Biodiversity effects of benthic ecosystem engineers on the spatial patterns of sediment CH$_4$ concentration in an urban Neotropical coastal lagoon

Efeitos da biodiversidade de espécies engenheiras bentônicas nos padrões espaciais da concentração de CH$_4$ no sedimento de uma lagoa costeira urbana Neotropical

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Abstract: Aim: Biodiversity of sediment bioturbators has been shown to be important for to the magnitude and stability of benthic-pelagic processes. However, no study to date has evaluated the importance of the biodiversity of benthic invertebrate bioturbators to the spatial patterns of sediment CH$_4$ concentration ([CH$_4$]). Here we conducted a laboratorial experiment to test the following predictions: (1) Bioturbator species richness will reduce the sediment [CH$_4$]; (2) individual bioturbator species (i.e. species composition) will have different effects on sediment [CH$_4$]; (3) and both the effects of bioturbator species richness and composition on sediment [CH$_4$] will be dependent on sediment depth. Methods: We manipulated the number and composition of three functional divergent benthic invertebrate bioturbators species that are widespread in South Atlantic coastal lagoons, in laboratorial sediment chambers containing the sediment and water of an urban impacted coastal lagoon Results: Bioturbator species richness had no overall significant effect on sediment [CH$_4$] when comparisons of sediment [CH$_4$] were made among species richness levels. However, bioturbator species richness significantly reduced sediment [CH$_4$] when species richness levels were compared to the control (defaunated treatments), but this effect was significant only at the deepest sediment layer. Furthermore, bioturbator species composition had significant, but distinct effects on the patterns of reduction in sediment [CH$_4$], depending on the sediment depth and the bioturbator species. Conclusions: We conclude that both the number and composition of bioturbator species are important to determine the effects of benthic bioturbators on spatial patterns of sediment [CH$_4$], but the strength of these effects depend on species traits that determine interspecific interactions strength across the sediment vertical niche space. Keywords: bioturbation, niche partitioning, ecosystem functioning, complementarity effects, methanotrophy, Brazil.
1. Introduction

Scientific evidence accumulated through the last two decades has now supported a general consensus that biodiversity loss has undesirable consequences to the functioning of ecosystems (Cardinale et al., 2012; Hooper et al., 2012; Naem et al., 2012). Unfortunately, efforts to slow the decline of biodiversity have largely failed, and this general degradation of the environment is set to continue for the foreseeable future (Perrings et al., 2011). This scenario is even more apprehensive for inland aquatic ecosystems because the human reliance on freshwater leads to a concentration of human activities on these systems (Sala et al., 2000). Among inland aquatic systems, tropical coastal lagoons may be considered the most threatened because of their high biodiversity and their distributions along densely-populated areas (Esteves et al., 2008).

Contrary to these compelling needs, the literature about the effects of biodiversity on ecosystem functioning has been largely biased to studies manipulating terrestrial vegetation in temperate systems, with studies frequently measuring the effects of biodiversity on acquisition of nutrients and/or on primary productivity (Caliman et al., 2010b). Much less attention has been devoted to evaluate processes driven by non-trophic interactions among aquatic mobile fauna (e.g., ecosystem engineering), but see (Caliman et al., 2013). It is surprising because ecosystem engineering is ubiquitous in nature (Jones et al., 1994) and the effects of biodiversity of ecosystem engineers may be stronger enough to propagate to different trophic levels and beyond habitat boundaries (Allen et al., 2012; Caliman et al., 2012).

Bioturbation, the biological reworking of soils and sediments, has been recognized as an archetypal example of ecosystem engineering, modifying physical habitat properties and resource availability to other species (Mermillod-Blondin and Rosenberg, 2006; Meysman et al., 2006). In aquatic ecosystems, bioturbation by benthic invertebrates is a key process altering microbial community structure and geochemical gradients of sediments and regenerating multiple nutrients across the benthic-pelagic interface (Kristensen et al., 2012). Although numerous studies have evaluated the importance of invertebrate bioturbators to the fluxes of inorganic nutrients (mostly N and P) across the sediment-water interface (Emmerson et al., 2001; Leal et al., 2003; Caliman et al., 2011), only a handful studies have tested the effects of invertebrate bioturbators on CH$_4$ cycling in sediments (see Kajani and Frenzel, 1999; Leal et al., 2007; Figueiredo-Barros et al., 2009) and no study to date has evaluated the effects of bioturbators biodiversity on sediment CH$_4$ concentration (hereafter [CH$_4$]). This neglect is important for some different reasons. First, CH$_4$ is a biogenic greenhouse gas and inland aquatic systems have been demonstrated to be important sources of CH$_4$ to the atmosphere (Bastviken et al., 2004, 2008; Barros et al., 2011). Second, effects of bioturbation by invertebrates may depend on characteristics associated to the species distribution and foraging strategies within the sediment (i.e., species functional-traits), as well as on mechanisms emerging from species interactions, such as niche partitioning (Mermillod-Blondin et al., 2002; Michaud et al., 2005; Caliman et al., 2011). Therefore, bioturbators biodiversity may impact the cycling of CH$_4$ in sediments through diverse facets such as species composition and richness. Understand whether the effects of bioturbator diversity on sediment CH$_4$ dynamics is preferentially mediated by the effects of individual species or by effects of species interactions is, therefore, important to improve our knowledge about the factors that potentially...
regulate the emission of biogenic greenhouse gases from aquatic ecosystems to the atmosphere (Figueiredo-Barros et al., 2009; Stief et al., 2009).

In this study, we used laboratory microcosms to test whether and how the effects of invertebrate bioturbator species richness and composition affect the spatial distribution of \([\text{CH}_4]\) in the sediment of a highly impacted Neotropical coastal lagoon. We manipulated the number and composition of three functionally distinct invertebrate bioturbators species that diverge in their mode of exploring sediment and that are major contributors to the invertebrate biomass of many South Atlantic coastal lagoons. Considering the results of previous studies which have demonstrated that bioturbation by invertebrates generally reduces \([\text{CH}_4]\) in sediments (for example by enhancing \(\text{CH}_4\) oxidation) and that the magnitude of this reduction may vary according to the sediment depth (Kajan and Frenzel, 1999; Leal et al., 2007; Figueiredo-Barros et al., 2009), we predicted that: (1) Bioturbator species richness will enhance the reduction of the sediment \([\text{CH}_4]\); (2) individual bioturbator species (i.e. species composition) will have different effects on sediment \([\text{CH}_4]\); and (3) both the effects of bioturbator species richness and composition on sediment \([\text{CH}_4]\) will be dependent on sediment depth.

2. Methods

Sediment and benthic invertebrates utilized in the experiment were collected near the littoral region (≈10 m distant from the macrophyte beds) of Imboassica lagoon (lat 22° 50’ S, long 44° 42’ W), an eutrophic, shallow, coastal brackish ecosystem located in Rio de Janeiro State, Brazil (Bozelli et al., 2009). This coastal lagoon is separated from the Atlantic Ocean by a narrow sandbar, and there are no tidal influences, as the lagoon is not directly connected to the sea. The total lagoon area is 326 ha, with a maximum volume of 3.56 × 10^6 m^3 and a mean depth of 1.1 m. The lagoon is subject to a wide range of anthropogenic impacts, including the discharge of untreated domestic sewage and occasional artificial breaching of the protective sandbar, reflecting changes in the physical, chemical, and biological features of the lagoon (Caliman et al., 2010a). The sediment at the sampling site is primarily silt and clay, with mean total C, N and P concentrations of 11.28 mg/g, 2.12 mg/g and 0.067 mg/g, respectively (Figueiredo-Barros et al., 2006).

We collected samples from the upper layer of sediment (0-5 cm) with a core sampler (8 cm internal diameter and 50 cm² surface area) modified from Ambühl and Bührer (1975), sieved them through 1-mm mesh, froze the sediment for 2 weeks, and then thawed the sediment to remove all metazoans and their resistant forms as specified in Emmerson et al. (2001). Next, we homogenized the sediment and allowed it to settle (~20-cm thick layer) for 10 days in a 30-L aquarium with a 10-cm deep layer of prefiltred (25-µm mesh) lagoon water to reduce the natural heterogeneity of the sediment and to permit the recovery of its microbial community and biogeochemical depth gradient (Leal et al., 2003; Caliman et al., 2011). During the sediment stabilization period we kept the aquarium darkened and under constant aeration.

We collected individuals of 3 species-larvae (0.5-0.7 cm long, 0.013-0.015 g wet mass) of *Chironomus* sp. Meigen (Diptera: Chironomidae), adults (3-4 cm long, 0.050-0.060 g wet mass) of *Heteromastus similis* Southern (Polychaeta: Cephalopidae), and adults (0.3-0.4 cm long, 0.010-0.012 g wet mass) of *Heleobia australis* D’Orbigny (Gastropoda: Hydrobiidae) – from the field 2 days before the experiment began and conditioned them in species-specific aquaria to allow them to acclimatize to laboratory conditions. The 3 species coexist locally and regionally (Esteves et al., 2008 and references therein) in coastal lakes across southeastern Brazil and are major contributors to the total benthic invertebrate biomass in Imboassica Lagoon (Alves et al., 2010). *Chironomus* sp. is a filter feeder and a tube dweller that oxygenates deep layers of sediment and pumps large amounts of dissolved and particulate material from the sediment to the overlying water (Caliman et al., 2007; Caliman et al., 2012). *Heleobia australis* ploughs the sediment surface and has little effect on vertical sediment geochemistry but can affect interfacial geochemical kinetics (Caliman et al., 2007). *Heteromastus similis* is a head-down subsurface-deposit feeder that builds extensive semipermanent galleries in the sediment throughout which it egests fecal pellets to the sediment surface (Figueiredo-Barros et al., 2009).

After the sediment stabilization period, we established experimental chambers (Plexiglas® tubes, 20 cm long × 5 cm internal diameter) containing a sediment-water interface (10 cm of sediment and 9 cm of overlying water), by gentling introducing the Plexiglas tubes into the stabilized sediment. After that, the overlying water of each chamber was drained and replaced by fresh 0.7-µm-filtered (GF/F Whatman) lagoon water to homogenize the starting conditions across
experimental chambers. Experimental design was constructed manipulating benthic invertebrate species richness and composition (1-3 species in all possible combinations resulting in 7 community treatments) to a constant community wet mass of 300 mg per experimental chamber in a full factorial replacement series design (Emmerson et al., 2001). Individuals of a given species and size were collected from the species-specific aquarium, rinsed to remove the attached sediment, weighed to the nearest 0.1 mg (wet mass after blotting excess water) and immediately distributed into the respective chambers. The biomass of the benthic invertebrates per chamber reflected a value near the mean invertebrate biomass observed for these species in Imboassica Lagoon (Caliman et al., 2007). We employed chambers with no invertebrates as controls. All macrofauna treatments and controls were replicated 4 times for a total of 32 experimental chambers.

Throughout the experiment, each chamber received constant gentle aeration to prevent the depletion or stratification of dissolved O₂. Experimental chambers were visually inspected several times a day and no dead organisms were found throughout the experiment. Experiment last for 48 hours, at a room temperature ranging from 24 to 26° C. At the end of experiment, overlaying water of each experimental chamber was siphoned out and the sediment was gentle sliced at 3 layers (0-2 cm, 2-4 cm and 4-6 cm). To determine sediment [CH₄], 5 ml of each sediment fraction was collected into 12-mL glass vials with 2 ml of NaOH (4%) and immediately sealed with rubber covers. For the determination of water content and sediment porosity, two subsamples of sediment from each layer were collected and weighed in ceramic vessels and weight loss was recorded after heating for 4 days at 60° C according to Dalsgaard et al. (2000). Vials containing sediment samples were stored in the dark at low temperature conditions (< 10° C) until analysis. To analyze [CH₄] in sediment fractions, head-space subsamples (1 mL) taken from the vials containing sediment samples were analyzed for CH₄ through gas chromatography using a Varian Star 3400 chromatograph equipped with a POROPAK-Q column (1 m, 60/100 mesh) at 85 °C, FID detector at 220° C, injection at 120° C, and N₂ was the carrier gas. The samples were injected using a Valvo C6W6 port loop valve (2.5 ml). A Star Chromatography workstation 5.51 (Varian, USA) was used to record data and for peak registration. Sediment [CH₄] (mM) was calculated dividing the values of [CH₄] obtained from gas chromatography analysis by the volume of sediment pore water registered for the respective sediment fraction.

We first used a multivariate analysis of variance (MANOVA) to analyze the individual and interactive effects of sediment depth (fixed within-subject factor) and bioturbator species richness and composition (fixed between-subject factors) on depth distribution of sediment [CH₄] (response variable). Because measures of [CH₄] at each sediment layer were not independent, we used MANOVA to avoid any problems of circularity inherent in repeated measured designs which is commonly used to test for treatment effects on interdependent response variables (Scheiner, 1993). To factor out the effects of species composition from the effects of species richness, species composition was treated as a nested factor within species richness (Schmid et al., 2002). We used Pillai's trace statistic as the test criterion in MANOVA because it is the recommended statistical test for significant effects on interdependent response variables (Scheiner, 1993). We then used univariate ANOVA to determine the effects of factors that were significant in the MANOVA on sediment [CH₄] at each depth. We used Tukey's Honestly Significant Difference (HSD) as a post hoc test to discriminate between different factor levels when univariate ANOVAs were significant. We also tested the significance of the bioturbator species richness on [CH₄] at each sediment layer with separate least squares linear regression analysis, because regression analysis are more powerful than ANOVA to detect statistical significance when the response variable varied monotonically with treatment factor (Cottingham et al., 2005). Prior to statistical analysis, we confirmed the assumptions of homogeneity of variances (for analyses of variance) by comparing the variance between levels of factors (sediment depth, species richness and composition) with a Bartlett test. The homogeneities of residuals (for linear regressions) were accessed by regressing the residual values for [CH₄] at each sediment depth on their respective estimated values.

To infer about the occurrence and magnitude of synergistic species interactions (i.e., the strength of nonadditive effects) on sediment [CH₄], we compared the observed [CH₄] in each sediment layer of experimental chambers containing the 2- and 3-species mixtures with expected [CH₄] calculated from the average among their respective component monocultures (Loreau, 1998).

significant linear relationship between bioturbator species richness and [CH\textsubscript{4}] on each sediment layer confirmed this result (Figure 1a-c). Bioturbator species composition had overall significant effects on sediment [CH\textsubscript{4}] (MANOVA, Table 1). However, nested factorial analysis of variance showed that the effects of species composition on sediment [CH\textsubscript{4}] occurred predominantly among monocultures and were dependent on the sediment depth (nested-ANOVA, Table 2; Figure 1a-c). The effects of species composition were significant at deeper sediment layers (2-4 cm and 4-6 cm), and were determined by the stronger relative reduction of sediment [CH\textsubscript{4}] in response to the bioturbational activities of the species \textit{H. similis} (Figure 1a-c). No significant synergistic effects were observed for the 2- and 3-species mixtures in any sediment layer (Figure 1d-f).

However, we observed different results when the effects of species composition were analyzed by comparing sediment [CH\textsubscript{4}] of individual treatments with their respective controls (defaunated sediment chambers). At the sediment surface (0-2 cm), only the gastropod \textit{H. australis} had no significant effects in [CH\textsubscript{4}] among species monocultures (Figure 1a). On the other hand, among the 2-species mixtures, both treatments containing the species \textit{Chironomus} sp. (Csp.+Ha and Csp.+Hs) showed significant reductions in sediment [CH\textsubscript{4}] compared to the control (Figure 1a). For the two deeper sediment layers, among monocultures, only the treatment containing the species \textit{H. similis} had significant lower sediment [CH\textsubscript{4}] than controls (Figure 1b and c). The results for the 2-species mixtures were

### 3. Results

Bioturbator species richness had no overall effect on sediment [CH\textsubscript{4}] (MANOVA, Table 1). No significant linear relationship between bioturbator species richness and [CH\textsubscript{4}] on each sediment layer confirmed this result (Figure 1a-c). Bioturbator species composition had overall significant effects on sediment [CH\textsubscript{4}] (MANOVA, Table 1). However, nested factorial analysis of variance showed that the effects of species composition on sediment [CH\textsubscript{4}] occurred predominantly among monocultures and were dependent on the sediment depth (nested-ANOVA, Table 2; Figure 1a-c). The effects of species composition were significant at deeper sediment layers (2-4 cm and 4-6 cm), and were determined by the stronger relative reduction of sediment [CH\textsubscript{4}] in response to the bioturbational activities of the species \textit{H. similis} (Figure 1a-c). No significant synergistic effects were observed for the 2- and 3-species mixtures in any sediment layer (Figure 1d-f).

### Table 1. Summary of the multivariate analysis of variance (MANOVA) showing the effects of benthic bioturbator species richness and composition (nested factor) on the concentrations of CH\textsubscript{4} in the bioturbated sediments. The distributions of CH\textsubscript{4} concentrations in each sediment layer were treated as spatially interdependent variables. Bold p-values indicate a statistically significant effect (p < 0.05).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Pillai’s trace</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0.97</td>
<td>3</td>
<td>227.23</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Richness (S)</td>
<td>0.41</td>
<td>6</td>
<td>1.72</td>
<td>0.14</td>
</tr>
<tr>
<td>Composition[S]</td>
<td>1.35</td>
<td>12</td>
<td>4.32</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

### Table 2. Summary of the nested-factorial analysis of variance testing the individual and interactive effects of sediment depth and bioturbator species composition on sediment CH\textsubscript{4} concentration. Bioturbator species composition was nested under species richness. Bold p-values indicate a statistically significant effect (p < 0.05).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>p</th>
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</thead>
<tbody>
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<td>1372.88</td>
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<tr>
<td>Sediment depth (D)</td>
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<td>204.99</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Composition[S]</td>
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<td>0.029</td>
<td>5.66</td>
<td>0.0002</td>
</tr>
<tr>
<td>Composition[S] × D</td>
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<td>0.014</td>
<td>2.79</td>
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<tr>
<td>Error</td>
<td>63</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Effects of bioturbator species richness and composition on sediment [CH$_4$]. Values are the mean ± 95% CI. (a, b, c) Linear regressions were performed by regressing measurements of [CH$_4$] from all bioturbated microcosms at each sediment depth (data not shown, $n = 28$) as functions of bioturbator species richness. The effects of species composition are shown by nested comparisons among the mean values of species monocultures or 2-species mixtures. Closed symbols represent individual monocultures and 2-species mixtures. Open symbols represent the average of sediment [CH$_4$] at each species richness level. Data points with different letters differ significantly within the same species richness level (Tukey test, $p < 0.05$). Grey shaded areas represent ± 95% CI for sediment [CH$_4$] in control chambers. Effects of bioturbation on sediment [CH$_4$] are significant if ± 95% CI do not overlap with grey shade area. (d, e, f) Comparisons between observed and expected values of sediment [CH$_4$] for 2- and 3-species mixtures. Csp = Chironomus sp., Hs = Heteromastus similis, Ha = Heleobia australis.
4. Discussion

Our results showed that bioturbation by benthic invertebrates significantly reduced sediment $[\text{CH}_4]$. Thirteen out of 21 (i.e. 62%) possible comparisons between treatments and their respective defaunated control showed significant reductions in sediment $[\text{CH}_4]$, corroborating the results of previous studies about the effects of invertebrate bioturbators on sediment $[\text{CH}_4]$ (Leal et al., 2007; Figueiredo-Barros et al., 2009). However, we found mixed support to our hypothesis. We observed that bioturbator species richness had no significant overall effect on sediment $[\text{CH}_4]$ when sediment $[\text{CH}_4]$ was tested among and across species richness levels. However, species richness had significant effects on sediment $[\text{CH}_4]$ when treatments were compared to the controls (i.e. bioturbation effect sizes), but only at the deepest sediment layer. Furthermore, accordingly to our predictions, bioturbator species composition had significant effects on sediment $[\text{CH}_4]$ and these effects were also dependent on the sediment depth. Our results highlight three important aspects concerning both the development of biodiversity and ecosystem functioning research and our understanding about the biotic factors that regulate $\text{CH}_4$ dynamics in sediments. First, our results are the first to demonstrate the importance of species functional attributes as a regulating factor of $\text{CH}_4$ dynamics in aquatic sediments. This result reinforces the need to preserve the functional diversity of benthic communities as the guarantee of maintenance of the heterogeneity in sediment biogeochemical processes (Snelgrove et al., 1997; Mermillod-Blondin et al., 2001; Vaughn and Hakenkamp, 2001; Covich et al., 2004; Norling et al., 2007). Second, both the effects of species composition and richness on sediment $[\text{CH}_4]$ were dependent on sediment depth, underscoring the importance to consider the habitat structure on the context-dependency of benthic biodiversity effects on sediment processes (Biles et al., 2003; Bulling et al., 2008; Godbold and Solan, 2009; Caliman et al., 2011). Finally, considering the results of previous studies that highlighted the potential of benthic bioturbators as remobilizers of $\text{CH}_4$ from the sediment to the overlaying water and to the atmosphere (Figueiredo-Barros et al., 2009), the observed effects of benthic bioturbators biodiversity on sediment $[\text{CH}_4]$ reduction may have far reaching cross-habitat impacts on aquatic ecosystem functioning.

Functional diversity, which can be expressed as the sum of the functional attributes of a community,
has been considered an important factor underlying the effects of biodiversity on ecosystem functioning (Scherer-Lorenzen, 2008; Griffin et al., 2009; Cadotte et al., 2011). Functional diversity has been considered to be high among benthic invertebrate bioturbators, because bioturbation of sediments by macrofauna can be determined by several species traits such as body size, feeding mode, distribution within sediment, activity, among others (Pearson, 2001; Reise, 2002; Solan et al., 2004). In addition, invertebrate bioturbators can be functionally plastic, altering for example, their functional attributes such as foraging behavior (i.e. filter feeder to selective feeder) and distribution within sediment in response to environmental change or species interactions (Dangles, 2002; Stief and Holker, 2006; Karlson et al., 2010). The discrepancy in functional traits among benthic invertebrate species has been considered an ad hoc criterion to preserve bioturbator diversity in aquatic ecosystems, given the disparate effects of species belonging to different functional groups may have on benthic-pelagic processes (Levin et al., 2001; Covich et al., 2004). Our results corroborate and expand that view by evidencing for the first time that benthic invertebrate bioturbator species belonging to different functional groups may have distinct effects on sediment [CH₄].

However, interestingly, the differences of species functional effects on sediment [CH₄] were apparently not complementary among species, because we did not observe any consistent synergistic interactions of species mixtures when compared to their respective monoculture. It may indicates that the biogeochemical mechanisms by which bioturbator species are affecting sediment [CH₄], either did not interact or the reduction of individual species biomass in mixtures [a consequence of the substitutive design – sensu (Jolliffe, 2000)] changed the interaction strength among species, so that, their interactions did not deviate from the expected sediment [CH₄] calculated from the average among constituent monocultures. In fact, previous studies have already observed that synergistic effects of biodiversity of bioturbators decreases as species biomasses decreases, a phenomenon that have been related to the reduction of the interaction strength among species (Emmerson and Raffaelli, 2000; Caliman et al., 2007; Caliman et al., 2012). Independent of these hypotheses, our results showed that species differing in their bioturbation mode consistently affected the sediment [CH₄] in quite different ways.

In general, the Polychaeta H. similis was the species that have the strongest effect on sediment [CH₄], because the sediment [CH₄] were consistently lower in all layers of sediment inhabited by this species compared to the controls. On the contrary, the surface deposit-feeder H. australis had no significant effect on the sediment [CH₄] in any sediment fraction. These two species differ largely in the way they explore and are distributed along the sediment profile, which determine their effects on sediment [CH₄]. For example, H. similis is a conveyor-belt deposit feeder which builds deep and irrigated semi-permanent galleries into the sediment, increasing the sediment surface that can be exposed to the downward flux of oxygenated water. The bioturbational activity promoted by H. similis may greatly intensify microbial metanotrophy reducing the sediment [CH₄] (Figueiredo-Barros et al., 2009). On the other hand, H. australis participates only on the surficial sediment diagenetic processes, with low impact on the sediment oxygenation, and consequently low impact on the sediment [CH₄].

A large number of studies have been focused on the investigation of the context-dependency of biodiversity effects, because it can inform us about the generalities of the biodiversity effects on ecosystem functions over different ecological scenarios, but also because such studies may help us to elucidate how the mechanisms underlying the biodiversity effects are altered in their strength and direction in different temporal and/or spatial scales (Cardinale et al., 2000, 2004). Our results, confirmed that biodiversity effects may vary depending on the spatial scale of inference. Both the effects of species richness (measured as the bioturbation effect sizes) and species composition on sediment [CH₄] were determined by sediment depth. Several non-mutually exclusive mechanisms may have been responsible for these patterns. The strength and directions of interspecific interaction may change along the sediment depth as bioturbator species differ in their distribution along the sediment biotope space. A previous study dealing with 3 bioturbator species that are functionally similar to the species used in this experiment demonstrated that synergistic species interactions that enhanced the flux of ammonium from the sediment to the water was only significant when sediment depth was sufficient to accommodate the spatial niche of all species (Caliman et al., 2011). However, interestingly, the fact that the bioturbation effect sizes on sediment [CH₄] were significantly affected.
by species richness only at the deepest sediment fraction is intriguing. The gastropod *H. australis* and the larvae of *Chironomus* sp. explore mainly the surficial fractions of the sediment (i.e. 0-4 cm depth), since these species have low tolerance to low oxygen concentrations. On the other hand, *H. similis* is better adapted to low oxygen and high sulphide concentrations, which are conditions more commonly associated to deeper depths of the sediment where oxygen availability is scarce. Therefore, *H similis* may explore a larger fraction of the total sediment vertical dimension, which indicates that the distributions of the three species overlap in a greater extent at the two surficial sediment fractions (0-2 and 2-4 cm) than at the deepest sediment fraction (4-6 cm). Corollary, we should expect that the effects of biodiversity on the reduction of sediment [CH$_4$] should be stronger at the two surficial sediment depths, a pattern that was not confirmed in our experiment since the significant effects of species richness on sediment [CH$_4$] was observed only at the deepest sediment fraction. A possible explanation of this pattern is that *H. similis* is a weaker competitor at the surficial sediment layers. As the interspecific competition increases with species richness, *H. similis* is outcompeted at surficial sediment layers concentrating their bioturbational activities at the deepest sediment layer, where the environmental conditions are too harsh to allow the presence of *H. australis* and *Chironomus* sp. Because *H. similis* has the strongest effect on the reduction of sediment [CH$_4$] among the three species, the competitive-mediated spatial segregation of *H. similis* to the deepest sediment layer may result in larger reductions of sediment [CH$_4$]. Therefore, the effect of bioturbator species richness on sediment [CH$_4$] may have been mediated by an inverse selection effect (sensu Loreau, 1998), when the species that have the disproportional effect on ecosystem processes is not the dominant competitor. Unfortunately, however, our experiment was not designed to test for specific mechanisms, which means that any attempts to explain the results are speculative. Further experiments may help us to disentangle the possible mechanisms regulating the effects of bioturbator diversity on the spatial patterns of sediment [CH$_4$].

Our results may have important repercussions to the understanding of the CH$_4$ dynamics in aquatic ecosystems with further ramifications to the interpretation of desirability of biodiversity effects for the restoration and conservation of aquatic ecosystems. For example, several interpretations could be made about the consequences of the reduction in sediment [CH$_4$] mediated by the number or composition of bioturbator species. In the context of the debate about inland aquatic systems as source of CH$_4$ to the atmosphere, if we assume that the main mechanism by which bioturbators reduced sediment [CH$_4$] was via active upward transport of CH$_4$ from the sediment pore water to the overlying water, the interpretations about the biodiversity effects may be negative considering that bioturbator diversity and/or species composition, in certain cases, may stimulate CH$_4$ emission to the atmosphere. An alternative interpretation, however, could lead to a positive conclusion about these effects. The reduction of sediment [CH$_4$] may prevent the formation of CH$_4$ bubbles, which can be formed as [CH$_4$] in the sediment exceeds saturation. Since CH$_4$ bubbles tend to pass through water column without being oxidized, bioturbator species diversity and composition may play an important role in decreasing net CH$_4$ emission to the atmosphere from aquatic environments by forcing CH$_4$ sediment diffusion and oxidation throughout the water column. Finally, if bioturbated CH$_4$ is an important energy subsidy to fueling pelagic food webs via metanotrophy (Bastviken et al., 2003; Ravinet et al., 2010), the effects of bioturbators diversity may be viewed as positive for ecosystem functioning.

In summary our results demonstrated that the biodiversity effects of benthic bioturbators on sediment [CH$_4$] were largely dependent on the identity of species, but species interactions were also important, depending on the sediment depth. Inland aquatic systems such as coastal lagoons have been considered one of the most threatened ecosystems worldwide (Esteves et al., 2008). Cultural eutrophication provoked by the disposal of untreated domestic and industrial sewage is the forefront problem in most of these systems. The effects of nutrient enrichment in aquatic system may cause anoxia which may stimulate methanogenesis, inhibit methanotrophy and cause benthic biodiversity loss. Therefore, further studies have to investigate the mechanisms by which bioturbators diversity may impact the CH$_4$ cycling in these systems if we want to generate enough information about how coastal inland aquatic ecosystems will respond to the ongoing anthropogenic impacts in the foreseeable future.
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