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VARIAÇÃO DA DIVERSIDADE E COMPOSIÇÃO DE MACROINVERTEBRADOS EM
RIACHOS DA BACIA HIDROGRÁFICA DO RIO DOS SINOS, RS, BRASIL

São Leopoldo

2014

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RIACHOS DA BACIA HIDROGRÁFICA DO RIO DOS SINOS, RS, BRASIL**

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Dedico esta tese a todas as pessoas que me apoiaram durante os quatro anos de trabalho, em especial, minha família. Também a dedico ao meu filho, ou filha, que está por vir.

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RESUMO

A comunidade de macroinvertebrados aquáticos pode ser estruturada por processos em nível de múltiplas escalas espaciais. A redução ou substituição da floresta ripária por diferentes usos do solo e o impacto nas condições físicas e químicas dos riachos pode afetar negativamente a comunidade de macroinvertebrados causando homogeneização da composição. Por isso, nós avaliamos as seguintes hipóteses. Artigo I) A riqueza de macroinvertebrados e a riqueza de EPT (Ephemeroptera, Plecoptera e Trichoptera) diminui com a redução da largura de floresta ripária; a composição de macroinvertebrados e dos grupos tróficos funcionais (GTF) variam com a redução da largura de floresta ripária; e a redução da largura de floresta ripária influencia similarmente a comunidade de macroinvertebrados em diferentes substratos do riacho. Artigo II) O uso do solo (floresta, agricultura, pastagem e urbanização) altera as variáveis físicas e químicas nos riachos; e a diversidade β diminui, ou a composição de macroinvertebrados é mais homogênea, em trechos de riachos impactados pelo uso do solo. Artigo III) A diversidade γ não é distribuída homogeneamente entre escalas, com diferenças entre o padrão observado no inverno e verão; e a diversidade β depende dos tipo de substratos diferentes, mas a maioria da dissimilaridade da composição ocorre em escalas espaciais mais amplas, independente da estação do ano analisada. Visando obter essas respostas, nós selecionamos trechos de três riachos de cabeceira na Bacia Hidrográfica do Rio dos Sinos, sul do Brasil. A composição de macroinvertebrados no substrato folhiço diferiu dos substratos encontrados em corredeira (pedra e seixo), mostrando proporcionalmente menor diversidade dentro da menor escala espacial (α_1) e maior diversidade na escala mais ampla (β_4). Nós encontramos diferenças na composição (diversidade β) entre a escala de trecho e escala de riacho no inverno e verão. Houve diferença significativa na composição de macroinvertebrados entre as larguras de florestas ripárias. A composição de macroinvertebrados e de GTF diferiu entre substratos, independente da largura de floresta ripária. As variáveis físicas e químicas dos riachos variaram de acordo com a intensidade do uso do solo. Houve homogeneização da comunidade, ou uma redução da diversidade β , com o aumento da velocidade do riacho, nitrogênio amoniacal e fósforo total, e uma maior heterogeneidade da comunidade ocorreu com o aumento do sombreamento e do pH. Nossos resultados revelaram que os riachos no sul do Brasil suportam uma comunidade de macroinvertebrados muito heterogênea e o papel da configuração espacial nesses riachos influencia fortemente a diversidade de macroinvertebrados. Uma largura de floresta ripária maior que 15 m é necessária para manter

a composição e as condições tróficas das famílias de macroinvertebrados em estados de conservação mais prístinas. Além disso, os resultados enfatizam a importância de estudos sobre a diversidade β para a avaliação da homogeneização da comunidade de macroinvertebrados em riachos impactados por diferentes usos do solo no sul do Brasil.

Palavras-chave: Escala espacial. Diversidade β . Composição de macroinvertebrados. Paisagem rural.

ABSTRACT

The aquatic macroinvertebrate community can be structured by processes in multiple spatial scales. Reduction or substitution of riparian forest by different land uses and the impacts on the physical and chemical conditions of streams can negatively affect the macroinvertebrate community causing homogenization of composition. Therefore, we assessed the following hypotheses. Article I) Macroinvertebrate richness and EPT (Ephemeroptera, Plecoptera and Trichoptera) richness decrease with reduced riparian zone width; macroinvertebrate and functional feeding group (FFG) composition vary with a reduced riparian zone width; and reduced riparian zone width similarly influence the macroinvertebrate community in different stream substrates. Article II) Land use (forest, agriculture, pasture and urbanization) alters physical and chemical variables in streams; and β diversity decreases, or the macroinvertebrate composition is more homogeneous, in stream reaches impacted by land use. Article III) γ diversity is not homogeneously distributed among scales, with differences between the pattern observed in winter and summer; and β diversity depends on different substrate types, but most composition dissimilarity occurs in larger spatial scales, regardless of the season analyzed. In order to obtain these responses we selected reaches of three headwater streams in the Sinos River watershed, northeastern southern Brazil. The macroinvertebrate composition on litter substrate differed from that on riffle (stone and gravel) substrata, showing proportionally lower diversity within the lowest scale (α_1) and higher diversity at the highest scale (β_4). We found differences in the composition (β diversity) between the reach scale and the stream scale in both winter and summer. There were significant differences in macroinvertebrate composition among riparian zone widths. The macroinvertebrate composition and FFG differed among substrates, independent of riparian zone width. The physical and chemical stream variables varied according to the land use intensity. A homogenization of the community, or reduced β diversity, occurred with increased stream velocity, ammoniacal nitrogen and total phosphorous, and higher heterogeneity occurred with more shade and higher pH. Our results revealed that streams in southern Brazil support a very heterogeneous macroinvertebrate community and the role of spatial configuration in these streams strongly influences macroinvertebrate diversity. A riparian width greater than 15 m is necessary to maintain composition and trophic conditions of macroinvertebrate families found in more pristine states of conservation. Furthermore, the results emphasize the importance of β diversity

studies for evaluating the homogenization of macroinvertebrate community in streams impacted by different land uses in southern Brazil.

Keywords: Spatial scale. β diversity. Macroinvertebrate composition. Rural landscape.

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APRESENTAÇÃO

Esta tese foi elaborada como parte dos requisitos necessários para a obtenção do título de Doutor em Biologia (Ciências Biológicas) no Programa de Pós-Graduação em Biologia - ênfase em Diversidade e Manejo da Vida Silvestre da Universidade do Vale do Rio dos Sinos - UNISINOS.

A tese está constituída por uma breve introdução geral, na qual foram consideradas informações sobre os riachos e suas matas ripárias, escalas espaciais e diversidade beta e a ecologia de macroinvertebrados aquáticos; e por três artigos científicos onde são apresentados os principais resultados obtidos de forma a atender aos objetivos e hipóteses do estudo.

O Artigo I, *Reduced riparian zone width compromises aquatic macroinvertebrate communities in streams of southern Brazil* objetivou avaliar se a riqueza de macroinvertebrados e de Ephemeroptera, Plecoptera e Trichoptera (EPT) diminuem com a redução da largura de floresta ripária; a composição de macroinvertebrados e dos grupos tróficos variam com a redução da largura de floresta ripária; e a redução da largura de floresta ripária influencia similarmente a comunidade de macroinvertebrados em diferentes substratos do riacho. Este artigo foi submetido ao periódico *Environmental Monitoring and Assessment*.

No Artigo II, *Does land use influence homogenization of macroinvertebrate communities in streams in southern Brazil?*, o qual foi submetido para o periódico Hydrobiologia, foi analisado se os usos do solo (floresta, agricultura, pastagem e urbanização) alteram as variáveis físicas e químicas nos riachos; e a diversidade β diminui, ou a composição de macroinvertebrados é mais homogênea, em trechos de riachos impactados pelo uso do solo.

No Artigo III, avaliamos as hipóteses de que a diversidade γ não é distribuída homogeneamente entre escalas, com diferenças entre os padrões observados no inverno e verão; e se a diversidade β depende dos tipos de substratos diferentes, mas a maioria da dissimilaridade da composição ocorre em escalas espaciais mais amplas, independente da estação do ano analisada. Este artigo intitulado *Additive partitioning of macroinvertebrate diversity across spatial scales in headwater streams in southern Brazil: a seasonal difference* ainda não foi submetido para periódico.

No modelo de formatação, citações e referências bibliográficas apresentadas nos artigos científicos seguem as normas específicas de cada periódico a ser submetido.

INTRODUÇÃO GERAL

Os Riachos e suas Matas Ripárias

Riachos de cabeceira formam o início do circuito da água de um rio de grande porte e representam ecossistemas de grande importância para a fauna aquática (Allan & Castillo, 2007). As cabeceiras de rios são influenciadas por impactos ocasionados por fontes não pontuais ou difusas. Esses impactos são gerados por diversos fatores associados principalmente à perda da mata ripária (Cooper, 1993; Neumann & Dudgeon, 2002).

A vegetação que ocorre adjacente aos cursos hídricos, chamada de mata ripária, tem sido uma das mais afetadas pela ação antrópica, como reflorestamento, agricultura e construções urbanas. A mata ripária possui papel extremamente importante para a manutenção da saúde dos rios e de suas comunidades biológicas (Sweeney, 1992; Castelle *et al.*, 1994; Lowrance *et al.*, 1997), com funções indispensáveis para os ecossistemas aquáticos, tais como: diminuição do aporte de nutrientes das áreas adjacentes; retenção do solo; diminuição da erosão; diminuição da temperatura da água; aumento do aporte de carbono nos sistemas aquáticos; estruturação do habitat para organismos aquáticos; promoção de microhabitats; e diminuição da amplitude nas variações de níveis de água. Dessa forma, a alteração da estrutura ou composição da mata ripária pode ter influências significativas sobre a qualidade da água e comunidades bióticas (Campbell & Doeg, 1989; Grown & Davis, 1991; Jackson *et al.*, 2001; Fortino *et al.*, 2004).

Os rios e suas matas ripárias estão intimamente ligados por fluxos recíprocos de materiais e organismos (Baxter *et al.*, 2005). Existem fluxos de emergência de insetos aquáticos para a mata ripária e entradas de invertebrados nos riachos, complementares entre as estações do ano (Nakano & Murakami, 2001). Além disso, muitos pesquisadores focaram seus estudos principalmente no papel da entrada de nutrientes, folhas e madeira das matas ripárias para dentro dos riachos (Likens & Bormann, 1974; Vannote *et al.*, 1980; Jackson & Fisher, 1986).

A ausência de mata ripária pode influenciar a disponibilidade de habitats para comunidades de macroinvertebrados aquáticos de diversas maneiras. A perda de vegetação terrestre (Swank *et al.*, 1988) e o aumento da área de superfícies impermeáveis (Changnon & Demissie, 1996) podem influenciar a evapotranspiração e a infiltração nos ecossistemas (Poff *et al.*, 1997). Muitas práticas de uso do solo aumentam as entradas de sedimentos nos riachos,

alterando as características do substrato e reduzindo a diversidade de macroinvertebrados (Quinn *et al.*, 1997). Com a remoção da mata ripária, a radiação solar que atinge o riacho aumenta, podendo haver um aumento na temperatura da água (Quinn *et al.*, 1997) e consequente alteração dos regimes térmicos críticos para a história de vida e ecologia dos macroinvertebrados (Vannote & Sweeney, 1980; Quinn *et al.*, 1994).

A interação de diversos fatores ocasionados pela substituição da mata ripária, como a alteração da hidrologia da bacia, entrada de nutrientes inorgânicos (Johnson *et al.*, 1997), aumento da disponibilidade de luz e aumento da temperatura, podem resultar em um aumento a produção primária do riacho (Webster *et al.*, 1983) e ocasionar alterações na estrutura trófica de comunidades bentônicas (Gurtz & Wallace, 1984).

Os resíduos agrícolas e as atividades urbanas podem aumentar as entradas de nitrogênio e fósforo ao riacho, levando à eutrofização e, portanto, afetar substancialmente a estrutura e funções de suas comunidades bentônicas (Nijboer & Verdonschot, 2004; Allan & Castillo, 2007; Bergfur *et al.*, 2007). Estudos sobre riachos e ecossistemas costeiros têm demonstrado que este aumento de nutrientes pode estimular a atividade e biomassa de microrganismos heterotróficos como bactérias e fungos (Suberkropp *et al.*, 2010). Além disso, a decomposição do folhiço provindo da mata ripária em riachos é estimulada pela condição da microbiota e pelos invertebrados consumidores em riachos com moderado enriquecimento de nutrientes (Rosemond *et al.*, 2002). A decomposição de folhiço por invertebrados parece ser mais sensível à poluição, comparado à decomposição realizada pela microbiota (Pascoal *et al.*, 2003). As densidades de invertebrados atingem seu pico em riachos moderadamente enriquecidos por nutrientes, decaindo sua densidade com o aumento da carga desses nutrientes (Friberg *et al.*, 2011).

A magnitude relativa aos efeitos do uso do solo pode depender da distribuição espacial nas bacias hidrográficas (Allan & Johnson, 1997). A hidrologia da bacia, bem como a disponibilidade de nutrientes inorgânicos em riachos, é muitas vezes relacionada a processos que ocorrem na paisagem terrestre (Hunsaker & Levine, 1995). Por outro lado, a disponibilidade de carbono orgânico em riachos, muitas vezes está relacionada a processos restritos à escala de vegetação adjacente ao riacho (Gregory *et al.*, 1991). Vários estudos têm mostrado que a relação entre as variáveis físico-químicas do riacho e elementos bióticos pode ser influenciada por propriedades da bacia que opera em diferentes escalas espaciais. Richards *et al.* (1996), identificaram que os melhores preditores para a estrutura da assembleia de macroinvertebrados foram características em escala de bacia (geologia e distribuição da

agricultura), morfologia do canal e hidrologia. Por outro lado, Richards *et al.* (1997) mostraram que, apesar da estreita relação entre propriedades em escala de bacia e estrutura do canal e hidrologia, traços característicos de espécies de macroinvertebrados foram correlacionados com fatores a nível de escala local.

Os riachos de cabeceira são compostos por diferentes substratos localizados em corredeiras, onde a velocidade do fluxo é mais alta, e remansos, onde a velocidade do fluxo é mais baixa. O fluxo é um fator de fundamental importância para a biota aquática (Biggs *et al.*, 2005; Brooks *et al.*, 2005), por criar, modificar e eliminar o habitat físico (Biggs *et al.*, 2005). As condições hidráulicas podem influenciar diretamente a biota, limitando os acessos de utilização de habitat (Davis, 1986) e, com isso, exercendo influência sobre a oferta de recursos alimentares particulados, gases dissolvidos e nutrientes para os processos metabólicos (Biggs *et al.*, 2005). Portanto, os macroinvertebrados são fortemente influenciados pela condição hidráulica, que modifica a composição e a distribuição desses organismos (Davis, 1986; Biggs *et al.*, 2005; Brooks *et al.*, 2005).

A complexidade estrutural também é um fator determinante para a distribuição das espécies (Minshall, 1984). Em riachos, diferentes substratos compõem essa complexidade. Alguns estudos indicam variação na comunidade de macroinvertebrados devido à natureza heterogênea de tamanhos de partículas de substrato e à variedade de tipos de substratos em riachos (Erman & Erman, 1984), sendo a distribuição de espécies e de grupos funcionais alimentares de macroinvertebrados determinados por diferentes microhabitats (Huamantinco & Nessimian, 1999; Wood & Sites, 2002).

O Novo Código Florestal Brasileiro (Lei Federal nº 12.651, 25 de maio de 2012)

A lei federal nº 12.651 é a lei brasileira que trata sobre a proteção da vegetação nativa. Desde a década de 90, a proposta de reforma do Código Florestal suscitou em polêmicas entre ruralistas e ambientalistas. Os parlamentares ruralistas, hegemônicos no congresso, vinham atuando em prol de uma redução das faixas mínimas de preservação previstas pelas Áreas de Preservação Permanente (APPs). Os ruralistas também desejavam obter permissão para realizar determinadas culturas em morros, o que é vedado pelas APPs. As zonas de Reserva Legal (RL) também foram focos de debate, uma vez que os ruralistas pretendiam favorecer uma redução das áreas de reserva. Por fim, ambientalistas questionaram a “Anistia dos

Desmatadores”, que deixariam de pagar multas referentes a desmatamentos realizados após a promulgação da Lei de Crimes Ambientais (22 de julho de 2008).

Diferente do Código Florestal de 1965, no novo código, imóveis de até quatro módulos fiscais (unidade de medida agrária variável para cada município) não precisam recompor a RL e há possibilidade de permissão de exploração econômica da RL. Mangues, várzeas, mata de encostas, topos de morro e áreas com altitude superior a 1800 metros podem ser utilizadas para determinadas atividades econômicas. Com relação às matas ripárias, a largura mínima para recomposição de mata reduziu-se a 15 m em rio com área consolidada de APP, além disso, permite a supressão de vegetação em APPs e atividades consolidadas até 2008, desde que por utilidade pública, interesse social ou de baixo impacto ambiental, incluídas atividades agrossilvipastoris, ecoturismo e turismo rural. O novo código também isenta proprietários rurais das multas e sanções previstas na lei em vigor por utilização irregular de áreas protegidas até 22 de julho de 2008.

A sociedade científica posicionou-se sobre a reforma do código florestal de 1965. No Brasil, críticos ou oponentes ao ativismo ambientalista não reconhecem a condição de conhecimento científico na área da ecologia e desqualificam qualquer manifestação de um ecólogo como uma posição farragosa. No caso do Código Florestal, o projeto de reforma foi nitidamente tendencioso e foi gestado sem a participação adequada de pesquisadores de áreas científicas cuja contribuição seria indispensável. Nos documentos da proposta de lei e nas atas de Comissão Especial, confundiu-se a autonomia da ciência ecológica com organizações ambientalistas (Lewinsohn, 2010).

Escalas Espaciais e Diversidade Beta

A escala de observação é um fator de grande importância na ecologia. A dimensão de um habitat ou o tamanho de uma unidade amostral (de uma partícula a uma bacia hidrográfica) e a freqüência ou duração das observações influenciam na identificação de respostas biológicas às variáveis ambientais, na percepção de padrões entre fatores bióticos e abióticos e em quais processos são mais importantes para o funcionamento de sistemas lóticos (Giller & Malmqvist, 1998).

Em riachos, as escalas espaciais e temporais estendem-se a aproximadamente 16 ordens de magnitude (Minshall, 1988), mas podem ser divididas em seis categorias hierárquicas principais, de partícula a sistema de riacho (Figura 1) (Frissel *et al.*, 1986). Grãos

de areia, rochas e folhiço são exemplos de microhabitats, nos quais são medidos em escala de centímetros e persistem por semanas a anos. A escala de habitat é representada pelo sistema de corredeira-remanso. As corredeiras são áreas de fluxo turbulento e os remansos são de fluxos lentos e atingem profundidades maiores. O sistema de trecho (*reach*) inclui de uma a várias seqüências de corredeira-remanso, que abrangem de metros a dezenas ou centenas de metros e persistem em seu local por dezenas ou centenas de anos. Escalas de trecho e secção de riacho mais ampla provavelmente persistem por centenas a milhares de anos, apesar de a posição exata alterar; por exemplo, canais de planície tendem a percorrer pelas planícies de inundação através do tempo. Bacias inteiras (sistemas de riacho) recobrem de dezenas a centenas de km quadrados provavelmente por uma longa história geológica (Giller & Malmqvist, 1998; Allan & Castillo, 2007).

Unidades hierárquicas mais amplas exercem uma considerável influência sobre unidades hierárquicas menores, mas não vice-versa. Por exemplo, sobre uma única rocha, a distribuição de algas é controlada por características de fluxo, turbulência e forças hidráulicas (Ward, 1989). Sob uma escala maior, a biomassa e a estrutura de comunidades de algas podem ser controladas pelos processos de limitação de nutrientes. Em um trecho de riacho mais longo, locais com alta concentração de nutrientes permitem um aumento na biomassa de algas para o consumo de invertebrados ou peixes raspadores (Fisher, 1994). Além disso, a biomassa de algas em trechos longos pode estar associada com o regime de distúrbio de inundação e sombreamento de vegetação ripária (Ward, 1989). O desflorestamento está relacionado com a interação raspador-alga através da luz, níveis de sedimento, etc. (Ward, 1989).

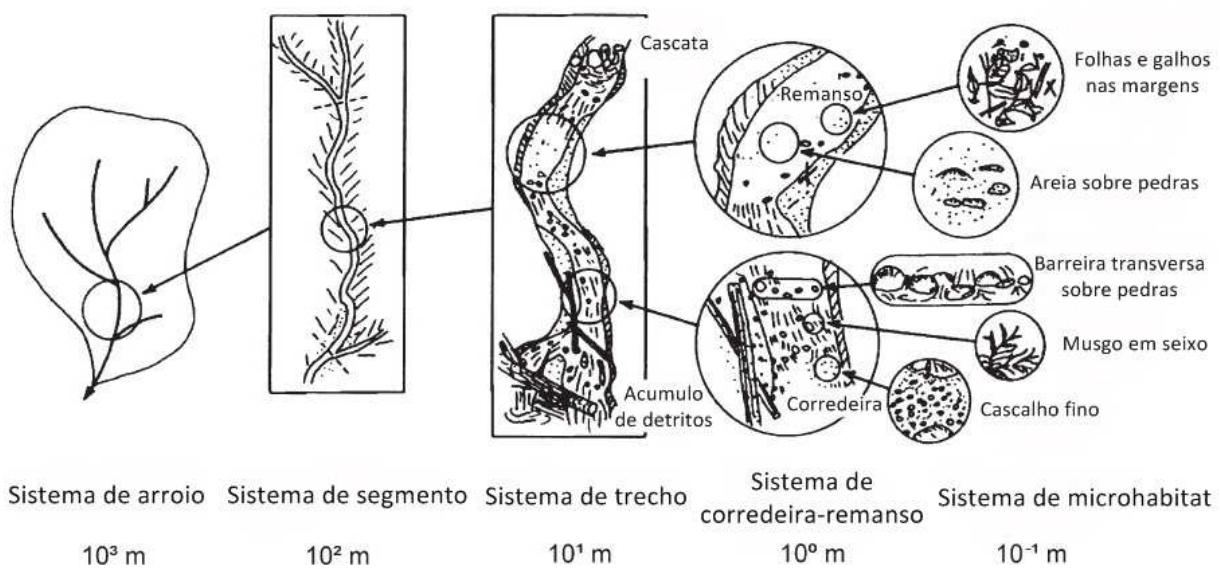


Figura 1. Organização hierárquica de um sistema de riacho ilustrando unidades menores aninhadas em unidades mais amplas (Fonte: Modificado de Frissel *et al.*, 1986).

A comunidade de macroinvertebrados aquáticos pode ser estruturada por processos em múltiplas escalas espaciais (Poff, 1997; Heino *et al.*, 2003; Ligeiro *et al.*, 2010), sendo que os padrões ecológicos observados dentro de uma comunidade local tende a ser diferente dos padrões observados em áreas maiores, como paisagens ou regiões (Crist *et al.*, 2003). Por essa razão, a estrutura da comunidade de macroinvertebrados em riachos tem sido associada com variáveis em escalas a nível local e regional (Heino *et al.*, 2003; Robson *et al.*, 2005; Ligeiro *et al.*, 2010).

Em riachos, existe uma relação do aumento da complexidade estrutural do substrato com o aumento da riqueza de macroinvertebrados (O'Connor, 1991). Muitos estudos têm mostrado que a maior fonte de variação na riqueza e densidade de táxons de macroinvertebrados está entre microhabitats ou unidades amostrais coletadas com Surber (Boyero, 2003; Heino *et al.*, 2004; Townsend *et al.*, 2004). Encontrar padrões de variação da comunidade de macroinvertebrados em outras escalas espaciais é mais variável, no entanto, alguns estudos demonstraram variações significativas entre corredeiras (Downes *et al.*, 1993; Boyero, 2003; Hepp & Melo, 2013), riachos (Hepp & Melo, 2013; Ligeiro *et al.*, 2010) e bacias hidrográficas (Hepp & Melo, 2013).

A diversidade beta tem sido usada para testar padrões dependentes da escala na diversidade de macroinvertebrados em riachos Neotropicais (Ligeiro *et al.*, 2010; Hepp & Melo, 2013). Esse cálculo de diversidade refere-se à diferenciação de comunidades ao longo

de gradientes ou a taxa de substituição de espécies entre determinados locais. A diversidade beta é uma medida de diferenças biológicas entre ambientes, ou seja, pode ser considerada a dissimilaridade entre amostras em termos de composição taxonômica. Tais diferenças podem ser causadas principalmente por limitações de dispersão, geralmente autocorrelacionadas no espaço, e por diferenças no ambiente físico (Legendre *et al.*, 2005).

Whittaker (1960), em um estudo utilizando amostras de vegetação com diferenças na formação geológica e condições de umidade local, definiu a diversidade beta como medida da "mudança na composição da comunidade, ou grau de diferenciação da comunidade, em relação a um gradiente complexo de ambiente, ou um padrão de ambientes" e mais tarde como *turnover* de espécies (Wittaker, 1972). Ele quantificou a diversidade beta de diferentes maneiras, sendo que uma delas foi através da razão entre a diversidade gama (diversidade em um conjunto de unidades amostrais, ou diversidade regional) com a diversidade alfa (diversidade média dentro de unidades amostrais, ou diversidade local) dentro de cada formação geológica.

Recentemente, métodos para o cálculo da diversidade beta têm levado em consideração não apenas as escalas local-regional, mas muitas escalas hierárquicas, como por exemplo, a análise de partição aditiva ou multiplicativa (Crist *et al.*, 2003). Além desses métodos que estimam a diversidade beta, métodos que estudam diretamente fatores que geram a diversidade beta têm sido aplicados em estudos ecológicos. Essas abordagens relacionam fatores ambientais com a diversidade beta com a utilização de, por exemplo, Análise de Correspondência Canônica Parcial e Análise de Redundância Parcial (Legendre *et al.*, 2005). Outra análise recente proposta por Anderson *et al.* (2006) testa diferenças na diversidade beta entre áreas através de uma medida de dissimilaridade média em um grupo de amostras, utilizando medidas de dissimilaridade, como Bray-Curtis e Sorensen.

As diferenças nas condições ambientais e a distância geográfica são os principais fatores que influenciam a diversidade beta de comunidades de macroinvertebrados em riachos. Essa influência ocorre devido à multiplicidade de habitats (corredeiras, raízes submersas, remansos e cachoeiras), submetidos a uma combinação de diversos fatores ambientais, existentes em diferentes escalas espaciais nos riachos, isso se torna fundamental para a determinação de assembleias de macroinvertebrados (Costa & Melo, 2008; Ligeiro *et al.*, 2010).

Ecologia de Macroinvertebrados Aquáticos

Os macroinvertebrados são organismos aquáticos pequenos (maiores que 0,2 milímetros) que vivem nos ecossistemas aquáticos (água doce e marinho) em diversos habitats, como o sedimento, a coluna d'água, as raízes de plantas aquáticas, pedras, galhos e folhas (APHA, 1989). Os macroinvertebrados estão representados por espécies de várias categorias taxonômicas, como por exemplo, platelmintos, anelídeos, moluscos, crustáceos, aracnídeos e insetos aquáticos (Figura 2). Esses organismos vêm sendo amplamente estudados no mundo devido ao seu papel no funcionamento e no diagnóstico da qualidade ambiental de ecossistemas aquáticos (Rosenberg & Resh, 1993).

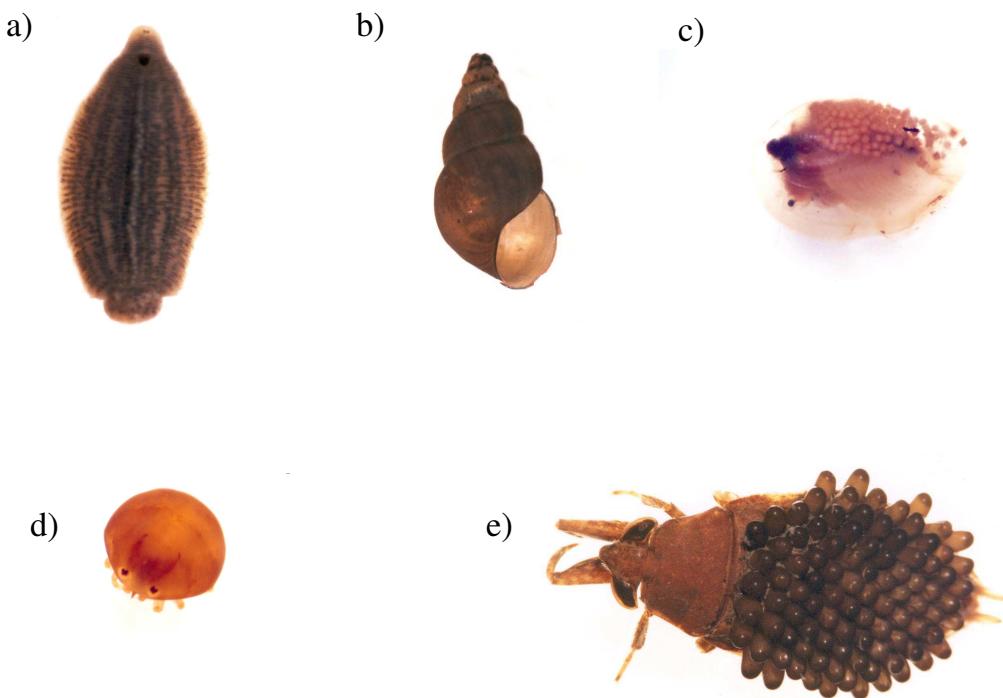


Figura 2. Exemplos da diversidade de grupos taxonômicos que representam os macroinvertebrados aquáticos: a) Anelídeos: Glossiphoniidae (Hirudinea) b) Moluscos: Hydrobiidae (Gastropoda); c) Crustáceos: Conchostraca (Branchiopoda); d) Aracnídeos: Hydracarina; e) Insetos: Belostomatidae

Muitos autores vêm destacando a importância da comunidade de macroinvertebrados no funcionamento dos ecossistemas aquáticos (Rosenberg & Resh, 1993; Esteves, 1998; Boulton & Jenkins, 1998, Richter, 2000).

Dentre as suas funções ecológicas, os macroinvertebrados:

- 1) participam dos processos de decomposição da matéria orgânica (folhas, troncos de madeira e outros organismos aquáticos);
- 2) facilitam a liberação de nutrientes do sedimento para a coluna d'água com seus movimentos. Os organismos remexem ou escavam o sedimento, acelerando a ciclagem de nutrientes;
- 3) são a base alimentar para muitas espécies de peixes e aves aquáticas. A comunidade constitui o elo entre os organismos produtores e consumidores.

Insetos aquáticos

Os insetos aquáticos representam mais de 50% da composição de macroinvertebrados, desempenhando um papel central nas redes tróficas dos ecossistemas aquáticos (Richter, 2000) (Figura 3). Essa grande representatividade deve-se principalmente às adaptações morfológicas e fisiológicas desses organismos, como por exemplo, resistência dos ovos, dieta alimentar variada nos diferentes estágios de vida e a presença de asas que facilitam a dispersão, o acesso ao alimento e a fuga de predadores (Ruppert & Barnes, 1996). Estima-se que existam aproximadamente 45.000 espécies de insetos aquáticos no mundo todo, representadas principalmente pelas ordens Diptera, Coleoptera, Trichoptera, Odonata, Hemiptera e Ephemeroptera (Brönmark & Hansson, 1998; Giller & Malmqvist, 1998).

Os insetos aquáticos podem apresentar metamorfose simples (hemimetábolos) ou completa (holometábolos). A metamorfose simples é dividida em três estágios (ovo, ninfa e adulto) e a completa em quatro estágios (ovo, larva, pupa e adulto). Na metamorfose simples, as ninfas são muito semelhantes aos adultos, como é o caso das ordens Ephemeroptera, Odonata e Hemiptera. Na metamorfose completa, os estágios imaturos (larva e pupa) são muito diferentes do estágio adulto, caracterizando as ordens Diptera, Coleoptera e Trichoptera (Brönmark & Hansson, 1998) (Figura 4).

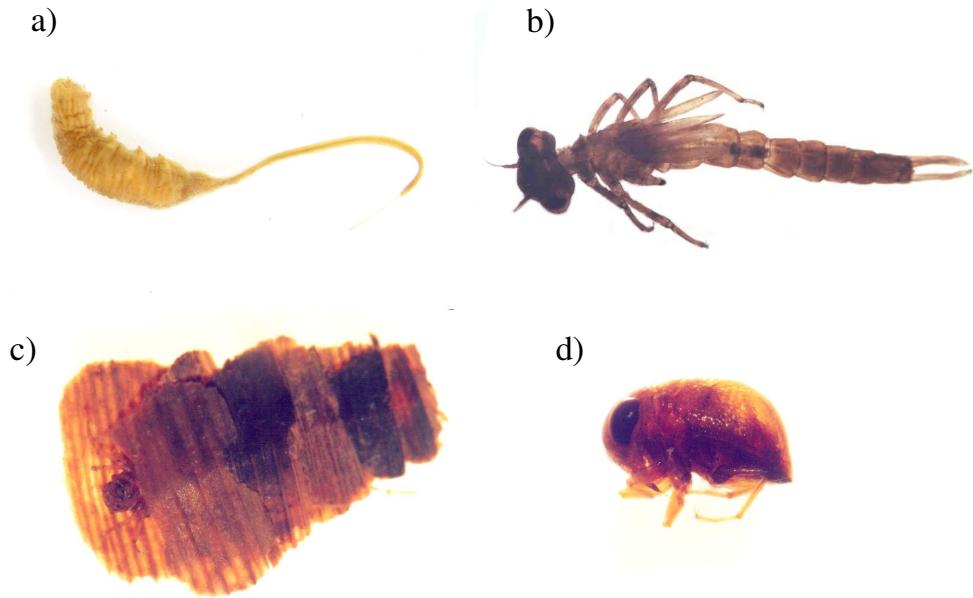


Figura 3. Alguns representantes de famílias de insetos aquáticos: a) Syrphidae; b) Coenagrionidae; c) Calamoceratidae; d) Pleidae

A maioria dos insetos aquáticos vive parte da vida nos ecossistemas aquáticos (estágios imaturos) e parte nos ecossistemas terrestres (estágio adulto) (Nakano & Murakami, 2001) (Figura 4). Entretanto, hemípteros e alguns coleópteros, por exemplo, vivem nos ecossistemas aquáticos durante todo o seu ciclo de vida (Brönmark & Hansson, 1998) (Figura 4).



Figura 4. Estágios imaturos: a) larva (Trichoptera) e b) pupa (Diptera); estágio adulto: c) Coleoptera, e d) Hemiptera

Crustáceos

A diversidade de crustáceos é alta nos ecossistemas aquáticos. Os microcrustáceos estão representados por três grupos principais: Cladocera, Copepoda e Ostracoda (Rocha & Güntzel, 1999). Os Cladocera ou pulgas d'água apresentam hábito bentônico ou planctônico. Enquanto as espécies bentônicas raspam a matéria orgânica da superfície de plantas, as espécies planctônicas são filtradoras, alimentando-se de algas, bactérias e partículas em suspensão (Rocha & Güntzel, 1999). Os copépodes são, na grande maioria, planctônicos, podendo ser filtradores (ordem Calanoida) ou predadores (ordem Cyclopoida). Os Ostracoda (Figura 5) são bastante abundantes em áreas úmidas, e podem ser encontrados no sedimento, na vegetação litoral ou em plantas aquáticas. Alimentam-se de algas, matéria orgânica depositada no sedimento ou de perifítion aderido à superfície de plantas.



Figura 5. Representante de Ostracoda



Figura 6. Dogielinotidae

Os macrocrustáceos mais freqüentemente encontrados nas áreas úmidas são os decápodos dulcícolas: camarões (Palaemonidae), caranguejos (braquiúros e aeglídeos) e lagostins (Parastacidae), e os anfípodos da família Dogielinotidae (Rocha, 2003) (Figura 6). Os representantes de Amphipoda habitam normalmente a vegetação litoral de lagoas e constituem uma importante fonte de alimento para peixes. Os representantes de Decapoda são em geral onívoros e bentônicos, e constituem um dos grupos de invertebrados de maior tamanho e ocorrência em áreas úmidas dulcícolas (Magalhães, 1999).

Ácaros

Os ácaros aquáticos constituem um grupo bastante diversificado que pode ser encontrado nas mais diferentes classes de áreas úmidas, desde rios, lagoas e lagos até represas e arrozais. O hábitat preferencial dos ácaros aquáticos é a região litoral das áreas úmidas, próximo a plantas flutuantes e submersas (Figura 7). As famílias Limnocharidae e Hydrachnidae podem apresentar formas



Figura 7. Ácaros aquáticos (Hydracarina) encontrados em áreas úmidas

parasitas e de vida livre (Forneris, 1999). O ciclo de vida dos ácaros aquáticos é complexo, compreendendo as fases de ovo, larva, ninfa e adulto. No estágio larval, estes organismos podem parasitar larvas de insetos de diversas ordens, principalmente Hemiptera, Diptera e Coleoptera.

Anelídeos

Os oligoquetas e hirudíneos (sanguessugas) caracterizam-se pela sua alta densidade em áreas úmidas. São muito importantes na dinâmica de nutrientes, com participação ativa na decomposição da matéria orgânica. Os anelídeos alimentam-se de algas e de todo tipo de detritos, sedimentos e vegetais (Lino *et al.*, 2004).

As famílias Tubificidae e Naididae são os representantes mais abundantes que compõem a fauna de oligoquetas aquáticos de lagos brasileiros (Figura 8). Os tubificídeos conseguem sobreviver em condições de baixa concentração de oxigênio devido à presença de pigmentos semelhantes à hemoglobina. Essa característica favorece sua predominância em ecossistemas com alta poluição orgânica. Enquanto os tubificídeos vivem preferencialmente no sedimento, os naidídeos encontram-se freqüentemente na vegetação submersa (Lino *et al.*, 2004).



Figura 8. A família Tubificidae representa, geralmente, a maior biomassa bentônica em substratos lodosos, argilosos ou arenosos

Os hirudíneos ou sanguessugas são freqüentes em áreas úmidas com grande quantidade de plantas aquáticas, podendo viver próximos ao sedimento ou na vegetação submersa (Lino *et al.*, 2004) (Figura 9). Esses animais são predadores ou ectoparasitas hematófagos de vertebrados. A hirudina é uma substância anticoagulante de importância farmacêutica produzida pelas glândulas das sanguessugas.

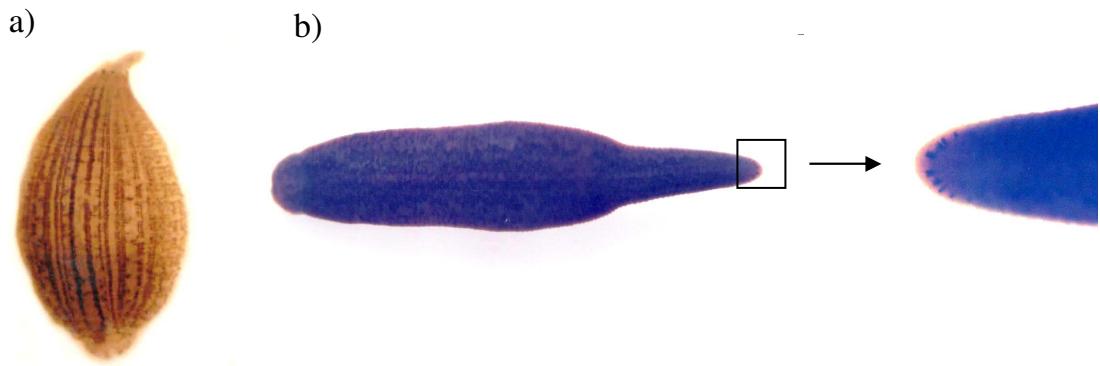


Figura 9. Hirudíneos: a) família Glossiphoniidae; b) família Hirudinidae, com destaque para a disposição dos olhos

Moluscos

Os moluscos de água doce são representados principalmente pelos gastrópodos e bivalves. Os gastrópodos estão entre os organismos mais importantes das áreas úmidas, tanto pela diversidade de espécies como pelo volume de biomassa (Simone, 1999). Os gastrópodos

alimentam-se de algas perifíticas e detritos aderidos aos substratos aquáticos. Os gastrópodos também são muito estudados em pesquisas médico-sanitárias, pois muitas espécies são vetores de doenças parasitárias, como por exemplo, a esquistossomose, doença transmitida por um verme que utiliza os caramujos do gênero *Biomphalaria* como



Figura 10. Planorbidae

hospedeiro intermediário (Figura 10). No Brasil, os bivalves de água doce estão representados principalmente por três famílias: Myctopodidae, Hyriidae e Sphaeriidae (Avelar, 1999) (Figura 11). Os bivalves são organismos filtradores, que se alimentam de algas microscópicas, bactérias e detritos suspensos na água, vivendo enterrados em fundos lodosos ou areno-lodosos de áreas úmidas lóticas e lênticas. Atualmente, muitas espécies de bivalves de água doce estão ameaçadas no Rio Grande do Sul devido à poluição e destruição dos mananciais (Fontana *et al.*, 2003).



Figura 11. Sphaeriidae

Platelmintos turbelários

Os turbelários são platelmintos de vida livre e, dentro deste grupo, os tricladídos dulceaquícolas são muito abundantes em áreas úmidas. Esses platelmintos desempenham papel como predadores, alimentando-se de outros invertebrados, tais como larvas de insetos, crustáceos e oligoquetas. A competição pelo alimento é um dos principais fatores que determina a distribuição e abundância das espécies de turbelários nos ecossistemas aquáticos (Reynoldson & Bellamy, 1971). Por serem sensíveis à luz, as formas de água doce podem freqüentemente ser encontradas aderidas a macrófitas ou na parte inferior de pedras em reservatórios, rios e lagos.

Grupos tróficos funcionais

De acordo com a natureza do alimento e seu modo de captura (relacionado ao tipo de aparelho bucal), os macroinvertebrados podem ser classificados em diferentes grupos tróficos funcionais (Cummins, 1973; Merritt & Cummins, 1996; Callisto *et al.*, 2001; Callisto & Gonçalves Jr., 2002, Lino *et al.*, 2004):

- 1) coletores (alimentam-se de matéria orgânica dissolvida na água ou no substrato): de acordo com o seu mecanismo de alimentação podem ser detritívoros, filtradores, coletores e catadores; as famílias Baetidae e Caenidae (Ephemeroptera) (Figura 12) são exemplos deste grupo trófico funcional;
- 2) raspadores (alimentam-se de material aderido ao substrato, como perifítion, que é formado por algas e microorganismos): podem ser raspadores de superfície mineral ou orgânica, como por exemplo, as famílias Ampullaridae (Figura 13) e Planorbidae (Gastropoda);
- 3) fragmentadores (alimentam-se de matéria orgânica particulada grossa, formada por fragmentos vegetais maiores, como folhas): podem ser herbívoros, detritívoros ou minadores; as famílias Tipulidae (Diptera) (Figura 14) e Calamoceratidae (Trichoptera) (Figura 15) são exemplos de macroinvertebrados fragmentadores;



Figura 12. Nálide



Figura 13. Ampullaridae



Figura 14. Tipulidae



Figura 15. Casinha de Leptoceridae

- 4) predadores (alimentam-se de organismos vivos): engolem as presas inteiras ou aos pedaços; os hemípteros (Figura 16), as náïades de todas as famílias de Odonata (Figura 17), as famílias Gyrinidae (Coleoptera) e Tabanidae (Diptera) são alguns exemplos de predadores. A maior parte dos hemípteros alimenta-se de matéria orgânica líquida, sendo bastante freqüentes em ecossistemas lênticos e em remansos de rios com vegetação aquática abundante;

16)



17)



Figuras 16 e 17. Representantes de predadores das famílias Gerridae e Aeshnidae

- 5) parasitas (alimentam-se de organismos vivos): vivem interna ou externamente no corpo de outros organismos vivos; os hirudíneos são exemplos de organismos ectoparasitas (Figura 18).



Figura 18. Representante de Glossiphoniidae

Algumas famílias de macroinvertebrados podem ter representantes de diferentes grupos tróficos, como é o caso das larvas da família Chironomidae (Diptera), que podem ser coletores (Figura 19a), raspadores, fragmentadores e predadores (Figura 19b), consumindo uma ampla variedade de alimentos (algas, detritos, macrófitas, animais, etc).

a)



Figura 19. Representante das subfamílias: a) Chironominae (coletor) e b) Tanypodinae

A composição e a diversidade de grupos tróficos funcionais são indicadores da disponibilidade dos recursos alimentares e da diversidade de habitats de um ecossistema aquático (Merritt & Cummins, 1996). Os grupos tróficos funcionais são freqüentemente usados em estudos de impacto ambiental em ecossistemas aquáticos (Silveira, 2004). Por exemplo, com a retirada da mata ripária e assoreamento do leito de um rio, alterações na abundância de determinados grupos são esperadas em função da menor disponibilidade de fonte de alimento alóctone provindo da vegetação ou da maior quantidade de matéria orgânica provinda das margens do rio. Após o desmatamento da vegetação ripária de um rio, macroinvertebrados coletores e filtradores tornam-se mais abundantes pela maior entrada de matéria orgânica suspensa causada pela erosão das margens e assoreamento do rio, e macroinvertebrados raspadores tornam-se mais abundantes pela maior incidência de calor e luminosidade (Cummins *et al.*, 1989; Cummins, 2002).

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ARTIGO I

Reduced riparian zone width compromises aquatic macroinvertebrate communities in streams of southern Brazil

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Abstract

Riparian buffer width is an important parameter for maintaining the functions and values of riparian forests and the health of aquatic communities. A reduced riparian zone can negatively affect the macroinvertebrate community. Therefore, the study assessed whether: I) macroinvertebrate richness and EPT (Ephemeroptera, Plecoptera and Trichoptera) richness decrease with reduced riparian zone width; II) macroinvertebrate and functional feeding group composition vary with a reduced riparian zone width and III) reduced riparian zone width similarly influence the macroinvertebrate community in different stream substrates. We selected three fragments with different riparian forest widths in three secondary streams in the Sinos River watershed, northeastern southern Brazil. A total of 42,633 individuals from 71 families were found throughout the study. Our results show that reducing the width of the riparian zone altered neither the number of families of macroinvertebrates nor families of EPT orders. However, EPT richness was greatest in the substrates stone and gravel, independent of riparian zone width. There was significant difference in macroinvertebrate composition among riparian zone widths. Furthermore, FFG (functional feeding group) composition differed marginally among the riparian zone widths. The macroinvertebrate composition and FFG differed among substrates, independent of riparian zone width. This study showed that reducing the riparian zone to between five and 15 m was enough to alter the macroinvertebrate community, and a width greater than 15 m is necessary to maintain composition and trophic conditions of macroinvertebrate families found in more pristine states of conservation.

Keywords: Riparian forest; Buffer; Aquatic community; Substrate; Conservation biology

Introduction

Riparian forest is vital to the maintenance and integrity of streams and their biological communities (Sweeney 1993; Allan and Castillo 2007). Riparian vegetation stabilizes soil on river banks, controls erosion and sedimentation, provides shade, moderates stream temperature and light, regulates primary production and filters sediments and nutrients leached from agriculture (Osborne and Kovacic 1993; Sweeney 1993). Furthermore, riparian forest provides food and habitat for aquatic communities through the input of fine and large organic debris (Sweeney 1993; Wallace et al. 1997; Allan and Castillo 2007).

Riparian buffer width is an important parameter for maintaining the functions and values of riparian forests and biodiversity conservation policies (Marczak et al. 2010). A buffer width of 100 m has been suggested as necessary to protect the ecological functions of riparian forests (Keller et al. 1993). However, a width of 30 m has become standard in many jurisdictions of Canada and the United States (Lee et al. 2004). Furthermore, a riparian forest width that protects aquatic resources may not be appropriate for maintaining terrestrial riparian species at levels comparable to undisturbed sites (FEMAT 1993). In general, widths recommended for protection of terrestrial riparian components were wider (100-200 m) than those recommended for aquatic ones (Lee et al. 2004). Many studies show that a width < 30 m is not enough to maintain viable populations of various terrestrial species, especially riparian fauna that prefer interior habitats, such as amphibians, birds and dung beetles (Marczak et al. 2010, Viegas et al. 2014).

In Brazil, the New Forest Law (Código Florestal nº 12.651, 2012) has reduced the legal riparian forest width of small farms from 30 m to 15 m or less (5 m). The critical point of this new law is that the conservation of riparian forest for small properties will not vary according to the width of the river, but according to the size of the rural property, which has caused great disagreement between farmers, ranchers and conservation biologists. The new legislation places the floodplains of large rivers at risk, including the many aquatic and terrestrial species that need these ecosystems to survive.

The ecological integrity of streams is affected by anthropogenic activities associated with the loss of riparian forest (Boyero and Bailey 2001; Neumann and Dudgeon 2002). Riparian forest removal changes the structure and physical-chemical water parameters of streams (Neumann and Dudgeon 2002; Allan 2004), which leads to changes in both

ecosystem processes and the composition and structure of aquatic communities (Lammert and Allan 1999; Niyogi et al. 2003; Johnson et al. 2007).

Aquatic macroinvertebrates play a central ecological role in streams and are fundamental to the dynamics of nutrients, transformation of organic material and energy flow (Callisto and Esteves 1995). Additionally, they are used as bioindicators of water quality since some groups respond differently to the level of contamination of aquatic ecosystems (Richter 2001). The absence of riparian forest could alter macroinvertebrate habitat conditions through erosive processes, sedimentation, modified hydrological regime, altered substrate (Quinn et al. 1997), entrance of inorganic nutrients (Johnson et al. 1997), increased light availability, increased temperature and increased primary productivity (Webster et al. 1983). These alterations change the macroinvertebrate trophic structure, which increases the abundance of periphyton consumers in streams whose riparian zones are 10 to 30 m wide (Kiffney et al. 2003). Reducing the riparian zone to less than 30 m could reduce the diversity of these organisms (Newbold et al. 1980). Studies in Brazil that have related the substitution of riparian zone by different land uses (urbanization, agriculture and pasture) have found reduced macroinvertebrate richness and modified composition (Roque et al. 2003; Nessimian et al. 2008; Hepp and Santos 2009; Hepp et al. 2010). However, studies that analyze the effect of a reduced riparian zone on macroinvertebrates in this region are still lacking. These works would be fundamental in guiding public politics related to reduced riparian zones in Brazil.

Assuming that reduced riparian zone width alters physical and chemical parameters of streams such as depth, width, shade, pH, temperature, water velocity, turbidity, dissolved oxygen and nutrients (N and P), and that this affects macroinvertebrate communities, our hypotheses are I) macroinvertebrate richness and EPT (Ephemeroptera, Plecoptera and Trichoptera) richness, which are often used as indicators of water quality (Rosenberg and Resh 1993), will decrease with reduced riparian zone width; II) macroinvertebrate and functional feeding group composition will vary with a reduced riparian zone width. The quantity of scrapers should increase because the absence of riparian vegetation leads to greater light incidence, which increases primary production, and the shredders will decrease since they feed on allochthonous plant fragments (Li and Dudgeon 2008). A reduced quantity of EPT families should also occur. We also tested whether reduced riparian zone width similarly influenced the macroinvertebrate community in different stream substrates (gravel, stone and leaf litter) since anthropogenic impacts on the macroinvertebrate community are more evident in some substrates or specific habitats (Roy et al. 2003; Buss et al. 2004).

Materials and Methods

Study area

The study took place in the Sinos River watershed, northeastern southern Brazil between 29° and 30° S. The watershed is in the most southern extent of the Atlantic Forest biome and is one of the main basins of southern Brazil. It has an area of approximately 4,000 km² and includes 32 towns and cities. Land use in the watershed consists mainly of fields and pastures (48.4%), forests (28.15%) and agricultural areas (8.78%) (Oliveira et al. 2013). The climate of this region is subtropical humid and the average annual rainfall varies from 1,200 to 2,000 mm. The Sinos River is located in one of the main regions of urban development (IBGE 2010). It is approximately 190 km long, and while its headwaters are at an elevation of 900 m, its mouth is 5 m above sea level (Rolon et al. 2003).

We selected three fragments with different riparian forest widths in three secondary streams for a total of nine 400 m long fragments. We classified the fragments according to the width of the riparian forest: 1) fragments wider than 40 m on both stream margins (Area > 40 m); 2) fragments of riparian vegetation with a width of 15 to 30 m on both stream margins (Area < 30 m); and 3) fragments of riparian vegetation with a width of 5 to 15 m on both stream margins (Area < 15 m) (Fig. 1).

In fragments with a riparian zone wider than 40 m on both stream margins (Area > 40 m), the riparian vegetation extends up to 1,000 m wide on one side of the stream and varies from 210 to 440 m wide on the other side. The fragments chosen for this study were non-contiguous, and the minimum distance between them was 1 km to increase the independence of the sampled areas. The land use type in all adjacent areas outside of the vegetation fragments is similar and includes small fields used for subsistence agriculture (crops and animals needed by the family to feed themselves during the year).

Sampling

Macroinvertebrates were collected in two sampling periods (winter of 2010 and summer of 2011) with a 30 x 30 cm Surber (250 µm mesh) and a 30 cm diameter frame dip net (250 µm mesh). The Surber was used to collect macroinvertebrates from the stones (20-28 cm) and gravel (1-6.5 cm) substrates. The Surber method consists of removing stones or gravel and collecting the material that is stirred up in the mesh. The dip net was used to collect from the leaf litter substrate. Two one-meter sweeps were carried out in the submerged

litter. A total of 36 samples were taken from each area from three different substrates (stone - 12, gravel -12 and litter - 12).

Macroinvertebrates were stored in 80% ethanol and taken to the laboratory, where the samples were washed in a 0.25 mm mesh sieve. The macroinvertebrates were identified to family level, which is a taxonomic resolution considered to be sufficient for evaluating different levels of anthropogenic impact (Heino and Soininen 2007; Melo and Hepp 2008). The functional feeding groups were based on Merritt and Cummins (1996) and Cummins et al. (2005). The specimens were processed and identified with an optical stereomicroscope, then placed in glass tubettes with 80% ethanol. A specialized bibliography was consulted for identification (Lopretto and Tell 1995; Merritt and Cummins 1996; Fernández and Dominguez 2001). The samples were stored in the Laboratory of Ecology and Aquatic Ecosystem Conservation in the Sinos River Valley University (Universidade do Vale do Rio dos Sinos – UNISINOS).

Environmental variables

Altitude, water depth, stream width, shade, pH, temperature, velocity, turbidity, conductivity and dissolved oxygen were measured *in situ* in the nine transects. Dissolved oxygen, temperature, pH, conductivity and turbidity were measured with a Horiba H-10 multi-parameter water quality checker. Water velocity was measured with a Marsh-McBirney Flow Mate 2000 portable velocity meter. Stream width and shade (classification by % canopy cover) means were calculated from transects placed along each sampling area. In each transect, three equidistant points and a point in the deepest part of the stream were determined. Water velocity and depth was measured at each point and the mean was calculated for each parameter. Water samples were collected from each stream fragment to analyze total phosphorus (P) and ammoniacal nitrogen (NH₃-N) according to Eaton et al. (2005).

Data analysis

Species accumulation curves based on the lowest number of individuals found in a sample were created to compare rarefied richness between stream fragments and substrates (Gotelli and Colwell 2001). Sample units obtained in a single type of substrate from a stream fragment with the same size riparian forest were combined into one sample. Since the

sampling method varied among substrates, the samples used in the multivariate analyses were standardized by dividing the abundance of each taxon in the sample by the total abundance of the sample.

Statistical analyses were performed with the means of the environmental variables between the two sampling periods. The environmental variables ammoniacal nitrogen ($\text{NH}_3\text{-N}$) and total phosphorus (P) were log-transformed to obtain normal data distribution. A one-way variance analysis (ANOVA) was used to verify differences in the environmental variables among the different riparian zone widths.

Differences in macroinvertebrate richness and Ephemeroptera, Plecoptera and Trichoptera (EPT) richness among riparian zone widths, type of substrate and sampling period were tested using repeated-measures ANOVA. Levene's test verified the homogeneity of variances, and Mauchly's sphericity test acknowledged the sphericity assumption. Assuming compound symmetry (homogeneity of the variance-covariance matrix), no adjustment was made for the F test. A *post hoc* Tukey test was used to verify which variations differed most.

Multivariate analyses were performed with the macroinvertebrate abundance means between the sampling periods for each stream fragment ($n=27$). A non-metric multidimensional scaling (NMDS) analysis was used to evaluate the variation of the macroinvertebrate community and functional feeding groups (FFG) among the samples from the nine stream fragments sampled. The analysis was performed with the Bray-Curtis distance with the least number of axes possible and a low stress. Variations of the macroinvertebrate composition among riparian zone widths and types of substrate were tested with a permutational multivariate two-factor variance analysis (PERMANOVA) with Bray-Curtis distance and 9999 permutations. The variation of each family among riparian zone widths was verified with an indicator species analysis (9999 interactions). All of the analyses were carried out in R ver 3.0.1 using the vegan and labdsv packages (R Development Core Team 2013), except for the repeated measures ANOVA and Tukey test, which were carried out on the program PASW version 18 (SPSS Inc. 2009).

Results

The environmental variables analyzed did not vary among streams with different riparian zone widths, except for altitude ($F=7.522$, $GL=2$, $P=0.023$), which varied from 63 to

189 m (Table 1). A total of 42,633 individuals from 71 families were found throughout the study. A total of 15,504 individuals from 62 families were collected from streams with the smallest riparian zone (<15 m), followed by 13,948 individuals from 65 families in streams with intermediate riparian zone (<30 m) and 13,181 individuals from 64 families in streams with the largest riparian zone (>40 m). EPT abundance was 7,090 individuals in streams with the smallest riparian zone. In streams with a riparian zone less than 30 m and greater than 40 m, EPT abundances were 8,076 and 7,701 individuals, respectively.

Insecta was the most representative class (88.6% of the individuals collected), followed by Gastropoda and Bivalvia, which each represented 9.5%. Proportions of individuals of macroinvertebrate groups were similar between streams with the largest riparian zone. Streams with the smallest riparian zone had a slightly higher proportion of Coleoptera and a considerably higher proportion of Mollusca than the other streams. We observed a reduced amount of Diptera (Ceratopogonidae and Simuliidae), Plecoptera and Trichoptera in streams with the least amount of riparian forest (Fig. 2).

Collectors accounted for 48.79% of the individuals found in the streams with the largest riparian zone, followed by shredders (19.56%) and scrapers (16.77%). In streams with an intermediate riparian zone, 53.41% of the individuals were collectors, followed by shredders (18.32%) and scrapers (15.50%). In streams with the smallest riparian zone, collectors accounted for 44.10% of the individuals, followed by scrapers (31.79%) and filterers (10.32%). All of the substrates had a greater proportion of collectors. The substrate gravel had a lower proportion of shredders (9%) than stone (14.3%) and litter (21.8%), and a greater proportion of shredders (26.6%) than stone (21.2%) and litter (18.4%). Stone had a higher proportion of filterers (12.3%) than gravel (7%) and litter (2%).

Macroinvertebrate richness did not vary among different riparian zone widths, substrates and sampling periods, and there was no interaction between variables (Table 2). However, we observed a tendency of reduced richness in streams with the smallest riparian zone, particularly in the leaf litter substrate (Fig. 3). EPT richness did not vary among riparian zone widths and between sampling periods, and there was no interaction between variables (Table 3). We also observed a tendency of reduced richness in streams with the smallest riparian zones. EPT richness was greatest in the substrates stone and gravel, independent of riparian zone width. EPT richness did not vary between stone and gravel substrates ($P=0.861$) (Fig. 4).

Dissimilarity of macroinvertebrate (*stress* = 0.1478) and FFG (*stress* = 0.0959) composition of the samples of the nine areas (n=27) was represented by two axes in the multidimensional scaling and organized by riparian zone width and substrate type by polygons (Fig. 5). The NMDS graph showed that macroinvertebrate composition varied among riparian zone widths, and streams with smallest and largest riparian zones differed the most in the multivariate space. Among the types of substrate, the macroinvertebrate composition of leaf litter was the most dissimilar of the three substrate types. There were also differences in FFG among riparian zone widths in the ordination graph, but dissimilarities overlapped more, and even so, streams with a <15 m riparian zone were more dissimilar than the other riparian widths. Dissimilarity of FFG in axis 2 of the graph showed that stone and gravel were the most distinct among the types of substrate (Fig. 5).

The PERMANOVA indicated significant differences in macroinvertebrate composition among riparian zone widths and substrates. There was no interaction between macroinvertebrate composition and riparian zone widths or types of substrate (Table 4), which means that composition varies among substrates regardless of riparian zone width and vice-versa, or that variation in composition among substrates is similar over the riparian zone gradient. FFG composition also differed among substrates. FFG differed marginally among the riparian zone widths. There was no interaction between FFG and riparian zone or substrate (Table 5).

Indicator Species analysis of macroinvertebrate composition indicated that gastropods of family Lymnaeidae ($IV=0.6635$; $P=0.001$) and tricopterans of families Helicopsychidae ($IV=0.7191$; $P=0.005$) and Hydrobiosidae ($IV= 0.5341$; $P=0.013$) were more associated with streams with a larger riparian zone. Tricopterans of family Odontoceridae ($IV= 0.5189$; $P=0.011$), hemipterans of family Gerridae ($IV=0.3926$; $P=0.023$) and ephemeropterans of family Baetidae ($IV= 0.4259$; $P=0.049$) were associated with streams with an intermediate riparian zone. Family Hydrobiidae ($IV=0.7630$; $P=0.006$) was associated with streams with the smallest riparian zone.

Discussion

Studies in neotropical streams indicate that removing the riparian forest or substituting the forest with different land uses reduces macroinvertebrate richness (Roque et al. 2003;

Nessimian et al. 2008; Hepp and Santos 2009; Mesa 2010). Our results show that reducing the width of the riparian zone altered neither the number of families of macroinvertebrates nor families of EPT orders. Studies that evaluate total or almost total substitution of the riparian zone by other land use in Neotropical streams have found significant differences in macroinvertebrate richness (Buss et al. 2004; Hepp and Santos 2009; Lorion and Kennedy 2009; Hepp et al. 2010). These studies showed that the removal and replacement of riparian vegetation by different land uses altered the physical and chemical parameters of streams related to water quality. In our study, the physical and chemical parameters remained similar among the different riparian zone widths. A greater impact on riparian forest (removal or total replacement) and resulting changes in physical and chemical characteristics of streams could reduce the macroinvertebrate richness.

The importance of the type of substrate in determining macroinvertebrate richness and composition in Neotropical streams is widely known (Buss et al. 2004; Costa and Melo 2008). Many families are able to colonize more than one substrate, but the results of this study show that these substrates had distinct macroinvertebrate communities. EPT richness also differed among types of substrate and was highest in stone and gravel. Differentiation of these families may have resulted from similar adaptations to habitat conditions such as quality and quantity of organic material and substrate stability, which is determined by the water current (Logan and Brooker 1983).

In southeastern Brazil, rapids substrates had greater macroinvertebrate richness, and habitat degradation influenced macroinvertebrates in different substrates (Buss et al. 2004). In the present study, there was no significant interaction between riparian zone width and type of substrate, which shows that macroinvertebrate community in the different riparian zone widths varied similarly among the substrates analyzed. Reduction of the riparian zone width had a similar impact on macroinvertebrate richness and composition among the substrates stone, gravel and litter.

Reducing the riparian zone altered macroinvertebrate composition, which indicates a greater impact on macroinvertebrate families in areas with smaller riparian zones. Streams with the smallest riparian zones had more scrapers, especially from family Hydrobiidae, which is an indicator of this type of disturbance. This FFG is a predominant group in deforested areas, where there is less allochthonous material and greater luminosity, which leads to increased production of autochthonous resources through primary production, such as

periphyton (Li and Dudgeon 2008). Although the shade of the study areas did not differ significantly, the gradient analyzed (variation from 79.5% to 20.5%) suggests a trend of increasing luminosity in areas with smaller riparian zones. Moreover, areas with large and intermediate riparian zones had three tricopteran families and one ephemeroptera family as indicators, which are more sensitive to degradation. There were also more shredders in the areas with larger riparian zones (Area > 40 m = 2.578, Area < 30 m = 2.555 and Area < 15 m = 1.283). Studies in southeastern Brazil have shown a lower quantity of shredders in areas with low availability of allochthonous materials due to deforestation (Silveira et al. 2006).

The effect of reduced riparian zone vegetation on the macroinvertebrate community was tested in tributaries within the watershed of this study. These tributaries were nearby and had similar geomorphology and water physical and chemical characteristics. Altitude was the only environmental parameter that differed among the study areas, even though the variation (between 63 and 189 m) is considered too low to influence our results. Macroinvertebrates do not usually respond to altitude variation of lower magnitude. A similar macroinvertebrate composition has been found in areas with low elevation (< 200 m) (Henriques-Oliveira and Nessimian 2010). Streams at altitudes of less than 200 m are characterized by taxa of potamal warm waters (Huamantinco 2004). All of the areas in our study were low altitude and the differences among the streams should be attributed mainly to the reduced riparian vegetation zone.

Since streams are organized in spatial-temporal hierarchies, species are influenced by local and riparian variables of the stream, watershed characteristics and regional environmental factors (Poff 1997). Riparian zone buffer functions vary according to land use patterns at a watershed level (Allan 2004). For this reason, this study focused on effects at a precise scale within the same watershed in order to eliminate the potential influence of diffused environmental factors on the macroinvertebrate community in the study areas. Many studies show that the macroinvertebrate community is more highly influenced by local riparian conditions than regional ones (Lammert and Allan 1999; Sponseller et al. 2001).

Studies in temperate streams relate different forest widths and morphologies, which vary according to specific conditions of the locations, with the conservation of biological communities (Lee et al. 2004; Chizinski et al. 2010). However, the vegetation width, composition and density that are most effective for conserving aquatic communities are still poorly understood (Broadmeadow and Nisbet 2004; Lee et al. 2004). The establishment and

maintenance of riparian forest buffers has been suggested as an important practice for avoiding negative effects on physical, chemical and biological conditions of streams (Sweeney 1993; Kiffney et al. 2003).

Our results suggest that a reduced riparian zone significantly modifies macroinvertebrate composition, and trophic conditions in these areas differ from those found in areas where the riparian zone is larger. The New Forest Law (Código Florestal nº 12.651, 2012) reduced the legal riparian forest width of small farms from 30 m to 15 m or less (5 m) in Brazil. However, this study showed that reducing the riparian zone to between five and 15 m was enough to alter the macroinvertebrate community, and a width greater than 15 m is necessary to maintain composition and trophic conditions of macroinvertebrate families found in more pristine states of conservation. However, further studies are necessary to understand how a reduced riparian zone influences aquatic macroinvertebrate community structure and dynamics. The development of management strategies for riparian forests is of the utmost importance to preserve these aquatic ecosystems since due to the loss of riparian forests, these are the most threatened ecosystems on the planet.

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Table 1 Mean values (range) of environmental variables in Area > 40 m, Area < 30 m and Area < 15 m in southern Brazil.

	Area > 40 m	Area < 30 m	Area < 15 m
Altitude (m a.n.m.)	146 (132-189)	108 (93-133)	65.7 (63-70)
Width (m)	9.1 (6.3-12.2)	11.5 (10.4-12.7)	14.0 (11.7-17-3)
Shade (%)	69.3 (51.7-79.5)	43.3 (35.5-53.3)	38.3 (20.5-53-5)
Depth (m)	0.38 (0.36-0.41)	0.40 (0.34-0.45)	0.43 (0.31-0.56)
Velocity (m/s)	0.32 (0.27-0.34)	0.26 (0.21-0.28)	0.26 (0.18-0.29)
Temperature (°C)	18.7 (15.7-20.3)	19.4 (16.8-20.6)	20.0 (17.9-21.4)
Dissolved oxygen (mg/L)	10.7 (10.3-10.9)	10.6 (9.7-11.8)	10.2 (9.8-10.4)
pH	7.4 (7.0-7.7)	7.4 (7.0-7.6)	7.6 (7.2-7.8)
Turbidity (NTU)	8.9 (6.2-11.8)	8.3 (6.3-10.5)	7.0 (5.9-8.5)
Conductivity (ms/cm)	0.04 (0.04-0.05)	0.05 (0.04-0.05)	0.05 (0.04-0.05)
NH ₃ -N (mg/L)	0.32 (0.23-0.42)	0.76 (0.3-1.42)	0.69 (0.19-1.46)
P (mg/L)	0.03 (0.02-0.03)	0.03 (0.02-0.03)	0.04 (0.03-0.06)

Table 2 Results of repeated measures ANOVA for macroinvertebrate richness in the study reaches, with area type (Area > 40 m, Area < 30 m and Area < 15), sample period (summer and winter) and substrate type (stone, gravel and litter).

Richness	d.f.	F	P
Sample period (P)	1	1.551	0.229
Area type (A)	2	1.147	0.34
Substrate type (S)	2	2.618	0.1
P * A	2	0.791	0.469
P * S	2	0.146	0.865
T * S	4	0.082	0.987
P * A * S	4	0.32	0.861

Table 3 Results of repeated measures ANOVA for Ephemeroptera, Plecoptera and Trichoptera (EPT) richness in the study reaches, with area type (Area > 40 m, Area < 30 m and Area < 15), sample period (summer and winter) and substrate type (stone, gravel and litter).

EPT richness	d.f.	F	P
Sample period (P)	1	0	0.994
Area type (A)	2	2.066	0.156
Substrate type (S)	2	6.784	0.006
P * A	2	0.72	0.5
P * S	2	0.038	0.963
T * S	4	0.416	0.795
P * A * S	4	0.544	0.706

Results in bold are significant at P<0.05.

Table 4 Results of PERMANOVA test for the effects of area type (Area > 40 m, Area < 30 m and Area < 15 m) and substrate type (stone, gravel and litter) on the taxonomic composition of macroinvertebrate community in study reaches in southern Brazil.

	d.f.	SS	MS	F	P
Area type (A)	2	0.25038	0.12519	1.8828	0.0296
Substrate type (S)	2	0.67384	0.33692	5.0669	0.0001
A * S	4	0.16832	0.04208	0.6328	0.9494
Residual	18	1.19688	0.06649		

Results in bold are significant at P<0.05.

Table 5 Results of PERMANOVA test for the effects of area type (Area > 40 m, Area < 30 m and Area < 15 m) and substrate type (stone, gravel and litter) on the functional feeding groups (FFG) composition of macroinvertebrate community in study reaches in southern Brazil.

	d.f.	SS	MS	F	P
Area type (A)	2	0.08414	0.042072	2.15034	0.0867*
Substrate type (S)	2	0.10199	0.050993	2.60629	0.0484
A * S	4	0.03936	0.009841	0.50297	0.8668
Residual	18	0.35217	0.019565		

Results in bold are significant at P<0.05 and * are marginally significant.

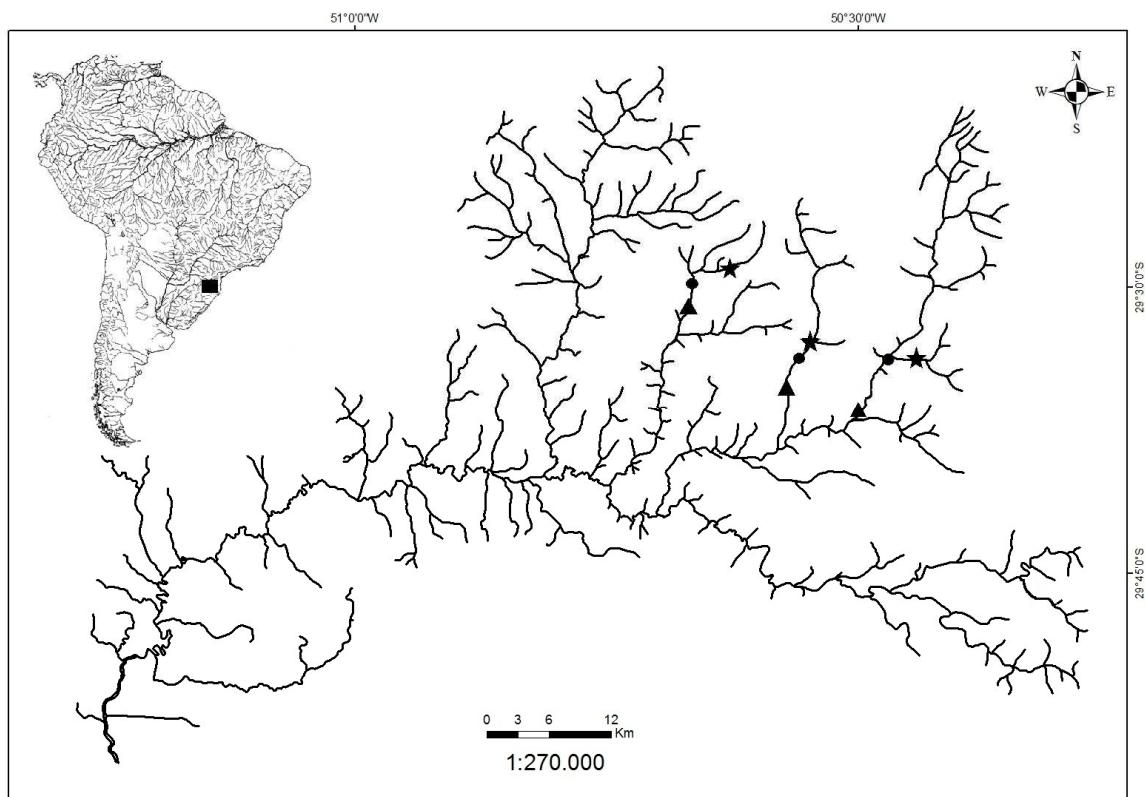


Fig. 1 Sampling areas ($n=9$) in three streams in the Sinos River watershed, northeastern southern Brazil. Symbols indicate Area > 40 m (\star), Area < 30 m (\bullet) and Area < 15 m (\blacktriangle).

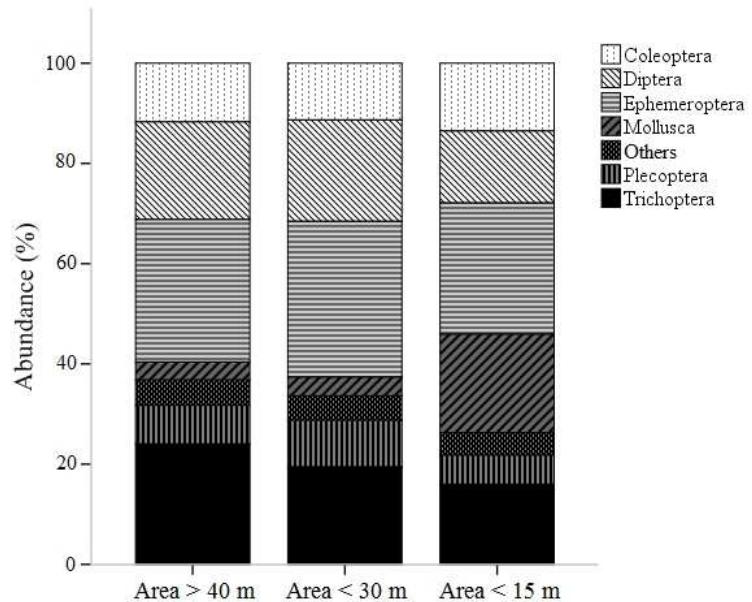


Fig. 2 Relative abundance of macroinvertebrate community in Area > 40 m, Area < 30 m and Area < 15 m based on the mean among sample periods of the streams studied in southern Brazil.

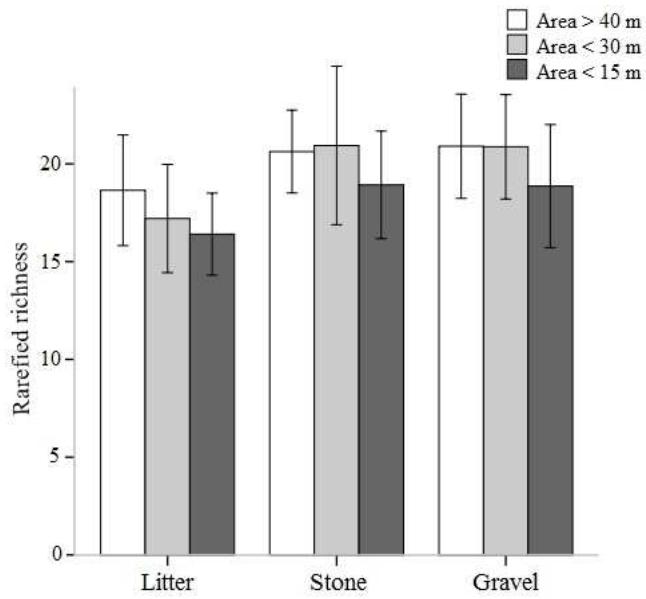


Fig. 3 Plots of macroinvertebrate richness in stone, gravel and litter in the three study area types based on the mean among sample periods of the streams studied in southern Brazil.

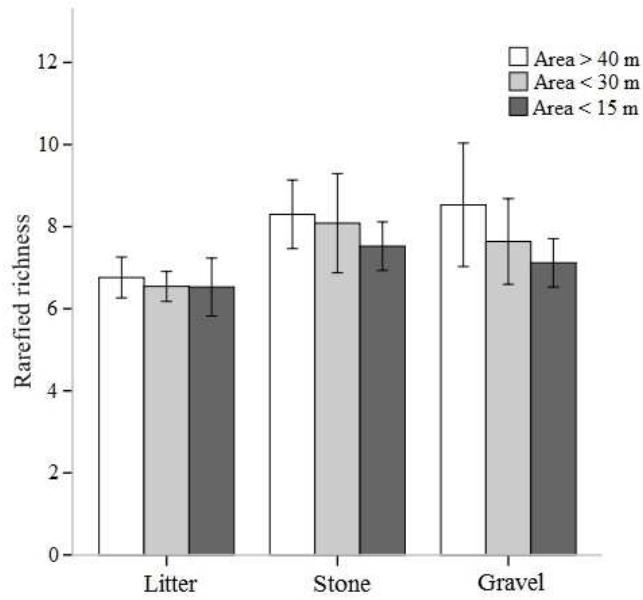


Fig. 4 Plots of Ephemeroptera, Plecoptera and Trichoptera richness in stone, gravel and litter in the three study area types based on the mean among sample periods of the streams studied in southern Brazil.

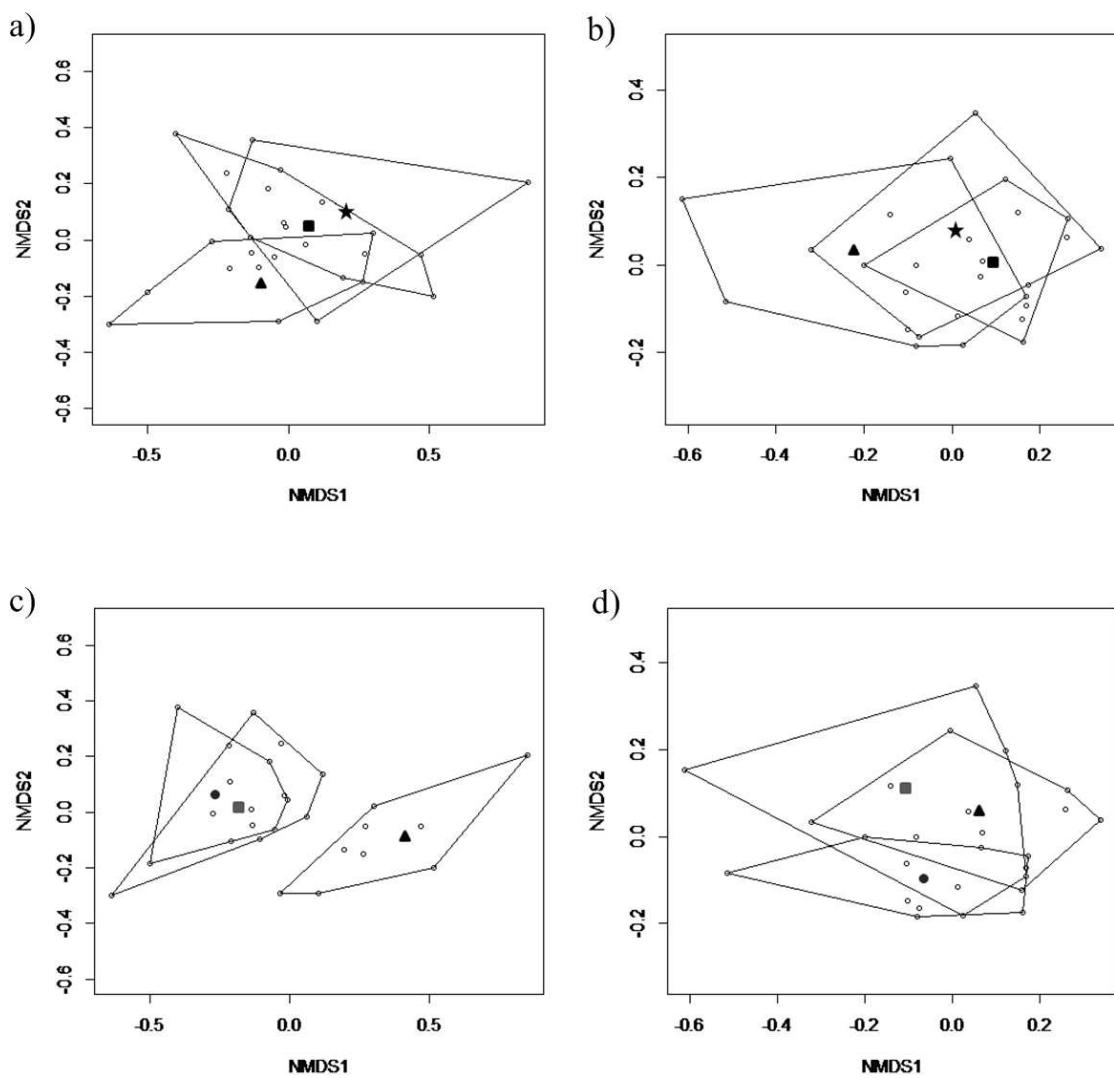


Fig. 5 **a)** Dissimilarity in macroinvertebrate composition and **b)** in functional feeding group composition among samples organized by area type and represented by: Area > 40 m (*), Area < 30 m (■) and Area < 15 m (▲). **c)** Dissimilarity in macroinvertebrate composition and **d)** in functional feeding group composition among samples organized by substrate type and represented by: stone (■), gravel (●) and litter (▲).

ARTIGO II

Does land use influence homogenization of macroinvertebrate communities in streams in southern Brazil?

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Abstract

Substitution of riparian zone by different land uses and the impacts on the physical and chemical conditions of streams has been associated with homogenization of macroinvertebrate composition. Biotic homogenization reduces β diversity, which increases the similarity between communities. We explore variation in β diversity of macroinvertebrates between sampling groups from different microhabitats in stream reaches impacted by land use. Samples were taken from three secondary streams in southern Brazil. In each stream, four stream reaches with different types of land use (forest, agriculture, pasture and urbanization) were evaluated. The physical and chemical stream variables varied according to the land use intensity. The two indices of dissimilarity (Bray-Curtis and Sorensen) were related only to the physical and chemical stream variables. β diversity (Bray-Curtis) was positively related to stream width, turbidity and P. Increased β diversity (Sorensen) was related to increased shade and pH and decreased stream velocity, NH₃-N and P. These results emphasize the importance of β diversity for evaluating the macroinvertebrate community in streams impacted by different land uses in southern Brazil.

Keywords: Beta diversity; Riparian vegetation; Forest fragment; Rural landscape

Introduction

The study of β diversity has been a key factor in understanding the functionality and health of ecosystems and biodiversity (Legendre et al., 2005; Anderson et al., 2006; Passy & Blanchet 2007; Tuomisto, 2010; Anderson et al., 2011). β diversity is a measure that quantifies the heterogeneity of species composition in sampling units in an area and spatial scale (Anderson et al., 2006), and it is better than species richness for analyzing biodiversity dynamics (Wang et al., 2012). Biotic homogenization reduces β diversity, which increases the similarity between biotic communities over time (McGoff et al. 2013). Many studies have emphasized the importance of understanding patterns of β diversity in aquatic ecosystems (Soininen et al., 2007; Al-Shami et al., 2013).

The concept of β diversity was introduced by Whittaker (1960), and there are now different definitions for this analysis (Legendre et al., 2005; Tuomisto, 2010; Anderson et al., 2011). β diversity as a concept of variation in community structure between groups of sampling units has been widely accepted in macroinvertebrate studies (Heino et al. 2013; Al-Shami et al., 2013). Limitations on organism dispersal, which are usually auto-correlated in space, and environmental factors are the principal causes that influence β diversity (Legendre et al., 2005). Some studies in Neotropical streams have considered variations in environmental factors highly important in determining β diversity of macroinvertebrates (Costa & Melo, 2008; Hepp et al., 2012; Molozzi et al., 2013)

Many environmental factors alter biological diversity in headwater streams (Clarke et al., 2008), and biotic homogenization is caused by human action (McKinney & Lockwood, 1999). Streams are influenced by anthropogenic impacts from diffuse sources throughout watersheds. These impacts are caused by land uses such as urbanization, industrial activities, deforestation and agriculture (Neumann & Dudgeon, 2002; Allan & Castillo, 2007). Substitution of riparian zone by different land uses and the impacts on the physical and chemical conditions of streams has been associated with variation in macroinvertebrate structure and composition (Townsend et al., 1997; Neumann & Dudgeon, 2002; Walling & Fang, 2003; Hepp et al., 2010). Altered stream habitats affect characteristics of stream flow, sedimentation (Quinn et al., 1997; Walling & Fang, 2003), light availability, temperature (Vannote & Sweeney, 1980; Quinn et al., 1994) and the entrance of inorganic nutrients (Bergfur et al., 2007; Bahar et al., 2008). These changes reduce species diversity and homogenize macroinvertebrate composition

(Huamantinco & Nessimian, 1999; Stevens & Cummins, 1999; Weigel et al., 2000; Burel et al., 2004).

Macroinvertebrate communities are highly associated with habitat diversity. Recent studies in Neotropical streams suggest that microhabitat degradation reduces β diversity in macroinvertebrate communities (Costa & Melo, 2008; Ligeiro et al., 2010; Hepp & Melo, 2013). Gutiérrez-Cánovas et al. (2013) demonstrated changes in β diversity with land use intensity, which reflected nesting of subsets of macroinvertebrate species. Increased β diversity in macroinvertebrates has also been observed with increased stream width (Al-Shami et al., 2013) and pH (Gutiérrez-Cánovas et al. 2013; Al-Shami et al., 2013). However, environmental factors that influence β diversity are still poorly known, especially in the Neotropics.

Therefore, we explore variation in β diversity of macroinvertebrates between sampling groups from different microhabitats in stream reaches impacted by land use in southern Brazil. We developed the following hypotheses: (1) Land use alters physical and chemical variables in streams; (2) β diversity increases with increased stream width, shade, riparian zone quantity, pH, dissolved oxygen and depth; (3) β diversity decreases, or the macroinvertebrate composition is more homogeneous, in streams with more intense land use and higher temperature, turbidity, stream flow and nutrients (N and P).

Materials and methods

Study area and sampling

The study area is located in the Sinos River watershed, which has an area of 3,820 km² (Figure 1). Current use of the watershed primarily includes pastures (48.8%), forest (28.15%) and agriculture (8.78%) (Oliveira et al., 2013). The Sinos River, the principal water course of the watershed, begins at an altitude of 600 m and extends for approximately 190 km (IBGE, 2010). According to the Köppen classification, the climate is humid subtropical, characterized by hot, humid summers and mild, cold winters. The average annual temperature is around 23°C and annual rainfall is 1250 to 2000mm, which is well-distributed among four seasons (Quadro et al., 1996).

The watershed is in the most southern portion of the Atlantic Forest biome, which is one of the world's hotspots (Myers et al., 2000; Silva & Casteli, 2003), and is globally recognized for its unique and threatened biodiversity. Intense fragmentation resulting from historical land uses has destroyed more than 90% of the original area of this biome (Campanili & Prochnow, 2006), which has led to species and ecosystems facing the threat of extinction (Myers et al., 2000; Ribeiro et al., 2009). The predominant vegetation in the riparian zones of the streams sampled includes *Nectandra megapotamica*, *Allophylus edulis* and *Cupania vernalis* (Oliveira et al., 2013).

Samples were taken from three secondary streams of the Sinos River watershed (Figure 1). In each stream, four stream reaches with different types of land use (forest, agriculture, pasture and urbanization) were evaluated. Samples were collected in riffles and pools from the four 100m reaches of each stream (n=12). The three dominant microhabitats of each stream reach were sampled. Twelve samples were collected from each microhabitat of each stream reach. Microhabitats in riffles were represented by stones (diameter of 20 to 28 cm) and pebbles (diameter of one to 6.5 cm). Habitats in pools were represented by leaf litter. Overall, 36 samples were collected from each stream reach. Samples were collected in winter (2010) and summer (2011), and the sum of all of the samples from the three streams was 432.

Stone and pebble sampling units were sampled with a 30x30cm Surber with 250 µm mesh. The method consisted of removing stones and carefully collecting the disturbed material in the net. Leaf litter sampling units were collected with a 30 cm diameter frame dip-net with 250 µm mesh. Each sampling unit consisted of two 1.0 m sweeps of the dip-net in the submerged leaf litter.

Material identification

The samples were fixed *in situ* with 80% ethanol and taken to the laboratory, where the macroinvertebrate samples were filtered with a 0.25 mm diameter mesh sieve. Most of the macroinvertebrates were collected and identified to the family level, a taxonomic resolution which is considered sufficient for evaluating different levels of anthropogenic impact (Heino & Soininen, 2007; Melo & Hepp, 2008). Mites, oligochates, leeches and nematodes were identified at a wider taxonomic resolution. Functional feeding groups were based on Merritt & Cummins (1996) and Cummins et

al. (2005). The specimens were separated and identified with an optical stereomicroscope and glass vials with 80% ethanol. Identification was based on a specialized bibliography (Lopretto & Tell, 1995; Merritt & Cummins, 1996; Fernández & Dominguez, 2001). The samples were stored in the Sinos River Valley University (Universidade do Vale do Rio dos Sinos – UNISINOS) Laboratory of Ecology and Aquatic Ecosystem Conservation.

Analysis of the physical and chemical conditions and landscape

Dissolved oxygen (DO), temperature, pH and turbidity were measured with a Horiba multiparameter water quality meter in each stream reach. Stream velocity was measured with a Flow Mate. Mean stream width and shade (a classification of % canopy cover) were calculated from measurements in 20 transects along a stream reach whose reach was equal to the mean stream width multiplied by 40. Additionally, in each transect, three equidistant points and one point in the deepest part of the stream were determined. Stream velocity and water depth were measured at these points. Chemical parameters, total phosphorus (P) and ammoniacal nitrogen ($\text{NH}_3\text{-N}$) were analyzed at each point. Three water samples were collected and placed in 500 mL plastic bottles to be analyzed according to the methodology of Eaton et al. (2005).

The 12 selected stream reaches were organized in the geographic information system ArcGis 10 in order to verify land use in each riparian zone. Image classification, based on vectors, interpreted the classes pasture, forest, water, agriculture and urbanization from Quikbird images extracted from Google Earth Pro 4.2.1 (images from April 2010). On each side of each stream, 50m buffers were defined within the concentric longitudinal 500 m windows over the sampling points and parallel to the water body. The area of each class (m^2) was calculated for each of the 12 stream reaches sampled, and the class water was not used in the analyses.

Data analysis

Stream reach scale was used to analyze differences in β diversity of the macroinvertebrate community. Macroinvertebrate richness and abundance corresponded

to total number of families and individuals collected, respectively, during the two samplings. The mean of each physical-chemical variable was calculated based on the two sampling events.

The test of homogeneity of dispersion betadisper, available in the vegan package (Oksanen, 2008) of the statistical program R, was used to examine multiple dispersions (β diversity) of the macroinvertebrate sampling groups represented by different microhabitats present in the streams. This test implemented the procedure proposed by Anderson (2006). Variation in β diversity, according to sampling unit dispersion in a multivariate space (Anderson et al., 2006), can compare community structure at different categorical levels and/or over a continuous gradient (Anderson et al., 2011). The concept of β diversity is flexible because it can be based on the selection of a variety of ecological measures of dissimilarity, both presence/absence and abundance (Anderson et al., 2006). The multiple dispersal (β diversity) of a group of samples is represented by calculating the average distance of the sample groups to the central group or spatial median in a multivariate space. To perform the betadisper tests, dissimilarity matrices were analyzed with two indices: Bray-Curtis for abundance data, which focus on richness, composition and abundance; and Sorensen for presence/absence data, which focuses on richness and composition. The Sorensen index is a measure of distance identical to Bray-Curtis dissimilarity when applied to quantitative data.

Pearson correlations were used to test correlations between environmental variables (land use, physical and chemical). Effects of land use on physical and chemical variables were analyzed with multiple regressions. β diversity was also associated with stream environmental variables through multiple regressions, which were performed with backward selection using the model with the lowest adjusted Akaike Information Criteria (AIC) value. Simple linear regressions were performed to manually select which physical and chemical variables should be incorporated in the multiple regression models to avoid multicollinearity. To ensure normality of the dependent and independent variables, a z-score standardization was performed before the multiple regression analyses. All of the statistical analyses were performed on program R ver 3.0.1 (R Development Core Team, 2013).

Results

A total of 59,641 individuals from 72 macroinvertebrate taxa were collected. Most of the taxa observed were rare (80% of the taxa were represented by less than 1% of the total individuals). Insects were the most abundant (88%) and diverse (79%) taxa. The insect orders Coleoptera and Trichoptera were represented by 11 families. There were 10 dipteran families, followed by Hemiptera (9), Odonata (8), Ephemeroptera (4), Plecoptera (2) and Megaloptera and Lepidoptera had one family each. Chironomidae, Elmidae and Leptohyphidae were the most abundant families in all of the microhabitats, with a total of 9,259, 7,689 and 7,096 individuals, respectively.

Mean and standard deviation values of the environmental variables for each point are available in Table 1. Among the land use variables, pasture and forest were correlated ($r=-0.647$, $P=0.023$). Among the physical and chemical variables, there was correlation between stream width and shade ($r=-0.667$, $P=0.018$), stream width and depth ($r=0.754$, $P=0.005$), stream velocity and dissolved oxygen ($r=0.696$, $P=0.012$) and turbidity and $\text{NH}_3\text{-N}$ ($r=-0.612$, $P=0.034$) (Table 2). Regarding the influence of land use on environmental variables, pasture was positively related with $\text{NH}_3\text{-N}$ and P ($\text{AIC}=-4.01$; $R^2\text{adj} = 0.421$, $F_{2, 9} = 4.997$, $p = 0.03$). Forest was positively related with shade, stream velocity and negative with $\text{NH}_3\text{-N}$ and P ($\text{AIC}=-23.49$; $R^2\text{adj} = 0.895$, $F_{4, 7} = 24.38$, $p < 0.001$). Urbanization had a marginally significant positive relationship with $\text{NH}_3\text{-N}$ and negative with stream velocity ($\text{AIC}=-2.07$; $R^2\text{adj} = 0.319$, $F_{2, 9} = 3.582$, $p = 0.071$). Agriculture was not related to any variable (model with $p>0.1$).

In the multiple regression models, the mean centroid distances (β diversity) of the indices of dissimilarity were not related to any of the land use variables. In other words, the land use activities did not influence beta diversity according to the Bray-Curtis index and the Sorensen index (all models with $p>0.1$). However, the two indices of dissimilarity were related to the physical and chemical stream variables. β diversity, according to the Bray-Curtis index, was positively related to stream width, turbidity and P ($\text{AIC}=-98.8$; $R^2\text{adj}=0.551$, $F_{3, 8}=5.517$, $p=0.023$). Increased β diversity, based on the Sorensen index, was related to increased shade and pH and decreased stream velocity, $\text{NH}_3\text{-N}$ and P ($\text{AIC}=-108.49$; $R^2\text{adj}=0.726$, $F_{5, 6}=6.846$, $p=0.018$) (Table 3).

Discussion

Various studies have demonstrated changes in nutrient concentrations and flow resulting from land use around streams (Pellerin et al., 2004; Poor & McDonnell, 2007; Gravelle et al., 2009). Land use influenced quantities of N and P at the sampling sites. Ammoniacal N and total P increased in areas with increased presence of pasture. Nutrient flow into bodies of water is directly related to livestock density in pastures (Carpenter et al., 1998). Waste from animals contributes to increased ammonia concentrations, and the use of manure and fertilizers on crops could increase the availability of all inorganic forms (Poor & McDonnell, 2007). Neill et al. (2001) and Silva et al. (2007) showed that there is an increase in inorganic forms of nitrogen in bodies of water due to the conversion of natural vegetation in pastures and plantations. Positive correlations between degree of watershed occupation and increase in inorganic N have been found in temperate watersheds (Pellerin et al., 2004; Gravelle et al., 2009).

The points with higher amounts of riparian zone also had more shade and less total P and ammoniacal N. Surface runoff is the most important pathway for nutrients associated with the soil in agricultural areas (Dillaha et al., 1988). In watersheds protected by vegetation, concentrations of phosphate tend to be low (Horbe et al., 2005). Nutrients reduction varies from 50 to 85% in the first few meters of the riparian zone (Vought et al., 1994). Furthermore, shade can alter nutrient transformation and cycling in streams. A stream with more shade should retain less dissolved inorganic nutrients in the form of plant biomass than a stream without shade and the same nutrient input (Rutherford et al., 1999). Our results showed that stream velocity was lower at the points with less riparian zone and more urbanized area. Removing the riparian zone can lead to increased runoff and erosion, which affects the supply of organic material and sediments to water bodies (Minshall, 1984). Not only do forests filter nutrients between higher grounds and aquatic ecosystems, they also stabilize stream banks. Therefore, sedimentation could have affected hydrology, reducing stream velocity in streams with less riparian vegetation.

Habitat degradation resulting from agricultural intensification could homogenize and change macroinvertebrate communities, which would lead to local communities being dominated by tolerant species (Burel et al., 2004). Although there was no relationship between macroinvertebrate β diversity and variation in area occupied by

different land uses in riparian zones, there was a relationship when the physical and chemical stream factors were considered directly. Physical and chemical factors were more important in determining variation in β diversity among stream microhabitats. Regarding β diversity calculated with the Sorenson index, dissimilarity in composition between microhabitats was greater when there was more shade and higher pH. This could occur because the riparian forest offers many quality resources for macroinvertebrates. Riparian forests provide allochthonous sources, such as leaf litter and wood, and shade, which create favorable water temperature for macroinvertebrate growth, survival and reproduction (Vannote & Sweeney, 1980; Sweeney, 1993; Quinn et al., 1994).

Natural and anthropogenic causes can alter stream acidity (Petrin et al., 2008). Naturally acidic streams seem to be less affected by species exclusion than streams made acidic by atmospheric pollution, and streams with a higher pH support a higher number of macroinvertebrate species (Allan & Castillo, 2007). pH varied from 7.0 to 7.8 in the present study. Although the range was low, it was enough to modify β diversity and increase the dissimilarity among microhabitats in the streams with more basic waters. A similar result was found by Al-Shami *et al.* (2013), who observed higher dissimilarity among streams with an increase in pH.

Increased β diversity (Sorenson index) was related to decreased stream velocity and nutrients (ammoniacal nitrogen and total phosphorus). Stream velocity is a physical force that directly affects organisms in the water column and substrate surface (Allan & Castillo, 2007). Increased stream velocity could reduce macroinvertebrate richness and abundance (Brooks et al., 2005). Moreover, a larger load of nutrients increases autotrophic biomass, especially if the amount of light also increases, and could cause reduced dissolved oxygen. Tolerant species would then substitute sensitive ones (Allan, 2004). Drastic changes in results can occur when relative abundance is included in the measures of dissimilarity (when the Bray-Curtis index is applied). In this case, common, numerically dominant species play an important role (Anderson *et al.* 2011). Variation in β diversity based on the Bray-Curtis index was positively related with stream width, turbidity and total phosphorus. In our study, stream width varied from 6.3 to 22.9 m. Macroinvertebrate species are usually highly associated with stream width, and macroinvertebrate richness and density tend to increase with stream size (Heino, 2009). Other studies of β diversity indicate a higher dissimilarity in composition in wider streams (Al-Shami *et al.*, 2013). Suspended fine sediments are one of the principal

factors that affect turbidity (Allan, 2004). High turbidity limits algae and macrophyte photosynthesis, which reduces the availability of resources for herbivorous macroinvertebrates (Ryan, 1991; Allan & Castillo, 2007). Sedimentation and nutrient input is caused by many types of land use (Lenat et al., 1981; Richards et al., 1996; Allan, 2004). Higher sedimentation (homogeneity) and nutrients in streams could alter the structure of the macroinvertebrate community (Allan, 2004; Burel et al., 2004).

Understanding how much of dissimilarity is attributed to differences in composition and how much is attributed to differences in relative abundance is an important question in ecology (Anderson *et al.* 2006; 2011). However, when species, and not quantity of individuals, is the conservation focus, a good method is to only focus on species identity, using presence/absence indices, such as the Sorenson index (Anderson *et al.* 2011). Based on this method, a homogenization of the community occurred with increased stream velocity, ammoniacal nitrogen and total phosphorous, and higher heterogeneity occurred with more shade and higher pH. These results emphasize the importance of β diversity for evaluating the macroinvertebrate community in streams impacted by different land uses in southern Brazil. Special attention must be paid to these ecosystems, and it should focus on diversity management and conservation practices since these ecosystems are considered among the most impacted in the world.

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Table 1 Mean and standard deviation values of the environmental variables for each sampled point (n=12) in southern Brazil.

	Mean	SD
Agriculture (ha)	1.392	1.525
Pasture (ha)	2.423	2.261
Forest (ha)	6.928	1.73
Urbanization (ha)	1.486	1.376
Width (m)	12.772	4.158
Shade (%)	42.646	19.996
Depth (m)	0.471	0.176
Velocity (m/s)	0.25	0.061
Temperature (°C)	19.433	1.763
DO (mg/L)	10.331	0.645
pH	7.43	0.277
Turbidity (NTU)	7.971	2.017
NH ₃ -N (mg NH ₃ /L)	0.654	0.513
P (mg P/L)	0.034	0.011

Table 2 Correlation between environmental variables in study reaches in southern Brazil.

	Agriculture	Pasture	Forest	Urbanization	Width	Shade	Depth	Velocity	Temperature	DO	pH	Turbidity	NH ₃ -N	P
Agriculture	1													
Pasture	-0.477	1												
Forest	0.063	-0.647*	1											
Urbanization	-0.276	0.075	-0.474	1										
Width	0.398	0.012	-0.443	0.186	1									
Shade	-0.072	-0.285	0.602*	-0.308	-0.667*	1								
Depth	0.354	0.013	-0.183	-0.196	0.754**	-0.483	1							
Velocity	0.173	-0.459	0.733**	-0.566	-0.379	0.554	-0.335	1						
Temperature	0.232	-0.159	-0.067	-0.057	0.506	-0.457	0.259	-0.262	1					
DO	0.179	-0.359	0.368	-0.339	-0.142	0.316	-0.356	0.696*	0.01	1				
pH	0.361	-0.278	0.081	0.057	0.05	-0.08	-0.183	0.528	-0.357	0.548	1			
Turbidity	0.098	-0.467	0.365	-0.274	0.375	0.013	0.18	0.381	0.571	0.551	0.003	1		
NH ₃ -N	-0.517	0.571	-0.312	0.527	-0.049	-0.148	-0.027	-0.35	-0.546	-0.482	0.052	-0.612*	1	
P	0.128	0.378	-0.534	0.049	0.045	0.128	-0.331	-0.03	-0.022	0.189	0.088	-0.086	-0.117	1

* P<0.05; ** P<0.01

Table 3 Results of multiple regressions between average distance to reach centroid (β diversity) and environmental variables, which were performed with backward selection.

	Coeficiente	SE	T	P	R ² ajustado
<i>Bray-Curtis</i>					-0.04
Pasture	-0.002	0.007	-0.32	0.757	
Agriculture	0.007	0.007	1.027	0.334	
Urbanization	0.007	0.006	1.133	0.29	
<i>Sorensen</i>					0.281
Agriculture	0.007	0.005	1.335	0.219	
Pasture	-0.007	0.005	-1.433	0.19	
Urbanization	0.002	0.004	0.528	0.612	
<i>Bray-Curtis</i>					0.551
Width	0.006	0.004	1.391	0.201	
Turbidity	0.009	0.004	2.067	0.072	
P	0.011	0.004	2.701	0.027	
<i>Sorensen</i>					0.726
Shade	0.011	0.004	2.488	0.047	
Velocity	-0.022	0.005	-3.978	0.007	
pH	0.014	0.004	3.122	0.02	
NH ₃ -N	-0.019	0.003	-5.674	0.001	
P	-0.008	0.003	-2.603	0.04	

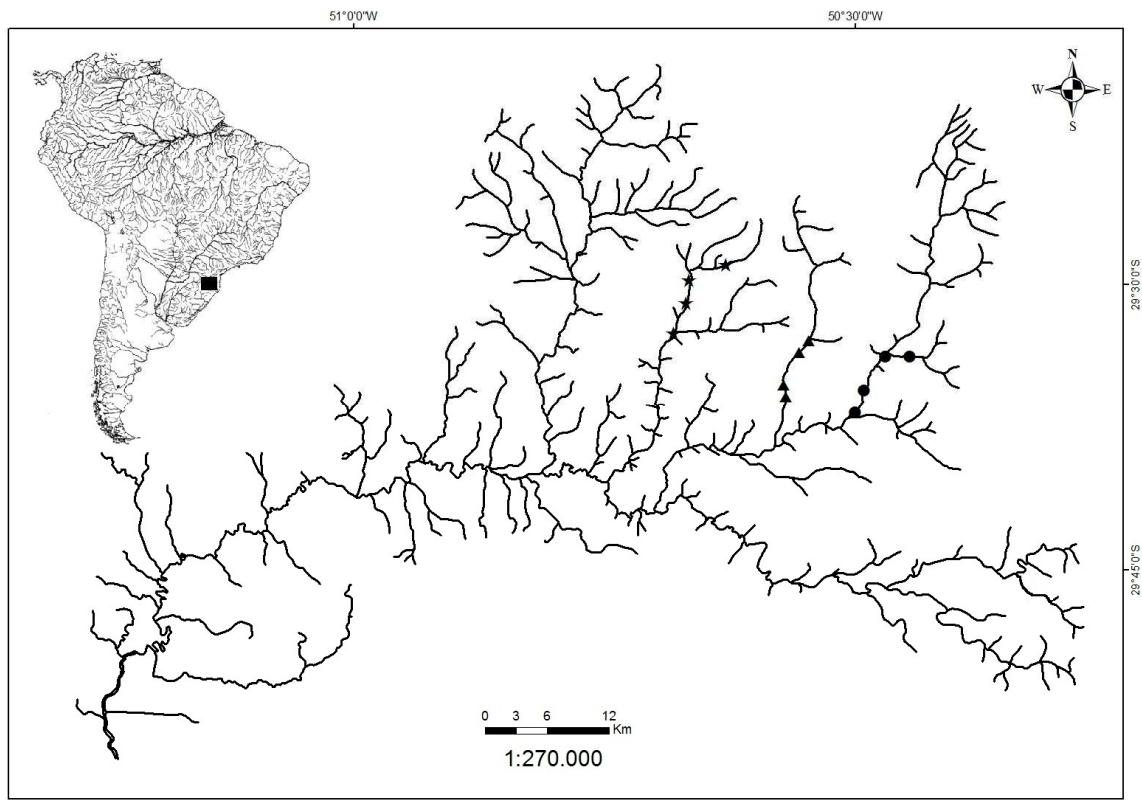


Fig. 1 Sampling areas ($n=12$) in three streams in the Sinos River watershed, northeastern southern Brazil.

ARTIGO III

Additive partitioning of macroinvertebrate diversity across spatial scales in headwater streams in southern Brazil: a seasonal difference

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ABSTRACT

Understand how patterns of species diversity change across spatial scales are a challenge for ecologists and conservation biologists. Our study aimed to: (1) assess the relative importance of the α diversity and β diversity of macroinvertebrate components between samples from different spatial scales in winter and summer and (2) assess the relative importance of the diversity components among three substrate types collected in pools and riffles. Sampling occurred during the winter (2010) and summer (2011) and was hierarchical, including microhabitats, habitats, stream reaches and stream segments. Additive partitioning of diversity was used to decompose the total variation in community composition (regional diversity) into alpha and beta components. A total of 27.129 individuals and 71 taxa of macroinvertebrates were collected. Partitioning of α and β diversity components showed similar patterns for both studied seasons (winter and summer). The macroinvertebrate community composition on litter in pool differed from that on riffle (stone and gravel) substrata, showing proportionally lower diversity within the finest scale (α_1) and higher diversity at the highest scale (β_4). We found differences in the family composition between the reaches and the streams studied in both winter and summer. However, the magnitude of β diversity component increased at the reach scale in winter, especially in riffle substrates (stone and gravel) and the magnitude of β diversity component increased at the broader scale (stream) in all substrate types in summer. Our results revealed that streams in southern Brazil support a very heterogeneous macroinvertebrate community and the role of spatial configuration in these streams strongly influences macroinvertebrate community diversity. Even reaches in the same stream may support different community composition. In this sense, diversity inventories should concentrate sampling in different microhabitats, stream reaches and stream sites.

Keywords: Beta diversity, Substrate, Macroinvertebrate composition, Riffles, Pools

INTRODUCTION

The spatial scale concept has received much attention in the study of diversity patterns in terrestrial and aquatic ecosystems (Li *et al.*, 2001; Stendera & Johnson, 2005; Bonada *et al.*, 2008). The community structure varies according to the spatial scale of study (Poff, 1997; Heino *et al.*, 2003; Ligeiro *et al.*, 2010.). The ecological patterns of a community on a small scale study (local) tend to be different from the patterns observed at larger spatial scales (landscapes or regions) (Crist *et al.*, 2003; Heino *et al.*, 2003). Streams are interesting ecosystems to verify ecological patterns in aquatic communities. Streams are organized in space-time hierarchies composed of nested hierarchical scales fragments and dependent on the scale of observation (Poff, 1997; Malmqvist, 2002). The stream species are influenced by local variables, riparian, watershed characteristics and regional environmental factors (Poff, 1997). Several studies have related the aquatic community structure in streams at different spatial scales (Heino *et al.*, 2003; Robson *et al.*, 2005; Rios & Bailey, 2006; Ligeiro *et al.*, 2010).

The streams present environmental factors and ecological events characteristic of each spatial scale (Whittaker *et al.*, 2001; Heino *et al.*, 2004; Hortal *et al.*, 2010). Habitat heterogeneity (Costa & Melo, 2008; Hepp *et al.*, 2012; Hepp & Melo, 2013), biotic interactions (Hildrew & Giller, 1994), disturbance (Gutierrez-Cánovas *et al.*, 2013), geographic distance (Mykrä *et al.*, 2007; Hepp *et al.*, 2012; Hepp & Melo, 2013), and seasonal fluctuations in the number of individuals (Cummins *et al.*, 1989, Cummins, 2002) are examples of factors that influence the macroinvertebrate community in streams.

Additive partitioning has been used to test hierarchical patterns and identify the scales that influence the macroinvertebrate diversity in Neotropical streams (Ligeiro *et al.*, 2010; Hepp & Melo, 2013). The calculation applies the Whittaker's terms concerning to partition of the total species diversity (γ) into additive components that represent the diversity within the community (α) and diversity among communities (β) (Lande, 1996). The diversity is measured as species richness, or by using either the Simpson or Shannon index, dividing the regional diversity in components that can be expressed at the same units and compared in temporal and spatial scales (Crist *et al.*, 2003).

β diversity is an ecological term originally introduced by Whittaker (1956, 1960) to describe changes in species composition and abundance through environmental changes, such as elevation and moisture. It is a measure of biological difference between environments and can be considered the turnover or dissimilarity between communities in terms of taxonomic composition (Legendre *et al.* 2005). The measure allows us to recognize the most important factors (regional and local) to the community heterogeneity in different habitat patches (Crist *et al.*, 2003; Legendre *et al.*, 2005). The context of β diversity of additive partitioning recognizes that β diversity can be measured and defined to α diversity, i.e. it is the average amount of species not found in a single sample (Lande, 1996).

Species richness increases as a new sampling unit is incorporated at local scale, increasing β diversity between habitats (Barton *et al.*, 2013). This occurs because the streams provide a variety of habitats (e.g. riffles and pools) subjected to several specific environmental factors. In this sense the determination of macroinvertebrates should be analyzed at different spatial scales (Robson & Chester, 1999; Lamouroux *et al.*, 2004; Costa & Melo, 2008). The relationship between substrate complexity and macroinvertebrates richness is positively correlated in streams (Hart, 1978; O'Connor, 1991). Studies that related the environmental heterogeneity with β diversity in Brazil, suggested the importance of different microhabitats for β diversity variation (Costa & Melo, 2008; Ligeiro *et al.*, 2010). However, despite the β diversity between microhabitats depend on the substrate type, Ligeiro *et al.* (2010) observed that larger scales (segment) are the most important to β diversity in all substrates types analyzed.

An increasing number of articles related to β diversity have been published in recent years (Anderson *et al.*, 2011; Melo *et al.*, 2011). Some studies have recently assessed the additive partitioning of diversity in streams in Brazil (Ligeiro *et al.*, 2010; Hepp and Melo 2013). However, the season effect was not evaluated in these studies, and it is of particular importance, since it provides greater heterogeneity in macroinvertebrates composition in streams. Our study aimed to: (1) assess the relative importance of the α diversity and β diversity components between samples from different spatial scales in winter and summer and (2) assess the relative importance of the diversity components among three substrate types collected in pools and riffles. We hypothesized that the γ diversity is not homogenously distributed among scales, with differences between the pattern observed in winter and summer. Furthermore, the β

diversity depends on different substrate types, but most composition dissimilarity occurs in the larger spatial scales, regardless of the season analyzed.

MATERIALS AND METHODS

Study area and sampling design

The study area is located in the Sinos River watershed, northeastern southern Brazil between 29° and 30° S (Figure 1). The Sinos River is the main course of the river basin. It is approximately 190 km long, and while its headwaters are at an elevation of 900 m, its mouth is 5 m above sea level (Rolon *et al.*, 2003). The average of annual temperatures is about 23°C and the rains are well distributed over the four seasons, about 1,250 to 2,000 mm of rain per year (Quadro *et al.*, 1996). The humid subtropical climate is characterized by hot, humid summers and mild to cool winters, according to the Köppen classification.

The watershed is in the most southern extent of the Atlantic Forest biome, recognized globally for its unique and threatened biodiversity and considered as a hotspot of the planet (Myers *et al.*, 2000; Silva & Casteli, 2003). There is an intense process of historical fragmentation (Campanili & Prochnow, 2006) that reduced by less than 90% of its original forest, threatening the existence of species and ecosystems (Myers *et al.*, 2000; Ribeiro *et al.*, 2009). The predominant vegetation in the riparian zones of the streams sampled includes *Nectandra megapotamica*, *Allophylus edulis* and *Cupania vernalis* (Oliveira *et al.*, 2013).

We selected three secondary streams of the Sinos River watershed, with similar physical and chemical parameters (Table 1), and natural vegetation along their margins and low human impact. Samplings occurred during winter (2010) and summer (2011) and were hierarchical, including microhabitats, habitats, reaches stream and stream segments (Frissell *et al.*, 1986; Li *et al.*, 2001; Allan & Castillo, 2007). In each stream segment, two reaches were selected and in each reach two habitats were defined (two riffles and two polls). Three sample units were collected in the finest spatial scale (microhabitat) within each habitat by season. The microhabitats were represented by the stones (20 to 28 cm) and gravels (one to 6.5 cm) in riffles. The microhabitats were

represented by litter substrate in pools. A total of 108 sampling units were collected in each season distributed among the different substrates (3 stream segments x 2 reaches in each stream x two habitats in each reach x three sampling units in each habitat). Habitats were at least 10 m long and 50 m distant from each other within each stream reach. Stream reaches was at least 1 km distant from each other.

The sampling units represented by stone and gravel were sampled using a 30 x 30 cm Surber (250 µm mesh). The Surber method consists of removing stones or gravel and collecting the material that is stirred up in the mesh. The sampling units represented by leaf litter substrate were collected with aquatic dip net (30 cm in diameter and 250 µm mesh). Two one-meter sweeps were carried out in the submersed litter.

Macroinvertebrate identification

Macroinvertebrates were stored in 80% ethanol and taken to the laboratory, where the samples were washed in a 0.25 mm mesh sieve. The macroinvertebrates were identified to family level. Mites, oligochates, leeches and nematodes were identified at a wider taxonomic resolution. The specimens were processed and identified with an optical stereomicroscope, then placed in glass tubettes with 80% ethanol. A specialized bibliography was consulted for identification (Lopretto and Tell, 1995; Merritt and Cummins, 1996; Fernández and Dominguez, 2001). The samples were stored in the Laboratory of Ecology and Aquatic Ecosystem Conservation in the Universidade do Vale do Rio dos Sinos – UNISINOS).

Data analysis

We performed cluster analysis to evaluate the main patterns of similarity among samples at different spatial scales in winter and summer. For this analysis, the six sampling units of each substrate present in riffle and pool within each reach were pooled, resulting in 18 samples per season. A hierarchical clustering method based on the Unweighted Pair-Group Average Method (UPGMA) was performed using arithmetic averages linkage and the Bray-Curtis distance calculated on log (x+1) abundance data. The analysis was performed using R statistical program version 3.0.1 (R Development Core Team, 2013).

Additive partitioning of diversity was used to decompose the total variation in community composition (regional diversity) into alpha and beta components. We organized our data according to the following hierarchical scheme: Sample unit (α_1), among sample unit (β_1), among habitats (β_2), among reaches (β_3), and among streams (β_4). Thus, the total taxon richness was partitioned into $\gamma = \alpha_1 + \beta_1 + \beta_2 + \beta_3 + \beta_4$. The additive partition analyzes were performed using a null model in a computer program, Partition version 2.0 (Veech and Crist, 2007) in which allocation of individuals are randomized among sample units. This procedure randomizes individuals and taxa simultaneously for all hierarchical levels (Gering et al., 2003). The randomization process was repeated 10,000 times to obtain null distributions of the alpha and β diversity at each hierarchical level (Crist et al., 2003). The test assess whether the observed values were higher or lower than expected by chance. A low p value (e.g. $\text{prop}_{\text{exp}>\text{obs}} < 0.05$) indicates that the observed diversity is significantly higher than that expected under the null model, and similarly, a high p value (e.g. $\text{prop}_{\text{exp}>\text{obs}} > 0.95$) indicates that the observed diversity is significantly lower than that expected (Crist et al. 2003). For this study, 5% ($P < 0.05$ for significantly high diversity; $P > 0.95$ for significantly low diversity) was taken as the level of significance. In order to assess whether the relative magnitude of diversity components differed among winter and summer and substrate types, we analyzed the data separately for each substratum per season.

RESULTS

A total of 27,129 individuals distributed in 71 macroinvertebrates taxa were collected. Insecta was the most representative class (94.7 % of the total abundance and 77.5 % of the richness). Both Coleoptera and Trichoptera orders were represented by 11 families. Diptera was represented by 10 families followed by Hemiptera (9) Odonata (8) Ephemeroptera (4) Plecoptera (2) Megaloptera (1) and Lepidoptera (1). Most of the taxa observed were rare (77.5 % of the taxa were represented by less than 1 % of total individuals). Chironomids, Leptohyphidae and Leptoceridae were the most abundant families in all microhabitats with a total of 17%, 12.4 % and 10.4 % of individuals observed, respectively.

UPGMA analysis defined groups according to the substrate type, reach and stream (Figure 2). The stream segment 1 represented a distinct group in winter, with

riffle habitats (stone and gravel) more similar within the same reach. The litter microhabitats were more similar to each other, regardless of the reach of the analyzed stream (Figure 2a). The reach scale seemed to be more important for the similarity of composition in stone and gravel microhabitats for both winter and summer (Figure 2). However, the litter substrate showed a very distinct group in summer, and the microhabitat scale was more important to the similarity of macroinvertebrates composition than reach and stream (Figure 2b).

Partitioning of alpha and β diversity components showed similar patterns for both winter and summer. The spatial partitioning of diversity showed that each sampling unit (α) presented less diversity than expected from the null model ($\text{prop}_{\text{exp}>\text{obs}} = 1$) in which individuals were randomized among sampling units for all three microhabitat types in both seasons (Tabela 1; Tabela 2). The α component for stone (29%) and gravel (27.8%) represented a smaller fraction of the total diversity in winter and a smaller fraction of the total diversity in summer (37.7% and 28.6%, respectively) (Table 2; Table 3). This result indicated a high aggregation among families on those microhabitats. However, the α component for litter represented a fraction of the total diversity in winter (22.3%) and summer (24.2%) smaller than stone and gravel, indicating a higher aggregation among families on litter microhabitat, reflecting similar spatial patchy distribution of individuals belonging to the same family.

The β_1 component (among sampling unit) represented a similar proportion to γ diversity in all microhabitat type in summer and winter (range: 14.5-16.2%). The relative magnitude of the β_1 component in relation to expectations under the null model were lower than expected for all microhabitat type in both seasons ($\text{prop}_{\text{exp}>\text{obs}} > 0.95$), excepted to β_1 for gravel in winter, which was similar to expected ($\text{prop}_{\text{exp}>\text{obs}} = 0.783$). The β_2 (among habitats) represented 10.3 to 13.5% of the total richness, the lowest proportion to γ diversity in all microhabitat type in both seasons. The β_2 component of most microhabitat type was similar to those expected, excepted to gravel in summer ($\text{prop}_{\text{exp}>\text{obs}} = 1$), which was significant lower than expected under the null model.

The observed β_3 component (among reaches) accounted for 14.1% to 19.1% of the total richness in all microhabitats types in both seasons, and was significant higher than expected under the null model of randomization of sampling units ($\text{prop}_{\text{exp}>\text{obs}} < 0.05$). However, the observed β_3 component on litter in summer was similar to those expected ($\text{prop}_{\text{exp}>\text{obs}} = 0.175$). The β_4 component (among streams) accounted for the highest fraction of β diversity (23.8% to 33.3%) in all microhabitats types in both

seasons. Furthermore the observed β_4 was higher than expected under the null model in all microhabitat types in both seasons ($\text{prop}_{\text{exp}>\text{obs}} < 0.05$) excepted to stone in winter, which the observed β_4 was similar to those expected ($\text{prop}_{\text{exp}>\text{obs}} = 0.183$) (Table 2; Table 3).

DISCUSSION

The macroinvertebrate community composition on litter in pool differed from that on riffle (stone and gravel) substrata, showing proportionally lower diversity within the finest scale (α_1) and higher diversity at the highest scale (β_4) for both summer and winter. The cluster analysis confirmed this result by differentiating the composition in litter. A similar result was observed in headwater streams of Serra do Cipó National Park in Brazil, where macroinvertebrate taxa composition on litter differed from stone and gravel, despite the samples collected on litter substrate were inserted in riffle habitats, not in pool habitats, as in our study (Ligeiro *et al.* 2010).

Many studies gave great importance to the substrate types for determining the macroinvertebrate composition in streams in Brazil (Buss *et al.*, 2004; Costa & Melo, 2008; Ligeiro *et al.*, 2010). The substrate provides the necessary space for the organisms settle, refuge, foraging and complete all or part of their life cycles (Allan & Castillo, 2007). In addition, macroinvertebrate achieve greater biomass and diversity in some substrates as compared to other (Buss *et al.*, 2004).

There is a great difference in resources for the macroinvertebrate community present in leaf litter, compared to stones and gravels. The litter is considered a primary food resource and serves as habitat for many aquatic macroinvertebrates and generally harbors a high density (Phillips, 2003). The litter is accumulated mainly in pools together with other debris, and is characterized as organic substrate consumed by specific functional feeding groups such as shredders (Merritt & Cummins, 1996). The substrate size and the heterogeneity of surface rocks are also important predictors for structuring macroinvertebrate community (Erman & Erman, 1984). Many species are commonly found in rocks of all sizes, but others are found associated with a particular size class (Cummins & Lauff, 1969). For example, species of mayflies, like *Ephemera danica* and *E. simulans* preferentially inhabit gravels, but *Hexagenia limbata* can not inhabit gravels, but lives in finer sediments (Eriksen, 1964).

Our results showed that alpha diversity was lower than β diversity and also lower than that expected by chance for all substrate type studied for summer and winter, thus indicating high aggregation among macroinvertebrates at fine spatial scales. This pattern may have resulted from similar abiotic characteristics or biotic interactions (e.g., competition, predation) across individual substrates and by the macroinvertebrates selecting for preferential or specific microhabitats (Robson & Chester, 1999; Downes et al., 2000; Costa & Melo, 2008). Similar results were found by Ligeiro et al. (2010) and Hepp & Melo (2013), studying families of macroinvertebrates collected on riffles in headwater streams of Brazil.

Hepp & Melo (2013) found a magnitude increase of the beta-diversity components with coarser scales, indicating that dispersal of macroinvertebrates tended to be reduced among streams and among microbasins. Similarly Ligeiro et al. (2010) found that diversity among streams (coarser scale) was higher than that expected by chance, and was usually the highest among the β diversity components studied. In our study, the β diversity components of coarser scales represented by reaches and streams were larger than expected in both seasons.

The high similarity in macroinvertebrates composition observed among habitats agrees with other studies (Parsons et al. 2003; Ciesielka & Bailey, 2007; Ligeiro et al. 2010). The drift of organisms may have favored the high similarity of composition at habitat spatial scale (Callisto & Goulart, 2005). According to Ligeiro et al. (2010), the result may be partially explained by the spatial extent of habitats, i.e., due to the short distance (50 m) between the habitats studied. However a distance of 150 m between riffles was enough for the β diversity component to be higher than expected in habitat scale in southern Brazil (Hepp & Mello, 2013).

The heterogeneity of macroinvertebrates composition generated by seasonal variation was enough to change the patterns of diversity partition among the four spatial scales evaluated. We found differences in the family composition between the reaches and the streams studied in both winter and summer. However, the magnitude of β diversity component increased at the reach scale in winter, especially in riffle substrates (stone and gravel) and the magnitude of β diversity component increased at the broader scale (stream) in all substrate types in summer. Seasonal variation in macroinvertebrate assemblages usually is attributed to environmental variability, like temperature or precipitation. Solar radiation values change seasonally with shifts in the angle of the sun and day length and by phenological changes in streamside vegetation (Hauer & Hill

2007). In our study, the precipitation is well distributed in the four seasons, about 1,250 to 2,000 mm of rain per year (Quadro *et al.*, 1996). Possibly the variation of light and temperature between summer and winter was the main variable that affected macroinvertebrate composition, modifying the magnitude of β diversity component between scales. The combination of changes in light and temperature between summer and winter shifts shredder macroinvertebrate dominance related to the type and timing of riparian litter inputs (Cummins *et al.*, 1989, Cummins, 2002). Furthermore, the greater light incidence increases stream primary production, changing the trophic structure with the increased scrapers macroinvertebrate (Li and Dudgeon, 2008)

Aquatic macroinvertebrate communities in streams are structured by a combination of dispersal limitation and environmental factors (Thompson & Townsend, 2006), so it is important to consider that macroinvertebrate communities respond to multiple environmental gradients, many of which are scale related (Stendera & Johnson, 2005). Although the study was conducted in streams of the same watershed, with similar environmental variations, there was heterogeneity in the composition between scales. Differences in environmental conditions not evaluated or a restriction in the macroinvertebrate dispersal may have favored a greater variation of β diversity.

Clarke *et al.* (2010) found that β diversity generally was higher at the reaches scale. β diversity at the reach scale may be generated by high-habitat heterogeneity among even very close sites. Small headwater streams are often structurally complex because their morphology is largely determined by the stochastic distribution of obstructions, such as rocks, tree roots and woody debris, which cannot easily be moved because of low stream power (Gooderham *et al.*, 2007). Furthermore, aquatic macroinvertebrate have limited dispersal capabilities (Collier & Smith, 1998). Hepp & Melo (2013) found that a great heterogeneity in the insect composition was only a result of dispersal limitation of these organisms; there was no correlation between composition and environmental variables in streams in southern Brazil.

We conclude that the relative importance of the beta component of diversity may change depending on the scale of study, season and substrate type. Understanding the scale dependence of macroinvertebrate diversity partitioning is critical to the application of streams management and conservation strategies. Our results revealed that streams in southern Brazil support a very heterogeneous macroinvertebrate community and the role of spatial configuration in these streams strongly influences macroinvertebrate community diversity. Even reaches in the same stream may support different

community composition. In this sense, diversity inventories should concentrate sampling in different microhabitats, stream reaches and stream sites. An efficient conservation gains are likely to be made by protecting unique streams, as we found a strong evidence of scale dependence on diversity partitioning of macroinvertebrate communities, with β diversity component at broader spatial scales (reach and stream) making a large contribution to total diversity in streams of southern Brazil.

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Table 1. Environmental variables (mean \pm standard deviation) of two reaches in three headwater streams studied in southern Brazil. Current velocity, temperature, dissolved oxygen, pH, turbidity and total dissolved solids were calculated as the mean and standard deviation of summer and winter in each stream (n=4).

	Stream 1	Stream 2	Stream 3
Altitude (m)	161 \pm 39.6	105 \pm 16.97	115 \pm 24.04
Width (m)	8.35 \pm 2.91	10.02 \pm 1.87	12.49 \pm 0.4
Shade (%)	64.87 \pm 16.44	60.37 \pm 27.05	43.63 \pm 11.49
Depth (m)	0.39 \pm 0.02	0.43 \pm 0.03	0.35 \pm 0.02
Current velocity ($m\ s^{-1}$)	0.31 \pm 0.09	0.12 \pm 0.09	0.21 \pm 0.2
Temperature ($^{\circ}C$)	16.34 \pm 6.81	20.43 \pm 4.4	20.33 \pm 3.33
Dissolved oxygen ($mg\ L^{-1}$)	10.56 \pm 2.22	10.04 \pm 3.0	11.37 \pm 1.62
pH	7.61 \pm 0.53	7.06 \pm 0.07	7.61 \pm 0.33
Turbidity (NTU)	3.82 \pm 4.83	7.91 \pm 9.17	5.77 \pm 6.62
Total dissolved solids (g/L)	2.47 \pm 2.82	0.41 \pm 0.6	5.47 \pm 6.37
NH ₃ -nitrogen ($mg\ L^{-1}$)	0.87 \pm 0.79	0.49 \pm 0.11	0.27 \pm 0.05

Table 2. Observed and expected total diversity partitioning into α (sampling unit), β_1 (among sampling units), β_2 (among habitats), β_3 (among reaches), and β_4 (among streams) during winter in three streams in southern Brazil.

	Diversity component	Observed	Expected	Prop _{exp>obs}
<i>Stone</i>	Sampling unit, α	16.31	20.16	1
	Among sampling units, β_1	8.11	9.75	1
	Among habitats, β_2	7.58	7	0.15
	Among reaches, β_3	10.67	6.74	<0.001
	Among streams, β_4	13.33	12.56	0.183
	Total, γ	56		
<i>Gravel</i>	Sampling unit, α	13.91	17.69	1
	Among sampling units, β_1	7.76	8.05	0.783
	Among habitats, β_2	6	6.53	0.833
	Among reaches, β_3	9	6.18	<0.001
	Among streams, β_4	13.33	11.55	0.026
	Total, γ	50		
<i>Leaf Litter</i>	Sampling unit, α	12.94	18.48	1
	Among sampling units, β_1	8.48	9.7	1
	Among habitats, β_2	7.08	7.17	0.582
	Among reaches, β_3	10.17	8.11	0.005
	Among streams, β_4	19.33	14.54	<0.001
	Total, γ	58		

Table 3. Observed and expected total diversity partitioning into α (sampling unit), β_1 (among sampling units), β_2 (among habitats), β_3 (among reaches), and β_4 (among streams) during summer in three streams in southern Brazil.

	Diversity component	Observed	Expected	Prop _{exp>obs}
<i>Stone</i>	Sampling unit, α	18.08	21.99	1
	Among sampling units, β_1	8.25	10.12	1
	Among habitats, β_2	5.34	5.96	0.888
	Among reaches, β_3	7.33	5.13	0.001
	Among streams, β_4	13	8.79	<0.001
	Total, γ	52		
<i>Gravel</i>	Sampling unit, α	15.49	19.53	1
	Among sampling units, β_1	8.93	10.63	1
	Among habitats, β_2	5.91	7.79	1
	Among reaches, β_3	9	7.51	0.026
	Among streams, β_4	14.67	8.54	<0.001
	Total, γ	54		
<i>Leaf Litter</i>	Sampling unit, α	12.37	16.74	1
	Among sampling units, β_1	8.3	9.21	0.996
	Among habitats, β_2	6.66	7.12	0.826
	Among reaches, β_3	8.34	7.74	0.175
	Among streams, β_4	15.33	10.2	<0.001
	Total, γ	51		

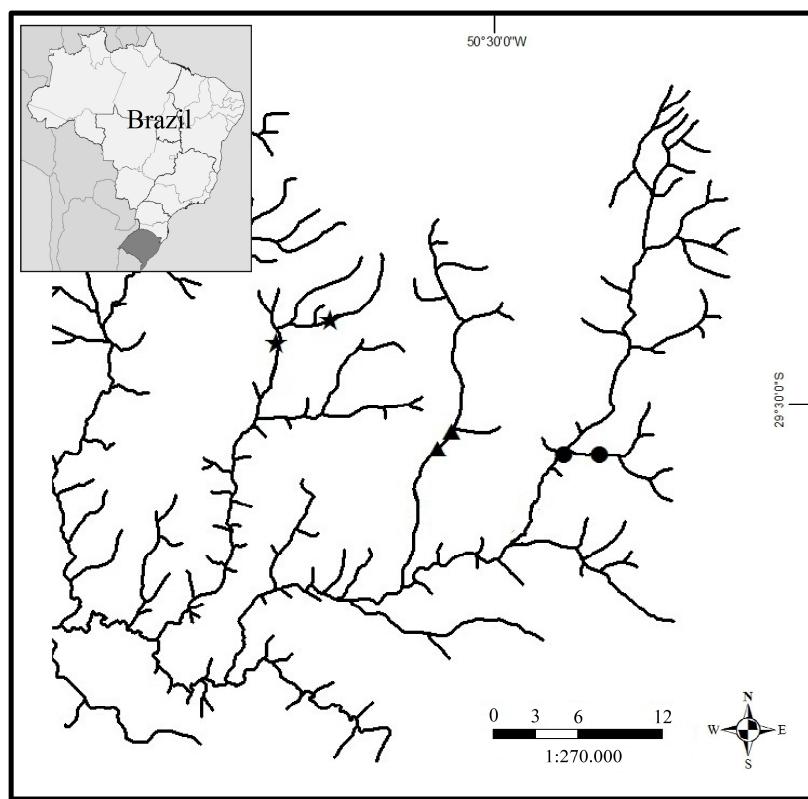


Figure 1. Location of study sites in southern Brazil, showing two reaches in stream 1 (★), stream 2 (●) and stream 3 (▲). Two habitats were sampled in each stream reach.

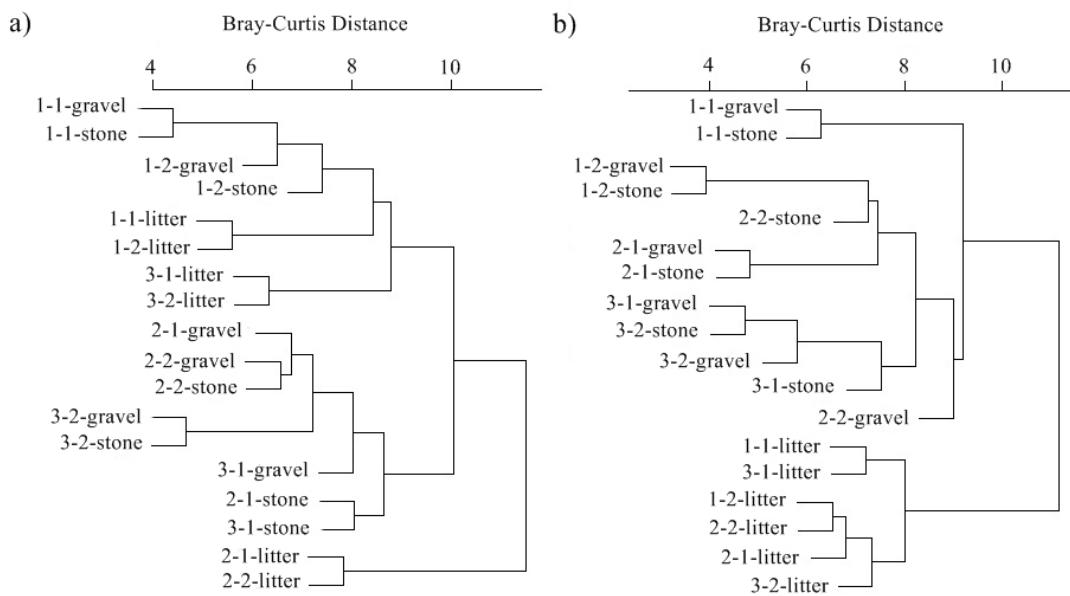


Figure 2. Dendrogram obtained using UPGMA as linkage method and Bray-Curtis dissimilarities of aquatic macroinvertebrate samples (combination of two habitats) obtained in a) winter and b) summer. The first number of the sample label indicates the stream segment. The second number indicates the reach.

CONSIDERAÇÕES FINAIS

Nossos resultados revelaram que as áreas mais preservadas dos riachos estudados suportam uma comunidade de macroinvertebrados heterogênea, sendo essa comunidade fortemente influenciada pela configuração espacial dos riachos. Além disso, a dissimilaridade da composição pode mudar dependendo da escala do estudo, estação do ano e tipo de substrato. O componente de diversidade beta em escalas maiores (trecho e riacho) contribui com a maior parte da diversidade total em riachos do sul do Brasil.

Em áreas com maior impacto sobre a mata ripária houve relação entre a intensidade do uso do solo e as características físicas e químicas dos riachos. O uso do solo influenciou as quantidades de nutrientes nos pontos amostrados. Os pontos com maior quantidade de mata ripária e maior sombreamento estiveram relacionados com a diminuição de nutrientes. Nos pontos com menos mata ripária e mais urbanização houve diminuição da velocidade da água, possivelmente relacionada à sedimentação de partículas.

Houve menor diversidade beta, ou homogeneização da composição, entre microhabitats dentro de trechos impactados pelo uso do solo. A dissimilaridade da composição, considerando o índice de presença e ausência de famílias, foi maior quando houve maior sombreamento, menor velocidade da água e dos nutrientes e um pH próximo do neutro. Considerando a abundância de famílias, a variação da diversidade β esteve relacionada positivamente à largura do riacho, turbidez e fósforo total. A utilização dos diferentes índices para análise de diversidade β tornou os resultados relacionados com os fatores físicos e químicos mais variáveis, porém todos demonstrando relações indiretas com os usos do solo.

Uma redução na largura de mata ripária para 15 m ou menos modificou significativamente a composição e as condições tróficas de macroinvertebrados nos riachos estudados. Larguras de mata ripária acima de 15 m são necessárias para manter a comunidade de macroinvertebrados em estados de conservação semelhantes às áreas de referência. Entretanto, o novo Código Florestal (Código Florestal nº 12.651, 2012) reduziu a largura de floresta ripária legal de pequenas propriedades de 30 m para 15 m ou menos (5 m) no Brasil.

Os resultados ressaltam a importância da preservação da mata ripária para a manutenção da diversidade e composição das comunidades de macroinvertebrados nos

riachos estudados. Nesse sentido, conhecimentos sobre estressores relacionados à comunidade de macroinvertebrados e à diversidade β são necessários para os estudos de conservação no sul do Brasil. Além disso, o desenvolvimento de estratégias de manejo para florestas ripárias é de extrema importância para preservar esses ecossistemas, uma vez que os riachos e rios estão criticamente ameaçados no mundo inteiro devido à perda de floresta ripária.