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Tese de Doutorado

Influência de fatores espaciais e temporais sobre a composição funcional da comunidade de insetos aquáticos em riachos

Silvia Vendruscolo Milesi

Porto Alegre, Março de 2016.

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Resumo

Influência de fatores espaciais e temporais sobre a composição funcional da comunidade de insetos aquáticos em riachos. Os estudos que envolveram os três capítulos da minha tese englobam padrões de distribuição dos insetos aquáticos, agregando informações sobre características funcionais dessas comunidades. No primeiro capítulo avaliei a influência da heterogeneidade do substrato sobre as características funcionais dos insetos bentônicos. Concluí que em ambientes naturais o substrato heterogêneo apresentou combinações específicas de atributos, associados principalmente ao tamanho do corpo e hábitos alimentares. Esses atributos exercem forte influência sobre o funcionamento do ecossistema dos riachos. No segundo capítulo verifiquei a variação temporal dos atributos funcionais dos insetos aquáticos, com enfoque para organismos especialistas e generalistas. Nos primeiros estágios da colonização organismos coexistem independente dos atributos, isso porque a comunidade mostrou atributos similares. Nos estágios mais avançados de colonização os organismos generalistas resistiram a fatores bióticos e abióticos, mostrando uma variedade de atributos superior à que foi encontrada no início da colonização. Conclui que a ausência ou diminuição de organismos especialistas pode ser um indicador de degradação ambiental. No terceiro capítulo avaliei se existe efeito da presença/ausência de cobertura vegetal considerando a distância entre os riachos sobre as comunidades de insetos. Concluí que a cobertura vegetal no entorno dos riachos é um fator determinante para a distribuição espacial dos insetos aquáticos. Em locais com cobertura vegetal, insetos aquáticos com maior habilidade de dispersão apresentaram decaimento da similaridade com a distância, contrário do que foi verificado para locais sem vegetação. Já para dispersores menos hábeis, riachos mais distantes apresentaram comunidades menos similares para locais come sem cobertura vegetal. Com isso, conclui que a presença de vegetação no entorno dos riachos pode ser uma barreira física que limita a dispersão dos insetos com maior capacidade de voo. Finalizando, este trabalho ressalta a influência de fatores locais (substrato) e regionais (vegetação/limitação de dispersão) sobre as características funcionais da fauna aquática. Além disso, a manutenção da heterogeneidade do substrato e da vegetação ripária, contribuiu para a manutenção da diversidade funcional das comunidades de insetos aquáticos.

Palavras chave: Atributos funcionais, generalistas e especialistas, vegetação ripária, colonização, heterogêneo, homogêneo.

Abstract

Influence of spatial and temporal factors on the functional composition of stream insect communities. Studies of my thesis include aspects of distribution patterns of aquatic insects and aggregate information on functional characteristics. In the first chapter, I evaluated the influence of substrate heterogeneity on the functional characteristics of benthic insects. I concluded that in natural environments, heterogeneous substrate select specific traits combinations associated mainly to body size and feeding habits, attributes that exert a strong influence on the functioning of ecosystems. In the second chapter, I verified the temporal variation of the functional attributes of aquatic insects, focusing on specialist and generalist *taxa*. In early stages of colonization, organisms coexist independent of the attributes, because the community showed similar attributes. In the last stages, specialists were able to resist to biotic and abiotic factors showing a variety of attributes that was not founded at the beginning of colonization. In conclusion, the absence or decrease of specialists can be an indicator of environmental degradation. In the third chapter, I used a functional approach with emphasis on attributes related to dispersion. We conclude that the canopy cover of streams is a determining factor for the distribution of aquatic insects. Aquatic insects with greater ability to disperse showed a weak pattern of distance decay of similarity, only in streams with grassland. For weak dispersers patterns of was similar for the two streams (open and forest riparian vegetation). Thus, we conclude that the presence of vegetation streams can be a physical barrier that limits the insect dispersal, especially *taxa* with greater abilities to disperse. Finally, this study highlights the influence of local (substrate) and regional (limiting dispersion) factors on the functional characteristics of the aquatic fauna. In addition, maintaining the diversity of substrate and riparian vegetation, contributes to the functional and taxonomic diversity of aquatic insect communities.

Key words: functional traits, specialists and generalists, riparian vegetation, colonization, heterogeneous, homogeneous.

Introdução geral

Os ecossistemas aquáticos são ambientes heterogêneos que apresentam uma fauna diversificada que varia tanto espacialmente como temporalmente (Clarke et al. 2008; Heino et al. 2010). A organização destas comunidades é influenciada por mudanças no habitat e no padrão de configuração espacial dos rios, o que pode ser verificado em escala local e regional (Allan e Castillo 2007, Tonkin et al. 2014). São esses fatores que geram diferentes configurações de distribuição e ocorrência, dependendo das condições ecológicas e de interações bióticas intra e interespecíficas. Com isso, essas comunidades de insetos aquáticos tornam-se ótimos modelos para a determinação de como e quais filtros ambientais agem na organização das mesmas (Cottenie 2005).

Ecólogos vivem em uma constante busca por padrões de distribuição das comunidades. Os estudos que envolveram os três capítulos da minha tese englobam esses aspectos, agregando informações sobre características funcionais das comunidades de insetos aquáticos. Estudos envolvendo abordagem funcional aumentaram notavelmente nos últimos anos, em todas as áreas da ecologia (Laureto et al. 2015). Nos ecossistemas aquáticos Cummins (1974) e Vanotte et al. (1980) foram pioneiros no uso de características funcionais dos organismos. Primeiramente, Cummins (1973) utilizou a descrição “grupos funcionais” para organismos que exploram recursos alimentares similares. Anos depois, Vanotte et al. (1980) desenvolveram a teoria do rio contínuo, levando em consideração a utilização de grupos funcionais e a distribuição dos mesmos ao longo do gradiente fluvial longitudinal.

A utilização da composição taxonômica de insetos aquáticos para diagnosticar padrões de organização de comunidades é comum, embora existam evidências de que não sejam suficientes para detectar padrões relacionados ao funcionamento do ecossistema (Dolédec et al.

2011). Características biológicas como tamanho do corpo, formas de dispersão e hábito alimentar podem ser utilizadas como indicadores da funcionalidade dos sistemas aquáticos (Vieira et al. 2006, Webb et al. 2010). Estes atributos podem ser comparados entre diferentes ambientes e entre regiões que diferem quanto à composição taxonômica (Statzner et al. 2004). A maioria dos estudos que abordam atributos de insetos aquáticos em sistemas lóticos foi desenvolvido no continente Europeu e na América do Norte (Vieira et al. 2006, Tachet et al. 2010).

Pesquisas envolvendo aspectos funcionais de invertebrados aquáticos na região neotropical são escassas (Ex. Tomanova e Usseglio-Polatera 2007). No Brasil foram desenvolvidos poucos estudos utilizando a abordagem funcional. Destaque para Colzani et al. (2013) que avaliaram a resposta de índices de diversidade funcional de insetos aquáticos frente a mudanças na paisagem. Nesta mesma linha e adicionando diversidade filogenética, Saito et al. (2015a) utilizaram os atributos funcionais de insetos na criação de índices multimétricos na avaliação do impacto ambiental. Cabe ressaltar que os estudos desenvolvidos no país são voltados para a utilização dos atributos funcionais no monitoramento da qualidade ambiental. Além disso, como na região neotropical esta abordagem é recente são praticamente inexistentes informações acerca da composição funcional de insetos aquáticos em ambientes naturais. Este fato dificulta comparações entre locais impactados ou modificados naturalmente, pois não existem estudos em áreas de referência.

Características funcionais são traços morfológicos ou fisiológicos dos organismos que afetam o desempenho dos mesmos em um determinado ambiente (Violle et al. 2007). Segundo Cianciaruso et al. (2009), o uso destas características em um índice de diversidade significa: “medir a diversidade de características funcionais, que são componentes dos fenótipos dos organismos que influenciam os processos na comunidade”. Essas características conferem às espécies a capacidade de se adaptar a condições ambientais específicas tornando-as aptas a sobreviver em um determinado local. Em outras palavras, o local onde os organismos vivem somado às restrições ecológicas aos quais eles estão expostos irão determinar os padrões de distribuição funcional das comunidades (Townsend e Hildrew 1994, Poff 1997).

A biodiversidade aquática diminui com o aumento do impacto antrópico, mostrando a necessidade da utilização de novas técnicas e que forneçam resultados mais rápidos, práticos e eficazes no que se diz respeito ao biomonitoramento e conservação desses ambientes. A abordagem levando em consideração aspectos funcionais das comunidades biológicas é crescente, porém o seu uso indiscriminado, sem critérios e apresentando lacunas teóricas torna sua utilização frágil e sem sentido para a ciência. O trabalho com atributos funcionais e insetos aquáticos está iniciando no Brasil e para que o seu futuro seja promissor é necessário que pesquisadores usem hipóteses bem consolidadas com bases teóricas adequadas. Além disso a escolha dos atributos pode ser um fator que complique a obtenção de informações de qualidade e que realmente auxiliem na manutenção da biodiversidade aquática.

Entre uma seleção de 100 questões fundamentais em ecologia, Sutherland et al. (2013) destacam a abordagem funcional e enfatizam a necessidade de estudos que revelem como a estrutura e a dinâmica de determinadas comunidades biológicas podem ser preditas a partir de atributos funcionais das espécies e qual a relação destes com a heterogeneidade espacial. Em relação a escala espacial local, entre os fatores que determinam a distribuição das comunidades de insetos aquáticos estão o tipo e a heterogeneidade do substrato (Poff e Ward 1990, Downes et al. 1998, Lamouroux et al. 2004). Habitats heterogêneos propiciam maior quantidade de recursos alimentares e refúgios que protegem os organismos de distúrbios, determinando padrões de colonização dos insetos aquáticos (Brown 2003). A quantidade de matéria orgânica que acumula no leito dos riachos também é fator preponderante para a organização da fauna aquática, pois além de ser recurso alimentar para os insetos aquáticos é utilizada como abrigo e proteção (Mackay 1992, Graça et al. 2004). Os efeitos da heterogeneidade do substrato sobre a estrutura funcional dos insetos aquáticos ainda não foram investigados experimentalmente, especialmente na região Neotropical e em riachos naturais (exceto estudos envolvendo sedimentação: Rabení et al. 2005, Descloux et al. 2014, Rasmussen et al. 2012). Para avaliar como a heterogeneidade do substrato influencia a composição funcional dos insetos aquáticos realizamos um experimento *in-situ* em riacho com pouco impacto antrópico utilizando amostradores artificiais (Figura 1).

Figura 1. Amostradores artificiais utilizados no experimento. (A) Substrato homogêneo com superfície lisa. (B) Substrato heterogêneo com pedaços colados para aumentar a heterogeneidade.

A abordagem funcional também pode ser útil para entender a colonização do substrato por insetos aquáticos. Para alguns autores os processos de colonização e sucessão de insetos aquáticos dependem de fatores chave como a heterogeneidade do substrato e a disponibilidade de recursos alimentares, como o acúmulo de matéria orgânica e de algas perifíticas (Mathooko 1995, Downes et al. 1998, Rae 2004). Considerando ecossistemas aquáticos, as habilidades de colonização e as características dos organismos podem determinar a organização das comunidades durante o período de colonização (Miyake et al. 2003). A dinâmica da colonização do substrato está vinculada a mobilidade (voo, nado e deriva) e ao tamanho do corpo dos organismos, além de aspectos da história de vida (Rosenberg e Resh 1982, Mackay 1992, Towsend et al. 1997). Essas caraterísticas das espécies podem limitar os organismos a condições ecológicas restritas, em outras palavras, especialização ecológica (Futuyma e Moreno 1988). Espécies especialistas apresentam uma aptidão elevada para colonizar habitats e recursos específicos, enquanto espécies generalistas podem tirar vantagens de diferentes tipos de habitats e, em alguns casos, se adaptar a áreas perturbadas (Clavel et al. 2010, DeVictor et al. 2010). A utilização desta abordagem em riachos é recente (Mondy e Usseglio-Polatera 2014) e se faz necessária, pois entender como ocorre a distribuição temporal de especialistas e generalistas em sistemas naturais é primordial, tanto para formulação de bases conceituais como para posterior utilização de informações na conservação desses riachos. Para verificar a proporção de insetos aquáticos especialistas e generalistas ao longo do tempo utilizei uma abordagem experimental, instalando amostradores artificiais em um riacho com pouca perturbação, durante um período de trinta dias (Figura 2).

Figura 2. Primeiro dia de instalação dos amostradores artificiais. Os amostradores foram retirados após 1, 3, 5, 10, 15 e 30 dias.

O sucesso na colonização do habitat está relacionado às características funcionais de cada organismo. Em escalas espaciais mais amplas, este sucesso depende mais intimamente de atributos ligados a dispersão dos insetos aquáticos (Petersen et al. 2004, Trekels et al. 2011, Saito et al. 2015b). A maioria dos insetos aquáticos dispersa ao longo do curso d’água, longitudinalmente (Wiens 2002), além disto, embora que de maneira menos efetiva, a colonização pode ocorrer por dispersão lateral (Brederveld et al. 2011). Em relação a distribuição espacial, quanto mais distante duas comunidades estão, menos similares devem ser. Esse padrão é conhecido como decaimento da similaridade com a distância (DDS: *distance decay of similarity*, Nekola e White 1999). Na região neotropical, Saito et al. (2015b) investigaram o padrão de decaimento da similaridade filogenética com a distância, utilizando atributos de insetos aquáticos relacionados a dispersão, nesse caso capacidade de voo, tamanho do corpo e voltinismo. Os autores sugerem que o voltinismo foi o principal fator que determinou o padrão de DDS da comunidade. No entanto, alguns fatores ambientais como a presença da vegetação ripária, a configuração espacial dos riachos e aspectos da paisagem do entorno da bacia hidrográfica podem interferir nestes padrões de distribuição (Fagan 2002, Petersen 2004, Brown e Swan 2010). Quanto à vegetação ripária estudos apontam que os insetos aquáticos acabam permanecendo próximo ao canal do rio na vegetação circundante, local de onde emergem e permanecem para reproduzir e realizar oviposição (Greenwood et al. 2011, Datry et al. 2016). Para verificar como ocorre o decaimento da similaridade da comunidade de insetos aquáticos com a distância (DDS) o trabalho foi realizado em locais com e sem vegetação ripária florestal no entorno dos riachos. Nesse caso, a ausência da vegetação florestal é decorrente da característica fitofisionômica natural da região de estudo (Figura 3).

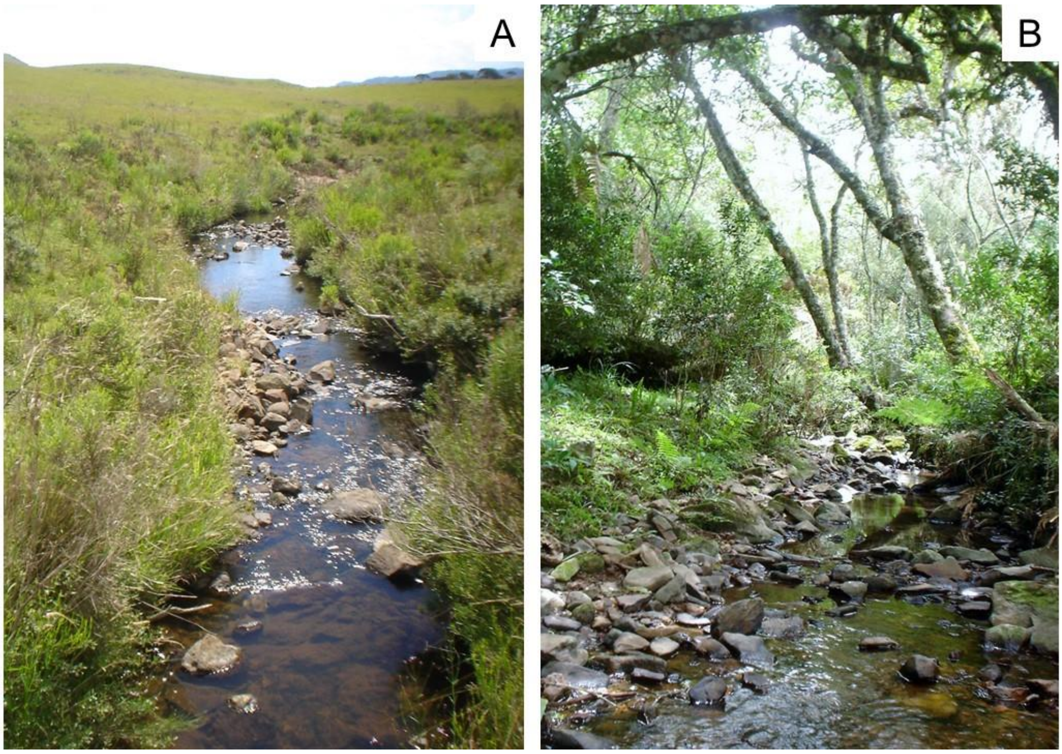

Figura 3 – Riachos com e sem cobertura vegetal florestal em São José do Ausentes, Rio Grande do Sul, Brasil.

Objetivos

Empreguei uma abordagem funcional para entender como ocorrem os padrões de distribuição espacial e temporal das comunidades de insetos aquáticos. No **capítulo um** avaliei as características funcionais dos organismos e suas respostas à heterogeneidade do habitat, utilizando amostradores artificiais com superfícies homogêneas e heterogêneas. No **capítulo dois**, usando características funcionais dos invertebrados, avaliei a ocorrência e distribuição de organismos especialistas e generalistas ao longo de trinta dias de colonização, para isso foram usados substratos artificiais. No **capitulo três** utilizei riachos com e sem cobertura vegetal florestal com a intenção de verificar se a similaridade entre as comunidades aumenta com o aumento da distância dependendo do tipo de cobertura. Além disso, avaliei se o padrão de decaimento da similaridade com a distância é diferente entre os dados funcionais e taxonômicos.

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Capítulo 1

Substrate heterogeneity influences the trait composition of stream insect communities: an experimental in-situ study

Este manuscrito foi submetido em novembro de 2015 para avaliação na revista Freshwater Science com co-autoria de Sylvain Dolédec[1] e Adriano Sanches Melo[2]. O manuscrito está formatado de acordo com as normas da referida revista.

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Functional structure of stream insect communities

Substrate heterogeneity influences the trait composition of stream insect communities: an experimental in-situ study

Abstract: Local stream insect distribution is influenced by abiotic factors such as substrate heterogeneity and amount of organic matter. We evaluated the influence of substrate heterogeneity on the functional structure and diversity of stream macroinvertebrate communities using four traits: feeding habits, body size, body shape and attachment to substrate. We found that the heterogeneous substrate supported communities with higher functional diversity and richness than the homogeneous substrate. In addition, heterogeneous substrate included a higher abundance of predators, shredders and filtering-collectors, and organisms with a large body size. In contrast, communities of homogeneous substrate showed a prominence of small-size organisms, and case-builders and scrapers. Our results suggest that in natural habitats substrate heterogeneity selects specific combination of traits especially associated to size and feeding habits, which have strong implication in stream ecosystem functioning. As a result one may anticipate that the ongoing homogenization of stream bottom substrate associated to human activities should result in a reduction or even a loss of important functions linked for example to the process of organic matter, which should greatly affect the stream ecosystem.

Keywords: benthic invertebrate traits, substrate heterogeneity, functional richness, functional diversity.

By filtering the functional attributes of organisms, local and regional factors control the biodiversity of stream ecosystems (Poff 1997). On a continental scale, several studies have shown that similar habitats yielded similar trait composition of communities and that biogeography was less of a barrier when considering traits in comparison to taxonomy (Statzner *et al.* 2001, Statzner *et al.* 2004, Bêche and Statzner 2009). On a local scale, by delineating a broad variety of habitat types, environmental factors such as substrate types, heterogeneity, and organic matter are known to greatly influence the distribution of aquatic organisms (Cummins and Lauff 1969, Erman and Erman 1984, Poff and Ward 1990) and insect traits have been shown to respond to variations in hydraulics (Lamouroux *et al.* 2004). More generally, spatial heterogeneity of the habitat is a fundamental feature for which trait predictions have been elaborated (Townsend and Hildrew 1994) and tested for aquatic insects (Usseglio-Polatera 1994). To our knowledge, the effects of substrate heterogeneity on stream insect functional structure have yet not been investigated experimentally, especially in Neotropical streams and the few studies on the effects of substrate on invertebrate traits rather consider substrate colmation (Rabení *et al.* 2005, Descloux *et al.* 2014) or sediment toxicity (Rasmussen *et al.* 2012).

Extensive evidence shows that heterogeneous substrates, i.e. including different sediment types, sizes, and/or textures, harbor high benthic invertebrate densities and richness than homogeneous ones (e.g., Lamberti and Resh 1979, Williams 1980, Erman and Erman 1984, Beisel *et al.* 2000, Poff *et al.* 2006, Palmer *et al.* 2010). Substrate heterogeneity, which allows more species to coexist, is thus postulated to result in higher resistance of stream communities to disturbance (O´Connor 1991, Brown 2003, Schneck *et al.* 2013). This is caused by a potentially higher availability of food resources and interstitial space in heterogeneous substrates, which provide protection from predators and refuge from disturbance (Townsend *et al.* 1997, Gjerløv *et al.* 2003, Hoover and Ackerman 2011, Hepp *et al*. 2012). Substrate heterogeneity can also be enhanced by the accumulation of coarse organic matter from riparian vegetation, which can increase the structural complexity of substrates (O´Connor 1991). In contrast, homogeneous substrates expose organisms to harsher microhabitat conditions (Erman and Erman 1984, Poff and Ward 1990). In addition, homogeneous surface imply less protection against predators and more exposition to physical disturbances (current velocity, shear stress). As a result, stream invertebrates have to adapt to resist high flow velocities on homogeneous substrate (Hoover and Ackerman 2011). Finally, homogeneous substrates can sustain high periphyton production (Hill and Knight 1988), which should have implication on trophic regime and functional feeding groups.

If we assume that heterogeneous substrates offer more resources to benthic invertebrates implying more species richness and abundance than homogeneous ones, we can predict that the functional attributes of invertebrates that we measure should occur in heterogeneous substrates. Heterogeneous substrates offering a greater availability of refuge allow invertebrates with different biological characteristics to coexist (Townsend and Hildrew 1994, Townsend *et al.* 1997). Supported by the habitat templet concept (Townsend and Hildrew 1994), we hypothesize that large body-size invertebrates should dominate in heterogeneous substrates since the predation risk decreases (availability of shelter) whereas the retention of food resources increases (Resh *et al.* 1994, Townsend and Hildrew 1994, Heino 2005, Table 1). In addition, because of the high amount of organic matter on heterogeneous substrates, they should shelter a great abundance of shredders and filtering-collectors (Encalada *et al.* 2010, Hepp *et al.* 2012).

Table 1. Predictions made for stream insect traits along with substrate heterogeneity.

	Heterogeneous	**Homogeneous**
Feeding habits	Shredder	Scraper
Body size	Large	Small
Body shape	All body shapes	Hydrodynamic
Locomotion and substrate relation	All types	Case building, silt gland and temporary attached

In contrast, homogeneous substrates, which include fewer refugia and higher exposition to flow, should harbor small-sized invertebrates with flattened bodies, which are biological characteristics that allow resistance to disturbance events (Vogel 1994, Townsend and Hildrew 1994, Gallardo *et al.* 2009). Consequently, invertebrates with specific adaptations, which, for example, allow attachment to substrate (e.g. silt glands) should dominate homogeneous substrates. In addition, the lack of shelter in homogeneous substrates should increase the number of insects that construct cases to avoid predation and/or physical disturbances (Lamouroux *et al.* 2004), and as a consequence of higher periphyton biomass on homogeneous substrates (Hill and Knight 1988, Biggs and Hickey 1994), scrapers should better develop.

Finally, since the substrate heterogeneity help supporting more organisms that share space and have different traits, functional richness and diversity should be higher in heterogeneous substrates. In contrast, homogeneous substrates should filter organisms that are able to resist harsher conditions and thus tend to be more functionally similar.

METHODS

Study area

Our experiment was set up in the Dourado stream, a tributary of the Uruguai river located in the north of Rio Grande do Sul, Brazil (29° 31′ 57″ S, 50° 14′ 55″ W). The region is situated in the south of the Atlantic Forest, with rain forests including *Araucaria angustifolia* (Bertol.) Kuntze and semideciduous seasonal forest (Oliveira-Filho *et al.* 2005). The stream has clear and fast-flowing water, is bordered by native riparian vegetation and the stream bed includes boulders, stones and gravel. The stream water is well oxygenated (dissolved oxygen > 9 mg L^{-1}), slightly acidic (pH = 6.4), and with low conductivity (< 30 μS cm^{-1}).

Field experiment

To evaluate the effect of substrate heterogeneity on stream insect traits, we designed 5 kg-weight artificial samplers using stone slate blocks. Each homogeneous substrate sampler consisted of an unmodified 50 x 29 x 2 cm stone slate block that presented a smooth surface (Fig. 1A). Each heterogeneous substrate sampler was obtained by adding pieces of different sizes of slate stones (two pieces of 5 x 2 x 5 cm, three pieces of 5 x 2 x 10 cm and two pieces of 10 x 2 x 10 cm) to a stone slate block. The pieces were attached to the top and bottom surfaces (Fig. 1B). To standardize the sampled area, the heterogeneous substrate samplers were smaller (50 x 21 x 2 cm). Thereby, the colonization surface of both substrates were equal.

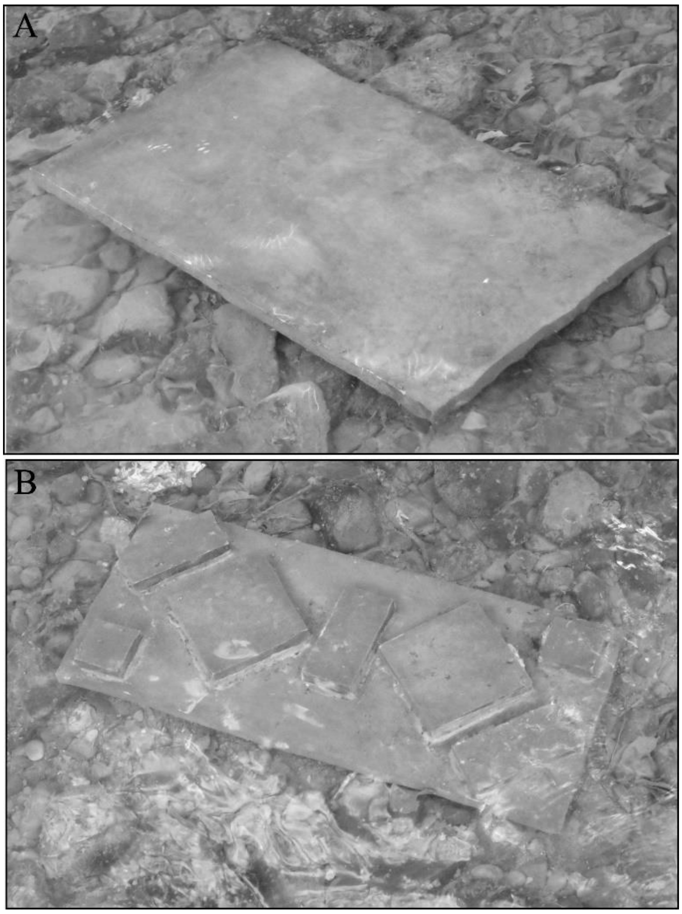

Figure 1. Artificial substrates used in the experiment. (A) Homogeneous substrate with smooth surface. (B) Heterogeneous substrate with complementary pieces glued to increase the heterogeneity of the stone.

One pair of artificial samplers (one heterogeneous and one homogeneous) was installed at least 2 m apart from the others pairs and 1 m between each other, totaling 20 pairs through a 2 km-long stream reach. Samplers were installed in riffles with similar flow velocity and bottom substrate in May 2013 and removed after 60 days. We used an adapted (0.5 x 0.5 m) Surber sampler (mesh size 250 µm) to retain organisms while collecting the substrates. The substrates were washed and carefully examined for attached organisms on both sides. Samples were fixed with 70% ethanol. In the laboratory, Coleoptera, Ephemeroptera, Plecoptera, Trichoptera, and Odonata were sorted at the genus level using the identification keys of Merritt and Cummins (1996), Fernández and Domínguez (2001), Salles *et al.* (2004), Pes *et al.* (2005), and Costa *et al.* (2006).

Flow velocity was measured before substrate removal, positioning a flow meter at the top of each substrate. We intended to assess the effect of substrate heterogeneity on invertebrate functional structure and, accordingly, we avoided other factors that could have affected the distribution of benthic invertebrates. In fact, current velocity was similar between homogeneous (0.71 ± 0.11) and heterogeneous (0.75 ± 0.13) substrates ($F_{1,19} = 0.43$, $p = 0.518$). Finally, we weighed the organic material accumulated upon the substrate and collected in the Surber sampler, after drying at 60 °C for 72 h (dry mass).

Stream insect traits

We considered four stream insect traits described by 16 states that could potentially be affected by substrate heterogeneity: feeding habits, body size, body shape and locomotion/substrate relation (adapted from Tomanova and Usseglio-Polatera 2007, Schmera *et al.* 2015) (Table 2). We gathered information from available literature on Neotropical streams (Bello and Cabrera 2001, Baptista *et al.* 2006, Tomanova *et al.* 2006, Tomanova and Usseglio-Polatera 2007, Gil *et al.* 2008, Reynaga and Santos 2012) and, when necessary, from the North American and European stream insect databases (Tachet *et al.* 2002, Poff *et al.* 2006, Vieira *et al.* 2006). Body size was obtained from direct measurements of all specimens (measured from head to abdomen end) and values were further classified into four states.

The affinity of each taxon for traits was described by fuzzy coding (Chevenet *et al.* 1994). The score for each taxon belonging to each trait category ranged from 0 to 3, with 0 indicating no link with the category, 1 indicating a weak link, 2 a moderately strong link and 3 a strong link (Table S1). This technique helps to compensate for different types and levels of information available for different taxa and for the inherent within-genus trait variability (Chevenet *et al.* 1994). In addition, it avoids the obligate assignment of a taxon to a single trait, which can lead to inaccurate characterization of the taxa because some traits (e.g. feeding habits) may change during the life cycle of insects. We multiplied the frequency of each trait category by the relative log-transformed abundances of taxa in a given sample (i.e. community weighted mean). The resulting trait-by-sample array contained the relative abundance of each trait category in each sample and was further analysed.

Statistical analysis

Stream insect fauna. In our experiment, we used paired design with 20 pairs of substrates, representing 20 replications for each one. We determined the influence of substrate heterogeneity on rarefied richness (Gotelli and Colwell 2001), abundance (log-transformed) of stream invertebrates, and accumulated organic matter (dry mass) with analyses of variance (ANOVA). The model was: y ~ F +block, where y is response variable, F is a variable of interest (substrate heterogeneity with two levels or organic matter) and block is a factor indicating each pair of substrates. Rarefied richness was obtained within pairs by estimating species richness in the substrate with more individuals for a subsample containing the same number of individuals in the substrate with fewer individuals (Gotelli and Colwell 2001).

Stream insect traits. To obtain multivariate sample scores based on overall trait composition we performed correspondence analysis on the table that contained trait-category relative abundance in each sample (also known as Fuzzy Correspondence

Analysis, Chevenet *et al.* 1994). This analysis allows the joint ordination of trait categories and samples. It also provides for each trait and each ordination axis a correlation ratio, which represents the percentage of variance accounted for among trait categories. In addition, to quantify the amount of variance in overall trait composition explained by substrate heterogeneity, we used between-class correspondence analysis (see e.g. Dolédec and Chessel 1989). The observed value of explained variance (between-substrate variance), characterizing the separation between homogeneous and heterogeneous substrates, was tested against simulated values obtained after 999 permutations of the rows of the trait composition table (Monte Carlo test). To account and test whether some traits were more influenced than others by substrate heterogeneity, we applied the same approach to the four traits separately.

We also used traits to calculate functional diversity and richness, by means of the “dbFD” function from FD package (Laliberté *et al.* 2014). Functional diversity metric use a distance-based framework that allows any type of traits and considers organisms relative abundances (Villéger *et al.* 2008). Functional diversity was calculated using Rao’s quadratic entropy, described as the sum of the dissimilarities among all possible pairs of species in the trait space weighted by the product of species relative abundances (Rao 1982). Functional richness (FRic) was computed as the amount of trait space filled by a community (Villéger *et al.* 2008). It is represented by the smallest convex set enclosing the volume of the n-dimensional trait space occupied by the organisms in a community (Cornwell *et al.* 2006). To evaluate whether substrate heterogeneity affected functional diversity and richness, we performed ANOVA as described in the previous subsection.

All analyses were carried out with the R environment (The R Development Core Team 2013), using vegan (Oksanen *et al*. 2010), FD (Laliberté *et al*. 2014) and ade4 (Thioulouse *et al*. 1997, Chessel *et al.* 2004) packages.

RESULTS

Stream insect fauna

Organic matter accumulated significantly more on heterogeneous than on homogeneous substrates ($F_{1,19}$ = 6.64, p= 0.018). The abundance of stream invertebrates followed the same pattern ($F_{1,19}$ = 38.85, p <0.001, Fig. 2, Table S2), being in addition significantly correlated to organic matter accumulation ($F_{1,19}$ = 5.37, p= 0.032). Contrastingly, richness (rarefied) was neither influenced by substrate heterogeneity ($F_{1,19}$ = 0.03, p = 0.855, Fig. 2) nor related to organic matter accumulation ($F_{1,19}$ = 0.67, p= 0.423).

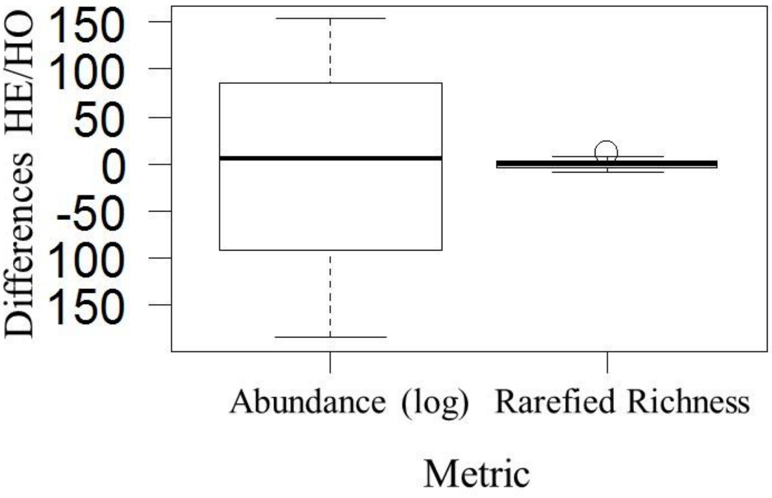

Figure 2. Differences of abundance (log-transformed) and rarefied richness of stream insect communities between heterogeneous (HE) and homogeneous substrates (HO).

Table 2. The 16 trait states and their code for 54 Neotropical stream insects

Trait	**Code**	**State**
Body size	Less_0.25	<0.25 mm
	0.25_0.50	>0.25-0.5 mm
	0.50_1.00	>0.50-1 mm
	More_1.00	>1 mm
Locomotion and substrate relation	Silt	Silt gland
	Swim	Swimmer
	Crawler	Crawler
	TempAtta	Temporary attached
	mat_case	Case building
Feeding habits	Coll_Gath	Collector-gatherer
	Shred	Shredder
	Scrap	Scraper
	Coll_Filt	Collector-filterer
	Pred	Predator
Body Shape	Flatt	Flattened/Streamlined
	Cylin	Cylindrical

Stream insect traits

The first two axes of a correspondence analysis (CA) performed on the stream insect trait composition took into account 72.7% of the overall variability (Table 3A). The first axis (48.8%, Table 3A) rather clearly separated heterogeneous and homogeneous substrates (Fig. 3) whereas the second axis (23.9%, Table 3A) separated samples within substrate types. Trait categories that showed the highest separation along axis 1 (i.e. highest percentage of variance accounted for among trait categories; see Table 3A) were body size and feeding habits (Fig. 3) whereas body shape and locomotion and relation to substrate better separated along axis 2 (Table 3A). The permutation test performed on the overall trait composition demonstrated a statistically significant difference between homogeneous and heterogeneous substrates (overall between-substrate variability = 27.1%, p = 0.001). Large body size and predators, shredders and collector-filterers were significantly more frequent on heterogeneous than on homogeneous substrate (Fig. 3, Table 3B, p = 0.001 for body size and feeding habits). In contrast, scrapers and case-building organisms were prominent on homogeneous substrates (Fig. 3, Table 3B, p = 0.001 for feeding habits p = 0.019 for locomotion and relation to substrate). The effect of substrate heterogeneity was not statistically significant for body shape (Table 3B, p = 0.055).

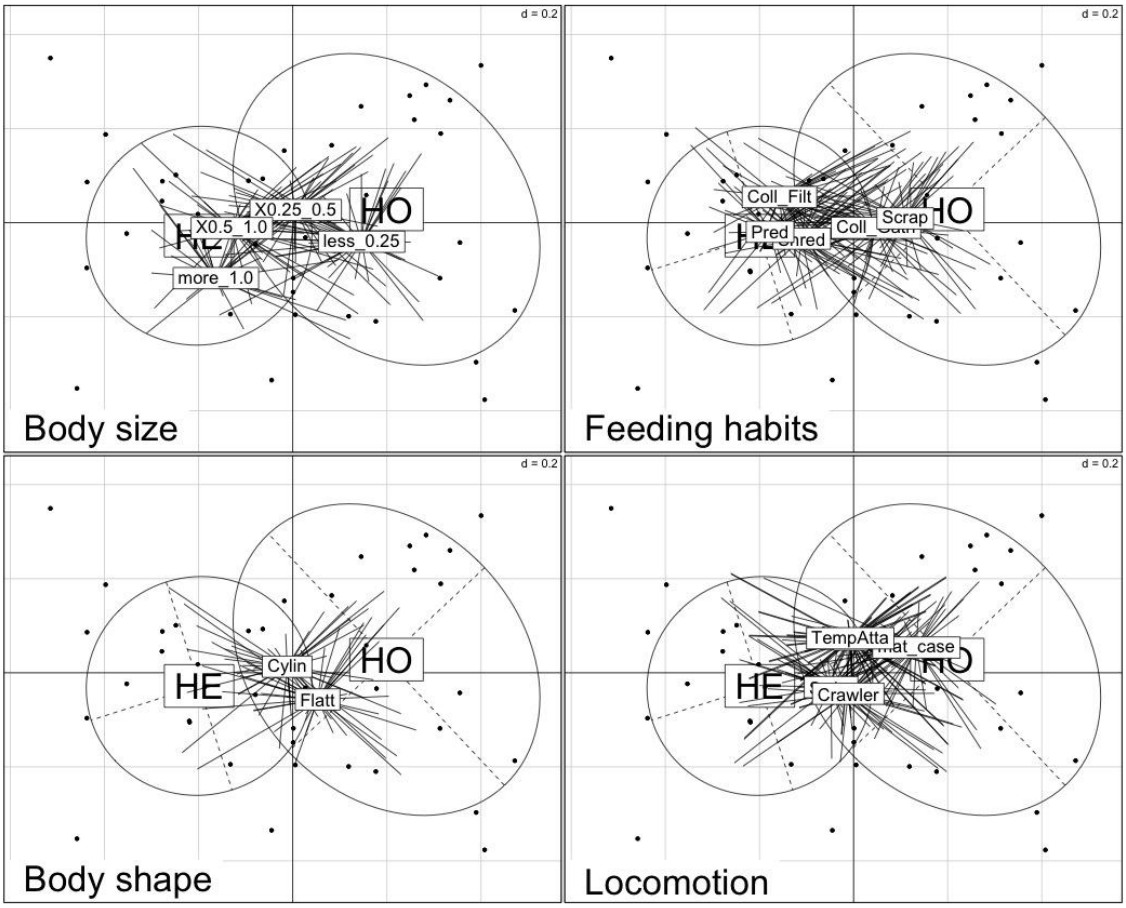

Figure 3. First two axes of correspondence analysis performed on the overall trait composition. For each trait, samples (dots) are grouped by substrate type (ellipses). Substrate labels (HE= heterogeneous, HO=homogeneous) are at the average of sample scores. Trait state labels (see code in Table 2) are at the weighted average of samples that have the state (note that for locomotion and relation to substrate temporary attachment and silt gland categories have fairly the same position). Lines link samples (dots) to their trait states but are only 50% of their total length for readability.

Table 3. Statistics of correspondence analysis and between-substrate correspondence analysis performed on the trait composition of samples A. Correlation ratios (i.e. the percentage of variance accounted for among categories) for four traits, eigenvalues and the percentage of variance along the first two axes of a correspondence analysis performed on all traits together. B. Between-substrate variability (i.e. Percentage of variance explained by substrate heterogeneity) and associated simulated probabilities (*p** based on 999 replicates) performed on each trait separately.

	A		B	
Trait	Axis 1	Axis 2	R^2	p^*
Body size	0.113	0.036	0.285	0.001
Feeding habits	0.154	0.017	0.333	0.001
Body shape	0.008	0.020	0.093	0.055
Locomotion/relation to substrate	0.029	0.076	0.112	0.019
Eigenvalue	0.076	0.037		
Percentage of variance	48.8	23.9		

Accordingly, heterogeneous substrate supported communities with a higher functional diversity and richness than homogeneous substrate ($F_{1,19} = 5.65$, $p = 0.02$ and $F_{1,19} = 41.20$, $p < 0.001$, respectively, Fig. 4).

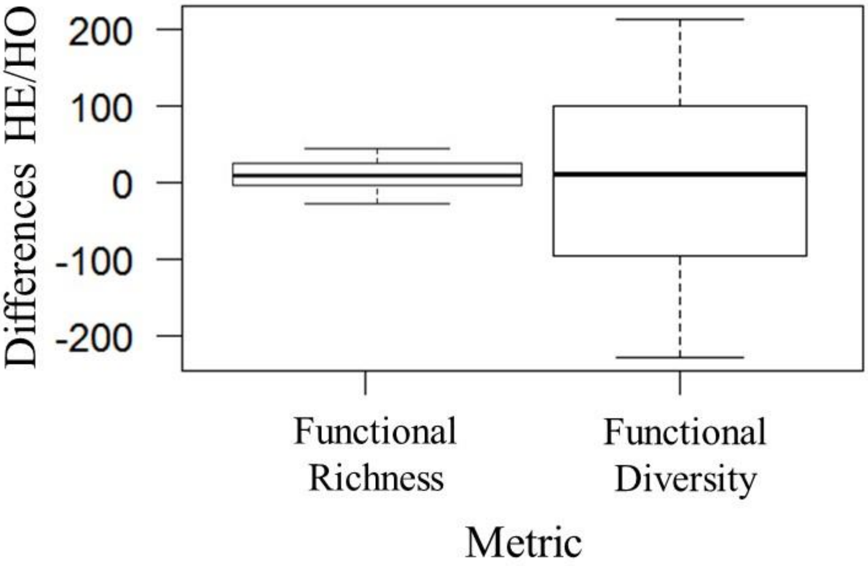

Figure 4. Differences of functional richness and functional diversity of stream insect communities between heterogeneous (HE) and homogeneous substrates (HO).

DISCUSSION

Stream insect fauna

Our observed pattern of stream insect abundance showing a higher value on heterogeneous than homogeneous substrates follows expectation (Poff *et al.* 2006, Downes *et al.* 2000). Heterogeneous habitats support higher species abundance because they allow more protection from predation, with distinct microhabitats and/or refuges, and greater amounts of food compared to homogeneous ones (Erman and Erman 1984, Townsend and Hildrew 1994, Gjerløv *et al.* 2003). In addition, previous studies have shown that the abundance of aquatic insects is influenced by food availability (Hildrew *et al.* 1980), and habitat structural complexity (Poff *et al.* 2006). In contrast and contrary to our expectation, taxonomic richness (rarefied) was not influenced by substrate heterogeneity. Accordingly, using artificial heterogeneous substrates, Hepp *et* *al.* (2012) showed that the substrate heterogeneity did not influence (rarefied) richness. This result differs from the findings of several previous studies, in which the relationship between richness and substrate heterogeneity was positive (Williams 1980, Downes *et al*., 2000, Poff *et al.* 2006). However, here we used rarefaction to calculate richness that was not applied in other studies, which may bias the comparison.

As anticipated we found a significantly higher amount of organic matter on heterogeneous than on homogeneous substrates. Heterogeneous substrate tends to accumulate leaves, wood and vegetal on the retentive structures arising from the substrate (Graça *et al.* 2004), which enhance the accumulation process. Previous studies showed that large amounts of organic matter on heterogeneous substrates yielded a high species richness of aquatic insects (Graça *et al.* 2004, Heino *et al.* 2004, González and Graça 2005). However, we must emphasize that these results may be related to the use of observed richness, which does not take into account organisms abundance and the different probability of collecting individual species whether it is or not abundant. In our study, stream insect abundance was indeed higher on substrate with greater amount of organic matter whereas we did not see any pattern for rarefied taxonomic richness.

Stream insect traits

Traits associated to high resilience and resistance, like small size or case-building behaviour were more common on homogeneous than heterogeneous substrates. This result is consistent with the ‘Habitat Templet’ concept (Townsend and Hildrew 1994) in which biological characteristics are shaped by local factors such as spatial heterogeneity, which lessen the effect of natural disturbance (Tomanova *et al.* 2008, Colzani *et al.* 2013). In contrast, the heterogeneous microhabitat creates conditions that can contribute to a variety of micro-habitats facilitating invertebrate colonization (Allan and Castillo 2007). Substrate heterogeneity may thus strongly selects the stream insect trait combinations. For example, the predicted small size (less than 0.25 cm) of organisms on homogenous substrate is a way for them to reduce dislodgment (Statzner 1988) and resist drag forces (Clifford et al. 1989, Lamouroux et al. 2004). Our predictions about the occurrence of feeding habits in homogeneous substrate were also confirmed. For example, stream insects that scrap on periphyton were more common on homogeneous substrate. Scrapers feed mainly on benthic biofilm (Campbell *et al.* 2012) and are generally associated with high periphyton biomass that develop well on homogeneous substrate (Biggs and Hickey 1994). In addition, homogeneous substrate favors the presence of organisms that construct cases/shelter that are generally less sensitive to drag forces due to case rigidity (Lamouroux *et al.* 2004). In addition, case-building is thought to be an antipredator adaptation for caddisflies (Wiggins and Mackay, 1977). Contrary to our expectations, organisms producing silt with a gland were not selected on homogeneous substrate. We expected that silt gland adaptation would be more common on homogeneous, because benthic invertebrates living in stressful conditions eventually would attach to the substrate (Lamouroux *et al*. 2004).

Our predictions about the trait responses expected in heterogeneous substrate were corroborated (Table 1). For example, in our study, large and intermediate body sizes of stream insects were more abundant on heterogeneous substrate. Moreover, associated to energy transfer, carnivorous are often the largest organisms in the community. Consistently, predators were more common on heterogeneous substrate. Corroborating our findings, Bispo *et al.* (2006) found that the abundance of predators was associated with organic matter accumulation. Shredders that feed mainly on organic detritus (Bispo *et al.* 2006, Allan and Castillo 2007), followed a similar prominence on heterogeneous substrate. In contrast, we found an unexpected higher proportion of filtering-collectors on heterogeneous substrate. Some filtering-collectors use organic material to construct their nets (*Smicridea* sp.) and there is some evidence that their larvae may use leaf/wood pieces as habitat rather than as a direct food source (Richardson 1992). Moreover, shredders are responsible for 30% conversion of the CPOM into FPOM (Petersen and Cummins 1974). Since filtering-collectors feed in majority on FPOM (Allan and Castillo 2007) the higher abundance of shredders may indirectly affect the abundance of filtering-collectors.

Finally, as predicted, functional richness and diversity were higher on heterogeneous than homogeneous substrates despite similar taxonomic richness suggesting that substrate heterogeneity allows the expression of a greater panel of trait states. Similarly, Gallardo *et al*. (2009) found a high functional richness in areas with a high spatial heterogeneity and a great variety of food resources.

In conclusion, substrate heterogeneity indeed influences stream invertebrate functional structure of communities allowing trait combination as predicted by the “Habitat Templet” concept (Townsend and Hildrew 1994). In addition, substrate heterogeneity affects positively the functional diversity and richness of communities, and selects a set of functionally different organisms. Finally, our results have implication for applied issues since we can anticipate that the homogenization of natural stream substrate will imply the reduction or the loss of important community functions in stream ecosystems.

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Supporting information

Table S1. Stream insect traits affinity described by fuzzy coding. The score for each taxon belonging to each trait category ranged from 0 to 3.

Trait	Body size				Feeding style					Locomotion and substrate relation					Shape	
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector Gatherer	Shredder	Scraper	Collector Filterer	Predator	Silt gland	Swimmer	Crawler	Temporary attached	Case building	Flattened Streamlined	Cylindrical
Coleoptera																
Heterelmis Adult	0	3	0	0	3	3	1	0	0	0	0	3	0	0	0	3
Heterelmis	0	2	1	0	2	3	0	0	0	0	0	3	0	0	0	3
Hexacylloepus Adult	0	3	0	0	3	1	1	0	0	0	0	3	0	0	0	3
Hexacylloepus	0	3	0	0	2	1	1	0	0	0	0	3	0	0	0	3
Neoelmis Adult	0	3	0	0	3	0	1	0	0	0	0	3	0	0	0	2
Neoelmis	0	2	1	0	2	1	2	0	0	0	0	3	0	0	0	3
Macrelmi Adult	0	1	1	1	2	1	2	0	0	0	0	3	0	0	0	3
Macrelmis	0	2	0	0	3	1	1	0	0	0	0	3	0	0	0	3
Microcyllopepus	0	3	0	0	3	0	1	0	0	0	0	3	0	0	0	3
Psephenus	0	0	3	0	2	0	3	0	0	0	0	3	0	0	3	0
Ephemeroptera																
Baetodes	2	1	0	0	3	0	3	0	0	0	3	2	0	0	0	3
Camelobaetidius	3	0	0	0	3	0	3	0	0	0	2	3	0	0	0	3
Americabaetis	1	1	0	0	3	1	2	0	0	0	3	2	0	0	0	3
Cloeodes	3	0	0	0	2	0	1	0	0	0	2	3	0	0	0	3
Caenis	0	2	1	0	3	1	1	0	0	0	1	3	0	0	1	2
Farrodes	3	0	0	0	2	1	1	1	0	0	1	2	0	0	2	0
Hagenulopsis	0	3	0	0	2	1	1	1	0	0	1	3	0	0	2	2
Simothraulopsis	0	1	0	0	2	1	2	1	0	0	1	3	0	0	2	2
Thraulodes	0	1	0	0	3	0	2	1	0	0	1	3	0	0	3	0
Leptohyphes	3	0	0	0	3	1	3	0	0	0	0	3	0	0	1	2

Table S1- Continuation

Trait	Body size				Feeding style					Locomotion and substrate relation					Shape	
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector Gatherer	Shredder	Scraper	Collector Filterer	Predator	Silt gland	Swimmer	Crawler	Temporary attached	Case building	Flattened Streamlined	Cylindrical
Trichoptera																
Traverhyphes	3	0	0	0	3	1	2	0	0	0	0	3	0	0	1	2
Tricorythopsis	3	0	0	0	3	1	3	0	0	0	0	3	0	0	1	2
Tricorythodes	3	0	0	0	3	0	1	0	0	0	0	3	0	0	1	2
Odonata																
Mnesarete	0	0	0	1	0	0	0	0	3	0	1	3	0	0	0	3
Hetaerina	0	0	0	2	0	0	0	0	3	0	1	3	0	0	0	3
Argia	0	0	2	1	0	0	0	0	3	0	1	3	0	0	1	3
Dythemis	0	0	1	2	0	0	0	0	3	0	0	2	0	0	2	3
Elga	0	0	0	3	0	0	0	0	3	0	0	2	0	0	2	3
Perithemis	0	0	1	2	0	0	0	0	3	0	0	2	0	0	2	3
Planiplax	0	0	3	0	0	0	0	0	3	0	0	2	0	0	2	3
Plecoptera																
Anacroneuria	0	0	2	1	1	1	0	0	3	0	1	3	0	0	2	0
Gripopteryx	0	1	2	0	1	1	0	0	2	0	1	3	0	0	2	0
Paragripoteryx	0	1	1	0	1	1	0	0	2	0	1	3	0	0	2	0
Tupiperla	0	1	2	0	1	1	0	0	2	0	1	3	0	0	2	0
Trichoptera																
Phylloicus	0	0	0	1	1	3	0	0	0	0	0	3	0	2	0	3
Itauara	1	2	0	0	3	0	3	0	0	2	0	3	3	3	0	3
Protoptila	1	2	0	0	3	0	3	0	0	2	0	3	3	3	0	3
Mortoniella	0	3	0	0	3	0	3	0	0	2	0	3	3	3	1	3
Helicopsyche	3	0	0	0	0	0	2	3	0	2	0	3	2	3	0	3

Table S1- Continuation

Trait	Body size				Feeding style					Locomotion and substrate relation					Shape	
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector Gatherer	Shredder	Scraper	Collector Filterer	Predator	Silt gland	Swimmer	Crawler	Temporary attached	Case building	Flattened Streamlined	Cylindrical
Trichoptera																
Leptonema	0	0	0	3	0	1	0	3	1	2	1	2	3	0	0	3
Smicridea R.	0	1	1	0	0	1	0	3	1	2	1	2	3	0	0	3
Smicridea S.	0	2	1	0	0	1	0	3	1	2	1	2	3	0	0	3
Alisotrichia	3	0	0	0	0	2	3	0	0	0	0	3	0	1	2	3
Metrichia	3	0	0	0	3	0	2	0	0	2	0	3	1	2	2	3
Neotrichia	3	0	0	0	1	1	3	0	0	1	0	3	1	3	0	2
Oxyethira	3	0	0	0	3	1	1	2	0	2	1	2	3	2	0	1
Nectopsyche	1	2	1	0	2	3	1	0	0	0	0	3	0	1	0	3
Triplectides	0	0	2	2	2	3	0	0	0	0	0	3	0	1	0	3
Marilia	0	1	0	3	3	1	3	0	1	0	0	3	0	3	0	3
Chimarra	0	0	2	0	0	1	0	3	0	2	0	3	3	0	0	3
Wormaldia	2	2	1	0	0	1	1	3	0	2	1	3	3	2	0	3
Cernotina	3	0	0	0	0	1	0	0	3	2	0	3	3	0	0	3

Table S2. Stream insect abundance (log) (Mean ± SD) on heterogeneous and homogeneous substrate.

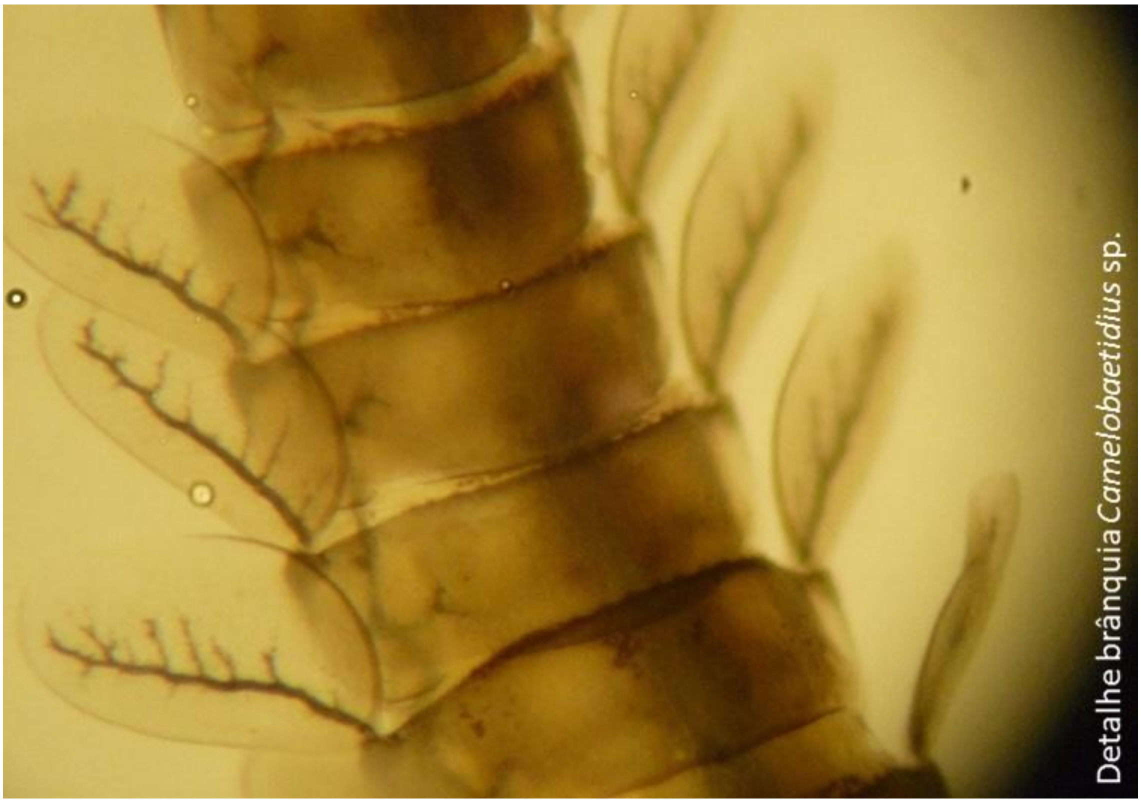
	Heterogeneous	Homogeneous
Coleoptera		
Heterelmis Adult	0.65 ± 1.83	-
Heterelmis	0.45 ± 0.90	-
Hexacylloepus Adult	0.50 ± 0.84	-
Hexacylloepus	0.75 ± 1.03	0.05 ± 0.23
Neoelmis Adult	1.45 ± 1.18	0.20 ± 0.54
Neoelmis	0.75 ± 0.79	0.10 ± 0.32
Macrelmis Adult	0.55 ± 0.90	0.05 ± 0.23
Macrelmis	0.50 ± 1.26	0.05 ± 0.23
Microcyllopepus	0.10 ± 0.32	-
Psephenus	-	1.00 ± 0.97
Ephemeroptera		
Baetodes	5.80 ± 6.84	2.85 ± 3.01
Camelobaetidius	-	0.65 ± 1.25
Americabaetis	1.40 ± 1.63	0.55 ± 0.77
Cloeodes	0.25 ± 0.92	-
Caenis	35.95 ± 22.13	4.05 ± 6.29
Farrodes	0.30 ± 1.38	0.90 ± 1.47
Hagenulopsis	0.15 ± 0.69	-
Simothraulopsis	0.35 ± 1.00	0.05 ± 0.23
Thraulodes	0.30 ± 1.00	1.60 ± 0.96
Leptohyphes	0.05 ± 0.23	0.75 ± 1.08
Traverhyphes	0.50 ± 1.17	-
Tricorythopsis	-	1.35 ± 1.40
Tricorythodes	0.65 ± 1.11	0.20 ± 0.71
Odonata		
Mnesarete	0.95 ± 2.29	0.05 ± 0.23
Hetaerina	0.60 ± 0.68	-
Argia	1.25 ± 1.24	0.10 ± 0.32
Dythemis	0.40 ± 0.61	-
Elga	0.35 ± 0.68	0.05 ± 0.23
Perithemis	0.45 ± 0.84	0.15 ± 0.23
Planiplax	0.85 ± 1.30	0.20 ± 0.32
Plecoptera		
Anacroneuria	0.60 ± 0.90	-
Gripopteryx	0.15 ± 0.23	0.15 ± 0.50
Paragripoteryx	0.55 ± 0.68	0.05 ± 0.23
Tupiperla	0.65 ± 1.06	0.10 ± 0.32
Trichoptera		
Phylloicus	2.45 ± 1.95	0.15 ± 0.37
Itauara	12.35 ± 14.06	15.30 ± 20.08
Protoptila	0.05 ± 0.23	0.70 ± 0.75
Mortoniella	10.70 ± 19.46	12.35 ± 19.30
Helicopsyche	0.25 ± 1.15	-
Atopsyche	0.10 ± 0.32	-

Table S2- Continuation.

	Heterogeneous	Homogeneous
Trichoptera		
Leptonema	-	0.15 ± 0.69
Smicridea R	22.05 ± 18.95	6.50 ± 14.77
Smicridea S.	15.55 ± 11.50	2.95 ± 3.78
Alisotrichia	-	0.40 ± 0.69
Metrichia	1.20 ± 1.73	0.35 ± 0.83
Neotrichia	0.90 ± 1.31	1.00 ± 1.31
Oxyethira	0.40 ± 1.61	0.15 ± 0.69
Nectopsyche	0.20 ± 0.54	-
Triplectides	0.55 ± 1.12	-
Marilia	-	0.20 ± 0.42
Chimarra	1.15 ± 1.74	0.35 ± 0.96
Wormaldia	-	0.45 ± 0.90
Cernotina	0.40 ± 1.17	-

Capítulo 2

Assessing community specialization during substrate colonization: an experiment using stream insects traits



Este manuscrito será submetido na revista Freshwater Biology com co-autoria de Sylvain Dolédec¹ e Adriano Sanches Melo². O manuscrito está formatado de acordo com as normas da referida revista.

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Community specialization substrate colonization

Assessing community specialization during substrate colonization: an experiment using stream insects traits

SUMMARY

1. Species may have a restricted ecological niche space driven by specific traits. Specialist species present a high fitness in few particular habitats and resources whereas generalist species can take advantages from different habitat types. We evaluated such dynamics of community specialization during a colonization experiment under natural conditions.
2. We tested the hypotheses that later stages of colonization should show higher community specialization (H1) and higher trait richness and diversity (H2) than early successional stages. As a result, during the colonisation of a new habitat, we expected an increase in the complementarity of species niche occupation, i.e. more specialists having different species traits, which should yield communities with a high trait richness and diversity. The reverse should occur in the initial stages where more generalists should have more redundant traits, which should yield communities with a lower trait richness and diversity.
3. Sixty samplers were installed in riffles, ten samplers substrates were removed after 1, 3, 5, 10, 15 and 30 days of colonization. We used the trait category profile of each taxon to calculate a taxon specialization index (TSI).Community specialization index was calculated for each trait by averaging the individual scaled TSIs (weighted abundances-log).
4. In early colonization stages, organisms can coexist regardless their traits, i.e., communities showed similar traits (low trait diversity and trait richness). In contrast, in late colonization stages, specialist taxon showed a greater variety of traits. The increase in specialist allows species coexistence and potentially the increase in functional complementarity, because each species has developed a specific function in the ecosystem. Finally, we highlighted that the trait selection is an important factor for better understanding the temporal functional dynamic of stream insect community.

Key words: specialists, generalists, functional composition, community specialization

Introduction

The use of multiple trait-based approaches in freshwater ecology has provided important insights into the assessment of the functional structure of communities and renewed community ecology (Townsend & Hildrew, 1994; McGill *et al.,* 2006; Verberk *et al.,* 2013). Loss of species with exclusive traits can cause the decline of ecological functions, which in turn may cascade to other interacting species (Poff *et al,* 2006; Clavel *et al.,* 2010). Species traits mediate the fitness of individuals to their environment, whereas each species is adapted to a particular set of abiotic conditions and biotic interactions (niche), which scale up to determine the structure of communities and ecosystem functioning (Violle, 2007). Accordingly, species traits can facilitate coexistence and allow organisms to cope with environmental variability (Hutchinson, 1957; Townsend & Hildrew, 1994).

Species may have a restricted ecological niche space driven by specific traits (ecological specialisation, Futuyma & Moreno, 1988). Specialist species present a high fitness in few particular habitats and resources whereas generalist species can take advantages from different habitat types and, in some cases, disturbed areas (Clavel *et al.,* 2010; Devictor *et al.,* 2010). The replacement of habitat specialists by more generalist species has been suggested as a response of communities to habitat degradation (Clavel *et al.*, 2010). For example, specialists have been demonstrated to present a higher abundance in less fragmented environments whereas generalists dominate fragmented ones (Devictor *et al.,* 2007). In stream invertebrate communities, Mondy & Usseglio-Polatera (2014) found that anthropogenic factors led to taxonomic and functional losses through the reduction of the number of specialised species. As a result, understanding the temporal distribution of habitat specialists and generalists in natural systems is critical both for basic science and conservation. The proportion of specialists and generalists in communities is sought to change in time according to fluctuations of food resources and natural or anthropogenic disturbances (Fisher & Ownes, 2004; Bellisario *et al.* 2013). However, the way in which community specialization changes in time in natural streams requires further colonization experiments.

Community response to substrate colonization is also linked to species traits of aquatic organisms. For instance, highly mobile aquatic insects will colonize substrate faster than less mobile ones (Myiake *et al.*, 2003). Moreover, during habitat colonization, the accumulation of organic matter and biofilm increase habitat complexity, which in turn should increase the abundance and richness of aquatic communities (Mackay, 1992; Graça *et al.*, 2004). In addition, the periphyton establishment can also promote an increase of physical habitat diversity through different algal structures (mucilaginous sheaths and stems for fixing to the substrate), which facilitates benthic invertebrate adhesion to the substrate (Miyake *et al.*, 2003; Barsanti & Gualtieri, 2006). As a result, the increase in food availability and diversity over the period of colonization likely affects specific attributes of individual aquatic insects, which in turn will determine the community succession.

Theory suggests that less diverse/complex communities of early successional stages should be dominated by generalists that utilize a broad range of habitat types (Munday *et al.*, 1997). Other concept was the trade-off competition–colonization were strong competitors are weak colonizers (Levins and Culver 1971). Species can coexist due to a trade-off, strong colonizers move faster, while strong competitors move intermittently, so dispersal abilities can influence directly on the colonization process (Cadotte *et al*., 2006).

As species accumulate over time, the proportion of specialists should increase to allow species coexistence on the substrate. Mapping the dynamics of ecological specialists and generalists in stream insect communities may thus provide a picture of the dynamics of community specialization.

We evaluated such dynamics of community specialization during a colonization experiment under natural conditions. Specifically, we tested the hypotheses that later stages of colonization should show higher community specialization (H1, Fig. 1) and higher trait richness and diversity (H2) than early successional stages. As a result, during the colonisation of a new habitat, we expected an increase in the complementarity of species niche occupation, i.e. more specialists having different species traits, which should yield communities with a high trait richness and diversity. The reverse should occur in the initial stages where more generalists should have more redundant traits, which should yield communities with a lower trait richness and diversity.

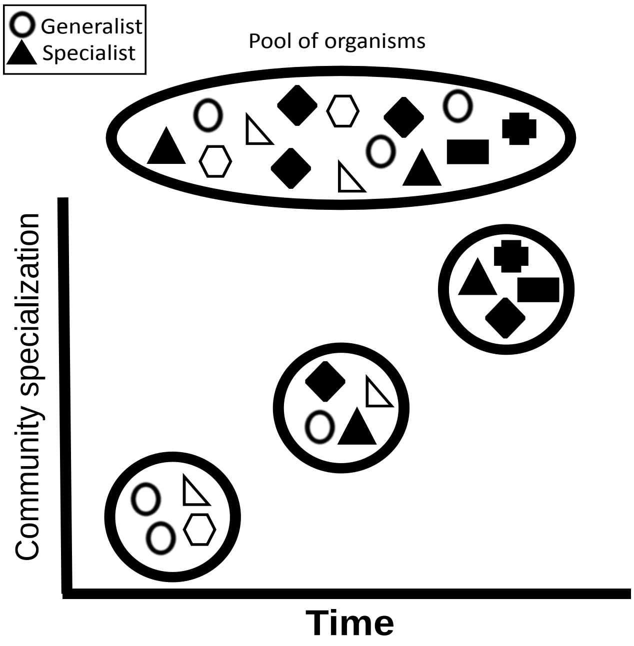

Figure 1. Conceptual presentation of community specialization versus time showing our prediction i.e. communities will show more generalists in the beginning and more specialists in the end of colonization.

Methods

Field experiment

Our experiment was set up in north of Rio Grande do Sul, Brazil (29° 31’ 57” S, 50° 14’ 55” W), in a fast-flowing third order stream with well oxygenated (dissolved oxygen > 9 mg L^{-1}), slightly acidic (pH = 6.4) and low conductivity (< 30 µS cm^{-1}) waters. We designed artificial samplers using stone slate blocks. Stone substrates were 50 x 25 x 2 cm and were left in the stream for 30 days to allow periphyton colonization. After this period, stream insects were removed and the substrates returned to the stream to start the colonization experiment, for another 30 days.

Sixty samplers were installed in riffles with similar flow velocity and bottom substrate, within a 2-km long stream reach. Ten substrates were removed after 1, 3, 5, 10, 15 and 30 days of colonization. We used a Surber (mesh size 250 µm) to collect the organisms and fixed them *in situ* with 70% ethanol. In the laboratory, Coleoptera, Ephemeroptera, Plecoptera and Trichoptera were sorted to the genus level using the identification keys of Merritt & Cummins (1996), Fernández & Domínguez (2001), Salles *et al.* (2004) and Pes *et al.* (2005). Only these orders were selected for the study because they are among the most abundant and species-rich groups of benthic macroinvertebrate communities in the Neotropical region (Melo, 2005; Hepp *et al.*, 2010; Milesi *et al.*, 2014).

Environmental variables

Coarse organic matter collected in the Surber sampler was weighted after drying at 60 °C for 72 h to provide AFDW organic matter. To estimate the periphyton growth during colonization, we quantified chlorophyll-a using three sub-sampling units obtained by scraping (4.7 cm² area) the top surface of each sampler. In the laboratory, we used the spectrophotometric ethanol method to calculate the concentration of chlorophyll-a (Biggs and Kilroy, 2000).

Biological traits

We considered four traits for our stream insects: feeding habits, body size, body shape and locomotion, which comprise a total of 15 trait categories (Table 1). Two of them can be considered effect traits i.e. functional traits (feeding habits and size) whereas the three other are rather response traits. The information was gathered from the literature on Neotropical stream insects (Baptista *et al.*, 2006; Tomanova & Usseglio-Polatera, 2007; Reynaga & Santos, 2012), except for body size that was obtained from the direct measurement of all specimens.

Table 1. Stream insect traits and their respective categories from 38 Neotropical stream insects.

Trait	**Category**	**Trait**	**Category**
Maximal body size (mm)	<0.25	Feeding habits	Collector
	>0.25-0.50		Shredder
	>0.50-1		Scraper
	>1		Predator
Body shape	Streamlined	Locomotion and relation to substrate	Flier
	Flattened		Swimmer
	Cylindrical		Crawler
			Temporally attached

Trait information available from literature was quantified by “fuzzy coding” technique (Table S1 - Supporting Information). The affinity of each *taxon* belonging for each trait category ranged from "zero" to "three", with "zero" indicating no affinity, "one" indicating a weak, "two" a moderate and "three" a strong affinity. Fuzzy coding helps to compensate for different types and levels of information available for different taxa and for the inherent within-taxon trait variability (Chevenet *et al.*, 1994). In addition, it avoids the obligate assignment of a taxon to a single trait state, which can lead to inaccurate characterization of taxa. For body size we calculate the fuzzy code following the methodology described in Chevenet *et al.* (1994). Based on a table of organism size, we produced a multidimensional response frequency table, processed by Correspondence Analysis (Fuzzy see Chevenet *et al.*, 1994). We use the positive scores to describe the affinity of species to four different size categories.

Statistical analysis

Stream insect richness and abundance

We used rarefaction to obtain the expected species richness for a standardized sample size taken as the minimum abundance at all the colonization period (13 individuals) (Gotelli & Colwell, 2001). In addition, we used linear models to evaluate whether rarefied richness and abundance (log[x+1]) values significantly increased with time.

Stream insect trait diversity and composition

We calculated trait diversity using the Rao's quadratic entropy (Rao, 1982) computed as:

$$FD = \sum_{i=1}^{s}\sum_{j=1}^{s} d_{ij} p_i p_j$$

where p_i and p_j are the proportions of species i and j, respectively, d_{ij} is the pairwise trait dissimilarity of species i and j, and S is the number of species in the community. Thus, functional diversity (FD) is the sum of the dissimilarities among all possible pairs of species in the trait space weighted by the product of species relative abundances. We also computed trait richness, a complementary component of trait diversity, which represents the amount of trait space filled by a community (Villéger *et al.,* 2008). It is expressed as the smallest convex set (or minimum convex hull) enclosing the volume of the n-dimensional trait space occupied by the species in a community (Cornwell *et al.*, 2006). From that, we evaluated our first hypothesis about the potential higher trait diversity (Rao) and richness (FRic) in late stages of colonization compared to early successional stages. We further examined the effect of time on the trait diversity measures with linear models.

Finally, we summarized the trait structure of aquatic insect communities along the colonization by first computing a traits-by-sites array. The array was obtained by multiplying the trait category profile of each taxon by its relative abundance (log) in each sample and summing the values per sample. We then performed Correspondence Analysis [known as "Fuzzy Correspondence Analysis" (FCA), Chevenet *et al.*, 1994] on the resulting community weighted mean trait array. We further used the first three FCA axes to evaluate the global influence of colonization time on combinations of aquatic insect traits and to select those trait categories that changed most with colonisation. We also tested the effect of colonization time on each trait category with regressions.

Community specialization index (CSI)

For each trait, we used the trait category profile of each taxon to calculate a taxon specialization index (TSI) as proposed by Mondy & Usseglio-Polatera (2014). This index corresponds to the Simpson (complement), of a given trait profile for a given taxon. A high TSI value corresponds to a truly specialist taxon (i.e. using only one trait category) whereas a truly generalist taxon (i.e. evenly using all the trait categories) should present a low TSI value. Community specialization index (CSI) was then calculated for each trait by averaging the individual scaled TSIs (i.e. according to the different number of categories for each trait), weighted by the respective taxa log-transformed abundances. Finally, the global CSI value of a sample was averaged across all traits. Scripts for TSI and CSI indices are available from Mondy & Usseglio-Polatera (2014). We ran simple regression to test our second hypothesis that later stages of colonization should show higher community specialization (CSI). All statistical analyses and graphics were created with the open source software package R (Development Core Team, 2013).

Results

We collected a total of 3809 stream insects distributed within 38 genera during the 30 days of colonization (Table S2 Supporting Information). Trichoptera was the richest order (18 genera), followed by Coleoptera (9). *Caenis* sp. (Ephemeroptera) showed the highest abundance representing 20% of the total, followed by *Itaura* sp. (Trichoptera) and *Baetodes* sp. (Ephemeroptera), both with 18% of the total. We sampled 429 individuals (11%) on the first day of colonization and reached 1145 individuals after 30 days. The availability of food resources on substrates measured by chlorophyll-a concentration and organic matter accumulation significantly increased during colonization ($F_{1,58} = 21.35$, $p < 0.001$ and $F_{1,58} = 8.24$, $p = 0.005$, respectively; Fig. S1 Supporting Information).

The abundance of stream insects increased with time by a factor of 0.036 (Fig. 2A; $F_{1,58} = 30.33$, $p < 0.001$) as well as rarefied richness that increased by a factor of 0.007 (Fig. 2B; $F_{1,58} = 6.34$, $p = 0.014$). Likewise, functional diversity (FD) and trait richness were significantly higher in late colonization (Fig. 2C; $F_{1,58} = 4.68$, $p = 0.029$ and Fig. 2D; $F_{1,58} = 17.86$, $p < 0.001$, respectively).

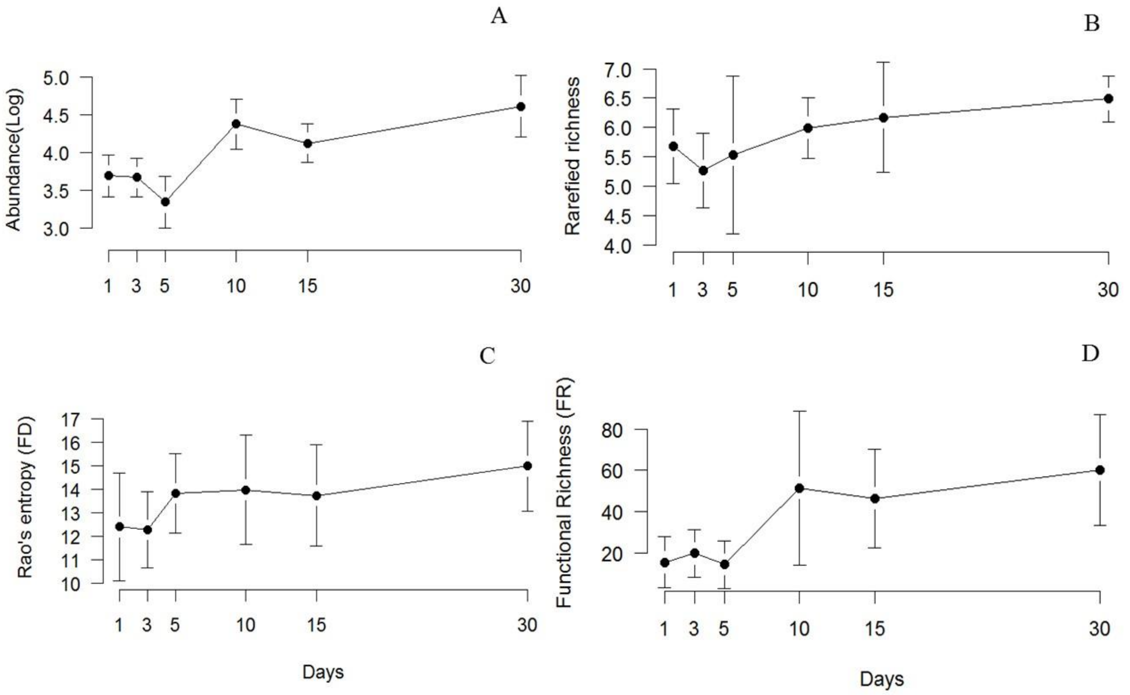

Figure 2. Relationship between mean values (dots ± 1SE) of stream insects community parameters and time of colonisation (Days) with (A) abundance (log), (B) rarefied richness, (C) functional diversity (FD) and (D) functional richness (FR).

The first three axes of a FCA performed on stream insect community traits represented 74.5% of the total variability. Only the third axis (16.5% of the variability) was significantly related with time ($F_{1,58}$ =5.38, $p < 0.001$). Large organisms (> 1cm, Fig. 3A, $F_{1,58}$ =21.84, $p < 0.001$) with cylindrical body shape (Fig. 3C; $F_{1,58}$ = 10.75, p = 0.001) and shredding habit (Fig. 3B; $F_{1,58}$ = 4.48, p = 0.038) were prominent in late colonization. In contrast, early colonists tended to have flattened body (Fig. 3D, $F_{1,58}$ = 13.26, $p < 0.001$). Finally, community was more specialized (CSI) in late colonization (Fig. 4).

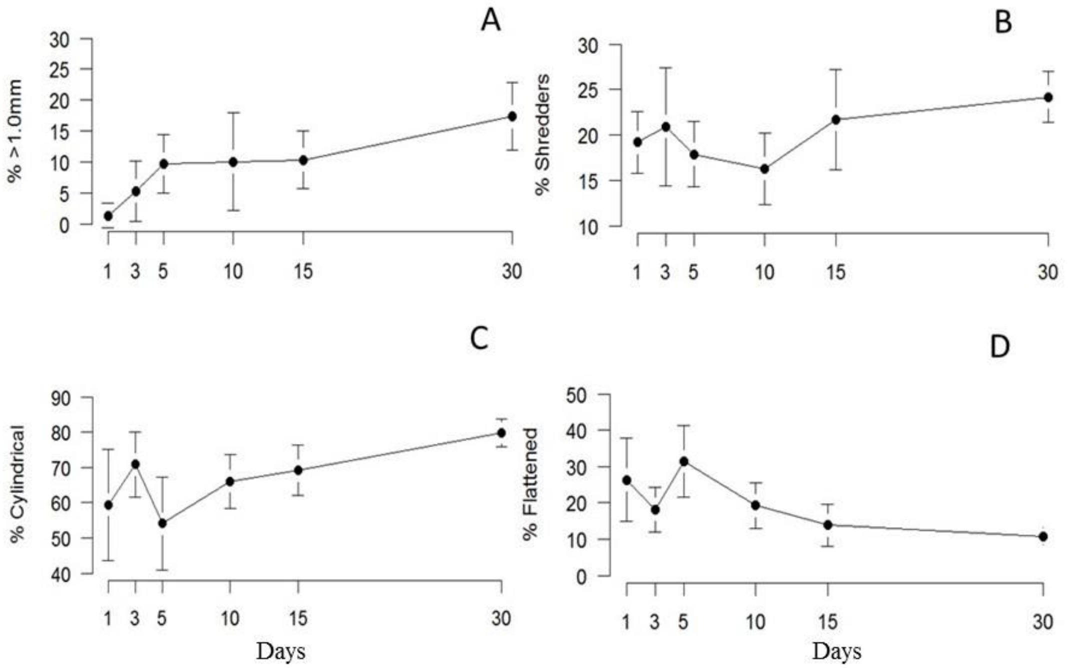

Figure 3. Relationship between mean values (dots ± 1SE) of the proportion of individuals having a given trait category (%) and time of colonisation (Days) for (A) maximal body size >1.0mm, (B) shredder, (C) cylindrical body and (D) flattened body.

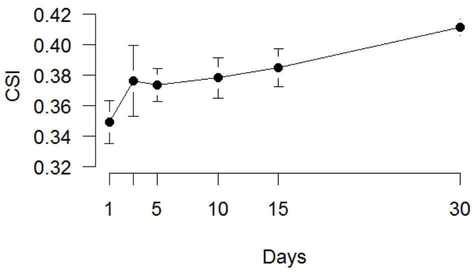

Figure 4. Relationship between mean values (dots ± 1SE) of Community Specialization Index (CSI) and time of colonisation (Days).

Discussion

In this colonization experiment, our aim was to test whether community got more specialized in late colonization than early successional stages and whether this specialization translated into a higher trait richness and diversity.

Abundance and richness showed the expected increase with time (Downes *et al.*, 2000; Cardinale *et al.*, 2001) in the line of the positive influence of available food resources (FPOM/CPOM and periphyton) found in previous experiments (Baer *et al.*, 2001; Miyake *et al.*, 2003; Heino *et al.*, 2004; Hepp *et al.*, 2013).

Temporal changes in trait composition

In our experiment, early colonists had flattened shape and disperse in water. These two traits have been shown to characterize pioneering colonizers (Mackay, 1992; Boyero & Bosch, 2004; Braccia *et al.*, 2014). In particular, at the beginning of colonization, the substrate surface is more susceptible to oscillation of water current velocity because the substrate is “clean”, without organic matter (no refuge). As a result, flattened body reduces the drag and contributes to effectiveness of crawling movements (Rader, 1997).

Besides, large organisms were more common in late colonization. A natural substitution of small to large organism generally occurs during the community succession (Braccia *et al.*, 2010). In addition, large-bodied organisms are generally associated to high spatial heterogeneity (Townsend & Hildrew, 1994). Therefore, we can infer that the accumulation of organic matter and the concomitant increase in substrate heterogeneity may facilitate the colonization of large-size organisms as seen in other studies (Tonin *et al.*, 2014; Boyero *et al.*, 2014). Additionally, food supplies (algae and organic matter) were highest at the end of colonization favouring large organisms.

Our FCA results showed that shredders, which are often large bodied organisms, dominated the end of the colonization. Shredders play an important role in organic matter processing in streams, converting coarse particulate organic matter (CPOM) to fine particulate organic matter (FPOM) (e.g. Wallace *et al.*, 1995) and large bodied shredders have shown great importance in the amount of leaf litter processed in streams

(Lecerf & Richardson, 2010). They were thus expected to be favoured by the accumulation of organic matter on substrates (Hepp *et al.*, 2012;Tonin *et al.*, 2014).

Temporal changes of functional trait richness and diversity

The higher trait richness and diversity occurring in late colonization in comparison to early successional stages followed our expectation. As organisms accumulate with time, the increasing diversity of traits allowed that more stream insects share the same substrate. In addition, the substrate becomes more heterogeneous and complex following algal colonization (Barsanti & Gualtieri, 2006). Consequently, during colonization, niche diversification increases due to more complex habitats thus allowing resource partitioning and coexistence (Schoener,1974). Functional diversity is associated to differences in resource use by organisms. As a result, higher niche complementarity should allow more complete use of the resources available (Tilman *et al.*, 1997; Díaz & Cabido, 2001), as observed in the last days of colonization. We detach that it is necessary to pay attention that some traits were more directly linked to the functioning of the stream than others, like body size and feeding habits traits.

Temporal changes in community specialization

The degree of specialization of stream insect community increased during the colonization period. As predicted, specialists were more common in late colonization (H1, Fig. 1). Specialists allow the division of resources and habitat, providing ecological opportunities that other organisms can exploit (Forister *et al.*, 2012). Specialists are generally favoured by environmental constancy and stable conditions, whereas generalists are more able to develop in less stable and disturbed habitats (Levins, 1968; Futuyma & Moreno, 1988; DeVictor *et al.*, 2008; Le Viol *et al.*, 2012).

In our experience, early colonization was characterized by a lower availability of resources (organic matter and chlorophyll) on substrate. Such conditions can be more favourable to generalists, which are more flexible and able to colonize any type of substrate. Contrastingly, in the last days of colonization, organic matter accumulation and biofilm growth could allow more favourable substrate conditions and subsequent could increase in specialists in the community. However, as the food availability increases during colonization, the space/niche decreases because there are more organisms coexisting in the same place. Therefore, being specialist confers an advantage in late colonization.

We conclude that in early colonization stages, organisms can coexist regardless their traits, i.e., communities showed similar traits (low trait diversity and trait richness). In contrast, in late colonization stages, specialist taxon that can resist to abiotic and biotic filters showed a greater variety of traits. The increase in specialist allows species coexistence and potentially the increase in functional complementarity, because each species has developed a specific function in the ecosystem. In addition, the dominance of specialists over generalists in late colonization can indicate that community of undisturbed areas will naturally show a similar pattern. Moreover, the abundance of specialists is crucial for stream ecosystems and their decline makes communities functionally more similar, which could affect ecosystem functioning, interrupting food webs and destabilizing process like nutrient cycle (large organisms and shredders). Finally, we highlighted that the trait selection is an important factor for better understanding the temporal functional dynamic of stream insect community.

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Supporting Information

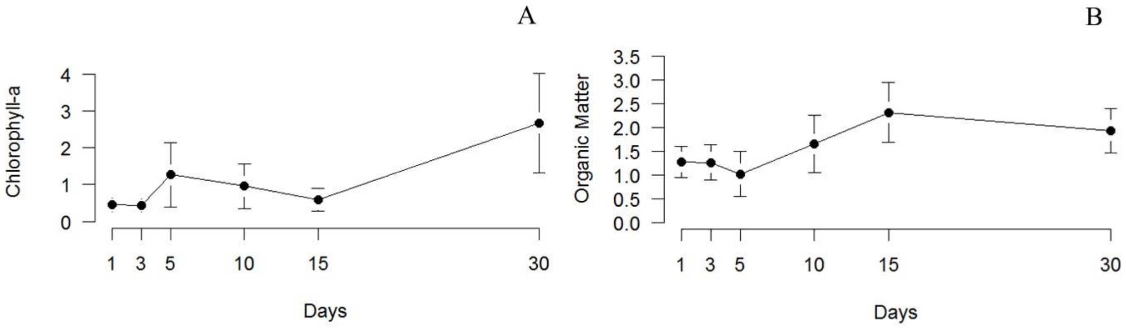

Figure S1. Relationship between mean values (dots ± 1SE) and time of colonisation (Days) for (A) chlorophyll-a (µg/cm²) and (B) organic matter (g).

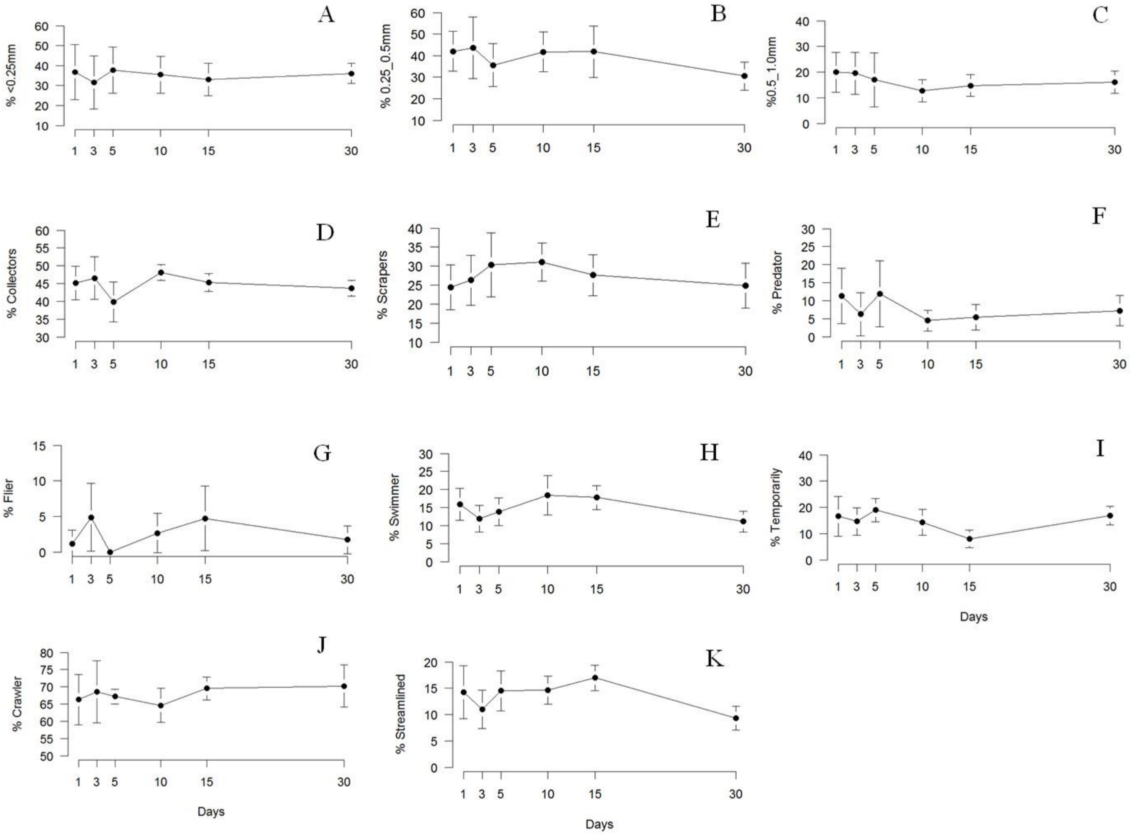

Figure S2. Relationship between mean values (dots ± 1SE) of the proportion of individuals having a given trait category (%) and time of colonisation (Days) for (A) <0.25 mm, (B) between 0.25 and 0.50 mm, (C) between 0.50 and 1.0 mm, (D) collector, (E) scraper, (F) predator, (G), flier, (H) swimmer, (I) temporally attached, (J) crawler and (K) streamlined.

Table S1. Stream insect traits affinity described by fuzzy coding. The score for each taxon belonging to each trait category ranged from 0 to 3.

Traits	Body size				Feeding habits				Locomotion and substrate relation				Shape		
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector Filterer	Shredder	Scraper	Predator	Flier	Swimmer	Temporally attached	Crawler	Streamlined	Flattened	Cylindrical
Coleoptera															
Heterelmis Adult	0	3	0	0	3	3	1	0	2	0	0	3	1	0	3
Heterelmis	2	2	1	0	2	3	0	0	0	0	0	3	0	0	3
Hexacylloepus Adult	0	3	0	0	3	1	1	0	2	0	0	3	0	0	3
Hexacylloepus	0	3	0	0	2	1	1	0	0	0	0	3	1	0	3
Hexanchorus Adult	1	3	1	0	3	1	1	0	2	0	0	3	0	0	3
Neoelmis Adult	0	3	0	0	3	0	1	0	2	0	0	3	0	0	2
Neoelmis	2	2	1	0	2	1	2	0	0	0	0	3	1	0	3
Macrelmi Adult	0	2	0	0	3	1	1	0	2	0	0	3	0	0	3
Macrelmis	0	1	1	1	2	1	2	0	0	0	0	3	1	0	3
Ephemeroptera															
Americabaetis	1	2	1	0	3	1	2	0	0	3	0	2	1	0	3
Baetodes	2	1	0	0	3	0	3	0	0	3	0	2	1	0	3
Camelobaetidius	3	0	0	0	3	0	3	0	0	2	0	3	1	0	3
Caenis	1	2	1	0	3	1	1	0	0	1	0	3	1	2	2
Farrodes	3	0	0	0	2	1	1	0	0	1	0	2	1	2	2
Thraulodes	1	1	0	0	3	0	2	0	0	1	0	3	1	3	0
Tricorythodes	3	0	0	0	3	0	1	0	0	0	0	3	0	1	2
Tricorythopsis	3	0	0	0	3	1	3	0	0	0	0	3	1	1	3

Table 1. Continuation.

Traits	Body size				Feeding habits				Locomotion and substrate relation				Shape		
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector Filterer	Shredder	Scraper	Predator	Flier	Swimmer	Temporally attached	Crawler	Streamlined	Flattened	Cylindrical
Plecoptera															
Anacroneuria	0	0	2	3	1	1	0	3	0	1	0	3	1	2	0
Paragripoteryx	0	2	1	1	1	1	0	2	0	1	0	3	1	2	0
Tupiperla	0	1	2	1	1	1	0	2	0	1	0	3	1	2	0
Trichoptera															
Phylloicus	0	0	0	1	1	3	0	0	0	0	0	3	1	0	3
Itaura	2	2	1	0	3	0	3	0	0	0	3	3	0	1	3
Protoptila	1	2	0	0	3	0	3	0	0	0	3	3	0	1	3
Helicopsyche	3	0	0	0	3	0	2	0	0	0	2	3	0	0	3
Leptonema	0	0	0	3	3	1	0	1	0	1	3	2	0	0	3
Smicridea	0	1	1	0	3	1	0	1	0	1	3	2	0	0	3
Hydroptila	3	1	0	0	3	2	2	0	0	0	1	2	0	3	3
Leucotrichia	3	1	0	0	2	2	2	0	0	1	2	2	0	3	3
Metrichia	3	0	0	0	3	0	2	0	0	0	1	3	1	2	3
Neotrichia	3	2	1	0	1	1	3	0	0	0	1	3	0	0	1
Oxyethira	3	0	0	0	3	1	1	0	0	1	3	2	0	0	1
Oecetis	1	2	2	0	2	3	1	0	0	0	0	3	0	0	3
Nectopsyche	0	0	2	2	2	3	0	0	0	0	0	3	0	0	3
Triplectides	0	1	0	3	3	1	3	1	0	0	0	3	0	0	3
Marilia	0	0	2	0	3	1	0	0	0	0	3	3	0	0	3

Table 1. Continuation.

Traits	Body size				Feeding habits				Locomotion and substrate relation				Shape		
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector Filterer	Shredder	Scraper	Predator	Flier	Swimmer	Temporally attached	Crawler	Streamlined	Flattened	Cylindrical
Trichoptera															
Chimarra	2	2	1	0	3	1	1	0	0	1	3	3	0	0	3
Wormaldia	3	0	0	0	0	1	0	3	0	0	3	3	0	0	3
Cernotina	1	2	1	0	2	3	1	0	0	0	0	3	0	0	3

Table S2. Stream insect abundance of the substrate during colonization experiment, totalizing 30 days.

Days	**1**	**3**	**5**	**10**	**15**	**30**	**∑**	**Days**	**1**	**3**	**5**	**10**	**15**	**30**	**∑**
Coleoptera								Trichoptera							
Heterelmis Adult	2	0	0	0	8	4	14	*Phylloicus*	2	5	9	23	23	40	102
Heterelmis	3	13	0	1	7	8	32	*Itaura*	98	94	141	164	37	188	722
Hexacylloepus Adult	0	2	0	0	0	10	12	*Protoptila*	33	4	3	21	3	21	85
Hexacylloepus	0	0	0	0	0	2	2	*Helicopsyche*	0	0	0	0	0	2	2
Hexanchorus Adult	0	0	0	0	2	0	2	*Leptonema*	0	0	0	2	1	4	7
Neoelmis Adult	0	1	0	5	3	0	9	*Smicridea*	81	99	53	152	58	182	625
Neoelmis	1	1	0	5	8	4	19	*Hydroptila*	0	0	3	2	0	5	10
Macrelmi Adult	0	2	0	2	0	2	6	*Leucotrichia*	0	0	0	0	0	3	3
Macrelmis	0	2	0	2	1	2	7	*Metrichia*	3	5	4	15	16	13	56
Ephemeroptera								*Neotrichia*	5	13	20	34	19	41	132
Americabaetis	12	9	11	43	34	40	149	*Oxyethira*	5	0	0	0	2	0	7
Baetodes	32	30	19	190	274	176	721	*Oecetis*	0	0	0	0	3	7	10
Camelobaetidius	0	0	0	4	2	0	6	*Nectopsyche*	15	1	0	0	0	9	25
Caenis	108	108	29	151	106	283	785	*Triplectides*	0	3	0	4	5	10	22
Farrodes	6	9	1	23	12	22	73	*Marilia*	0	0	0	0	2	10	12
Thraulodes	3	2	6	16	6	1	34	*Chimarra*	0	1	1	4	1	12	19
Tricorythodes	2	0	0	0	0	0	2	*Wormaldia*	1	0	0	5	5	9	20
Tricorythopsis	7	5	4	1	9	15	41	*Cernotina*	0	0	0	0	0	13	13
Plecoptera															
Anacroneuria	0	0	4	3	2	3	12								
Paragripoteryx	5	0	2	2	2	2	13								
Tupiperla	5	6	3	4	5	2	25								

Capítulo 3

Canopy influence on distance decay of similarity in stream insect communities

Este manuscrito será submetido na revista Insect Conservation and Diversity com co-autoria de Luiz Ubiratan Hepp[1] e Adriano Sanches Melo[2]. O manuscrito está formatado de acordo com as normas da referida revista.

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Distance decay of similarity in stream insect

Canopy influence on distance decay of similarity in stream insect communities

Abstract. 1. The distance decay of similarity (DDS) has been widely used in ecological studies, because the procedure provides hints on community spatial distribution. The way stream insect disperse can be expected to affect DDS, decreasing the relationship with the increase of insect dispersal abilities. Basically, neutral and niche theories were used to explain the mechanisms that shape the rates of distance decay in biological communities. However, besides the geographical distance, landscape factors can influence DDS pattern, including physical barriers like canopy cover.

2. We hypothesized that 1) higher decrease in similarity as function of distance for taxonomic data than using functional composition data, and 2) communities composed of strong dispersers on forest riparian vegetation streams will show a steeper distance decay relationship than streams with grassland. However, 3) DDS will be strong on both types of streams for weak dispersers.

3. We collected stream insects from 44 small streams of southern Brazil differing in canopy structure (open and closed). We used aquatic insect ecological and morphological characteristics to measure the functional diversity of the community. To assess whether the rates of distance decay on similarity between matrices (functional *vs*. taxonomic and grassland *vs*. forest riparian vegetation) were different, we use a Monte Carlo randomization procedure.

4. As we predict functional data showed weaker distance decay relationship than taxonomic data. For strong dispersers the forest riparian vegetation showed higher rate of distance decay of similarity than grassland streams. Weak dispersers were unaffected by canopy cover. Our results suggest two processes: 1) significant distance decay of similarity, and 2) the configuration of physical barriers (type of riparian vegetation) increase rates of distance decay. We conclude that forest riparian vegetation is a crucial factor for stream invertebrate spatial distribution.

Key words. Functional traits, spatial distribution, insect dispersal ability, riparian vegetation.

Introduction

Communities that are geographically distant are different from communities nearby. This pattern has been recognized early in vegetation studies (Whittaker 1960, 1972) and become popular with Nekola &White (1999). Since that, the pattern has been recognized for a wide variety of groups including terrestrial (plant, mammal, insect, microbial) and aquatic communities (insect, fish parasite, diatom, fish) (Tuomisto *et al.*, 2003, Poulin, 2003, Novotny *et al.*, 2007, Soininen & Hillebrand, 2007, Leprieur *et al.*, 2009, Maloney & Munguia, 2011, Wetzel *et al.*, 2012). The distance decay of similarity has been widely used in ecological studies, because the procedure provides hints on community spatial distribution (Soininem *et al.*, 2007, Morlon *et al.*, 2008). These patterns are directly linked to beta diversity that consider changes on communities' composition between sites.

Basically, two mechanisms would be primarily responsible for the pattern known as Distance Decay of Similarity (DDS) (Nikola & White, 1999, Soininem *et al.*, 2007, Astorga *et al.*, 2012, Wetzel *et al*., 2012). Firstly, the niche theory states that organisms are closely related to ecological conditions and resources and, as they are spatially autocorrelated. Sites far from each other probably will be environmentally different and communities will respond to these differences. Spatial variation of environmental conditions would function as filter for communities, selecting species able to survive in a particular site (Leibold *et al.*, 2004, Maloney & Mungria, 2011). Secondly, the neutral theory states that organisms are usually dispersal-limited independent of environmental characteristics (Hubbell, 2001). Accordingly, stream insect dispersal can produce autocorrelated patterns of community spatial distribution among distant sites even within homogeneous environmental areas. Furthermore, the two mechanisms are not mutually incompatible and, thus, both mechanisms may operate simultaneously on communities (Thompson & Townsend, 2006). Besides these theories, the recent metacommunity theory recognized that local (environmental conditions and species interactions) and regional (organism dispersal) processes could affect the stream insect distribution (Winegardner *et al.*, 2012, Heino *et al.*, 2015).

Communities become less similar with increased geographical distances, elevating the beta diversity. Similarly, community composition diverges as environmental differences increase (Nekola & White, 1999). Studies using stream invertebrate communities to evaluate the DDS pattern were developed using taxonomic information and report a strong negative relationship between geographic distance and species similarity (Thompson & Townsend, 2006, Maloney & Munguia, 2011). These studies on stream fauna usually focus on taxonomic composition and relative abundances (Maloney & Munguia, 2011, Astorga *et al.*, 2012).

Stream insects differ in a number of life histories traits as well as ways of dispersal (Sheldon, 1984, Maloney & Munguia, 2011). Stream insect functional composition can be compared between distant sites in different landscapes regions, because organisms share the same traits yet in different states. This possibility contrasts with species taxonomic compositions where distant communities may share no species (Thompson & Townsend, 2006). The functional approach has been widely used to evaluated stream insect fauna distribution and structure (Townsend & Hildrew, 1994, Statzner *et al.*, 2004, Tomanova & Usseglio-Polatera, 2007) and as a biomonitoring tool (Charvet *et al.*, 2000, Statzner *et al.*, 2001, Dolédec *et al.*, 2011, Feio & Dolédec, 2012), however, scarcely explored as pattern for spatial distribution based on distance decay relationship (Thompson & Townsend, 2006).

Stream invertebrates interact with both aquatic and terrestrial environments during their life cycle (Cushing & Allan, 2001). These insects can disperse by drift during their immature stages or as winged adults through the stream corridor. In a few cases, they can fly laterally and reach distant sites (Finn & Poff, 2008, Ridley *et al.,* 2011). Invertebrates that are strong active dispersers showed a weak distance decay relationship because they were able to colonize distant sites and, therefore, homogenize the community composition between sites (Brown *et al.*, 2011, Heino *et al.*, 2015). The way stream insect disperse can be expected to affect distance decay relationships, decreasing the relationship with the increase of insect dispersal abilities (Thompson & Townsend, 2006, Brown & Swan, 2010, Bonada *et al.*, 2012). Based on insects potential to disperse, the landscape characteristics are an important factors for determine patterns of DDS (Briers & Gee, 2004, Macneale *et al.*, 2005, Smith *et al.*, 2015).

Besides geographical distance, other environmental factors could influence DDS pattern, physical barriers like riparian vegetation, landscape structure and stream network configuration (Fagan, 2002, Brown & Swan, 2010). One of the most important factor that can influence the spatial distribution of stream insects is the presence of canopy cover. Streams with forest riparian vegetation can work as a barrier to wind, limiting the distance travelled passively by stream insects (Briers & Gee, 2004).

Besides, stream insects that disperse via flight may use the adjacent riparian vegetation as a place for resting (Kovats *et al.*, 1996). In addition, these insects can use the forest riparian vegetation and corridors to disperse as the habitat were more climatic favourable compare to open areas (Petersen *et al.* 2004). Therefore, organisms should remain near the place of origin (emergence site), reducing distances travelled by them (Bilton *et al.*, 2001). Additionally, riparian vegetation can benefits flying insects and increase the fly persistence of adult insects (Greenwood *et al.*, 2011, Datry *et al.*, 2016) by providing suitable microclimate (cool and moist), one of the main factor that affect flying adults (Briers & Gee, 2004). Studies evaluating the influence of grassland areas on invertebrate stream fauna are scarce. The few existing studies assess sites where the natural vegetation was removed (clear-cut, cropland and urbanization). These studies generally found an effect of grassland on the aquatic insect communities. Collier & Smith (1998) found that adult aquatic insects with strong dispersal abilities travelled long distances in areas without riparian vegetation (open cropland areas). However, conclusions regarding dispersal may be confounded by anthropic impacts. In addition, even passive and weak dispersers organisms can be carried by wind and colonize distant places (Briers *et al.*, 2003). Moreover, on streams without canopy cover, adult insect where more exposed to predation, it is easy for birds, for example, to distinguish insects on open areas (Whitaker *et al.*, 2000). We detached that our study include streams on areas that are naturally unforested (i.e grassland), contrary to most studies, where the lack of riparian vegetation is artificial (anthropic activity).

Based on these studies we could say that grassland or forest riparian vegetation cover could modify DDS patterns. Delettre & Morvan (2000) using Chironomidae stream communities highlighted that the influence of riparian vegetation cover as barrier or corridor for organisms dispersal need to be explored. However, on one hand, the presence of riparian vegetation can protect stream insects but at the same time limit species dispersal. On the other hand, the absence of canopy can generate predation risk, provide adverse conditions (high temperature and less humidity), but facilitate passive dispersal by wind (Whitaker *et al.*, 2000, Bilton *et al*., 2001, Petersen *et al*. 2004).

We assessed distance decay relationships of Neotropical stream insects communities on small spatial scale (<100km). We hypothesized that 1) taxonomic similarity should decrease as a function of distance. On the other hand, functional similarity should not decrease with distance, as species with the same set of traits should replace each other. We also hypothesized that 2) communities composed of strong dispersers on forest riparian vegetation streams will show a steeper distance decay relationship than on streams with grassland. However, 3) DDS will be strong on both types of streams for weak dispersers.

Material and methods

Study area

We carried out the study on streams located in high altitude grasslands (~1200 m a.s.l.) of southern Brazil (28°36’S, 49°56’W). The climate of the region is influenced by the ocean and with uniform rainfall through the year. The yearly average rainfall oscillates between 1400 to 2200 mm and the temperature varies from 12 to 18°C (Alvares *et al.*, 2013). The vegetation consists of a mosaic of grassland with patches of Araucaria forest. We sampled streams with tree riparian vegetation as well as open streams harbouring grasses and shrubs on their margins. Forest streams were shaded by *Araucaria angustifolia* (Bertol.) Kuntze, *Drimys brasiliensis* Miers, *Podocarpus lambertii* Klotzsch, *Ilex paraguariensis* A. St.-Hill., *Acca sellowiana* (O.Berg) Burret and at least 40 other species (Bond-Buckup, 2010).

The studied area encompassed around 100 km, where we selected 44 1st-2nd order streams. The streams were categorized during fieldwork as open or forest riparian vegetation. Open streams included grass and scrubs usually less than 0.5 m tall in the 20 m wide riparian area. Closed streams included at least 20 m of forest on both sides of the channel. The streambed was similar among stream types, composed of boulders, stones and pebbles. All the streams sampled were under natural vegetation and undisturbed or weakly disturbed areas.

In each stream site, we collected stream insects using Surber sampler (area: 0.09 m^2; mesh 250 µm; 3 sample units). The organisms were identified until genus level using identification keys of Merritt & Cummins (1996), Fernández & Domínguez (2001), Salles *et al.* (2004), Pes *et al.* (2005), and Costa *et al.* (2006). Only Ephemeroptera, Plecoptera and Trichoptera (EPT) families were studied as previous studies in the region (Melo, 2005) indicated they were not only among the most species-rich groups but also representative of macroinvertebrate community and, for the purposes of this study, encompass a wide variety of functional traits.

Stream insect traits

We used the following ecological and morphological characteristics of stream insects to estimate the functional diversity of studied communities: body size and shape, dispersal mode, specific adaptation and mobility (Table S1 for trait categories). We use a "fuzzy" code approach to minimize the differences between insect stages and changes along development. The fuzzy code consisted of affinity levels of the taxa to a particular trait, with scores ranging from 0 to 3. High scores indicate high affinity of the taxa to the trait. The information on traits was obtained from literature on the Neotropical fauna (Tomanova & Usseglio-Polatera, 2007) (Supporting information Table S1). We separate stream insect communities in two matrices one with strong dispersers and other with weak dispersers based on literature using functional attributes (aerial and aquatic dispersal) (Poff *et al.,* 1997; Townsend *et al.*, 1997; Malmquist *et al.*, 2000; Astorga *et al.*, 2012; Landeiro *et al.*, 2012; Colzani *et al.*, 2013; Heino, 2013; Saito *et al.*, 2015) (Supporting information Table S2).

Environmental characteristics

At each site, we measured the width, depth, flow velocity, water temperature, electrical conductivity, pH, and dissolved oxygen. We also recorded information about presence of bryophytes, periphytic algae and rocks on streambed. To construct the environmental matrix we standardized the variables by their ranges because they were on different scales. We determine the geographic distance between each pairs of sites based on geographical coordinates (latitude and longitude) recorded during fieldwork.

Statistical analyses

We evaluated the hypotheses that taxonomic similarity should decrease as a function of distance using a Mantel test with similarity matrices of log abundance and presence/absence data (Bray–Curtis dissimilarity). The same test was used to evaluate correlation of the community functional composition and geographic distance.

For the hypothesis that strong dispersers communities on forest riparian vegetation streams will show a steeper distance decay relationship than on streams with grassland we run a Mantel test. We use the same test to test the hypothesis that for weak dispersers the DDS will be steep on both streams (closed and grassland).

To assess if the rates of DDS between matrices (functional *vs.* taxonomic (abundance and presence absence) and grassland vs. forest riparian vegetation (with two matrices: weak dispersers and strong dispersers) were different, we use a randomization test. Briefly describing the procedure, we calculate the rate of decay with the distance to the observed data and the slope of each distance decay relationship was registered. Thereafter, these slope values were compared with the slope values of the DDRs gradient generated by randomization samples (10000 times). To estimate the difference between the slopes we evaluated the ratio among the number of times from randomized sample were equal or greater than the observed data (for more details see Wetzel *et al.*, 2012).

We used Euclidian distance to estimate environmental differences between pairs of sites. To assess if geographic distance and environmental distance were correlated we used a Mantel test. All analyses were carried out with the R software (The R Development Core, 2013), using the package "vegan" (Oksanen *et al.*, 2010) and "ade4" (Thioulouse *et al.*, 1997, Chessel *et al.*, 2004) packages.

Results

Environmental distance vs. geographical distance

The stream sites did not show a spatial pattern based on morphological and limnological characteristics. We did not observe correlation between geographical distance and environmental dissimilarity (r = 0.04; p = 0.897; Fig. 1).

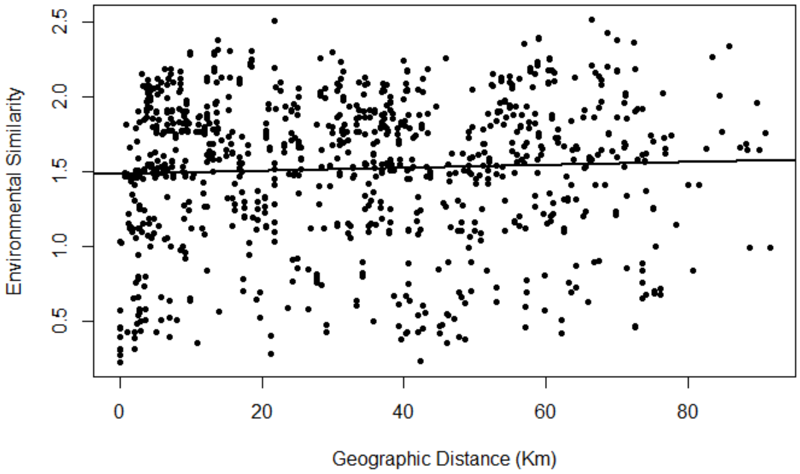

Figure 1. Distance decay of environmental similarity (morphological and limnological) and geographic distance of the streams in southern Brazil.

Taxonomic vs. functional insect communities

Community similarity decreased with geographical distance for functional, abundance, and presence/absence taxonomic data sets (r = -0.37; p = 0.001; r = -0.35; p = 0.001; r = -0.10; p = 0.030, respectively; Fig. 2). Distance decay relationship observed from aquatic insects abundance and presence/absence were compared and similar relationships between them were verified (randomization: p = 0.527). As hypothesized, functional traits showed weaker DDS compared to abundance and presence/absence data sets (randomization: $p < 0.001$ and p = 0.023, respectively).

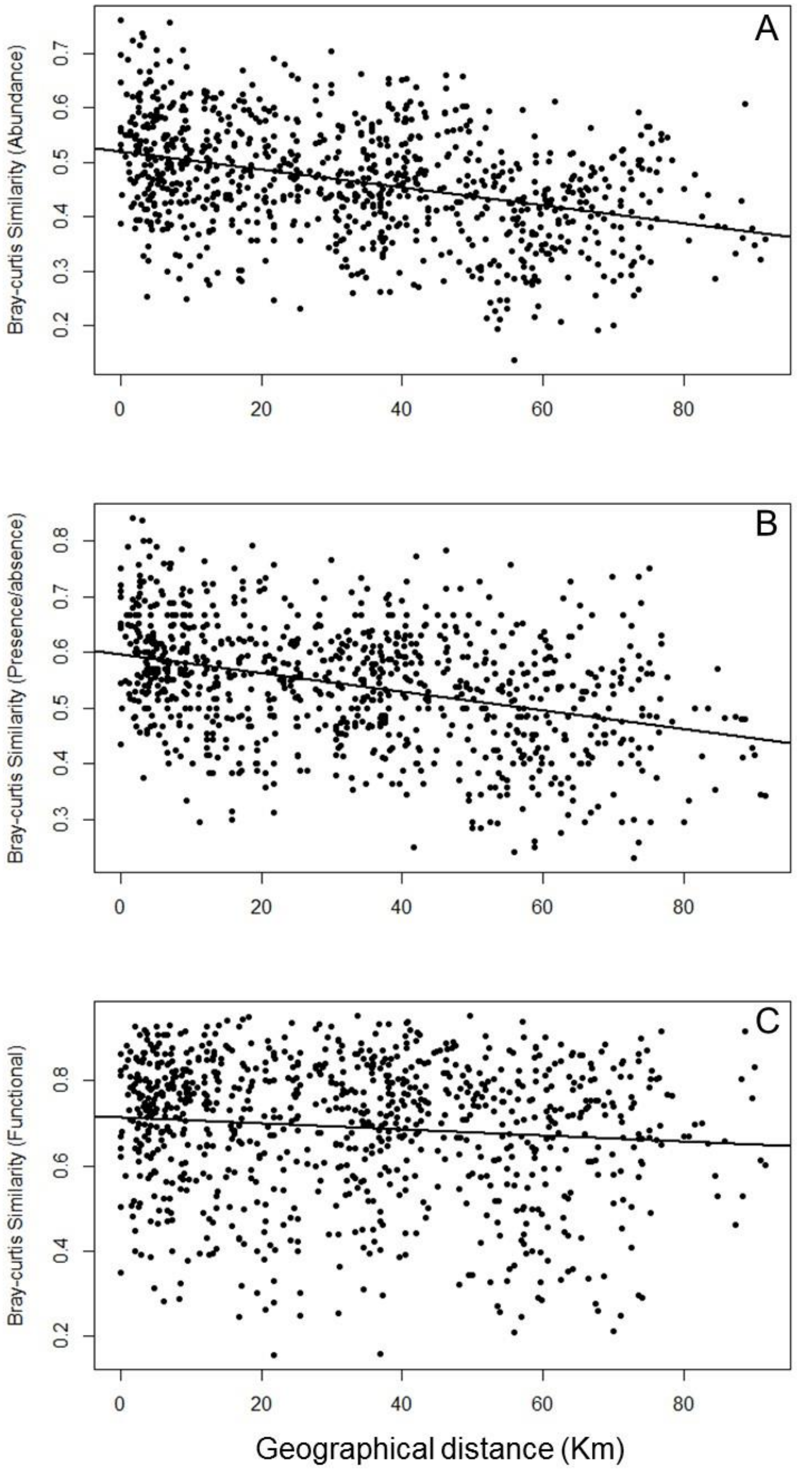

Figure 2. Distance decay of similarity (Bray-Curtis distance) for abundance (A), presence/absence (B) and functional (C) data sets of stream insect communities in southern Brazil.

Open vs forest riparian vegetation streams community

Aquatic insects with high dispersal abilities on streams with forest riparian vegetation showed a negative and significant DDS (r = -0.365, *p* = 0.001, Fig. 3A). In contrast, these high-dispersal insects did not showed significant DDS on streams with grassland (r = 0.002, *p* = 0.487, Fig. 3B). We confirm that the DDS of strong dispersers on streams with forest riparian vegetation showed a significantly higher slope than the DDS from areas grassland with the randomization test (*p* =0.012).

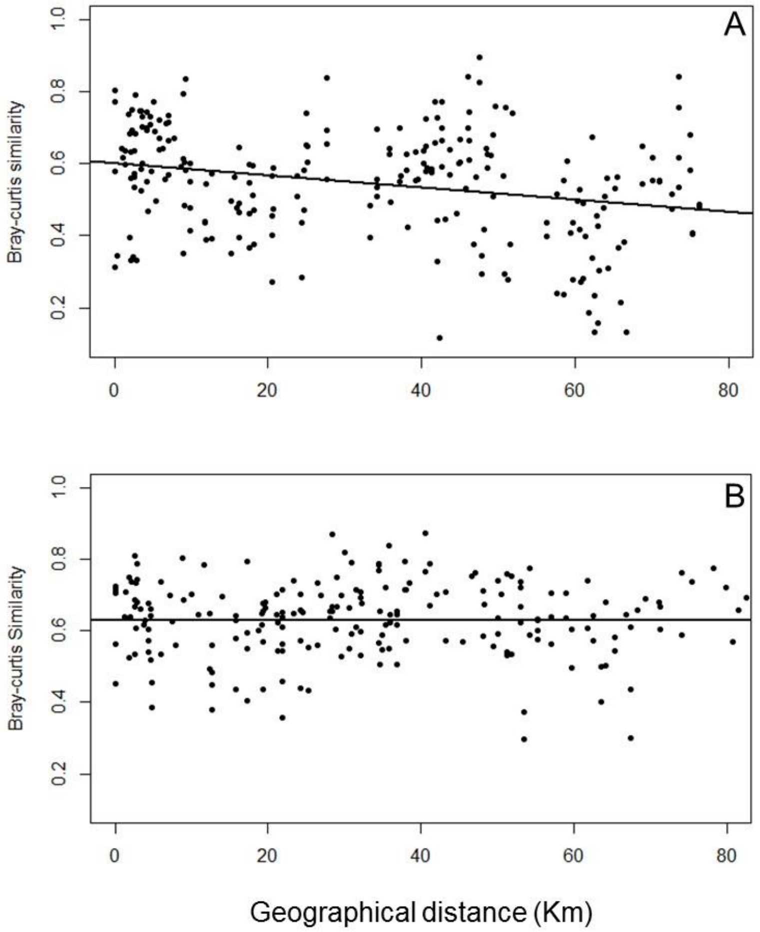

Figure 3. Distance decay of similarity (Bray-Curtis similarity) for strong disperser stream insects on streams with forest riparian vegetation (A) and grassland (B).

For weak dispersers similarity decreased with distance for both streams on closed and grassland communities (r = -0.461, *p* = 0.001, r = -0.470, *p* = 0.001, respectively. Fig. 4). In addition, the steep of the DDS were similar in streams with closed and grassland (randomization: *P* = 0.871).

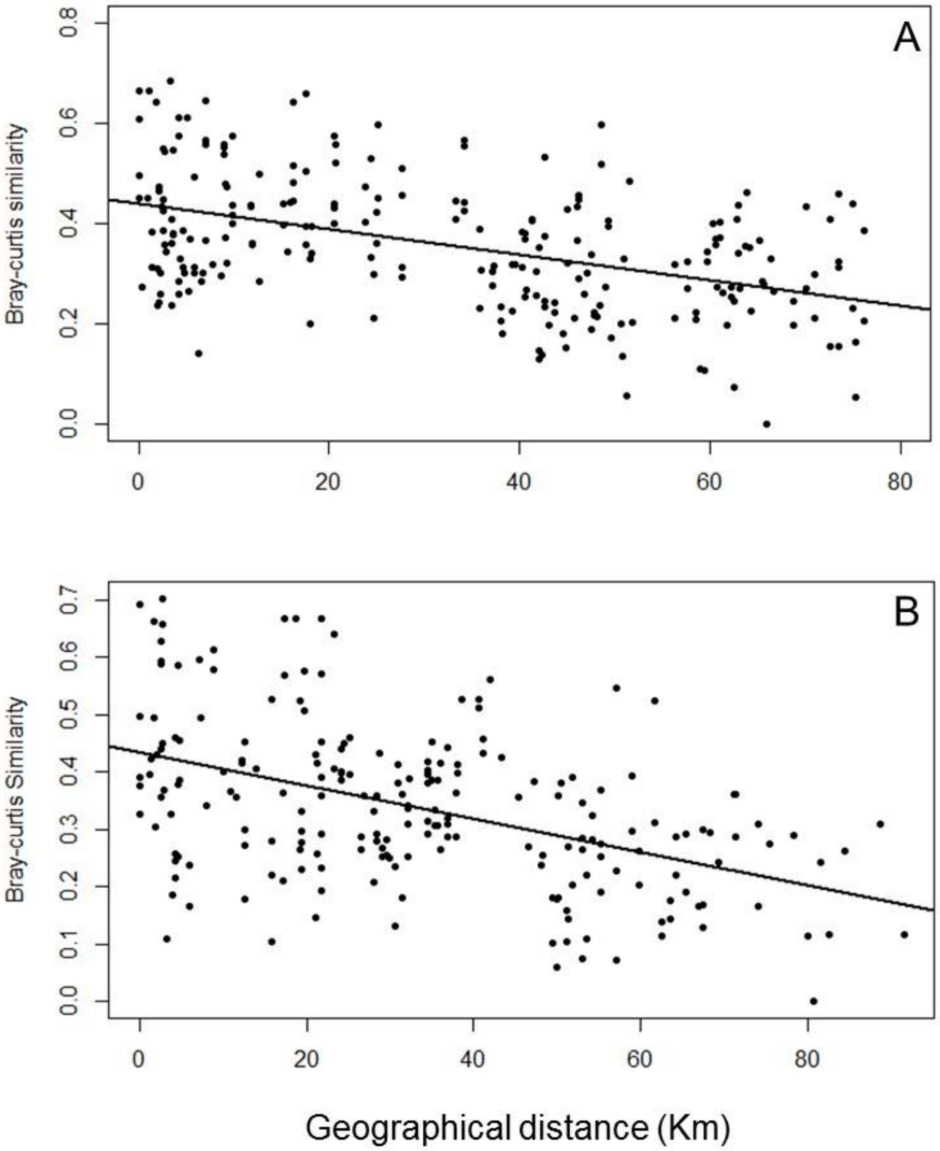

Figure 4. Distance decay similarity (Bray-Curtis distance) for weak disperser insect on streams with forest riparian vegetation (A) and grassland (B).

Discussion

Environmental distance vs. geographical distance

The geographical distance of streams sites was not correlated to the similarities in environmental conditions. The absence of correlation between the environmental and geographical distance showed that there are no spatial pattern between sites. Environmental characteristics could be a confounding factor when spatial distribution were evaluated (Hepp & Melo, 2013). Our result allows us to detect community patterns that were exclusively influenced by distance.

Taxonomic vs functional streams communities

As we hypothesized, the taxonomic data sets showed significant and steeper distance decay than functional data set. Although weaker, the functional similarity showed a significant DDS pattern. The strength of the correlation between community similarity and geographical distance could be interpreted as evidence for processes such as dispersal limitation (Thompson & Townsend, 2006). Natural aquatic environments generally show high species richness and great variety of functional traits. However, functional characteristics were less diverse when compared to the number of taxa. The opposite should be expected for composition and abundance, because *taxa* change between distant sites but the functional attributes remains similar (same traits, different *taxa*). The functional redundancy can also contribute to weak DDS step for functional composition. High dispersal potential, highlighted indirectly by traits related to dispersal, like body size and insects mobility, can homogenize communities decreasing beta diversity (Mouquet & Loreau, 2002, Wetzel *et al.*, 2012). Astorga *et al.* (2012) found a strong DDS between low dispersal ability insects and geographic distance, whereas for high dispersal insects the DDS was higher with environmental distance. As our sites did not showed a spatial pattern for environmental characteristics, allow us to infer that distance between sites and dispersal abilities of each group of invertebrates drives the DDS pattern.

Closed vs grassland streams

As we hypothesized for strong disperser insects, the decline in similarity as a function of distance on streams with forest riparian vegetation was steeper than on grassland streams. The presence of forested riparian vegetation was a determinant factor to the correlation between biological similarity and geographical distance. Probably, because stream invertebrate can use the riparian vegetation as windbreaks for protection and persistence (Briers & Gee, 2004, Greenwood *et al.*, 2011). In addition, these organisms could be saving energy and spend it on reproduction and oviposition sting near to stream channel (Petersen *et al.* 2004). The canopy cover of stream corridor can influence directly the microenvironment (relative humidity and air temperature), increasing the chances of survival for organisms. Contrary to our results, studies pointed out that dispersal can be facilitated by canopy cover, because these microclimatic conditions favour individuals with high-flight ability that travel long distances between streams (Briers & Gee, 2004). This means that forest riparian vegetation areas can acts as a physical barrier to the aerial dispersal organisms (Petersen *et al.*, 1999).

Results pointed that organisms with high flight ability were able to disperse farther on grassland streams than on streams with forest riparian vegetation. Physical barriers can influence the distribution and the dispersal of stream insects (Kneitel & Miller, 2003). Studies showed that strong dispersers showed weak distance decay relationships and were more homogeneous even across distant sites (Townsend *et al.*, 2003, Leibold *et al.*, 2004). However, according to our results, it depends on physical barriers like riparian vegetation. In addition, studies showed that high dispersal stream insects could go through the limitation imposed by dispersal barriers like riparian vegetation, high mountains, cliffs and valleys (Macneale *et al.*, 2005,Brown & Swan, 2010, Bonada *et al.*, 2012, Grönroos *et al.*, 2013). Our findings provide evidence that distance-decay patterns in stream insect communities on small scales are driven by dispersal limitation and depend on physical barriers.

On the other hand, weak dispersers show similar distance decay relationships between communities on streams with forested riparian vegetation and grassland streams (Kovats *et al.*, 1996, Bilton *et al.*, 2001, Briers & Gee, 2004). These findings suggest the physical barrier imposed by riparian vegetation did not influence on the distance decay relationships of both strong and weak dispersers. Even on open areas the weak dispersers, that could be carried by wind to long areas, were more representative closer to stream channel (Delettre & Morvan, 2000, Vanschoenwinkel *et al.*, 2008). In addition, less mobile insects were dependent from the place they first colonize, which can contribute to dispersal limitation (Thompson & Townsend, 2006). Contrary to our results, Saito *et al.* (2015) found no significant distance decay relationships for weakly dispersers, they affirm that insects were carried by the wind to other sites. However, using organisms with lower flight capacity we found similar steep distance decay relationship on streams with closed and grassland cover. These organisms can be less influenced by riparian vegetation and more sensitive to stream channel parameters, because they do not have the ability to flight and search for better areas for reproduction and oviposition (Blakely *et al.*, 2006, Smith *et al.*, 2009), and disperse more longitudinally on stream corridor than laterally between sites.

We found that the communities on stream with forest riparian vegetation cover showed stepper DDS than grassland streams. Riparian cover near to stream channel can generate favourable conditions for stream insects, like refuge to oviposit, safe places for reproduction, food supply and higher diversity of microhabitats (Macedo *et al.*, 2014). Therefore, the presence of vegetation near to streams influence aquatic insect distribution and increase beta diversity, which is crucial for community persistence and resilience, even for strong dispersers (Winterbourn *et al.*, 2007; Macedo *et al.*, 2014). Our results highlighted the importance of riparian vegetation determining the distance decay pattern of stream insect communities. Finally, we identified that regional forces, based on dispersal abilities, were shaping the distribution of stream insect communities.

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Supporting information

Table S1. Stream insect traits affinity described by fuzzy coding. The score for each taxon belonging to each trait category ranged from 0 to 3.

Traits	Body size				Feeding habits				Locomotion and substrate relation			Dispersal				Shape		
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector	Shredder	Scraper	Predator	Swimmer	Crawler	Temporary attached	Aquatic passive	Aquatic active	Aerial passive	Aerial active	Streamlined	Flattened	Cylindrical
Ephemeroptera																		
Americabaetis	1	1	0	0	3	1	2	0	3	2	0	3	2	1	3	1	0	3
Apobaetis	2	1	0	0	2	0	0	0	2	3	0	3	2	1	3	1	0	3
Askola	3	0	0	0	0	0	2	0	3	1	0	2	1	1	2	3	1	1
Baetodes	2	1	0	0	3	0	3	0	3	2	0	3	2	1	3	1	0	3
Caenis	1	2	1	0	3	1	1	0	1	3	0	2	1	1	1	1	2	2
Camelo	3	0	0	0	3	0	3	0	2	3	0	2	1	1	2	1	0	3
Cloeodes	3	0	0	0	2	0	1	0	2	3	0	3	2	1	3	1	0	3
Farrodes	3	0	0	0	2	1	1	0	1	2	0	2	1	1	3	1	2	2
Hagenulopsis	2	1	0	0	2	1	1	0	1	3	0	3	1	1	3	1	2	2
Leptohyphes	2	1	0	0	3	1	3	0	0	3	0	2	1	1	2	0	1	2
Massartella	3	2	1	0	0	0	3	0	2	1	0	3	1	1	2	1	1	1
Simothraulopsis	0	2	0	0	3	0	2	0	1	3	0	1	1	1	2	1	3	0
Traverhyphes	3	0	0	0	3	1	2	0	0	3	0	1	1	1	2	0	1	2
Tricorythodes	3	0	0	0	3	0	1	0	0	3	0	2	1	1	2	0	1	2
Tricorythopsis	3	0	0	0	3	1	3	0	0	3	0	2	1	1	2	1	1	3

Table S1. Continuation.

Traits	Body size				Feeding habits				Locomotion and substrate relation			Dispersal				Shape		
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector	Shredder	Scraper	Predator	Swimmer	Crawler	Temporary attached	Aquatic passive	Aquatic active	Aerial passive	Aerial active	Streamlined	Flattened	Cylindrical
Ephemeroptera																		
Traulodes	0	1	0	0	3	0	2	0	1	3	0	1	1	1	2	1	3	0
Tupiara	2	1	0	0	2	0	0	0	3	2	0	3	1	1	3	1	0	3
Plecoptera																		
Anacroneuria	0	0	2	1	1	1	0	3	1	3	0	1	2	0	1	1	2	0
Gripopterix	0	0	0	3	1	1	0	2	1	3	0	2	2	0	1	1	2	0
Kempnyia	2	2	0	0	1	1	0	3	1	3	0	1	2	0	1	1	2	0
Paragripopterix	0	1	1	0	1	1	0	2	1	3	0	2	2	0	1	1	2	0
Tupiperla	0	1	2	0	1	1	0	2	1	3	0	2	2	0	1	1	2	0
Trichoptera																		
Alisotrichia	1	2	0	0	0	2	3	0	0	3	0	1	1	2	1	1	2	3
Atopsyche	2	0	0	0	0	1	0	3	1	3	0	1	2	1	1	0	0	3
Ceratotrichia	1	2	0	0	0	2	3	0	0	3	0	1	1	2	1	1	2	3
Helicopsyche	3	0	0	0	0	0	2	0	0	3	2	0	0	1	1	0	0	3
Itaura	1	2	0	0	3	0	3	0	0	3	3	1	2	1	1	0	1	3
Leptonema	0	0	0	3	0	1	0	1	1	2	3	3	1	1	3	0	0	3
Macrostemum	0	1	2	3	1	1	0	1	0	2	3	1	1	2	3	0	0	3
Machairocentron	0	3	0	0	2	0	0	0	0	2	1	0	1	1	1	0	1	3
Marilia	1	2	1	0	3	1	3	1	0	3	0	3	1	1	1	0	0	3

Table S1. Continuation.

Traits	Body size				Feeding habits				Locomotion and substrate relation			Dispersal				Shape		
Categories	Less_0.25	0.25_0.5	0.5_1.0	1.0_2.0	Collector	Shredder	Scraper	Predator	Swimmer	Crawler	Temporary attached	Aquatic passive	Aquatic active	Aerial passive	Aerial active	Streamlined	Flattened	Cylindrical
Trichoptera																		
Metrichia	3	0	0	0	3	0	2	0	0	3	1	1	1	2	1	1	2	3
Nectopsyche	2	2	1	0	2	3	1	0	0	3	0	2	1	1	3	0	0	3
Neotrichia	1	2	0	0	1	1	3	0	0	3	1	1	1	2	1	1	0	2
Oecetis	3	2	0	0	2	2	1	1	2	2	0	1	1	1	3	0	0	3
Oxyethira	3	0	0	0	3	1	1	0	1	2	3	1	1	2	1	0	0	1
Phylloicus	0	0	0	1	1	3	0	0	0	3	0	1	1	0	3	1	0	3
Polyplectropus	0	2	0	0	2	0	0	0	0	2	1	1	1	1	1	0	1	3
Smicridea	0	1	1	0	0	1	0	1	1	2	3	3	2	1	3	0	0	3
Wormaldia	2	2	1	0	0	1	1	0	1	3	3	1	2	1	1	0	0	3
Chimara	1	2	0	0	0	1	0	0	0	3	3	1	1	1	1	0	0	3
Protoptila	1	2	0	0	3	0	3	0	0	3	3	1	2	1	1	0	1	3

Table S2. Stream insect classification on strong and weak dispersers based dispersal traits.

Strong Dispersers	**Weak Dispersers**
Ephemeroptera	Ephemeroptera
Americabaetis	*Askola*
Apobaetis	*Caenis*
Baetodes	*Camelobaetidius*
Cloeodes	*Cyrnellus*
Farrodes	*Leptohyphes*
Hagenulopsis	*Massartella*
Tupiara	*Simothraulopsis*
Leptonema	*Traverhyphes*
Trichoptera	*Tricorythodes*
Macrostemum	*Tricorythopsis*
Nectopsyche	*Traulodes*
Phylloicus	Plecoptera
Smicridea	*Anacroneuria*
Oecetis	*Gripopterix*
	Kempnyia
	Paragripopterix
	Tupiperla
	Trichoptera
	Alisotrichia
	Atopsyche
	Ceratotrichia
	Helicop
	Itaura
	Machairoc
	Marilia
	Metrichia
	Neotrichia
	Oxyethira
	Polyplec
	Wormaldia
	Chimara
	Protoptila

Conclusões gerais

Os resultados da minha tese mostraram que o uso de características funcionais dos organismos é uma ferramenta útil na determinação de padrões de distribuição e ocorrência dos insetos aquáticos. Sendo que, processos locais (heterogeneidade do substrato) e regionais (limitação de dispersão) afetaram a organização funcional das comunidades de insetos aquáticos. Além disto, de maneira geral, determinei como a estrutura funcional das comunidades variou temporalmente.

No primeiro capítulo avaliei a influência da heterogeneidade do substrato sobre as características funcionais dos insetos bentônicos. Concluí que em ambientes naturais o substrato heterogêneo seleciona combinações específicas de atributos, associados principalmente ao tamanho do corpo e hábitos alimentares. Esses atributos exercem forte influência no funcionamento dos ecossistemas aquáticos. Além disto, posso ressaltar que a homogeneização do substrato do rio associado a atividades humanas, resulta na redução e até na perda de funções importantes, as quais estão lincadas a processos (entrada de matéria orgânica) que afetam o funcionamento do ecossistema.

No segundo capítulo verifiquei a variação temporal dos atributos funcionais dos insetos aquáticos, com enfoque para organismos especialistas e generalistas. Nos primeiros estágios da colonização os organismos coexistem independente dos atributos, isso porque a comunidade mostrou atributos semelhantes (baixa diversidade e riqueza funcional). Já nos últimos estágios os táxons especialistas resistiram a fatores bióticos e abióticos mostrando uma variedade de atributos superior à que foi encontrada no início da colonização, consequentemente o aumento de especialistas permitiu a coexistência entre os organismos. Além disso, ressalto que a ausência ou diminuição de organismos especialistas pode ser um indicador de degradação ambiental. A abundância de especialistas é crucial para o ecossistema do riacho e o seu declínio torna a comunidade funcionalmente mais similar, o que pode afetar o funcionamento do ecossistema, desestabilizando cadeias tróficas e processos, como o ciclo de nutrientes.

No terceiro capítulo utilizei uma abordagem funcional, porém em uma escala espacial mais ampla. A cobertura vegetal no entorno dos riachos é um fator determinante para a distribuição espacial dos insetos aquáticos. Em riachos sem vegetação ripária, os insetos aquáticos com maior habilidade para dispersão apresentaram um padrão fraco de DDS. Para dispersores menos hábeis o padrão DDS foi similar para os dois riachos (com e sem cobertura vegetal). Com isso, concluí que a presença de vegetação no entorno dos riachos pode ser uma barreira física que limita a dispersão dos insetos aquáticos, principalmente dos organismos com maiores habilidades para dispersar. Por um lado, a vegetação ripária pode agir como barreira na dispersão dos insetos aquáticos e por outro lado pode favorecer o aumento da diversidade entre os diferentes locais.

Diante dos resultados obtidos com esse trabalho ainda existem lacunas no que se refere ao uso da abordagem funcional em estudos sobre aspectos ecológicos dos insetos aquáticos. Outro fator que merece destaque é a mensuração e a escolha dos atributos funcionais dos invertebrados bentônicos, essa etapa pode determinar o êxito para alcançar objetivos quando a abordagem funcional é utilizada. Com isso, concluí que a utilização da temática funcional auxiliou na identificação de padrões ecológicos das comunidades estudadas, juntamente com características taxonômicas dos organismos.

Além disso, ressalto que a avaliação de outras características do substrato seria um complemento interessante para o entendimento de padrões de distribuição funcional dos insetos aquáticos. Ou seja, questões relacionadas a complexidade, estabilidade e rugosidade do substrato e a relação com a diversidade funcional das comunidades subsidiariam e complementariam os estudos realizados nessa tese. Considerando a importância dos fatores locais e regionais para a fauna aquática, posso afirmar que a heterogeneidade do substrato e da vegetação ripária contribuem com a manutenção da diversidade funcional das comunidades de insetos aquáticos. Em virtude de perturbações antrópicas ininterruptas e crescentes a fauna de insetos aquáticos de rios e riachos é modificada constantemente, tanto funcional quanto taxonomicamente. Com isso concluí que compreender como ocorrem essas modificações por meio da determinação de padrões ecológicos é primordial para investir em ações de conservação da fauna aquática.