Benthic bioturbator enhances CH$_4$ fluxes among aquatic compartments and atmosphere in experimental microcosms

Marcos Paulo Figueiredo-Barros, Adriano Caliman, João J. F. Leal, Reinaldo L. Bozelli, Vinicius F. Farjalla, and Francisco A. Esteves

Abstract: We utilized laboratory microcosms to evaluate the effects of a benthic sediment bioturbator (Heteromastus similis; Polychaeta; conveyor-belt deposit feeder) on vertical distributions of CH$_4$ in sediment and net CH$_4$ fluxes across sediment–water–air interfaces. The effect of $H$. similis on sediment CH$_4$ concentration ([CH$_4$]) varied depending on sediment depth and was strongest at higher animal densities. In comparison with defaunated controls, microcosms with the highest density of $H$. similis exhibited an increase in [CH$_4$] of 3.7-fold, on average, at the sediment surface (0–2 cm), but these concentrations decreased by ~2-fold in deeper sediment layers (2–8 cm). However, irrespective of sediment depth, the density of $H$. similis resulted in an overall nonlinear reduction of bulk sediment [CH$_4$]. Most of the observed CH$_4$ losses from the sediment were due to CH$_4$ oxidation, but the bioturbatory activities of $H$. similis also promoted significant increases in [CH$_4$] in both the water column and the microcosm headspace. These results suggest that benthic invertebrates can mediate CH$_4$ turnover between compartments in aquatic ecosystems, with further consequences for the coupling between benthic–pelagic food chains via the methanotrophic-mediated microbial loop, as well as increase CH$_4$ emissions to the atmosphere.

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Introduction

Inland aquatic ecosystems such as wetlands, rice fields, and shallow lakes are major sources of the CH$_4$ that is released into the atmosphere (Bartlett and Harriss 1993; Whit- ing and Chanton 1993; Bastviken et al. 2004). The importance of aquatic communities in CH$_4$ emissions from aquatic ecosystems to the atmosphere has been reinforced by recent studies, but with a focus on aquatic macrophytes as the primary mediators (Joabsson et al. 1999). A few studies have recently demonstrated that the productivity of benthic invertebrates may rely strongly on foraging for methanotrophic bacteria (Eller et al. 2005). However, the effects of benthic invertebrates on CH$_4$ cycling through nontrophic, indirect mechanisms (e.g., bioturbation) still remain poorly understood (but see Kajan and Frenzel 1999; Leal et al. 2007).

Bioturbation is a biogenic process that has been considered an “archetype” of ecosystem bioengineering because it alters geophysical gradients and redistributes resources and organisms within sediment layers, as well as between the sediment and other benthic and pelagic compartments in aquatic ecosystems (Meyssman et al. 2006). Bioturbation is an important process that alters sediment characteristics, and the production, oxidation (i.e., methanotrophy), and flux of CH$_4$ through the sediment–water interface may be strongly affected by invertebrate sediment reworking (Kajan and Frenzel 1999; Leal et al. 2007). Such changes in the sediment enhance benthic–pelagic coupling with great implications for ecosystem functioning, particularly in shallow aquatic ecosystems where the sediment is relatively important for sustaining pelagic communities and ecosystem-level processes (Schindler and Scheuerell 2002). For instance, recent studies have demonstrated that CH$_4$ released from sediment into the water column has the potential to sustain a considerable fraction of energy flux through planktonic food chains via carbon incorporation by methanotrophic bacteria (Bastviken et al. 2003). Moreover, the CH$_4$ released from the sediment into the water column may not be completely oxidized, and the extent amount may then diffuse into the atmosphere. However, no study to date has investigated whether the effects of bioturbation mediated by benthic invertebrates on CH$_4$ flux across the sediment–water interface translates into a significant increase in CH$_4$ emission into the atmosphere.

Furthermore, only a handful of studies has evaluated the effects of benthic infauna on CH$_4$ flux through the sediment–water interface, both focusing on filter-feeder tube dwellers (Kajan and Frenzel 1999; Leal et al. 2007). However, benthic invertebrate species may differ with respect to their distribution and foraging strategies (i.e., different functional groups), which may lead to different sediment–water interfacial processes rates such as material and nutrient advection, as well as microbial activity (Heilskov and Holmer 2001; Mermilолод-Blondin et al. 2001; Caliman et al. 2007). Thus, bioturbator invertebrates that belong to diverse functional groups must have different effects on CH$_4$ cycling across the sediment–water interface. Moreover, the density of invertebrates may also be an important factor because density-dependent effects may modify benthic–pelagic processes in very complex ways, depending on the effects of density on the foraging behavior and (or) intraspecific interactions of animals (Mermilолод-Blondin et al. 2002; Marinelli and Williams 2003).

The aim of this study was to evaluate whether the presence and population density of Heteromastus similis (Polychaeta: Capitellidae), which is a conveyor-belt deposit feeder, alters the concentration of CH$_4$ (hereafter [CH$_4$]) in the sediment layer and if such changes affect the pelagic compartment and atmosphere. A laboratory experiment was designed to determine if the sediment bioturbation promoted by H. similis will result in the following: (i) altered sediment [CH$_4$]; (ii) increased [CH$_4$] in the overlying water and atmosphere; (iii) increased net CH$_4$ oxidation rates; and (iv) a dependence of these processes on the density of H. similis.

Material and methods

Study site and species characteristics

Individuals of H. similis, as well as sediment and water utilized in this experiment, were sampled from the central area (~0.9 m depth) of Imboassica Lagoon (latitude 22°50'S, longitude 44°42'W), which is a tropical, shallow, coastal freshwater ecosystem located in the state of Rio de Janeiro, Brazil. 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 $\times$ 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 (Figueiredo-Barros et al. 2006; Santangelo et al. 2007).

Regarding the benthic community, the main species and groups present are Hydroobiidae gastropods (Figueiredo-Barros et al. 2006), chironomid midges, and Polychaeta (Calisto et al. 1998). Among the Polychaeta, H. similis is the most abundant species, with densities ranging from 132.6 to 9481.0 individuals m$^{-2}$. Heteromastus similis is a conveyor-belt subsurface-deposit feeder that builds extensive semipermanent galleries in the sediment (Rao 1980). This species modifies the distribution of sediment organic matter and intensifies benthic–pelagic coupling by sediment advection and upward egestion of fecal pellets (Caliman et al. 2007).

Sediment and organism sampling and pre-incubation procedures

Sediment and individuals were sampled with a “corer” (8 cm internal diameter, 50 cm$^2$ surface area), modified from Ambühl and Bührer (1975). Water was collected with polyethylene bottles at the subsurface. In the laboratory, the sediment was washed with lagoon water on a sieve (0.5 mm mesh size) to select H. similis individuals and exclude other benthic infauna from the sediment. Individuals of H. similis were maintained for 10 days in an aerated aquarium that contained sediment and water from the sampling site, which allowed the animals to acclimatize to laboratory conditions. Concurrently, defaunated sediment was homogenized and allowed to settle (~20 cm thick layer) for 10 days in a 30 L aquarium with a layer of prefiltered (GF/C Whatman,
1.2 μm pore size) lagoon water with a depth of 10 cm. This procedure reduces the natural heterogeneity of the sediment and permits recovery of the microbial community and sediment biogeochemical depth gradient (Svensson 1998; Leal et al. 2007). After the stabilization period, 20 microcosms were established by introducing Plexiglas tubes (20 cm long × 4 cm internal diameter) into the stabilized sediment to a depth of 10 cm and maintaining overlaying water 9 cm deep. The microcosms were equipped with a rotating magnetic stirrer connected to the microcosm internal wall at 5 cm above the sediment surface. Microcosms were kept open at the top and placed around a rotating magnetic system according to the methods of Leal et al. (2007), and the whole system was submerged into a tank filled with filtered lagoon water (GF/C Whatman, 1.2 μm pore size), which was maintained under constant aeration for 12 h (Fig. 1a). This procedure was employed to homogenize starting abiotic conditions across all experimental microcosms.

**Experimental design and incubations**

After homogenization of the microcosms, five 8 mL water samples were collected from the tank to determine the initial [CH₄] in the water. Four microcosms were randomly chosen for determination of initial [CH₄] in sediment pore water across different sediment depths (0–2, 2–4, 4–6, and 6–8 cm). Three levels of *H. similis* density (4, 6, and 8 individuals per microcosm) and a defaunated control were replicated four times across the remaining 16 microcosms. The experimental range of *H. similis* density was comparable with the range of natural density observed for this species in Imboassica Lagoon (3175–6349 individuals·m⁻²; M.P. Figueiredo-Barros, unpublished data). After addition of the animals, 20 mL of water was sampled from each microcosm to establish an internal headspace (atmosphere) ~2 cm high. The atmospheric [CH₄] in the experimental room was considered to correspond to initial [CH₄] in the atmosphere of the microcosms. The microcosms were then tightly sealed with silicon stoppers and incubated for 12 h (Fig. 1b). This incubation period was determined after a pilot experiment, where oxygen saturation in microcosms with sediment and eight animals were observed do not deplete less than 50% of the initial oxygen saturation. Hence, neither infaunal activity nor microbial metanotrophy was oxygen-limited in the experiment.

**CH₄ samplings and analyses**

At the end of the experiment, headspace air samples were taken with 1 mL syringes from each microcosm and immediately analyzed (see below). Water samples (8 mL) were collected from the overlaying water of the microcosms and injected into 12 mL glass capped vials at negative pressure to a final concentration of 20% NaCl by volume. To determine [CH₄] in sediment pore water, 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, subsamples of fresh sediment were 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 both water and sediment samples were stored in the dark at low temperature conditions (<10 °C) until analysis.

Syringes containing atmosphere samples and headspace subsamples (1 mL) taken from the vials containing sediment and water 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
Table 1. Results of bifactorial repeated-measures analysis of variance (RM-ANOVA) for individual and interactive effects of invertebrate density and sediment depth on sediment [CH$_4$].

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
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<tr>
<td>Density</td>
<td>3</td>
<td>2369.41</td>
<td>35.03</td>
<td>&lt;0.0001</td>
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<td>Error</td>
<td>12</td>
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<td>Within subject factors (Huynh–Feldt adjusted)</td>
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<tr>
<td>Sediment depth</td>
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<td>5881.79</td>
<td>56.50</td>
<td>&lt;0.0001</td>
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<tr>
<td>Density × sediment depth</td>
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<td>827.82</td>
<td>7.95</td>
<td>&lt;0.0001</td>
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<td>Error</td>
<td>36</td>
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We used bifactorial repeated-measures analysis of variance (RM-ANOVA) to test the individual and interactive effects of *H. similis* density (between-subject factor) and sediment depth (within-subject factor) on sediment [CH$_4$]. The data were log$_{10}$-transformed to improve variance homogeneity and adjusted (for the within-subject factor) by the Huynh–Feldt method to meet the assumption of circularity. Tukey’s pairwise comparisons were performed to test if differences in sediment [CH$_4$] were among density levels or between the control and any density level (presence effect). Separate unifactorial analyses of variance (ANOVA) followed by Tukey’s pairwise comparisons were used to test the effects of *H. similis* density on [CH$_4$] of the overlaying water and atmosphere. A significance level of $\alpha = 0.05$ was kept for all statistical analysis, using the software STATISTICA (version 7.0; StatSoft Inc., Tulsa, Oklahoma).

**Results**

Both the density of *H. similis* and sediment depth had significant individual and interactive effects on sediment [CH$_4$] (RM-ANOVA; Fig. 2; Table 1). For all treatments, sediment [CH$_4$] was a unimodal function of sediment depth, with the highest sediment [CH$_4$] observed at a depth of 4–6 cm. *Heteromastus similis* density caused an overall nonlinear reduction in sediment [CH$_4$], which was more pronounced (2.1× lower than control) in the highest density treatment (Fig. 3c; Tukey’s post hoc test $P < 0.05$). Despite this significant overall negative effect of *H. similis* density on sediment [CH$_4$], the strength and direction of the effect of *H. similis* on sediment [CH$_4$] was also dependent on sediment depth, as indicated by the significant interactive effects (RM-ANOVA; Table 1). In comparison with controls, the bioturbational activities promoted by *H. similis* increased [CH$_4$] in the sediment surface (0–2 cm) on average 3.7 times but decreased [CH$_4$] over all deeper sediment layers (2–8 cm) on average 2.5 times (Fig. 2). Additionally, Tukey’s pairwise comparisons demonstrated that with respect to sediment depth, the effect of *H. similis* density on sediment [CH$_4$] was consistent in the 4–6 cm layer, as both treatments with six and eight individuals per microcosm were significantly different from the treatment with only four individuals and the control (Fig. 2; Tukey’s post hoc test $P < 0.05$). However, the presence of *H. similis* affected sediment [CH$_4$] at all sediment depths, which can be assessed by the significant differences between at least one *H. similis* treatment and controls.
controls (Fig. 2; Tukey’s post hoc test P < 0.05). The strong effects of *H. similis* density on sediment [CH$_4$] at the sediment depth of 4–6 cm mirrored the effect observed in the bulk sediment [CH$_4$] (Fig. 3c), which suggests that complex interactions between density-dependent bioturbational behavior and depth-dependent microbial activities can substantially drive the overall effect of *H. similis* on bulk sediment [CH$_4$].

As observed for the sediment, *H. similis* density had a strong effect on both water (one-way ANOVA; $F = 325.8$, $P < 0.0001$) and air (one-way ANOVA; $F = 72.75$, $P < 0.0001$) [CH$_4$] (Figs. 3a–3b). The density of *H. similis* also had an increasingly positive effect on the [CH$_4$] for both overlaying water and air (Tukey’s post hoc, $P < 0.05$). On average, [CH$_4$] for water and air were 150 times and 106 times higher, respectively, in the highest density treatment compared with controls.

The density of *H. similis* also had positive significant effects on net CH$_4$ oxidation rates. However, such effects exhibited a saturating response as density increased (Fig. 4). Confidence intervals were significantly different only for pairwise comparisons performed against the lowest density treatment.

**Discussion**

These results demonstrate that bioturbation promoted by *H. similis* affected the processes related to CH$_4$ turnover in the sediment and between sediment–water–atmosphere interfaces. The presence of *H. similis* significantly altered the profiles of [CH$_4$] in the sediment (supporting hypothesis i), enhanced CH$_4$ flux from the sediment to water column and atmosphere (supporting hypothesis ii), and increased the net CH$_4$ oxidation rates (supporting hypothesis iii). Moreover, macroinvertebrate density also had significant effects on these processes (supporting hypothesis iv).

**Effects of bioturbation on spatial dynamics of sediment [CH$_4$]**

*Heteromastus similis* bioturbation had contrasting effects...
on vertical sediment [CH4], increasing [CH4] at the sediment surface (0–2 cm), but promoting overall reductions in [CH4] at the three deeper sediment layers. Such polarization of sediment [CH4] may be attributed to a set of non-mutually exclusive mechanisms mediated by the functional mode by of H. similis foraging within the sediment. First, bioadvection of surface particles (i.e., sediment mixing as a result of particle ingestion within the sediment and egestion at surface) might have decreased oxygen availability at the sediment boundary layer, limiting local CH4 oxidation and (or) favoring methanogenesis at this sediment portion. Second, bioirrigation processes resulting from H. similis might have translocated CH4-rich interstitial water from deeper sediment layers to the sediment surface and promoted CH4 oxidation within deeper sediment layers (Fig. 5). Third, it has been recently shown that anaerobic microbial activity in the gut of benthic invertebrates may account for significant releases of nitrous oxide via fecal deposition by animals (Stief et al. 2009). Release, via fecal pellet deposition, of CH4 produced in the gut of invertebrates might also have been important for the increase in [CH4] at the sediment surface. Although, the deposition rates of fecal pellets was not monitored in our work, similar studies conducted with conveyor-belt deposit feeders have exhibited significant deposition of fecal pellets after relatively short-term incubation periods (Van de Bund et al. 1994; Mermillod-Blondin et al. 2005). In addition, a recent study conducted with H. filiformes has also demonstrated that under nonflowing water conditions, oxygen consumption rates were 10 times higher within freshly egested fecal pellets than in the bulk surrounding sediment (Wild et al. 2005). Other studies have also reported significant increases in the abundance of active sediment bacteria associated with fecal pellets of tubificid worms, which generally have higher organic content than the surrounding sediment (Van de Bund et al. 1994; Mermillod-Blondin et al. 2002). In fact, a greater amount of fecal mounds at the sediment surface of microcosms containing individuals of H. similis was observed at the end of incubation time, and the amount of fecal mounds was proportional to animal density. Therefore, the deposition of fecal pellets by H. similis might have intensified surficial diagenetic processes and promoted the formation of spatially heterogeneous microsites at the sediment surface characterized by anoxic conditions that favor methanogenesis and (or) limit CH4 oxidation, thereby increasing [CH4] at the sediment surface. A less obvious mechanism that might have contributed to the increase of [CH4] at the sediment surface is that fecal pellet deposition on surficial sediments may have decreased the rates of diffusive processes across the sediment–water interface (Van de Bund et al. 1994). Such a geochemical barrier might have retained part of the CH4 transported upward by H. similis bioturbation.

In comparison, the observed decrease of [CH4] at the 2–8 cm sediment fractions in the presence of H. similis may be attributed to the stimulation of methanotrophy, as well as an upward transport of CH4 to upper sediment layers. Bioturbatory activities of benthic invertebrates that build tubes or galleries are known to increase oxygen diffusion into the sediment (Svensson 1997; Leal et al. 2003), which affects metabolic processes due to alteration of the sediment redox potential (Kristensen 2000). Eller et al. (2005) reported that

Fig. 5. Conceptual diagram illustrating the postulated causal connection between bioturbation by Heteromastus similis and spatial vertical dynamics of CH4 cycling within the microcosms. 

Chironomus plumosus might lead to a threefold increase of the sediment–water interface area. Furthermore, tubes built by invertebrate bioturbators have been proposed as an important microsite for CH4 oxidation within the sediment (Kajan and Frenzel 1999). The increase in the sediment–water interface area in treatments with invertebrates may explain the density-dependent reduction of [CH4] observed in deeper sediment layers (2–8 cm) in the presence of H. similis.

In accordance with recent studies regarding the nonconsumptive effects of benthic fauna on nutrient fluxes (Mermillod-Blondin et al. 2004; Michaud et al. 2006; Caliman et al. 2007), our results suggest that benthic invertebrate species from different functional groups can also differentially affect [CH4] within sediment when compared with another study that evaluated the effects of invertebrate bioturbation on sediment [CH4]. In a study of mayfly nymphs (Campsurus notatus), Leal et al. (2007) found that invertebrate bioturbation decreased [CH4] in all sediment fractions. Such differences may be attributed to functional differences between H. similis and C. notatus. Campsaurus notatus builds U- or J-shaped burrows within the sediment, which promotes a strong flux of water. However, this species does not deposit fecal pellets in the sediment surface like H. similis. Further studies should investigate how benthic bioturbator identity and diversity can affect the outcome of CH4 turnover in the sediment of lake ecosystems, as benthic invertebrate species may differ in their impact upon sediment biogeochemical processes (Emmerson et al. 2001).
Effects of bioturbation on CH₄ flux through sediment–water–atmosphere interfaces

The observed effect of *H. similis* bioturbation on the spatial distribution of sediment [CH₄] was sufficient to cross sediment–water and water–atmosphere interfaces, as significant increases of [CH₄] in the overlaying water and headspace of microcosms inhabited by *H. similis* were observed. These results suggest that benthic invertebrates may affect CH₄ emissions from aquatic systems, and to our knowledge, this is the first study to experimentally demonstrate such an effect. However, the observed absolute [CH₄] in the water and atmosphere of microcosms was strikingly low in relation to the net reduction of bulk sediment [CH₄], as observed in the respective density treatments. Such a result shows that the impact of bioturbation promoted by *H. similis* was much greater on CH₄ oxidation than on CH₄ release to the water and headspace. The density of *H. similis* intensified net CH₄ oxidation. However, the main pathways of the net CH₄ oxidation could not be identified as CH₄ oxidation rates were not monitored at local scales (within sediment or in overlaying water). A great number of studies have demonstrated the importance of pelagic methanotrophy in the reduction of CH₄ emissions from water to the atmosphere (Utsumi et al. 1998; Sundh et al. 2005; Bastviken et al. 2008), but there is evidence that methanotrophy by sediment bacteria, mainly at the sediment–water interface, may greatly reduce the CH₄ flux from sediment to the water (Kajan and Frenzel 1999; Murase and Frenzel 2007). Both pathways of CH₄ oxidation were likely to have occurred in our experiment. As previously discussed, sediment biodvection promoted by *H. similis* via surficial deposition of fecal pellets may translocate accumulated CH₄ in the sediment that is available for methanotrophy to the water column, while stimulating metanotrophy by sediment bacteria through oxygenating sediments via bioirrigation (Fig. 5). An additional complexity relies on the coupling of methanogenesis and methanotrophy, which greatly complicates our ability to derive conclusive pathways by which sediment reworking may affect CH₄ dynamics across aquatic compartments. Further studies regarding the effects of benthic infauna on CH₄ flux through benthic–pelagic compartments should investigate the mechanisms behind the spatial coupling between the origin and fate of CH₄ in aquatic systems.

Consequences of bioturbation to CH₄ cycling in aquatic systems and emissions from atmosphere

Our results demonstrated that bioturbatory activities by benthic infauna play an important role to the sediment CH₄ turnover and flux across sediment–water–atmosphere interfaces. These results suggest that bioturbation may be an important factor contributing to the coupling of carbon cycling between aquatic compartments, which has been demonstrated with nutrients such as N and P (Emmerson et al. 2001; Lohrer et al. 2004; Meysman et al. 2006). Recent studies have indicated a high reliance of secondary production of lake pelagic communities upon carbon and energy derived from CH₄ via trophic links among methanotrophic bacteria and higher trophic levels such as protozoa and zooplankton (Bastviken et al. 2003; Sundh et al. 2005; Taipale et al. 2008). For example, Taipale et al. (2007) have estimated that methanotrophic bacteria contributed 64%–87% of carbon incorporation by zooplankton during autumn in Mekkojavä Lake. Therefore, mechanisms that enhance CH₄ transport from benthic to pelagic compartments, such as bioturbation, may indirectly affect carbon availability for pelagic food webs.

Regarding the effects of bioturbation on CH₄ emissions from aquatic systems to the atmosphere, this study demonstrated that sediment bioturbator organisms might contribute to aquatic systems as a source of greenhouse gases in the atmosphere. Considering the estimation of CH₄ emissions observed in the microcosms of intermediate invertebrate density (four individuals), bioturbation by *H. similis* might indirectly mediate the efflux of approximately 7.6 tonnes of CH₄ per year in Imboassica Lagoon. However, we are aware that important caveats associated with our experiment regarding the observed positive effects of *H. similis* on CH₄ emission should be considered. First, *H. similis* may prevent the formation of CH₄ bubbles by reducing CH₄ concentration in the sediment, as CH₄ bubbles can form when CH₄ concentration in the sediment exceeds saturation. According to Nielsen et al. (2004), the maximum predicted [CH₄] in pore water is 900 μmol·L⁻¹ at 29 °C and 1 atm, which increases with increasing pressure (higher depths) and decreasing temperatures. In our experiment, the [CH₄] observed in the sediment of microcosms was significantly lower than saturation values, which hinders bubble formation. Because CH₄ bubbles tend to pass through the water column without being oxidized, bioturbation by benthic invertebrates may play an important role in decreasing net CH₄ emission to the atmosphere from aquatic environments by forcing CH₄ sediment diffusion and oxidation throughout the water column. Second, CH₄ emissions may have been underestimated in our experiment due to the short water column of our microcosms, which decreases the probability of pelagic CH₄ oxidation. However, we believe that such an experimental artifact does not invalidate our results, as littoral regions of lakes and small ponds, which generally act as sources of CH₄ in the atmosphere (Bastviken et al. 2004; Bussmann 2005; Parsons et al. 2006), may exhibit water depths comparable with those of our experimental microcosms. Third, the generality of our results are constrained by the short-term experiment duration, and therefore, we are unable to assert to what extent the experimental results represent a transitional effect following colonization of sediment by benthic infauna or a more stable long-term effect of bioturbation. However, many studies have shown that the effects of invertebrate bioturbators on sediment reworking and materials and solute transfers across the sediment–water interface are consistent over temporal scales ranging from days to months (Emmerson et al. 2001; Marinelli and Williams 2003; Lohrer et al. 2004), which reinforce the validity of our results. However, to obtain a more precise knowledge about the effects of benthic infauna on CH₄ dynamics across sediment–water interfaces, further experiments should be conducted over larger temporal scales.

This study suggests that benthic invertebrate bioturbators may indirectly mediate CH₄ turnover among aquatic compartments and the atmosphere. Considering the effects of *H. similis* on CH₄ oxidation, these results demonstrate that bioturbatory activities promoted by invertebrate organisms may also indirectly subsidize carbon transformation and energy.
availability through enhancing methanotrophy, in addition to enhancing nutrient availability to pelagic production. These findings may be particularly important in understanding CH₄ turnover in shallow aquatic systems, where benthic-derived processes may be important drivers of ecosystem functioning.

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