante (Storey et al., 2011; Suter and Cormier, 2015). Além da fonte de recurso e das características hidrológicas/hidráulicas, a estrutura e o funcionamento dos córregos de cabeceira florestais dependem da comunidade de invertebrados aquáticos que atua como um elo entre os recursos basais e os níveis tróficos superiores (herbívoros e predadores) (Covich et al., 1999; Gessner et al., 1999).

Como a produtividade primária nesses ecossistemas é limitada pela reduzida incidência de luz no corpo hídrico, a serapilheira (folhas e detritos lenhosos) proveniente da zona ripária é a principal fonte de matéria e energia (Vannote et al., 1980). Sua incorporação na cadeia trófica é intermediada pelos invertebrados fragmentadores que tanto consomem a matéria orgânica particulada grossa (CPOM) quanto disponibilizam detritos em menores frações na coluna d’água (Gessner et al., 1999; Graça, 2001). Além de alimento, a serapilheira acumulada no leito do córrego pode constituir habitat ou ambiente remanescente de colonização após eventos de distúrbio (Dobson and Hildrew, 1992).

Em condições naturais, distúrbios nos bancos de serapilheira podem decorrer de mudanças temporais na produção, como observado em ambientes temperados que apresentam pulsos de queda de folhas no outono (Wallace et al., 2015). Já em ambientes tropicais com produção de serapilheira contínua e em pequenas quantidades (Bambi et al., 2017), o distúrbio é resultado do regime hidrológico associado à capacidade de retenção da matéria orgânica alóctone no córrego. Nesse caso, eventos rápidos de vazão intensa (picos de vazão) gerados pela chuva modificam o acúmulo de serapilheira (Kiffney et al., 2014; Speaker et al., 1984), podendo refletir na estrutura e nas respostas ecológicas da comunidade de invertebrados em córregos de cabeceira florestais (White et al., 2018).

Como preconiza o conceito do “*Habitat Templet”* (Southwood, 1977), as respostas ecológicas são o resultado do ajuste evolutivo dos traits funcionais dos organismos a um modelo (*templet*) fornecido pelos habitats. Nesse conceito, estabilidade e previsibilidade das variáveis ambientais, nível e constância de recursos são as características que definem o templet de cada habitat (Southwood, 1977); sob influência das interações bióticas (Greenslade, 1983). Essas características, denominadas "forças seletivas", atuam como "filtro ambiental" diante de organismos cujos traits não foram ajustados à sua variabilidade espacial ou temporal (Poff et al., 1997). Com isso, entender a heterogeneidade dos habitats pode favorecer a caracterização da comunidade de invertebrados (composição, abundância e diversidade) e a avaliação do papel dos traits para estrutura e funcionamento dos ecossistemas.

Com relação à variabilidade temporal, Tonkin et al (2017) classificaram o regime hidrológico sazonal (ocorrência de eventos em períodos específicos do ano) e previsível (elevada recorrência de eventos) como a força seletiva preponderante para a comunidade de invertebrados de córregos em diferentes biomas. Aqueles sob clima sazonal apresentaram maior diversidade de invertebrados (Tonkin et al., 2017), em função da possibilidade de coexistência de diferentes espécies durante determinado período de tempo, cada uma em suas condições ótimas e nenhuma sob condições precárias por longo período de tempo (Hutchinson, 1961). Na escala espacial, os invertebrados respondem a forças seletivas hierárquicas, desde grandes bacias, influenciadas pelo clima, geologia e cobertura do solo; até os microhabitats, cujo templet é definido pela combinação de características do substrato, velocidade e profundidade da água (Poff et al., 1997; Townsend and Hildrew, 1994).

Segundo Huet (1954), um rio da cabeceira até o mar, pode ser discretizado em zonas sucessivas, resultantes da ação combinada da temperatura e da velocidade da água, que condicionam ótimos de vitalidade para as populações de peixes. Denominado como conceito da "*Zonação*", essa foi a primeira tentativa de descrever por completo o ecossistema fluvial, sendo posteriormente aplicada para a comunidade de invertebrados (Illies and Botosaneanu, 1963). O conceito do "*Contínumm Fluvial*" (RCC, Vannotte et al., 1980) expandiu o conceito da Zonação para incluir as relações funcionais, permitindo descrever a estrutura e o funcionamento das comunidades ao longo de um sistema fluvial. A estrutura física acoplada ao ciclo hidrológico define transporte, armazenamento e uso de matéria orgânica pelos grupos funcionais de alimentação (GFA) dos macroinvertebrados (Vannote et al. 1980).

Apesar das divergências entre os padrões de zoneamento da fauna, conforme Huet (1954), e os gradientes abióticos da cabeceira à foz, apresentados por Vannote et al.

(1980), Statzner e Higler (1986) sugerem o conceito de "*Hidráulica de Córregos*" como uma alternativa ponderada. Nesse caso, apesar da possibilidade de interferência por outras variáveis abióticas e bióticas, variações espaciais e temporais dos parâmetros hidráulicos irão determinar mudanças na estrutura biótica dos córregos (Statzner e Higler, 1986). Essa abordagem requer medições de velocidade e profundidade da água e rugosidade do substrato, permitindo a estimativa de parâmetros hidráulicos, como o número de Froude (turbulência gerada pelo escoamento no leito do corpo hídrico). Merigoux and Doledec (2004) demonstraram que a distribuição de cerca de 70% dos táxons de invertebrados foi significativamente relacionada à tensão de cisalhamento (atrito do escoamento com o leito do rio) e ao número de Froude em um trecho do rio Ardèche, no sul da França.

Parâmetros hidráulicos, em conjunto com mecanismos de retenção física e a disponibilidade de nutrientes, são considerados importantes para definição dos grupos funcionais de alimentação em ambientes aquáticos, conforme observado pelo conceito do "*Espiralamento*" (Wallace et al., 1977). Este descreve a ciclagem de nutrientes à medida que são assimilados da coluna d'água no formato de biomassa bentônica, temporariamente retidos e mineralizados de volta para a coluna de água (Newbold et al., 1981; Wallace et al., 1977). Como inclui o transporte de nutrientes a jusante, essa ciclagem é considerada parcialmente aberta ou "em espiral" (Wallace et al., 1977). O comprimento do espiral, uma medida integrada entre velocidade da água e demanda bioquímica, indica a eficiência na utilização dos nutrientes (Newbold et al, 1981). No caso das cabeceiras florestais, que tendem a conservar e armazenar recursos devido à elevada atividade biológica e capacidade de retenção de matéria orgânica, o comprimento do espiral tende a ser reduzido (Lorenz et al., 1997).

Além da eficiência no uso dos recursos, córregos de cabeceira apresentam condições variáveis de habitat, permitindo sustentar uma comunidade de invertebrados diversa e, portanto, favorecer a biodiversidade dos ecossistemas a jusante (Dobson, 1994; Heaston et al., 2017; Meyer et al., 2007). Dobson (1994) atribuiu à variabilidade das condições do microhabitat o controle da diversidade de invertebrados em córregos de cabeceira. Ligeiro et al. (2010) verificaram que a amostragem em diferentes microhabitas em um único ponto de corredeira seria suficiente para coletar 75% dos táxons observados em um trecho de córrego de cabeceira, reduzindo o esforço amostral para representação da diversidade.

No entanto, o papel das condições ambientais nas estratégias de sobrevivência e alimentação, definindo a largura do nicho trófico dos táxons de invertebrados dominantes, permanece pouco explorado. Heino et al. (2009) apontaram que a avaliação da comunidade de invertebrados em áreas protegidas pode ser fundamental para predizer alterações em sua distribuição em decorrência das mudanças climáticas. Nesse caso, ênfase considerável tem sido dada aos córregos de cabeceira, devido às possíveis alterações no regime hidrológico somadas à elevação na temperatura da água (Callisto et al., 2012; Stanfield and Jackson, 2011).

Além disso, por apresentarem bacias hidrográficas com tamanho reduzido, córregos de cabeceira são considerados sensíveis às alterações na cobertura do solo, principalmente em função da dependência da vegetação ripária (Meyer et al., 2007), com consequências tanto para o regime hidrológico, com a intensificação do escoamento superficial, quanto para a alteração dos gradientes de luz, devido a remoção da vegetação ripária (Kaushal et al., 2014). Bechtold et al. (2017) verificaram, por meio análise de isótopos estáveis (SIA, *Stable Isotope Analysis*) de carbono (C), que o incremento na disponibilidade de luz favorece a produtividade primária (atividade fotossintética das algas), com potencial de alteração no metabolismo e no controle do fluxo de energia nos córregos de cabeceira (Bumpers et al., 2017). Sturt et al (2011) demonstram como o aumento na incidência de luz, associado a maiores concentrações de nutrientes, pode favorecer o desenvolvimento de algas filamentosas que, na ausência de herbívoros invertebrados, acumulam rapidamente nos córregos.

A projeção é de que mudanças climáticas e na cobertura do solo reduzam a resiliência e a complexidade biótica em córregos de cabeceira, contribuindo para a perda da integridade ecológica e de importantes serviços (Stanfield and Jackson, 2011). Em função disso, é necessário identificar as características que mantem a estrutura dos habitats e que devem ser manejadas ou recuperadas. Esse conhecimento permitirá entender como as oscilações climáticas, representadas principalmente pelas mudanças hidrológicas, e a remoção da vegetação ripária, com reflexos na transferência de energia e matéria, poderão moldar as funções desempenhadas pelos macroinvertebrados, com reflexo no funcionamento dos córregos de cabeceira.

Metodologia de isótopos estáveis carbono e nitrogênio

O fluxo de energia e as relações tróficas entre os organismos em um ecossistema podem ser avaliados por meio da metodologia de isótopos estáveis de carbono e nitrogênio (Layman et al., 2012). Isótopos estáveis são variações do mesmo elemento que têm uma massa atômica diferente, mas não são radioativas. A maioria dos elementos usados pelos seres vivos existem como vários isótopos. O carbono, por exemplo, existe em duas formas estáveis; ^{12}C e ^{13}C. A abundância relativa dos isótopos de carbono é usada para estudar as vias fotossintéticas nas plantas.

Plantas C_3 (como trigo) possuem enzimas que reagem mais rapidamente com o ^{12}C, de modo que resulta em mais produtos: açúcares, etc. As plantas C_4 (por exemplo, cana e milho) utilizam o caminho Hatch-Slack que é menos seletivo para ^{12}C e consequentemente mais enriquecido em ^{13}C (Martinelli et al., 2009). Dessa forma, a razão entre os isótopos estáveis ^{13}C e ^{12}C (expresso como $\delta^{13}C$) pode ser usada para diferir as fontes basais e como a composição isotópica do carbono do tecido dos animais é similar à de sua dieta, é um dos isótopos mais utilizados em estudos em teias alimentares aquáticas ou terrestres (Cifuentesl et al., 1988). Portanto, a utilização dos isótopos estáveis de carbono são empregados como um rastreador de fontes de carbono para consumidores ao longo da cadeia trófica (Perkins et al., 2014; Post, 2002).

As cadeias alimentares em sistemas lóticos são sustentados por duas fontes de carbono: autóctones (algas do biofilme, musgos e plantas aquáticas ligadas a um substrato) e alóctones (ou seja, serapilheira e invertebrados terrestres) (Nakano and Murakami, 2001). Razões de isótopos estáveis de carbono ($\delta^{13}C$) são amplamente utilizadas para discriminar o biofilme e a serapilheira em sistemas lóticos (Finlay, 2001). Muitos pesquisadores têm apontado a importância de se compreender o padrão de variabilidade nos valores $\delta^{13}C$ do biolfime para fins de análise precisa da cadeia alimentar (Doi et al., 2007; Finlay et al., 1999; Hill et al., 2008; MacLeod and Barton, 1998; Singer and Battin, 2007).

Embora a importância de entender a variabilidade dos valores de $\delta^{13}C$ do biofilme seja bem reconhecida, os fatores que determinam os valores $\delta^{13}C$ do biofilme são complexos devido às influências da heterogeneidade espacial em múltiplas escalas espaciais, tais como habitat local, trecho de córrego e bacia hidrográfica (Doi et al., 2007). Ishikawa e colaboradores (2012) desenvolveram um modelo hierárquico através de escalas espaciais mostrando que os controles locais para o biofilme $\delta^{13}C$ foram controlados por fatores regionais, o que sugere que a produtividade e a disponibilidade de CO_2 determinam o $\delta^{13}C$.

Para o nitrogênio, o fracionamento isotópico, ou seja, o enriquecimento ou empobrecimento do isótopo pesado da amostra em estudo (produto) em relação a sua fonte (substrato), do $\delta^{15}N$ (^{15}N e ^{14}N) varia geralmente de 2 a 4 ‰ a cada nível trófico

(Caut et al., 2009; Mccutchan et al., 2003), facilitando portanto caracterização da cadeia alimentar e a posição de um organismo na mesma (Layman et al., 2012; Vander Zanden and Rasmussen, 1999). Portanto, as relações isotópicas de $\delta^{13}C$ e $\delta^{15}N$ em tecidos animais podem refletir informações sobre o uso de habitats físicos, origem da matéria orgânica, relações tróficas, tamanho nichos e se estes se sobrepõem (Bearhop et al., 2004; Clapcott et al., 2010).

Recentemente, estudos tem utilizado as razões isotópicas (^{13}C:^{12}C e ^{15}N:^{14}N) dos tecidos dos consumidores para representar o nicho trófico de uma espécie (Bearhop et al., 2004), formalizando o conceito de "*Nicho Isotópico*" (Newsome et al., 2006). Porém, como mencionado por Jackson et al (2011), o nicho isotópico guarda uma estreita relação com o nicho trófico, porém eles não devem ser considerados como sinônimos, mas sim como uma aproximação (*proxy*) que permite descrever e traçar inferências sobre aspectos chave do nicho ecológico de uma espécie ou comunidade.

O delineamento dos espaços de nichos isotópicos (*isotope space* - biplot $\delta^{15}N$-$\delta^{13}C$) e as medidas de sobreposição interespecífica desses nichos podem fornecer um meio para quantificar nichos tróficos em comunidades naturais e inferir sobre o quão semelhantes são as dietas entre as espécies concorrentes (Jackson et al., 2011). Essa ferramenta pode ser utilizada para prever o potencial para a coexistência ou exclusão competitiva das espécies que utilizam dos mesmos recursos alimentares. Isso porque a variação isotópica de $\delta^{15}N$ e $\delta^{13}C$ nos indivíduos de cada espécie pode ser utilizada como uma medida de amplitude de forrageamento, além de definir os limites do nicho isotópico ocupado (Jackson et al., 2011; Layman et al., 2012).

No entanto, as cadeias alimentares são complexas e o número de fontes alimentares excede o número de marcadores de isótopos em mais de um. Neste caso, o modelo não gera valores exatos para as contribuições proporcionais de cada fonte, mas em vez disso oferece uma gama de possíveis contribuições (Layman et al., 2012). Atualmente, modelos de mistura aplicam a inferência Bayesiana para avaliar os dados de isótopos estáveis (Kadoya et al., 2012; Parnell et al., 2010), caracterizar as incertezas das fontes alimentares e os valores isotópicos do consumidor, bem como estimar as contribuições das diferentes fontes alimentares (Layman et al., 2012).

Com o intuito de investigar aspectos da estrutura trófica, tais como diversidade da dieta, recursos basais e níveis tróficos explorados e área do nicho isotópico, existem as métricas de Layman (Layman et al., 2007) derivadas dos valores isotópicos de $\delta^{13}C$ e

δ^{15}N dos consumidores. As métricas, adaptadas de Layman et al. (2007) e estimadas a partir da abordagem Bayesiana (Jackson et al., 2011), foram denominadas:

i) Amplitude de δ^{13}C (CR – "δ^{13}C *range*"): representa a diversidade da fonte basal;

ii) Amplitude de δ^{15}N (NR – "δ^{15}N *Range*"): descreve o comprimento trófico;

iii) Distância média ao centroide (CD – "*Mean distance to centroid*"): distância Euclidiana média de cada componente da comunidade ao centroide; mede a largura do nicho da comunidade (relacionada à diversidade trófica) e o espaçamento entre espécies na área isotópica;

iv) Distância média dos vizinhos mais próximos (MNND – "*Mean nearest neighbor distance*"): distância euclidiana média de cada grupo ao seu vizinho mais próximo no espaço do biplot δ^{13}C-δ^{15}N; indica a densidade e o agrupamento de espécies dentro da comunidade.

v) Desvio padrão da distância do vizinho mais próximo (SDNND - "*Standard desviation of nearest neighbor distance*"): mede a uniformidade do espaçamento dos grupos no espaço biplot.

Tanto o MNND quanto o SDNND informam sobre redundância trófica. Valores reduzidos de MNND indicam maior redundância trófica, ou seja, que existem muitos grupos com ecologias tróficas similares; e valores reduzidos de SDNND significam uma distribuição mais uniforme das espécies, sugerindo maior redundância trófica com diferentes grupos, porém, com ecologias tróficas semelhantes.

vi) Área total (TA – "*Total area*"): informa a área que engloba todas as espécies no espaço bi-plot δ^{13}C- δ^{15}N, como uma medida da quantidade total de espaço de nicho ocupado e, portanto, uma aproximação para a extensão total da diversidade trófica dentro de uma teia alimentar.

2 Objetivos e Estrutura

O objetivo do presente estudo foi verificar como a estrutura e as interações tróficas da comunidade de macroinvertebrados são influenciadas pelas características do habitat em córregos de cabeceira florestais no Brasil Central. Para atender esse objetivo, foram realizados os seguintes estudos:

No capítulo 1 investigamos os principais fatores que podem alterar a estrutura da comunidade de macroinvertebrados em córregos do Cerrado, considerando o efeito das mudanças sazonais (estação chuvosa e seca) do regime hidrológico na escala de microhabitats (Figura 1). Hipotetizamos que (I) na estação de chuva aumenta a importância de traits tolerantes ao alto fluxo, por exemplo, de tamanho corporal pequeno, alta flexibilidade corporal e com adaptações específicas de permanência e mobilidade; (II) na estação seca, a condição de fluxo estável favorece traits sensíveis a variação do fluxo sazonal, por exemplo, tamanho corporal maior, baixa flexibilidade corporal e sem adaptações de mobilidade e permanência a fluxos elevados; (III) a serapilheira é a principal variável de microhabitat para a comunidade de macroinvertebrados bentônicos e apresenta uma alta vulnerabilidade quanto a capacidade de retenção e acúmulo no leito do córrego em condições de alto fluxo.

Esse estudo foi realizado por meio da coleta de dados em quatro córregos prístinos do Distrito Federal, durante três campanhas de coleta na estação chuvosa (março-abril) e três campanhas na estação seca (julho-agosto), no ano de 2017. Foram amostrados invertebrados e características hidráulicas associadas ao substrato (tipo de substrato, profundidade e velocidade de fluxo) em 528 microhabitats. A análise dos dados consistiu principalmente da relação entre os traits dos macroinvertebrados e o estresse hidráulico, representado pelo número de Froude, na escala de microhabitat.

Figura 1. Efeito do pico de vazão (distúrbio de fluxo) observado na vegetação ripária do córrego Capetinga, um dos córregos de cabeceira monitoramento neste estudo.

No capítulo 2, com o objetivo de avaliar o efeito da sazonalidade na transferência de energia dos recursos basais para a comunidade dos macroinvertebrados bentônicos (Figura 2), testamos as hipóteses de que mudanças sazonais nas características hidráulicas reduzem i) a diversidade de recursos basais assimilados pelos invertebrados; e ii) e a complexidade da cadeia alimentar na estação chuvosa. As variações nas assinaturas isotópicas de carbono e nitrogênio dos recursos alimentares (biofilme, seston e serapilheira), consumidores e predadores foram medidas em escala de microhabitat, no mesmo arranjo experimental utilizado no capítulo 1, e as características dos nichos isotópicos dos macroinvertebrados foram comparadas entre as estações chuvosa e seca.

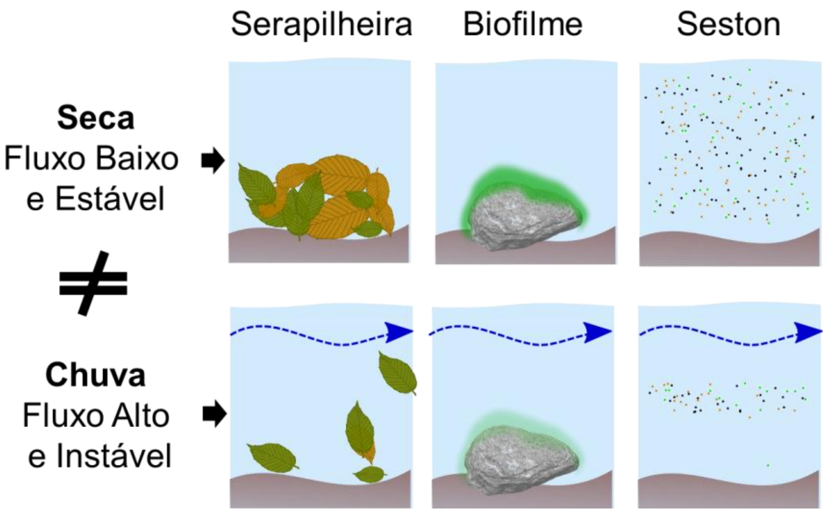

Figura 2. Alteração esperada dos recursos basais predominantes nos córregos de cabeceira florestais em resposta à variação do regime hidrológico.

No capítulo 3, avaliamos como os fatores *top-down* (pastejo) e *bottom-up* (luz e nutriente) atuam no biofilme e se a atividade fotossintética determina o fracionamento isotópico do carbono no biofilme (Figura 3). Nesse caso, o trabalho experimental proposto por Sturt et al. (2011), com foco na colonização pelo biofilme em estruturas eletrificadas (exclusão de herbívoros) e não eletrificadas (com herbívoros), foi implementado em áreas abertas e sombreadas em quatro córregos de cabeceira, dois oligotróficos e dois enriquecidos em nutrientes. Assim como os invertebrados coletados nas estruturas não eletrificadas, o biofilme extraído em ambas as estruturas (eletrificadas e não eletrificadas) após 15 dias de colonização foi submetido à análise do isótopo estável carbono. Matéria seca livre de cinzas (AFDM) e as concentrações de clorofila-a do biofilme também foram analisadas em laboratório. O capítulo 3 encontra-se em processo de revisão para publicação na revista Freshwater Biology.

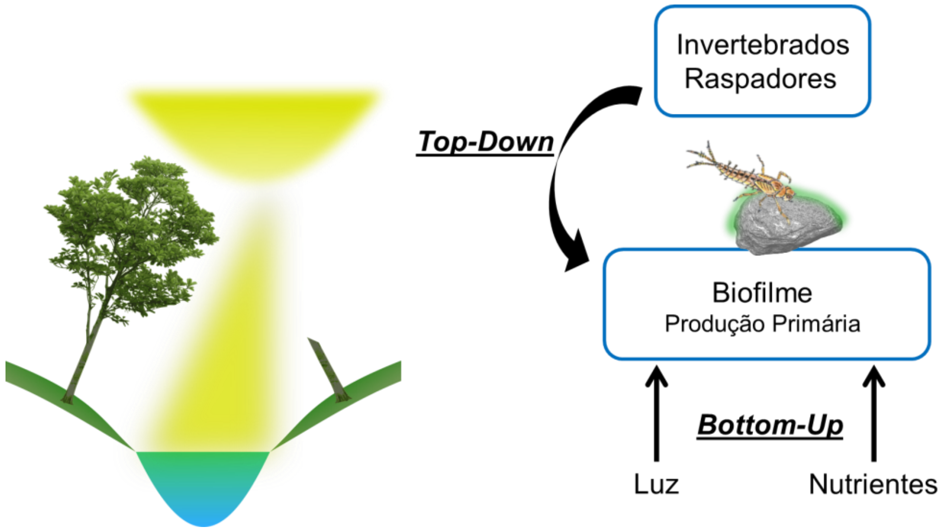

Figura 3. Fatores top-down e bottom-up associados ao desenvolvimento do biofilme em córregos de cabeceira florestais.

3 Resultados e Discussão Geral

No **capítulo 1** identificamos traits tolerantes ao estresse hidráulico significativamente superior na estação chuvosa, conforme observado pela comparação do número de Froude entre as estações ($W = 37655$, $p < 0,001$). O acúmulo de serapilheira no leito dos córregos foi significativamente menor na estação chuvosa (0,08 ±0,22 $m^2.m^{-2}$) em relação à estação seca (0,17 ± 0,25 $m^2.m^{-2}$) e inversamente relacionada ao número de Froude ($r = -0,27$, $p = 0,001$, $n = 528$), indicando que o estresse hidráulico, intensificado com a chuva, diminui a capacidade de retenção de serapilheira no leito dos córregos. Além disso, dentre as fontes de matéria orgânica oriundas da vegetação ripária (raiz, tronco e serapilheira), a serapilheira não se correlacionou com nenhuma métrica ou índice da comunidade de macroinvertebrados na estação chuvosa, diferentemente dos troncos, que apresentaram correlação positiva com a riqueza e diversidade taxonômica. A presença de troncos é um elemento estrutural chave em córregos florestados, pois aumenta a complexidade do córrego, a diversidade de habitats e oferece refúgio para muitas espécies (Faustini and Jones, 2003; Reid et al., 2008).

O modelo de seleção forward, aplicado à análise de redundância (RDA) com as variáveis do microhabitat, identificou o número de Froude como o principal preditor dos traits da comunidade na estação chuvosa (R^2 ajustado = 0,04; $p = 0,001$), apresentando correlação positiva com a diversidade de traits e a densidade de invertebrados. Em ordem de importância, os invertebrados também foram influenciados na estação chuvosa pelos substratos do tipo matacão (R^2 ajustado = 0,02; p = 0,005), seixo (R^2 ajustado = 0,01; p = 0,030), raízes (R^2 ajustado = 0.01; p = 0,029) e serapilheira (R^2 ajustado = 0,63; p = 0.004). A RDA explicou aproximadamente 10% da variação dos dados (13% não ajustado; F = 5,21; p = 0,001) e apenas o primeiro e o segundo eixo foram significativos.

Número de Froude e os seixos foram correlacionados negativamente com o primeiro eixo da RDA, explicando 60% da variação. Os traits de aderência ao substrato, alta flexibilidade corporal, coletores-filtradores, raspadores, com ventosas, escavadores endobentônicos, com garras tarsais e tamanho de corpo entre 2,5 e 10 mm foram também negativamente correlacionados com o primeiro eixo da RDA, indicando relação com o número de Froude e com os seixos. Esses traits indicam adaptações específicas em condições de alto fluxo.

Os filtradores-coletores aumentaram a importância na estação de chuva. Durante o período de alto fluxo o transporte de material orgânico fino na coluna de água foi favorecido, podendo indicar o aumento da abundância relativa de coletores-filtradores passivos (Georgian and Thorp, 1992). A importância de pequenos organismos com alta flexibilidade corporal também foi destacada na estação chuvosa, mostrando a capacidade potencial dos indivíduos encontrarem refúgio (de la Fuente et al., 2018; Robinson et al., 2004). Os traits nadadores na superfície d’água, sem adaptação ao fluxo, escavador epibentônico e de tamanho corporal entre 2,5 e 40mm foram positivamente correlacionados com o primeiro eixo, indicando relação inversa com o número de Froude e seixo.

A relação entre os traits adaptados ao estresse hidráulico, Froude e seixo pode ser resultado do uso de refúgios de fluxo na estação chuvosa, com o seixo oferecendo um abrigo mais adequado em comparação ao substrato fino (Theodoropoulos et al., 2017; Townsend et al., 1997). A superfície coberta por seixo ocupou mais de 70% do leito dos córregos e não foi alterada entre as estações. Esse substrato confere heterogeneidade de habitats e resistência à comunidade dos córregos diante de perturbações, permitindo que diferentes espécies coexistam (Brown, 2003), devido à disponibilidade de recurso alimentar e proteção contra predadores (Gjerløv et al., 2003; Hoover and Ackerman, 2011; Townsend et al., 1997). Substratos heterogêneos suportam comunidades com alta diversidade e riqueza funcional quando comparados com substratos homogêneos em córregos brasileiros (Milesi et al., 2016).

A ordenação ao longo do segundo eixo da RDA, que explicou 30% da variação total ajustada, foi determinada principalmente pelo tipo de substrato, havendo correlação com a serapilheira e o matacão. No entanto, de acordo com o segundo eixo, a serapilheira apresentou correlação negativa, enquanto o matacão apresentou correlação positiva. A maior parte dos traits foram correlacionados negativamente ao segundo eixo, indicando que traits não adaptado ao fluxo, predadores, com baixa flexibilidade e tamanho corporal e ganchos anais foram influenciados pela serapilheira.

Apesar de apresentar alta correlação com o segundo eixo da RDA, nenhum trait se mostrou correlacionado ao matacão. Esse substrato foi negativamente correlacionado com a riqueza taxonômica e representou menos de 5% do leito dos córregos. Certamente, sob condições de fluxo instável na estação chuvosa, os matacões podem expor organismos a condições severas (Erman and Erman, 1984; Poff and Ward, 1990), tornando-os vulneráveis à predação e aos distúrbios de fluxo (por exemplo, aumento na velocidade da água e na tensão de cisalhamento).

Seguindo nossa terceira hipótese, o modelo de seleção forward identificou que a serapilheira foi o principal preditor dentre as variáveis de microhabitat para os macroinvertebrados bentônicos na estação seca (R^2 ajustado = 0,08; p = 0,001), seguida pelo número de Froude (R^2 ajustado = 0,05; p = 0,001), e os substratos cascalho (R^2 ajustado = 0,03; p = 0,001), raízes (R^2 ajustado = 0,007; p = 0,028) e tronco (R^2 ajustado = 0.006; p = 0,037). A RDA explicou aproximadamente 18% da variação dos dados (20% não ajustada; F = 12,77; p = 0,001) e apenas o primeiro e o segundo eixo foram significativos.

A serapilheira foi correlacionada positivamente com o primeiro eixo da RDA que explica 70% dessa variação. A maioria dos traits também foi positivamente correlacionada ao primeiro eixo. Na estação seca, a condição de fluxo estável pode ter favorecido traits sensíveis (ou seja, indivíduos maiores com baixa flexibilidade corporal), influenciados, em sua a maioria, pela serapilheira. Foram eles: predadores, com ganchos anal, fragmentadores, flexibilidade corporal menor que 45° e tamanho corporal entre 10 e 20mm.

Os predadores foram influenciados pela serapilheira nas duas estações, apresentando maior importância na estação seca. Estudos sugerem que os invertebrados predadores estão diretamente relacionados ao acúmulo de serapilheira no leito e, assim, interações bióticas como a predação podem ser suprimidas durante períodos de instabilidade ambiental (Dudgeon, 1993). Experimentos de longo prazo mostraram que a exclusão do acúmulo de serapilheira em córregos resultou em diferenças significativas nas abundâncias de invertebrados detritívoros e predadores quando comparados aos córregos de referência (Wallace, 1997).

Na estação seca, a ordenação ao longo do segundo eixo da RDA explicou 20% da variação total ajustada e foi determinada principalmente pelo número de Froude, correlacionado positivamente com este eixo. Os traits com aderência temporária ao substrato, com ventosas e coletores-filtradores também foram correlacionados positivamente ao segundo eixo, sendo influenciados pelo número de Froude.

O cascalho e o substrato fino (areia, silte e argila) mostraram uma correlação negativa com a riqueza e a diversidade taxonômica na estação seca, respectivamente. O seixo apresentou correlação positiva com a equitabilidade de traits na estação seca, indicando que hábitats com esse substrato, em geral associados à elevado estresse hidráulico, podem restringir a distribuição de macroinvertebrados (Statzner and Higler, 1986).

Diversidade de substrato, raiz e cascalho não apresentaram correlação significativa com nenhuma métrica ou índice da comunidade. A área coberta por troncos, embora tenha sido pequena (< 2%) com relações aos outros tipos de substrato, foi significativamente maior na estação seca e apresentou correlação positiva com a riqueza e diversidade taxonômica em ambas as estações. Além de favorecer a complexidade do canal e a diversidade de habitats (Faustini and Jones, 2003; Scealy et al., 2007), esse substrato pode incrementar a capacidade de retenção de matéria orgânica alóctone no leito do rio (Tejeda, 2018).

Em comparação com a chuva, a estação seca apresentou maior densidade, riqueza taxonômica e riqueza funcional, além do dobro de serapilheira acumulado no córrego. Conforme a RDA de ambas as estações, a serapilheira demonstrou importância para os traits da comunidade de invertebrados, principalmente aquelas sensíveis à variação sazonal do fluxo. Além disso, todas as métricas e índices da comunidade foram correlacionados significativamente com a serapilheira, exceto pelo índice equitabilidade de traits na estação seca. Nesse caso, o acúmulo de serapilheira favorecido pelo fluxo estável pode ter condicionado o uso intensivo de traits específicos (por exemplo, nenhuma adaptação ao alto fluxo, predadores, tamanho corporal elevado), diminuindo a equitabilidade de traits.

Concluímos neste capítulo que o acúmulo de serapilheira, além da presença de troncos, pode representar um recurso crucial para a comunidade de macroinvertebrados em córregos neotropicais florestais (Baptista et al., 2001; Bruder et al., 2014; Ríos-Touma et al., 2009). Nos córregos de savana neotropicais, a entrada mensal de serapilheira a partir da vegetação ripária é contínua e em pequenas quantidades, com picos ocorrendo durante a transição da estação seca para a chuvosa (Bambi et al., 2017). Assim, hipotetizamos que a retenção de serapilheira depende principalmente da alteração sazonal no regime hidrológico (Speaker et al., 1984; Tejeda, 2018), que neste estudo foi representada a partir do número de Froude, com uma medida do estresse hidráulico em cada microhabitat..

No **capítulo 2**, identificamos a serapilheira como principal recurso assimilado pela comunidade em ambas as estações. No entanto, a disponibilidade serapilheira foi significativamente menor na estação chuvosa ($W = 41332$, $p < 0,00$). Os demais recursos alimentares (seston e biofilme) também foram reduzidos em comparação com a seca. Observamos redução significativa na amplitude dos valores de $\delta^{13}C$ (dCR) da comunidade na estação chuvosa, sugerindo menor diversidade de recursos basais assimilados.

Embora a entrada mensal de serapilheira nos córregos de cabeceira florestais do Cerrado seja contínua e em pequenas quantidades, com picos ocorrendo na transição entre as estações seca e chuvosa (Bambi et al., 2017), os bancos de serapilheira mantidos nos córregos foram menores na estação chuvosa, provavelmente devido a maior ocorrência de picos de vazão. No entanto, a serapilheira remanescente pode representar um recurso alimentar estratégico para a comunidade, responsável pela manutenção da complexidade da cadeia alimentar na estação chuvosa.

Dentre os tipos de material alóctone acumulados nos córregos, os bancos de serapilheira, serapilheira fina, raízes e troncos representam 53%, 36%, 6% e 5%, respectivamente, na estação chuvosa. Na estação seca, os bancos de serapilheira, serapilheira fina, raízes e troncos representam 72%, 19%, 2% e 8%, respectivamente. Além de menores proporções de serapilheria acumulada, foi evidenciado um aumento na razão C:N da serapilheira na estação chuvosa, implicando maior dificuldade de assimilação desse recurso alimentar pela biota. A entrada de serapilheira nos córregos na estação chuvosa é provavelmente composta por folhas "fresca", sendo, portanto, uma fração menos mineralizada (Peipoch et al., 2012).

Comparando-se os recursos alimentares, na estação seca, as assinaturas do $\delta^{13}C$ e $\delta^{15}N$ do seston foram enriquecidas em comparação ao biofilme (Kruskal $X^2 = 40,83$; $p < 0.0001$), e a serapilheira não diferiu significativamente entre o biofilme e o seston. Na estação chuvosa, a assinatura $\delta^{13}C$ entre os recursos alimentares foi significativamente diferente (Kruskal $X^2 = 0{,}66$, valor de $p < 0{,}0001$), com valores mais depletados na serapilheira, seguida pelo seston e o biofilme. Na comparação entre as estações do ano, as assinaturas da serapilheira e do biofilme δ^{13}C não diferiram, porém o seston foi significativamente enriquecido na estação chuvosa ($W = 1127{,}0$, $p < 0{,}0001$). A comparação de assinatura de δ^{15}N ainda entre as estações, mostrou maior valor de δ^{15}N no seston na estação chuvosa ($W = 998$, $p = 0{,}0001$). Para o biofilme ($W = 596{,}5$; $p = 0{,}7043$) e a serapilheira ($W = 727{,}5$; $p = 0{,}1765$) as assinaturas de δ^{15}N não diferiram entre as estações.

As concentrações de clorofila-a no seston e no biofilme foram significativamente maiores na estação seca em comparação com à estação chuvosa ($W = 23{,}5$ e $p < 0{,}001$; $W = 175{,}5$ e $p < 0{,}001$; respectivamente), porém a AFDM não diferiu entre as estações, para ambos os recursos. Concentrações de clorofila-a no seston, principalmente na estação seca, podem indicar a presença de microrganismos fotoautotróficos. Provavelmente, o seston foi composto não apenas de detritos resultantes da decomposição de matéria orgânica alóctone, mas também, por organismos microscópicos autotróficos (diatomáceas) e heterotróficos (flagelados, ciliados e rotíferos), que podem incrementar a qualidade nutricional. Esse resultado pode ser ressaltado por diferenças nas razões C:N e isotópicas entre o seston e a serapilheira, sendo observada uma significativa redução da razão C:N para o seston em ambas as estações.

A palatabilidade do seston pode ter tido relação com o maior número de invertebrados coletores como Hydropsychidae, Odontoceridae e Simuliidae, Tipulidae e Chironominae. Houve, portanto, uma afinidade comum dos táxons por detritos finos, indicando a importância desse recurso alimentar e atividade intensa de invertebrados filtradores-coletores em ecossistemas neotropicais de água doce (Palmer et al., 1993; Tomanova et al., 2006). Para o Tipulidae, Chironominae e Hydropsychidae, o seston contribuiu significativamente na estação chuvosa; e para Odontoceridae na estação seca. Esse recurso contribuiu para a dieta do Simuliidae em ambas as estações, contudo o biofilme de modo semelhante.

A área isotópica ("*isotope space*") dos Simuliidae foi maior na estação seca em relação à estação chuvosa, provavelmente devido à ingestão de fragmentos de serapilheira fina e de microrganismos fotoautotróficos acoplados no seston (Majdi and Traunspurger, 2017). Além do hábito alimentar de filtrador e coletor, os Simuliidae são raspadores (Ramírez and Gutiérrez-Fonseca, 2014). Essa característica pode estar relacionada ao fato de identificarmos correlação positiva entre esses indivíduos, o número de Froude e o substrato grosseiro, indicando adaptações que permitem aos Simuliidae se alimentarem do biofilme.

Na estação chuvosa, a contribuição elevada do seston como alimento para os Hydropsychidae corrobora com seu hábito alimentar coletor. Além disso, houve correlação positiva entre a densidade de Hydropsychidae e o número de Froude, provavelmente em função de suas características morfológicas (garras anais e alta flexibilidade corporal) que garantem resistência sob altas vazões e permitem o consumo do seston. Na estação seca, os Hydropsychidae tiveram a serapilheira como principal recurso alimentar. Nos córregos de savanas neotropicais foi observado que as larvas de Hydropsychidae em seus estágios iniciais se alimentam de sedimentos e algas capturados em sua rede, no entanto, em seu último estágio de vida podem ingerir pequenos invertebrados aquáticos e fragmentos de plantas, sendo então classificados como generalistas (Cordeiro-Bentes et al., 2008). Assim, a diferença na massa seca média individual de Hydropsychidae entre as estações, com indivíduos significativamente maiores na estação seca do que na estação chuvosa, corrobora com a maior área isotópica e o predomínio da serapilheira como principal fonte de recurso na estação seca.

Na estação chuvosa, Odontoceridae apresentou maior nicho isotópico e a serapilheira como seu principal recurso alimentar. Por apresentar relação inversa com o número de Froude, teorizamos que esses indivíduos podem ter sido deslocados para áreas de menor força de cisalhamento na estação chuvosa, onde os bancos de serapilhera podem ter sido alocados. Desta forma, podem ter utilizado a serapilheira remanescente como recurso estratégico e acesso a um recurso mais diversificado (por exemplo, seston). Na estação seca, em função do fluxo da água estável e por habitarem principalmente o sedimento arenoso dos córregos (Celina Reynaga et al., 2014), o seston representa a principal fonte alimentar dos Odontoceridae, como indicado na SIA.

O biofilme, apesar da elevada concentração de clorofila-a e a menor razão C:N entre os demais recursos alimentares, apresentou contribuição reduzida para a comunidade. Foi considerado importante para Dipteras (Tipulidae e Chironominae) na estação seca, no entanto, sua contribuição não diferiu claramente do do seston. Vale ressaltar que os Tipulidae e os Chironominae apresentaram grande variação nos valores de δ^{15}N. Muito possivelmente porque essas duas famílias são consideradas detritívoras, habitam e se alimenta da matéria orgânica depositada no sedimento, que geralmente é um material mais mineralizado enriquecido em ^{15}N.

Psephenide tiveram valores de δ^{13}C significativamente depletados comparado com todos os outros indivíduos da comunidade, indicando o consumo de microrganismos metanotróficos e/ou quimioautotróficos. Esses microrganismos apresentam valores significativamente reduzidos de δ^{13}C (<-40 ‰) em relação às fontes fotoautotróficas. O carbono derivado de metano parece entrar nas comunidades de córregos por meio de habitats anóxicos, que podem ocorrem em remansos e zonas hiporéicas (Kohzu et al., 2004). No entanto, não identificamos um predador em potencial e mais estudos devem verificar se a cadeia alimentar de córregos neotropicais é parcialmente sustentada pelo carbono.

Em síntese, as métricas NND ("*Mean nearest neighbor distance*" distância euclidiana média do vizinho mais próximo de cada grupo) e SDNND ("*Standard deviation of nearest neighbor distance*" desvio padrão do NND) da comunidade de invertebrados indicaram redundância trófica na estação chuvosa, ou seja, as famílias ocuparam posições tróficas menos distintas. Na estação seca, a massa seca de toda a comunidade foi duas vezes maior em relação à estação chuvosa. No entanto, a razão entre a massa seca de predadores versus consumidores foi equivalente, com 1,3 (768,3 mg / 564,4 mg) e 1,2 (1650,0 mg / 1944,5 mg) na estação chuvosa e de seca, respectivamente.

Com base nesses dados, sugerimos que as relações tróficas entre consumidores e predadores não foram afetadas significativamente pela sazonalidade. Além disso, apesar do menor intervalo em δ^{15}N (dNR – "δ^{15}N *Range"* amplitude do δ^{15}N*)* entre os consumidores na estação chuvosa, que sugere uma cadeia alimentar mais compacta e menos diversificada, a probabilidade de divergência para essa métrica em relação a estação de seca é baixa. Assim, diferentemente dos córregos temperados (Wallace et al., 2015), o acúmulo de serapilheira em córregos Neotropicais florestais é influenciado principalmente pela variação sazonal do fluxo, com reflexos na produção secundária.

No **capítulo 3**, evidenciamos o papel dos fatores *bottom-up* (nutrientes e luz) e *top-down* (invertebrados pastejadores) como limitantes para o desenvolvimento do biofilme algal em córregos de cabeceira oligotróficos (5,44 ± 1,71 µS cm^{-1}) e enriquecidos em nutrientes (15,58 ± 8,49 µS cm^{-1}). A massa seca livre de cinzas (AFDM – ash-fre dry mass) duplicou nos córregos enriquecidos em nutrientes e foi maior em condição de sombreamento. Além disso, houve uma correlação positiva, mas não significativa, entre o AFDM e a clorofila-a (n = 48, r = 0,27, p = 0,06). Níveis de clorofila-a foram influenciados positivamente pelos nutrientes e pela abertura do dossel, bem como pela interação entre ambos.

Mesmo nos córregos oligotróficos, a abertura do dossel resultou em aumento significativo de aproximadamente dez vezes na concentração de clorofila em comparação com ambiente sombreado. O gradiente de luz, conforme observado para ambientes temperados, desempenha papel predominante no controle da biomassa de algas (Bechtold et al., 2017; Mosisch et al., 2001). Desta forma, aberturas no dossel típicas de florestas bem desenvolvidas ou causadas por perturbações antrópicas, favorecem o crescimento de algas bênticas e, consequentemente a retenção e absorção de nutrientes (Bechtold et al., 2017).

As ninfas de Baetis e os ácaros Hydrachnidia foram os invertebrados mais abundantes (> 90% da comunidade) encontrados nas placas em todos os tratamentos onde não houve exclusão. Apenas as ninfas de Baetis são consideradas pastoras (Merritt et al., 2008), enquanto os ácaros são considerados parasitas e / ou predadores (Di Sabatino et al., 2014). As placas com pulsos elétricos de alta voltagem não exibiram colonização por macroinvertebrados bentônicos em nenhum dos quatro córregos. Além disso, as placas localizadas em ambientes sombreados apresentaram significativamente menos raspadores do que as placas localizadas nas regiões de abertura de dossel. A densidade de Baetis nas placas variou entre 12 a 237 ind. m-^{2} entre os córregos, sendo maior nas placas sem eletrificação com enriquecimento de nutrientes e sob dossel aberto.

O efeito do pastoreio na biomassa do biofilme ocorreu apenas em córregos enriquecidos com nutrientes, consistindo em uma redução de 50% na AFDM. As ninfas de Baetis indicam uma pressão negativa sobre a biomassa do biofilme, constituindo um controle *top-down* nos córregos enriquecidos com nutrientes (Garcia et al., 2015; Sturt et al., 2011). Ao contrário do que foi observado para a biomassa, o modelo indicou que os raspadores não tiveram efeito negativo na concentração de clorofila-a. Houve correlação positiva e significativa entre a densidade de ácaros e a densidade de ninfas de Baetis (inclinação 0,37, $R^2 = 0,68$, $p <0,001$, $n = 48$). Estudos sugerem que o enriquecimento de nutrientes favorece o aumento da densidade de invertebrados devido ao aumento de produtores primários (Singer and Battin, 2007).

Quanto às razões isotópicas, os valores médios (± DP) de $\delta^{13}C$ para ninfas de Baetis, ácaros e biofilme nos córregos Onça e Gama (enriquecidos com nutrientes) foram de -31,40 ± 1,20 ‰, -30,54 ± 1,34 ‰; e -27,20 ± 0,81 81 ‰ -28,10 ± 1,28 ‰, respectivamente. Quanto as assinaturas de $\delta^{15}N$, os valores médios (± DP) foram de 4,00 ± 1,16 ‰, 5,96 ± 0,67 ‰, 3,08 ± 0,79 ‰ e 2,42 ± 1,00 ‰ para as ninfas de Baetis, ácaros e biofilme dos córregos Onça e Gama, respectivamente. A análise isotópica indicou que as ninfas de Baetis são presas dos ácaros e possivelmente um efeito cascata trófico de baixo para cima ocorreu (Hunter and Price, 1992). Nesse caso, o aumento da biomassa do biofilme facilitou o aumento da população de ninfas de Baetis que, consequentemente, é um recurso alimentar (presas) para os ácaros.

O biofilme pode ser considerado um recurso de alta qualidade cujo sinal isotópico é identificado em níveis desproporcionais nos consumidores à medida que aumenta sua disponibilidade nos córregos, sugerindo o aumento na importância relativa da matéria orgânica autóctone em suportar o nível trófico superior (Warren et al., 2016). De modo contrário ao esperado, a relação entre a concentração de clorofila-a e os valores de δ^{13}C foi significativamente negativa (inclinação de 0.39, $R^2 = 0,32$, $p <0,0001$, $n = 48$).

Essa relação foi significativa mesmo sob condições de enriquecimento de nutrientes e abertura do dossel. Apesar do aumento da concentração de clorofila-a nos córregos enriquecimentos em nutrientes, o intervalo de variação foi limitado (0-2 mg m^{-2}). Assim, a biomassa do biofilme pode ter sido insuficiente para a ocorrência do efeito de difusão do CO_2. O biofilme pouco espesso com maior proporção de células próximas à interface água/biofilme, pode estar associado a menores restrições de carbono e, resultando, portanto, na discriminação do ^{13}C (Hill and Middleton, 2006).

A variação das assinaturas do δ^{13}C em relação a mudança significativa nas concentrações de clorofila-a indica que a atividade fotossintética das algas determina o fracionamento isotópico entre o carbono inorgânico dissolvido e células algais, mesmo considerando o refinamento da resolução espacial (córregos de cabeceiras). Provavelmente, sob condições limitadas de luz e nutrientes, houve uma quantidade muito reduzida de algas no biofilme e, como essas condições primárias foram alteradas em função da incidência de luz solar e do aumento na concentração de nutrientes, a ocorrência de algas foi beneficiada.

4 Conclusões

A sazonalidade e o gradiente de luz foram identificados como forças seletivas para a comunidade de macroinvertebrados bentônicos nos córregos de cabeceira florestais. No primeiro caso, a alteração hidrológica sazonal refletiu a dependência dos macroinvertebrados com relação à conformação dos microhabitats, favorecendo traits tolerantes ao estresse hidráulico na estação chuvosa (Figura 4). A redução na disponibilidade de alimentos na estação chuvosa refletiu em redução na diversidade de recursos alimentares assimilados pelos macroinvertebrados, além do aumento na redundância funcional da comunidade. Na estação seca, traits sensíveis apresentaram condições adequadas de desenvolvimento, preditas principalmente pelo acúmulo de serapilheira. Desse modo, o estresse hidráulico mostrou-se importante para a composição da comunidade de invertebrados, principalmente em função da dependência da serapilheira no fornecimento de alimento, habitat e refúgio.

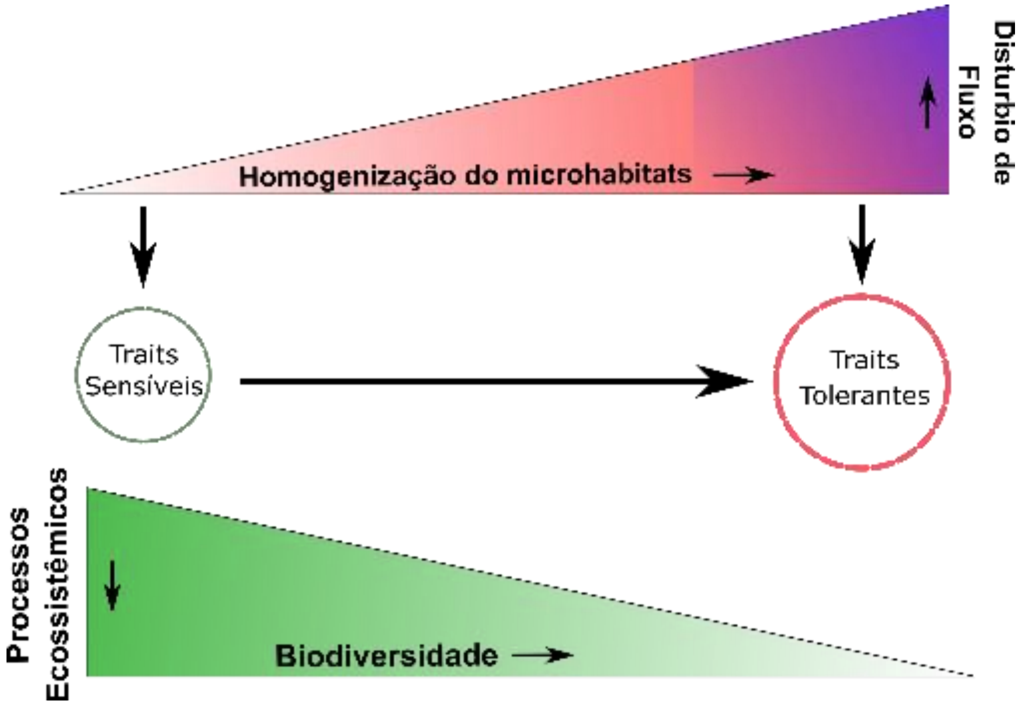

Figura 4. Efeito do distúrbio de fluxo nas características dos microhabitats, com o favorecimento de traits tolerantes e redução dos traits sensíveis, podendo acarretar em perda da biodiversidade e, consequentemente, no funcionamento dos ecossistemas aquáticos.

Com relação ao gradiente de luz, invertebrados pastejadores foram indiretamente favorecidos pelas condições ambientais que resultaram no aumento da produtividade primária e em mudanças no fracionamento isotópico do carbono no biofilme. É importante destacar que esses córregos são naturalmente sombreados e oligotróficos e as mudanças na cobertura florestal ripária podem promover fluxos de energia a partir de recursos autóctones, afetando as características e o funcionamento da teia alimentar.

5 Perspectivas Futuras

O presente teve como objetivo verificar como a estrutura e as interações tróficas da comunidade de macroinvertebrados na escala de micro-habitat em dois períodos hidrológicos sazonais. Contudo o estudo teve como foco córregos de cabeceira florestais não inundáveis no Brasil Central localizados em rochas com minerais predominantemente silicatados. Dessa forma, há a necessidade em investigar estes mesmos efeitos em outros grupos geológicos e em florestas ripárias inundáveis.

Florestas inundáveis podem apresentar respostas hidrológicas sazonais diferentes em função da diferença na topografia do qual está inserida. Além disso, diferentemente de rochas silicatadas, rochas carbonáceas estão associadas a altas vazões em virtude da dissolução cárstica que favorece a circulação da água subterrânea, contribuindo para o escoamento de base (Campos, Lineu and Rodrigues, 2006). A bacia limite à bacia do São Bartolomeu, Bacia do Rio Preto, pertence ao grupo geológico Bambuí do qual é composta por rochas carbonáceas com granulometria fina (Campos, Lineu and Rodrigues, 2006).

Por fim, é importante considerar que mudanças no código florestal brasileiro, que reduziram a necessidade de preservação de matas ripárias, diminuindo a largura a ser preservada, podem causar alterações não lineares no metabolismo e funcionamento dos córregos de cabeceira, reduzindo a capacidade desses em fornecer serviços ecossistêmicos associados à manutenção da qualidade da água (Figura 5).

Figura 5. Representação esquemática da relação entre mudanças climáticas e de uso e cobertura do solo nas características ambientais dos córregos de cabeceira.

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Capítulo 1 - Effects of seasonal flow disturbances on the relationship between macroinvertebrate community and microhabitat in neotropical savanna streams

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O presente capítulo será submetido para apreciação do periódico Hydrobiologia.

Todos os autores contribuíram criticamente para a interpretação dos resultados e com a versão final do manuscrito

Title: Effects of seasonal flow disturbances on the relationship between macroinvertebrate community and microhabitat in neotropical savanna streams

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Abstract

1. The interaction between water flow and substrate can determine the impact of seasonal flow disturbances on lotic communities. Thus, we measured the response of macroinvertebrate communities to natural flow disturbances in neotropical savanna (Cerrado) streams under the microhabitat scale in silicate geological streams located in the Central Plateau of Brazil.

2. The study was carried out in four pristine streams, and the samplings were conducted during periods of high and unstable flow (wet season) and reduced and stable flow (dry season) in 2017. The accumulated rainfall in 2017 was approximately 1201 mm. We collected a total of 4534 macroinvertebrate samples in 528 microhabitats and performed associated hydraulic and substrates characterization. Nine microhabitat variables were evaluated: boulder, cobble, gravel, fine substrate (i.e., sand, silt and glay), root, wood, leaf litter, Froude Number and substrate diversity.

3. In the wet season, the Froude Number, which indicates the hydraulic stress in microhabitat, and cobble substrate had crucial importance on the selection of tolerant functional traits with specific adaptation and mobility to flow constrain. In the dry season, the proportion of leaf litter covering the surface of the stream bed doubles and showed a negative correlation with Froude Number ($r = -0.27$, $p = 0.001$, $n = 528$). The metrics and indices of the macroinvertebrate community were strongly influenced by the leaf litter in the dry season.

4. The impact of seasonal flow variation on the structural and functional attributes of macroinvertebrate assemblages in Neotropical savanna streams depends on the interaction between the water flow and the substrate type, fulfilling the river habitat templet concept. In the dry season, the stable flow condition favors sensitive traits to turbulent flows and was greatly influenced by leaf litter accumulation. Our findings demonstrate that seasonal changes in Neotropical savannas act as strong filters of species traits of benthic macroinvertebrates communities in forest streams.

Keywords: Habitat templet; Functional traits; Seasonality.

1. Introduction

Ecological responses provided by the invertebrates are the result of the evolutionary adjustment of functional traits to a templet provided by habitats, according to the Habitat Templet concept (Southwood, 1977). In this concept, stability and predictability of the environmental variables, resource level and constancy are the characteristics that define the templet of each habitat (Southwood, 1977) under the influence of biotic interactions (Greenslade, 1983). These characteristics, called "selective forces," act as "landscape filter" for organisms whose traits have not been adjusted to their spatial or temporal variability (Poff, 1997). Thus, understanding the habitat variability can favor the characterization of the community (composition, abundance and diversity) and the evaluation of the role of traits in ecosystem structure and functioning.

In the temporal scale, the periodicity of environmental variables is a central component of global ecosystems. Tonkin et al. (2017) classified the seasonal (occurrence of events in specific periods of the year) and predictable (high recurrence of events) hydrological regime as the preponderant selective force for the community of stream invertebrates. Seasonal climate regions showed a greater diversity of invertebrates (Tonkin et al., 2017), because they favor the coexistence of different species during a certain period of time, each in its optimal conditions and none under precarious conditions for long period of time (Hutchinson, 1961).

Neotropical streams have a pronounced seasonal variation between the dry and wet seasons (Flecker and Feifarek, 1994), with higher diversity invertebrates community in the dry season (Yokoyama et al., 2012), probably due the stream channel accumulates more leaf litter in this period (Baptista et al., 2001). In environments with continuous standing crop litter, like the riparian forest of Brazilian neotropical savannas (Bambi et al., 2017), the leaf litter accumulation may depends mainly on the magnitude and variability of the seasonal flows on determining the leaf litter retention (Speaker et al., 1984).

Studies suggest that predatory invertebrates are directly related to leaf litter accumulation within the stream and thus, biotic interactions such as predation can be suppressed during periods of environmental instability (Dudgeon, 1993). Pearson (2014) suggested that stormflows events in the wet season may cause the decline and mortality of many invertebrates, reducing the importance of predation and competition as structuring forces in the tropical stream food web. In addition, the increased flow velocity, favors the transport of fine organic material within the water column and thus justifying the increased relative abundance of passive filter feeders (Georgian and Thorp, 1992; Wallace and Merritt, 1980), or the high relative abundance of grazers/scrapers (Pastuchova et al., 2010).

The presence of a variety of refuges is known to be responsible for greater resilience of macroinvertebrate communities to flow disturbances (Townsend et al., 1997). Without such refuges, high flow disturbances in streams may induce rock tumbling and abrasion by suspended sediments, causing fauna dislodgment or damage (Flecker and Feifarek, 1994; Townsend et al., 1997). Experiments shown the active search by macroinvertebrates for shelter during high-flow events where the immature Ephemeroptera and Trichoptera had a small movement between the layers of the substrate as the flow increased (Holomuzki and Biggs, 2000).

Reduced body size is favored in adverse hydraulic conditions because smaller larvae can penetrate substrates of low porosity, in small interstitial spaces that serve as refugia against current displacement (Townsend and Thompson, 2007). Increasing hydrological unpredictability has been shown favor species with traits that confer resilience (e.g., smaller body size, high mobility and use of the flow refuge) or resistance (e.g., tarsal and anal claws, aerodynamic and flexible body shape) in a predictable way (Townsend and Hildrew, 1994; Townsend et al., 1997).

Interaction between water flow and substrate can determine the impact of seasonal flow disturbances on lotic communities (Pearson, 2014; Reice et al., 1990; Townsend and Scarsbrook, 1997). The understanding of this interaction is essential to assist the development of models that allow predicting community dynamics in response to natural disturbances (Lytle and Poff, 2004). This evaluation is possible in the microhabitat scale, whose templet is defined by the combination of substrate characteristics, stream velocity and depth (Merigoux and Doledec, 2004; Poff, 1997; Sagnes et al., 2008).

Lamouroux et al. (2004) demonstrated that microhabitat variables (e.g., Froude number and substrate size) simultaneously influenced several functional traits of aquatic organisms (e.g., drag resistance and foraging strategies). These variables can also promote the selection of ecological strategies that improve the local species persistence (Poff and Ward, 1990). In Brazilian forest streams, adjacent microhabitats in a single stream site harbor macroinvertebrate community more dissimilar than those found in a single microhabitat at different stream sites (Costa and Melo, 2008).

Generally, the seasonal patterns in Neotropical streams ecosystems are not quantified and are extrapolated from landscape-scale patterns. Given the uncertainties of climate change, the understanding of seasonality encompassing gradients of climate variability is a critical factor for basic science and ecosystem management (Tonkin et al., 2017). The goal of this study was to characterize the primary influencing factors structuring macroinvertebrate community changes in Neotropical savanna (Cerrado) streams, considering the microhabitat scale during two hydrological periods (i.e., wet season - instable flows and dry season - stable flows). Here we identify complementary functional traits, at the community level, that are most sensitive and tolerant to seasonal flow changes

We hypothesize (I) in the wet season selects tolerant functional traits, e.g., small body size, high body flexibility, low profile and with specific adaptation and mobility to flow constraints; (II) in the dry season, the stable flow condition favors sensitive traits, e.g., larger body size, low body flexibility, high profile and with low flow adaptation and / or mobility; (III) leaf litter as the main microhabitat variable predictor to the benthic macroinvertebrates that, however, are straightforwardly reduced by seasonal high flows.

2. Material e Methods

2.1 Study area

The study was carried out in four streams located in the Conservation Units of the Federal District, Brazil. The Tortinho and Três Barras streams are situated in the Brasilia National Park (15°38'17.69"S, 48°0'20.82"W) and the Cabeça-de-Veado and Capetinga streams are placed in the Environmental Protection Area (APA) of Gama and Cabeça-de-Veado (15°53'23.94"S, 47°50'36.68"W) (Figure 1). The nutrient concentrations reported from nearby streams are relatively low when compared to data collected from reference areas in Brazil or temperate zone (Fonseca et al., 2014). Predominant land cover in the watersheds is natural vegetation, with untouched evergreen riparian forests. The physiognomy is evergreen, barely exhibiting leaf fall in the dry season. Tree height varies between 20 and 30 meters, and the position of the treetops provides coverage of 70% to 95% (Ribeiro and Walter, 2001).

Climate is Aw (wet tropical, according to Köppen classification), marked by strong seasonality, with a wet season from October to April concentrating up to 80% of annual precipitation (mean temperature around 29 °C), and a dry season from May to September when precipitation ranges from zero to below 50 mm (mean temperature around 18 °C). According to Instituto Brasília Ambiental (2015), annually in the Federal District precipitation varies between 1,200 mm and 1,700 mm, with average temperature between 18ºC and 22ºC. The accumulated rainfall in 2017 was approximately 1201 mm, with about 51% of this volume distributed between the months of January and April, and the rest (49%) between October and December.

The geology of the four watersheds is silicate and belongs to the Paranoá Group, with clay-sandy metarrhythmic and quartzite units (Campos et al., 2013). The soils of the study area are mainly Oxisols according to the US soil classification. Unless for the water temperature, which varied between 3 and 4 °C between the seasons with measurements always in the early morning, the most water quality variables did not considerably oscillate between seasons (Table 1). Other characteristics of the streams are given in Table 1.

2.2 Field sampling

Samples were collected during the wet season (March-April), demonstrating high and unstable flows, and during the dry season (July-August), showing lower and more stable flows, in 2017. Three sampling campaigns were carried out during each season in the four streams totaling 24 collections. Precipitation (mm) and water level (cm) data were monitored at Capetinga stream equipped with a telemetric monitoring station (FAL-UnB, nº 60478482) (Figure 2). These data are made publicly available through the HIDROWEB platform maintained by the Brazilian National Water Agency (ANA-Agência-Nacional-de-Águas, 2017).

In each stream (n=4) we selected a 100-meter stream section and 11 equidistant sampling points. Each sampling point was composed of a 1-meter square of bed substrate divided into four rows orthogonal to flow comprising the right and left margins and center of the stream, yielding 12 microhabitats (Surber area = 0.09 m^2) per sampling point (labelled from 1 to 11) (Figure 3). At each season and stream, there was an interval of two weeks between the sampling campaigns. Thus, to avoid the influence of previous sampling campaign the microhabitats sampled never repeated and in each sampling campaign two microhabitats per sampling point (from 1 to 11) were randomly sampled, totaling 22 microhabitats per stream in each sampling campaign (Figure 3). For each season and considering the four streams we ended up with 264 microhabitats. Considering the microhabitat area equal to 0.09 m² (Surber area), a total area of 23.76 m² of stream bed was sampled per season.

Macroinvertebrates were collected with a Surber sampler (30 cm × 30 cm, 100 µm mesh size) (Anexo A, Figure S1). We separated the macroinvertebrates in the field using forceps and a sieve with a diameter of 19 cm and 100 µm mesh in the field. For Surber sample, we spend on average 15-20 mins in th(e separation and classification of the macroinvertebrates (totaling approximately 8 hours in the field). Previous studies have shown that at the family level, the separation and classification of macroinvertebrates in the field does not bias taxonomic richness values in comparison to the sampling protocol using the "D" net sampling and a separation and classification of organisms in the laboratory (Stein et al., 2008). In the laboratory, the macroinvertebrates were preserved at -20°C before being counted and identified at the family level using the available identification keys (Hamada et al., 2014; Mugnai et al., 2010). We evaluated common community metrics, including, density (individuals per square meter), taxonomic richness, diversity (Simpson's index) and evenness (Pielou index).

The characterization of the substrate was done with pictures of the sampled area (30 cm x 30 cm) using a waterproof camera (GoPro Hero 5). The pictures were further analyzed with ImageJ™, to classify substrate according to grain size and type (i.e., organic and inorganic) (Figure 3). In ImageJ software, we set the scale according to the known Surber area (0.09m²). The result of the substrate classification was given in substrate surface area of the substrate type per square meter (m².m-2). The substrates were classified as boulder, cobble, gravel, fine substrate (i.e., sand, silt and glay), root, wood and leaf litter.

In addition, at each microhabitat, we measured water column depth (m) and water flow velocity (m.s-1) and for each sample we measured pH, dissolved oxygen (mg.L-1), electrical conductivity (µS.cm-1), turbidity (NTU) and temperature (°C). A total of nine microhabitat variables were evaluated: boulder, cobble, gravel, fine substrate (i.e., sand, silt and glay), root, wood, leaf litter, Froude Number and substrate diversity. The substrate diversity was measured through Shannon-Weaver diversity index using all substrate types and the Froude number was calculated, as an indicator of hydraulic stress for invertebrates in each microhabitat, considering the depth (D) and water velocity (v) from the equation Fr=v/(g*D)0.5, where g is gravitational acceleration.

2.3 Community traits

We collected available trait information from literature about Neotropical streams (Hamada et al., 2014; Reynaga and Santos, 2012; Tomanova et al., 2008, 2006). For the families Corduliidae, Culicidae Dytiscidae, and Tabanidae it was necessary to use the insect traits database of North American and European streams (Merritt et al., 2008; Tachet et al., 2002). In this case, the trait scores were computed for each family that was sampled by averaging the trait scores of genera present in the database and belonging to the same family. We ended up with information on seven traits (feeding habits, body size, body flexibility, body shape, specific adaptation to flow restrictions, and mobility and attachment to the substrate) described by 31 modalities and known to respond to substrate characteristics and flow changes (Table 2).

The affinity of each family for trait category was done using the fuzzy coding (Chevenet et al. 1994). Affinity scores varied from 0 to 3, with 0 for no, 1 for weak, 2 for moderately strong and 3 for the strong affinity of the family with the trait category. Lastly, we calculated trait richness (FRic), which measures the general distribution of traits of all taxa in the community (Villéger et al., 2008), functional evenness (FEve) and the trait diversity with the functional dispersion index (FDis), which represents the dispersion of species in the trait space of the centroid of all species, weighted by their relative abundances (Laliberté et al., 2015).

2.4 Statistical analysis

For data analysis, normality was evaluated using the Shapiro-Wilk W-test. Comparisons between dry and wet season related to substrate variables and community metrics and indexes were performed using the nonparametric Mann-Whitney U-test. To examine relationships between community metrics and substrate variables, we assessed the non-parametric Spearman rank correlation for each season separately. Spearman rank correlation was also used to assess the relationship among Froude Number and leaf litter cover in the stream bed.

To get a trait-by-samples matrix, we multiply the frequency of each trait modality by the relative abundance of taxa. We then check the relationships between the traits selected in the macroinvertebrate community and the habitat variables (Froude Number, substrate diversity, boulder, cobble, gravel, leaf litter, root, wood) through Redundancy

Analysis (RDA) in dry and wet season separately. Model selection was performed using stepwise regression (forward selection). The best model was chosen based on the highest adjusted R^2 and lowest AIC.

Only statistically significant variables (p-value<0.05 after 999 random permutations and partial $R^2 \geq 0.01$) were retained in final models (using function 'forward.sel' in the packfor package for R), as described by Blanchet et al. (2008). Variance inflation factors (VIF) were assessed for each selected variable to prevent multicollinearity between the model's variables. For RDA, the substrate variables data were log transformed, using log(1+x). The Monte Carlo random permutation test (999 random permutations) with Bonferroni correction was used to determine the significance of the redundancy analysis.

3. Results

The Froude Number was significantly higher in the wet season compared to the dry season (Figure 4A). The leaf litter was significantly lower in the wet season (Figure 4G), showing a negative correlation with the Froude Number ($r = -0.27$, p-value = 0.001, $n = 528$). In addition, in the wet season, the leaf litter did not correlate with any metric or index of the macroinvertebrate community (Table 3). We collected 4534 macroinvertebrates (3332 in the dry season and 1202 in the wet season), distributed in 45 families.

Density, trait and taxonomic richness and diversity of macroinvertebrate community were significantly lower in the wet season compared to the dry season (Figure 5 A-D; G). Dry season showed a lower taxonomic and functional evenness of macroinvertebrate community compared to the wet season (Figure 5 E-F). The leaf litter surface area was negative correlated to the taxonomic evenness. In the dry season, the proportion of litter covering the stream bed doubled (Figure 4J) and was significantly correlated with all macroinvertebrate metrics and indexes, except for trait evenness (Table 4).

Surface covered by cobble, boulder and gravel occupied more than 90% of the stream bed and did not change between seasons. However, the area covered by wood, although it was low (<2 %) compared to other substrate types, was significantly higher in the dry season. The presence of wood along the stream showed to have a positive correlation with the richness and taxonomic diversity for both seasons and important for the diversity of trait in the wet season.

The boulder and fine substrate (i.e., sand, silt and glay) in the dry season showed a negative correlation with richness and taxonomic diversity, respectively. The cobble had a positive correlation with the trait evenness in the dry season. The Froude Number showed a positive correlation with the diversity of traits in both seasons and also showed a positive correlation with the macroinvertebrate density in the wet season. The variables substrate diversity, root and gravel showed no significant correlation with any metric or index of the macroinvertebrate community. For gravel, root and substrate diversity there was no significant correlation with the metrics and indexes of the macroinvertebrate community.

A forward selection model applied to the redundancy analysis (RDA) of microhabitat variables identified Froude Number as the best predictor of trait in the wet season (adjusted $R^2 = 0.04$; p-value = 0.001) following by boulder (adjusted $R^2 = 0.02$; p-value = 0.005), cobble (adjusted $R^2 = 0.01$; p-value = 0.030), root (adjusted $R^2 = 0.01$; p-value = 0.029) and leaf litter (adjusted $R^2 = 0.63$; p-value = 0.004). All of these variables selected had a VIF below 2. The RDA explained approximately 10% of the data variation (13% not adjusted; F=5.21; p-value = 0.001) and only the first and second axis were significant (Figure 6).

The Froude Number and cobble substrate were negative correlated with the first axis of the RDA that explains 60% of this variation. The traits temporarily attached to substrate (attach), high body flexibility (Flex.45), collectors-filterers (CF), scrapers (SC), collectors-gatherers (CG), suckers (Suc), endobenthic burrower (enb), climbers (TH) and with body size between 2.5 and 10 mm are also negatively correlated to the first axis, thus indicating the relationship of Froude Number and cobble substrate. The surface swimmer (sws), no flow adaptation (NA), epibenthic burrower (epb), body size between 2.5 and 40mm were positively correlated with the first axis, indicating the inverse relationship with Froude number and cobble substrate.

Ordination along the second RDA, that explained 30% of the adjusted total variation, was mainly determined by substrate type which leaf litter and boulder substrate were the most correlated to this axis. However, according to the second axis, the leaf litter had a negative correlation whereas boulder substrate had a positive correlation. The most part of the traits are negative correlated to the second axis indicating that the traits related to no flow adaptation (NA), predators (PR), body flexibility < 10° (Flex.10), body size between 10 and 40mm and anal hooks (AH) were influenced by leaf litter.

In the dry season, a forward selection model identified leaf litter as the best predictor (adjusted $R^2 = 0.08$; p-value = 0.001) following by Froude Number (adjusted $R^2 = 0.05$; p-value = 0.001), gravel (adjusted $R^2 = 0.03$; p-value = 0.001), root (adjusted $R^2 = 0.007$; p-value = 0.028) and wood (adjusted $R^2 = 0.006$; p-value = 0.037). All of these variables selected had a VIF below 3. The RDA explained approximately 18% of the data variation (20% not adjusted; F=12.77; p-value = 0.001) and only the first and second axis were significant (Figure 7).

Leaf litter was positive correlated with the first axis of the RDA that explains 70% of this variation. The most of the traits are also positive correlated to the first axis. Predators (PR), anal hooks (AH), shredders (SH), body flexibility lower than 45° and body size between 10 and 20mm were the traits more influenced by leaf litter. In the dry season, the ordination along the second RDA explained 20% of the adjusted total variation and was mainly determined by Froude Number that was positive correlated to this axis. Temporary attached to substrate (attach), suckers (Suc) and collectors-filterers (CF) were also positive correlated to the second axis and were highly influenced by Froude Number.

4. Discussion

Following our first hypothesis, in the wet season selects tolerant functional traits with specific adaptation and mobility to flow constrain (i.e., temporarily attached to substrate, suckers and climbers) and small individuals with high body flexibility. Following our second hypothesis, in the dry season, the stable flow condition favors sensitive traits (i.e., larger individuals with a low body flexibility) which most of the trait species were influenced by leaf litter. Lastly, following our third hypothesis, the leaf litter was the main microhabitat variable predictor for the benthic macroinvertebrates in the dry season where showed a higher density, taxonomic and functional richness compared to the wet season. Additionally, the leaf litter was significant lower in the wet season compared to the dry season and inversely related to the Froude Number, indicating that in the wet season decrease the leaf litter retention capacity because of the increased seasonal flow.

The association between Froude Number, cobble substrate and tolerant traits in wet season could be related to refuges use in the wet season, where the cobble substrate provides a more adequate flow shelter than fine substrates (Theodoropoulos et al., 2017; Townsend et al., 1997). The cobble substrate was also positive correlated to the trait evenness in the dry season indicating that habitats with cobble and high flows may restricts the macroinvertebrate distribution (Statzner and Higler, 1986). The cobble substrate was the predominant substrate type covering more than 70% of the streams bed surface which means to a heterogeneous surface. Substrate heterogeneity, by allowing more species to coexist, confers resistance to the community of the streams in the face of disturbances (Brown, 2003).

This resistance can be caused by the high availability of food resources and interstitial spaces which, in turn, promote protection against predators and flood refuges (Gjerløv et al., 2003; Hoover and Ackerman, 2011; Townsend et al., 1997). The importance of small organisms with high body flexibility increased in the wet season, showing the potential ability to individuals find refuge (de la Fuente et al., 2018; Robinson et al., 2004). Milesi et al. (2016) showed that heterogeneous substrates support communities with high diversity and functional richness when compared with homogeneous substrates in Brazilian streams.

Boulder substrate correlated negatively with the taxonomic richness and represents less than 5% of stream bed surface area. In RDA from the wet season, despite showed a high correlation with the second axis, none trait was correlated. Certainly, under instable flow conditions in the wet season, the boulder substrates may expose organisms to severe conditions (Erman and Erman, 1984; Poff and Ward, 1990) where it can promote less protection from predators and more exposure to physical disturbances (e.g., current velocity, shear stress) (Hoover and Ackerman, 2011).

The leaf litter surface area in the stream bed doubles in the dry season. In the RDA of both seasons showed that the leaf litter was an important variable of microhabitat that most influenced traits, mainly to the traits sensitive to the variation of the seasonal flow. However, in the dry season, the importance was higher when compared to the wet season. Unless for trait evenness, which showed a negative correlation, the metrics and indices of the macroinvertebrate community were strongly influenced by the leaf litter in the dry season. Due to the stable flow conditions in the dry season where increased the leaf litter accumulation, we speculate intensive use of some specific trait (e.g., no adaptation to high flow, predators, high body size) decreasing the trait evenness.

Therefore, the leaf litter input represent for Neotropical forest streams the critical resource for the benthic macroinvertebrate community (Baptista et al., 2001; Bruder et al., 2014; Ríos-Touma et al., 2009; Tank et al., 2010). In neotropical savanna streams, the monthly leaf litter input by riparian is continuous and in small quantities, with peaks occurring during the transition from dry to wet season (Bambi et al., 2017). Thus, we hypothesize that the leaf litter retention depends mainly on the magnitude and variability of the seasonal flow (Speaker et al., 1984; Tejeda, 2018).

We also observed that the area covered by wood, although low in relation to other substrates (<2%), was significantly higher in the dry season and showed a significant correlation for the taxonomic richness and diversity of traits in both seasons. Just as the leaf litter, the wood substrate is a key structural element in forested streams, as it enhances channel complexity, increases habitat diversity and offers refuge for many species (Faustini and Jones, 2003; Scealy et al., 2007). Wood substrate can also increase the retention of allochthonous organic matter (Tejeda, 2018).

It was also verified the importance of the functional groups of feeding that changed with the seasonality. The predators were influenced by litter in both seasons, showing a highest importance in the dry season. Studies suggest that predatory invertebrates are directly related to leaf litter accumulation within the stream bed and thus, biotic interactions such as predation can be suppressed during periods of environmental instability (Dudgeon, 1993). Long-term experiments have shown that the exclusion of litter accumulation in streams resulted in significant differences in the abundances of dendritic and predatory invertebrates when compared to the reference streams (Wallace, 1997). In addition, collectors-filterers increased the importance in the wet season. Water flow increase favors the transport of fine organic material in the water column, involving the increase of the relative abundance of passive collectors-filterers (Georgian and Thorp, 1992).

Our findings demonstrate that seasonal changes on Neotropical savannas act as strong filters of species traits of benthic macroinvertebrates communities in forest streams. In addition, in Neotropical savanna streams, the impact of seasonal flow variation on the structural and functional traits of macroinvertebrate assemblages depends on the microhabitat features, which fulfilling the Habitat Templet concept (Southwood, 1977; Townsend and Hildrew, 1994). In high seasonal flow the macroinvertebrate community may adjust to flow disturbance (i.e., increasing the resistant and resilient traits) and the organic debris becomes more susceptible to losses. Our results also suggests a greater dependence of Neotropical forest streams in relation to the riparian vegetation which promotes a food resource, habitat and refuge for the benthic macroinvertebrates community through the input of organic debris (Vannote et al., 1980).

In a scenario of riparian vegetation removal, increased flow disturbance due to increase impervious surface and / or the number of intensity rains related to the global environmental change, the retention of organic detritus inside streams can be impairment, in addition to the streams bed being buried via the input of fine sediment that consequently will affect the entire aquatic biodiversity. The contributions of species to ecosystem properties and services as well as their vulnerabilities depend not on the individual traits, but a combination of these.

Moreover, the headwater streams (catchment size <10 km2) are essentially link to terrestrial environment making them vulnerable to disturbances in the surrounding anthropogenic catchment (Lowe and Likens, 2005). The importance of headwater streams for biodiversity at catchment scale and their vulnerability to anthropic disturbances are often neglected in management strategies (Baattrup-Pedersen et al., 2018). Our study was limited in silicate geology that underlines the need to consider others geologies and the scale, both spatial and temporal, in comparing or assessing the resilience of streams.

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Table 1. General information about the investigated streams.

Stream name	Três Barras (A)		Tortinho (B)		Capetinga (C)		Cabeça-de-Veado (D)	
Altitude (m)	1116		1146		1088		1075	
Stream order	2nd		2nd		2nd		2nd	
Stream Width (m)	2.0±0.7		2.2±0.7		3.6±1.0		2.8±0.5	
Season	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**
Electrical conductivity (µS.cm^{-1})	3.9±0.3	3.4±0.3	5.6±0.5	6.1±0.3	3.9±0.2	4.4±0.6	4.7±0.3	5.1±1.3
Ph	6.4±0.3	5.9±0.1	6.1±0.2	6.2±0.1	5.9±0.1	5.5±0.1	5.8±0.1	5.7±0.1
Dissolved oxygen (mg.L^{-1})	6.3±1.1	6.7±0.2	5.6±0.5	6.6±0.2	8±3.7	5.9±0.3	5.3±0.3	6.3±0.1
Turbidity (NTU)	2.9±0.9	4.0±1.2	4.0±0.6	4.2±1.3	2.5±0.7	3.9±2.5	1.2±0.5	2.2±0.6
Temperature (ºC)	20±1.2	16±0.5	20±0.2	17±0.5	20±0.7	15±1.6	21±0.2	17±1.5
Discharge (m^3.s^{-1})	0.3±0.1	0.2±0.1	0.2±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.0

Table 2. Traits and their modalities and abbreviations used for 45 aquatic family insects collected in neotropical savanna streams

Traits name	Number of modalities	Modalities (abbreviation)
Feeding Habits	6	collectors-gartheners (CG), shredders (SH), scrapers (SC), collectors-filterers (CF), piercers (PI), predators (PR)
Body Size	6	≤2.5, 2.5-5, 5-10, 10-20, 20-40, 40-80
Body Flexibility	3	smaller than 10° (Flex<10), between 10° and 45° (Flex10_45), higher than 45° (Flex>45)
Body Form	4	streamlined (streamli), flattened (flat), cylindrical (cylin), spherical (spherical)
Specific adaptation to flow constraints	6	suckers (Suc), silt gland (SG), mineral case (MM), anal hooks (AH), climbers (TH), no adaptation (NA)
Mobility and attachment to the substratum	6	surface swimmer (sws), full water swimmer (sww), crawler (crawler), epibenthic burrower (epb), endobenthic burrower (enb), temporarily attached (attach)

Table 3. Spearman rank correlations between trait and taxa metric and microhabitat substrate variables for each season separately. For gravel, root and substrate diversity there was no significant correlation. Only significant correlations are shown ($p < 0.05$) with the metrics and indexes of the macroinvertebrates community.

	Froude Number	Boulder	Cobble	Fine Substrate	Leaf Litter	Wood
Wet Season						
Taxa Richness	-	-	-	-	-	0.17
Trait Richness	-	-	-	-	-	-
Taxa Diversity		-	-	-	-	0.22
Trait Diversity	0.23	-	-	-	-	0.17
Taxa Evenness		-	-	-	-	-
Trait Evenness		-	-	-	-	-
Density	0.2	-	-	-	-	-
Dry Season						
Taxa Richness	-	-0.13	-	-	0.28	-
Trait Richness	-	-	-	-	0.24	0.13
Taxa Diversity	-	-	-	-	0.21	-
Trait Diversity	0.2	-	-	-0.13	0.13	0.15
Taxa evenness	-	-	-	-	-0.21	-
Trait evenness	-	-	0.22	-	-	-
Density	-	-	-	-	0.24	-

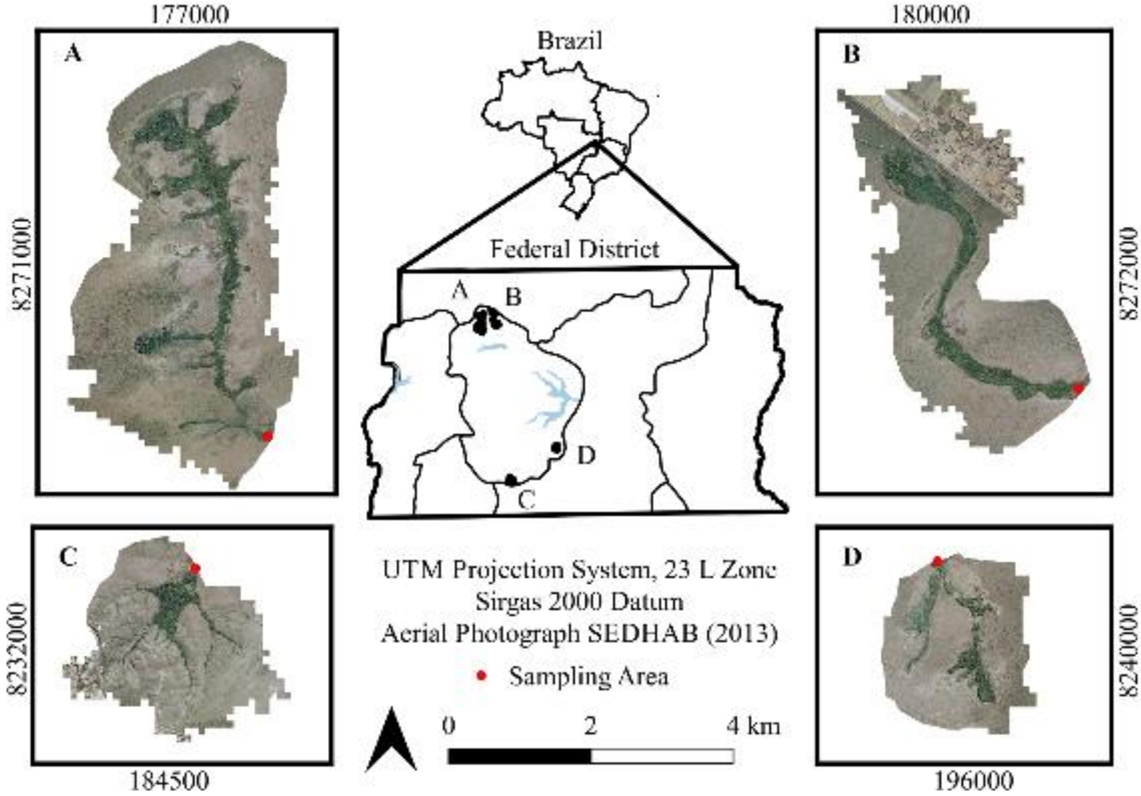

Figure 1. Location of catchments in the Federal District, Brazil. A) Três Barras and (B) Tortinho streams belong to the National Park of Brasilia. D) Cabeça-de-Veado and (C) Capetinga streams belong to the Environmental Preservation Area Gama Cabeça-de-Veado. Red dots represent the sampling location in each basin.

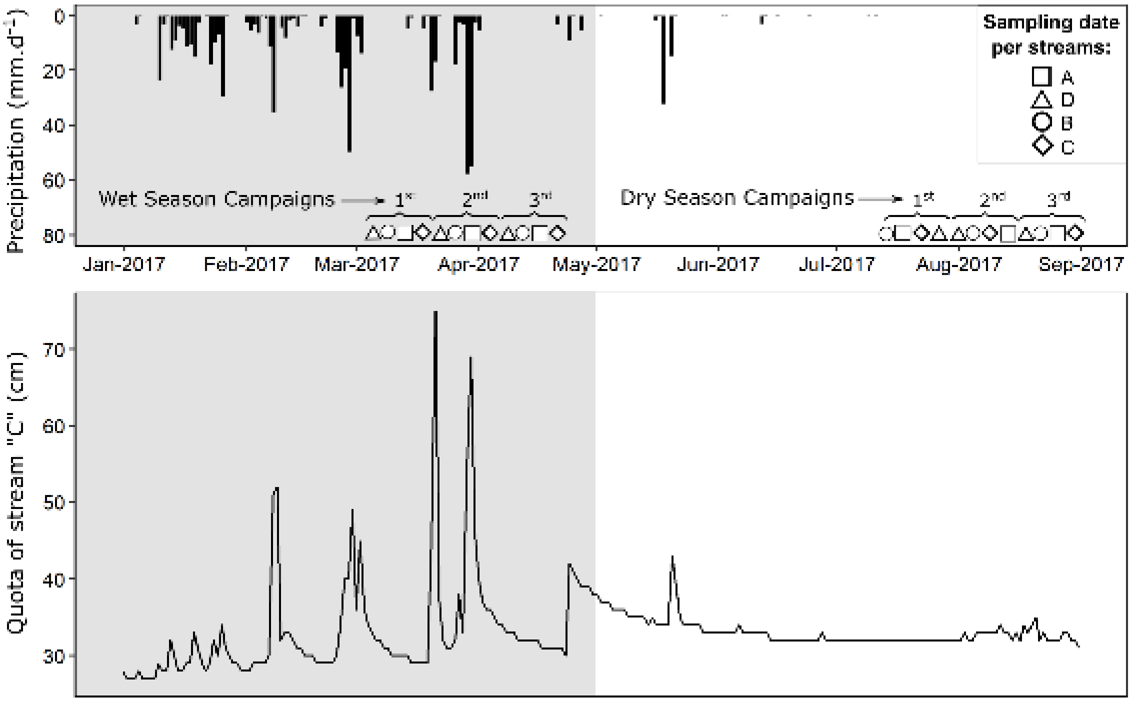

Figure 2. Precipitations and water levels monitored at the Capetinga stream (C). The shaded area corresponds to the wet season whereas the unshaded area represents the dry season. At each season, sampling times are shown.

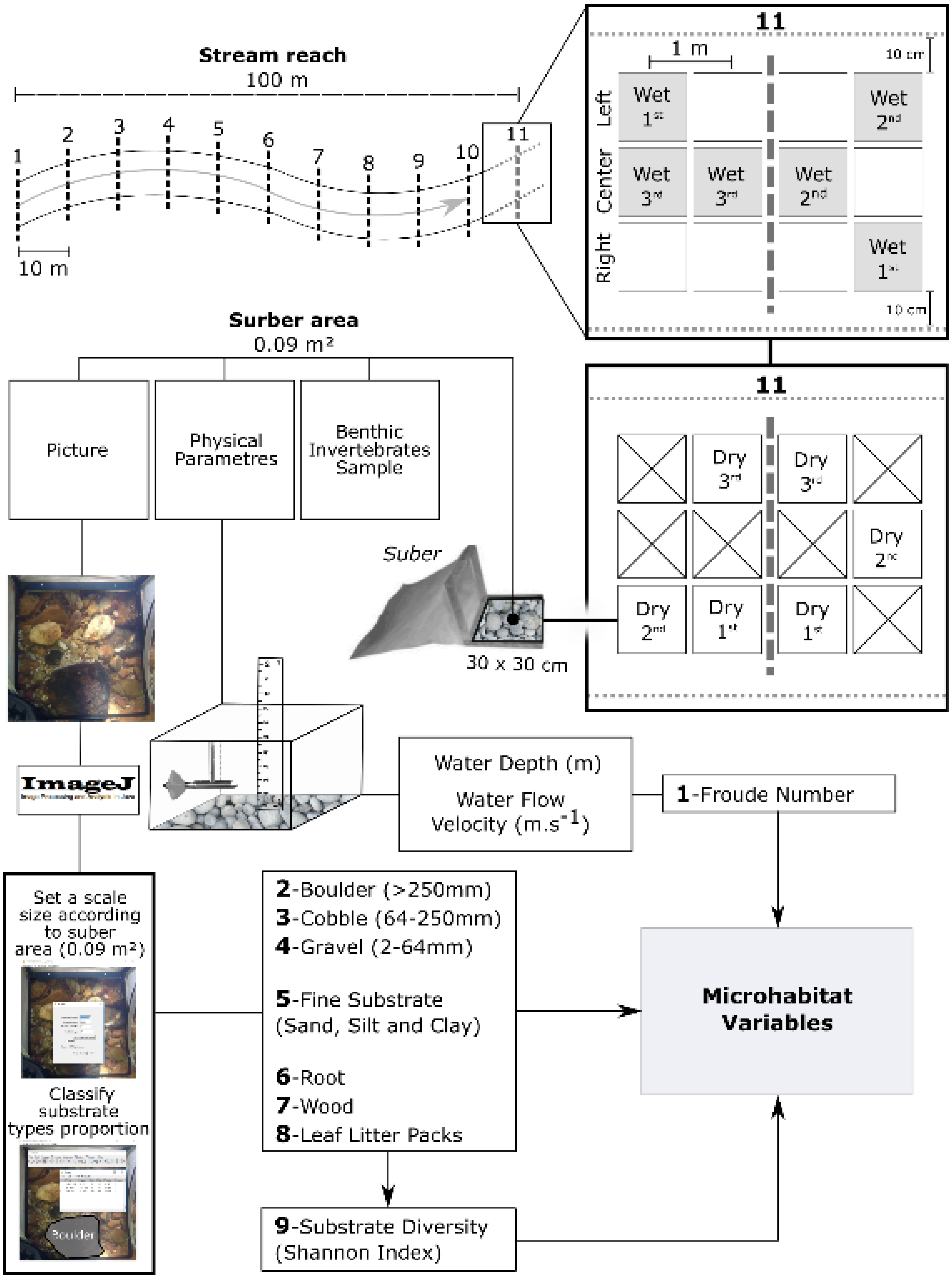

Figure 3. Schematic diagram showing the sampling design and nine microhabitat variables measured.

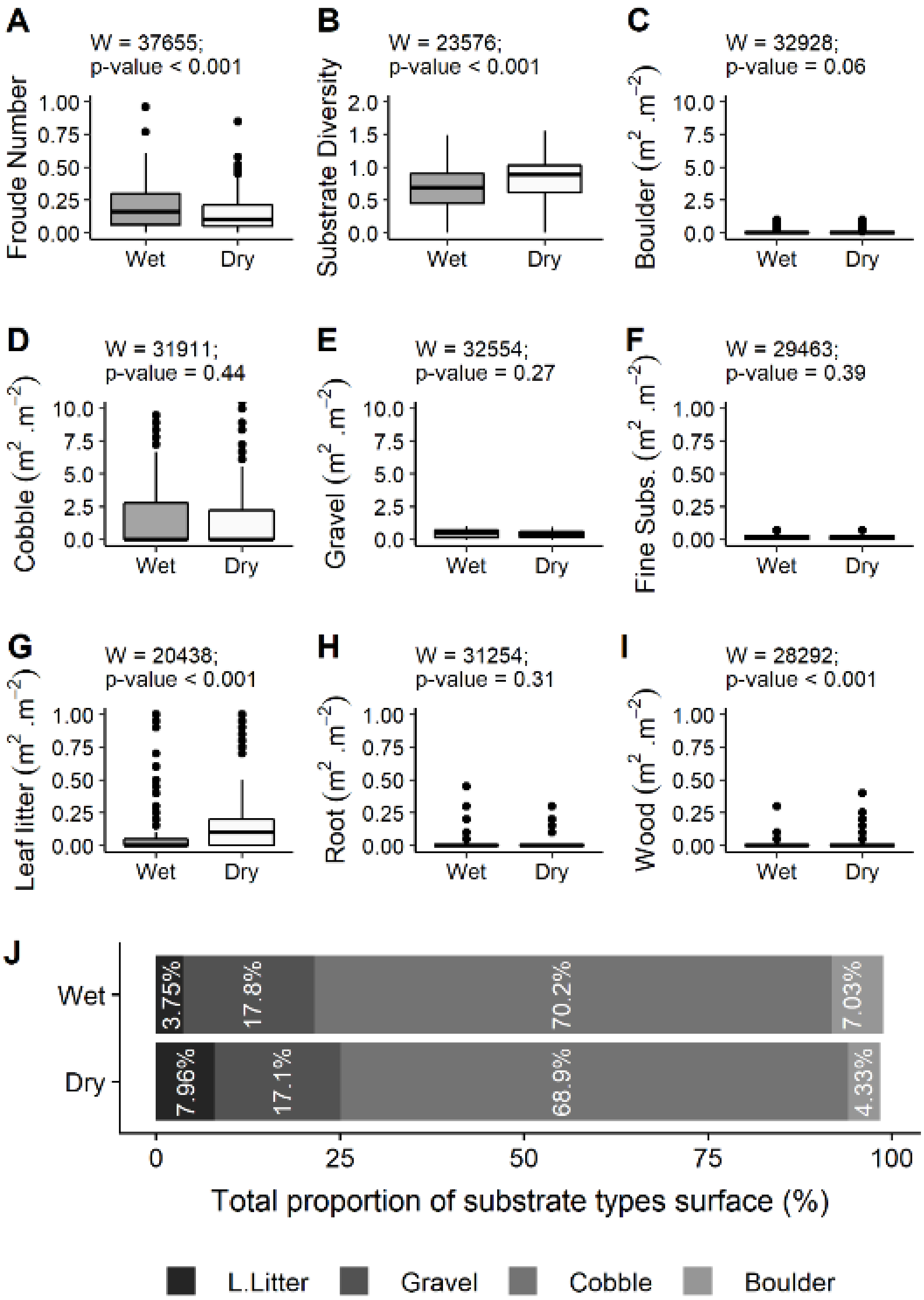

Figure 4. Boxplot comparison of the substrate variables (A-I) for the dry and wet season. The statistical comparison (W-Wilcoxon rank test) of the dry and wet season is reported in each graph. Were also showed the total proportion of substrate types surface (J). The total proportion surface of root, wood and fine substrate were not represented in the proportion graph (J) because they represent together less than 2%, making it difficult to visualize them.

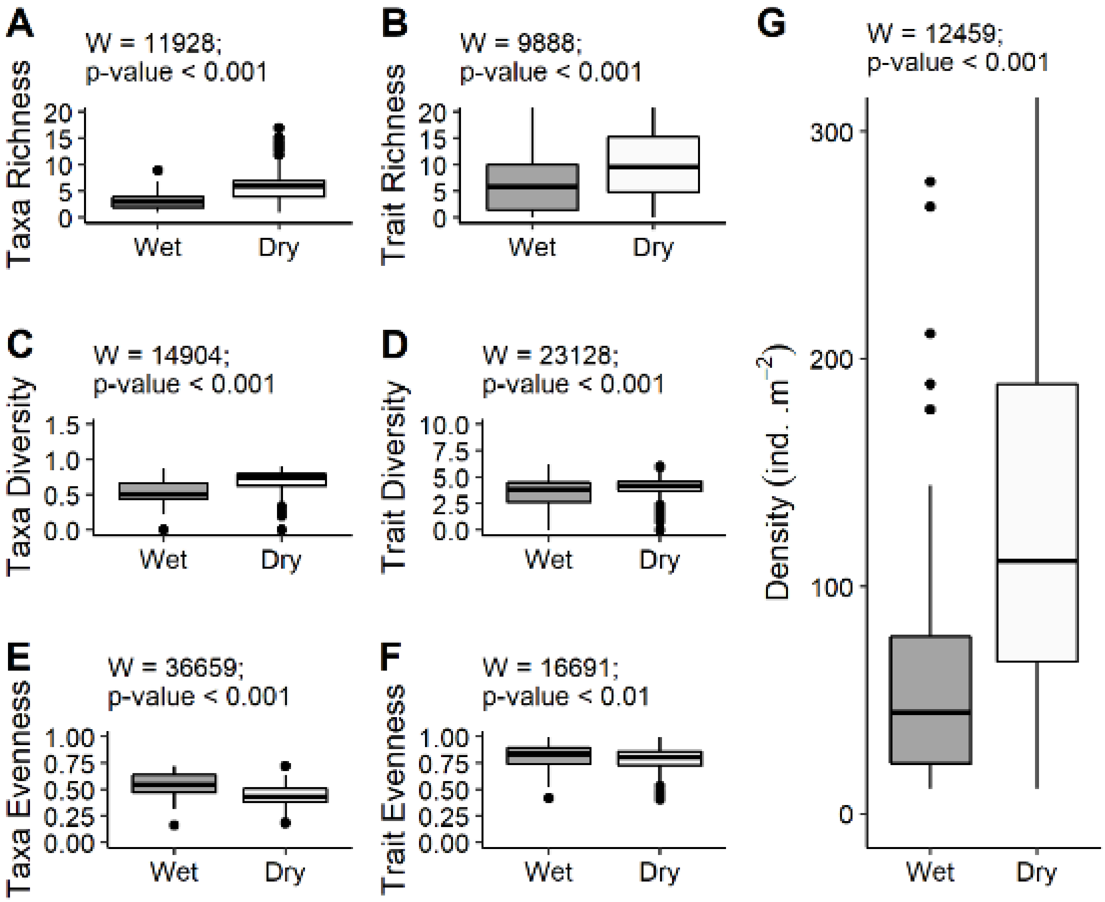

Figure 5. Boxplot comparison of the macroinvertebrate metrics and indexes (A-G) for the dry and wet season. The statistical comparison (W-Wilcoxon rank test) of the dry and wet season is reported in each graph.

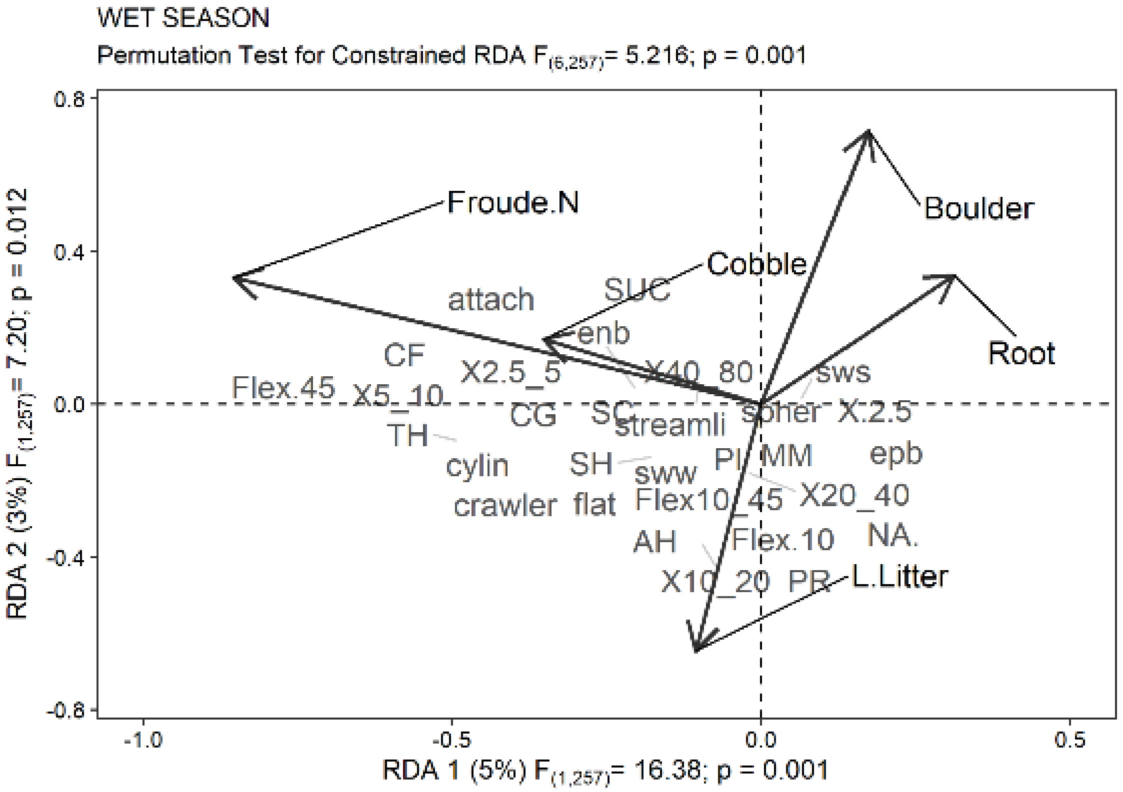

Figure 6. RDA ordination diagram based on six substrate variables and 38 invertebrate traits weighted by abundance for the wet season. The “F” refers to permutation test for constrained correspondence analysis for both axis and the overall model. The traits are in grey scale letters following by: collectors-gartheners (CG), shredders (SH), scrapers (SC), collectors-filterers (CF), piercers (PI), predators (PR), body size ≤2.5 (X.2.5), body size 2.5-5 (X2.5_5), body size 5-10 (X5_10), body size 10-20 (X10_20), body size 20-40 (X20_40), body size 40-80 (X40_80), flexibility smaller than 10° (Flex<10), flexibility between 10° and 45° (Flex10_45), flexibility higher than 45° (Flex>45), streamlined (streamli), flattened (flat), cylindrical (cylin), spherical (spherical), suckers (Suc), silt gland (SG), mineral case (MM), anal hooks (AH), climbers (TH), not adapted to high flow, (NA), surface swimmer (sws), full water swimmer (sww), crawler (crawler), epibenthic burrower (epb), endobenthic burrower (enb) and temporarily attached (attach).

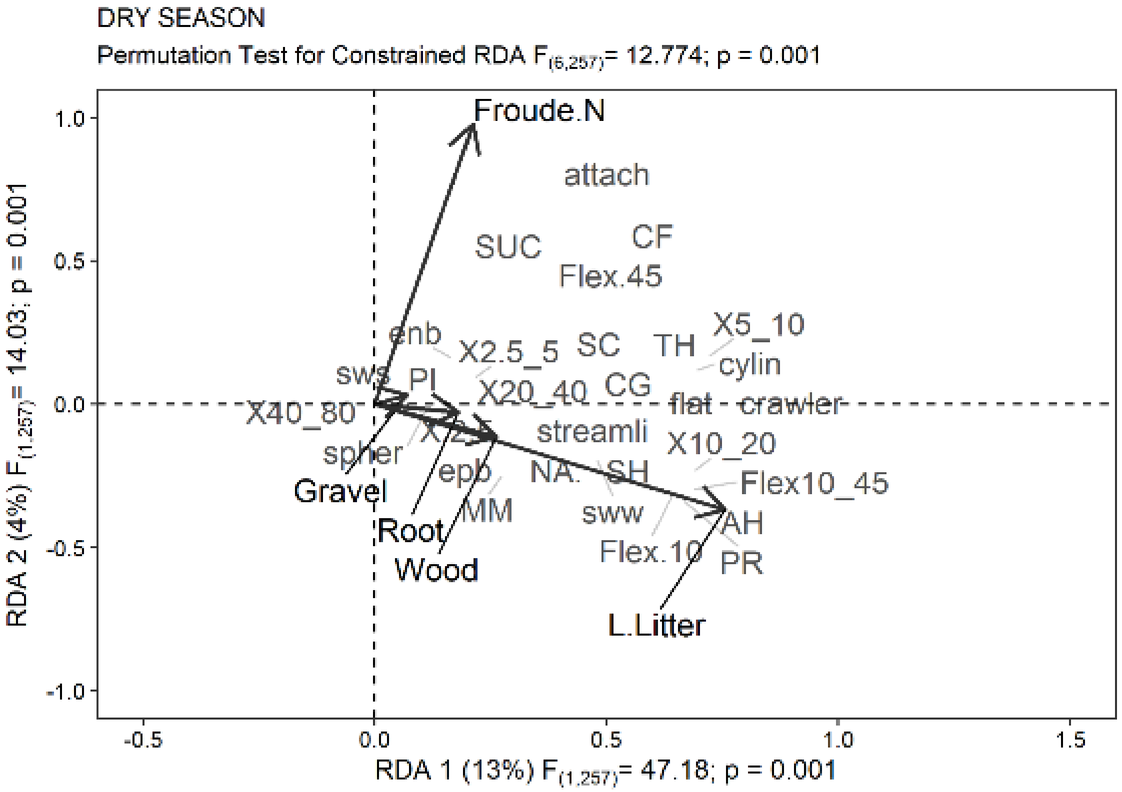

Figure 7. RDA ordination diagram based on six substrate variables and 38 invertebrate traits weighted by abundance for the dry season. The “F” refers to permutation test for constrained correspondence analysis for both axis and the overall model. The traits are in grey scale letters following by: collectors-gartheners (CG), shredders (SH), scrapers (SC), collectors-filterers (CF), piercers (PI), predators (PR), body size ≤2.5 (X.2.5), body size 2.5-5 (X2.5_5), body size 5-10 (X5_10), body size 10-20 (X10_20), body size 20-40 (X20_40), body size 40-80 (X40_80), flexibility smaller than 10° (Flex<10), flexibility between 10° and 45° (Flex10_45), flexibility higher than 45° (Flex>45), streamlined (streamli), flattened (flat), cylindrical (cylin), spherical (spherical), suckers (Suc), silt gland (SG), mineral case (MM), anal hooks (AH), climbers (TH), not adapted to high flow (NA), surface swimmer (sws), full water swimmer (sww), crawler (crawler), epibenthic burrower (epb), endobenthic burrower (enb) and temporarily attached (attach).

Capítulo 2 - Seasonal effects on the food web structure of benthic macroinvertebrates community in Neotropical forest streams

Tiago Borges Kisaka, Diego Marcel Parreira de Castro e Gabriela Bielefeld Nardoto

O presente capítulo será submetido para apreciação do periódico *Food Webs*

Todos os autores contribuíram criticamente para a interpretação dos resultados e com a versão final do manuscrito.

Title: Seasonal effects on the food web structure of benthic macroinvertebrates community in Neotropical forest streams

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Abstract

1. Seasonality in Neotropical forest streams has great potential to affect the availability of resources and consequently the trophic web of aquatic biota. Despite this potential disturbance, community-level responses of seasonal flow variations over food resources and trophic relationships are inconsistent. We investigate the seasonal effects on the energy flow from basal resources to benthic macroinvertebrates community in Neotropical forest streams.

2. The study was carried out in four low order forested streams located in Conservation Units at the Paranoá sub-basin, Central Brazil. We monitored the resources and the trophic structure of the benthic macroinvertebrates community. Natural variations in the carbon and nitrogen isotopic signatures of food resources, consumers, and predators were measured and the characteristics of isotopic niches in dry vs. wet season were compared.

3. Chlorophyll-a concentrations of biofilm and seston and the percentage of organic debris were significant higher in the dry season compared to the wet season. In the dry season, dry mass of the entire community was twofold higher in relation to the wet season. Bayesian estimation of community metrics in dry vs. wet season showed a 13.6% larger δ^{15}N range in the dry season and a 23.2% larger δ^{13}C range. The trophic redundancy metric was 36.4% smaller in the wet season, indicating the trophic redundancy across community members.

4. We conclude that in the wet season food availability is reduced with consequences on the diversity of food resources assimilated by the macroinvertebrates, in addition to increasing the functional redundancy in the community. Leaf litter accumulation was a significant predictor of the food web, but the seston also contributed as a food resource for the macroinvertebrate community. Lastly, a large amount of energy transferred along the trophic structure was severely reduced in the wet season.

Keywords: Seasonal flow; Trophic relationship; Isotope niche, Dry mass.

1. Introduction

Neotropical streams have a pronounced seasonal variation between dry and wet seasons (Flecker and Feifarek, 1994), where the more turbulent flows present in the wet season may impact the availability of food resources and consequently the trophic relationships established in the aquatic biota (Davis et al., 2018; Frauendorf et al., 2013). Despite this potential disturbance to aquatic biota, community-level responses of seasonal flow variations over food resources availability are inconsistent for neotropical forest streams. Given the uncertainties of climate change, the understanding of seasonality encompassing gradients of climate variability is a critical factor for both basic science and ecosystem management (Tonkin et al., 2017).

As in temperate streams, the main source of energy in tropical rainforest headwater streams are from the riparian forest (Neres-lima et al., 2017). The allochthonous source has fundamental importance for the functioning of these ecosystems being represented mainly by the particulate organic matter in the leaf litter form (Colón-Gaud et al., 2008). In temperate streams, the litter stocks accumulated in the streams depend mainly on the forest primary production, with leaf fall pulses in autumn (Wallace et al., 2015). However, in tropical environments where the leaf litter input into the streams is continuous and in small quantities (Bambi et al., 2017), the litter accumulation in streams depends mainly on the magnitude and variability of the water flow determining the leaf litter retention capacity in the streams (Speaker et al., 1984).

Temporal changes in the hydrology of the tropical streams with recurrent stormflows events (Salemi et al., 2013) result in great hydraulic power and may decrease the retention capacity and consequently the accumulation of litter in the streams (Larrañaga et al., 2003). The increase in water velocity can produce transport of fine particulate organic material in the water column from which it can be related to the increase of the abundance of invertebrates with collector-filtering feeding habits (Georgian and Thorp, 1992). It is suggested that the fine organic debris have great importance for neotropical streams as litter decomposition in tropical streams is fast and continues throughout the year (Palmer et al., 1993). Therefore, it suggests that the activity of collectors-filter invertebrates should be regular, indicating fine debris as an important food resource in neotropical forest streams (Tomanova and Usseglio-Polatera, 2007).

Besides leaf litter, there are some evidence showing the importance of algae in the food webs of tropical streams (Neres-Lima et al., 2016). However, stormflow events occurring in the wet season may reduce the food resources in streams (e.g., leaf litter and biofilm) which can influence the types of resources consumed by the aquatic community and limit the secondary production (Colón-Gaud et al., 2009; Dudgeon, 2000a; Yang et al., 2009). Thus, it is expected an omnivorous predominant behavior of aquatic invertebrates in neotropical streams compared to temperate streams due to changes in the relative availability of resources that fluctuate with seasonality (Covich, 1988).

Studies suggest that predatory invertebrates are directly related to litter accumulation within the stream and thus, biotic interactions such as predation can be suppressed during periods of environmental instability (Dudgeon, 1993). Pearson (2014) suggest that stormflows events in the wet season may cause the decline and mortality of many invertebrates, reducing the importance of predation and competition as structuring forces in tropical stream food webs. In the dry season may also cause the stream contraction that could increase individuals densities and enhanced the likelihood of inter- and intra-specific interactions of the macroinvertebrates community (Benson and Pearson, 1987).

Long-term experiments have shown that the exclusion of litter accumulation in streams resulted in significant differences in the abundances of detritivores and predators invertebrates when compared to pristine streams (Wallace, 1997). These observations provide support for the productivity hypothesis (Pimm, 1982), in which the availability of basal resources determines the number of trophic levels (Thompson and Townsend, 2005).

Therefore, this study aimed to investigate the effects of seasonality on the energy flow from basal resources to benthic macroinvertebrates community in Neotropical forested streams. The isotopic ratios of carbon and nitrogen of resources, consumers, and predators were measured and the characteristics of the isotopic niches in dry vs. wet season were compared. Following the seasonal flow changes in the neotropical savannas, we expected less multiple basal resources assimilated by the community in the wet season that would be visible at the community-level through a greater focus of carbon isotope signatures. We also expected a decrease of the isotopic space in the wet season, that would be visible through a smaller range of nitrogen isotope signatures in the wet season.

2. Material e Methods

2.1 Study area

The study was carried out in Conservation Units in the Paranoá basin located of the Federal District, Brazil (Figure 1). Four streams were selected according to the geology that is silicate and belongs to the Paranoá Group, with clay-sandy metarythmic and quartzite units (Campos et al., 2013). The substrate bed of streams is mainly composed by gravel cobble, boulder and leaf litter. The forest formation that accompanies these streams forms closed corridors (galleries) over the watercourse. The physiognomy is evergreen, barely exhibiting leaf fall in the dry season. The height of the trees varies between 20 and 30 meters, and the position of the treetops provides coverage of 70% to 95% (Ribeiro and Walter, 2001).

The climate of the region is marked by strong seasonality, with a wet season from October to April and a dry season from May to September. The average annual rainfall is about 1.500 mm. The soils of the study area are mainly Oxisols according to the US soil classification. Unless for the water temperature, which varied between 3 and 4 degrees Celsius between the seasons with measurements always in the early morning, the most water quality parameters did not show a significant difference between seasons (Table 1). Other characteristics of the streams are given in Table 1.

2.2 Study area

Samples were collected during the wet season (March-April), demonstrating high and unstable flow, and during the dry season (July-August), showing lower and more stable flow, in 2017 (Figure 2). Three sampling campaigns were carried out during each season in the four streams totaling 24 samplings. A continuous segment of 100 meters was selected in each stream and subdivided into 11 equally sections (10m between each one). In each sampling campaign and section, two samples were collected, totaling 22 samples per stream. In each season was collected 66 samples per stream, totaling 256 samples per season. In each campaign and stream, five samples of food resources: biofilm, seston (suspended particulate organic matter) and leaf litter were randomly collected between sections. Therefore, were obtained a total of 360 food resource samples (4 streams x 5 points/stream x 3 resources/point x 2 seasons x 3 sampling campaigns/season) and 528 macroinvertebrates samples (4 streams x 11 sections/stream x 2 microhabitats/section x 2 seasons x 3 sampling campaigns/season).

Macroinvertebrates were collected with a Surber sampler (30 cm × 30 cm, 100 µm mesh size) covering all substrates. We separated the individuals in the field using forceps and a sieve with a diameter of 19 cm and 100 µm mesh. Pictures of the Surber sampled area (30 cm x 30 cm) using a waterproof camera (GoPro Hero 5) were analyzed with ImageJ™ to classify and quantify the organic substrate as leaf litter packs, fine litter, wood and roots and coarse substrate (i.e., boulder and cobble). We also calculated the Froude number, as indicator of hydraulic stress for invertebrates in each microhabitat, considering the depth (D) and water velocity (v) from the equation Fr=v/(g*D)0.5, where g is gravitational acceleration.

Biofilm was collected by cleaning rocks (eight rocks per sample) with a soft bristle brush and distilled water (Anexo B, Figure S1). Seston was collected with a phytoplankton net (0.45 mm) set for 2 min upstream of each site (Anexo B, Figure S2). The biofilm and the seston samples were stored in amber containers. The litter samples were sampling in accumulation zones characterized by low flows with tube corer (20 cm in diameter). All food resource samples were immediately placed on ice in a cool box and transported to the laboratory. For the macroinvertebrate samples, each individual was kept separate and alive in thermal boxes for transportation and in the laboratory were kept alive in cooled water for 24 h to clear their digestive tracts of residues that could distort the isotopic analyses.

2.3 Sample processing

Invertebrates and food resources samples were not treated with preservative to avoid bias in measures of δ^{13}C and δ^{15}N isotopic signatures. After the gut clearance, the invertebrate samples were preserved at -20°C. Then, the organisms were counted, taxonomically identified at the family level using the available identification keys (Hamada et al., 2014; Mugnai et al., 2010) and classified into functional feeding groups (FFG) (Merritt et al., 2008; Ramírez and Gutiérrez-Fonseca, 2014). For stable isotope analyses (SIA), we selected the taxa according to feeding habit and their relation with microhabitat variables (i.e., Froude Number, coarse substrate and leaf litter pack) (Table 2). We also select the families of which their abundances represent about 70% of the total of individuals sampled. Each taxon was considered a consumer and biofilm, seston and leaf litter were considered resources. Macroinvertebrates that were not predators were considered as food resources for predators.

The individuals were ground and dried at 60°C for 48 hours for SIA, and different numbers of invertebrates were used to reach a minimum amount of material for isotope analysis. Because 1.5–2.0 mg dry weight of the sample is required for SIA, in all cases individuals had to be assembled to meet the detection limits of the mass spectrometer. Thus, 10–20 Leptophlebiidae, 6-10 Simuliidae, 2–4 Elmidae (Adult), 4-10 Chironomidae, 2–4 Perlidae, 9–15 Baetidae, 1-3 Calamoceratidae, 1-4, Tipulidae, 1-4 Psephenidae, 2-5, Odontoceridae, 1-5, Hydropsychidae, 1-2, Gomphidae and 1-3 Libellulidae had to be sorted, cleaned from adhering particles in distilled water, and encapsulated together to make up enough biomass for SIA. Thus, from 702 individuals designated for SIA were encapsulated 194 samples. We also matched the same number of samples between the seasons, that is, 97 samples in the dry season and 97 samples in the wet season, with the same proportion per family (Table 2).

Biofilm and seston samples were immediately vacuum filtered using pre-combusted glass-fiber filters (pore-size 45 µm, Whatman GF/C) for the determination of chlorophyll-a, ash-free dry mass (AFDM) and SIA, from different aliquots of the collected volume. The filters for the determination of chlorophyll-a were placed in centrifuge tubes containing 10 ml of 100% ethanol, (Sartory and Grobbelaar, 1984). The absorbance values of the supernatant were measured at 750 and 665 nm, and the chlorophyll-a concentrations were calculated following Marker et al. (1980). To determine the AFDM, each sample was transferred to pre-labeled aluminum plates and dried at 60°C until a constant mass was reached. These dried samples were weighed and then combusted at 550°C for 6 hours and then weighed again.

For the stable isotope analysis, the filtered biofilm and seston samples were dried in an oven at 60°C for 48 h before ground to a fine and homogeneous powder using a mortar and pestle for SIA (Anexo B, Figure S3). The leaf litter samples, preserved at -20°C in plastic containers, were rinsed with deionized water, dried at 45°C for 48h and pulverized to a fine powder using a knife-mill grinder for the SIA. Approximately 1.5–2.0 mg of dried animal tissue and resources was encapsulation in tin cups for the isotopic analysis.

We measure the dry mass of macroinvertebrates as an estimation of biomass. The macroinvertebrates dry mass was estimated trough length-mass equations (Benke et al., 1999). Twenty randomly individuals in each taxon and having the entire body parts were selected. The length of those specimens was measured using LCD digital caliper Clockwise Tools™ (DCLR-0605, accuracy: ±0.001 mm). Body length in insects included the distance between the anterior part of the head and the posterior part of the last abdominal segment. Subsequently, we dried at 60 °C until constant weight was reached and directly weighed on the precision microbalances Radwag™ (MYA 21.4Y, readability 0.001 mg).

The body mass relationships were assessed assuming that weight and length are related by the general power equation $\ln DM = \ln a + b \ln L$ (where: a and b are constants, DM = dry mass, and L = length). The linearized model was fitted to the log-transformed data by standard regression techniques according to Miserendino (2001). Finally, after measured the length of all individuals, the biomass was estimated through length-mass equation.

2.4 Stable isotope analysis

All the samples were sent to the Isotope Ecology Lab at the Centre for Nuclear Energy in Agriculture (CENA) of the University of São Paulo, Piracicaba, Brazil, to determine the values of $\delta^{13}C$ and $\delta^{15}N$. Isotopic ratio analyses were processed through the combustion of samples under continuous flow ultra-pure helium in an elemental analyzer (Carlo Erba, CHN-1110) which was connected to a Thermo Finnigan Delta Plus mass spectrometer. The results were expressed in delta notation (δ), in parts per thousand (‰), in relation to the standard international references (V-PDB - Vienna Pee Dee Belemnite for C and atmospheric air for N). The analytical precision values estimated by replicates of the working standards of $\delta^{13}C$ and $\delta^{15}N$ were ± 0.15 ‰ and ± 0.30 ‰, respectively.

2.5 Data analysis

Descriptive statistics were used to investigate the background behaviors of variables under seasonal contrast. For data analysis, normality was evaluated using the Shapiro-Wilk W-test. Wilcoxon independent tests were used to compare the results between seasons and between food resources, and consumers were compared using a Kruskal–Wallis test and Kruskal Multiple Comparisons (kruskalmc) post-hoc analysis from the pgirmess package in R (Giraudoux et al., 2018). Considering that there was no significant difference for the signatures of $\delta^{13}C$ and $\delta^{15}N$ values of the food resources between streams and to increase the accuracy of the analyses, we assembled the samples from all streams for the isotopic analyzes comparison between seasons.

The first impression of trophic positioning in dry vs. wet was obtained by plotting the $\delta^{13}C$ vs. $\delta^{15}N$ isotopic signatures of the resources and invertebrates. Trophic levels were identified using $\delta^{15}N$, and food sources using $\delta^{13}C$ (Peterson and Fry, 1987). SIAR v 4.2., a Bayesian stable isotope mixing model under the R statistical framework, was used to estimate the relative contributions of putative food sources to the assimilated diet of consumers and predators ('SIAR' package; Parnell and Jackson, 2013; Parnell et al., 2010). SIAR provides a true probability distribution of dietary contributions and directly compares estimated diets between groups of consumers. SIAR inferences are based on the assumption that the isotopic proportions from a food source undergo modification (expressed as a trophic enrichment factor, TEF) when assimilated by a consumer, as light isotopes are lost during protein assimilation. In our study, constant TEF values of +0.4 ±1.23 SD $\delta^{13}C$ and +2.3 ±1.61 SD $\delta^{15}N$ were used. These values corresponded to the mean TEF estimates in aquatic ecosystems (Mccutchan et al., 2003).

To increase the accuracy of mixing models, we assembled Tipulidae and Chironominae as "Diptera" and Baetids and Leptophlebiid as "Ephemeroptera." The signatures of primary consumers were therefore merged based on the following body-size, phylogeny, and related feeding habit criteria. Similarly, to reduce the number of putative sources for a diet of predators, in order to increase the accuracy of mixing models, Simuliidae were assembled in Diptera and we also disconsider the Calamoceratidae and Psephenidae, since this taxon was not evenness between streams. Thus, we consider Diptera, Elmidae, Ephemeroptera, Hydropsychiidae, and Odontoceridae as possible prey for predators.

The stable isotope Bayesian ellipses in R ('SIBER' package; Jackson et al., 2011) were used to estimate and compare the isotopic niche of single groups and whole communities in dry vs. wet. A series of metrics relating to the characteristics of the isotopic space filled by single groups and communities was proposed by Layman et al. (2007) and consisted of the following: CR (maximum $\delta^{13}C$ range); NR (maximum $\delta^{15}N$ range); TA (total area of the convex hull encompassed by all groups in the $\delta^{13}C$–$\delta^{15}N$ bi-plot space); CD (mean Euclidean distance of each group to the centroid of the community's $\delta^{13}C$–$\delta^{15}N$ bi-plot space); NND (mean Euclidean distances to each group's nearest neighbor, i.e., small NND values indicate high trophic redundancy in a community); and SDNND (standard deviation of NND, measuring the evenness of trophic redundancy). Since the reliability of those metrics can be affected by differences in the sample size (Jackson et al., 2011), they were reformulated using SIBER under a

Bayesian framework (qualitatively similar to bootstrapping approaches) to take into account errors and uncertainties in data sampling.

First, the assumption that the isotope data were well-described by a multivariate distribution was tested using the Shapiro-Wilk test. Posterior means, calculated by combining priors and likelihoods in an iterative simulation process using JAGS freeware (Plummer, 2003), were used to obtain unbiased, multivariate ellipse-based metrics, which allowed robust quantitative comparisons of isotopic niches among and within communities. The estimated values were then compared directly in a probabilistic manner regarding their similarity (or lack thereof).

3. Results

3.1 Resources

Chlorophyll-a concentrations of biofilm and seston were significantly higher in the dry season compared do the wet season, but the AFDM did not differ between dry and wet season (Figure 3). The organic debris was significantly lower in the wet season when compared to the dry season (Figure 3). Among the organic debris, leaf litter packs, fine litter, roots, and wood represents 53%, 36%, 6% e 5%, respectively, in the wet season. In the dry season, leaf litter packs, fine litter, roots, and wood represents 72%, 19%, 2% e 8%, respectively.

The %C, %N, and C:N content of the biofilm and seston did not differ significantly between wet and dry season. Leaf litter showed a significant decrease of C:N ratio in dry season compared to the wet season. The stoichiometric differences in the C:N ratio between resources were significant (Figure 3).

Comparing the food resources in the dry season, the δ^{13}C and δ^{15}N of seston were higher than the biofilm (Kruskal X^2=40.83 p-value < 0.0001) and the leaf litter isotope ratios did not differ significantly among biofilm and seston. In the wet season, the δ^{13}C between food resources were significantly different (Kruskal X^2=0.66, p-value < 0.0001) with lower values in the leaf litter following by seston and biofilm. In the comparison between the seasons, the leaf litter and biofilm δ^{13}C did not differ, but seston was significantly higher in the wet season (W = 1127, p-value < 0.0001). The δ^{15}N comparison between the seasons showed a higher δ^{15}N of seston in the wet season (W = 998, p-value

=0.0001) and the biofilm (W = 596.5, p-value = 0.7043) and leaf litter (W = 727.5, p-value = 0.1765) δ^{15}N did not differ between seasons.

3.2 Standing stocks invertebrate

Invertebrate dry mass varied within groups and at the community-level between dry and wet season (Table 3). The average dry mass per taxon was significantly higher in the dry season for Hydropsychidae, Perlidae, Baetidae, and Psephenidae compared to the wet season. Only the average dry mass of Simuliidae was higher in the wet season compared to the dry season. The total dry mass of invertebrates in the wet season was 0.05 g.m-2 and in the dry season was 0.15 g.m-2. Odonata and Hydropsychidae were the heaviest invertebrates and had the greatest contribution terms of total biomass.

3.3 Trophic positioning and diet estimation

From the distribution of taxonomic groups along the δ^{15}N axis, typical predatory groups were distinguished (filled gray circles in Figure 4), in agreement with their known feeding traits. The stable isotope mixing models showed that in the dry season most of the consumers primarily derived their biomass from the assimilation of litter. Litter diet had a 95% probability of making up 36-96% and 24-80% in the dry and wet season, respectively, of the Calamoceratidae diet (Table 4). In the wet season, seston was the predominant resource for Simuliidae (13-63%), Hydropsychdae (28-57%) and Diptera (16-71%).

The trophic shift occurred in Diptera larvae from a biofilm-based diet in the dry season (8-76%) towards a seston-based diet in the wet season (16–71%) and with Odontoceridae from a seston-based diet in the dry season (8-86%) towards a litter-based diet in the wet season (16–71%). Simuliidae did not show an evident specificity related to the food resource assimilated in both seasons being, despite the greater consumption of seston. In the wet season, Odontoceridae also did not have a clear distinction with the assimilated food resource. Ephemeropterans were the predominant prey of predatory Libelulidae but contributed less to the diets of Gomphidae (Table 5). Diptera were the predominant prey of predatory Gomphidae. The trophic shift occurred in Perlidae predator from Odontoceridae-based diet in the dry season (5-40%) towards an Ephemeroptera-based diet in the wet season (5-40%).

3.4 Groups isotopic niches

Qualitatively, the isotopic niche of most invertebrate groups did not show high variation in their relative positions in the food web from wet to dry season (Figure 5). The shifts were notable for Calamoceratidae, Diptera and Odontoceridae. Quantitatively, the area of the isotopic niche (SEA.B) showed highly probable differences among different members of the communities (Hydropsychidae and Simuliidae had the smallest SEA.B in the wet season, and Gomphidae the smallest SEA.B in the dry season). The SEA.B changed from wet to dry season, either widening, as was the case for Psephenidae, Perlidae, and Odontoceridae, or contracting, as was the case for Hydropsychdae and Gomphidae (Figure 6).

3.5 Community isotopic niche

A trophic shift between dry and wet season emerged at the community level which for all isotope space metrics the dry season was higher. Bayesian estimation of community metrics in dry vs. wet season revealed a 13.6% larger δ^{15}N range in the dry season (from 4.96 to 5.72; the probability of divergence >80%) and a 23.2% larger δ^{13}C range (from 8.65 to 11.28; the probability of divergence >98% see Figure 7). Together, this resulted in a 24.1% larger isotopic space area (TA) occupied by the invertebrate community in the dry season (from 19.8 to 26.1; the probability of divergence >90%). Trophic diversity as estimated by CD was 12.3% larger in the dry season (from 2.00 to 2.40; the probability of divergence >95%). The mean Euclidean distance to each group's nearest neighbor (NND) was 11.8% larger in the dry season, which implied a decrease in trophic redundancy related to the wet season (from 1.30 to 1.54; the probability of divergence >85%). The SDNND was 36.4% larger in the dry season (from 1.32 to 2.15; the probability of divergence >99%), indicating that trophic redundancy across community members was greatly more the wet season.

4. Discussion

As expected, there was a higher incorporation of litter-derived material by the community of benthic invertebrates in both seasons. However, the availability of resources measured for the community was lower in the wet season, with a decrease in the δ^{13}C range of the community (dCR) suggesting lower diversity of basal resources assimilated by the community in this period of the year. Both MNND and SDNND suggested an increase of trophic redundancy in the wet season, that is, species have less distinct trophic positions (smaller distance among them in niche space) and suggest a more competitive interaction. Despite the lower range in δ^{15}N among consumers (dNR) in the wet season, that suggests a compact and less complexity food web, with a short trophic length, the probability of divergence for this metric between the dry season was low.

In the wet season, besides reducing the amount of litter was showed a greater C:N ratio of leaf litter that implies in a more difficult material to be assimilated by the biota. The input of leaf litter inside stream in the wet season is probably composed of “fresh” litter that is a less-mineralized fraction of detrital material (Peipoch et al., 2012). Although monthly leaf litter input in the streams is continuous and in small quantities, with peaks occurring in the transition between dry and wet seasons (Bambi et al., 2017), the leaf litter packs retained in the stream were lower in the wet season, probably because of the highest frequency of stormflows. However, even at flow instability period, the leaf litter was not totally taken downstream, and remnants were still available in the stream bed elucidating the greater contribution of leaf litter as a food resource for the community in the wet season. It can, therefore, hypothesize that these fragments of leaf litter packs maintain the food chain length (FCL) in the wet season.

In the dry season, the dry mass of the entire community was twofold higher in relation to the wet season. However, the ratio between the dry mass of predators vs. consumers was very close with 1.3 (768.3mg / 564.4 mg) e 1.2 (1650.0 mg / 1944.5 mg) in the wet and dry season, respectively. Due to the proximity between the dry mass ratio of consumers vs. predators in the wet and dry season, we suggest that the global trophic relationships between consumers and predators were not remarkably affected by seasonality. This is following the low probability of divergence of a δ^{15}N range of the community (dNR) between seasons. Thus, diverging from temperate streams (Wallace et al., 2015), the accumulation of leaf litter packs coalescing in neotropical forest streams might increase the complexity of in-stream habitat structures and seemed to be a stronger determinant of secondary production than standing crop of litter.

The isotopic ratios of seston and litter were not similar as expected and did not support its provenance mainly by the breakdown products of coarse plant material that fell into the stream, where it was subjected to leaching, microbial decomposition, and mechanical fragmentation by invertebrate shredders. Chlorophyll-a concentrations in the seston, mainly in the dry season evoking the presence of photoautotrophic microorganisms. Probably the seston was made up not only of decaying pieces of organic matter but also, to a much lesser extent, of microscopic autotrophic (diatoms) and heterotrophic (flagellates, ciliates, and rotifers) organisms, which likely improved the nutritional quality (Majdi and Traunspurger, 2017).

The lower C:N ratio of seston suggests, according to the stoichiometry, a higher nutritional quality than leaf litter. Seston was important for filters and collectors-gatherers invertebrates such as Hydropsychidae, Odontoceridae, Simuliidae, Tipulidae and Chironomidae. Seston contributed significantly to the Simuliidae diets in both seasons. For Tipulidae, Chironominae and Hydropsychidae the major contribution of seston was in the wet and for Odontoceridae in the dry season. As predicted by Tomanova et al. (2006) and Palmer et al. (1993), we observed a common affinity of taxa to fine organic detritus, indicating the importance of this food resource and a high collectors-gatherers invertebrates activity in neotropical freshwater ecosystems.

The isotopic space of the Simuliidae was higher in the dry season in relation to the wet season probably due to the greater ingestion of fine litter fragments and photoautotrophic microorganisms couple in the seston (Majdi and Traunspurger, 2017). However, according to stable isotope analyses (SIA), the biofilm contribution as food resource assimilated was also almost equally. Besides filters and collectors-gatherers feeding habit, the Simuliidae are scrapers. We also identified that the Simuliidae had a positive correlation with Froude number and coarse substratum being able therefore to feed biofilm from the stones and pebbles.

In the wet season, the high contribution of seston as a food resource to the Hydropsychidae are allied to the collectors-gatherers feeding habit. Also, the positive correlation with a Froude number assisted by their anal claws and high body flexibility, allowed these individuals to persist on the substrate even under high flows being able therefore to feed on seston. In the dry season, the Hydropsychidae had the leaf litter as the main food resource. For Neotropical savannas streams, Cordeiro-Bentes et al. (2008) observed that the larvae of Hydropsychidae in their early stages feed on sediments and algae caught in their net. However, their last stage ingests small aquatic macroinvertebrates and fragments of plants, classifying them as generalists. Thus, the differences in the average individual dry mass between seasons of Hydropsychidae, where in the dry season individuals were significantly larger than in the wet season, that is, possibly in theirs last stage, corroborate with the greater isotopic niche size and the leaf litter as the main resource in the dry season.

Despite the highest chlorophyll-a concentrations and the lowest C:N ratio among the resources measured, biofilm implied in a low contribution for the community. Biofilm was an important food resource for Diptera (Tipulidae and Chironominae) in the dry season. However, seston contributed almost equally. It is worth mentioning that the Tipulidae and the Chironominae had a great variation in the values of $\delta^{15}N$ quite possibly because these two families are considered detritivores from which most of them inhabit buried and feed on the organic matter deposited in the sediment, that is usually enriched in ^{15}N.

In the wet season, Odontoceridae had a larger isotopic niche and leaf litter as the main food resource. Since these organisms have an inverse relationship with the turbulent flows (Table 2), we theorize that these individuals may have been sheltered in areas of lower shear force in the wet season, where the remaining leaf banks may also be allocated. In this way, these organisms may have had the litter as main resource and access to a more diversified resource (e.g., seston). In the dry season, the stable water flow and since these organisms lived mainly in sandy bottoms of streams corroborates the seston as the main food source indicated by SIA. In neotropical streams, Odontoceridae inhabits sandy bottoms of streams and show a diet dominated by leaf litter and fine particulate material (Celina Reynaga et al., 2014).

Some Psephenide invertebrates had values of $\delta^{13}C$ significantly depleted indicating the consumption of methanotrophic and/or chemoautotrophic microorganisms. These microorganisms have significantly depleted values of $\delta^{13}C$ (< -40‰) relative to the photoautotrophic sources. Kohzu et al. (2004), shows that methane-derived carbon seems to enter stream communities around anoxic habitats such as backwater pools and hyporheic zones. Nevertheless, we did not identify a potential predator, and more studies should check if food webs are partly sustained by methane-derived carbon in neotropical streams.

For adult Elmidae we did not identify a potential predator and the instable flow in the wet season probably did not impact them, since their total dry mass was not affected and showed a positive correlation with the turbulent flows. Segura et al. (2014) verified that Elmidae has great phenotypic plasticity that allows them to persist in streams along a gradient of flow disturbance. However, according to SIA, the leaf litter was the main resource to Elmidae in both seasons which produces a contradiction related to their absence in leaf litter packs. Thus, we assume that these organisms forage in leaf litter packs, which according to the literature some taxa are herbivorous shredders (Seagle, 1982), but do not settle on these habitats.

The position of top-predators in neotropical savanna streams food web was consistent with the literature. In the aquatic environment, the Odonata larvae are efficient predators due to the presence of the prehensile lip, which is designed to capture the prey by bringing it to the mouth (Neiss and Hamada, 2014). Larvae feed on aquatic insects, small fish, tadpoles, crustaceans and other invertebrates in general, so they are important in structuring the food webs in the aquatic ecosystems where they live (Neiss and Hamada, 2014). Perlidae nymphs are predators with potential influence on benthic invertebrates in some tropical streams (Alencar; et al., 1999; Dudgeon, 2000b). The isotopic space of Perlidae was higher in the wet season related to the dry season, indicating that these individuals may be preying on distinctive prey due to the impairment of resources in the instable flow period. According to the contribution isotopic analysis and unlike the Odonata, the Perlidae changed their prey according to the season.

We conclude that in the wet season the food availability is reduced with consequences on the diversity of food resources assimilated by the benthic macroinvertebrate community, in addition to increasing the functional redundancy in the community. We also verified that leaf litter packs are key components for the neotropical forest streams since they contributed as a food resource in both seasons. The remaining leaf litter accumulation within the stream bed, even during periods of seasonal high flows, was an important factor to the trophic structure persists in the wet season. In this way, our results suggest that leaf litter accumulation was a significant predictor of the food web in Neotropical forest streams. Furthermore, seston as a mixture of allochthonous and autochthonous source contributed as a food resource for the macroinvertebrates community. Finally, we verified through biomass estimation that a large amount of energy transferred along the trophic structure was severely reduced in the wet season.

It is worth mentioning, therefore, that the leaf litter packs in these Neotropical forest streams, besides hotspot for aquatic biodiversity, is a stock of energy and matter which possibly maintains the structure and functioning of these ecosystems even in periods of flow instability. Knowing this importance, the removal of riparian vegetation, an increase of stormflows, due to the increase of impermeable surfaces, and extreme events of precipitation, due to climate change, might generate important implications for input, retention and accumulation of the leaf litter within stream. Consequently, much of the ecosystem services provided by these ecosystems, such as water quality and delivery of organic matter and nutrients downstream, would be lost.

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Table 1. General information about the investigated streams

Stream name	Três Barras (A)		Tortinho (B)		Capetinga (C)		Cabeça-de-Veado (D)	
Altitude (m)	1116		1146		1088		1075	
Stream order	2nd		2nd		2nd		2nd	
Stream Width (m)	2.0±0.7		2.2±0.7		3.6±1.0		2.8±0.5	
Season	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Electrical conductivity (µS.cm^{-1})	3.9±0.3	3.4±0.3	5.6±0.5	6.1±0.3	3.9±0.2	4.4±0.6	4.7±0.3	5.1±1.3
Ph	6.4±0.3	5.9±0.1	6.1±0.2	6.2±0.1	5.9±0.1	5.5±0.1	5.8±0.1	5.7±0.1
Dissolved oxygen (mg.L^{-1})	6.3±1.1	6.7±0.2	5.6±0.5	6.6±0.2	8±3.7	5.9±0.3	5.3±0.3	6.3±0.1
Turbidity (NTU)	2.9±0.9	4.0±1.2	4.0±0.6	4.2±1.3	2.5±0.7	3.9±2.5	1.2±0.5	2.2±0.6
Temperature (ºC)	20±1.2	16±0.5	20±0.2	17±0.5	20±0.7	15±1.6	21±0.2	17±1.5
Discharge (m^3.s^{-1})	0.3±0.1	0.2±0.1	0.2±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.1±0.0

Table 2. Invertebrates selected to stable isotope analyses. The feeding traits are classified according to Merritt et al. (2008) and Ramírez and Gutiérrez-Fonseca (2014). L=Larvae, CG=Collectors-Gatherers, Ft= Filters, Sh=Shredders, Sc=Scrapers, Dt=Detritivores and Hb=Herbivores. Only significant Spearman correlation values (r) were shown ($p < 0.05$).

Taxa	Code	Feeding trait	nº of invertebrates selected to SIA and the effective samples analyzed ()	Abundance and contribution (%) in the total of invertebrates sampled		Spearman correlation between microhabitat variables (r)		
				Wet	Dry	Froude Number	Coarse substrate	Organic Debris
Consumer group								
Calamoceratidae	cal	Sh-Dt; Sc	18 (10)	10 (1%)	63 (2%)	-0.18	-0.13	0.22
Tipulidae	tip	Sh-Dt; CG	23 (8)	28 (2%)	52 (2%)	-	-	-
Psephenidae	pse	Scraper	28 (10)	56 (5%)	45 (1%)	-	-	-0.15
Odontoceridae	odo	Shreder	58 (20)	65 (5%)	61 (2%)	-	-	0.10
Chironomidae	chi	CG; Ft	49 (8)	16 (1%)	161 (5%)	-0.18	-	0.26
Baetidae	bae	CG; SC	91 (8)	51 (4%)	171 (5%)	-	0.17	-
Hydropsychidae	hyd	Ft; Pr; Sc	48 (26)	110 (9%)	166 (5%)	0.31	-	-
Simuliidae	sim	Ft; Sc	84 (10)	99 (8%)	271 (8%)	0.37	0.15	-
Elmidae (Adult)	elm	CG, Sc, Sh-Hb	84 (28)	213 (18%)	251 (8%)	0.32	-	-0.16
Leptophlebiidae	lep	CG; Sc; Ft	111 (8)	133 (11%)	822 (25%)	-0.20	-0.20	0.26
Predator group								
Gomphidae	gom	Predator	13 (10)	30 (2%)	44 (1%)	-0.21	-0.17	0.28
Libellulidae	lib	Predator	31 (26)	25 (2%)	54 (2%)		-0.11	-
Perlidae	per	Predator	64 (22)	100 (8%)	181 (2%)	-	-	0.11
Total			702 (194)	936 (78%)	2342 (70%)			

Table 3. The dry mass of invertebrates in the wet and dry season at the Paranoá sub-basin streams. Asterisks highlight the significant differences between seasons.

	Average (± SD) dry mass per taxon (mg ind^{-1})		Total dry mass (mg) per taxon and their contribution (%)		Dry mass (mg) of consumer and predator and their contribution (%)	
	Wet	Dry	Wet	Dry	Wet	Dry
Consumer group						
Calamoceratidae	1.60 ± 2.54	2.84 ± 5.18	16.0 (1.20%)	179.0 (4.98%)	565.4 (42%)	1650.0 (45%)
Tipulidae	1.54 ± 2.52	1.62 ± 2.55	44.9 (3.36%)	84.5 (2.35%)		
Psephenidae	1.37 ± 1.11	1.99 ± 1.38*	76.9 (5.76%)	89.7 (2.49%)		
Odontoceridae	0.71 ± 0.41	0.82 ± 0.51	48.0 (3.60%)	50.10 (1.39%)		
Chironomidae	0.14 ± 0.31	0.05 ± 0.05	2.24 (0.16%)	8.43 (0.23%)		
Baetidae	0.09 ± 0.06	0.15 ± 0.13*	5.14 (0.38%)	26.6 (0.74%)		
Hydropsychidae	2.14 ± 5.98	3.27 ± 9.62*	240. (18.0%)	543.0 (15.1%)		
Simuliidae	0.79 ± 1.00*	0.45 ± 0.61	78.9 (5.91%)	124.0 (3.46%)		
Elmidae (Adult)	0.29 ± 0.16	0.26 ± 0.12	20.1 (1.51%)	17.3 (0.48%)		
Leptophlebiidae	0.24 ± 0.33	0.64 ± 9.64	32.3 (2.42%)	526.0 (14.6%)		
Predator group						
Gomphidae	17.9 ± 36.8	18.1 ± 53.4	537.0 (40.3%)	781.0 (21.7%)	768.3 (57%)	1944.5 (54%)
Libellulidae	4.45 ± 8.07	16.9 ± 54.3	106.0 (8.02%)	898.0 (24.9%)		
Perlidae	1.22 ± 2.28	1.46 ± 2.22*	123.0 (9.26%)	264.0 (7.35%)		
Total			1333.8	3594.5		

Table 4. Results of a Bayesian mixing model showing the estimated relative contribution of resources to the isotopic signature of consumers' tissues. The highest contributions in a single row are highlighted in bold. The 95% confidence interval for the contribution values are shown in parentheses.

Primary consumers	Season	% Contribution to biomass (95% confidence interval)					
		Biofilm		Leaf Litter		Seston	
Calamoceratidae	Dry	14	(0-39)	**69**	(36-96)	16	(0-41)
	Wet	18	(0-43)	**52**	(24-80)	29	(2-51)
Diptera	Dry	**44**	(8-76)	15	(0-35)	41	(3-73)
	Wet	33	(1-61)	22	(16-71)	**43**	(16-71)
Elmidae	Dry	22	(0-43)	**46**	(31-61)	30	(2-54)
	Wet	24	(1-46)	**52**	(1-43)	23	(1-43)
Ephemeroptera	Dry	27	(1-49)	**46**	(27-67)	25	(0-49)
	Wet	35	(8-59)	**43**	(0-43)	21	(0-43)
Hydropsychidae	Dry	27	(5-47)	**48**	(34-63)	23	(1-44)
	Wet	21	(4-38)	35	(28-57)	**42**	(28-57)
Odontoceridae	Dry	33	11-53)	23	(7-38)	**43**	(20-67)
	Wet	34	(8-58)	**39**	(4-45)	25	(4-45)
Psephenidae	Dry	21	(0-45)	**51**	(27-79)	26	(0-52)
	Wet	28	(0-56)	**47**	(0-51)	24	(0-51)
Simulidae	Dry	34	(6-60)	28	(5-48)	**36**	(7-65)
	Wet	35	(5-60)	26	(13-63)	**38**	(13-63)

Table 5. Results of a Bayesian mixing model showing the estimated relative contribution of prey categories to the isotopic signature of predators' tissues. The highest contributions in a single row are highlighted in bold. The 95% confidence interval for the contribution values are shown in parentheses.

Predators		% Contribution to biomass (95% confidence interval)									
		Diptera		Elmidae		Ephemeroptera		Hydropsychidae		Odontoceridae	
Gomphidae	Dry	**26**	(1-46)	18	(0-36)	12	(0-30)	16	(0-34)	25	(1-46)
	Wet	**23**	(1-41)	18	(0-35)	14	(0-31)	**23**	(1-42)	19	(0-37)
Libelulidae	Dry	6	(0-19)	22	(0-45)	**26**	(0-50)	**26**	(0-50)	7	(0-21)
	Wet	8	(0-22)	27	(1-49)	**31**	(11-51)	14	(0-31)	18	(0-38)
Perlidae	Dry	20	(3-36)	20	(2-35)	20	(2-36)	20	(2-36)	**23**	(5-40)
	Wet	15	(0-31)	20	(0-36)	**24**	(5-40)	19	(0-36)	20	(0-38)

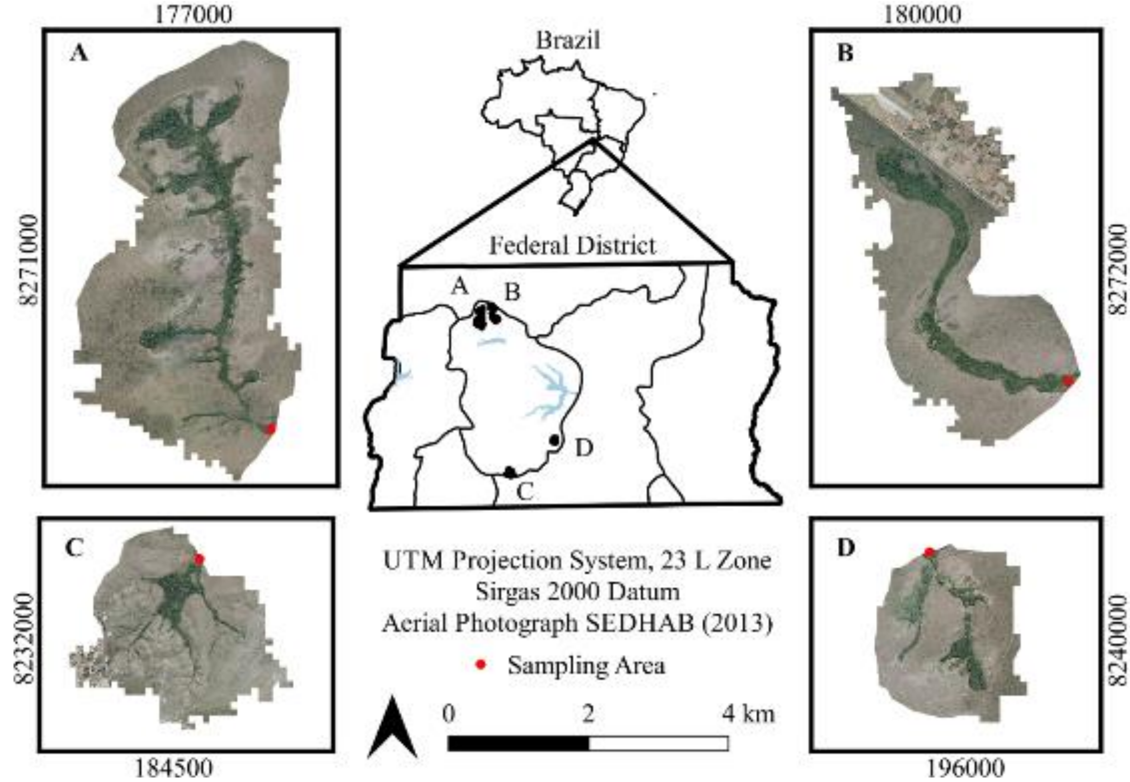

Figure 1. Location of Paranoá sub-basin streams in the Federal District, Brazil. A) Três Barras and (B) Tortinho streams belong to the National Park of Brasilia. (C) Capetinga and D) Cabeça-de-Veado and streams belong to the Environmental Preservation Area Gama Cabeça-de-Veado. Red dots represent the sampling location in each basin.

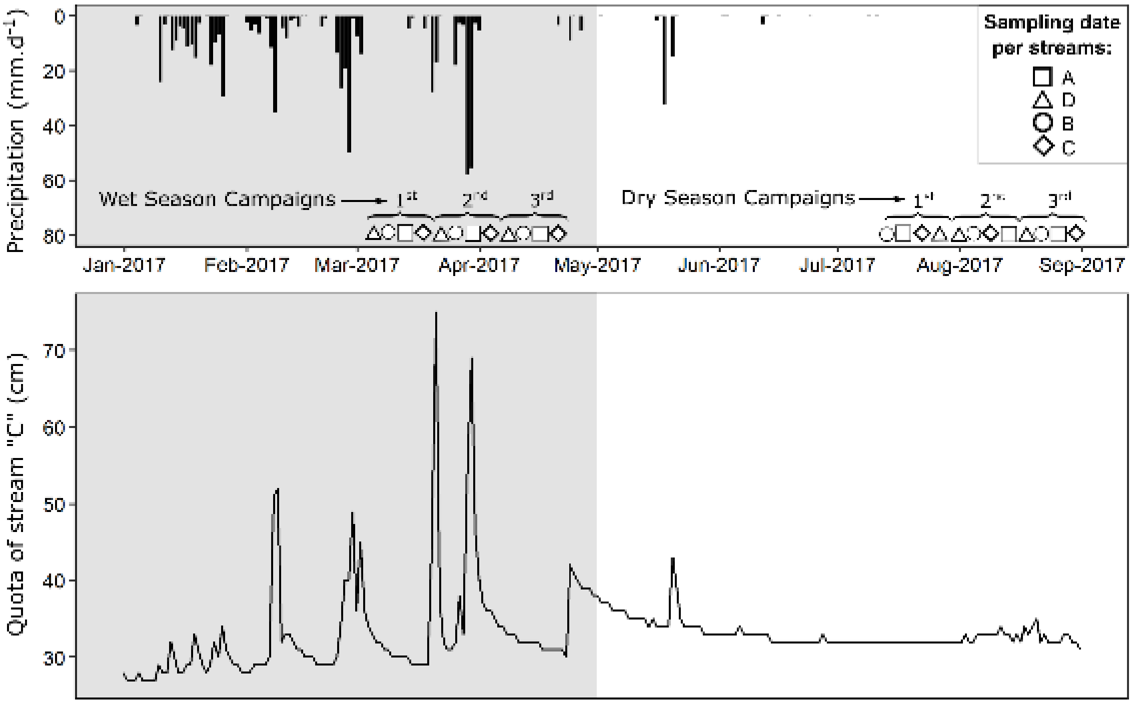

Figure 2. Precipitations and water levels monitored at the Capetinga stream (C). The shaded area corresponds to the wet season whereas the unshaded area represents the dry season. At each season, sampling times are shown.

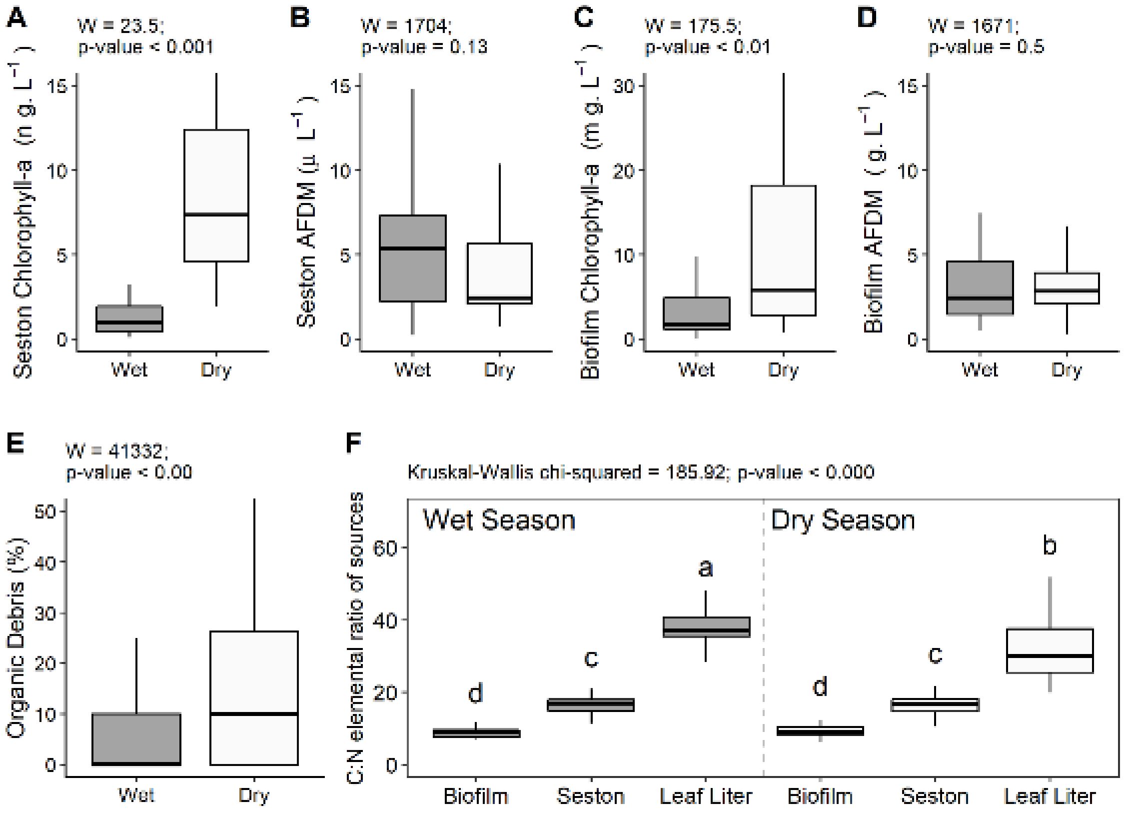

Figure 3. Boxplot comparison between wet and dry season of seston clorophill-a concentration (A) seston AFDM (B), biofilm chlorophyll-a concentration (C), biofilm AFDM (D), percentage of organic debris (E) and resource stoichiometry (F). The statistical comparison (W-Wilcoxon rank test) between dry and wet season is reported in A to E and Kruskal Multiple Comparisons (kruskalmc) post-hoc analysis is reported in F.

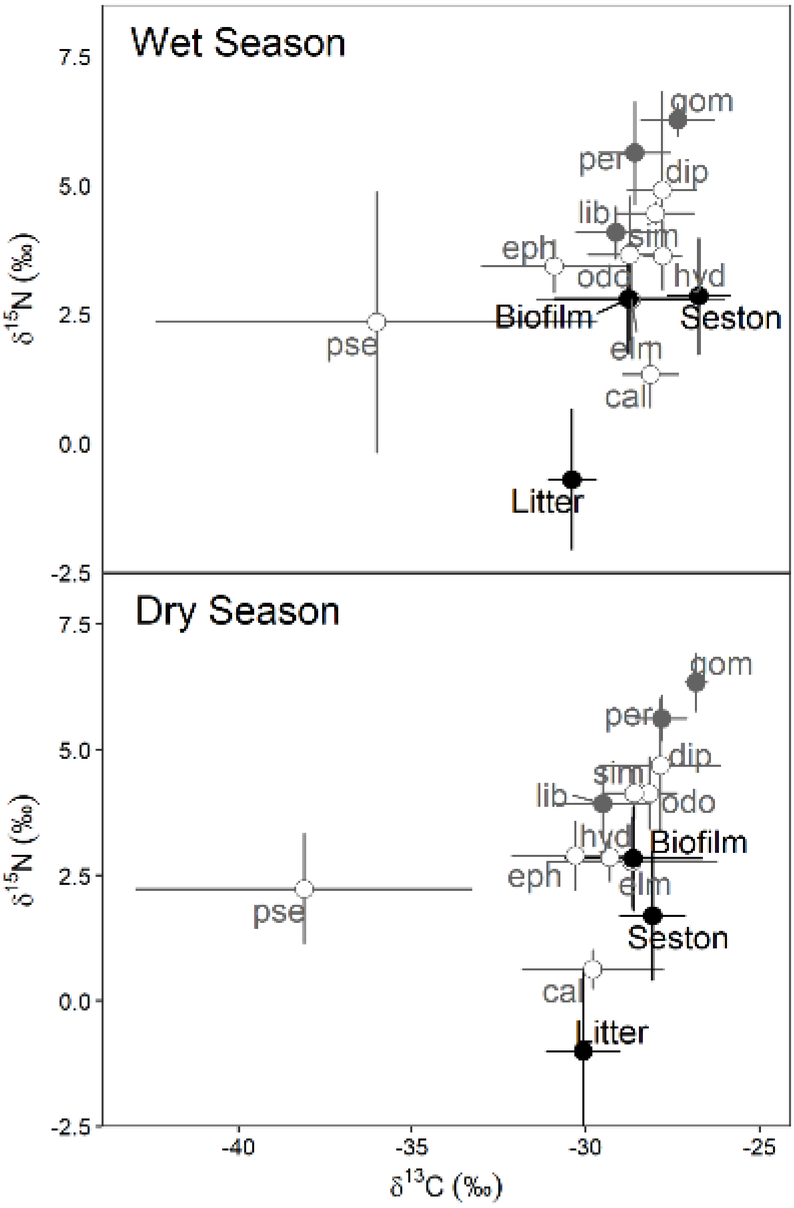

Figure 4. Mean (±SE) δ^{13}C and δ^{15}N signatures of basal resources (black circles), primary consumers (open circles), and predators (gray circles) at the Paranoá sub-basin streams in the wet and dry season. Leptophlebiidae (lep), Simuliidae (sim), Elmidae (elm), Chironomidae (chi), Perlidae (per), Baetidae (bae), Calamoceratidae (cal), Tipulidae (tip), Psephenidae (pse), Odontoceridae (odo), Hydropsychidae (hyd), Gomphidae (gom) and Libelulidae (lib).

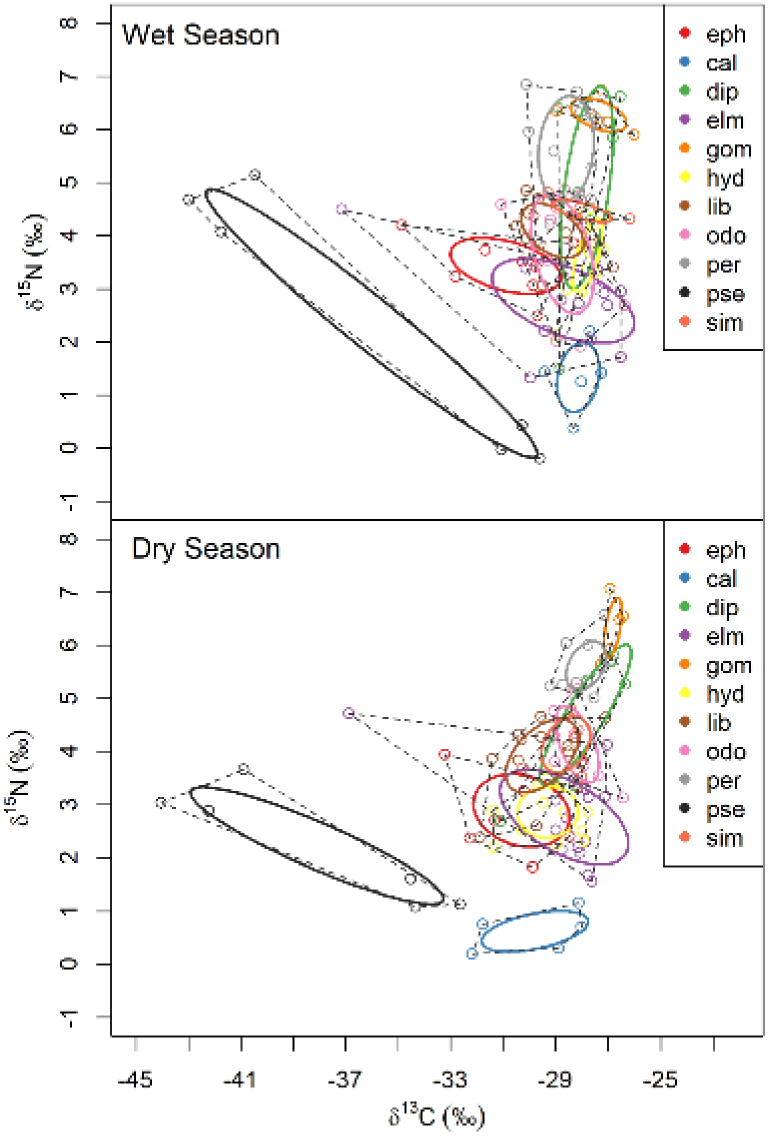

Figure 5. Distribution of invertebrate community samples in isotopic space in the Paranoá sub-basin streams in the wet and dry season. Group ellipses show the 95% confidence interval of the bivariate mean. Leptophlebiidae (lep), Simuliidae (sim), Elmidae (elm), Chironomidae (chi), Perlidae (per), Baetidae (bae), Calamoceratidae (cal), Tipulidae (tip), Psephenidae (pse), Odontoceridae (odo), Hydropsychidae (hyd), Gomphidae (gom) and Libelulidae (lib).

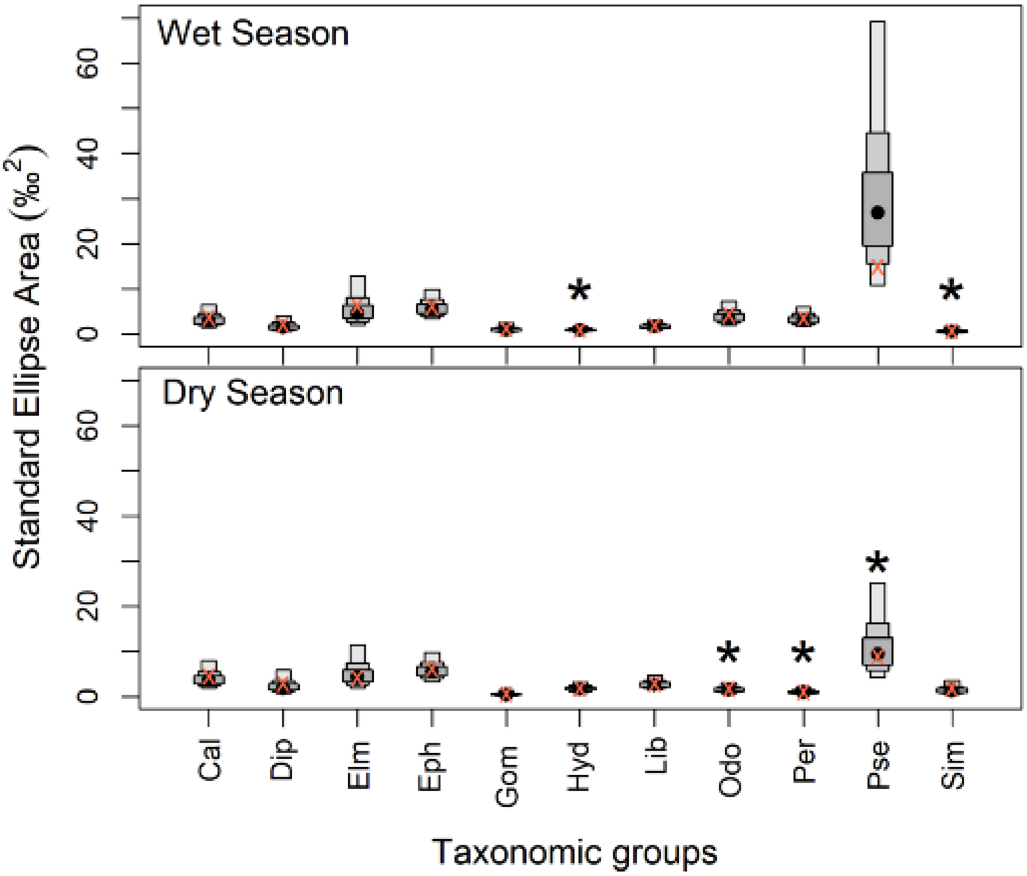

Figure 6. Bayesian standard ellipse area (SEA.B), a measure of the isotopic niche of groups within and among communities. SEA.B values can be compared quantitatively and the difference stated with reference to the robust probability associated with that difference. Black dots represent SEA.B modes; red crosses indicate the true population values as (maximum likelihood estimates: SEA.c), and the shaded boxes represent (from dark to light gray) the 50%, 75%, and 95% confidence intervals. Asterisks highlight a highly probable (<95%) seasonal change in the isotopic niche area of one taxonomic group. Abbreviations in Table 2.

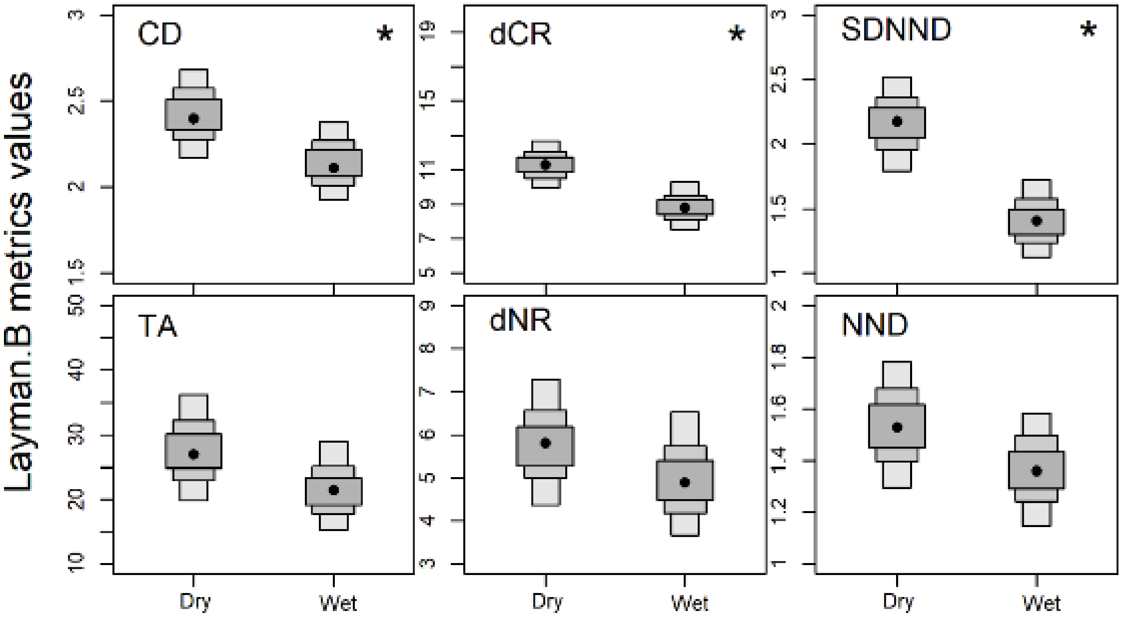

Figure 7. Layman's metrics reformulated using Bayesian estimates (Layman.B), describing the isotopic niche properties of the whole invertebrate community dwelling in the Paranoá sub-basin streams in the wet and dry season. dNR: δ^{15}N range, dCR: δ^{13}C range, TA: area of the community hull in isotopic space. The metrics can be compared quantitatively, with the difference referring to the probability associated with that difference. Black dots represent the modes of the Layman.B metrics, and the shaded boxes (from dark to light gray/ochre) the 50%, 75%, and 95% confidence intervals

Capítulo 3 - Understanding the controlling factors over biofilm as an autochthonous resource in shaded oligotrophic neotropical streams

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O presente capítulo foi submetido para apreciação do periódico *Freshwater Biology* no dia 2 outubro de 2018. No dia 12 de novembro de 2018 o manuscrito voltou com as considerações dos revisores como "*major revision*". No dia 5 de janeiro de 2019 o artigo foi resubmetido e encontra-se novamente em processo de revisão por pares.

Todos os autores contribuíram criticamente para a interpretação dos resultados e com a versão final do manuscrito.

Title: Understanding the controlling factors over biofilm as an autochthonous resource in shaded oligotrophic neotropical streams

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Abstract

1.In shaded, low nutrient headwater streams, the main source of energy is of allochthonous origin. With changes in light and nutrient dynamics, however, the top-down vs bottom-up controls on stream biofilms can change and thus have repercussions to the metabolism of the ecosystem. Moreover, the increase in primary productivity may yet result in greater variability in the carbon isotope ratios of the biofilm.

2.The objective of this study was to evaluate how the top-down (grazing) and bottom-up (light and nutrient) factors act on the biofilm and whether biofilm photosynthetic activity determines the isotopic fractionation of δ^{13}C. The density of grazing macroinvertebrates was controlled with electric pulses in four headwaters of Central Brazil, two oligotrophic and two enriched in nutrients. Electrified (exclusion of grazers) and not-electrified (with grazers) grids were implemented in open and shaded areas in each stream.

3.The ash-free dry mass (AFDM) doubled in nutrient-enriched streams. The effect of grazing on the biomass of the biofilm was significant for the nutrient-enriched streams, with a 50% reduction in AFDM. Shading influenced chlorophyll-a even in pristine streams, increasing the concentration under open canopy conditions approximately ten-fold. The relationship between chlorophyll-a concentration and δ^{13}C values was significantly negative.

4.Our study provides evidence that biofilm productivity increased as a result of changes in bottom-up controls and the top-down control plays an important role in a food web based on autochthonous primary production. Mutually, the algal photosynthetic activity determines isotopic fractionation between dissolve inorganic carbon and algal cells even in forest headwater streams.

Keywords: Stable Isotopes, Invertebrates, Experimental ecology, Biofilm, Eutrophication

1. Introduction

The headwaters of the Brazilian savannas (locally called Cerrado) have been altered through intensification of the input of nutrients from agriculture (e.g., fertilisers) and urban areas (e.g., sewage effluent discharge) and with the removal of riparian vegetation (Gücker, Boëchat & Giani, 2009; Fonseca et al., 2014; Hunke et al., 2015; Strassburg et al., 2017). These impacts are stressors that can affect the availability of basal resources (e.g., detritus versus algae) and generate changes in the metabolism, and control of energy flow in these ecosystems (Bumpers et al., 2017). These implications for ecosystem services depend not only on species richness and composition but also on food interactions (Worm & Duffy, 2003; Estes et al., 2011; Rooney & McCann, 2012).

Headwater stream ecosystems are known for having their main source of carbon consisting of basal allochthonous resources (e.g., from riparian vegetation) (Vannote et al., 1980; Goncalves, França & Callisto, 2006). When compared to rivers, headwater streams generally have low nutrient concentration and reduced light availability, limiting photosynthetic activity (Vannote et al., 1980). The headwaters of the Cerrado, in turn, have naturally lower nutrient concentrations compared to other regions of Brazil (Fonseca & de Mendonça-Galvão, 2014; Fonseca et al., 2014). Thus, nutrient enrichment and increased incidence of sunlight can promote energy flows from autochthonous resources, with changes associated with the functioning and characteristics of the associated food web (Bumpers et al., 2017).

This fact underscores the need for research regarding how the effects of nutrient enrichment and light conditions (bottom-up process) and grazing invertebrates (top-down process) controls the primary productivity in tropical oligotrophic streams, which are potentially more vulnerable to increased nutrient loading (Garcia, Townsend & Douglas, 2015) and deforestation of riparian vegetation (Sturt, Jansen & Harrison, 2011). Studies suggest that the enrichment of nutrients provides the increase of biofilm that consequently increase the density of grazing invertebrates (Singer & Battin, 2007) and possibly, increases the occurrence of predator (Slavik et al., 2004; Bumpers et al., 2017). In addition, the increase of the canopy opening, typically from well-developed forests or caused by anthropic disturbances, can also promote the increase of benthic algal growth, increase nutrient retention and uptake (Bechtold et al., 2017) and production of higher trophic levels (Warren et al., 2016).

Sturt et al. (2011) demonstrated that for temperate streams, in addition to nutrients and forest cover (bottom-up process), the action of grazing invertebrates (top-down process) can control the increase of primary productivity. Although bottom-up controls have been shown to be relatively more important in controlling primary productivity (Gruner et al., 2008; Garcia et al., 2015; Bumpers et al., 2017), top-down forces can act in conjunction with the bottom-up forces, determining the primary productivity control by means of grazing invertebrates (Sturt et al., 2011; Lourenço-Amorim et al., 2014).

The increase of primary productivity from lotic ecosystems may also determines changes in isotopic fractionation between dissolved inorganic carbon (DIC) and algal cells (Ishikawa, Doi & Finlay, 2012). The global meta-analysis performed by Ishikawa et al. (2012) demonstrated that the δ^{13}C values for the biofilm increase with increasing size of the catchment and the canopy opening, in addition, a positive relationship was observed between chlorophyll-a density and biofilm δ^{13}C. This pattern shows that algal cells photosynthesizing within thick biofilm are likely to become enriched in ^{13}C because impedes the DIC diffuse into the inner layer of biofilm and results in relatively high biofilm δ^{13}C (Hill & Middleton, 2006).

Mainly in headwater forested streams, the knowledge of algal δ^{13}C should be highlighted because algae biofilm may be important food resource for higher trophic levels and the stable-carbon isotopes are able to identify the potential carbon sources for stream food webs. (Finlay, 2001). Currently, stable carbon isotope measurements are powerful food webs and ecosystem tracer which promotes the understanding of the mechanisms that regulate the ecological integrity of those environments.

Given the natural low nutrient concentrations of Cerrado streams (Fonseca et al., 2014) and the limited understanding of biofilm as a food resource for tropical oligotrophic headwaters, the objective of this study was to test the following hypotheses in a scenario of altered pristine conditions: (i) the increase in algal photosynthetic activity increases the δ^{13}C values of the biofilm; (ii) riparian shading acts as a limiter and prevents biofilm growth; (iii) macroinvertebrate grazers reduce biofilm biomass.

2. Material e Methods

2.1 Study area

This study was conducted in four headwater streams located in the Federal District, in Central-Western Brazil (Figure 1). Based on the work of Fonseca and Mendonça (2014), the following streams were selected: Capetinga (Lat: 15°57'40.1"S, Long: 47°56'36.5"W), Gama (Lat: 15°56'51.03"S, Long: 47°57'38.91"W), Onça (Lat: 15°57'18.00"S, Long: 47°57'45.40" W) and Cabeça-de-Veado (Lat: 15°53'20.4"S, Long: 47°50'33.6"W). They are low-order streams, with a catchment area of between 10 and 15 km^2 and a stony riverbed substrate (Fonseca et al., 2014) (Figure 1).

Regarding soils, the four basins are dominated by Latosol (Oxisols, according to the U.S. Soil Taxonomy), with a predominance of clay fraction (up to 80%), (Reatto & Martins, 2005). The climate of the region is marked by strong seasonality, with a wet season from October to April and a dry season from May to September. The annual mean precipitation is about 1,500 mm. The forest formation that accompanies these streams forms closed corridors (galleries) over the watercourse. The physiognomy is evergreen, barely exhibiting leaf fall in the dry season. The height of the trees varies between 20 and 30 meters, and the position of the treetops provides coverage of 70% to 95% (Ribeiro & Walter, 2001).

The four streams are located within the Gama and Cabeça-de-Veado Environmental Preservation Area (EPA), whereas the Capetinga and Cabeça-de-Veado streams are located, respectively, at the Ecological Stations of the University of Brasília and the Brasília Botanical Garden, which consist of conservation units (natural areas protected by the state). Land use and land cover is dominated by native Cerrado vegetation with a gallery forests for three of the four streams.

In contrast, the riparian zone of the Gama stream was impacted due to urban land use (Fonseca & de Mendonça-Galvão, 2014). In addition, the headwaters of the Onça stream are located in a rural area, where family-based agriculture predominates. Nonetheless, the specific stream reaches where we conducted the field experiment were located within a preserved area. The probable interference of anthropic activities, with indication of nutrient enrichment in the Gama and Onça streams, can be verified by the comparison of average nutrient concentrations for each site, available in Fonseca & de Mendonça-Galvão (2014) (Table 1).

2.2 Biofilm colonisation experiment

In order to evaluate the influence of nutrient concentration and the incidence of sunlight (bottom-up factors), as well as grazing (top-down factor) for the development of biofilm in tropical headwater streams, the experimental work performed by Sturt et al. (2011) in southwest Ireland was replicated in the four streams described earlier. Differing from the original work, which considered three stretches of the same river (Owennagearagh River) with variable concentrations of nutrients due to the discharge of treated wastewater, this study evaluated two sets of streams classified as oligotrophic (Capetinga and Cabeça-de-Veado) and enriched in nutrients (Onça and Gama) (Table 1). In each stream, four points were defined for the installation of colonization plates, in triplicate. Two were used as controls for the natural grazing conditions of biofilm, and two others were treated, with the exclusion of grazing organisms. The plates were comprised of eight tiles, each tile with an area of 10x10 cm, attached using cable ties to plastic-coated metal grids (Anexo C, Figure S1).

The exclusion of grazing organisms was carried out by means of high-power electrification equipment (Model: ZK-80, 11.5 kV, 3.9 joules of output; ZEBU), which transferred electrical pulses to the colonisation plates. Isolated wires connected the anode and cathode of the colonisation plates to the electrifier positioned on the stream bank. In addition to the effect of grazing, electrified and not-electrified sites were arranged in shaded (closed canopy) and unshaded (open canopy) patches along the gallery forest. The percentage of canopy opening was estimated from hemispherical photographs obtained with a digital camera (GoPro HERO 5) mounted on a tripod. The camera was levelled and positioned in the center of the stream channel. After analysing the images, using Gap Light Analyzer software, canopy opening values were defined.

The sampling points were also standardised in terms of flow velocity (10 - 20 cm/s), stream depth (15 - 30 cm) and substrate composition (cobble and gravel). The width of the stream channels varied between 2 and 3 m in the stretches studied. We installed the plates in the stream using steel stakes. Initially, we allowed the tiles to incubate within the stream for 15 days. We then scraped the tiles to remove any significant growth, leaving only a surface which would rapidly accrue new biofilm. Finally, we placed them back into the stream for another 15 days of the experiment. The experiment occurred between 25 June and 7 July 2017. In this period, the most abundant invertebrates (Baetis nymphs and Hydrachnidia mites) on the plates were monitored in situ at the not-electrified sites (shaded and not shaded) and at the electrified sites (shaded and not shaded) to assess the degree of exclusion of grazers.

An LCD screen and a waterproof camera (GoPro HERO 5) connected wirelessly were used to monitor the colonisation of the plates. After 15 days of colonisation, four tiles were randomly selected from each of the three plates installed at the four monitoring sites in each stream. After the invertebrates were removed, the surfaces of the tiles were cleaned with a soft bristle brush and distilled water to remove the biofilm. The samples were stored in amber containers and polystyrene boxes until it was taken to the laboratory for the determination of chlorophyll-a and ash-free dry mass (AFDM). Water temperature, dissolved oxygen, turbidity, pH and electrical conductivity were measured in the field every three days using standard electrodes.

2.3 Laboratory analysis

In the laboratory, invertebrates and organic detritus were removed with tweezers, and the biofilm slurry was observed using a stereomicroscope (50x magnification) and diluted in distilled water. The biofilm samples were subsequently vacuum filtered using pre-combusted glass fiber filters (Whatman GF/C). The filters were placed in centrifuge tubes containing 10 ml of 100% ethanol, (Sartory & Grobbelaar, 1984). The absorbance values of the supernatant were measured at 750 and 665 nm, and the chlorophyll-a concentrations were calculated following Marker et al. (1980). To determine the AFDM, each sample was transferred to pre-labelled aluminium plates and dried at 60°C until a constant mass was reached. These dried samples were weighed and then combusted at 550°C for 6 hours and then weighed again.

All the invertebrates sampled were stored on ice for transport to the laboratory. In the laboratory, the organisms collected were washed in distilled water and taxonomically identified. For the isotopic analysis, the invertebrates collected on the plates from the streams enriched with nutrients (i.e. Gama and Onça) were used, since there were not enough invertebrates in the oligotrophic streams to gather the minimum weight for this analysis.

Thus, we collected several specimens of the same taxon and used composite samples for isotopic analysis. In all, four samples composed of mites and four samples with Baetis nymphs were analysed. On average, for each composite sample, 10 individual mites and 6 individual Baetis nymphs were grouped. The isotopic analysis of the biofilm was performed from samples from the four streams, and was preceded by the filtering step, as was conducted for the AFDM analysis, but using another aliquot from the biofilm material.

For both the invertebrates and for the biofilm material (resulting from the scraping of the glass fiber filters), the samples for isotopic analysis were obtained by drying at 60°C for 48 hours, grinding to a fine powder and homogenisation using a mortar and pestle, and then stored in Eppendorf tubes. Approximately 1.5-2 mg of this material was used for isotopic analysis. All the samples were sent to the Isotope Ecology Lab at the Centre for Nuclear Energy in Agriculture (CENA) of the University of São Paulo, Piracicaba, Brazil, to determine the values of δ^{13}C and δ^{15}N.

Isotopic ratio analyses were processed through the combustion of samples under continuous flow ultra-pure helium in an elemental analyser (Carlo Erba, CHN-1110) which was connected to a Thermo Finnigan Delta Plus mass spectrometer. The results were expressed in delta notation (δ), in parts per thousand (‰), in relation to the standard international references (V-PDB - Vienna Pee Dee Belemnite for C and atmospheric air for N). The analytical precision values estimated by replicates of the working standards of δ^{13}C and δ^{15}N were ± 0.15 ‰ and ± 0.30 ‰, respectively.

2.4. Data analysis

The comparison of the physical and chemical variables of the water between the oligotrophic and nutrient-enriched streams was performed using the Student's t-test. For the analyses in which the residues did not follow a normal distribution, the Mann-Whitney-Wilcoxon test was performed. The three-way variance analysis (three-way ANOVA) was then applied to evaluate the differences in the densities of Baetis nymphs (nutrient enrichment and oligotrophic), grazer exclusion (excluded and grazed) and the opening of the canopy (open and closed). Normality and homoscedasticity were tested a priori by the Shapiro-Wilk Test and Fligner-Killeen Test, respectively.

Then, after testing the assumptions, a multiple linear regression was performed with AFDM, δ^{13}C, and chlorophyll-a as dependent variables and the treatment of nutrient enrichment, canopy cover and the exclusion of the grazers as explicative terms. For the nutrient enrichment and canopy opening variables, continuous electrical conductivity and canopy opening percentages were used, respectively. Two simple linear regressions were used to verify i) the influence of the density of mites as a result of the density of Baetis nymphs, and ii) the influence of the chlorophyll-a concentration on the $\delta^{13}C$ values of the biofilm.

Finally, the standard ellipse area (SEAc, in ‰2) was estimated as a bivariate measure of the central mean of the isotopic niche (Jackson et al., 2011). For this analysis, the $\delta^{13}C$ values of the samples from the nutrient-enriched streams were used, since they were the streams where the mites appeared. The SEAc allows us to calculate the degree of niche overlap (in percentage, where 100% indicates total overlap) and can be used as a quantitative measure of diet similarity between the different trophic groups (Hill et al., 2015).

3. Results

Significant difference for electrical conductivity (t = 3.70, d.f. = 18, p-value = 0.001) was identified between the oligotrophic streams (5.44 ± 1.71 µS cm^{-1}) and the nutrient-enriched streams (15.58 ± 8.49 µS cm^{-1}). For water temperature, turbidity, dissolved oxygen and pH variables, there was no significant difference between the streams. The dissolved oxygen concentration remained above 90% of the saturation level throughout the experiment in all of the streams. The streams had mean values (± SD) of water temperature, turbidity and pH of 15.4 ± 1.3°C, 2.21 ± 0.90 NTU, and 6.2 ± 0.3, respectively.

The *Baetis* nymphs and Hydrachnidia mites were the most abundant invertebrates (> 90% of the community) found on the plates in all treatments where there was no electrification. Only the *Baetis* nymphs are considered to be grazers (Merritt et al., 2008), while the mites are considered to be parasites and / or predators (Di Sabatino et al., 2008). The plates with high voltage electric pulses did not exhibit colonisation by benthic macroinvertebrates in any of the four streams, (Figure 2; Table 2). In addition, the plates located in shaded environments had significantly fewer herbivores than the plates located in open canopy regions (Figure 2; Table 2). The highest densities of macroinvertebrates were found on the plates without electrification with nutrient enrichment in open canopy condition (Figure 2). The *Baetis* density on the plates ranged from 12 to 237 ind. m^{-2} between the streams.

In the excluded invertebrate plates, the AFDM was significantly higher under riparian shading conditions and doubled in nutrient-enriched streams. There was a positive but non-significant correlation between the AFDM and chlorophyll-a (n = 48, r

= 0.27, p = 0.06). In addition, the AFDM was reduced in the presence compared to the absence of grazers in streams with nutrient enrichment. The main effect of grazing on the biomass of the biofilm occurred only in nutrient-enriched streams, consisting of a 50% AFDM reduction (Table 3; Figure 3).

Chlorophyll-a levels were positively influenced by nutrients and the canopy opening, as well as by the interaction between the two. The Chlorophyll-a concentration increased approximately 10-fold under open canopy condition for oligotrophic streams. The biofilm δ^{13}C values were negative influenced by nutrients and the canopy opening, as well as by the interaction between the two (Table 3). Unlike what was observed for the biomass, the model indicated that grazers had no effect on the chlorophyll-a concentration (Table 3; Figure 3).

As for isotope ratios, the mean values (± SD) of δ^{13}C for *Baetis* nymphs, mites, Onça-biofilm and Gama-biofilm (streams enriched with nutrients) were -31.40 ± 1.20 ‰, -30.54 ± 1.34 ‰ and -27.20 ± 0.81 ‰ and -28.10 ± 1.28 ‰, respectively. As for δ^{15}N, the mean values (± SD) were 4.00 ± 1.16 ‰, 5.96 ± 0.67 ‰, 3.08 ± 0.79 ‰ and 2.42 ± 1.00 ‰ for the *Baetis* nymphs, mites, Onça-biofilm and Gama-biofilm, respectively (Figure 4). There was a significant positive correlation between the density of mites and the density of *Baetis* nymphs (slope 0.37, $R^2 = 0.68$, $p < 0.001$, n = 48, Figure 4). Finally, the relationship between chlorophyll-a concentration and the δ^{13}C values were significantly negative (slope 0.39, $R^2 = 0.32$, $p < 0.0001$, n = 48, Figure 5).

4. Discussion

Unlike the global meta-analysis by Ishikawa et al. (2012), the δ^{13}C values for the biofilm obtained in this study decreased as the concentration of chlorophyll-a of the biofilm increased. This effect was significant even under conditions of nutrient enrichment and canopy opening, which were considered important factors in the control of algal biofilm growth. The biofilm biomass had significant pressure from the grazing invertebrates in cases where there was an increase in nutrient concentration.

Possibly, the relationship between biofilm chlorophyll-a concentration and δ^{13}C values was opposite from the model proposed by Ishikawa et al. (2012) because of the low range of chlorophyll concentration. Despite the increase of chlorophyll-a concentration in a nutrient enrichment streams, the range of variation was limited (0-2 mg m^{-2}) compared to that obtained by Ishikawa et al. (2012) (1-100 $mg.^{-2}$). Subsequently, the biofilm biomass may have been insufficient for the diffusion effect occur. The thin biofilm, with less total photosynthesis and a larger proportion of cells close to the water/biofilm interface, should have fewer carbon constraints and therefore a lower proportion of ^{13}C (Hill and Middleton, 2006).

Nevertheless, as in Ishikawa and collaborators (2012), which encompasses a broad spatial scale, our results showed that the variation in ^{13}C relative to the smaller change in chlorophyll-a concentrations indicates that the algal photosynthetic activity also determines isotopic fractionation between DIC and algal cells. Probably, under limited light and nutrient conditions there was a reduced the amount of algae in the biofilm and as these pristine conditions were altered, increased the occurrence of algae from C_3 photosynthetic pathway. Notwithstanding, our results also indicate, as in Ishikawa et al. (2012), low biofilm δ^{13}C from headwater streams in function of the lowest productivity compared to large rivers (Finlay, 2001).

As the nutrients and light increased, the concentration of biofilm chlorophyll-a was higher (Hill et al., 1995; Lourenço-Amorim et al., 2014; Sturt et al., 2011). Thus, the present study showed that nutrients and light act as limiting factors for the development of biofilm algae in shaded ecosystems within the Cerrado. It is therefore worth emphasizing the importance that these variables have in altering the bottom-up forces and the metabolism of the ecosystem, with the possibility of altering heterotrophic conditions in autotrophs.

Studies in temperate streams, which manipulated the light gradient and nutrients, found that light was the predominant factor in the control of algal biomass, while nutrients had a smaller role (Bechtold et al., 2017; Mosisch et al., 2001). Due to differences in algal community composition of the biofilm, with diatoms predominating in shaded streams and filamentous algae predominating in streams with open canopy (DeNicola et al., 1992), there may be changes in their response to carbon sources, which may influence nutrient dynamics (Bechtold et al., 2017). In this way, patches of open canopy, typically from well-developed forests or caused by anthropic disturbance, increase benthic algal growth by increasing light and can subsequently increase nutrient retention and uptake (Bechtold et al., 2017). Thus, the increased of electrical conductivity in nutrient enriched streams may be a response of changes in light gradient and / or the input of nutrient-enriched waste (e.g., sewage and agricultural fertilizer).

In addition, the increase of light incident on the stream can also promote the production of higher trophic levels (i.e., bottom-up process) (Warren et al., 2016). For all of the streams, the Baetis nymphs were predominant, being found in higher density on the plates without electricity and with increased nutrients and an open canopy. The increase in grazer density may be directly related to the quantity of the resources. Studies suggest that nutrient enrichment also facilitates the increase in invertebrate density because of the increase in primary producers (Singer and Battin, 2007). Beyond that, the Baetis nymphs indicate a negative pressure on the biofilm biomass, constituting a top-down control in the streams enriched with nutrients (Garcia et al., 2015; Sturt et al., 2011).

Furthermore, it was possible to observe a direct relationship between the increase in density of mites and Baetis nymphs. Isotope analysis indicated that the Baetis nymphs are prey for the mites and possibly a bottom-up trophic cascade effect is occurring (Hunter and Price, 1992) in which the increase of the biofilm biomass facilitates the increase of the Baetis nymph population and, consequently, it is a food resource for increasing the density of mites.

Biofilm can be considered a high-quality resource and the isotopic signal can be found in consumers at disproportionate levels as their availability in the streams increases, suggesting the increase in relative importance of autochthonous organic matter in supporting higher trophic level (Warren et al., 2016), coinciding with the patterns of changes found in neotropical streams. The presence of mites seems to have established a top-down trophic cascade on the biofilm biomass that, even with the presence of grazers, was not exhausted. Larval stage mites (Hydrachnidia) are parasites for adult insects and, as adults, they are predators of invertebrate eggs and larvae (Di Sabatino et al., 2008). Thus, these organisms deserve more attention in aquatic food web studies (Di Sabatino et al., 2008; Stegen and Black, 2011).

In the present study, it was possible to identify that autochthonous productivity increased as a result of changes in bottom-up controls (e.g., nutrient enrichment and canopy opening). As a result of this change, the grazing invertebrates exhibited greater relevance, i.e., the top-down control may play an important role in a food web based on autochthonous primary production. Accordingly, changes in land cover of headwaters that lead to changes in the availability of nutrients and light in headwater streams can promote energy flows based on autochthonous resources. Particularly, even in streams, with low range of chlorophyll concentration (0-2 mg.m-2), may increase the relative importance of autochthonous organic matter in supporting higher trophic level.

Mutually, the carbon isotope signature from biofilm followed these changes which can be reflected in the highest trophic levels. In this way, efforts in headwater streams to increase the knowledge of algal $\delta^{13}C$ should be highlighted, since the algae may be disproportionately more important for higher trophic levels than terrestrial detritus (Finlay, 2001). Without investigation of the controls that act over algae $\delta^{13}C$ there may be uncertainty in isotopic baseline determinations of food webs and consequently influence the accuracy of food web analyses. In addition to light conditions, different water current velocities (standardized in the present study) may produce changes in the biofilm $\delta^{13}C$ (Finlay et al., 1999; Ishikawa et al., 2012).

Complementary studies are needed, since the changes evaluated in the present study were much smaller than those that usually occur in the region (i.e., lower nutrient enrichment and sun spots and no sewage inflow and total deforestation) (Fonseca et al., 2014). Furthermore, the results reinforces the fact that, in addition to the relationship between algae, nutrients and light, attention should be paid to the role of grazing invertebrates in the control of biofilm growth (Hart, 1990; Sturt et al., 2011).

Apparently, this type of system operates more in heterotrophic metabolism, which maintains a good water quality in general. It is important to note that these streams are naturally shaded and oligotrophic and changes in riparian forest cover and / or the increase of nutrient-enriched waste can promote energy flows based on autochthonous resources, affecting the characteristics and the functioning of the food web. Changes in the Brazilian forest code, which reduced the need for preservation of riparian forests, decreasing the width to be preserved, may cause non-linear changes in the metabolism and functioning of neotropical streams ecosystems, reducing the capacity of streams to provide ecosystem services as water quality.

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Table 1. Limnological variables (median, minimum and maximum) of the experimental points located at the Fazenda Água Limpa (FAL) and the Brasília Botanic Garden (BBG) (APA Gama and Cabeça-de-Veado, Federal District, Brazil).

Variable	Oligotrophic Streams						Nutrient Enrichment Streams					
	Cabeça -de-Veado Stream			Capetinga Stream			Gama Stream			Onça Stream		
	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max
Ec (μS cm.$^{-1}$)	5.20	4.70	5.70	5.20	3.60	8.30	10.30	6.50	11.30	14.00	5.60	17.20
SRP (μg .L^{-1})	8.00	6.00	10.00	2.80	<1.00	5.00	13.30	5.00	25.00	13.50	6.40	25.00
NH4+(μg. L^{-1})	6.50	5.00	8.00	3.50	2.00	17.00	9.20	7.00	27.00	47.50	5.00	69.00
NO3- (μg. L^{-1})	26.50	25.00	28.00	19.10	9.70	33.50	38.20	13.10	81.00	130.90	11.20	163.00

Table 2. Results of the three-way ANOVA of the Baetis nymph density with canopy (open / shaded), grazer exclusion (grazed / grazers excluded) and nutrient concentration (oligotrophic / nutrient enrichment) as independent variables.

Baetis Density	d.f.	F-value	Pairwise comparisons
Canopy	1	9.045**	Open > Shaded
Grazer exclusion	1	27.135***	Grazed > Excluded
Nutrient concentration	1	25.555***	Nutrient Enrichment > Oligotrophic
Canopy: Grazer exclusion	1	3.015·	
Canopy: Nutrient concentration	1	4.459*	
Grazer exclusion: Nutrient concentration	1	8.518**	
Canopy: Grazer exclusion: Nutrient concentration	1	1.486	
Residuals	40		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 3. Results of the multiple regression of ash-free dry matter (AFDM) responses (A) and chlorophyll-a (B) density for treatments with nutrient concentration in the water (nutrient enrichment and oligotrophic), grazer exclusion (grazed and excluded) and canopy cover (open and shaded).

	Estimate	SE	t value
(A) AFDM			
Intercept	0.709	0.326	2.175*
Grazed	0.176	0.364	0.483
Nutrient	0.115	0.022	5.320***
Open Canopy	-0.011	0.006	-1.766
Grazed:Nutrient	-0.099	0.021	-4.794***
Grazed:Open Canopy	0.003	0.006	0.511
Nutrient:Open Canopy	0.000	0.000	0.803

Residual standard error: 0.4956 on 39 degrees of freedom. Multiple R^2: 0.7356, Adj-R^2: 0.6915. F-statistic: 16.69 on 6 and 39 d.f., p-value: 4.27e-09

	Estimate	SE	t value
(B) Chlorophyll a			
Intercept	-4.148	0.675	-6.143***
Grazed	-0.18	0.753	-0.239
Nutrient	0.154	0.045	3.432**
Open Canopy	0.042	0.012	3.389**
Grazed: Nutrient	-0.015	0.043	-0.347
Grazed:Open Canopy	0.002	0.012	0.13
Nutrient:Open Canopy	0.002	0.001	2.091*

Residual standard error: 1.026 on 39 degrees of freedom. Multiple R^2: 0.5487, Adjusted R^2: 0.4735 F-statistic: 7.294 on 6 and 39 d.f., p-value: 3.764e-05

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

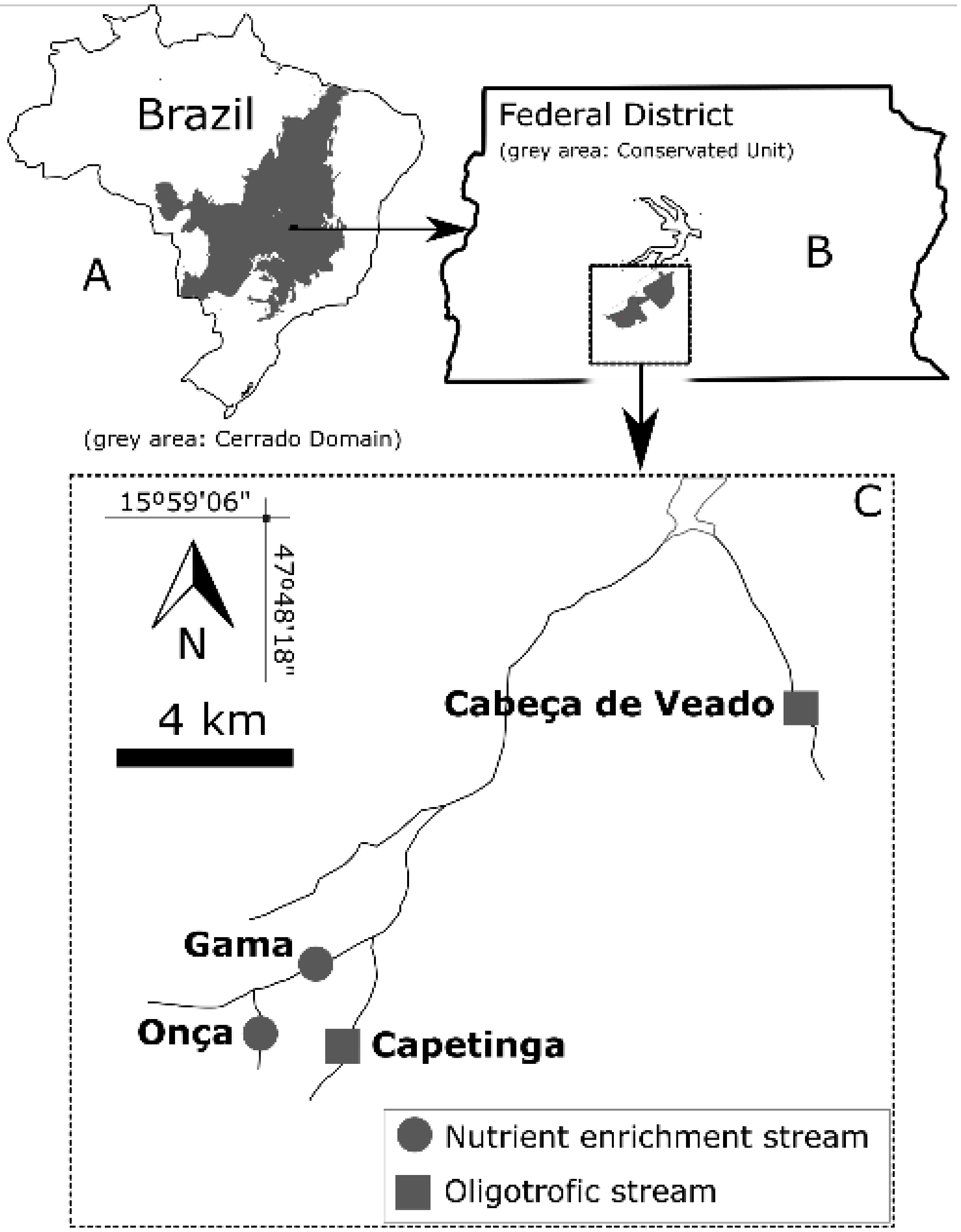

Figure 1 Location of study areas in the Federal District, Brazil. The gray scale in the area of Brazil (A) represents the Cerrado domain. The gray scale in the Federal District (B) arearepresents the Federal Conservation Units. C - circle points represent the location of nutrient enrichment streams and square points represent oligotrophic streams

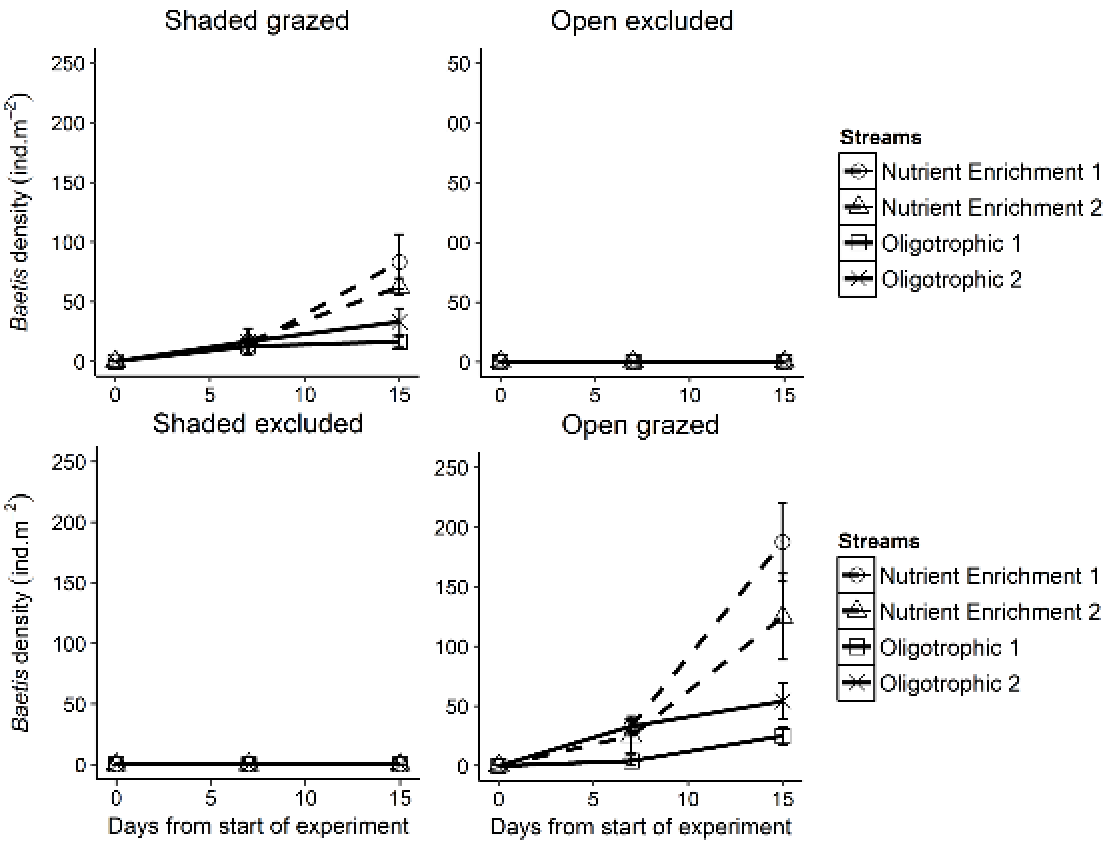

Figure 2 Mean (± 1 SE) (N = 3) of Baetis nymph density on the electrified (excluded) and non electrified (grazed) plates from open and closed canopy areas for oligotrophic streams (1 Cabeça-de-Veado and 2- Capetinga) and streams with nutrient enrichment (1-Onça and 2-Gama)

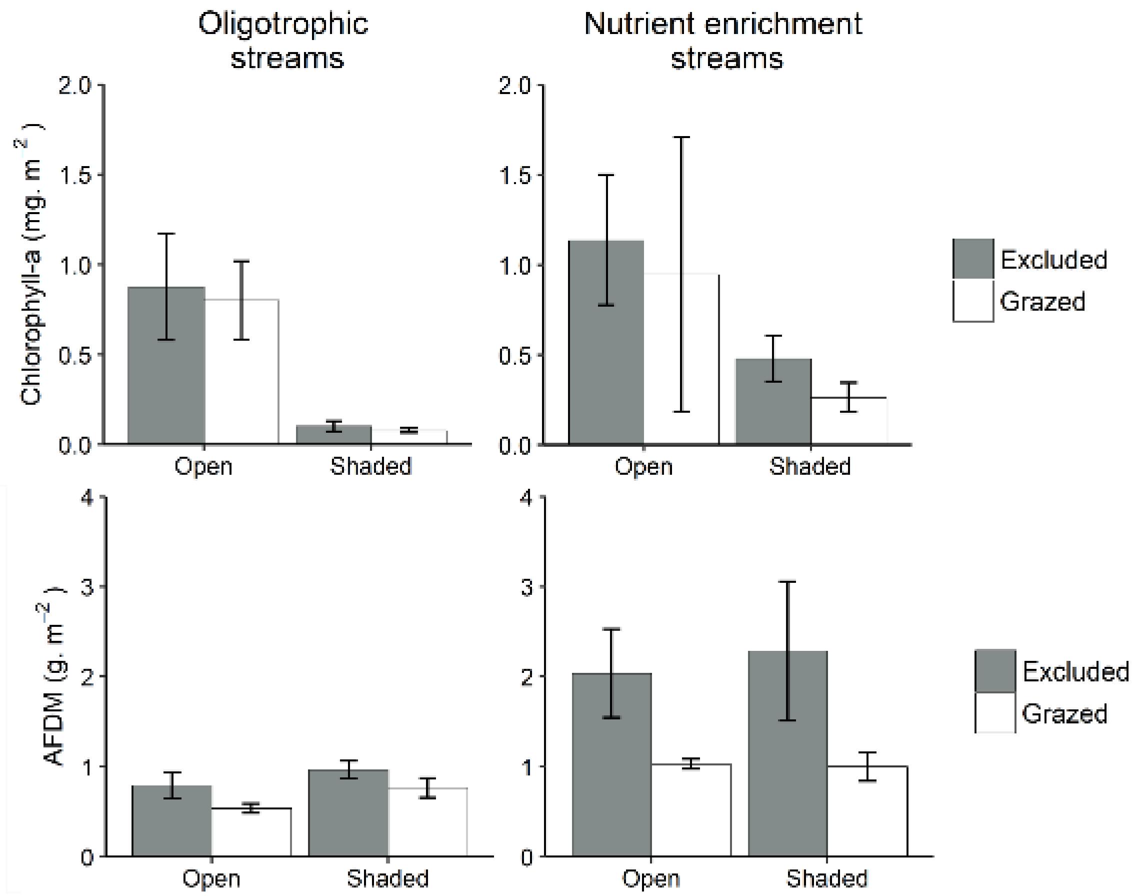

Figure 3. Mean (± SE) (n = 3) of ash-free dry matter and chlorophyll-a from shaded areas with and withoutthe exclusion of invertebrates and from open canopy areas with and without exclusion, for oligotrophic streams and streams with nutrient enrichment.

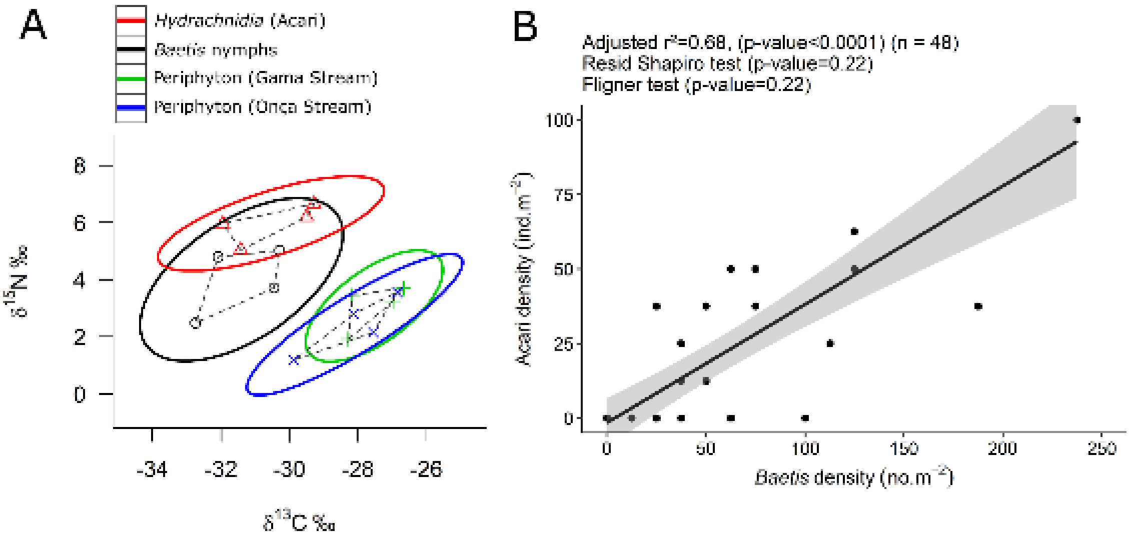

Figure 4. A - Standard Ellipse Area (SEA, solid lines), representing the isotopic niche of the biofilm, of the Baetis nymphs and of the mites, as determined by the SIBERmodel. B - Analysis of the linear regression of the density of mites as a result of the density of the Baetis nymphs.

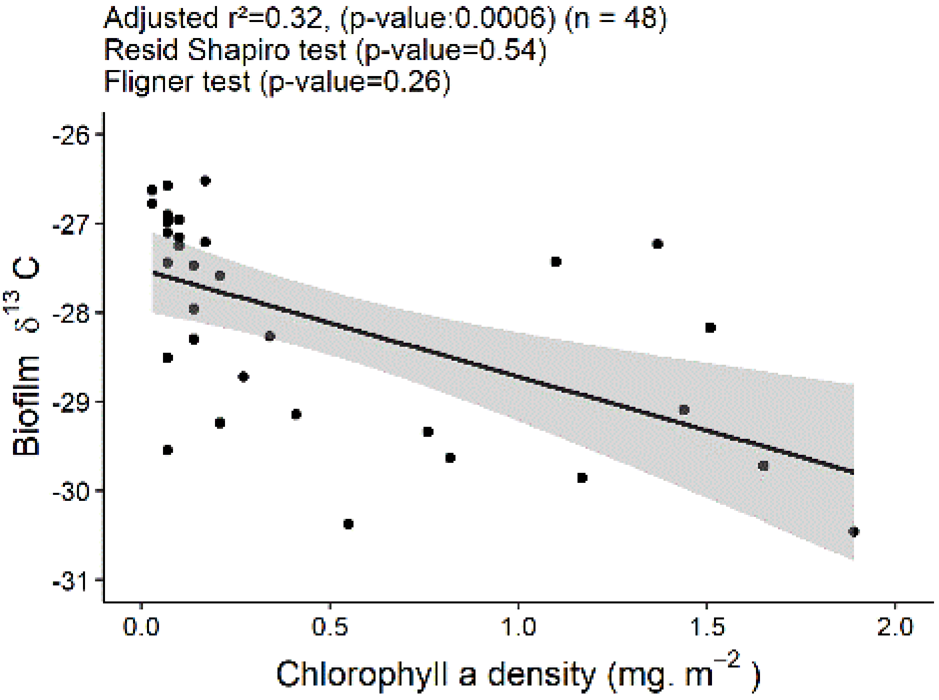

Figure 5. Linear regression analysis of the δ^{13}C values for biofilm as a result of chlorophyll-a density.

ANEXO A

Title: Effects of seasonal flow disturbances on the relationship between macroinvertebrate community and microhabitat in neotropical savanna streams

Authors: Tiago Borges Kisaka*, Elisa Araújo Cunha Carvalho Alvim, Luciana Mendonça-Galvão, Vinícius Tirelli Pompermaier, Sylvain Dolédec and Gabriela Bielefeld Nardoto
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Figure S1. Sampling with surber net and storage of macroinvertebrates in the field.

Table S1. Taxonomic composition and abundance of the sampled community.

Order	Family	Abundance
Coleoptera	dytiscidae	57
	elmidae	464
	gyrinidae	10
	noteridae	82
	psephenidae	101
	scirtidae	16
Diptera	ceratopogonidae	34
	chironominae	177
	simuliidae	370
	tabanidae	11
	tanypodinae	190
	tipulidae	80
Ephemeroptera	baetidae	222
	euthyplociidae	48
	leptohyphidae	68
	leptophlebiidae	955
Hemiptera	naucoridae	55
	veliidae	52

Lepidoptera	pyralidae	66
Megaloptera	corydalidae	27
	sialidae	6
Odonata	aeshnidae	5
	coenagrionidae	114
	corduliidae	22
	gomphidae	74
	libellulidae	79
	megapodagrionidae	38
	perilestidae	6
Plecoptera	perlidae	281
Trichoptera	calamoceratidae	73
	hydrobiosidae	21
	hydropsychidae	276
	leptoceridae	136
	odontoceridae	126
	polycentropodidae	107
	sericostomatidae	65

ANEXO B

Title: Seasonal effects on the food web structure of benthic macroinvertebrates community in Neotropical forest streams

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Figure S1. Biofilm sampling in a quadrat area of 4 x 4 cm and storage.

Figure S2. Sampling with phytoplankton net and storage.

Figure S3. Preparation of the seston and biofilm samples for the analysis of stable isotopes.

ANEXO C

Title: Understanding the controlling factors over biofilm as an autochthonous resource in shaded oligotrophic neotropical streams

Authors: Tiago Borges Kisaka*, Andréia de Almeida, Luciana Mendonça-Galvão, Luiz Felippe Salemi and Gabriela Bielefeld Nardoto
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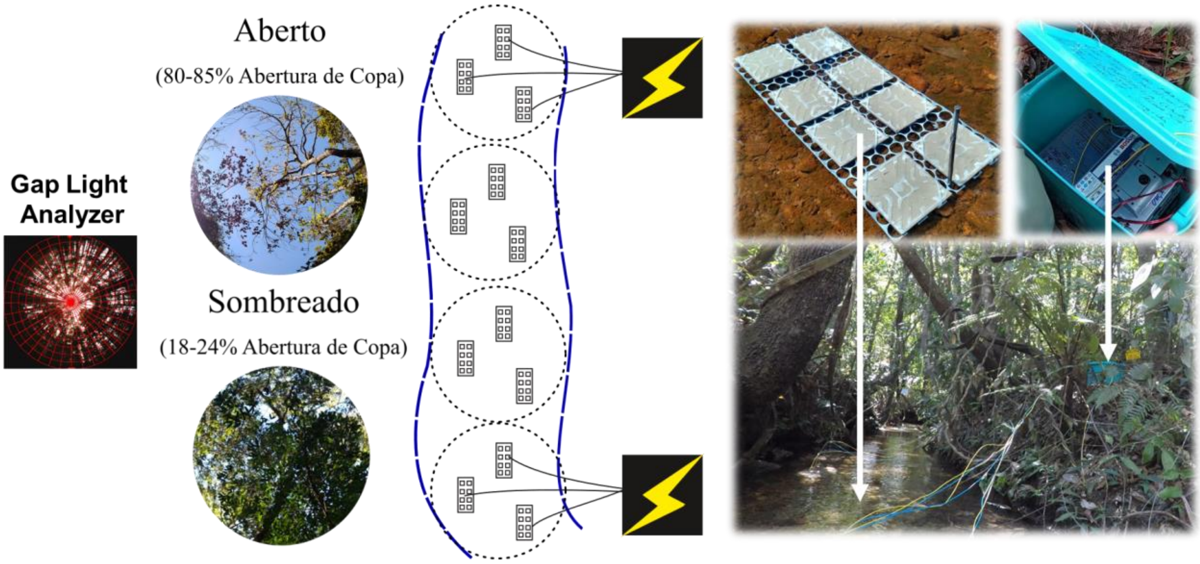

Figure S1. Experimental arrangement used to evaluate the effect of light, nutrients and grazing on biofilm colonization.