

RESEARCH ARTICLE

Using topsoil translocation from natural wetlands to restore rice field systems

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Wetlands provide vital habitat for biodiversity and relevant ecosystem services to human population. The rapid transformation of freshwater wetlands to grow food crops became the wetlands strongly threatened by human activities. Restoration efforts related to wetlands have been widely attempted; however, science-based guidelines are relatively scarce. Here, we analyzed whether different levels of topsoil addition from natural wetlands in the sediment from rice fields influence the zooplankton communities. Our hypothesis was that the richness and abundance of hatchlings would be higher in treatments with high topsoil addition from natural wetlands, followed by treatments with low addition. In the laboratory, eight field sediment samples were incubated for zooplankton hatching in four treatments: rice fields, rice fields added with low (5%) and medium (30%) quantities of sediment from natural wetlands, and natural wetlands. A total of 4,493 hatchlings belonging to 24 taxa were observed in the experiment. While zooplankton richness and abundance did not differ between sediment treatments, significant differences in species composition associated with topsoil addition were observed. SIMPER analysis showed that topsoil addition from natural wetlands enhanced the abundance of cladocerans and larval copepods. Our results showed that sediments from natural wetlands have a bank of zooplankton dormant eggs that can help the recovery of wetlands disturbed by rice fields. The addition of natural wetland sediments in areas disturbed by rice fields modifies the zooplankton composition of the rice fields, aiding the recovery of disturbed natural wetlands for rice production.

Key words: dormant stages, hatchlings, sediment addition, wetland restoration, zooplankton

Implications for Practice

- Sediments from natural wetlands have a bank of zooplankton dormant stages that can help the recovery of wetlands disturbed by rice fields.
- Addition of topsoil from natural wetlands can lead to significant changes in the zooplankton composition of rice fields.
- Seedling and dormant stage introduction may be used as wetland restoration techniques to accelerate succession and ecosystem development.
- It is urgent for further exploration to study in situ if the addition of topsoil from natural wetlands may be used as wetland restoration techniques.

Introduction

Wetlands provide vital habitat for biodiversity and highly relevant ecosystem services (Millennium Ecosystem Assessment 2005; Costanza et al. 2014). Although foodstuffs and other provisioning services are well perceived by human populations, essential regulating services associated to wetlands, such as water purification or climate regulation, are not often recognized by general public and policy-makers. Over the past decades, crops replaced substantial areas of native wetlands, leading to biodiversity erosion and changes in water quality (Schiesari & Corrêa 2016; Stenert et al. 2018; Moreira et al. 2021). Brazil

currently ranks among the top crop producers in the world, with large cultivated areas of soybean, sugarcane, and rice (FAOSTAT 2020). In this sense, rapid transformation of freshwater wetlands to grow food crops puts at risk the high biodiversity and the ecosystem services these wetlands provide.

While restoration efforts related to wetlands have been widely attempted, science-based guidelines are still relatively new and

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incomplete (Moreno-Mateos et al. 2012; Craft 2016). Water quality improvement, species enhancement, and compensation associated with developmental activities are the main drivers associated with wetland restoration projects (Comín et al. 2001; Yamada et al. 2007; Melo et al. 2015; Craft 2016). However, wetlands are quite variable in terms of hydrology, vegetation, and soil (Junk et al. 2014). It is well appreciated that such diversity has consequences on ecological functions played by different types of wetlands, which are constrained by stressors originated on-site or off-site. In particular, areas converted to rice fields have high inputs of sediments and agrochemicals, altered hydrology associated to ditching/tile drainage, and susceptibility to invasive species (Wilson et al. 2008; Van Leeuwen et al. 2013; Moreira & Maltchik 2015). Even so, rice fields are considered important supplementary habitats for natural wetlands in farmland landscapes worldwide (Elphick & Oring 2003; Stenert et al. 2009; Machado & Maltchik 2010; Maltchik et al. 2017; Koshida & Katayama 2018). Life history traits, mainly related to dispersion and reproduction, are particularly relevant to determine which species will persist in agricultural areas (Ávila et al. 2015; Moreira et al. 2016, 2021; Pincheel et al. 2016). Consequently, enhancing larval recruitment of freshwater organisms would play an important role in accelerating the restoration of degraded wetlands.

Although biological processes develop in faster time scales than physicochemical ones, restoration of wetlands usually involves reintroducing hydrology followed by passive natural colonization (Craft 2016; Koshida & Katayama 2018). But whether and how available propagules (plants and animals) would benefit wetland development is still a key gap in our knowledge about ecosystem recovery (Eskinazi-Sant'anna & Pace 2018). Restoration techniques such as seedling and dormant stages introduction may be used to accelerate succession and ecosystem development (Angeler & García 2005; Klimkowska et al. 2007; Kettenring & Tarsa 2020). While restoration interventions are essential in many situations, a growing scholar interest has raised concerns about species reintroduction. Such criticisms are related to when reintroducing is needed, propagules origin, failure of the native species return, and possible damage of the donor ecosystems (Lorimer et al. 2015; Pilon et al. 2019; Kettenring & Tarsa 2020). Therefore, understanding colonization processes and appropriate keystone species is essential for management purposes in wetlands converted to agricultural uses.

In general, zooplankton species provide the trophic link between primary producers and many larger organisms, including fish, amphibians, and waterfowls (Hornung & Foote 2006; Kattel 2012). Some zooplankton animals, in particular rotifers and crustaceans, have dormancy capability (i.e. long-term resistant stages) that enables them to survive periods of unfavorable conditions (Fontaneto 2019; Vargas et al. 2019). Thus, such dormant stages are crucial for colonization processes and community dynamics in freshwater wetlands, especially temporary ones (Shurin 2000; Badosa et al. 2017; Brendonck et al. 2017; Olmo et al. 2020). Seemingly isolated temporary wetlands may serve as source of zooplankton propagules via surface

water flow, wind, and animals. However, both dispersal limitation and environmental gradients seem important to zooplankton community structure, with evidence for species sorting in some habitats and mass effects in others (Shurin et al. 2009; De Bie et al. 2012). Besides, some agricultural activities used in rice fields, like changes in the hydrological regime and agrochemical application, have substantial negative effects on dormant stages of zooplankton species (Stenert et al. 2010; Ávila et al. 2015). In systems that lack nearby source of propagules and with depleted egg bank, enhancing dormant stages supply can be a strategic management intervention. Topsoil transplantation from natural to degraded wetlands has been used to improve species richness in plant communities (Craft 2016; Kettenring & Tarsa 2020), but the consequences of such addition for zooplankton communities are still largely unknown.

With this study, we set out to evaluate the consequences of topsoil translocation on zooplankton communities' structure in an ex-situ experiment with wetlands used for agriculture. Our goal was to assess whether increasing amount of topsoil addition from natural (non-cultivated) wetlands in the sediment of rice fields could influence the taxonomic richness, abundance, and composition of the zooplankton communities. We hypothesized that: (1) richness and abundance of hatchlings would be higher in treatments with high topsoil addition from natural wetlands, followed by treatments with low addition, and lowest in rice field sediments; and (2) rice fields and natural wetland sediments would have a different taxa composition, but topsoil addition would make community composition similar between cultivated areas and natural wetlands.

Methods

Study Area and Sediment Sampling

The study area is located in a portion of the Coastal Plain of the southern Brazil (30° 15' S and 31° 06' S, 50° 30' W, and 50° 55' W), an important region of rice production in South America. This region is characterized by a mosaic of grasslands and shrubby vegetation (Marques et al. 2015) and encompasses different natural wetlands, such as marshes, coastal lagoons, inland lagoons, and floodplains. For the experiment, we sampled sediment from four natural wetlands and four rice fields in October 2017, during the off season of the rice cultivation cycle. Each rice field was close to a natural wetland (~500 m). Each pair of rice field—natural wetland ($n = 4$) was located at least 15 km distant from each other to minimize spatial autocorrelation. During this period, most of the natural wetlands start to dry or to reduce significantly in surface water. Some wetlands of the region dry up completely and others may decrease a lot their surface area (60–80%). The natural wetlands analyzed were intermittent, with similar sizes (1 ha), water depth (0.5 m on average), and composition of aquatic macrophyte (herbaceous and emergent). During the cultivation cycle, the water level is controlled in the rice fields (about 10 cm for 130 days), and the application of agrochemicals (herbicide glyphosate—2 L/ha) is concentrated in

the initial growth period stage (November–December) to control weeds, especially sedges and grasses.

In each of the eight selected sites, we randomly collected 10 subsamples during a dry period using a core sampler (7.5 cm diameter) inserted to a depth of 5 cm into the dry substrate (Brendonck & De Meester 2003). So, sediment subsamples were dehydrated in a dark oven for 96 hours at 40°C, sieved (1-mm mesh size) for the removal of roots and leaves and then pooled into 1 sample/site (around 1.5 kg of dry sediment) (Vendramin et al. 2021). The dry sediment was stored in black plastic bags for 1 year (23°C) before the beginning of the experiment (November 2018).

Experimental Set-up

For the experimental procedures, 250 g aliquots from dry topsoil were used to create four treatments with four replicates each: rice fields (250 g from rice fields), rice fields 5% (12 g from natural wetlands + 238 g from rice fields), rice fields 30% (75 g from natural wetlands + 175 g from rice fields), and natural wetlands (250 g from natural wetlands). These different proportions of topsoil addition were chosen to include less costly and more viable proportions for wetland restoration projects developed in-situ. In the mixed sediment treatments, natural wetland sediment was added to rice field sediment belonging to the same pair.

The 16 sediment samples were incubated in trays (30.3 × 22.1 × 7.5 cm), kept aerated, and submersed under a depth of 2 cm of distilled water. Water level, temperature (23 ± 2°C), photoperiod (12 hours light/12 hours dark), and dissolved oxygen (>6.5 mg/L) were kept constant (Ávila et al. 2015). The experiment was maintained in the laboratory for 4 weeks, and hatchlings were collected three times per week ($n = 13$ per tray), to avoid as much as possible that the individuals collected are from parthenogenetic reproduction. The sampling intervals of 2–3 days were used in other studies to minimize the chance of parthenogenetic reproduction (Brock et al. 2005; Nielsen et al. 2013). Hatchlings were sampled using a 22.1 cm wide net (53 µm mesh) stirring up the sediment and then sweeping above the overlying water (three times at each tray). The content of the sample was then transferred to a Bogorov chamber for sorting of hatchlings using a stereomicroscopic (Zeiss Stemi 2000-C). Non-hatched dormant eggs were returned to their respective trays. Identification was performed to the species level whenever possible using literature (Lopretto & Tell 1995; Elmoor-Loureiro 1997, 2000; Gazulha 2012), and took additional help from taxonomists. Samples of the identified organisms are kept at the Laboratory of Ecology and Conservation of Aquatic Ecosystems of UNISINOS.

Data Analyses

To compare richness and abundance of zooplankton across sediment treatments, we used generalized linear models (GLM). Response variables were taxa number and number of hatchlings, and the model was adjusted for the Poisson distribution for

richness and negative binomial distribution for abundance (because of major overdispersion of residuals). Topsoil class (four levels) was included in the models as a fixed factor. Area identity was determined for each of the four pairs of rice field/natural wetland, and it was also included as a fixed factor in the statistical models to investigate differences among sampling areas. Statistical significances were assessed using the ANOVA function which performs Wald Chi-Square Test. Significant interactions were investigated with a Tukey's post hoc test.

A permutational variance analysis (PERMANOVA) was used to assess differences in zooplankton composition among sediment treatments and sampling area id, based on Bray–Curtis dissimilarity. In case of significant differences, we conducted separated PERMANOVAs for pairs of natural wetlands and disturbed sediments (rice field, rice field 5%, and rice field 30%). Before PERMANOVA, zooplankton data were log transformed. Because this analysis is sensitive to data dispersion and may therefore confuse within group variation with among-group variation, we performed an analysis of multivariate homogeneity (PERMDISP), as recommended by Anderson and Walsh (2013). We used a Bray–Curtis dissimilarity matrix for PERMDISP and a permutation test (999 runs) to assess the significance. Similarity percentages (SIMPER) analysis was used to investigate the sources of variation (taxa) responsible for composition dissimilarity identified in the PERMANOVA. A non-metric multidimensional scaling (NMDS) was used to assist with interpretation. We used the ordihull function to represent the projected two-dimensional ordination space occupied by each sediment class. All analyses were performed using vegan (Oksanen et al. 2019) and lme4 (Bates et al. 2015) packages for R (R Development Core Team 2020).

Results

Over the course of 4 weeks experiment, we registered 4,493 hatchlings belonging to 24 taxa. The complete data for zooplankton abundance and diversity hatched during the experiment are provided in Table 1. Two cladocerans (*Alona gutatta* Sars and *Macrothrix elegans* Sars) dominated the abundance of the hatchlings. Seven rotifers and two cladocerans hatched exclusively in one sediment class, but at low abundance. Only 10 copepods hatched from the experiment (Table 1). Zooplankton richness did not differ between sediment treatments (Wald $\chi^2 = 2.637$, $df = 3$, $p = 0.451$; Fig. 1A) and sampling area id (Wald $\chi^2 = 0.097$, $df = 3$, $p = 0.992$; Fig. S1A). Zooplankton abundance was affected both by sediment treatments (Wald $\chi^2 = 10.736$, $df = 3$, $p = 0.013$; Fig. 1B) and sampling area id (Wald $\chi^2 = 12.150$, $df = 3$, $p = 0.007$; Fig. S1B). Post hoc tests indicated lower values of abundance in rice fields 5% when compared to rice fields 30% ($p = 0.025$) and natural wetlands ($p = 0.014$; Fig. 1B), and significant differences between Area 2 and Area 4 ($p = 0.002$; Fig. S1B). However, considering that dominance of two cladocerans species could influence variation in abundance, we excluded these species and reanalyzed the data. In fact, after species exclusion, variation in zooplankton abundance did not differ between sediment treatments (Wald $\chi^2 = 5.151$, $df = 3$, $p = 0.161$; Fig. 2A), but abundance

Table 1. Zooplankton taxa hatching from sediment of four categories. $n = 4$ for each category.

	Rice Fields	Rice 5%	Rice 30%	Wetlands
Rotifera				
<i>Adineta vaga</i> (Davis, 1973)	31	27	6	39
<i>Asplanchna sieboldi</i> (Leydig, 1854)	2		3	4
<i>Balanus</i> sp.				3
<i>Brachionus plicatilis</i> (Müller, 1786)	73	1	10	7
<i>Colurella obtusa</i> (Gosse, 1886)				1
<i>Euchlanis dilatata</i> (Ehrenberg, 1832)		1	2	19
<i>Filinia longiseta</i> (Ehrenberg, 1834)	1			
<i>Keratella cochlearis</i> (Gosse, 1851)				2
<i>Lecane lunaris</i> (Ehrenberg, 1832)	17	1		
<i>Notholca</i> sp.	1			
<i>Philodina</i> sp.	1		7	6
<i>Platyias</i> sp.		1		
<i>Synchaeta</i> sp.	3			
Cladocera				
<i>Alona guttata</i> (Sars, 1862)	8	322	1,087	970
<i>Chydorus eurynotus</i> (Sars, 1901)	272	12	125	89
<i>Diaphanosoma</i> sp.	1			
<i>Ilyocryptus spinifer</i> (Herrick, 1882)		7	8	4
<i>Moina micrura</i> (Kurz, 1875)	50	36	78	35
<i>Macrothrix elegans</i> (Sars, 1901)	59	10	197	819
<i>Oxyurella ciliata</i> (Bergamin, 1939)	4		3	11
<i>Simocephalus</i> sp.				1
Platyhelminthes				
<i>Mesostoma</i> sp.	1	1		4
Copepoda				
Copepodite		3	1	
Calanoida nauplii			2	4
Total richness	15	13	13	17
Mean richness \pm standard error	7 \pm 1.08	6.3 \pm 0.63	8 \pm 0.71	9.3 \pm 0.95
Total abundance	524	422	1,529	2,018
Mean abundance \pm standard error	131 \pm 68.20	105.5 \pm 73.34	382.3 \pm 202.77	504.5 \pm 216.78

differences related to sampling area id remained (Wald $\chi^2 = 12.001$, $df = 3$, $p = 0.007$; Fig 2B).

Comparison of hatchlings between treatments showed significant differences in species composition associated with sediment (PERMANOVA, $F_{3,9} = 1.840$, $p = 0.032$; Fig. 3), and sampling area id (PERMANOVA, $F_{3,9} = 1.996$, $p = 0.011$; Fig. S2). However, the association with sediment was modest, explaining only 27% of the variability observed. The PERMANOVA results were not affected by multivariate dispersion within sediment groups ($F_{3,12} = 0.392$, $p = 0.749$) or sampling areas ($F_{3,12} = 0.209$, $p = 0.9$). Natural wetlands have a different species composition, when compared to rice fields ($F_{1,7} = 1.671$, $p = 0.05$) and rice fields 5% ($F_{1,7} = 2.92$, $p = 0.018$). No differences in zooplankton hatchling composition were detected between rice fields 30% and natural wetlands ($F_{1,7} = 0.76$, $p = 0.694$). SIMPER analysis showed that 10% of overall dissimilarity between wetlands and rice fields was because of *M. elegans* abundance ($p = 0.03$). Differences between wetlands and rice fields 5% were mainly related to three taxa: two cladocerans (*M. elegans*, $p = 0.03$; *Oxyurella ciliata* Bergamin, $p = 0.012$) and Calanoida nauplii ($p = 0.031$). Together, these taxa contributed to 17% of overall dissimilarity between the two groups. Overall, many species were more

abundant in rice fields 30% and natural wetlands (Fig. 3). Five out of the 13 rotifer species hatched at more abundance in rice fields, while among cladocerans only *Chydorus eurynotus* Sars was more abundant in rice fields than natural wetlands (Table 1; Fig. 3).

Discussion

In short-term experiment, we found that 30% of topsoil from donor natural wetlands can lead to significant changes in the zooplankton composition from areas used for rice production. Although there were no differences in richness and abundance (after exclusion of dominant species) related to topsoil addition, different taxa were associated to rice fields and natural wetlands. According to our results, the transplant of zooplankton dormant stages associated to topsoil can help to recover community composition in wetlands degraded by rice agriculture. Areas with a long agricultural history are often habitat sinks that are subject to disturbances associated to tillage and crop harvesting, plus the periodical pulses of nutrients, sediment, and agrochemicals (Schiesari & Corrêa 2016). Associated to these predictable disturbances, we may expect dormant bank erosion (Brendonck et al. 2017; Stenert et al. 2018). Such erosion would result in

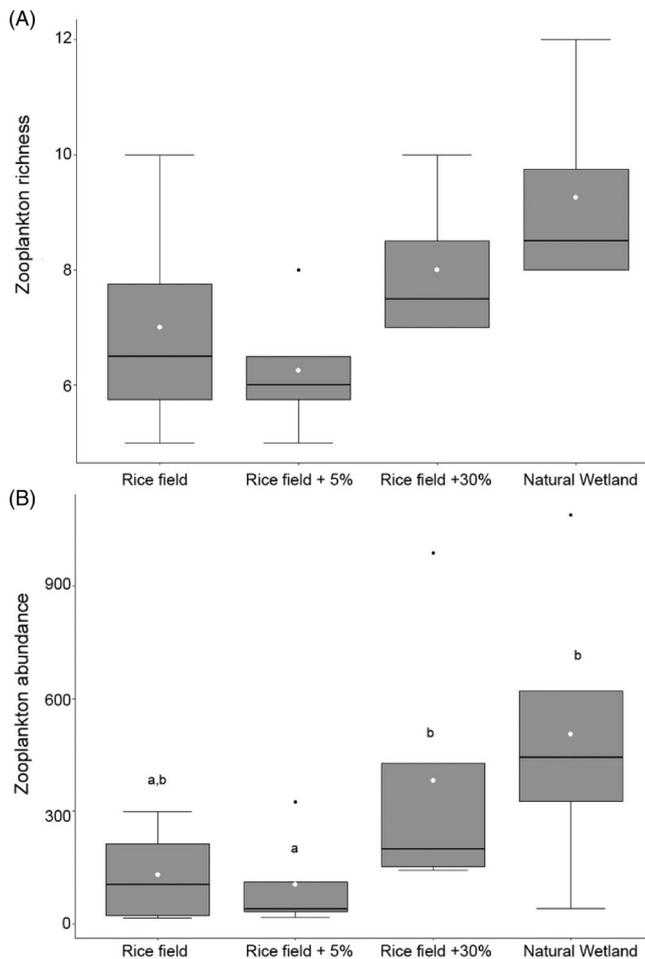


Figure 1. Richness (A) and abundance (B) of zooplankton hatchlings from four classes of sediment. Black dots represent outliers. White dots represent mean values. Different lower case letters mean statistically significant differences.

changes in dominance of active zooplankton, as the species that recolonize agricultural areas are often disturbance specialists that thrive on these conditions.

Contrary to the prediction from hypothesis 1, all rice field treatments show similar levels of zooplankton emerging from sediment. Late hatching may bias richness/abundance estimates in short incubation periods, mainly for cladocerans (Ávila et al. 2015; Olmo et al. 2016). However, the richness of taxa in our study was similar to that found in other experiments with longer incubation periods in similar habitats (18–25 spp.; Araújo et al. 2013; Ávila et al. 2015; Freiry et al. 2020). Although the emergence of zooplankton might be a good indicator that communities are resilient to stressors in the rice fields, this must be heeded cautiously. Because the water used for crop irrigation comes from streams or permanent ponds, active zooplankton might have originally occurred in other flooded areas and then ended up in the rice fields via irrigation water. Another important point here is that active freshwater zooplankton richness in the region is three times higher than dormant stages

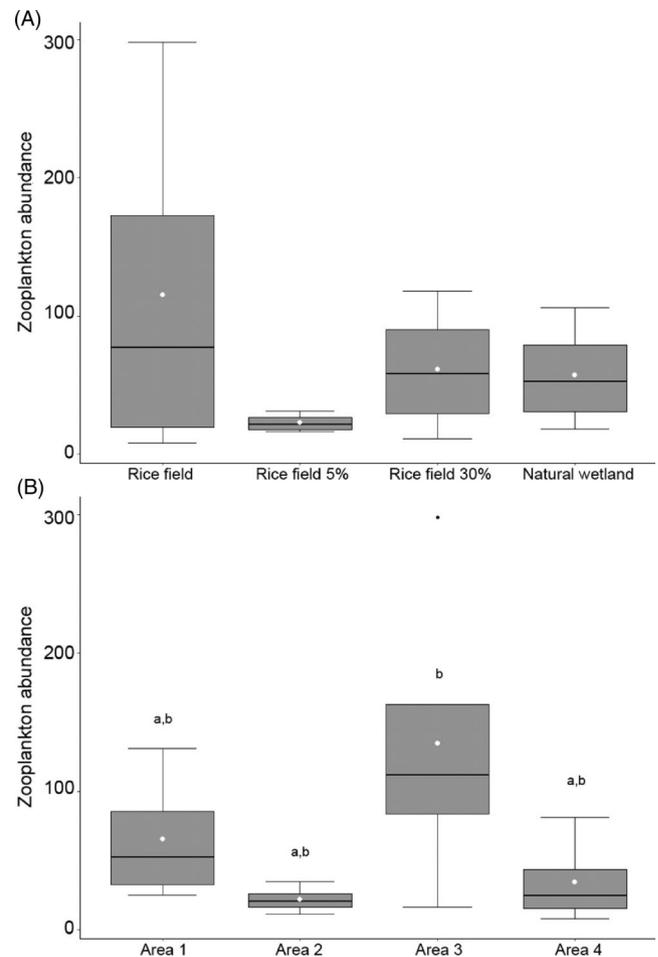


Figure 2. Abundance of zooplankton hatchlings by topsoil class (A) and sampling area id (B). Two dominant cladoceran species were excluded from the analysis (see main text for details). Black dots represent outliers. White dots represent mean values. Different lower case letters mean statistically significant differences.

(Freiry et al. 2020). Although active zooplankton communities are species rich, some studies have showed some degrees of functional redundancy in the species pool (Thompson & Shurin 2012; Benedetti et al. 2019). Extend our experiment by assessing traits related to drought/contamination tolerance and feeding mode might be particularly instructive considering a global expansion of short-term crops area.

It is well established that agricultural activities have substantial effects on zooplankton species (i.e. decreases in species richness and biomass, imbalances in the hatching dynamics; Dulić et al. 2014; Gutierrez et al. 2017). So, changes in species composition are prone to happen between rice fields and natural wetlands. Here, dominant species that emerged from rice fields were thermophilic character (genera *Brachionus*, *Chydorus*, and *Lecane*). The topsoil translocation from donor sites enhanced the emergence of some groups in rice fields, mainly cladocerans and copepods. The most affected species by topsoil addition (*M. elegans* and *M. micrura*) seem particularly sensitive to temperature and dissolved oxygen (Rojas et al. 2001;

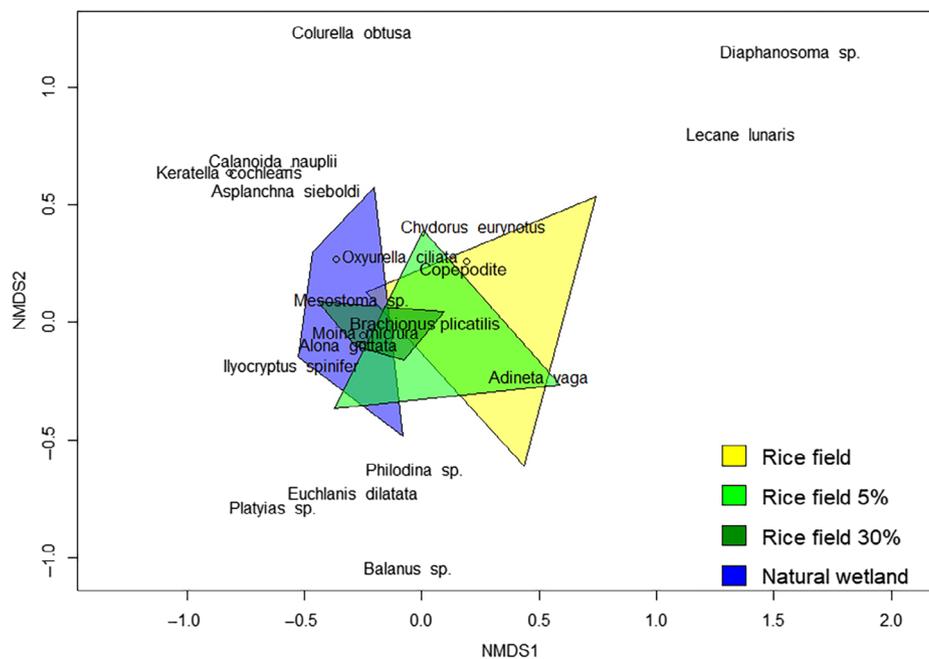


Figure 3. Multidimensional scaling ordination for zooplankton hatchlings from four classes of sediment (stress = 0.196).

Diniz et al. 2013). However, we can only speculate which factors in the rice fields would constraint some cladocerans and copepods. Unlike cladocerans and rotifers, copepods have obligatory sexual reproduction. If their egg banks are low-density, as in our study, maybe the topsoil translocation is not capable to form viable copepod populations in the water column of restored wetlands due to the Allee effect via mate limitation (Kramer et al. 2008). Of course, dispersal from source (wetlands) to sink habitats (rice fields) could affect dormant species bank through mass effects (Silva et al. 2018, 2019). The coastal plain of southern Brazil is characterized by high occurrence of temporary wetlands (Maltchik et al. 2003). Although these wetlands may allow dispersal of dormant stages to agricultural areas (via animal vectors, wind, or water), passive dispersal is pointed as stochastic, constraining re-colonization of areas with frequent local population crashes (Brendonck et al. 2017).

While native wetland loss owing to crop conversion itself is of paramount importance, rice cultivation stressors are often periodic (i.e. flow mistiming, excessive sediment loading, agrochemical use). In addition, stressors off-site in agricultural landscapes are known to affect wetlands far downstream (Marchesan et al. 2007; Rasmussen et al. 2016). Our results were based on sediment samplings located 15–112 km apart that were collected during off season. So, topsoil translocation has the potential to restore communities under different land management practices. Pesticides used in rice fields belong to a wide diversity of chemical classes, having half-lives that range from few hours to months (Fantke et al. 2014; Pervinder et al. 2017; Stenert et al. 2018). Still, cross-resistance to agrochemicals has been reported for some species in the zooplankton communities near from agricultural fields (Bendis & Relyea 2014, 2016; Hébert

et al. 2021). The dominance of *C. eurynotus* emerging from rice sediments was therefore not unexpected, because species in the *Chydorus* genus seems particularly resilient to glyphosate (Hébert et al. 2021), a common herbicide in the rice cultivation. Pesticide tolerant species may have an increase in their biomass, which potentially jeopardize aquatic food webs and have consequences for (re-)colonization of wetlands near agricultural fields or abandoned crops (Bendis & Relyea 2014; Hébert et al. 2021). In a restoration context, ameliorating the stressors impact is the critical first step to ensure proper topsoil translocation effects.

Our results showed that the topsoil addition of 30% in the rice fields made the zooplankton composition resemble the composition of the reference wetlands. However, smaller quantities should be initially tested in the field, since the hatching of organisms would not be restricted to the incubation period and could be influenced by different environmental variables throughout the ecological succession in the restored area. For practical purposes, it is important to highlight that some natural wetlands may not be good donors of topsoil sediment to restore areas subjected to mining activities (Vendramin et al. 2021). In this sense, the quality of topsoil, water and habitat structure and the area of donor wetlands must also be considered when using the sediment translocation methodology for ecological restoration purposes. Besides, the type of matrix surrounding the rice fields, the proximity to other wetlands, and the age, size, and the farming system (conventional, organic) of the rice fields must also be considered in in situ studies. In our experimental study, we verified that the addition of natural wetland sediments in areas disturbed by rice fields modifies the zooplankton composition of the rice fields, aiding the recovery of disturbed natural wetlands

for rice production. These results show that the translocation of sediment from natural wetlands can be a promising methodology to be used in the recovery of rice fields, even used for other wetlands impacted by anthropic pressures, such as mining and pollution. This methodology could help to reduce the impacts of rice fields on natural wetlands and improve the recovery of degraded wetlands.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Richness (A) and abundance (B) of zooplankton hatchlings by sampling area id.

Figure S2. Multidimensional scaling ordination for zooplankton hatchlings by sampling area id (stress = 0.196).