UNIVERSIDADE DO VALE DO RIO DOS SINOS
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA

MARINA SCHMIDT DALZOCHIO

PODEM AS LAVOURAS DE ARROZ AUXILIAREM NA CONSERVAÇÃO DE MACROINVERTEBRADOS AQUÁTICOS DO SUL DO BRASIL? UNINDO O SISTEMA PRODUTIVO COM CONSERVAÇÃO DA BIODIVERSIDADE.

SÃO LEOPOLDO
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Tese apresentada ao curso de Pós-Graduação em Biologia, da Universidade do Vale do Rio do Sinos, como parte das exigências para a obtenção do título de Doutor em Biologia.

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Orientador: Prof. Dr. Leonardo Maltchik

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A tese intitulada **'Podem as lavouras de arroz auxiliarem na conservação de macroinvertebrados aquáticos do sul do Brasil? Unindo o sistema produtivo com conservação da biodiversidade'**, elaborada por Marina Schmidt Dalzochio, foi julgada adequada e aprovada por todos os membros da Banca Examinadora, para obtenção do título de DOUTORA EM BIOLOGIA, com área de concentração: Diversidade e Manejo de Vida Silvestre.

São Leopoldo, 25 de fevereiro de 2014.

Membros da Banca Examinadora da Tese:

Prof. Dr. Fabio de Oliveira Roque - Universidade Federal de Mato Grosso do Sul

Prof. Dr. Luiz Ubiratan Hepp - Universidade Regional Integrada do Alto Uruguai e das Missões

Profa. Dr. Juliano Morales de Oliveira – Universidade do Vale do Rio dos Sinos

Profa. Dr. Everton Nei Lopes Rodrigues - Universidade do Vale do Rio dos Sinos

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MUITO OBRIGADO!!!

“So wake me up when it’s all over
When I'm wiser and I'm older
All this time I was finding myself
And I didn’t know I was lost”

Avicii

APRESENTAÇÃO

Esta tese foi elaborada em duas partes a fim de facilitar a publicação dos resultados obtidos. Primeiramente, apresenta-se um marco teórico sobre os assuntos abordados nos demais capítulos, com objetivo de fornecer base teórica acerca da temática desta tese. Essa primeira parte, inicia-se com os aspectos gerais que compõe uma área úmida, suas funções e status de conservação destes ecossistemas. A seguir, apresentamos informações sobre o cultivo do arroz irrigado e como estas áreas de produção podem gerar algum benefício para a biodiversidade. Traremos também informações gerais sobre a biologia e a ecologia da comunidade de macroinvertebrados aquáticos nesses ecossistemas, bem como as principais ameaças atuais à este grupo. Por fim, complementaremos essa seção com os objetivos, hipóteses, metodologia do trabalho desenvolvido, conclusões e considerações finais.

Na segunda parte, temos os capítulos que resumem os resultados obtidos durante o estudo, estruturados sob a forma de artigos científicos. O primeiro manuscrito teve como objetivo principal comparar a estrutura da comunidade de invertebrados aquáticos entre áreas úmidas naturais e lavouras de arroz irrigadas, cultivadas sob o manejo orgânico e convencional, ao longo de um ciclo de cultivo. Porém, medidas tradicionais de diversidade que levam em conta apenas o número de espécies e suas contribuições relativas, podem expressar apenas uma parcela da biodiversidade dos ecossistemas. Pensando nisso, o segundo manuscrito foi elaborado levando em conta aspectos funcionais da comunidade dos insetos aquáticos, através da comparação dos índices de diversidade funcional das áreas úmidas naturais e lavouras de arroz irrigadas cultivadas sob o manejo orgânico e convencional, ao longo de um ciclo de cultivo. Desta forma, acredita-se, que será incorporado informações adicionais da biodiversidade nesses sistemas, podendo revelar padrões mais precisos do para conservação das áreas úmidas.

Por fim, o terceiro manuscrito aborda como a intensificação da agricultura pode influenciar a biodiversidade de invertebrados aquáticos. Avaliou-se de que forma arrozais com diferentes idades de cultivo podem afetar a estrutura das comunidades de macroinvertebrados em relação às áreas naturais.

As referências da introdução seguem as normas vigentes da Associação Brasileira de Normas Técnicas (ABNT) e os manuscritos seguem as normas das seguintes revistas:

- Capítulo 1: Agriculture, Ecosystems & Environment
- Capítulo 2: Ecological Entomology
- Capítulo 3: Environmental Management

SUMÁRIO

RESUMO

As áreas úmidas são ecossistemas prioritários para a conservação em vista de sua grande diversidade biológica e produtividade, além de suas inúmeras funções e valores. No Sul do Brasil, dados conservativos apontam que aproximadamente 90% das áreas úmidas originais já foram destruídas principalmente devido à expansão agrícola, especialmente de lavouras de arroz irrigado. Por outro lado, o arroz é o cereal mais importante cultivado em países em desenvolvimento, sendo o principal alimento para mais da metade da população mundial. Além disso, uma alta diversidade de espécies de plantas e animais tem sido encontrada nessas áreas agrícolas. O objetivo geral deste estudo foi avaliar a estrutura e a diversidade de macroinvertebrados em lavouras de arroz com diferentes tipos de manejo (orgânico e convencional) e com diferentes tempos de cultivo (3, 10 e 20 anos) nas diferentes fases hidrológicas do ciclo de cultivo em uma importante região orizícola do Rio Grande do Sul, visando a conservação da biota nestes agroecossistemas. As coletas foram realizadas ao longo de dois ciclos de cultivo (agosto de 2010 a junho de 2012) em 17 lavouras de arroz e oito áreas úmidas naturais na Planície Costeira do Rio Grande do Sul. Foram obtidas amostras qualitativas, utilizando um puçá aquático. Cada amostra consistiu em 5 varreduras de 1 metro do sedimento e da coluna d'água ao longo de seus gradientes de distância da margem. Os principais resultados obtidos foram: as amostragens resultaram em um total de 37.035 indivíduos distribuídos em 82 *taxa* de macroinvertebrados coletados nas lavouras de arroz e áreas úmidas naturais; a agricultura orgânica afeta negativamente a comunidade de macroinvertebrados aquáticos tanto quanto a convencional, mesmo em termos funcionais e; os primeiros anos de cultivo das lavouras de arroz são os mais impactantes para a comunidade de macroinvertebrados aquáticos. Esses resultados podem ser utilizados em planos de manejo que busquem conciliar a produção agrícola com a conservação da biodiversidade no Rio Grande do Sul.

Palavras-chave: biodiversidade, macroinvertebrados bentônicos, cultivo de arroz, manejo de agroecossistemas, conservação de áreas úmidas.

ABSTRACT

Wetlands are prioritary ecosystems for conservation due to their vast biological diversity and productivity, besides their many functions and value. In southern Brazil conservative data show that about 90% of the original wetlands are already destroyed due to agricultural expansion, mainly the irrigated rice fields. On the other hand, rice is the most important cereal grown in developing countries, being the main food source for more than half of the world population. Also, a high diversity of plants and animals has been found in these agricultural areas. The main goal of this study was to assess the structure and diversity of macroinvertebrates in rice fields with different rice cultivation systems (organic and conventional) and ages (3, 10 and 20 years) over the different hydrological phases of the cultivation cycle in an important rice cultivation area in Rio Grande do Sul to preserve the biota in these agroecosystems. Sampling was conducted over two cultivation cycles (August 2010 to June 2012) in 17 rice fields and eight natural wetlands in the coastal plain of Rio Grande do Sul. Qualitative samples were obtained using a dip net. The samples were collected by kicking up the substrate and then sweeping above the disturbed area to capture dislodged or escaping macroinvertebrates. Five random sweeps of 1 m each were performed in each area. The main results were: sampling resulted in a total of 37,035 individuals in 82 *taxa* of macroinvertebrates collected in rice fields and natural wetlands; organic agriculture negatively affects aquatic macroinvertebrate community as much as conventional, even in functional terms and the first years of cultivation of rice crops are the most impactful for the community of aquatic macroinvertebrates. These results can be used in management plans which aim at reconciling agricultural production and biodiversity conservation in Rio Grande do Sul.

Key-words: biodiversity, benthic macroinvertebrates, rice cultivating, agroecosystem management, wetland conservation.

1. MARCO TEÓRICO

1.1. Áreas úmidas naturais

Área úmida é um termo genérico utilizado para caracterizar ecossistemas de solo saturado de água, como pântanos, banhados, mangues e áreas similares (TINER, 1999; ELDREDGE, 2002). São áreas ecotonais entre ecossistemas aquáticos e terrestres cuja estabilidade e diversidade de espécies estão estreitamente condicionados pela hidrologia e fluxos de materiais (BEDFORD et al., 2001).

Esses ecossistemas ocorrem em todos os continentes (exceto Antartico), bem como em uma grande variedade de tipos climáticos (BRINSON; MALVARÉZ, 2002). Há áreas úmidas no deserto, no Ártico, em florestas úmidas e em regiões de grande densidade populacional (ROTH, 2009), cobrindo cerca de 6% da surperfície da Terra (BEDFORD et al., 2001), ocorrendo principalmente nos trópicos e nos subtropicos.

Por ocorrer em uma gama de combinações climáticas, topológicas e biogeográficas, as áreas úmidas suportam riqueza extraordinária de plantas e animais (KEDDY, 2000, BRINSON; MALVARÉZ, 2002, GETZNER, 2002, JANTKE; SCHNEIDER, 2010). A rica biota é adaptada à tolerar periodos alternados de presença de água, salinidade, pH e nutrientes, formando um ecossistema altamente produtivo e funcionalmente diversificado (BENSTEAD; JOSÉ, 2001).

Ao lado de sua importancia para vida selvagem, áres úmidas são essenciais por uma série de benefícios que dispõem à sociedade humana (BENSTEAD; JOSÉ, 2001, ELDREDGE, 2002). As áreas úmidas naturalmente regulam o fluxo de água nas bacias hidrográficas, controlando a erosão e as inundações. Fornecem ainda, solos ferteis à agricultura, recurso energético, de pesquisa e recreação. A biota presente nesses ecossistemas, atuam como um filtro, absorvendo poluentes e purificando a água antes desta chegar à outros ambientes aquáticos (RAMSAR CONVENTION SECRETARIAT- RAMSAR, 2013). Nesse sentido, as áreas úmidas são ecossistemas prioritários para a conservação (JANTKE; SCHNEIDER, 2010, MALTICHIK et al., 2012).

Diante deste cenário, em 1971, na cidade iraniana de Ramsar, um dos primeiros tratados de caráter intergovernamental foi assinado, visando a conservação e o uso racional das áreas úmidas (ROTH, 2009). A Convenção sobre Zonas Úmidas de Importância Internacional, ou apenas Convensão de Ramsar, entrou em vigor em 1975 e tem como objetivo construir pilares norteadores da conservação desses ecossistemas sob três aspectos: uso racional dos serviços ecossistêmicos, criar lista de Sítios Ramsar (áreas úmidas de importancia internacional) e a cooperação internacional para uma conservação concreta das áreas úmidas (RAMSAR, 2013).

Existem hoje, segundo relatório oficial (RAMSAR, 2013), 2.127 áreas úmidas de importancia internacional. Ao ser incluida como um Sítio Ramsar, a região tem prioridade na implementação de políticas governamentais, além de notável conhecimento público. Até o momento, 168 países assinaram, este tratado de cooperação internacional para o proteção das áreas úmidas (RAMSAR, 2013).

O Brasil integrou oficialmente a Convenção de Ramsar em 1996, através do decreto nº 1.905 de 16 de maio de 1996. Atualmente, 11 áreas úmidas brasileiras estão incluídas na Lista de Ramsar (Tabela 1) e coicidem com Unidades de Conservação, já protegidas pelo Sistema Nacional de Unidades de Conservação (SNUC). A extensão total de áreas úmidas protegidas no País perfaz 6.500.000 hectares. Além de reconhecimento internacional, esses sítios possuem acesso facilitado aos fundos de doação específicos (MINISTÉRIO DO MEIO AMBIENTE- MMA, 2013).

No Rio Grande do Sul, o Parque Nacional da Lagoa do Peixe é o único sítio protegido pela Convenção de Ramsar. É um dos mais importantes refúgios de aves migratórias da América do Sul e berçário para aves residentes e espécies marinhas, como tainha e linguado. Além disso, é a última área do estado em que há predominância da restinga litorânea (LOEBMANN; VIEIRA, 2005).

Entretanto, esforços de conservação não são suficientes enquanto a demanda por terra e comida aumenta sem precedentes. Áreas úmidas são ecossistemas sensíveis e pequenas mudanças podem alterar seu funcionamento (MOORE, 2006). Continuamente, estatísticas bem fundamentadas acusam perdas em vários países (RAMSAR, 2013, RUSSI et al., 2013). As perdas variam de 53% (Estados Unidos) a 90% (Nova Zelândia). Diante disto, é razoável supormos que globalmente já perdemos 50% e continuamos perdendo, particularmente nos países em desenvolvimento (RUSSI et al., 2013). No Brasil, inventários sobre a perda de áreas úmidas são escassos, porém somente o Pantanal, uma das maiores áreas alagáveis do Planeta, já tem 40% da sua área original perdida (HARRIS et al., 2005). Segundo Moore (2006), as principais forças motoras que impulsionam a perda e degradação dessas áreas úmidas são a conversão de áreas para fins agrícolas, desenvolvimento urbano e industrial, tomada excessiva de água doce para uso na agricultura e poluição das águas por agrotóxicos e metais pesados.

As áreas úmidas são formações comuns na paisagem do Rio Grande do Sul e, no passado, ocupavam grandes extensões da zona costeira e também de regiões mais internas do Estado (CARVALHO;OZÓRIO, 2007). Segundo Fundação Zoobotânica do Rio Grande do Sul (2002), o estado possuía originalmente 5,3 milhões de hectares de áreas úmidas, porém, a grande parte delas foi drenada para o cultivo do arroz irrigado.

1.2. Arrozais

O arroz está entre os cereais mais consumidos do mundo, sendo a base alimentar de mais de três bilhões de pessoas (COMISSÃO TÉCNICA SUL BRASILEIRA DE ARROZ IRRIGADO- CTAR, 2010). Na América Latina são consumidos em média, 30 kg desse grão por pessoa/ano. Em decorrência, desempenha papel estratégico na solução de questões de segurança alimentar (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 2013).

Atualmente o arroz é a cultura com maior potencial de aumento de produção no mundo e cresce 3% a cada ano (FAO, 2013). Cerca de 165 milhões de hectares das terras cultiváveis do mundo são destinadas à produção do grão, representando 11% da área arável total do planeta.

Apenas duas espécies de arroz são utilizadas como alimento: *Oryza sativa* L., originária da Índia e China, cultivada mundialmente e *Oriza glaberrima* Steud., originária e ainda cultivada no Oeste Africano (SWAMINATHAN, 1984). É uma planta muito adaptável, podendo ser cultivada em áreas secas e irrigadas, com grande variação na profundidade da água (FASOLA; RUIZ, 1996).

O arroz, classificado primariamente como uma cultura tropical e subtropical, é atualmente produzido por mais de 100 países. O continente asiático destaca-se por 90% da produção mundial do grão (FAO, 2013), onde somente a China e a Índia contribuem com 50% de todo arroz consumido no mundo (BANCO NACIONAL DO DESENVOLVIMENTO-BNDES, 2008). O Brasil é o nono produtor mundial (MINISTÉRIO DA AGRICULTURA, PECUARIA E ABASTECIMENTO-MAPA, 2013), com cerca de 2 milhões e 400 mil hectares de área plantada e uma produção de cerca de 12 milhões de toneladas/ano (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATISTICA-IBGE, 2013).

No Mercosul, o Brasil participa com cerca de 80% da produção de arroz, sendo que esta produção está distribuída nos estados do Rio Grande do Sul, Santa Catarina e Mato Grosso (CTAR, 2010, MAPA, 2013). A produção de arroz no Brasil, até o final do século XIX, foi oriunda, exclusivamente, de lavouras de sequeiro. Na última década do século, paralelamente, surgiram as primeiras lavouras com cultivo de arroz irrigado, principalmente no Sul do Brasil, as quais mostraram sensível ganho de produtividade em relação ao cultivo de sequeiro (AZAMBUJA et al., 2004).

A cultura do arroz irrigado, em função de suas características, estabeleceu-se e desenvolveu-se no Rio Grande do Sul sobre os denominados solos de várzea, encontrados nas planícies de rios e lagoas, que apresentam como característica comum, o hidromorfismo (GOMES et al., 2004). Outro aspecto importante é que parcelas de arroz, no Estado, são cultivadas desde muitos anos. Segundo Ferreiro e Villar (2003), desde meados de 1970 registra-se aumento da área cultivada do arroz irrigado no Brasil. Como forma de diminuir a pressão ambiental sobre a biodiversidade, atualmente, essas parcelas são submetidas às novas tecnologias afim de aumentar o rendimento. Entretanto, a pressão de demanda é tão grande, que faz-se necessário a conversão de outras parcelas, anualmente, para aumentar a quantidade de grão. Estimativas apontam que até 2030, a safra mundial de arroz precisará aumentar em 50% (FAO, 2013).

O cultivo de arroz irrigado contribui, em média, com 70% da produção nacional (IBGE, 2013), sendo o Rio Grande do Sul o maior produtor brasileiro. O arroz é produzido no estado em 133 municipios, onde aproximadamente 230 mil pessoas vivem direta ou indiretamente da exploração dessa cultura (CTAR, 2010). Segundo a classificação utilizada pelo Instituto Riograndense do Arroz (CTAR, 2010), o Rio Grande do Sul está dividido em seis regiões orizícolas: a maior região produtora de arroz é a Fronteira Oeste, com aproximadamente 29% da produção, seguido pela Zona Sul (20%), Campanha (18%), Depressão Central (15%) e Planície Costeira externa e interna à Laguna dos Patos (cerca de 10% cada).

As lavouras de arroz do Rio Grande do Sul são cultivadas sob diferentes regimes, sendo que os principais são: convencional, plantio direto, cultivo mínimo, orgânico e biodinâmico. Enquanto o sistema convencional de cultivo do arroz irrigado baseia-se em intensa mecanização, os sistemas de cultivo mínimo e plantio direto são sistemas conservacionistas de manejo do solo. Entretanto, esses três sistemas de cultivo do arroz irrigado utilizam agrotóxicos e fertilizantes sintéticos (CTAR, 2010). Já o sistema orgânico diferencia-se dos demais por não utilizar agro-químicos e por incluir a rotação de culturas e o uso de esterco animal e restos vegetais como fertilizantes. O sistema biodinâmico ainda, faz o uso de plantas fixadoras de nitrogênio e o manejo do volume de água da área cultivada para controle de plantas daninhas (ANDERSSON et al., 2012).

A irrigação das lavouras de arroz no Rio Grande do Sul é realizada através do sistema de submersão do solo, sendo que a água utilizada é captada, principalmente, de rios, açudes, lagoas e barragens (GOMES et al., 2004). O uso da água para cada hectare de arroz cultivado no Rio Grande do Sul varia entre 9.000 a 25.000 metros cúbicos/ano (LORENSI et al., 2010).

1.3. Ecologia de arrozais

Ecologicamente, arrozais são áreas úmidas artificiais caracterizadas por perturbações hidrológicas periódicas (BAMBARADENIYA et al., 2004). Um único ciclo de cultivo abrange três condições hidrológicas diferentes: 1) aquática; 2) semi-aquática; e 3) terrestre (FERNANDO, 1995). Fisicamente, a fase aquática constitui-se de um ambiente de lamina d´água rasa, que varia de 5 a 30 cm, necessários para a manutenção dos estágios vegetativo e reprodutivo da planta do arroz. A fase semi-aquática correponde ao estágio de pós-colheita do arroz e entressafra, onde não há manutenção da lâmina d´água, apenas a permanencia do solo bastante enxarcado. A fase terrestre corresponde ao estágio de preparo do solo, onde o solo está completamente drenado e há ausência de vegetação (BAMBARADENIYA et al., 2004).

Segundo Bambaradeniya e Amerasinghe (2003), além do regime hidrológico, fatores como condições climáticas e práticas agrícolas também são fatores importantes para a ecologia dentro das lavouras de arroz. Fatores climáticos, como radiação solar, temperatura, umidade relativa e velocidade do vento, controlam a evaporação, enquanto a precipitação controla as caracteristicas fisico-químicas da água utilizada para a irrigação. Ainda segundo esses autores, as práticas agrícolas são fatores primordiais na ecologia dessas áreas úmidas artificiais, uma vez que, as intervenções humanas controlam o pulso de inundação, o crescimento da planta de arroz, a fertilizadade dos solos e a qualidade da água, consequentemente controlando toda biodiversidade em arrozais. Estudos anteriores (STENERT et al., 2009, MACHADO; MALTCHIK, 2010, ROLON; MALTCHIK, 2010) demonstram claramente que as práticas agrícolas, alteram as condições físicas, químicas e biológicas dos arrozais.

1.3.1. Biodiversidade em arrozais

A conversão das áreas úmidas naturais em arrozais tornou estes sistemas refúgios estratégicos para muitas espécies de plantas e animais aquáticos em todo o mundo (Stenert et al., 2012). Bambaradeniya et al. (2004), encontrou mais de 490 espécies de invertebrados, 100 espécies de vertebrados e 89 espécies de macrófitas associados à arrozais no Sri Lanka. Hayasaka et al. (2012) cerca de 180 espécies de invertebrados e vertebrados em arrozais no Japão. Nesses trabalhos, entre os vertebrados, há destaque para grande número de espécies de aves. Sundar e Subramanya (2010) identificaram mais de 350 espécies de aves nidificando ou forrageando em arrozais. Os arrozais têm um importante papel nas rotas de migração de aves, bem como na conservação das populações de mamíferos (Rizo-Pátron et al., 2013). No Brasil, há poucos trabalhos que resumem a biodiversidade associadas às lavouras de arroz. No sul do Brasil, Maltchik et al. (2011) encontrou, em canais utilizados para irrigação desses arrozais, mais de 160 *taxa* de invertebrados, macrófitas e anfíbios. Amostrando lavouras de arroz com diferentes tipos de manejo da água de irrigação, Machado e Maltchik (2009) coletaram 12 espécies de anfíbios anuros e Rolon e Maltchik (2010) identificaram 88 espécies de plantas aquáticas.

A ocorrência de determinados grupos de organismos, tanto da flora quanto da fauna, apresenta padrões relacionados com as diferentes fases hidrológicas e de crescimento da planta do arroz ao longo do ciclo de cultivo (período com água e sem água) (BAMBARADENIYA; AMERASINGHE, 2003). Em geral os organismos que habitam as lavouras de arroz irrigado caracterizam uma biota oportunista, e altamente resiliente, capazes de reagir através de suas características fisiológicas e comportamentais às mudanças temporais drásticas que ocorrem nesses sistemas, recuperando-se rapidamente após os diferentes tipos de perturbações às quais as lavouras de arroz são submetidas (BAMBARADENIYA, 2000). A alta diversidade biológica verificada nesses arrozais é mantida pela rápida colonização, bem como pelas altas taxas de reprodução e crescimento dos organismos encontrados nesses agroecosistemas (FERNANDO, 1995; 1996). Muitos desses organismos colonizam os arrozais por meio de estruturas de resistência mantidas no solo, pelo ar e pela água via irrigação (STENERT et al., 2010).

a) Macroinvertebrados Aquáticos

Os macroinvertebrados aquáticos são invertebrados de tamanho relativamente grande (1-8 cm), visíveis ao olho humano. Compreendem principalmente artrópodes, dos quais a Classe Insecta é a mais representativa, especialmente com suas formas larvais. Há também representantes de outros grupos taxonômicos, como platelmintos, anelídeos e moluscos (ALBA-TECEDOR et al., 2005). São os organismos mais abundantes dentro dos corpos d´água (BOUCHARD, 2004).

Macroinvertebrados aquáticos podem ser encontrados próximos à qualquer habitat aquático, desde poças temporárias até grande lagos e de pequenos córregos até grandes rios. Os ambientes mais extremos habitados por esses animais incluem águas salinas, poças de petróleo, lagoas de tratamento de efluentes e águas termais (MERRITT et al., 2008). Em águas paradas, ocorrem no fundo de lagos, ao longo das margens vegetadas e em águas abertas. Em ambientes lóticos, ocorrem debaixo de pedras, galhos e troncos, enterrados na areia ou sedimentos, e rastejando sobre rochas e folhas (BOUCHARD, 2004).

Os macroinvertebrados aquáticos se alimentam de uma grande variedade de tipos de alimentos, incluindo as folhas das árvores, algas, madeira, detritos, outros invertebrados e mesmo alguns vertebrados, como pequenos peixes e girinos. A comunidade de macroinvertebrados é um importante componente em ecossistemas aquáticos continentais, sendo fundamental para a dinâmica de nutrientes, para a transformação da matéria e para o fluxo de energia (CALLISTO; ESTEVES, 1995).

Os macroinvertebrados aquáticos constituem um dos grupos biológicos mais amplamente usados como indicadores da qualidade da água, sobretudo em ambientes lóticos (VANNOTE et al., 1980, CUMMINS et al., 1984, CALLISTO et al., 1998, BIS et al., 2000, CALLISTO et al., 2001, GOULART; CALLISTO, 2003, CUMMINS et al., 2005, NESSIMIAN et al., 2008; MILESI et al., 2008; HEPP et al., 2010). Apenas com a recente preocupação em torno da perda de áreas úmidas e de suas funções, os estudos sobre macroinvertebrados de áreas úmidas vêm aumentando em todo mundo (STENERT et al., 2004, STENERT; MALTCHIK, 2007; STENERT et al., 2008; MALTCHIK et al., 2012).

As áreas úmidas apresentam uma série de desafios à sobrevivência dos organismos aquáticos, entre os quais o hidroperíodo parece ser o mais importante. Diversos estudos citam a importancia do hidroperiodo na composição da comunidade de macroinvertebrados (WHILES; GOLDOWITZ, 2005, STENERT; MALTCHIK, 2007). Em áreas úmidas permanentes, a diversidade de macroinvertebrados é tipicamente maior e dominadas por Diptera, Amphipoda e Hemiptera (ZIMMER et al., 2001). Em áreas úmidas temporárias tendem ser numericamente dominadas por culicídeos e Chironomidae (NICOLET et al., 2004). Outros fatores como presença de predadores (MCPARLAND; PASZKOWSKI, 2007) e de fitotóxicos (ROTH, 2009), temperatura e profundidade da água (STUDINSKI; GRUBBS, 2007), oxigenação e salinidade (ROTH, 2009) e altitude (JACOBSEN, 2004) também demonstraram ser determinantes da composição da comunidade em áreas úmidas.

No sul do Brasil, Stenert et al. (2004) analisaram a diversidade e estrutura da comunidade de macroinvertebrados em 146 áreas úmidas, e observaram um total de 84 famílias distribuídas em quatro filos (Arthropoda, Mollusca, Annelida e Platyhelminthes) e 11 classes. Além disso, Stenert e Maltchik (2007) identificaram que o tamanho da área úmida e o hidroperíodo como sendo os fatores ambientais que mais influenciaram a riqueza e composição de macroinvertebrados nesses ecossistemas. Stenert et al. (2008) e Ávila et al. (2011) também avaliaram a importância da diversidade de hábitats para a comunidade de macroinvertebrados e constataram que a riqueza de macroinvertebrados em áreas úmidas da Planície Costeira do Rio Grande do Sul estava diretamente relacionada a essa variável ambiental. Maltchik et al. (2012), utilizando ferramentas biogeográficas, identificaram 32 áreas prioritárias para conservação de macroinvertebrados no Rio Grande do Sul, localizadas principalmente na Planície Costeira e no Planalto da Campanha.

Como as áreas úmidas naturais, a variedade de macroinvertebrados aquáticos que colonizam arrozais são evidentes na literatura, indicando a dominância de amplo espectro da fauna desses sistemas. Suhling et al. (2000) e Leitão et al. (2007) estudando arrozais europeus identificaram em torno de 84 espécies de macroinvertebrados. Wilson et al. (2008) avaliando arrozais australianos de manejo orgânico e convencional amostraram 90 morfoespécies de macroinvertebrados. Bambaradeniya et al. (2004) amostrando arrozais no Sri Lanka registraram 179 espécies de macroinvertebrados. No sul do Brasil, acredita-se que mais de 150 *taxa* de macroinvertebrados colonizam esses ambientes (MOLOZZI et al., 2007, STENERT et al., 2009, 2012).

O principal desafio para a comunidade de macroinvertebrados em lavouras de arroz está na sobrevivência diante às condições impostas pelo manejo do solo e aplicação de fertilizantes e pesticidas em lavouras convencionais. Essas práticas alteram o pH, temperatura, concentração de oxigênio, salinidade e turbidez da água, exigindo fortes adaptações para a sobrevivência (SUHLING et al., 2000, WILSON et al., 2008).

Ambientes lênticos, como lagos, lagoas e áreas úmidas são ambientes fechados, onde as variáveis ambientais são governadas pelas condições climáticas, pela área e forma do corpo d´água. Os macroinvertebrados, sobretudo os insetos apresentam muitas adaptações a estas restrições de habitat, que combinadas têm um grande impacto sobre a estrutura da comunidade (WALLACE; ANDERSON 2008). Além disso, numerosos fatores tróficos e interespecíficos interagem com essas limitações influenciando a estrutura e a dinâmica da comunidade de insetos aquáticos nos ambientes lênticos (HERSHEY et al., 2010).

Dentro das áreas úmidas, os insetos aquáticos, são funcionalmente e taxonomicamente os mais diversos, incluindo formas que ocorrem nos mais variados microhábitats (BEDFORD et al., 2001). Quironomídeos, com suas formas larvais, são geralmente os mais abundantes invertebrados aquáticos presentes nas áreas úmidas naturais. Outros grupos ecológicamente importantes incluem os odonatos, efêmeros, mosquitos, percevejos, besouros aquáticos (WISSINGER, 1999). Dentre os diferentes tipos de áreas úmidas, os arrozais, com períodos bem marcados de seca e cheia, exibem uma nítida sucessão na composição de espécies de insetos aquáticos, que vai da dominância de quironomídeos e culicideos à grandes *taxa*, como Odonata, Coleoptera e Heteroptera (SIMPSON et al., 1994, GHAHARI et al., 2008).

A maioria das informações disponíveis sobre os insetos presentes em arrozais refere-se a insetos considerados pragas do arroz (ROGER, 1996). Por outro lado, Bambaradeniya et al. (2004) registrou um total de 65 espécies de insetos aquáticos em uma lavoura de arroz no Sri Lanka. Suhling et al. (2000), Leitão et al. (2007) e Lupi et al.

(2013) encontraram cerca 140 espécies de insetos aquáticos, em arrozais europeus. Alguns trabalhos em arrozais na Ásia (LI et al., 2007, ZHANG et al., 2013), Austrália (WILSON et al., 2008), Costa Rica (RIZO-PATRON et al., 2013) também demonstram alta diversidade desses organismos.

No Brasil, os trabalhos de Molozzi et al. (2007) e Stenert et al. (2009, 2012) demonstram a capacidade dos arrozais de suportar alta biodiversidade de insetos. Esses trabalhos salientam ainda que as práticas de manejo utilizadas e a fase do ciclo de cultivo da planta causam redução da biodiversidade desses organismos, através da redução de habitats e simplificação de ecossistemas.

1.4. Agricultura Organica

A agricultura orgânica foi proposta com o intuito de aliviar a pressão sobre a biodiversidade em paisagens agrícolas (BENGTSSON et al., 2005, HOLE et al., 2005, RUNDLÖF et al., 2010). Este tipo de manejo agrícola difere-se da agricultura convencional por não permitir uso de pesticidas e de fertilizantes sintéticos, necessitando rotações de culturas mais elaboradas, como por exemplo com o uso de plantas fixadoras de nitrogênio (STOCKDALE et al., 2001).

Diversos estudos demonstram que a gestão de fazendas orgânicas favorece o aumento de biodiversidade em relação às de cultivo convencional e de fato tendem a apoiar um maior número de espécies e abundância total entre os táxons (BENGTSSON et al., 2005, HOLE et al., 2005, FULLER et al., 2005). Andersson et al. (2012) e Batáry et al. (2013), encontraram maior riqueza de espécies em lavouras de trigo e morango orgânicas, demonstrando que esse tipo de manejo favorece a diversidade e o sucesso dos polinizadores. Hodgson et al. (2010) encontrou que paisagem orgânicas suportam maior diversidade de borboletas do que as de cultivo convencional. Rizo-Patron et al. (2013), demonstrou que as lavouras de arroz de cultivo orgânico permitem o estabelecimento de grande número de famílias conhecidas como sensíveis à poluição, diferentemente das lavouras convencionais.

Em contrapartida, Klaus et al. (2013), estudando a diversidade de plantas vasculares, coleópteros, aranhas e hemípteros, concluiram que, apesar de manejo orgânico ser uma forma razoável para apoiar a agro-biodiversidade, ele não aumenta automaticamente a diversidade dos táxons estudados. Bengtsson et al. (2005) e Hole et al. (2005) demonstraram que pássaros, insetos predadores, organismos de solo e plantas responderam positivamente à agricultura biológica, enquanto que os insetos não-predadores e pragas, não. Fuller et al. (2005) mostraram que plantas tendem a responder com consistencia às modificações de manejo utilizado em lavouras agrícolas, no entanto, invertebrados, aves e morcegos não mostram resultados tão consistentes.

1.5. Diversidade Funcional

Em sistemas agrícolas, a biodiversidade desempenha serviços ecossistêmicos que vão além da produção de alimentos. A reciclagem de nutrientes, controle do microclima local, regulação dos processos hidrológicos e da abundância de organismos indesejáveis são em grande parte de origem biológica, e portanto, a sua persistência depende da manutenção da biodiversidade (ALTIERI, 1994). Quando esses serviços naturais são perdidos devido à simplificação biológica, os custos econômicos e ambientais pode ser bastante significativos.

O funcionamento de um ecossistema pode ser influenciado por diversos fatores, sendo um deles as características funcionais dos organismos presentes. Entretanto, essas influências não se dão de forma linear, sendo algumas espécies mais responsáveis por determinadas funções que outras (HOOPER et al., 2005; BALVANERA et al., 2006). Adicionalmente, pode ocorrer uma complementaridade entre espécies, aumentando a taxa de alguma função e a resistência e a resiliência de um ambiente (LOREAU, 2004; HOOPER et al., 2005).

A influência do indivíduo nas funções pode variar de acordo com os atributos (*traits*) das espécies. Esses atributos são propriedades bem definidas, mensuráveis, geralmente medidas no nível de indivíduo que podem ser de caráter morfológico, fisiológico ou comportamental (MCGILL et al., 2006). São selecionados pelo ambiente em função do impacto positivo que proporcionam no *fitness* (ou adaptabilidade) do organismo no ambiente (WEBB et al., 2010). Esta abordagem, conhecida como diversidade funcional, incorpora as similaridades ecológicas entre as espécies coexistentes em uma comunidade (TILMAN, 2001).

Medidas de diversidade funcional podem ser utilizadas para entendermos como as comunidades locais são estruturadas de acordo com o banco regional de espécies (WEIHER; KEDDY, 1999; PETCHEY et al., 2007; GÓMEZ et al., 2010). Essa estruturação é usualmente interpretada como um resultado de dois mecanismos distintos: ***filtro ambiental***, em que espécies coexistentes tendem a ser mais similares do que se esperaria ao acaso, pois as condições ambientais atuam como um filtro, selecionando e possibilitando a persistência de um espectro relativamente pequeno de traços funcionais das espécies (KEDDY, 1992); e ***similaridade limitante***, que previne as espécies de serem funcionalmente muito similares, assumindo a maior coexistência de espécies ecologicamente diferentes entre si (MACARTHUR; LEVINS, 1967).

A teoria do nicho considera que a composição de espécies é influenciada pelas características ecológicas das espécies e, portanto, competição interespecífica, seleção de habitats e diversidade de recursos seriam determinantes para a co-ocorrência de espécies (WEIHER; KEDDY, 1999; GÓMEZ et al., 2010). Valores de diversidade funcional diferentes do esperado ao acaso após perturbações apoiariam a teoria do nicho, pois padrões não randômicos da diversidade de traços funcionais indicariam a importância de processos ligados nichos das espécies (filtro ambiental e similaridade limitante) para a composição de espécies das comunidades (PETCHEY et al., 2007). Por outro lado, a teoria neutra de biodiversidade (HUBBEL, 2001) assume que as espécies coexistem nas comunidades independentemente de suas características ecológicas. Nesse caso, a composição de espécies resultaria da dispersão, reprodução e morte dos indivíduos (OSTLING, 2005). Valores de diversidade funcional iguais ao esperado ao acaso apoiariam as premissas da teoria neutra, pois o nicho das espécies não seriam importantes para a definição da composição de espécies das comunidades analisadas.

A diversidade funcional de comunidades poderia ser também relacionada com a estrutura do habitat. Sabemos que habitats complexos contêm mais espécies do que habitats mais simples (BELL et al., 1991). Um mecanismo que explica esse padrão é a diversificação de nichos: habitats complexos oferecem melhor particionamento de recursos e, consequentemente, suportariam mais espécies ecologicamente diferentes entre si (SCHOENER, 1974). Organismos podem também variar de acordo com a forma com que são afetados pelas estruturas ambientais. Por exemplo, o risco de predação de aves quando forrageiam no solo é menor em habitats com menos vegetação do que em habitats com mais vegetação (WHITTINGHAM; EVANS, 2004). Sendo assim, torna-se importante a análise das relações entre as estrutura ambiental e a diversidade funcional das espécies, pois distúrbios da paisagem podem afetar indiretamente a diversidade funcional por meio de modificações na estrutura do ambiente.

2. OBJETIVOS E HIPÓTESES

2.1. Objetivo Geral

O objetivo geral deste trabalho foi avaliar a estrutura da comunidade de macroinvertebrados aquáticos em lavouras de arroz irrigado com diferentes sistemas de manejo e idades de cultivo nas diferentes fases hidrológicas do ciclo de cultivo em uma região orizícola do Rio Grande do Sul, visando a conservação da biota nestes agroecossistemas.

2.2. Objetivos específicos

1) Realizar um inventário da diversidade de invertebrados aquáticos em lavouras de arroz irrigado na Planície Costeira do sul do Brasil;

2) Analisar a dinâmica da comunidade de macroinvertebrados aquáticos, avaliada através das variações na riqueza, abundância e composição, ao longo das diferentes fases hidrológicas do ciclo de cultivo em lavouras de arroz irrigado de cultivo orgânico e convencional em relação às áreas úmidas naturais;

3) Comparar a diversidade funcional de insetos aquáticos, avaliada através de quatro índices funcionais, em áreas úmidas naturais, lavouras de arroz orgânicas e lavouras de arroz convencionais;

4) Analisar a dinâmica da comunidade de macroinvertebrados aquáticos, avaliada através das variações na riqueza, abundância e composição, ao longo das diferentes fases do ciclo de cultivo em lavouras de arroz irrigado de cultivo com 3, 10 e 20 anos de cultivo em relação às áreas úmidas naturais.

2.3. Hipóteses

Assumindo que a riqueza, abundância e composição de macroinvertebrados aquáticos são fortemente influenciadas pelas práticas agrícolas adotadas em lavouras de arroz (controle de nível de água, aplicação repetitiva de pesticidas e maquinário para preparo de solo) (ROGER et al., 1991; MESLÉARD et al., 2005; WILSON et al., 2008), bem como alteram a funcionalidade dos sistemas, testamos as seguintes hipóteses:

1) Uma redução da riqueza e abundância de macroinvertebrados e uma composição modificada nas lavouras de arroz em relação áreas úmidas naturais;
2) Uma redução da riqueza e abundância de macroinvertebrados e uma composição modificada nas lavouras convencionais em relação as lavouras orgânicas;
3) Uma redução gradativa dos valores de índices funcionais entre as áreas úmidas naturais, lavouras de arroz orgânicas e convencionais;
4) Uma redução da riqueza e abundância de macroinvertebrados e uma composição modificada nas lavouras mais antigas (10 e 20 anos) em relação as lavouras mais novas (3 anos).

Além disso, supondo que a ausência de água durante o preparo suprime a ocorrência e abundância de alguns táxons de macroinvertebrados aquáticos com baixa tolerância à seca (WILLIAMS, 1996), esperamos:

5) Uma redução da riqueza e abundância de macroinvertebrados e uma composição modificada nas lavouras durante as fases de crescimento do arroz em relação as áreas úmidas naturais.

3. METODOLOGIA GERAL

3.1. Área de estudo

O estado do Rio Grande do Sul está localizado na região Sul do Brasil com uma área de 282.184 km^2 (Figura 1). A Planície Costeira estende-se por 640 km à margem do Oceano Atlântico, com área correspondente a 10,6% da área total do Estado, sendo sua principal característica hidrológica a ausência de grandes rios e a presença de várias lagoas distribuídas em toda sua extensão. A Planície Costeira é uma das regiões do Rio Grande do Sul com a maior concentração de áreas úmidas e também é uma importante região produtora de arroz no Estado (MALTCHIK, 2003, AZAMBUJA et al., 2004).

O estudo foi realizado no município de Sentinela do Sul, localizada na Serra do Sudeste, fazendo parte da Planície Costeira do estado do Rio Grande do Sul (30º36'44,0" Sul, 51º34'52,0" Oeste) (Figura 1). Sentinela do Sul, é umas das cidades integrantes da décima maior regional produtora de arroz irrigado no Rio Grande do Sul (INSTITUTO RIO GRANDENSE DO ARROZ – IRGA, 2013). O município contribui com cerca de 10% da área plantada total desta regional, que atualmente é de 20.307 hectares (IRGA, 2013). O clima predominante é do tipo tropical temperado, apresentando temperaturas que variam entre 10 e 32ºC. As quatro estações são bem definidas, com médias de 15ºC no inverno e de 27ºC no verão. Em termos de relevo, a altitude média está entre 40 e 50 m, havendo 70% de áreas onduladas e 30% planas. A agricultura é a atividade principal, com as culturas temporárias, como o arroz irrigado, constituindo o maior segmento, tanto em termos de área cultivada como de quantidade produzida (SITE OFICIAL DA CIDADE DE SENTINELA DO SUL, 2011).

Nesse estudo, foram selecionadas quatro propriedades rurais, uma que utilizava o sistema de cultivo orgânico-biodinâmico e as outras três que utilizavam o sistema de cultivo convencional (Figura 3; 5). No sistema biodinâmico, nenhum agroquímico é aplicado às lavouras de arroz irrigado, em nenhum estágio da produção. Arrozais orgânicos são plantados com o auxílio de maquinário leve, o que fornece alguma proteção contra as pragas. Uma alternância no regime de inundação é feita para eliminar espécies pragas e manter a fertilidade do solo. No cultivo convencional, a semeadura do arroz é feita com auxílio de maquinário. Assim que as plântulas estão estabelecidas (5-10 cm de altura), os campos são permanentemente inundados. Aplicação de fertilizantes artificiais, inseticidas e herbicidas é concentrada no início da fase de crescimento. Na região, as lavouras do tipo convencional representam 95% do total de área plantada e há lavouras cultivadas há 3, 10 e 20 anos.

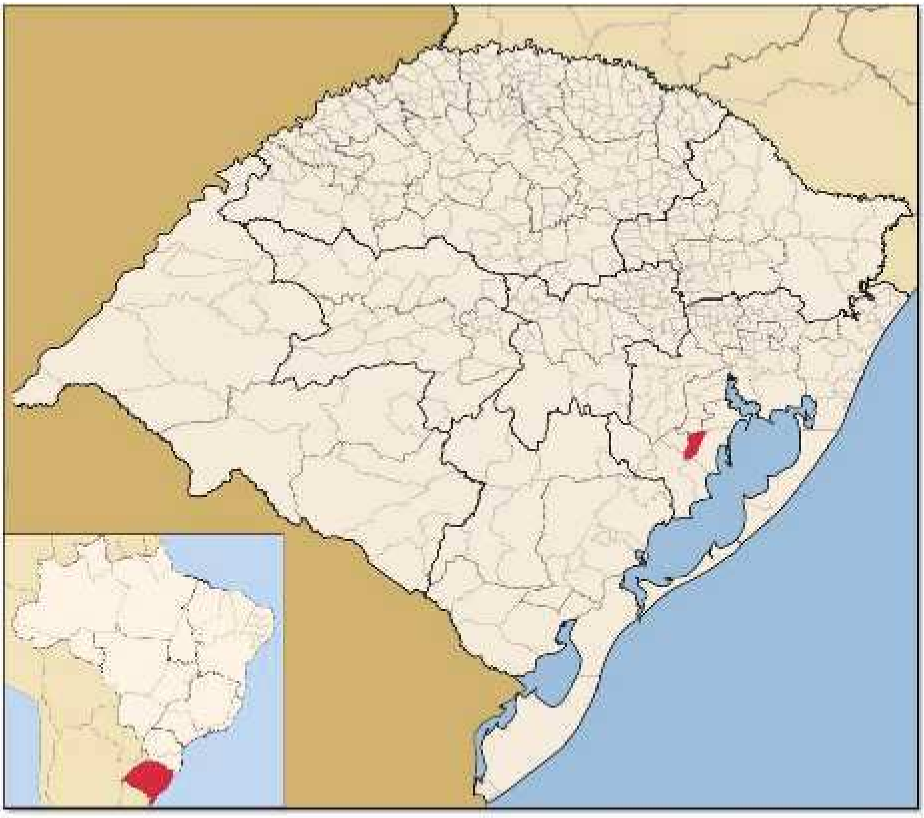

Figura 1- Localização da área de estudo: Sentinela do Sul, Planície Costeira, Rio Grande do Sul, Brasil.

A área de cada lavoura era de aproximadamente 1 ha, interconectadas por estradas vicinais e canais de drenagem (Figura 2a). A água captada pelos canais de drenagem durante o período de crescimento do arroz era oriunda do Arroio Velhaco (Figura 2b) e áreas úmidas adjacentes (Figura 2c; d). A largura dos canais de irrigação estudados era de aproximadamente 2,5 metros e 1 metro de profundidade.

Figura 2 - Área de estudo: a) canais de irrigação; b) Arroio Velhaco; c) àrea úmida amostrada; d) área úmida utilizada para irrigação. Fotos: Leonardo Maltchik

Foram desenvolvidos dois tipos de estudo nessas propriedades rurais, descritos a seguir:

1) *Estudo realizado em lavouras de arroz irrigado orgânicas e convencionais*

Um total de quatro áreas úmidas naturais e 8 lavouras de arroz divididas em orgânicas (4) e convencionais (4) foram coletadas aleatoriamente (Figura 3).

Figura 3- Áreas amostradas para o estudo da estrutura e dinâmica de macroinvertebrados aquáticos em lavouras de arroz orgânicas e convencionais.★ propriedade orgânica-biodinâmica e ◆ propriedade convencional 20 anos (e.g. figura 1); O: lavouras orgânicas; C: lavouras convencional; B: áreas úmidas naturais.

Em cada área de estudo, um total de 6 coletas foram realizadas distribuídas nas principais fases do ciclo do arroz: 1 expedição no período de entressafra anterior ao cultivo (Figura 4a); 1 expedição durante o preparo do solo (Figura 4b); 1 expedição no início do crescimento inicial das plantas (Figura 4c); 1 expedição no período de crescimento final das plantas (Figura 4d); 1 expedição no pós-colheita (4e); e 1 expedição no período de entressafra posterior ao cultivo (Figura 4f).

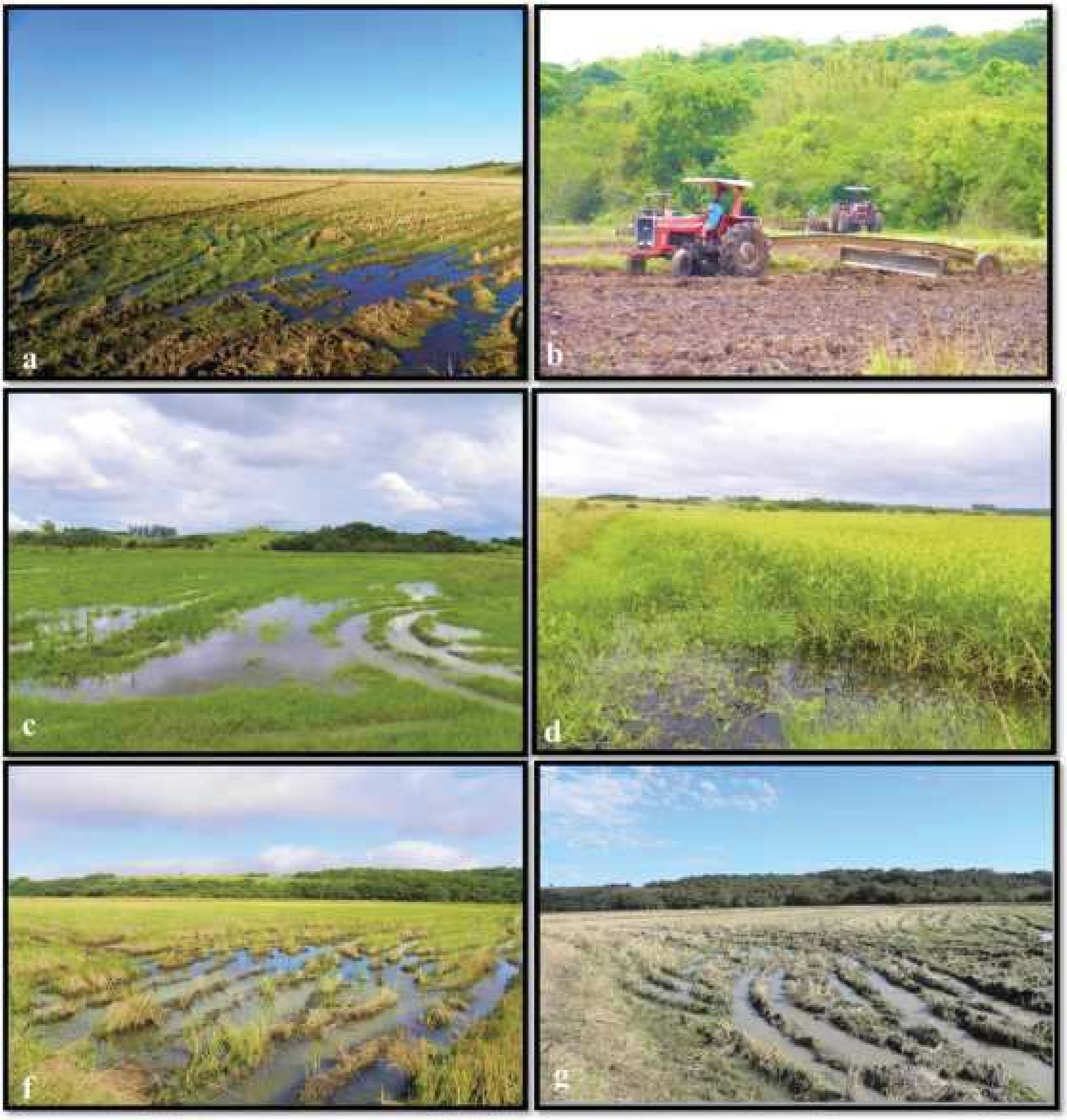

Figura 4 - Fases de amostragem: a) entressafra anterior ao cultivo; b) preparo do solo; c) crescimento inicial da planta; d) crescimento final da planta; e) pós-colheita; f) entressafra posterior ao cultivo.

2) Estudo realizado em lavouras de arroz irrigado com diferentes idade de cultivo

Um total de três áreas úmidas naturais e 9 lavouras de arroz divididas em 3 anos de cultivo (3), 10 anos de cultivo (3) e 20 anos de cultivo (3) foram coletadas aleatoriamente (Figura 5).

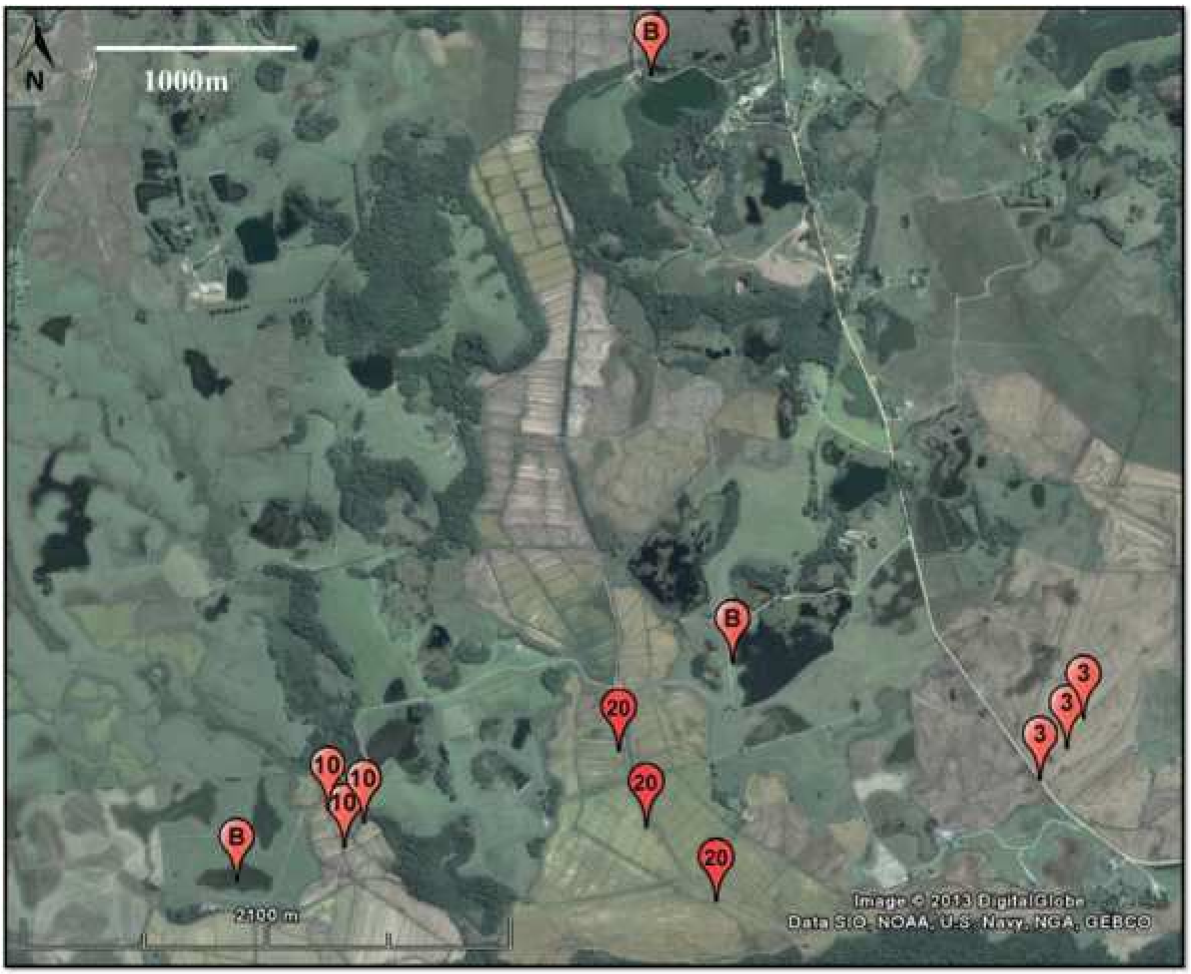

Figura 5- Áreas amostradas para o estudo da estrutura e dinâmica de macroinvertebrados aquáticos em lavouras de arroz com diferentes idades de cultivo. 3: lavouras de 3 anos; 10: lavouras de 10 anos; 20: lavouras de 20 anos; B: áreas úmidas naturais.

Em cada área de estudo, um total de 4 coletas foram realizadas distribuídas nas principais fases do ciclo do arroz: 1 expedição no período de entressafra anterior ao cultivo (Figura 4a); 1 expedição no início do crescimento inicial das plantas (Figura 4c); 1 expedição no período de crescimento final das plantas (Figura 4d); e 1 expedição no pós-colheita (4e).

3.2. Amostragem de macroinvertebrados

Os invertebrados aquáticos foram coletados através de um "puçá aquático" ("frame dip-net"), seguindo a metodologia proposta para levantamentos biológicos rápidos desta comunidade (CONVENTION ON BIOLOGICAL DIVERSITY, 2003) lavoura e/ou área natural foi representada por uma amostra qualitativa, que consistiu em 5 varreduras de 1 metro do sedimento e da coluna d'água ao longo de seus gradientes de distância da margem.

As amostras foram acondicionadas em frascos plásticos (500 mL), fixadas in situ com formaldeído a 10% e levadas ao laboratório. No laboratório, as amostras foram lavadas com o auxílio de uma peneira de 0,25 mm de diâmetro para remoção do barro e restos vegetais. Os invertebrados aquáticos foram triados em um Microscópio Estereomicroscópio e guardados em tubetes de poliestireno com álcool a 80%.

Os macroinvertebrados aquáticos triados foram classificados tanto quanto possível até nível genérico. A classificação taxonômica foi baseada em bibliografia especializada (BOUCHARD, 2004, COSTA; SIMONKA, 2006 e MERRITT et al., 2008) e as amostras foram armazenadas na coleção de referência do Laboratório de Ecologia e Conservação de Ecossistemas Aquáticos da Universidade do Vale do Rio dos Sinos – UNISINOS.

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5. RESULTADOS

Aquatic macroinvertebrate responses to different rice cultivation systems in southern Brazil

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ABSTRACT

Agriculture is one of the leading anthropological activities responsible for declining biodiversity. The use of agrochemicals, water management and intense mechanization has compromising species diversity and the availability of high-quality natural habitats. Organic agriculture has been proposed as way to reduce the pressure placed on biodiversity in agricultural landscape. Therefore, we tested if organic rice fields benefit aquatic macroinvertebrate richness, abundance and composition in relation to conventional crops. Furthermore, we predicted a more similar composition between organic rice crops and wetlands and a reduction of richness and abundance and a modified composition in the tillage and the initial growth phases of the rice cultivating cycle. For this, a set of twelve study sites (four temporary wetlands, 4 organic and 4 conventional rice fields) was randomly selected in Southern Brazil. In each site, five sample collection events were carried out over the rice cultivation cycle (August 2010 to August 2011). A total of 25,449 individuals from 73 different *taxa* during the rice cultivation cycle in all of the study areas. The richness and abundance were higher in wetlands than in rice crops in the anterior and posterior off-season and in the soil preparation. There was difference between organic and conventional rice crops only in the post-harvest stage for richness (μ=18.75, SE±1.10; μ=9.5, SE±1.55, respectively) and in the initial growth stage for abundance (μ=1.40, SE±0.48; μ=2.55, SE±0.14, respectively). Both conventional and organic rice crops affect wetland macroinvertebrate communities by decreasing richness (wetlands = 70 taxa, organic crops = 58 taxa, conventional crops= 50 taxa) and abundance (wetlands = 16,487 individuals, organic crops = 4,413 individuals, conventional crops = 4,549 individuals). The composition differed only between rice crops and wetlands. However, the presence of many *taxa* that only occur in organic crops and wetlands indicates that this organic management favors the establishment of some wetland macroinvertebrate *taxa* in rice agroecosystems, mainly predators. Macroinvertebrate predators have a beneficial role for the control of rice pests and vectors of human and animal diseases. Our results should be seen as an incentive for more sustainable production with less impact on the environment.

Keywords: agroecosystems, biodiversity, cultivation cycle, macroinvertebrates, organic farming, rice fields.

1. Introduction

Agriculture is one of the leading anthropological activities responsible for declining biodiversity (Batáry et al., 2013). The conversion of natural areas into agricultural land has compromised species diversity and the availability of high-quality natural habitats (Benton et al., 2003; Foley et al., 2005; Fuller et al., 2005). Wetlands are some of the most impacted ecosystems on the planet (Craig et al., 2008; RAMSAR, 2013). Inland and coastal wetlands together cover a minimum of 12.8 million km^2 in the world (Finlayson et al., 1999; Spiers, 2001). However, almost half of the world's wetlands disappeared in the last century due to agricultural and urban development (Shine and Klemm, 1999). Rice field expansion is one of the main human activities responsible for the decline of natural wetlands throughout the world (Czech and Parsons, 2002; Russi et al., 2013).

On the other hand, irrigated rice fields have been defined as man-made wetlands by the Ramsar Convention for grain produce (RAMSAR, 2013). Rice is grown in over 100 countries today on every continent except Antarctica and from sea level to an altitude of 3,000 m (Juliano, 1993; Pathak and Khan, 1994). Currently, 165 million hectares are occupied by rice fields, which comprise 11% of arable land on Earth (FAO, 2013). Asia accounts for 89% of the total area harvested with rice in the world, and 50% of world production is related to China and India (FAO, 2013). Brazil, the ninth largest rice producer, has a cultivated area of 2.5 million hectares and production of 12 million tons/year (MAPA, 2013). In terms of national production, southern Brazil stands out with a total contribution of 70% of irrigated rice production in the country (about 8 million tons/year) (IBGE, 2013). In the last six decades, rice fields have been responsible for draining most of the wetlands in southern Brazil and have become typical in the region's landscapes.

Some characteristics of rice fields, such as periodic flooding, make them hydrologically similar to temporary wetlands (Bambaradeniya et al., 2004; Lupi et al., 2013). Rice fields help to maintain regional biodiversity of many vertebrate and invertebrate species (Lawler, 2001), and they act as supplemental habitats for many species of aquatic plants, invertebrates, amphibians and birds throughout the world (Elphick, 2010; Schoenly et al. 2010; Piatti and Souza, 2011; Lupi et al, 2013; Rizo-Patrón et al., 2013; Zhang et al., 2013), included in southern Brasil (Rolon and Maltchik, 2010; Stenert et al., 2009; Machado and Maltchik, 2010; Guadagnin et al., 2012). An important question in terms of biodiversity conservation is to know whether agricultural wetlands can maintain high levels of biodiversity and the intrinsic mechanisms involved. The development of new management practices which reconcile the sustainability of rice fields and the conservation of species could help to maintain a rich biodiversity outside protected areas.

Rice fields in southern Brazil are cultivated under different systems, which differ in the use of agrochemicals, water management and mechanization. Conventional systems involve intense mechanization and the irrigation water contains pesticides and chemical fertilizers (CTAR, 2010). Organic systems do not utilize agrochemicals and instead use animal manure and plant debris as fertilizers. These systems also use elaborate crop rotation techniques, including the use of nitrogen-fixing plants and manage the water level in cultivated areas to control aquatic plants and weeds (Andersson et al., 2012).

Organic agriculture has been proposed as way to reduce the pressure placed on biodiversity in the agricultural landscape (Brittain et al., 2010; Andersson et al., 2012).

Moreover, various studies have shown that organic farming enhances biodiversity more than conventional farms (Freemark and Kirk, 2001; Hutton and Giller, 2003; Bengtsson et al., 2005; Hole et al., 2005; Fuller et al., 2005; Rundlöf and Smith, 2006). However, the extent of response to different practices varies among different groups of organisms such as plants, invertebrates, birds and bats (Fuller et al., 2005; Purtauf et al., 2005; Brittain et al., 2010).

Aquatic macroinvertebrates are the most diverse and abundant organisms in wetlands, providing food for several wildlife species, such as fish and waterfowl (Wissinger, 1999) and they have been used to monitor physical and chemical conditions of water resources for decades (Resh, 2007). In rice fields, the presence of macroinvertebrate taxa has a beneficial role for the soil fertility, because they significantly contribute to the nutrient cycling, besides playing the roles of predators, competitors and parasites of rice pests and vectors of human and animal diseases (Roger et al., 1991).

Some studies in southern Brazil identified important environmental predictors of diversity patterns and community structure of aquatic macroinvertebrates in natural wetlands (Stenert et al., 2008; Maltchik et al., 2010; Maltchik et al., 2012; Crippa et al., 2013) and rice fields (Stenert et al., 2009; Maltchik et al., 2011; Stenert et al., 2012). However, few studies have focused on the macroinvertebrate community differences between conventional and organic rice crops worldwide (Hesler et al., 1993; Wilson et al., 2008; Rizo-Patrón et al., 2013) and none have been developed in southern Brazil. Direct exposure to pesticides and other agrochemicals can affect macroinvertebrate diversity by reducing species richness, changing species composition and increasing dominance by common groups (Leitão et al., 2007; Wilson et al., 2008; Schäfer et al., 2011; Rasmussen et al., 2012; Batáry et al., 2013, Rizo-Patrón et al., 2013). Moreover, soil tillage caused by intense mechanization and hydric stress from hydrological fluctuations impair the establishment of late-successional and long-lived species (Suhling et al., 2000; Bambaradeniya et al., 2004).

Assuming that aquatic macroinvertebrate richness, abundance and composition are strongly influenced by the adopted agricultural practices in rice fields (water level control, herbicide application, and machinery usage) (Roger et al., 1991; Mesléard et al., 2005; Wilson et al., 2008), we tested the following hypotheses: 1) richness and abundance of macroinvertebrates are lower in rice fields than in natural wetlands; 2) conventional rice crops decrease macroinvertebrate richness and abundance compared with organic crops; 3) macroinvertebrate composition in organic rice crops is more similar to the composition in natural wetlands than conventional fields. Furthermore, assuming that the absence of water during tillage suppressed the occurrence and abundance of some aquatic macroinvertebrate taxa with low tolerance to drought (Williams, 1996), we expected a reduction of macroinvertebrate richness and abundance and a modified composition in the tillage and the initial growth phases of the cultivating cycle in organic and conventional rice fields.

2. Materials and methods

2.1. Study area

Rio Grande do Sul is the southernmost state in Brazil (49º42’-57º38’ W; 27º04’-33º45’ S), with an area of approximately 282,000 km^2. The state’s coastal plain is an important rice production area in South America, with a cultivated area of 2.5 million hectares and an annual production of about 12,000 kg/ha (CTAR, 2010). This region concentrates one of the highest densities and diversities of wetlands in southern Brazil

(Maltchik et al., 2003) and it has high macrophyte diversity (Irgang and Gastal, 1996). The climate is subtropical humid and the average annual temperature is 17.5 ºC, ranging between 4.6 ºC in winter and 22.2 ºC in summer. The mean annual rainfall reaches 1,250 mm, ranging between 1,150 mm in summer to 1,450 mm in winter. Although there have been occasional droughts during summer, historical accounts indicate regular rainfall throughout the year (Tagliani, 1995).

The study took place in Sentinela do Sul (51º38'-51º41'W; 30º42'-30º45'S), which stands out for its organic rice production in Rio Grande do Sul. Sentinela do Sul integrates the tenth largest region producer of irrigated rice in southern Brazil, with an annual production of 5,625 kg/ha and an area of approximately 2,600 ha (IRGA, 2013). In organic rice fields, the crop is cultivated without applying agrochemicals (chemical fertilizers and herbicides) and weeds are controlled before and during the cultivation period by managing the flooding and draining regime of the area. This water management stimulates the aquatic plant seed bank to germinate, and then the plants die when the water is drained. The organic material from these plants is then incorporated into the soil during tillage. In conventional crops, weeds, especially sedges and grasses, are controlled during soil preparation (4L/ha) and at the beginning of rice emergence (2L/ha) by applying glyphosate (Trade name Only). Conventional rice crops were fertilized with NPK ratio of 16-3-4 (3L/ha) before irrigation and after rice emergence.

2.2. Study design

Rice plantations are divided into multiple 1ha plots that are interconnected by secondary roads and drainage canals. These drainage canals (2-5 m wide and 0.5-1.5 m deep) are filled with water from nearby streams and provide water to the cultivated plots (~ 10 cm water for 130 days) during the rice cultivation cycle. We classified the rice fields in the study area according to the system of cultivation used on two different rural properties (conventional and organic). We identified all temporary wetlands in the study region (10) that had the following characteristics: 1) close to rice fields; 2) hydroperiod between 8 and 10 months; 3) maximum water depth of 40 cm; and 4) area of 0.8 to 1.2 ha. Then, we randomly selected a subset of four conventional crops, four organic crops and four wetlands for the study from a larger set of 10 wetlands, 20 organic crops and 25 conventional crops. The spatial independence of the twelve studied sites was tested using Principal Coordinates of Neighbor Matrices analysis (PCNM) using vegan package (Oksanen, 2013) in R statistical program version 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria) (R Development Core Team, 2013). As the global PCNM analysis did not detect significant spatial structure (P = 0.68), it was not included in the statistical analysis.

We sampled each area at six different times throughout the rice cultivation cycle (August/2010-2011). The sampling periods coincided with the main stages of rice cultivation: off-season (August/2010 and August/2011), soil preparation (October/2010), initial growth (January/2011), final growth (March/2011) and post-harvest (May/2011). The off-season represents the period when the agricultural land remains without rice culture. Soil preparation is the period in which the soil is prepared for planting and includes plowing, herbicide application (conventional), fertilizer application and sowing. In the crops, the presence of standing water was associated with rice growth and the water depth during the initial growth period was 20 cm, while it reached up to 40 cm during the final growth period. The fields were drained during soil preparation and post-harvest. The presence of water during the off-season was associated with the frequent rains that kept the rice crops with water mainly during the winter months (June to August). Organic and conventional crops had similar water regimes and depths during the rice crop cycle (Figure 1).

Wetlands (0.8-1.2 ha) dried up or had a significantly decreased floodplain area from December to March, which coincided with summer and flooding of the rice fields. Water depth varied from 20 to 50 cm in the autumn and winter and dried up in the summer (December to March). The natural hydrology of wetlands was not similar to that of rice fields from October to May. During the soil preparation (October) and post-harvest (May) periods, rice crops were drained and wetlands were inundated. Additionally, rice crops were inundated in the initial (January) and final growth (March) periods while wetlands dried up or had lower water depth (0 to 5 cm). However, the hydroperiod of crops and wetlands was similar (8 months). The distinct habitats of wetlands were characterized by the presence of hydrophytes and rushes. The species *Luziola peruviana*, *Nymphoides indica*, *Myriophyllum aquaticum*, *Salvinia minima*, *Heteranthera reniformis*, *Eleocharis* spp. and *Utricularia gibba* were abundant in wetlands (Linke et al., 2014). The mean values of environmental variables (pH, water conductivity, oxygen dissolved in water and organic matter) in wetlands, organic and conventional rice crops during the cultivation cycle is resume in Table 1.

2.3. Macroinvertebrate sampling

In each sampling period, macroinvertebrates were sampled with a dip-net (30 cm wide, 250-μm mesh). The samples were collected by kicking up the substrate and then sweeping above the disturbed area to capture dislodged or escaping macroinvertebrates (Rosenberg et al., 1997). Five random sweeps of 1 m each were performed in each area. Sweeps were deposited in 3.5-L plastic buckets and preserved with 10% formalin. In the laboratory, the samples were washed through a 250-μm sieve and leaves, stems and other debris were removed. The resulting material was preserved with 80% ethanol. Macroinvertebrates were identified to the level of genus according to Bouchard (2004), Costa and Simonka (2006), Mariano and Froehlich (2007), Souza et al. (2007) and Merrit et al. (2008). The macroinvertebrates were deposited in a Reference Collection of the Laboratory of Ecology and Conservation of Aquatic Ecosystems of University of Vale do Rio dos Sinos (UNISINOS).

2.4. Data analysis

Macroinvertebrate richness and abundance corresponded to the number of taxa and individuals, respectively. Differences in macroinvertebrate richness and abundance between rice crops (organic and conventional) and natural wetlands over time were tested using Repeated Measures Analysis of Variance (ANOVA), performed using SPSS software, version 17.0 (Polar Engineering and Consulting, Nikiski, U.S.A.) (SPSS Inc., 2008). Tukey tests were applied for multiple comparisons of the richness and abundance among the different sampling periods and sites (n = 4 areas X 3 site types X 6 sampling periods = 72).

Non-metric multidimensional scaling (NMDS) was used to assess the variation of macroinvertebrate composition between natural wetlands and rice crops over the rice cultivation cycle. The analysis was performed with the Bray-Curtis dissimilarity index on two axes. A scree plot of stress scores against different numbers of dimensions indicated that two axes were sufficient for a significant reduction in stress, which accounted for most of the goodness-of-fit (Supplementary figure 1) (Gotelli and Ellison, 2004). A two-way Permutational Multivariate Analysis of Variance (PERMANOVA) was used to compare differences in the macroinvertebrate composition among organic and conventional rice crops, and natural wetlands as well as among the different sampling periods. NMDS and PERMANOVA analyses were carried out in the statistical software PAST 2.17c (Hammer et al., 2001).

3. Results

We collected 25,449 individuals from 73 different *taxa* (orders, families and genera) during the rice cultivation cycle in all of the study areas (Supplementary Table 1). Insecta was the most abundant class (n = 15,626), followed by Oligochaeta (n = 7,017) and Hirudinea (n = 1,734). The most abundant family was Chironomidae (n = 5,952), followed by Libellulidae (n = 1,312) and Corixidae (n = 1,236). Most families were represented by only one genus (Supplementary Tables 1 and 2).

A total of 16,487 individuals from 70 different *taxa* were collected from wetlands during the study period. *Forcepsioneura* (n=11), *Coryphaeshna* (n=3), *Hydrovatus* (n=6), *Suphis* (n=9), *Helochares* (n=1), *Bezzia* (n=22), *Gelastocoris* (n=9), *Ambrysus* (n=10), *Ranatra* (n=5) and Stratiomyidae (n=12) were only found in wetlands. The most abundant *taxa* were oligochaetes and chironomids with 5,334 and 3,048 individuals, respectively. A total of 4,413 individuals from 58 *taxa* were found in organic crops. The genus *Anax* (n=4) was only found in organic fields and the most representative family was Chironomidae (n=986). A total of 4,549 individuals from 50 taxa were collected from conventional crops, and Chironomidae represented 42.2% of the individuals collected in this rice system (Supplementary Table 2).

Pomacea (organic n=12, conventional n= 41) and Isopoda (organic n= 13, conventional n=33) were only found in rice fields. *Eurysternus* (conventional n=3, wetlands n=7), *Pelonomus* (conventional n=1, wetlands n=5), *Tramea* (conventional n=1, wetlands n=18), *Hyalella* (conventional n=4, wetlands n=39) and Ephydridae (conventional n=3, wetlands n=1) were not collected in organic fields. Other taxa that were present in organic crops and natural wetlands included *Ilybius* (organic n=2, wetlands n=6), *Enochrus* (organic n=17, wetlands n=22), *Celina* (organic n=1, wetlands n=8), *Chrysops* (organic n=6, wetlands n=12), *Tabanus* (organic n=30, wetlands n=15), *Derallus* (organic n=4, wetlands n=46), *Lestes* (organic n=2, wetlands n=42), *Scirtis* (organic n=1, wetlands n=24), *Aeshna* (organic n=3, wetlands n=13), *Atrichopogon* (organic n= 2, wetlands n=7), *Physa* (organic n=2, wetlands n=17), and Elmidae (organic n=2, wetlands n=6) (Supplementary Table 2).

Wetlands had the highest richness (s=55) and abundance (n=4,769) during the first collection period (August/2010). The period with the lowest number of taxa (s= 36) and individuals (n= 951) in these wetlands was during the post-harvest period (June/2011). The highest richness in organic and conventional rice fields (46 and 37 *taxa*, respectively) was during the final rice growth period (March/2011). While the highest macroinvertebrate abundance in organic rice fields (n=1,726) was during the final growth period (March/2011), the highest abundance in conventional rice fields was during the initial growth period (January/2011). In the rice crops, the lowest richness and abundance (organic s=6/n=9, conventional s=2/n=2) were found during the soil preparation period (October/2010) (Figure 2; Supplementary Table 1).

Aquatic macroinvertebrate richness varied between wetlands and rice fields throughout the study period (Sites: $F_{2,9}$=19.337, p<0.001; Time: $F_{5,45}$=5.072, p<0.001; Interaction: $F_{10,45}$=4.973, p<0.001). Mean richness was higher in wetlands than conventional crops during the anterior (μ=36, SE±4.52; μ=16.75, SE±1.49, respectively) and posterior off-season (μ= 23.25, SE±4.93; μ=11,SE±4.30, respectively) (Tukey, p<0.05), soil preparation (μ= 35.5, SE±2.53; μ=1, SE±0.40, respectively, Tukey,

$p<0.001$) and post-harvest ($\mu=18.25$, SE±5.39; $\mu=9.5$, SE±1.55, respectively, Tukey, $p<0.05$). Mean richness was higher in wetlands than organic crops during anterior off-season ($\mu=36$, SE±4.52; $\mu=21.5$, SE±3.27, respectively, Tukey, $p<0.05$) and soil preparation ($\mu=35.5$, SE±2.53; $\mu=2.5$, SE±0.64, respectively, Tukey, $p<0.001$) (Figure 2). In the other periods of cultivation cycle, richness did not differ between wetlands and rice fields. Mean richness was similar between organic and conventional crops throughout cultivation cycle except for post-harvest ($\mu=18.75$, SE±1.10; $\mu=9.5$, SE±1.55 respectively, Tukey, $p<0.05$) (Figure 2). Regarding variation in richness among the cycle stages, significant differences were only observed in the crops sampled, when the soil preparation had the lowest values (organic crops, $\mu=2.5$, SE±0.40; conventional crops, $\mu=1.0$, SE±2.53, Tukey, $p<0.05$) compared to other periods (Figure 2).

Aquatic macroinvertebrate abundance varied between wetlands and rice fields throughout the rice cultivation cycle (Sites: $F_{2,9}=20.994$, $p<0.001$; Time: $F_{5,45}=3.093$, $p<0.01$; Interaction: $F_{10,45}=2.280$, $p<0.01$). Mean abundance was higher in wetlands than organic and conventional crops during anterior off-season ($\mu=3.05$, SE±0.07; $\mu=2.37$, SE±0.18; $\mu=2.38$, SE±0.16, respectively, Tukey, $p<0.01$) and soil preparation ($\mu=2.90$, SE±0.07; $\mu=0.47$, SE±0.10; $\mu=0.15$, SE±0.08, respectively, Tukey, $p<0.01$). During the initial growth stage, mean abundance was higher in wetlands ($\mu= 2.75$, SE±0.20) and conventional crops ($\mu=2.55$, SE±0.14) than organic crops ($\mu=1.40$, SE±0.48) (Tukey, $p < 0.05$). Mean abundance was higher in wetlands ($\mu=2.74$, SE±0.22) than conventional crops ($\mu=1.62$, SE±0.56) during the posterior off-season period (Tukey, $p<0.05$). Abundance in wetlands and crops was similar during the other cultivation periods (Figure 3). Significant differences in the variation in abundance among the cycle periods were only observed in the rice fields and the soil preparation period had the lowest values (organic crops, μ=0.47, SE±0.08; conventional crops, μ=0.15, SE±0.07, Tukey, $p<0.05$) compared to other periods. In the organic crops, the abundance during the final growth period (μ=2.49, SE±0.43) was significantly higher than during initial growth (μ=1.40, SE±0.14; Tukey, $p<0.05$) (Figure 3).

The first NMDS axis indicated a gradient of composition variation from natural wetlands to conventional rice crops and the organic crops were plotted in intermediate positions of the ordination gradient (NMDS, stress=8.9) (Figure 4). The composition of wetlands and rice crops differed mainly in the lack of some taxa in the crops (i.e. *Coryphaeshna, Hydrovatus, Suphis, Bezzia, Gelastocoris, Ambrysus* and *Ranatra*) and the fact that some *taxa* occurred more frequently and were more abundant in natural areas (*Physa, Hyallela, Acanthagrion, Micrathyria, Buenoa* and *Notonecta* (Supplementary Table 1). On the other hand, many *taxa* only occurred in organic crops and wetlands (*Ilybius, Notonecta, Enochrus, Celina, Chrysops, Tabanus, Derallus, Lestes, Scirtis, Aeshna, Atrichopogon, Macrobdella, Physa,* Elmidae and Nematoda). Some taxa were more abundant in conventional crops compared to organic crops such as *Pomacea*, Isopoda and Chironomidae (Supplementary Table 1).

The macroinvertebrate composition varied significantly between the natural wetlands and crops throughout the rice cultivation cycle, and this variation was not independent of the sampling period (Treatment: $F_{2,10}=4.25$, $p<0.001$; Time: $F_{5,54}=3.55$, $p<0.001$; Interaction: $F_{10,54}=0.9400$, $p<0.001$). The composition was more similar between rice crops (organic and conventional) and wetlands in the final rice growth period, and it was more distinct mainly in the soil preparation, posterior off-season, post-harvest and initial rice growth periods (Figure 5). In both types of rice crops the composition in the anterior off-season was different from the other periods of the rice cultivation cycle (Figure 4).

4. Discussion

The predominance of chironomid larvae in our study was also verified in Philippine rice fields, with about 8,000 to 10,000 individuals/m^2 (Grant et al., 1986; Simpson et al., 1994). Furthermore, populations of larvae up to 18,000 individuals/m^2, corresponding to 7 genera, were recorded in California rice fields (Clement et al., 1977). According to Roger (1996), chironomids were reported to be the most numerous insects in the rice fields of the Republic of Korea. Additionally, oligochaetes are also considered important components of the benthic fauna in rice fields, where they play important roles in organic material mineralization, biological soil stratification and nutrient transfer between soil and water (Bambaradeniya et al., 2004). Tubificid populations of about 10,000 and 40,000 individuals/m^2 were recorded in Philippines and Japan rice fields, respectively (Roger, 1996).

One of the techniques used in conventional rice fields is the application of herbicides such as glyphosate and clomazone, and insecticides such as chlorantraniliprole and carbofuran, which cause mortality and changes in the behavior, reproduction and development of aquatic organisms (Rizo-Patrón et al., 2013). Organic management arose as a more sustainable production strategy with the aim of minimizing the negative effects of agricultural intensification and the use of agrochemicals on biodiversity (Klaus et al., 2013). In Australia, rice cultivated without agrochemicals had higher aquatic macroinvertebrate diversity than rice conventionally cultivated with pesticides (Wilson et al., 2008). Several studies have documented that organic farming increases the biodiversity in agroecosystems (Bengtsson et al., 2005; Brittain et al., 2010; Andersson et al., 2012; Rizo-Patrón et al., 2013). However, in our study, organic and conventional rice fields showed similar richness and abundance.

The similar macroinvertebrate richness and abundance between organic and conventional rice fields could be primarily associated with the water level management used to control weeds in organic crops. Although organic farms do not use agrochemicals during any stage of production, weed control is performed before (soil preparation) and after planting (cultivation) by intentionally flooding and draining the crop area. The natural water cycle in rice fields is strongly altered because the paddies, which are flooded during the rice growing period, become dry in autumn and winter. Therefore, the amount of standing water time can affect the survival of species with longer life cycles such as Heteroptera, Coleoptera and Odonata (Bazzanti et al., 2003; Caramujo and Boavida, 2010).

Moreover, physical changes caused by the use of machinery for soil preparation are known to reduce invertebrate diversity in rice fields (Suhling et al., 2000). Therefore, intentional fluctuations in the surface water level and soil tillage could have led to similar macroinvertebrate richness and abundance between organic and conventional rice crops. The adopted agricultural practices in rice fields were probably responsible for the reduction of richness and abundance in rice crops compared to wetlands. These results support the first hypothesis which states that richness and abundance are lower in rice fields than in wetlands, but refute the second hypothesis of our study which predicts that conventional rice crops decrease richness and abundance compared with organic ones.

In our study, the reduction of the richness and abundance during soil preparation compared to rice growing stages may be a result of agricultural practices such as soil drainage, mechanized plowing and sowing in both cultivation systems, and chemical pesticide and fertilizer applications in conventional crops. On the other hand, the increase of the richness and abundance during the rice growing stages may be related to the rapid increases in abundance of chironomids and oligochaetes. These *taxa* are usually the first organisms to appear with the return of water and after fertilization application, which stimulates primary production providing food for them (Simpson et al., 1994, Roger, 1996). Thereafter, chironomids and oligochaetes serve as food of predator *taxa* such as Heteroptera adults and Odonata larvae, increasing the community richness during the rice growing stages. A similar successional pattern of non-target macroinvertebrates throughout the cultivation cycle has been seen in European, Australian and Asian rice fields, with chironomid larvae appearing before other *taxa* (Suhling et al. 2000, Bambaradeniya et al. 2004, Leitão et al. 2007; Wilson et al., 2008; Lupi et al., 2013; Zhang et al., 2013) and in rice fields of southern Brazil (Stenert et al., 2009).

The macroinvertebrate composition differed only between rice crops and wetlands. However, the presence of many *taxa* that only occur in organic crops and natural wetlands indicates that this organic management favors the establishment of some wetland macroinvertebrate *taxa* in rice agroecosystems, mainly predators. The most abundant aquatic insect species in the pesticide-free rice fields of Southeast Asia are predators (Heckman, 2005). The pesticide use can result in the reduction of beneficial insects like parasites and predators, thereby reducing the effectiveness of pest control strategies that attempt to minimize pesticide use in rice fields (Pingali, 1995). Moreover, some dominant *taxa* in the conventional crops such as Chironomidae are known for their resistance to agrochemicals (Suhling et al., 2000; Bambaradeniya et al., 2004). Takamura and Yasuno (1986) reported the development of large populations of chironomids in pesticide-treated rice fields in Japan, while the number of their natural predators decreased. Another sidelight of pesticide use is the propagation of pests, including invertebrate vectors of dangerous diseases (Heckman, 2005). For example, *Pomacea* sp. is a well-known pest of irrigated rice. However, our third hypothesis was refuted, which postulates that macroinvertebrate composition in organic rice crops is more similar to the composition in wetlands than conventional fields.

On the other hand, a plausible explanation for the similar composition between conventional and organic systems may be related to the reduced persistence of pesticides in conventional crops since the pesticide application occurs only at the beginning of rice emergence. Pingali and Roger (1995) showed that the rice pesticides are degraded by chemical and biological processes which determine the pesticide persistence in the rice crops over time. For example, the half-life of glyphosate in soils is about 60 days (Newton et al., 1994).

5. Conclusions

This study indicates that rice fields are used as a complementary habitat for wetland macroinvertebrate communities in southern Brazil. Although our study showed similar macroinvertebrate responses between organic and conventional rice fields, some *taxa*, mainly predators, only occur in organic crops and wetlands. Macroinvertebrate predators in rice fields have a beneficial role for the control of rice pests and vectors of human and animal diseases (Roger et al., 1991). In this sense, the organic techniques favor the presence of wetland predators and can be efficient in undesirable plant control, reducing the use of herbicides in agricultural areas (Linke et al., 2014). Pesticides certainly do significantly impact on the majority of the aquatic community in rice fields even at recommended application rates. In this sense, rice farmers should minimize the use of chemical substances because their repeated use will affect the structural and functional integrity of the aquatic community. To maintain the ecological integrity some strategies in pesticide usage must take into consideration not only their effects on target species but also on ecosystems. Moreover, the negative effects of agrochemicals on other macroinvertebrate community parameters such as physiological and morphological responses should also be included in future laboratory toxicity studies, particularly in southern Brazil, where more than 90% of wetland systems have already been lost and the remaining ones are still at high risk due to the expansion of rice production. Since only two percent of Brazilian agricultural properties are organic (IBGE, 2013), our results should be seen as an incentive for more sustainable production with less impact on the environment.

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Figures Caption

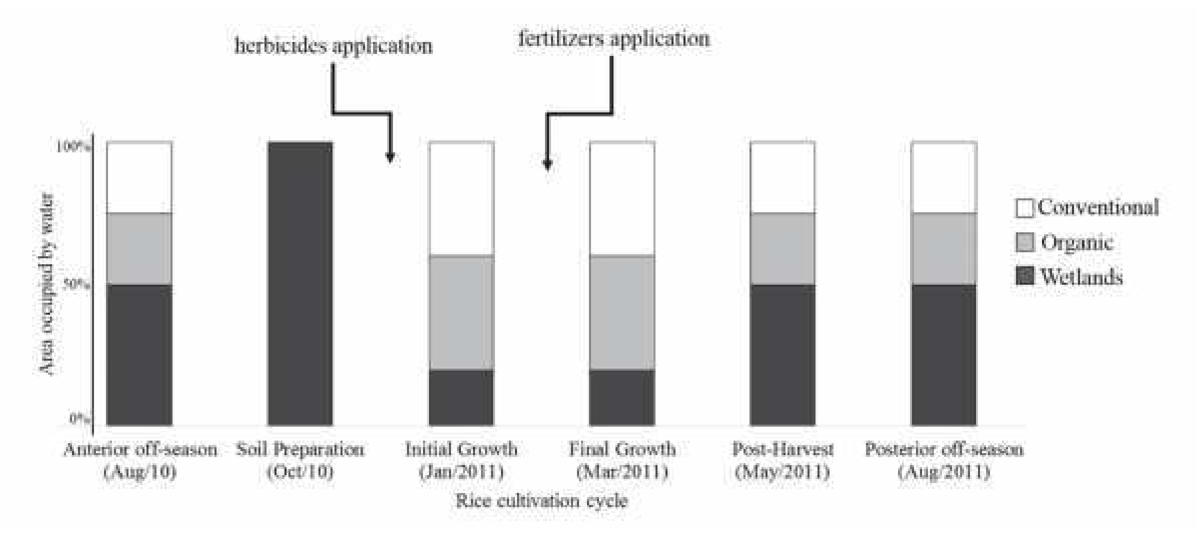


Figure 1. Steps of the rice cultivation in wetlands and rice fields (organic and conventional) over six phases of rice cultivation cycle in Southern Brazil (2010-2011).

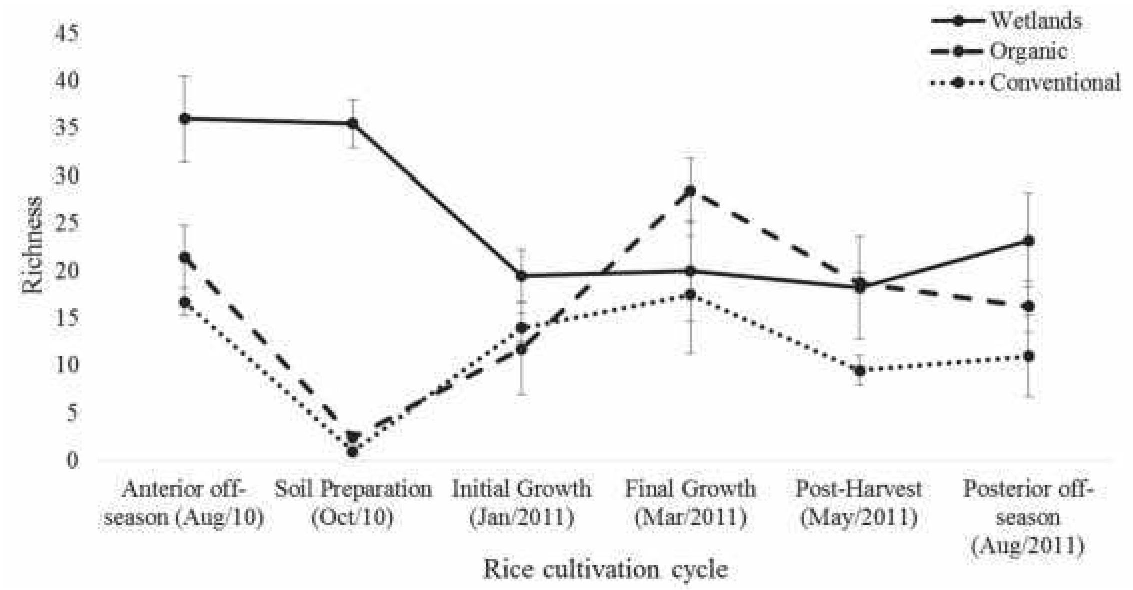


Figure 2. Mean richness (±SE) of macroinvertebrates in wetlands and rice fields (organic and conventional) over six phases of rice cultivation cycle in Southern Brazil (2010-2011).

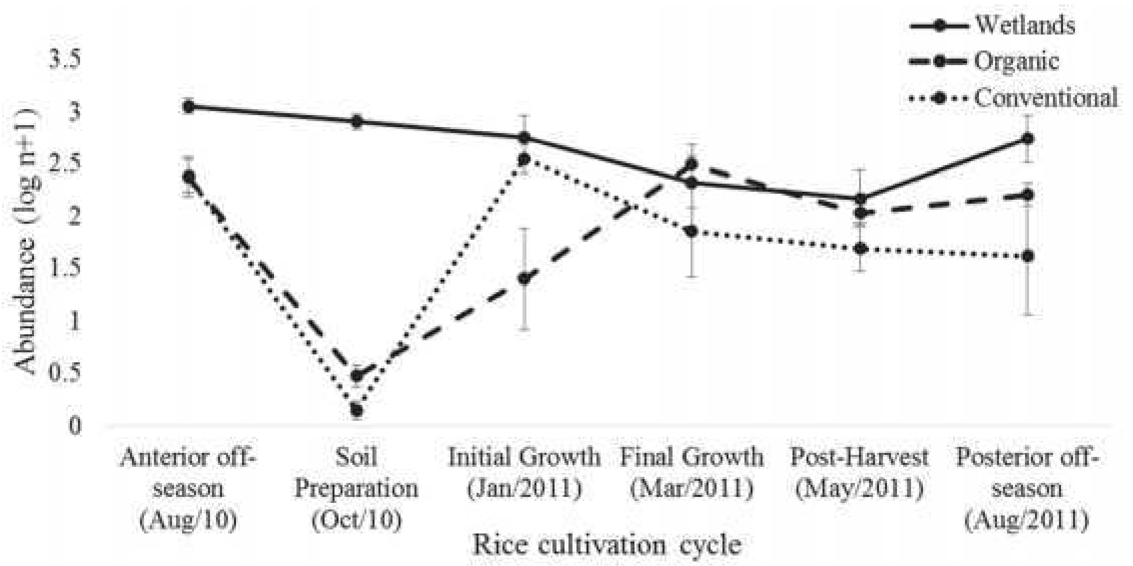


Figure 3. Mean abundance (±SE) of macroinvertebrates in wetlands and rice fields (organic and conventional) over six phases of rice cultivation cycle in Southern Brazil (2010-2011).

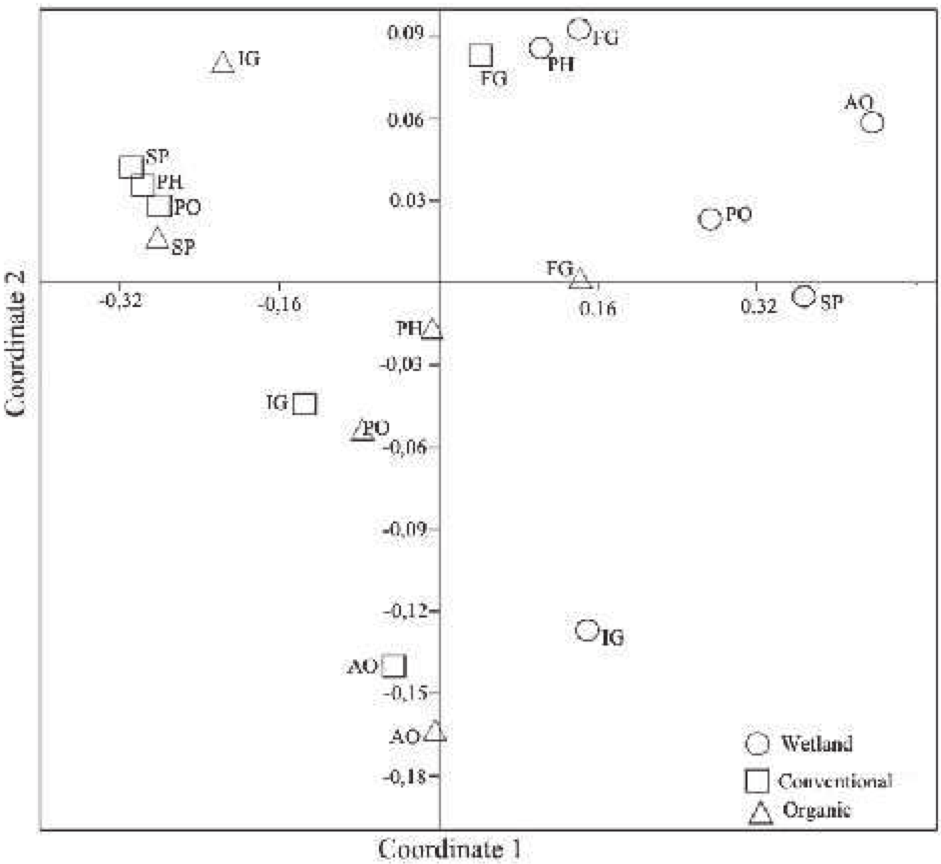


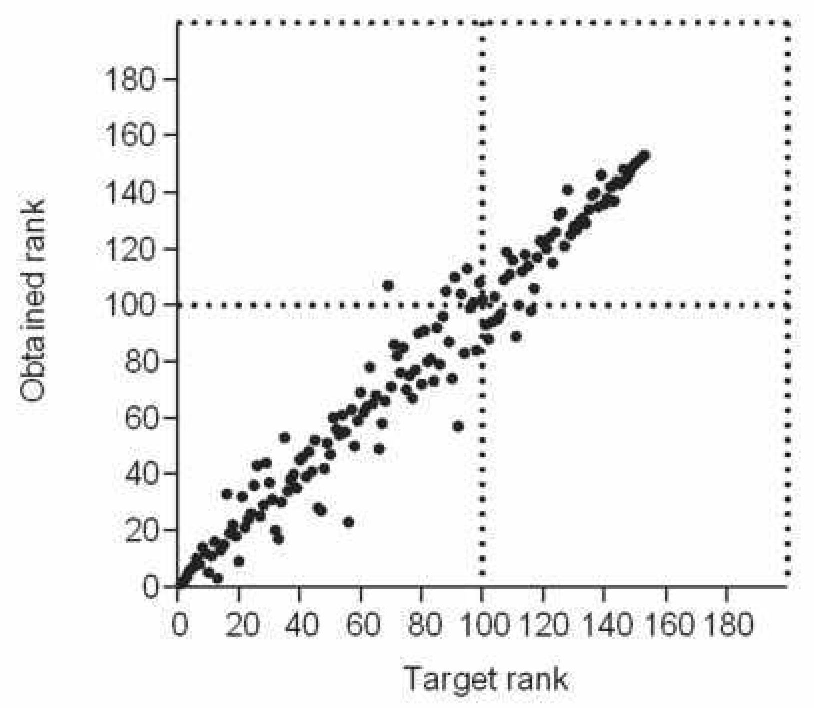
Figure 4. Multidimensional scaling ordination for macroinvertebrates assemblages (stress=8.9) in wetlands and rice fields (organic and conventional) over six phases of rice cultivation cycle in Southern Brazil (2010-2011). AO: Anterior off-season (Aug/10); SP: Soil preparation (Oct/10); IG: Initial Growth (Jan/11); FG: Final Growth (Mar/11); PH: Post-Harvest (May/11) and; PO: Posterior off-season (Aug/11).

Table caption

Table 1. Mean values ± standard error of environmental variables (pH, water conductivity, oxygen dissolved in water and organic matter) in wetlands, organic and conventional rice crops during the cultivation cycle in southern Brazil (August/2010-2011).

	Wetlands	Organic	Conventional
pH	6.18±0.33	6.17±0.18	6.29±0.12
Water conductivity (µS/cm)	10±2.03	3±1	10.1±0.54
xygen dissolved in water (p.p.n	8.31±3.00	11.81±1.07	9.56±1.98
Organic matter (g)	5.77±3.17	4.42±2.18	4.51±2.10

Supplementary data



Supplementary figure 1. Scree plot of stress scores against different numbers of dimensions of Multidimensional scaling ordination for macroinvertebrates assemblages (stress=8.9) in wetlands and rice fields (organic and conventional) over six phases of rice cultivation cycle in Southern Brazil (2010-2011).

Supplementary Table 1. Aquatic macroinvertebrate taxa in wetlands and rice fields (organic and conventional) over six phases of rice cultivation cycle in Southern Brazil (2010-2011). RA: Anterior off-season (Aug/10); PS: Soil preparation (Oct/10); CI: Initial Growth (Jan/11); CF: Final Growth (Mar/11); PC: Post-Harvest (May/11) and; RP: Posterior off-season (Aug/11).

Class	Order	Family	Genus	Wetlands						Organic						Conventional						
				AO	SP	IG	FG	PH	PO	AO	SP	IG	FG	PH	PO	AO	SP	IG	FG	PH	PO	Total
Nematoda				3	1	1	0	3	0	0	0	23	1	0	0	0	0	0	0	0	1	33
	Haplotaxida			1,094	1,041	1,996	549	93	561	181	2	0	481	59	54	431	0	76	154	54	191	7,017
Clitellata	Arhynchobdellida	Hirudinidae	*Macrobdella*	5	2	0	0	0	2	7	0	0	2	0	2	0	0	0	0	0	3	23
	Rhynchobdellida	Glossiphoniidae	*Haementeria*	5	2	0	2	2	3	0	0	15	0	3	1	0	0	0	1	0	0	34
			Helobdella	112	130	9	32	44	67	247	1	0	19	78	443	286	1	0	66	80	62	1,677
	Basommatophora	Planorbidae	*Drepanotrema*	2	0	0	0	1	0	91	0	2	75	42	7	7	0	1	5	8	13	254
			Biomphalaria	14	114	109	4	11	58	9	0	0	28	4	15	1	0	2	23	1	22	415
		Physidae	*Physa*	0	0	0	16	1	0	0	0	1	1	0	0	0	0	0	0	0	0	19
	Architaenioglossa	Ampullaridae	*Pomacea*	0	0	0	0	0	0	0	0	3	6	1	2	1	0	1	33	0	6	53
	Veneroidea	Sphaeriidae	*Pisidium*	37	0	0	3	0	41	0	0	2	0	1	0	0	0	0	1	0	0	85
			Hydracarina	4	2	7	4	17	7	2	0	0	6	1	0	0	0	0	13	0	2	65
	Isopoda			0	0	0	0	0	0	13	0	0	0	0	0	33	0	0	0	0	0	46
	Amphipoda	Hyalellidae	*Hyalella*	21	16	0	0	0	2	0	0	0	0	0	0	0	0	4	0	0	0	43
		Isotomidae	*Isotomurus*	3	1	0	0	0	1	12	0	4	4	8	6	4	0	2	10	0	4	59
	Ephemeroptera	Baetidae	*Callibaetis*	336	134	15	12	2	151	92	0	0	22	6	4	101	0	19	20	0	22	936
		Caenidae	*Caenis*	145	122	62	65	71	302	7	0	9	145	11	1	13	0	2	91	2	0	1,048
		Coenagrionidae	*Ischnura*	319	99	21	28	21	109	1	0	1	31	12	2	17	0	11	18	5	0	695
			Acanthagrion	13	2	0	1	34	7	1	0	0	2	5	0	7	0	4	14	0	0	90
		Protoneuridae	*Forcepsioneura*	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
		Lestidae	*Lestes*	10	7	4	1	1	19	0	0	0	2	0	0	0	0	0	0	0	0	44
			Aeshna	7	1	0	2	2	1	2	0	0	1	0	0	0	0	0	0	0	0	16
		Aeshnidae	*Coryphaeshna*	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
	Odonata		*Anax*	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	4
			Orthemis	17	2	0	3	2	1	3	0	0	0	0	0	5	0	0	0	0	0	33
			Perithemis	0	9	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	0	13
Insecta		Libellulidae	*Tramea*	10	8	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	19
			Micrathyria	56	136	0	8	51	2	0	0	1	9	4	1	0	0	0	15	4	0	287
			Erythemis	10	20	1	0	23	9	0	0	7	1	5	3	1	0	0	2	2	0	84
			Erythrodiplax	360	134	3	34	3	228	31	0	4	8	23	8	7	0	5	6	0	4	858
		Belostomatidae	*Belostoma*	15	39	9	34	15	11	4	0	4	60	8	2	0	0	0	18	1	0	220
		Corixidae	*Sigara*	236	60	71	5	95	394	105	0	0	2	44	33	100	0	6	1	6	74	1,232
		Notonectidae	*Buenoa*	64	20	2	0	18	32	2	0	0	0	5	5	2	0	1	1	0	0	152
			Notonecta	4	31	0	1	0	2	0	0	1	0	0	0	0	0	0	0	1	0	40
	Hemiptera	Pleidae	*Neoplea*	73	74	47	87	100	86	0	0	3	8	1	0	0	0	0	10	0	1	490
		Mesovellidae	*Mesovellis*	0	0	0	27	6	4	0	0	1	11	0	0	1	0	0	7	0	0	57
		Gelastocoridae	*Gelastocoris*	1	0	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	9
		Naucoridae	*Ambrysus*	0	0	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
		Nepidae	*Ranatra*	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	5

Supplementary Table 1(continued). Aquatic macroinvertebrate taxa in wetlands and rice fields (organic and conventional) over six phases of rice cultivation cycle in Southern Brazil (2010-2011). RA: Anterior off-season (Aug/10); PS: Soil preparation (Oct/10); CI: Initial Growth (Jan/11); CF: Final Growth (Mar/11); PC: Post-Harvest (May/11) and; RP: Posterior off-season (Aug/11).

Class	Order	Family	Genus	Wetlands						Organic						Conventional						
				AO	SP	IG	FG	PH	PO	AO	SP	IG	FG	PH	PO	AO	SP	IG	FG	PH	PO	Total
Insecta	Coleoptera	Dytiscidae	*Laccophilus*	34	16	9	8	3	27	26	0	1	12	12	2	8	0	2	7	0	4	171
			Bidessonotus	3	3	1	3	0	2	8	2	0	0	1	0	8	0	0	0	2	0	33
			Celina	0	3	3	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	9
			Hydrovatus	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
			Ilybius	0	6	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	8
			Termonecthus	3	0	6	5	2	1	3	0	0	2	1	0	3	0	3	1	0	0	30
		Noteridae	*Suphisselus*	76	34	34	20	8	9	13	0	0	5	11	11	5	0	0	0	27	2	255
			Hydrocanthus	60	9	3	31	8	34	5	0	0	8	21	0	0	0	0	13	0	1	193
			Suphis	6	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
			Pronoterus	4	3	0	1	2	0	1	0	0	2	0	1	1	0	0	0	1	1	17
		Hydrophilidae	*Berosus*	32	25	190	10	17	22	51	0	5	14	18	27	9	0	6	4	7	5	442
			Tropisternus	7	9	43	12	9	3	12	0	0	28	7	1	7	0	29	1	1	7	176
			Enochrus	6	4	5	2	0	5	1	1	0	13	2	0	0	0	0	0	0	0	39
			Helochares	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
			Derallus	19	19	1	6	0	1	0	0	4	0	0	0	0	0	0	0	0	0	50
		Curculionidae	*Lissorhoptrus*	4	5	36	4	9	3	4	1	0	13	4	2	0	0	2	0	0	0	87
		Elmidae		1	2	2	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	8
		Scirtidae	*Scirtis*	19	0	0	4	0	1	0	0	0	1	0	0	0	0	0	0	0	0	25
		Scarabeidae	*Eurysternus*	1	0	6	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	10
		Dryopidae	*Pelonomus*	0	0	5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	6
		Haliplidae	*Haliplus*	1	2	0	0	0	1	1	0	2	0	0	0	0	0	0	7	0	1	15
	Diptera	Ceratopogonidae	*Culicoides*	29	18	14	1	0	11	6	0	0	19	1	12	7	0	11	1	0	15	145
			Bezzia	3	3	2	5	0	9	0	0	0	0	0	0	0	0	0	0	0	0	22
			Atrichopogon	2	0	0	0	0	5	0	0	0	1	0	1	0	0	0	0	0	0	9
		Culicidae	*Culex*	216	9	0	5	4	9	2	0	3	0	0	0	0	0	0	3	6	1	258
			Anopheles	24	11	5	4	2	4	1	0	1	6	0	0	0	0	12	8	0	0	78
		Tabanidae	*Chrysops*	2	4	5	0	0	1	3	0	0	3	0	0	0	0	0	0	0	0	18
			Tabanus	5	4	0	1	1	4	2	0	0	25	0	3	0	0	0	0	0	0	45
		Chaoboridae	*Chaoborus*	13	23	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	40
		Stratimionidae		6	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
		Chironomidae		1,089	731	283	141	151	653	302	2	147	441	46	48	159	0	1,450	232	3	74	5,952
		Ephrydidae		0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	1	0	0	4
	Lepidoptera	Pyralidae		8	5	3	6	3	5	0	0	1	5	0	0	0	0	3	4	0	0	43
	Trichoptera	Leptoceridae	*Oecetis*	35	116	28	130	101	90	0	0	0	177	14	6	0	0	0	106	5	23	831
		Hydroptilidae	*Oxyethira*	63	87	0	3	4	23	0	0	1	32	0	0	0	1	1	8	0	0	223
			Total	**4,752**	**3,376**	**3,053**	**1,335**	**946**	**3,025**	**1,255**	**9**	**248**	**1,736**	**460**	**705**	**1,224**	**2**	**1,660**	**907**	**217**	**539**	**25,449**

Supplementary Table 2. Aquatic macroinvertebrate taxa in four wetlands and eight rice fields (four organic and four conventional) in Southern Brazil (2010-2011). 1-4: studied sites from each type of wetland/rice field.

Class	Order	Family	Genus	Wetlands				Organic				Conventional				
				1	2	3	4	1	2	3	4	1	2	3	4	Total
Nematoda				2	0	3	3	14	0	4	6	1	0	0	0	**33**
	Haplotaxida			1,456	2,310	717	851	64	57	524	132	273	425	139	69	**7,017**
Clitellata	Arhynchobdellida	Hirudinidae	*Macrobdella*	0	2	2	5	4	0	7	0	0	0	3	0	**23**
	Rhynchobdellida	Glossiphoniidae	*Haementeria*	7	2	3	2	12	0	4	3	1	0	0	0	**34**
			Helobdella	100	68	97	129	83	157	271	277	108	251	66	70	**1,677**
	Basommatophora	Planorbidae	*Drepanotrema*	3	0	0	0	10	57	28	122	5	1	13	15	**254**
			Biomphalaria	45	103	124	38	12	9	10	25	34	1	8	6	**415**
		Physidae	*Physa*	16	1	0	0	0	0	1	1	0	0	0	0	**19**
	Architaenioglossa	Ampullaridae	*Pomacea*	0	0	0	0	5	1	1	5	9	5	2	25	**53**
	Veneroidea	Sphaeriidae	*Pisidium*	21	4	9	47	0	0	1	2	1	0	0	0	**85**
			Hydracarina	18	4	9	10	2	0	2	5	14	0	1	0	**65**
	Isopoda			0	0	0	0	0	0	12	1	0	8	9	16	**46**
	Amphipoda	Hyalellidae	*Hyalella*	9	13	2	15	0	0	0	0	0	1	3	0	**43**
		Isotomidae	*Isotomurus*	3	0	0	2	9	14	5	6	14	0	2	4	**59**
	Ephemeroptera	Baetidae	*Callibaetis*	203	189	153	105	0	8	102	14	77	25	51	9	**936**
		Caenidae	*Caenis*	257	135	327	48	30	16	121	6	50	4	5	49	**1,048**
		Coenagrionidae	*Ischnura*	214	172	144	67	13	6	23	5	32	5	3	11	**695**
			Acanthagrion	33	0	10	14	2	2	3	1	20	0	0	5	**90**
		Protoneuridae	*Forcepsioneura*	7	4	0	0	0	0	0	0	0	0	0	0	**11**
		Lestidae	*Lestes*	13	9	15	5	1	0	1	0	0	0	0	0	**44**
		Aeshnidae	*Aeshna*	2	0	4	7	0	0	3	0	0	0	0	0	**16**
			Coryphaeshna	1	0	2	0	0	0	0	0	0	0	0	0	**3**
	Odonata		*Anax*	0	0	0	0	2	1	1	0	0	0	0	0	**4**
		Libellulidae	*Orthemis*	2	0	0	23	0	2	0	1	1	0	2	2	**33**
			Perithemis	0	0	9	0	0	1	0	1	2	0	0	0	**13**
Insecta			*Tramea*	5	4	7	2	0	0	0	0	1	0	0	0	**19**
			Micrathyria	85	114	35	19	2	0	13	0	0	3	1	15	**287**
			Erythemis	12	6	32	13	7	5	2	2	0	3	0	2	**84**
			Erythrodiplax	99	87	227	349	9	14	34	17	3	10	5	4	**858**
		Belostomatidae	*Belostoma*	28	45	39	11	32	10	27	9	16	1	1	1	**220**
		Corixidae	*Sigara*	146	131	201	383	4	44	75	61	68	52	25	42	**1,232**
		Notonectidae	*Buenoa*	48	16	52	20	0	5	6	1	3	0	1	0	**152**
			Notonecta	4	8	3	23	0	0	1	0	1	0	0	0	**40**
	Hemiptera	Pleidae	*Neoplea*	185	142	140	0	0	0	12	0	10	0	0	1	**490**
		Mesovellidae	*Mesovellis*	6	9	22	0	0	3	0	9	6	0	1	1	**57**
		Gelastocoridae	*Gelastocoris*	0	0	2	7	0	0	0	0	0	0	0	0	**9**
		Naucoridae	*Ambrysus*	0	1	9	0	0	0	0	0	0	0	0	0	**10**
		Nepidae	*Ranatra*	1	0	3	0	0	0	0	0	0	1	0	0	**5**

Supplementary Table 2(continued). Aquatic macroinvertebrate taxa in four wetlands and eight rice fields (four organic and four conventional) in Southern Brazil (2010-2011). 1-4: studied sites from each type of wetland/rice field.

Class	Order	Family	Genus	Wetlands				Organic				Conventional				
				1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**Total**
			Laccophilus	17	30	37	13	16	4	23	10	8	2	4	7	**171**
			Bidessonotus	3	4	2	3	2	0	7	2	4	0	4	2	**204**
		Dytiscidae	*Celina*	1	3	1	3	0	0	1	0	0	0	0	0	**42**
			Hydrovatus	4	0	1	1	0	0	0	0	0	0	0	0	**15**
			Ilybius	2	1	1	2	1	0	0	1	0	0	0	0	**14**
			Termonecthus	2	0	4	11	0	1	4	1	3	0	3	1	**38**
			Suphisselus	77	88	8	8	5	8	7	20	2	1	3	28	**285**
		Noteridae	*Hydrocanthus*	54	26	60	5	7	3	12	12	12	0	0	2	**448**
			Suphis	4	5	0	0	0	0	0	0	0	0	0	0	**202**
			Pronoterus	6	3	1	0	0	0	2	2	0	2	1	0	**26**
	Coleoptera		*Berosus*	51	37	95	113	13	14	42	46	4	11	10	6	**459**
			Tropisternus	21	9	17	36	14	7	24	3	10	7	19	9	**618**
		Hydrophilidae	*Enochrus*	8	7	2	5	1	2	10	4	0	0	0	0	**215**
			Helochares	1	0	0	0	0	0	0	0	0	0	0	0	**40**
			Derallus	18	13	15	0	0	0	2	2	0	0	0	0	**51**
		Curculionidae	*Lissorhoptrus*	17	6	20	18	8	6	5	5	0	1	0	1	**137**
		Elmidae		1	1	3	1	0	0	1	1	0	0	0	0	**95**
Insecta		Scirtidae	*Scirtis*	9	1	14	0	1	0	0	0	0	0	0	0	**33**
		Scarabeidae	*Eurysternus*	1	0	3	3	0	0	0	0	2	0	0	1	**35**
		Dryopidae	*Pelonomus*	0	0	2	3	0	0	0	0	1	0	0	0	**16**
		Haliplidae	*Haliplus*	2	1	1	0	0	0	1	2	8	0	0	0	**21**
			Culicoides	35	23	14	1	5	13	4	16	7	8	19	0	**160**
		Ceratopogonidae	*Bezzia*	3	7	12	0	0	0	0	0	0	0	0	0	**167**
			Atrichopogon	2	0	5	0	1	0	0	1	0	0	0	0	**31**
		Culicidae	*Culex*	163	24	54	2	2	0	2	1	4	2	1	3	**267**
			Anopheles	10	12	26	2	1	0	7	0	7	1	0	12	**336**
	Diptera	Tabanidae	*Chrysops*	0	6	5	1	0	4	1	1	0	0	0	0	**96**
			Tabanus	6	7	2	0	15	5	6	4	0	0	0	0	**63**
		Chaoboridae	*Chaoborus*	10	28	0	0	1	0	0	1	0	0	0	0	**85**
		Stratimionidae		9	3	0	0	0	0	0	0	0	0	0	0	**52**
		Chironomidae		944	879	988	237	117	105	696	68	474	475	750	219	**5,964**
		Ephrydidae		0	0	0	1	0	0	0	0	0	1	2	0	**5,956**
	Lepidoptera	Pyralidae		7	6	11	6	1	0	5	0	6	0	0	1	**47**
	Trichoptera	Leptoceridae	*Oecetis*	167	78	223	32	44	54	62	37	109	5	4	16	**874**
		Hydroptilidae	*Oxyethira*	73	67	26	14	2	0	28	3	4	0	0	6	**1,054**
			Total	4,759	4,954	4,055	2,719	574	638	2,246	955	1,415	1,312	1,161	661	**25,449**

Do organic and conventional rice fields alter aquatic insects' functional diversity in wetland systems?

Running title: Aquatic Insects functional diversity in wetlands

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Abstract

1. Agriculture is one of the leading anthropological activities responsible for declining biodiversity. Biodiversity changes have been assessed mainly using diversity indexes that take into account taxonomic metrics. Although these measures are efficient, a trait-based metrics can be more significant in response to land-use change.
2. In this study, we evaluated functional diversity of aquatic insects utilizing four indices: functional redundancy (FG), functional richness (FRic), functional evenness (FEve) and functional divergence (FDiv). Our goal was to compare how functional diversity of aquatic insects changed along a gradient of agricultural intensification in Southern Brazil.
3. Our analysis suggests the conversion of wetlands to rice fields results in shifts toward less specialized aquatic insect communities with altered proportions of functional groups. This may affect the ecosystem services provided by insects in irrigated rice fields and other agricultural landscapes. Both conventional and organic rice crops negatively affect aquatic insects' communities by decreasing functional redundancy (resilience) and FRic, altering how resources are used.
4. Our analysis of functional composition also adds an important caution to previous studies focusing on taxonomic diversity. We suggest this approach as a basis towards developing a more complete framework to analyse the impacts of land use changes, including multiple disturbances and drought events, across natural environments.

Keywords: biodiversity, organic farming, agroecosystems, traits

Introduction

Biodiversity changes have been assessed mainly using diversity indexes that take into account the number of species present (species richness), abundance and composition. Although these measures are efficient, recent studies have emphasized that trait-based metrics can be more significant than taxonomic metrics in response to land-use change (Clarke & Warwick, 1998; Petchey & Gaston, 2002; Heino, 2008; Statzner *et al.,* 2001; Kinzig *et al.,* 2002; Petchey & Gaston, 2006). Functional ecology is based on the use of functional traits, which are defined as biological attributes that influence organismal performances (Violle *et al.,* 2007). Basically, functional traits have been related to ecosystem processes (effects traits) or to ecosystem stability through resistance and resilience (response traits). It can be physical (body size), biochemical (presence of secondary metabolities), behavioral (period of foraging) and temporal (duration of larval stage) (Mayfield *et al.,* 2010). Depending on the exact nature of the traits, it can influence environmental tolerance and habitat requirements, determining where a species can live, as it interact with one another; competition rates, consumption efficiency and differences in nutrient use or storage.

Functional characteristics are good indicators of the individual effect of an organism's function in environment. A trait-based approach, which scales-up traits from the species level to the community level, can improve our understanding of how ecological communities respond to environmental changes (Mayfield *et al.,* 2010). Changes in environmental condition following disturbance may well act as a filter, structuring the community with functionally similar species (Poff *et al.,* 2006; Cardinale *et al.,* 2012; Fauset *et al.,* 2012).

Wetlands are some of the most productive ecosystems on Earth (Eldredge, 2002) and they are under increasing pressure due to drastic changes in land's use caused by agriculture, acceleration of urbanization and global warming (Moore, 2006). Rice fields are wetlands managed by humans and suffer modifications from grain production. The expansion of rice fields is one of the main human activities that has led to the loss of natural wetlands (Czech & Parsons, 2002). Rice fields are abundant component of the Southern Brazil landscape and are responsible for simplification of natural habitats from this region. These agroecosystems use a great volume of water for irrigation as well as fertilizers and pesticides (Rizo-Patrón *et al.,* 2013). Conversion of wetlands into rice fields has changed richness and composition of several aquatic communities (Maltchik *et al.,* 2010; Stenert *et al.,* 2012), altered the quality of associated biodiversity and the loss of some ecosystem functions (Micheli & Halpern, 2005; Villéger *et al.,* 2010). However, the effect of the rice fields at the functional diversity has not been studied yet.

One important agri-environmental scheme is organic farming, where synthetic fertilization and pesticide treatments are not applied, while both are common in conventional farming systems (Krauss *et al.,* 2011**)**. Many studies (Bengtsson *et al.,* 2005; Hole *et al.,* 2005; Fuller *et al.,* 2005; Rundlöf & Smith, 2006) have showed that biodiversity is clearly enhanced on organic fields compared to conventional fields, although results vary among taxonomic groups and regions (Linke et al. 2014).

Aquatic insects are abundant in most freshwater habitats and often exhibit high diversity (Hershey *et al.,* 2010). Aquatic insect communities have been long used for understanding ecological patterns and environmental quality (Heino, 2009; Cereghino *et* *al.,* 2011). However, most of these studies are limited to the lotic envirnonment (Doledec and Statzner 2010). In Brazil, Colzani *et al.* (2013) seems to be pioneer at understand functional diversity from these organisms in streams from Atlantic Forest. In Wetlands, the fauna is dominated by aquatic insects that play an important role in nutrient flows (Wissinger, 1999). These communities generally have aquatic insect representatives from most aquatic orders, mostly Dipteran (Keiper *et al.,* 2002). In Brazil, taxonomic aquatic insect diversity is well known in wetlands as much (Stenert *et al.,* 2008; Maltchik *et al.,* 2010; Maltchik *et al.,* 2012; Crippa *et al.,* 2013), as in rice fields (Stenert *et al.,* 2009; Maltchik *et al.,* 2011; Stenert *et al.,* 2012). However further efforts are needed to determine the factors that maintain or threaten the biodiversity of aquatic insects' communities.

Our aim was to compare how functional diversity of aquatic insects changed along a gradient of agricultural intensification. Here we focus on functional diversity metrics, which demonstrated to be more sensible than taxonomic diversity to habitat degradation (McGill *et al.,* 2006; Mouillot *et al.,* 2013). More specifically, our goal was to compare metrics used to assess functional diversity among wetlands, organic and conventional rice fields. We expected that the organic farming would favor the presence of some functional traits when compared to conventional crops. We also expected that those wetlands and organic rice fields have more similar patterns between each other than the conventional ones. We also test the hypothesis that disturbance acts as an environmental filter, selecting species more functionally similar than expected by chance. In this way, we expected that conventional rice fields will have lower functional diversity than organic crops and wetlands.

Materials and methods

Study area

Rio Grande do Sul is located in the southern Brazil (49º42'-57º38' W; 27º04'-33º45' S), with an area of approximately 282.000 km^2 (Figure 1). The climate is subtropical humid and the average annual temperature is 17.5 ºC, and ranges between 4.6 ºC in winter and 22.2 ºC in summer. The average rainfall reaches 1250 $mm.yr^{-1}$ and ranges from 1150 to 1450 $mm.yr^{-1}$. Although there have been occasional drought periods in summer, historical dates indicate regular rainfall throughout the year (Tagliani, 1995).

The study took place in Sentinela do Sul, which stands out for its biodynamic organic rice production in Rio Grande do Sul. Sentinela do Sul integrates the tenth largest region producer of irrigated rice in southern Brazil, and rice fields contributing with an area of approximately 2.600 ha (IRGA, 2013). In biodynamic organic rice fields, the crop is cultivated without applying agrochemicals (chemical fertilizers and herbicides), and weeds are controlled before and during the cultivation period by managing the flooding and draining regime of the area. This water management aims to stimulate germination of the plant seed bank and cause subsequent death when the water is drained. The organic material from these plants is then incorporated into the soil. In conventional crops, weeds, especially sedges and grasses, are controlled during soil preparation and at the beginning of rice emergence by applying glyphosate.

Study design

We classified the rice fields in the study area according to the system of cultivation used (conventional and organic). We randomly selected four conventional crops, four organic crops and four wetlands (Figure 1) for the study from a total of 10 wetlands, 20 organic crops and 25 conventional crops. The spatial independence of the twelve studied sites (rice fields and wetlands) was tested using Principal Coordinates of Neighbor Matrices analysis (PCNM), by PCNM function of Vegan R package (Oksanen, 2013). As the global PCNM analysis did not detect significant spatial structure ($P = 0.68$), it was not included in the statistical analysis. We sampled each area (rice crops and wetland) at six different times throughout the rice cultivation cycle (August/2010 to August/2011).

The sampling periods coincided with the main stages of rice cultivation: off-season (August/2010 and August/2011), soil preparation (October/2010), initial growth (January/2011), final growth (March/2011) and post-harvest (May/2011). The off-season period is the period in which the areas do not produce rice. Soil preparation is the period in which the soil is prepared for planting and includes plowing, herbicide application (conventional), fertilizer application and sowing. In the crops, the presence of standing water was associated with rice growth, and the water depth during the initial growth period was 20 cm, while it reached up to 40 cm during the final growth period. The fields were drained during soil preparation and post-harvest. The presence of water during the off-season was associated with precipitation and the declivity of the land, and water pooled intermittently in the lower portions of the land. Organic and conventional crops had similar water regimes and depths during the cultivation phase.

Wetlands (0.8-1.2 ha) dried up or had a significantly decreased floodplain area, from December to March, which coincided with summer and flooding of the rice fields. Water depth varied from 20 to 50 cm in the autumn and winter and dried up in the summer (two to four months). The natural hydrology of wetlands was not similar to that of rice fields from October to May. During the soil preparation (October) and post-harvest (May) periods, rice crops were drained and natural wetlands were inundated. Additionally, rice crops were inundated in the initial (January) and final growth (March) periods while natural wetlands dried up or had lower water depth (0 to 5 cm). However, the hydroperiod of crops and natural wetlands was similar (8 months).

Aquatic insects sampling

In each sampling period, aquatic insects were sampled with a dip-net (30 cm wide, 250-μm mesh). The samples were collected by kicking up the substrate and then sweeping above the disturbed area to capture dislodged or escaping aquatic insects (Rosenberg *et al.,* 1997). Five random sweeps of 1 m each were performed in each area. Sweeps were deposited in 3.5-L plastic buckets and preserved with 10% formalin. In the laboratory, the samples were washed through a 250-μm sieve, and leaves, stems, and other debris were removed. The resulting material was preserved with 80% ethanol. Aquatic insects were identified to the level of genus according to Bouchard (2004), Costa & Simonka (2006) and Merrit et al. (2008). The specimens were deposited in a Reference Collection of the Laboratory of Ecology and Conservation of Aquatic Ecosystems of University of Vale do Rio dos Sinos (UNISINOS).

Measuring functional diversity

For functional categorization, we used twelve attributes: voltinism, respiration, habit, trophic group, food size, maximal potential size, aquatic stages, physiological sensitiveness to pesticides, dispersion, dissecation resistance forms, exoskeleton and reproduction (Table 1). The attributes were collected based on the database published by Cummins *et al.* (2005), Poff *et al.* (2006), Tomanova & Usseglio–Polatera (2007), Merritt *et al.* (2008), Ippolito *et al.,* (2012), beyond specific works. Only the main traits were categorized for analysis, since some taxa can present secondary and terciary ecological habits (eg. alimentary habits). Most aquatic insect groups have only larval instars in the aquatic environment, but, for groups that have aquatic larvae and adults (*e.g.* Coleoptera) we considered functional differences between them (Supplementary table 1).

After obtaining the traits matrix, four complementary functional diversity metrics were calculated: (1) Functional Redundancy (FRed)): Species richness within a functional group shows the number of species that have traits with similar effects on the ecosystem. Thus, higher species richness within a FG implies greater redundancy (FRed) (more species performing similar functions) and this may provide insurance to the system if species show compensatory response to disturbance (Allen *et al.,* 2005). (2) Functional richness (FRic) quantify the volume of the functional space that is occupied by the species in the community; (3) Functional evenness (FEve), describe the regularity in which the functional space is occupied by one species. This index ranges from 0, complete unevenness, to 1, complete evenness and decreases when relative abundance of species is less evenly distributed and when distances among species are irregular; (4) Functional divergence (FDiv) incorporates species abundance to quantify the volume of functional space occupied by the community. According to Mouchet et al. (2010) and Baratolo et al. (2012), FDiv can give information that are in contrast to those from FRic, as it discriminate among cases where the majority of the abundant species are specialists and cases where the generalist species are more abundant. In these methods, traits act as coordinates in functional space, thus identifying a species' functional niche (Villéger *et al.,* 2008). Traits were given equal weighting and species were weighted by their relative abundance. These statistical routines were developed in the statistical software FDiversity (Casanoves *et al.,* 2011).

Data Analysis

Observed Functional diversity

Differences in aquatic insects' functional diversity between rice crops (organic and conventional) and wetlands were tested using a one-way ANOVA, with post hoc Tukey tests for individual differences. A matrix containing genus richness in each functional group was submitted to Non-parametric Multivariate Analysis of Variance (NPMANOVA) in order to determine the functional redundancy (FRed) in each studied area. A PCA Biplot was used to assess the variation of functional composition between wetlands and rice crops, corroborated with NPMANOVA test. The analysis was performed with the Bray-Curtis dissimilarity index on two axes. The analyses were carried out in the statistical software PAST 2.17c (Hammer *et al.,* 2001).

Differences from expected functional diversity

The Standardized Effect Size (SES) from three out of four metrics of functional diversity (FRic, FEve and FDiv) across studied areas were compared to test if the functional diversity values were more or less similar form what would be expected by chance. We defined SES as **SES=(valor.obs – valor.rand)/sd** (see in Edwards *et al.,* 2013)**.** Where *valor.obs* is the values correspondent to the measure of diversity in the communities, *valor.rand* is the mean value found for the randomized communities, and *sd* is the standard deviation for 1000 randomizations. Communities were generated by chance using the algorithm "independent swap", keeping species richness and abundance in the null communities (Gotteli and Entsminger, 2001). We used simple t test to test if the standardized effects were different from those expected by chance (mean = 0). The statistical routines were elaborated in EcoSimR package, from the software R 3.02 (Gotelli & Ellison, 2013) and FDiversity (Casanoves *et al.,* 2011).

Difference between the observed and random results can be interpreted using the standardized effect size (SES): SES values > 0 show that traits are more divergent than expected by chance, suggesting species interaction structures communities. If SES values<0, traits are more convergent than expected by chance, suggesting environmental conditions structure communities. Finally, if SES ~ 0, then trait values aren't different from random.

Results

We recorded 15,606 individual aquatic insects distributed into 61 genus: 10,127 individuals in wetlands, 2,536 individuals in organic farms and 2,943 individuals in conventional fields. A mean of 48 (± 5,24) genus per wetland, 32,7 (± 3,76) genus per organic farm and 26,7 (± 4,45) genus per conventional farm were sampled.

Sixteen functional groups (FG) were identified and grouped by traits related to alimentation (trophic group and food size), physiological sensitivity, respiration and dispersion (Table 2). Wetlands and organic fields presented all functional groups. Conventional fields, groups 3 and 11 were not present, totalizing 14 functional groups. FRed has varied significantly among the studied areas (F=5,806; p>0,01). Wetlands had the biggest functional redundancy (μ=3), followed by organic fields (μ=2,04) and conventional fields (μ=1,67) (Fig. 2A).

Conventional and organic fields presented FRic (μ=4,24; 13,37 respectively) smaller than wetlands (μ=84,01; $F_{2,9}$=11,53; p<0.01) (Fig. 2B). FEve was similar among wetlands (μ=0,46), organic fields (μ=0,47) and conventional crops (μ=0,50), ($F_{2,9}$=0,63; p<0,55) (Fig. 2C). FDiv in the conventional fields (μ=0,855) were higher than in the organic fields (μ=0,765) ($F_{2,9}$=4,71; p<0,05; Tukey=0,03). Wetlands presented FDiv (μ=0,812) values similar to the rice fields (Fig. 2D).

FRic observed values in the wetlands and organic fields were not different than expected by chance, as indicated by SES-FRic values. In the conventional fields, observed FRic values were smaller than expected by chance. Observed FEve and FDiv values were not different than expected by chance in all studied areas (Table 3).

PCA biplot with NPMANOVA (F=15,98; p<0,01) shows clear distinction in functional composition of wetlands and rice fields (Fig. 3). Wetlands differed from organic and conventional rice fields along traits spectra. The most important traits in wetlands are body size and food type. Traits related to the organic fields were respiration, habits, trophic group and exoskeleton. On the other hand, in the conventional fields, the most related traits were voltinism, dispersion and resistance forms.

Functional group 13 composed by genus *Culicoides, Bezzia* and by the subfamily Tanypodinae were dominant in these wetlands and organic fields. Functional group 14 composed by genus *Atrichopogon* and by subfamilies Orthocladiinae and Chironominae were more abundant in the conventional fields. In the wetlands, functional group 1 and 4 were also abundant (Supplementary Table 1).

Discussion

Functional traits in ecological studies have been investigated through a dozen of papers in freshwater ecology (e.g. Statzner *et al.,* 2001; Bonada *et al.,* 2006; Diaz *et al.,* 2008). In freshwater environments, Charvet *et al.* (1998) showed that effluents from wastewater treatment plant significantly changed the trait composition benthic invertebrate communities from a small stream. Similar investigations at the European scale showed significant differences in the trait composition between communities impacted by sewage and natural reference communities (Statzner *et al.,* 2001). Our results are among the first assessments of how irrigated rice fields affect functional diversity of aquatic insects in Southern Brazil. We found a great reduction at functional redundancy and richness of aquatic insects following the conversion of wetlands to rice fields. Although they are not significant, these reductions tend to be minor at conventional rice fields. The decline observed in Functional redundancy and FRic following the conversion of wetlands (FRic = 84,01; 16 FGs) to organic (FRic = 13,37; 16 FGs) and conventional rice fields (FRic= 4, 24; 14 FGs) indicated that the loss of species in the wetlands is not compensated by an increase of species tolerant to disturbances (Edwards *et al.,* 2013). These results supported our first hypothesis, since organic fields present higher values of FRic compared with conventional ones and they present the same composition of wetlands. These results showed that the cultivation practices applied in rice fields increase significantly the vulnerability of the functional groups to future disturbances and consequently, decreases the resilience of the community. The loss of functional redundancy (or richness within FGs) and decrease of FRic may imply in the loss of traits that contribute to ecosystem function, or even in the complete loss of function, increasing the vulnerability of the ecosystems and potential shifts to undesirable states (Chillo *et al.,* 2011).

The standardized FRic in conventional fields (SES<0) have shown strong evidence of environmental filters in structuring the community. This result show that techniques used in the rice fields act like a filter, selecting and allowing the persistence of a relatively small spectrum of functional traits (Keddy, 1992), what is not observed in organic fields or wetlands. One of the techniques used in conventional rice fields is the application of pesticides, which cause mortality and changes in the behavior, reproduction and development of aquatic organisms (Rizo-Patrón *et al.,* 2013). Organic management arose as a more sustainable production strategy with the aim of minimizing the negative effects of agricultural intensification and the use of agrochemicals on biodiversity (Klaus *et al.,* 2013). These results reinforced by the values of FRic from organic fields and wetlands attributed to chance, that support the premises that the species composition would be a result of dispersion, reproduction and death of individuals (Ostling, 2005) within the communities.

FEve values found for the three studied areas showed that the relative abundance of genus is not uniformly distributed within the traits functional space and point out a great proportion of abundances concentrated in a small part of the functional space (Villéger *et al.,* 2008). These values are related with the overutilization of some resources as well as the underutilization of others (Mason *et al.,* 2005). Also, the FEve and FDiv values showed overutilization of some resources and availability of others. High FDiv values as those observed in the conventional rice fields, demonstrate an aggregation of species (weighted by their abundances) in some regions of the functional space (Mason *et al.,* 2005). Small values suggest a better use of the resources. Although smaller than in conventional rice fields, the FDiv obtained values at the wetlands and organic fields still demonstrate irregularity in the resource utilization. In wetlands, especially the temporary ones, as the rice fields, insects have several different strategies to avoid desiccation, like resistance forms, multivoltinism, long-distance dispersion, etc. These general strategies interact with life history such that wetland insect communities exhibit a succession following the drought cycle with dominance of same groups, like mosquitoes and chironomids (Hershey *et al.,* 2010). These groups reproduce fast and have big offsprings.

Because of this, some resources are overutilized while others remain available. All FEve and FDiv values were not different from what would be expected by chance, showing that degradation generated by implementation of rice fields, or even the techniques used in the conventional fields did not act as a filter promoting irregularity in resource utilization.

The most important traits in wetlands are body size and food type. Wetlands have larger individuals than those found in rice fields. It also exhibit more predatory behavior, usually of invertebrates and small vertebrates, different from the rice fields. The body size structure of communities can be directly influenced by ecological drivers including size-selective predation (particularly by fish), size-related risk of dislodgement by hydraulic forces, and the provision of refuge that may counter predation or dislodgement risks. But, we can also expect indirect links between body size and habitat dimensions because body size is related to many other traits. For example, carnivores and shredders are over-represented in bigger size classes, reflecting the larger size of their food particles (Hildrew *et al.,* 2007). Furthermore, body size may be positively correlated with competitive status (Bourassa & Morin, 1995) and negatively correlated with the vulnerability to environmental contaminants (Hendricks & Heikens, 2001). In conventional crops, more related traits are voltinism, dispersion and resistance forms. The most abundant functional groups into conventional crops quickly respond to hydrologic fluctuations that occur in these crops. According Townsend *et al.* (1997), species that are resilient has short life cycles, rapid growth, high reproductive rates, high reproductive rates and use of dormancy during the period without water.

Functional composition did differ between natural wetlands, organic and conventional rice crops. The composition of natural wetlands and organic rice crops differed mainly in the lack of some taxa in the crops (i.e. *Coryphaeshna, Gelastocoris,* *Ambrysus* and *Ranatra*), decreasing richness within functional groups and decreasing functional redundancy. Conventional crops differ from the other areas studied mainly by the absence of two functional groups. Furthermore, the dominant *taxa* in the conventional crops (*Caenis* and Chironomidae) are known for their resistance to agrochemicals and generalist behaviors (Suhling *et al.,* 2000; Bambaradeniya *et al.,* 2004).

Our analysis suggests the conversion of wetlands to rice fields results in shifts toward less specialized aquatic insect communities with altered proportions of functional groups. This may affect the ecosystem services provided by insects in irrigated rice fields and other agricultural landscapes. Both conventional and organic rice crops negatively impact aquatic insects communities by decreasing functional redundancy (*e.g.* resilience) and FRic, altering how resources are used. These trait shifts underline the need to cautiously manage the rate of conversion to these habitats, especially if we consider that they may be reinforced with subsequent cutting cycles in areas that are already functionally degraded (Foley *et al.,* 2007). Nevertheless, this result should not be seen as a negative aspect of organic rice crops and lower impact organic techniques can effectively control undesirable weeds, which reduces the use of agrochemicals (Linke *et al.*, 2014). Our analysis of functional composition also adds an important caution to previous studies focusing on taxonomic diversity. We suggest this approach as a basis towards developing a more complete framework to analyses the impacts of land use changes, including multiple disturbances and drought events, across natural environments.

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Figures Caption

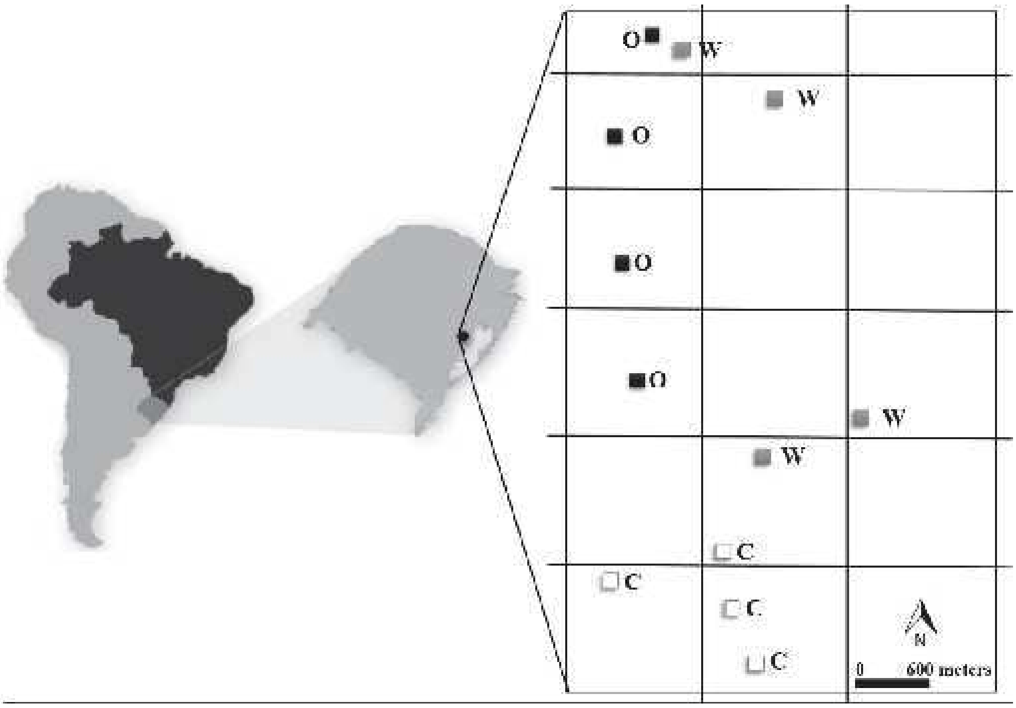

Figure 1. Study area (black circle) in the coastal plain of Rio Grande do Sul, Brazil. Gray square (**W**): Wetlands; black square (**O**): Organic rice fields; white squares (**C**): Conventional rice fields.

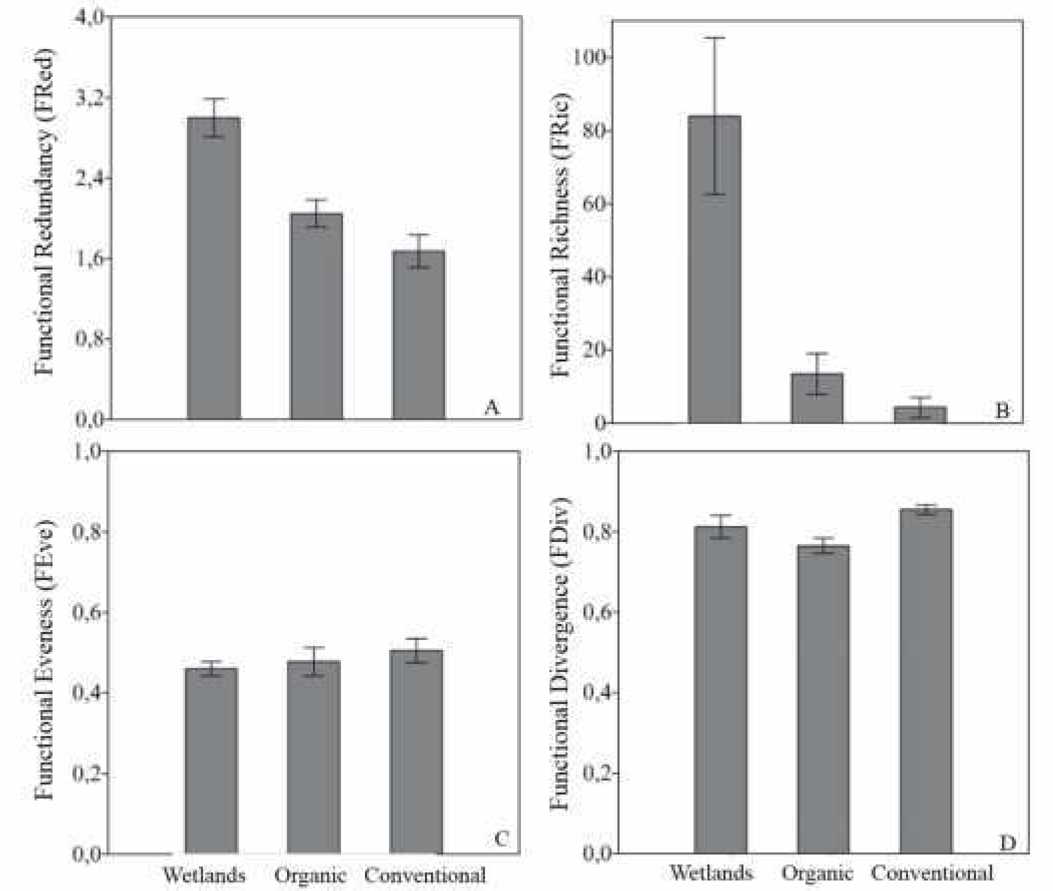

Figure 2. Mean of Functional metrics (±SE) of Aquatic Insects in wetlands and rice fields (organic and conventional) in Southern Brazil (2010-2011). A) FRed; B) FRic; C) FEve; D) FDiv.

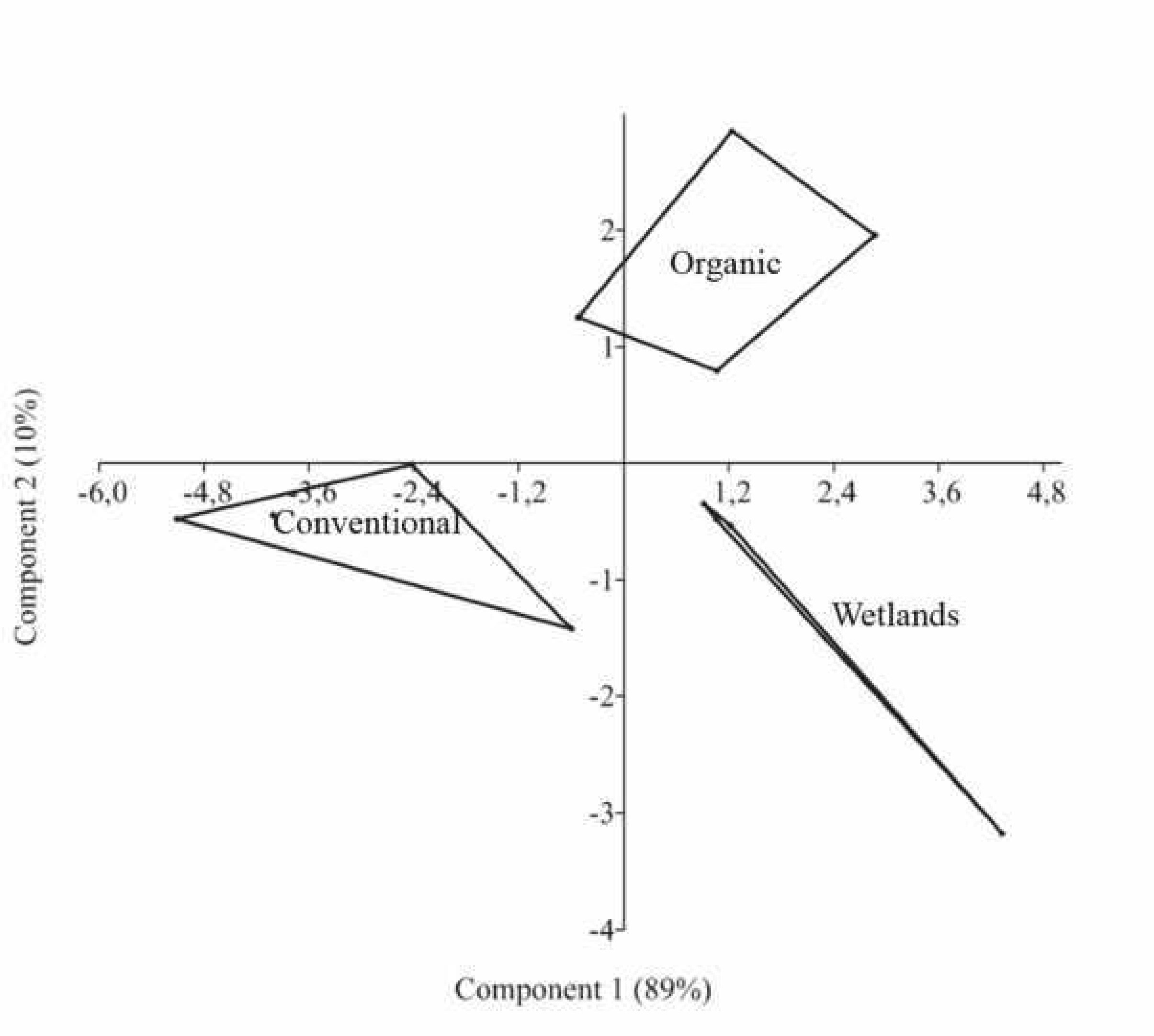

Figure 3. PCA Biplot of Functional Composition of aquatic insects in wetlands and rice fields (organic and conventional) in Southern Brazil (2010-2011).

Table 1. Functional traits examined, adapted from Cummins (1973), Cummins et al. (2005), Poff et al. (2006), Tomanova and Usseglio–Polatera (2007), Merritt et al. (2008), Ippolito et al., (2012).

Trait	State	code
Voltinism (volt)	< 1 generation per year	1
	1 generation per year	2
	2 generation per year	3
	> 2 generation per year	4
Respiration (resp)	Integumentary	1
	Gills	2
	Spiracles	3
Habit (habi)	Burrow	1
	Climb	2
	Sprawl	3
	Cling	4
	Swim	5
	Skate	6
Trophic group (Trop)	Collector-gatherer	1
	Collector-filterer	2
	Herbivore Shredder	3
	Detritivore Shredder	4
	Mineral Scraper	5
	Organic Scraper	6
	Piercer	7
	Engulfer	8
Food Size (Food)	Fine sediment	1
	detrits < 1mm	2
	plant detrits > 1mm	3
	Living microphytes	4
	Living macophytes	5
	dead animal > 1 mm	6
	Living microinvertebrates	7
	Living macroinvertebrates	8
	vertebrates	9
Maximal potential size (maxs)	<0,25 cm	1
	>0,25-0,5 cm	2
	>0,5-1 cm	3
	>1-2 cm	4
	>2-4 cm	5
	>4-8 cm	6
	>8 cm	7

Table 1 (continued). Functional traits examined, adapted from Cummins (1973), Cummins et al. (2005), Poff et al. (2006), Tomanova and Usseglio–Polatera (2007), Merritt et al. (2008), Ippolito et al., (2012).

Trait	State	code
Aquatic stages (Aqst)	egg	1
	larva	2
	pupate	3
	adult	4
	egg/larva	5
	egg/pupate	6
	larva/adult	7
	all of them	8
	egg/larva/adult	9
	egg/larva/pupate	10
	larva/pupate	11
Physiological sensitiveness to pesticides (Psep)	(-2 a -1)	1
	(-1 a 0)	2
	0 a 1	3
Dispersion (disp)	Aquatic passive	1
	Aquatic active	2
	Aerial passive	3
	Aerial active	4
	Aquatic and aerial passive	5
	Aquatic and aerial ative	6
	Aquatic passive and aerial active	7
	Aquatic active and aerial passive	8
Dissecation resistance forms (Dirf)	eggs	1
	cocoons	2
	Cells against desiccation	
	Diapause or dormancy	
	None	5
Exoeskeleton (exoe)	Soft	1
	Lightly	2
	Strong	3
Reprodution (repr)	Ovoviviparity	1
	Isolated eggs, cemented	2
	Clutches cemented or fixed	3
	Assexual reproduction	4
	Isolated eggs, free clutches	5
	Clutches free	6
	Eggs or Clutches on vegetation	7

Table 2. Functional Groups (FG) and traits state in it. For code see Table 1.

	Volt	Resp	Habi	Trop	Food	Maxs	Aqst	Psep	Disp	Dirf	Exoe	Repr
FG 1	2/3	2	3/4/5	1/3	4/5	3/4	2/5	3/2	7	5	1/2	3/4
FG 2	2	1	2	8	7	4	2	2	1/4	5	2	6
FG 3	1/2	1/2	2	8	7/9	4/5/6	2	2	4	1	2	6
FG 4	3/4	2	1/2/3	8	8	4/5	5	1	4	4/5	2	3
FG 5	2	3	2/4/5	7	8	3/4/5	2/5/7	2	6	4	1/2	4/6
FG 6	3	3	5	7/8	5/8	1/2/3/4	7/8/9	2	6	1/3/4	2/3	2/3
FG 7	1/3	2/3	5/6	2/7	3/8	2/3	7/10	2	2/4	1	1/2	2/5/6
FG 8	1	3	5	7	8	1/2/3	7/8	2	6	5	3	4/6
FG 9	2	3	2	7	5	5/6	7	1	6	2	3	4
FG 10	2	3	2	7	5	1/2	8/9	1	6	2	3	4
FG 11	2	3	5	1	5	1/2	9	2	6	5	3	4
FG 12	1/2	2/3	2/4	1/2/3	2/4/5	2/4	5/9/10	2	6/7	5	3	4/6/7
FG 13	2	1/2	3	8	7/8	3/4	10	2	7	5	1	4
FG 14	2/3	1/2	1/3	1	2/3	3/4	10	2	7	5	1	4/5
FG 15	2/3	1/2	3/5	4/8	5/8	3	10/11	2	2/4	4/5	1	5/7
FG 16	2	2	1/4	2/8	2	2/3	10	3	7/5	5	2	4

Table 3. Standardized Effect Size (SES) values of Functional Richness (FRic), Functional Eveness (FEve) and Functional Divergence (FDiv) in each studied area.

	Wetlands	**Organic**	**Conventional**
SES-FRic	5,39	-0,54	**-1,04**
SES-FEve	-0,27	0,04	0,58
SES-FDiv	-0,07	-1,07	0,89

Supplementary data

Supplementary Table 1. State of traits in each genus of Aquatic Insects in wetlands and rice fields (organic and conventional) in Southern Brazil (2010-2011).

Genus	volt	resp	habi	grut	tial	tama	esaq	sfpe	disp	fore	exoe	repr
Callibaetis	3	2	5	1	4	4	5	3	7	5	2	4
Caenis	3	2	3	1	4	3	5	2	7	5	2	3
Ischnura	2	1	2	8	7	4	2	2	1	5	2	6
Acanthagrion	2	1	2	8	7	4	2	2	4	5	2	6
Forcepsioneura	2	1	2	8	7	4	2	2	4	1	2	6
Lestes	2	1	2	8	7	5	2	2	4	1	2	6
Aeshna	1	2	2	8	9	6	2	2	4	1	2	6
Coryphaeshna	2	2	2	8	9	6	2	2	4	1	2	6
Anax	2	2	2	8	9	6	2	2	4	1	2	6
Orthemis	3	2	1	8	8	5	5	1	4	4	2	3
Perithemis	4	2	3	8	8	4	5	1	4	5	2	3
Tramea	3	2	3	8	8	5	5	1	4	5	2	3
Micrathyria	3	2	3	8	8	4	5	1	4	5	2	3
Erythemis	4	2	3	8	8	4	5	1	4	5	2	3
Erythrodiplax	4	2	2	8	8	4	5	1	4	5	2	3
Belostoma	2	3	5	7	8	4	7	2	6	4	2	6
Sigara	3	3	5	7	8	3	7	2	6	4	2	4
Buenoa	2	3	5	7	8	3	7	2	6	4	2	6
Notonecta	2	3	5	7	8	4	7	2	6	4	2	6
Neoplea	3	3	5	7	8	1	7	2	6	4	3	6
Mesovelia	3	3	6	7	8	2	7	2	2	1	2	6
Gelastocoridae	3	3	5	7	8	3	7	2	6	1	3	6
Naucoridae	2	3	5	7	8	3	2	2	6	4	2	6
Ranatra	2	3	2	7	8	5	2	2	6	4	2	6
Laccophilus	1	3	5	7	8	2	7	2	6	5	3	4
Bidessonotus	1	3	5	7	8	1	7	2	6	5	3	4
Celina	1	3	5	7	8	2	7	2	6	5	3	4
Hydrovatus	1	3	5	7	8	2	7	2	6	5	3	4
Ilybius	1	3	5	7	8	3	7	2	6	5	3	6
Termonecthus	1	3	5	7	8	3	8	2	6	5	3	4

Supplementary Table 1 (continued). State of traits in each genus of Aquatic Insects in wetlands and rice fields (organic and conventional) in Southern Brazil (2010-2011).

Genus	volt	resp	habi	grut	tial	tama	esaq	sfpe	disp	fore	exoe	repr
Suphisselus	2	3	2	7	5	5	8	1	6	2	3	4
Hydrocanthus	2	3	2	7	5	6	8	1	6	2	3	4
Suphis	2	3	2	7	5	2	8	1	6	2	3	4
Pronoterus	2	3	2	7	5	1	9	1	6	2	3	4
Berosus	2	3	5	7	5	2	8	2	6	3	3	6
Tropisternus	2	3	5	7	5	3	9	2	6	3	3	6
Enochrus	2	3	5	1	5	2	9	2	6	5	3	4
Helochares	2	3	5	1	5	2	9	2	6	5	3	4
Derallus	2	3	5	1	5	1	9	2	6	5	3	4
Lissorhoptrus	2	3	2	3	5	2	9	1	7	5	3	4
Elmidae	1	3	4	1	5	2	9	1	7	5	3	4
Scirtis	2	3	2	2	2	4	5	1	6	5	3	7
Eurysternus	3	3	5	8	8	4	10	1	6	5	3	4
Pelonomus	1	3	2	3	4	2	10	1	6	5	3	7
Haliplus	2	2	4	3	5	2	10	1	6	5	3	6
Culicoides	2	1	3	8	8	4	10	2	7	5	1	4
Bezzia	2	1	3	8	8	4	10	2	7	5	1	4
Atrichopogon	3	1	3	1	3	4	10	2	7	5	1	4
Culex	3	2	5	2	3	3	10	2	4	1	1	5
Anopheles	1	2	5	2	4	3	10	2	4	1	1	2
Chrysops	1	3	4	7	8	4	5	2	6	5	1	4
Tabanus	2	3	4	7	8	4	5	2	6	5	1	4
Chaoborus	2	1	5	4	8	3	10	2	2	4	1	5
Stratimionidae	2	3	5	1	5	5	5	2	4	4	1	2
Orthocladiinae	2	2	1	1	2	3	10	2	7	5	1	4
Chironominae	3	2	1	1	2	3	10	2	7	5	1	5
Tanypodinae	2	2	3	8	7	3	10	2	7	5	1	5
Ephrydidae	3	1	3	8	5	3	11	2	4	5	1	7
Pyralidae	2	2	4	3	4	4	2	2	7	2	1	4
Oecetis	2	2	4	8	2	3	10	3	7	5	2	4
Oxyethira	2	2	1	2	2	2	10	3	5	5	2	4

Does crop aging compromise macroinvertebrate communities in agricultural landscapes? A case study in southern Brazil

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Abstract Rice is one of the most highly consumed cereals worldwide and is a food staple for about three billion people. Annual rice production has increased by about 3% and it is predicted that by 2030, the world rice yield will need to increase by 50% to sustain the human population. The expansion of plantations throughout the landscape has created an environmental mosaic in natural and cultivated areas with different cultivation ages and disturbance histories. Aquatic invertebrates are the most diverse and abundant organisms in wetlands and they have been used to understand the effect of anthropogenic impacts on these ecosystems. Few studies have focused on how crop age affects aquatic biota. Therefore, we asked the following question: Does crop aging compromise macroinvertebrate communities in agricultural landscapes? We also analyze seasonal changes in the aquatic macroinvertebrate community throughout aquatic phases of rice cultivation cycle. For this, a set of twelve study sites (three wetlands, three short-therm crops, three mid-therm crops and three long-therm rice fields) was randomly selected in Southern Brazil. In each site, four sample collection events were carried out over the rice cultivation cycle (August/2011 to June/2012). A total of 42 taxa were identified in our study, including 37 at natural wetlands, 31 at short-therm rice crops, 39 at mid-therm crops and 37 at long-therm crops. Richness, abundance and Shannon index were similar between natural wetlands and mid-therm and long-therm rice fields. Short-therm rice fields had lower richness and Shannon index than the other areas. Our results indicated that the impacts caused in the first years of cultivation highly influence the macroinvertebrate community. In the first years of cultivation (i.e. short-term crops), the impacts caused by the conversion of natural areas, along with mechanized soil preparation and agrochemical application, drastically reduce the macroinvertebrate community. Macroinvertebrare richness, abundance and Shannon index did not change during the rice cultivation cycle, and they were constant throughout the cultivation periods, reinforce the importance of a layer of surface water in natural wetlands adjacent to rice fields for maintaining local biodiversity.

Keywords rice fields; natural wetlands; conservation; agroecosystems

Introduction

Agricultural expansion fragments and homogenizes natural landscapes, which leads to the loss of habitat and species (Sala et al. 2000; Donald 2004; Green et al. 2005; Hoffmann et al. 2010). Population growth has placed even more pressure on producers to increase agricultural production (Green et al. 2005). Rice is one of the most highly consumed cereals worldwide and is a food staple for about three billion people (FAO 2013). Currently, 165 million hectares are dedicated to rice production, which has increased by 15 million hectares over the last decade (FAO 2013). Annual production has increased by about 3% and it is predicted that by 2030, the world rice yield will need to increase by 50% to sustain the human population (FAO 2013).

The expansion of plantations throughout the landscape has created an environmental mosaic in natural and cultivated areas with different cultivation ages and disturbance histories. As plantations produce repeated crops, biodiversity in the agricultural matrix suffers from the cumulative effects of soil tillage and agrochemical application, which affect community structure and distribution in different ways (Isbell et al. 2013). Rice fields and wetlands are integral components of the current landscape in southern Brazil (Lupatini et al. 2013). Agricultural expansion has fragmented the natural wetlands of the region and harmed biodiversity (Phalan et al., 2013), but recent studies have shown the importance of these agroecosystems in providing habitat for many species of invertebrates, plants, fish, amphibians and aquatic birds (Rolon and Maltchik 2010; Stenert et al. 2009; Machado and Maltchik 2010; Guadagnin et al. 2012). The high species richness observed in rice fields demonstrates the need to include this productive system in biodiversity conservation programs in southern Brazil, especially since 90% of wetland systems in this region have already been lost. However, no studies have analyzed whether cultivation age could harm species richness.

Aquatic invertebrates are the most diverse organisms in wetlands (Wissinger 1999), and they have been used to understand the effect of anthropogenic impacts on these ecosystems (Callisto 2000; Goulart and Callisto 2003). Diversity of macroinvertebrates, and especially aquatic insects, could be affected by direct exposure to pesticides and other agrochemicals. These chemicals lead to reduced species richness, altered composition and increased dominance by generalist groups like Chironomidae (Suhling et al., 2000; Rizo-Patrón et al. 2013). Soil tillage caused by annual management throught cultivation cycle impair the establishment of late-sucessional species (Bambaradeniya et al. 2004).

Few studies have focused on how crop age affects aquatic biota. Cultivation and repetitive application of agrochemicals could have long-term affect on invertebrate diversity and abundance (Simpson and Roger 1995). As fields produce repeated crops, macroinvertebrates suffer from the cumulative effects of soil tillage and agrochemical application (Isbell et al. 2013). Thus, understanding how irrigated rice field age affects species diversity is essential for guiding correct public policies related to biodiversity conservation in wetlands that have been converted to rice fields.

Therefore, we tested the following hypotheses: 1) richness, abundance and Shannon Index of aquatic macroinvertebrates will be greater in short-term crops than in mid- and long-term ones; 2) aquatic macroinvertebrate composition in short-term crops will be more similar to the composition of natural wetlands than mid- and long-term crops; and 3) natural wetlands will have different aquatic macroinvertebrate richness, diversity, biomass and composition than irrigated rice fields. We also analyze seasonal changes in the aquatic macroinvertebrate community throughout the cultivation cycle.

Methods

Study area

The state of Rio Grande do Sul produces 64.4% of the rice produced in Brazil, and the Coastal Plain region is an important area for irrigated rice production in South America (Azambuja et al. 2004). The study occurred between August 2011 and August 2012 in an agricultural area (30.705° to 30.755°S ; 51.630° to 51.700°W) dominated by rice production, in the central-west portion of the coastal plains. The climate is mildly humid subtropical and the average temperature is 18.5°C, with an average of 11°C in winter and 26°C in summer. Annual rainfall ranges from 1500 to 1700 mm/year.

Site selection and data collection

Rice plantations are divided into multiple 1ha plots that are interconnected by secondary roads and drainage channels. These drainage channels (2-5 m wide and 0.5-1.5 m deep) are filled with water from nearby streams and provide water to the cultivated plots (~ 10 cm water for 130 days) during the rice cultivation cycle. We classified the rice fields according to three crop ages: long-term (areas cultivated for at least 20 years), mid-term (areas cultivated for up to 10 years) and short-term (areas cultivated no more than three years). Three replicas of rice parcels for each cultivation age were sampled within a 10 km radius (Fig. 1). We sampled three wetlands to compare the composition between natural and modified areas. The natural wetlands were the same size as and had the same hydroperiod as the rice fields (less than five months). We sampled five times during the rice cultivation cycle: once during the off season (August/2011), two times during the growth period (January/2012 and March/2012) and once during post-harvest (June/2012).

Macroinvertebrate sampling

During each sampling period, macroinvertebrate collections were carried out with a dip-net (30 cm wide, 250-µm mesh). The samples were collected by kicking up the substrate and then sweeping above the disturbed area to capture dislodged or escaping macroinvertebrates (Rosenberg et al. 1997). Five random sweeps of 1 m each were performed in each area. Sweep contents were pooled into 3.5-L plastic buckets and preserved *in situ* with 10% formalin. In the laboratory, the samples were washed through a 250-µm sieve and leaves, stems and other debris were removed. The resulting material was preserved with 80% ethanol. Macroinvertebrates were identified to the level of genus according to Bouchard (2004), Costa and Simonka (2006) and Merrit et al. (2008). The macroinvertebrates were deposited in a Reference Collection of the Laboratory of Ecology and Conservation of Aquatic Ecosystems of University of Vale do Rio dos Sinos (UNISINOS).

Data analysis

Macroinvertebrate richness, abundance and diversity index corresponded to the number of taxa, number of individuals and Shannon index (for formula see Hammer et al., 2001), respectively. Richness values were transformed by square root and abundance values were transformed by log (x+1) to reduce discrepancies among samples.

Differences in macroinvertebrate richness, abundance and diversity between rice crops (different crop ages) and natural wetlands over time were tested using repeated measures Analysis of Variance (ANOVA), with post hoc Tukey tests for multiple comparisons of the richness, abundance and diversity among the different sampling periods. The spatial independence of the twelve study sites was tested using Principal Coordinates of Neighbor Matrices analysis (PCNM), with the PCNM function of R Vegan package (Oksanen 2013). Since the global PCNM analysis did not detect significant spatial structure ($P = 0.77$), it was not included in the statistical analysis.

Non-metric multidimensional scaling (NMDS) was used to test the variation of macroinvertebrate composition between natural wetlands and rice crops over the rice cultivation cycle. The analysis was performed with the Bray-Curtis dissimilarity index on three axes. A two-way Permutational Multivariate Analysis of Variance (PERMANOVA) was used to compare differences in the macroinvertebrate composition among rice crops and natural wetlands, and among the different sampling periods. All analyses were carried out in the statistical software PAST 2.17c (Hammer et al. 2001).

Results

A total of 14,310 individuals from 42 *taxa* (orders, families and genera) were collected during the rice cycle (Table 1). Insecta was the richest (s=30) and most abundant (n=9,872), followed by Clitellata (s=3 and n=3,155). Mollusca was represented by 849 individuals from five families. The other groups sampled included mites, springtails and nematodes (3% of the total). Chironomidae was the most abundant family (n=5,952), followed by Corixidae (Heteroptera; n=682) and Caenidae (Ephemeroptera; n=602). Coleoptera, Heteroptera and Diptera were the most diverse orders, with seven families each (Supplementary Table 1).

A total of 5,069 individuals from 37 *taxa* were sampled in wetlands during the cultivation cycle. In rice fields, 8,961 individuals from 41 *taxa* were sampled during the cultivation cycle. Mid-therm crops had the highest number of *taxa* (s=39) and abundance (n=3,500). Short-therm crops had 3,361 individuals from 31 *taxa*, and long-therm crops had 2,100 individuals from 37 taxa. Chironomidae was the most dominant group in wetlands and rice fields except for long-therm fields, which was dominated by Class Clitellata (n= 879).

Ampularidae, Physidae and Isotomidae only occurred in rice fields. Aeshnidae, Naucoridae and Nepidae only occurred in wetlands. Hydroptilidae and Lestidae were found in wetlands and mid-therm rice fields. Sphaeriidae was found in wetlands and long-therm rice fields. No species occurred exclusively in short-therm rice fields and no species occurred in both these fields and wetlands. The highest richness in wetlands and rice fields occurred during the final growth period (Março/2012) (s=40), followed by the off-season (Agosto/2011; s=39), initial growth (Janeiro/2012; s=33) and post- harvest periods (Junho/2012; s=31). The highest number of individuals in wetlands and rice fields was found during initial growth (n=4,308), followed by off-season (n=3,891), post-harvest (n=3,173) and final growth (n=2.938).

Mean richness varied among study areas, but it did not vary over the rice cultivation cycle (Treatment: $F_{3,8}$=7.397, p<0.05; Time: $F_{3,24}$=2.701, p=0.097; Interaction: $F_{9,24}$=2.522, p=0.155) (Fig. 2a; 2b). Richness was higher in wetlands and mid-therm crops than in short-therm crops (Tukey, p<0.05). Richness was similar between natural wetlands and mid and long-therm crops (Tukey, p=0.29), and between short-therm and long-therm crops (Tukey, p=0.15).

Mean abundance varied neither among study areas nor throughout the cultivation cycle (Treatment: $F_{3,8}$=1.487, p=0.290; Time: $F_{3,24}$=0.913, p=0.450; Interaction: $F_{9,24}$=1.503, p=0.203) (Fig. 2c; 2d). The Shannon index varied among study areas, independent of cultivation period (Treatment: $F_{3,8}$=4.406, p<0.05; Time: $F_{3,24}$=2.478, p<0.088; Interaction: $F_{9,24}$=1.497, p=0.208). The Shannon index was lower in short-therm rice fields than in natural wetlands and mid and long-therm rice fields (Tukey, p<0.01) (Fig. 2e;f).

Similarity in aquatic macroinvertebrate community composition was represented by three axes in the ordination analysis (NMDS, stress=11) (Fig. 3). Composition varied significantly among the study areas throughout the cultivation cycle (Treatment: $F_{3,9}$=3.15, p<0.001; Time: $F_{3,9}$=3.72, p<0.001; Interaction: $F_{9,47}$= 1.84, p<0.001).

Macroinvertebrate composition differed between wetlands, short and long-therm rice throughout the cultivation cycle. Macroinvertebrate composition differed between wetlands and mid-therm crops only during the off-season and initial growth periods. During the post-harvest period, macroinvertebrate composition in the short-therm rice fields differed from all other areas.

Discussion

Various studies have shown that rice fields provide habitat for many species of aquatic macroinvertebrates around the world (e.g. Bambaradeniya et al. 2004; Leitão et al. 2007; Sánchez-Guzmán et al. 2007; Picazo et al. 2010; Rizo-Patron et al. 2013; Zhang et al. 2013). We collected 41 families of aquatic macroinvertebrates in rice fields. The number of *taxa* corroborated with other similar studies performed in southern Brazil (Stenert et al. 2009; 2012). Rice fields supported more than 50% of the macroinvertebrate families observed in wetlands of southern Brazil (74 families—Stenert and Maltchik, 2007; and 61 families—Stenert et al. 2008).

The most abundant organisms were the aquatic insects, particularly Chironomidae. Predominance of aquatic insects has also been observed in wetlands in southern Brazil (Stenert and Maltchik 2007; Stenert et al. 2008), the Pantanal (Heckman 1998), Costa Rica (Rizo-Patron et al. 2013), Australia (Finlayson et al. 2006; Wilson et al. 2008), Europe (Suhling et al. 2001; Leitão et al. 2007) and Asia (Bambaradenyia et al. 2004; Zhang et al. 2013). Insects exhibit diverse trophic functionality and adapt well to conditions imposed by rice field management (Schoenly et al. 1996; Bambaradeniya et al. 2004). Chironomidae was abundant among these groups (Leitão et al. 2007; Wilson et al. 2008). Increased amounts of organic matter in the soil of tropical rice fields causes the chironomid larvae population density to rapidly increase (Simpson et al. 1994). Another dominant taxon was Oligochaeta, especially in the long-therm rice fields. Oligochaetes are also important components of the benthic fauna in rice fields (Bambaradeniya et al. 2004), where they respond quickly to added fertilizers (Leitão et al. 2007) and play important roles related to nutrient availability (Suhling et al. 2000).

Our results showed that richness, abundance and Shannon index were similar between natural wetlands and mid and long-therm rice fields. Short-therm rice fields had lower richness and Shannon index than the other areas. This result refutes our original hypothesis that the richness, abundance and Shannon index of short-thermrice fields would be more similar to that of natural wetlands than mid and long-therm crops. Several studies have shown the negative effects of agrochemical application and management techniques in crops (i.e. fertilizer application, soil tillage and livestock trampling) on soil fertility and organism diversity (Roger et al. 1991; Simpson and Roger 1995; Wilson et al. 2008; Geiger et al. 2010; Hayasaka et al. 2012a; Imfeld and Vuilleumier 2012). However, few studies have focused on the repeated effect of these disturbances on aquatic communities. Our results indicated that the impacts caused in the first years of cultivation highly influence the macroinvertebrate community. In the first years of cultivation (i.e. short-term crops), the impacts caused by the conversion of natural areas, along with mechanized soil preparation and agrochemical application, drastically reduce the macroinvertebrate community. These unpredictable disturbances heavily stress populations, thus reducing and/or eliminating them. Some generalist and more resistant species, such as Chironomidae and Oligochaeta larvae, rapidly recolonize an environment, and their populations increase in the absence of predators (Leitão et al. 2007). As these disturbances become periodic (i.e. mid- and long-term crops), species are able to respond to disturbance, resisting and recovering quickly after disturbance events through physiological characteristics to temporal changes will be selected (Bambaradeniya et al. 2004). One of the main responses to these alterations is the formation of resistant structures. A study on the recovery capacity of aquatic invertebrates through egg banks in irrigated rice fields that remained dry for up to two years in southern Brazil found that many invertebrates, and especially microcrustaceans, produce resistant eggs that remain viable in these agroecosystems (Stenert et al. 2010). Therefore, egg banks in the sediments of rice fields are an ecological and evolutionary reserve fundamental to environmental fluctuations (Hairston 1996), and they allow the recovery of communities in these ecosystems (Boulton and Lloyd 1992; Brock et al. 2003; Jenkins and Boulton 2007).

Our results showed that macroinvertebrate richness, abundance and Shannon index did not change during the rice cultivation cycle, and they were constant throughout the cultivation periods. Macroinvertebrate dynamics are highly influenced by hydroperiod (Stenert et al. 2008; Stenert et al. 2009). In this study, the lack of difference over time could be a result of the samples only having been collected during cultivation periods, when there was surface water. Stenert et al. (2012) showed that the main differences in macroinvertebrate dynamics in rice fields occurred between the periods with and without surface water, particularly during soil preparation, when the environment is completely dry.

Macroinvertebrate composition varied among the study areas over the cultivation cycle. The composition of the short and long therm rice crops differed from that of the wetlands during each cultivation period. The mid-therm rice fields had a similar composition to that of the wetlands except during off-season and initial growth periods. Macroinvertebrate composition of wetlands differed from the rice fields by the presence of many families that occurred only in wetlands, such as Aeshnidae, Naucoridae and Nepidae. Furthermore, some taxa were more frequent and abundant in wetlands (Ceratopogonidae, Hydroptilidae, Lestidae, Libellulidae, Leptoceridae, Notonectidae and Pleidae – see Table 1). The reduced macroinvertebrate diversity in rice fields could be due to the loss of microhabitats and the application of pesticides and fertilizers (Wilson et al. 2008; Leitão et al. 2007; Rizo-Patrón et al. 2013). Studies show that pesticide use reduces aquatic macroinvertebrate diversity, particularly during the initial plant growth period (Amarante et al. 2002). Moreover, the use of disc plows and pesticide application in these fields leads to simplification of the number of habitats resulting from a lack of plant cover and the dominance of only one species (*Oryza sativa*) while the rice is growing. Many studies in wetlands have focused on the relationship between aquatic macrophyte and macroinvertebrate occurrence (Stenert et al., 2008). High plant richness provides a high number of habitats, which provide food and shelter for the macroinvertebrate community. Practices that lead to reduced macrophyte stands and consequently simplified habitat, negatively affect the macroinvertebrate community.

The proximity of the studied rice fields to wetlands influenced our results. Wetlands are sources for species recolonization in habitats that suffer repeated disturbances (Maltchik et al. 2011). The mid and long-therm rice fields shared several taxa with the natural wetlands. Short-therm crops and wetlands only shared generalist, disturbance-resistant taxa (Oligochaeta) with good dispersal ability (Chironomidae, Corixidae, Caenidae, Libellulidae, Dytiscidae, Hydrophilidae) and fewer in number.

These results highlight the importance of adjacent wetlands for biodiversity maintenance in irrigated rice fields.

This study showed that rice fields are used as complementary habitats to wetlands by aquatic macroinvertebrate communities in southern Brazil. However, our results indicate that aquatic macroinvertebrate communities tend to suffer more in the first few years from impacts caused by soil and water management in these crops. Our results reinforce the importance of a layer of surface water in wetlands adjacent to rice fields for maintaining local biodiversity.

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Figures Caption

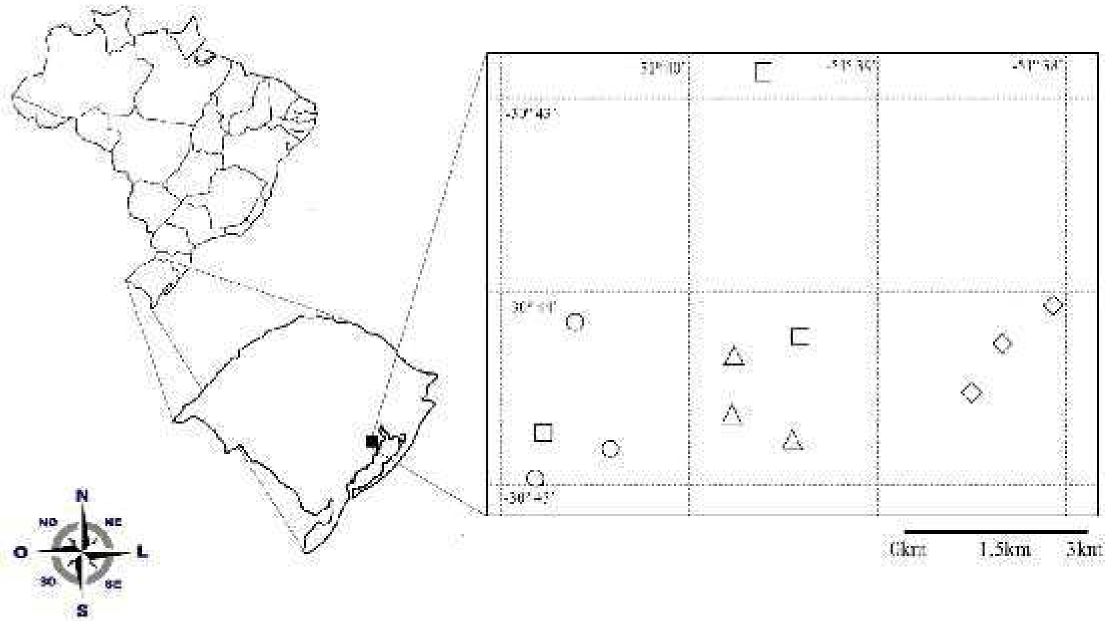

Figure 1. Study area (black square) in the coastal plain of Rio Grande do Sul, Brazil (2011-2012). White Squares: natural wetlands, triangles: Long-therm rice fields, circles: Mid-therm rice fields, diamonds Short-therm Rice fields.

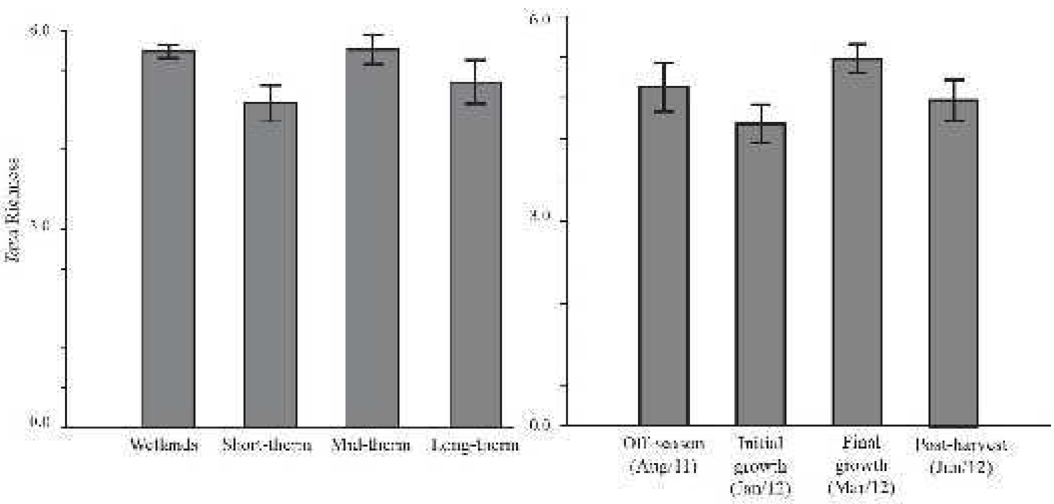

Figure 2. Mean richness (±SE) of macroinvertebrates in wetlands and rice fields over four phases of rice cultivation cycle in Southern Brazil (2011-2012).

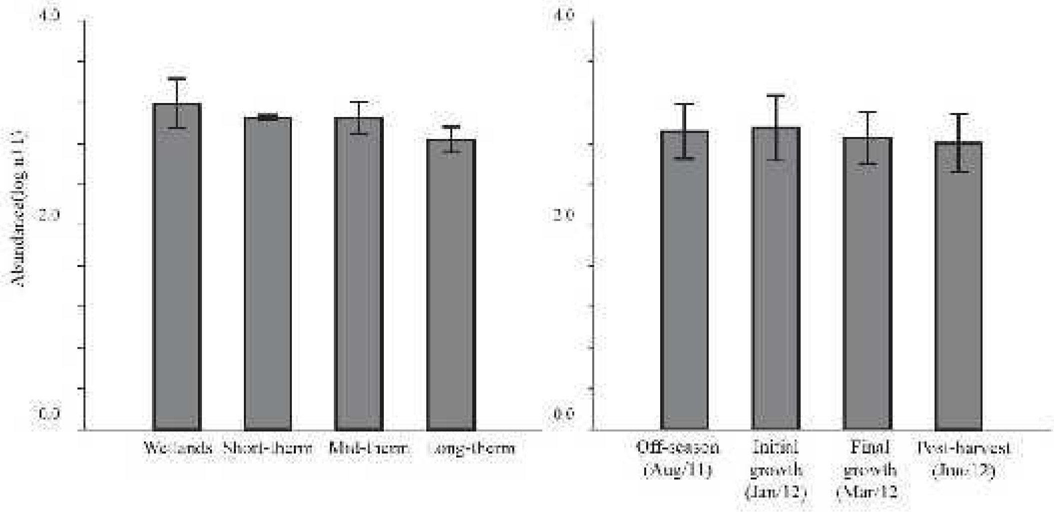

Figure 3. Mean abundance (±SE) of macroinvertebrates in wetlands and rice fields over four phases of rice cultivation cycle in Southern Brazil (2011-2012).

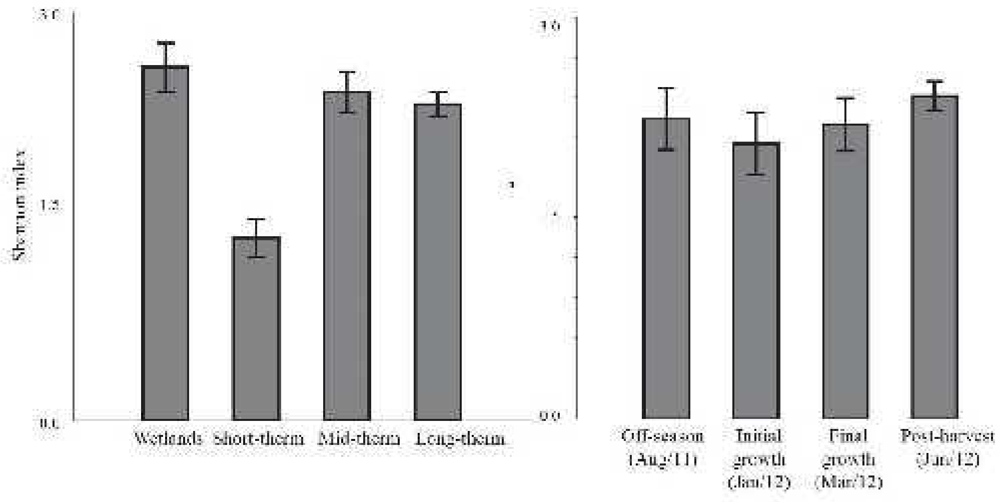

Figure 4. Mean Shannon index (±SE) of macroinvertebrates in wetlands and rice fields over four phases of rice cultivation cycle in Southern Brazil (2011-2012).

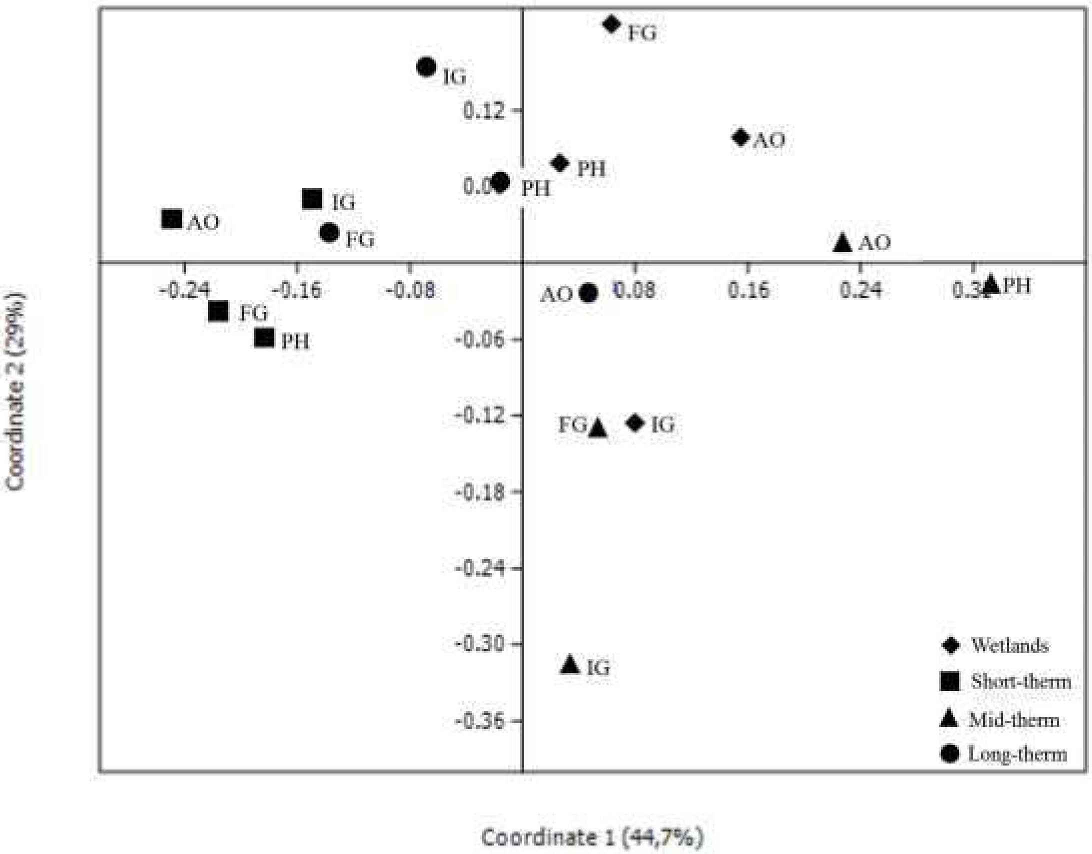

Figure 5. Multidimensional scaling ordination for macroinvertebrates assemblages (stress=11) in wetlands and rice fields over four phases of rice cultivation cycle in Southern Brazil (2011-2012). AO: Anterior off-season (Aug/11); IG: Initial Growth (Jan/12); FG: Final Growth (Mar/12); PH: Post-Harvest (Jun/12).

Supplementary Table 1. Aquatic macroinvertebrate *taxa* in wetlands and rice fields (short, mid and long-therm) over six phases of rice cultivation cycle in Southern Brazil (2011-2012). AO: Anterior off-season (Aug/11); IG: Initial Growth (Jan/12); FG: Final Growth (Mar/12); PH: Post-Harvest (Jun/12).

Class	Sub-Class	Order	Family	Genus	Wetland				Short-therm				Mid-Therm				Long-Therm				Total
					AO	IG	FG	PH	AO	IG	FG	PH	AO	IG	FG	PH	AO	IG	FG	PH	
Turbellaria		Tricladida	Dugesiidae	*Dugesia*	0	0	51	0	0	0	0	4	0	0	0	0	0	0	0	0	**55**
Nematoda					4	2	1	5	1	1	2	0	1	0	0	0	2	11	1	0	**31**
Clitellata	Hirudinea	Rhynchobdellida	Glossiphoniidae	*Helobdella*	25	60	37	27	341	1	17	0	209	1	25	200	47	51	52	138	**1,231**
		Arhynchobdellida	Hirudinidae		5	5	2	0	19	0	1	0	1	1	0	0	1	0	2	4	**41**
	Oligochaeta				34	58	88	34	27	75	78	1	188	15	91	11	69	53	20	156	**998**
Bivalvia	Heterodonta	Veneroida	Sphaeriidae	*Psidium*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
Gastropoda	Prosobranchia	Architaenioglossa	Ampullariidae	*Pomacea*	0	0	0	0	4	4	8	0	1	0	5	0	15	0	3	0	**40**
		Neotaenioglossa	Hydrobiidae		0	0	0	0	0	0	18	3	0	0	1	0	1	0	3	2	**28**
	Pulmonata	Basommatophora	Lymnaeidae		0	0	0	0	0	0	0	5	0	2	0	1	0	0	0	0	**8**
			Physidae	*Physa*	0	0	0	0	0	8	0	20	1	0	0	1	0	0	0	0	**30**
			Planorbidae		140	18	0	0	19	0	3	0	33	154	41	147	35	0	34	44	**668**
Arachnida	Acari (Hydracarina)	Trombidiformes			20	18	9	4	0	2	1	1	0	12	8	2	0	3	1	13	**94**
Entognatha	Collembola	Entomobryomorpha	Isotomidae	*Isotomurus*	0	0	0	0	4	17	24	2	1	51	6	6	9	1	73	28	**222**
		Poduromorpha	Poduridae	*Podura*	0	0	0	0	4	0	0	0	0	0	1	0	26	0	0	6	**37**
		Symphypleona	Sminthuridae		0	0	0	0	0	0	0	0	0	0	3	0	0	0	8	0	**11**
Insecta	Pterygota	Ephemeroptera	Baetidae	*Callibaetis*	44	15	49	8	6	1	16	1	62	0	56	7	3	0	0	0	**268**
			Caenidae	*Caenis*	189	69	18	9	1	0	5	0	13	0	112	19	0	1	1	0	**437**
		Odonata	Aeshnidae		0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	**3**
			Coenagrionidae		86	6	40	6	0	653	12	26	5	0	85	19	0	0	6	0	**944**
			Lestidae	*Lestes*	12	0	2	8	0	0	0	1	1	0	2	0	0	0	0	0	**26**
			Libellulidae		84	7	24	11	1	0	12	0	30	0	63	15	1	0	4	0	**252**
		Hemiptera	Belostomatidae	*Belostoma*	6	3	48	1	0	0	2	5	1	2	8	8	1	0	1	1	**87**
			Corixidae	*Sigara*	58	28	53	3	26	3	0	0	36	139	7	8	7	41	0	19	**428**
			Hebridae		0	0	0	0	0	0	1	0	0	0	0	0	0	0	6	0	**7**
			Mesoveliidae	*Mesovelia*	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	**3**
			Naucoridae		33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**33**
			Nepidae	*Ranatra*	1	4	1	0	0	1	0	0	0	0	0	0	0	0	0	0	**7**
			Notonectidae		20	8	19	2	0	0	0	2	3	2	1	1	0	1	0	3	**62**
			Pleidae	*Neoplea*	17	54	34	1	0	2	0	0	0	1	1	0	0	0	0	1	**111**
			Veliidae		1	1	1	0	0	0	0	0	0	0	2	0	0	0	0	0	**5**

Supplementary Table 1 (continued). Aquatic macroinvertebrate *taxa* in wetlands and rice fields (short, mid and long-therm) over six phases of rice cultivation cycle in Southern Brazil (2011-2012). AO: Anterior off-season (Aug/11); IG: Initial Growth (Jan/12); FG: Final Growth (Mar/12); PH: Post-Harvest (Jun/12).

Class	Sub-Class	Order	Family	Genus	Wetland				Short-therm				Mid-Therm				Long-Therm				
					AO	IG	FG	PH	AO	IG	FG	PH	AO	IG	FG	PH	AO	IG	FG	PH	**Total**
			Curculionidae		2	0	4	1	0	4	0	8	0	16	4	0	0	1	0	0	**40**
			Dytiscidae		1	0	46	1	3	9	4	0	0	76	7	6	0	4	0	18	**175**
			Elmidae		3	2	1	0	0	2	0	1	0	0	2	0	0	0	0	0	**11**
		Coleoptera	Haliplidae	*Haliplus*	0	0	0	0	0	0	0	191	0	0	16	0	0	0	1	0	**208**
			Hydrochidae		1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	**2**
			Hydrophilidae		4	20	26	10	7	36	5	0	17	163	16	4	10	31	2	42	**393**
			Noteridae		15	6	23	1	0	0	2	0	2	16	21	57	0	0	1	13	**157**
			Scirtidae	*Scirtis*	0	0	2	0	0	1	1	0	0	1	0	0	0	0	0	0	**5**
			Ceratopogonidae		3	18	26	17	6	0	6	0	8	0	50	22	3	20	12	75	**266**
Insecta	Pterygota		Chaoboridae	*Chaoborus*	0	0	7	1	0	0	0	0	0	0	1	0	0	0	0	0	**9**
			Chironomidae		278	234	354	108	56	1,242	172	0	144	221	299	233	32	93	64	50	**3,580**
		Diptera	Culicidae		12	2	40	0	1	15	8	30	0	0	46	22	0	0	2	72	**250**
			Ephydridae		1	0	0	0	0	1	0	0	0	0	1	2	0	1	0	0	**6**
			Psychodidae		0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	**4**
			Tabanidae		2	0	1	0	1	0	7	0	1	0	6	0	1	0	0	0	**19**
			Tipulidae		6	0	0	0	1	0	0	0	0	0	0	0	4	0	0	0	**11**
		Lepidoptera	Pyralidae		10	1	13	1	0	0	0	0	0	11	8	0	1	1	4	0	**50**
		Trichoptera	Hydroptilidae	*Oxyethira*	12	13	8	1	0	0	0	0	1	0	15	0	0	0	0	0	**50**
			Leptoceridae	*Oecetis*	74	6	13	27	2	4	17	2	17	0	18	0	0	0	2	0	**182**
				Total	**1,205**	**658**	**1,047**	**287**	**530**	**2,082**	**422**	**304**	**776**	**885**	**1,030**	**791**	**268**	**313**	**303**	**685**	**11,586**

6. CONCLUSÕES GERAIS

Este trabalho sugere que as lavouras de arroz são capazes de conservar uma parcela importante da diversidade de macroinvertebrados aquáticos, funcionando como refúgios estratégicos de biodiversidade. Entretanto, esses agroecossistemas não são capazes de substituir os remanescentes naturais de áreas úmidas na conservação da biodiversidade regional. É importante destacar que as áreas úmidas naturais possuem inúmeras funções e valores reconhecidos internacionalmente (recarga de aqüíferos, estabilidade climática, armazenamento e purificação da água, controle de inundações, recreação, pesquisa, entre outros). Além disso, esses ecossistemas apresentam uma alta diversidade de espécies de plantas e animais. Embora muitos *taxa* de macroinvertebrados encontrados nas lavouras de arroz irrigado também estejam presentes em áreas úmidas naturais, observou-se que alguns grupos, tais como, oligoquetas, quironomídeos e glossifonídeos, predominaram em relação a outros *taxa* amostrados nas áreas agrícolas.

O presente estudo também constatou que as flutuações hidrológicas que ocorrem durante o ciclo de cultivo do arroz são importantes na resiliência da comunidade de macroinvertebrados aquáticos nesses agroecossistemas. A medida que o ambiente passa das fases semi-aquática (entressafra e pós-colheita) para terrestre (preparo do solo) e aquática (crescimento do grão), observam-se flutuações drásticas na riqueza, abundancia, diversidade e composição da comunidade de macroinvertebrados, diminuindo ao longo das fases secas e se restabelecendo nas fases aquáticas e semi-aquáticas. É interessante ressaltar, que a presença de lâmina d´água durante os períodos de entressafra e pós-colheita favorecem a biodiversidade nesses sistemas, principalmente em relação aos fases de crescimento do grão, que apresentam lâmina d´água mais profunda, porém utilizam do manejo da água e da aplicação de agrotóxicos.

Os resultados relacionados ao experimento realizado em lavouras com manejo orgânico e convencional mostraram que ambas lavouras de arroz irrigado impactam negativamente a comunidade de macroinvertebrados, diminuindo a riqueza, abundancia e a diversidade, além de alterar a composição. No entanto, este resultado não deve ser visto como um aspecto negativo da cultura do arroz orgânico e técnicas orgânicas de baixo impacto podem efetivamente controlar organismos indesejáveis sem o uso de agrotóxicos. Diversos estudos demonstram os malefícios dos agrotóxicos para a saúde humana e para outros aspectos da ecologia dos ecossistemas, sobretudo no desempenho de suas funções, assim como observado no segundo manuscrito. Embora não significativas, o manejo orgânico favoreceu funcionalmente muito mais a comunidade de insetos aquáticos do que o cultivo convencional, evidenciando, ainda que de forma sútil, que o uso de agrotóxicos é determinante na manutenção da funcionalidade dos ecossistemas.

Por fim, o presente estudo traz informações de como a idade da lavoura influencia a estrutura da comunidade de macroinvertebrados. Diferentemente do que se esperava, as lavouras mais jovens (3 anos) foram mais impactantes à comunidade estudada do que as mais antigas (10 e 20 anos). Em termos de conservação, esses resultados sugerem a capacidade de se adaptar que esta comunidade apresenta, frente a repetidas perturbações. É interessante salientar, a importância da comunidade de macroinvertebrados aquáticos na cadeia trófica em áreas úmidas e que sua capacidade de resiliência após fortes perturbações, como a conversão de áreas para a agricultura, pode ser fundamental no estabelecimento de outros organismos e na manutenção das funções ecossistêmicas.

7. CONSIDERAÇÕES FINAIS

Esses resultados podem auxiliar nas pautas de manejo de áreas agrícolas capazes de ampliar a capacidade de proteger a biodiversidade, principalmente em regiões onde grande parte das áreas úmidas naturais já foi destruída e as áreas remanescentes continuam sendo ameaçadas. Dessa forma, estudos ecológicos que investiguem os efeitos de diferentes alternativas de manejo sobre as comunidades biológicas nas lavouras de arroz irrigado são fundamentais para conciliar a produção agrícola com a conservação da biodiversidade no Rio Grande do Sul. Os agricultores podem colaborar com a conservação da biodiversidade protegendo remanescentes naturais em suas propriedades e manejando adequadamente o sistema de cultivo. Além disso, os resultados obtidos nesse estudo podem subsidiar futuros planos de restauração de áreas úmidas naturais a partir de parcelas de arroz abandonadas ou improdutivas.