Assessment of CH$_4$ oxidation in macroinvertebrate burrows of tidal flats

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Abstract

In tidal flats that lack plants, methane (CH$_4$) fluxes are both positive (gas emission) and negative (gas "sinking") in nature. The levels of methanotroph populations significantly affect the extent of CH$_4$ sinking. This preliminary study examined CH$_4$ flux in tidal flats using a circular closed-chamber method to understand the effects of macroinvertebrate burrowing activity. The chamber was deployed over decapods (mud shrimp, Laomedia astacina and crab, Macrophthalmus japonicus) burrows for ~ 2 h, and the CH$_4$ and CO$_2$ concentrations were continuously monitored using a closed, diffuse CH$_4$/CO$_2$ flux meter. We found that Laomedia astacina burrow (which is relatively long) site afforded higher-level CH$_4$ production, likely due to diffusive emission of CH$_4$ in deep-layer sediments. In addition, the large methanotrophic bacteria population found in the burrow wall sediments has CH$_4$ oxidation (consumption) potential. Especially, nitrite-driven anaerobic oxidation of methane (AOM) may occur within burrows. The proposed CH$_4$-oxidation process was supported by the decrease in the $\delta^{13}$C of headspace CO$_2$ during the chamber experiment. Therefore, macroinvertebrate burrows appear to be an important ecosystem environment for controlling atmospheric CH$_4$ over tidal flats.

Key words: Methane oxidation, Stable carbon isotopes, Bioturbation, Tidal flat

1. Introduction

Macroinvertebrates can affect sediment chemistry via bioturbation (e.g., burrowing activity, tube--building, ingestion, defecation, respiration, and mucus secretion), which affects material cycles (e.g., oxygen, nutrients, and metals) and the purification (i.e., organic carbon decomposition) of tidal flats (Hughes et al., 2000; Otani et al., 2010). This burrowing activity enhances chemical mass transfer at the burrow walls due to oxidation, and consequently affects mineral phases in the sediments and the activity and community structure of...
2. Materials and Methods

2.1 CH4 and CO2 measurements using the closed-chamber method

The closed chamber (Fig. 1b) was made of transparent acrylic with an internal volume of 12.968 \times 10^{-3} m^3 and basal area of 5.64 \times 10^{-2} m^2. It was placed on the surface sediments with sufficient firmness to isolate the internal volume of the chamber from the outside. A fan was installed inside the top wall to enhance gas mixing when the chamber was closed. The CH4 and CO2 fluxes were measured using a closed, diffuse CH4/CO2 flux meter (West Systems, Pisa, Italy) (e.g., Fridriksson et al., 2016; Schroder et al., 2016). Gas was continuously extracted from the chamber, sent to the flux meter, and then reinjected into the chamber (i.e., circular chamber technique; Chiodini et al., 1998).

Three measurements were conducted over ~ 2 h (once each at L. astacina and M. japonicus burrows: L. astacina burrows are large [up to 150 cm in depth and 18,160 mL in volume] and M. japonicus burrows are small [up to 20 cm in depth and 440 mL in volume] and once at a non-burrow reference site), and the gas concentrations of the headspace were recorded every second.

2.2 Gas sampling and $\delta^{13}$C analysis

Another chamber (Fig. 1c) was designed for collecting gas into a 10-mL Vacutainer through a vent that was opened and closed by an automatic switch. The vent between the chamber headspace and vacutainer was opened for 10 min to allow equilibrium to be attained. During one measurement cycle at the L. astacina burrow, four samples were obtained to determine the $\delta^{13}$C of CH4 and CO2.

The carbon isotope ratios of CH4 and CO2 were measured using a trace gas–isotope ratio mass spectrometer (TG–IR/MS) system (Isoprime 100, UK). Gas (250 μL) was directly introduced via the injection port of the instrument using a gas–tight syringe. The CH4 and CO2 mixture was then cryofocused twice using liquid N2. Subsequently, the purified gas was introduced into the TG–IR/MS, where the CH4 and CO2 components were separated chromatographically.

Isotope ratios are presented as $\delta$ values (‰) expressed relative to the Vienna PeeDee Belemnite (VPDB) carbon standard, and were calculated as follows: $\delta X = ((R_{sample} - R_{std})/R_{std}) \times 1000$ (‰), where $X = ^{13}$C and $R = ^{13}$C/$^{12}$C. The reference materials were IAEA–CH6 ($\delta^{13}$C = $-10.45 \pm 0.2‰$) and IAEA–CH3 ($\delta^{13}$C = $-24.72 \pm 0.1‰$). Analytical precision was within 0.2‰ for carbon. In addition, standard CH4 (UN1956, $-69$ and $-45‰$: Air Liquid, USA)
and CO₂ (UN1956, –39, –25, and 0‰; Air Liquid, USA) gases were analyzed to normalize the δ¹³C values; the required normalizations were < 0.1‰.

2.3 Sediment sampling in burrow walls

A hexagonal closed observatory (60 cm in length × 100 cm in height) made of transparent acrylic was buried in sediment to allow direct observation of an L. astacina burrow. The space within the observatory became filled with seawater during rising tides. Sediment sampling of the burrow wall was conducted with aid of pre-drilled silicone-filled holes in the acrylic walls, allowing direct measurements of burrow sediment after the seawater had been pumped out. Sediment samples from the wall of a deep L. astacina burrow were obtained at depths of 15, 20, and 25 cm. Surface sediment samples of L. astacina burrow walls, and samples at 10-cm depths of M. japonicus burrow walls, were collected directly. All sediment samples were stored in polyethylene (PE) vials and frozen prior to bacterial analysis in the laboratory.

2.4 Analysis of methanogenic and methanotrophic bacteria in sediments

Methanogenic and/or methanotrophic microorganisms were identified by amplicon analysis. Total genomic DNA was extracted using a PowerMax® Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) from 0.25 g (wet weight) of each sediment sample, according to the manufacturer’s instructions. DNA extracts were purified using a PowerClean® DNA cleanup kit (MoBio Laboratories) and DNA aliquots stored at ~20°C in 1× TE buffer prior to analysis. 16S rRNA genes were amplified...
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using the universal primer pair 341F and 805R (Takahashi et al., 2014), followed by a second round of PCR to introduce tags. Amplicons were sequenced on an Illumina Miseq GA platform and BLAST-identified by Chunlab Inc. The sequences were then analyzed with the aid of the CLCommunity browser (ver. 4.3). Initially, 100,000 reads/sample were randomly subsampled to search for known methanotrophs and methanogens, and their proportions calculated.

3. Results and Discussion

3.1 Macroinvertebrate burrowing activity and CH₄ emission

For each experimental site, the CH₄ concentration in the chamber headspace was plotted against the measurement time (at 1-min intervals), revealing wave–like shapes that were more pronounced at the burrow sites than the reference site (Fig. 2a). The CH₄ concentration (max. 2.5 ppm) was higher at the L. astacina burrow than at the M. japonicus burrow (max. 2.2 ppm) and reference (max. 2.1 ppm) sites. The duration of maximum values may reflect an equilibrium state at each site, and this circumstance could inhibit CH₄ emission. Therefore, the wave–like shapes seem to be associated with variation in CH₄ emission rates during the chamber experiments. In comparison, CO₂ decreased gradually at the M. japonicus and reference sites over the duration of the experiment, which was likely attributable to primary production (Migné et al., 2002). However, at the L. astacina burrow site, the CO₂ concentration increased somewhat but then gradually decreased after only ~1.1 h (Fig. 2b). Generally, the CO₂ variations supported the idea that the CH₄ headspace concentration was not influenced by outside air: the chambers were efficiently sealed.

Table 1. Proportions of methanogens and methanotrophs in burrow wall sediments and the surface sediment of the reference site, together with the proportions of Candidatus methylomirabilis group bacteria among the methanotrophs (in parentheses)

<table>
<thead>
<tr>
<th>Sites</th>
<th>Depth (cm)</th>
<th>Methanogens (%)</th>
<th>Methanotrophs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference surface</td>
<td>0.009</td>
<td>0.36 (15.3)</td>
<td></td>
</tr>
<tr>
<td>M. japonicus burrow</td>
<td>10</td>
<td>0.013</td>
<td>1.18 (71.4)</td>
</tr>
<tr>
<td>L. astacina burrow</td>
<td>surface</td>
<td>0.009</td>
<td>0.64 (3.6)</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.102</td>
<td>1.26 (24.1)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.088</td>
<td>0.51 (31.7)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.116</td>
<td>1.26 (73.6)</td>
</tr>
</tbody>
</table>

Fig. 2. Variations in CH₄ and CO₂ concentrations with time in the chamber headspace at the ① reference site, and ② Macrophthalmus japonicus and ③ Laomedia astacina burrows: and the CH₄ emission rates (by the equation, slope (ppm hr⁻¹) × (chamber volume m³/surface area m²), with correction for the barometric pressure and air temperature) calculated by reference to the initial slope at each site.

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In Fig. 2a, the step-like shape of the curve reflects a typical low-level CH4 flux. Based on the initial slope, the CH4 emission rate was one order of magnitude higher at the L. astacina burrow (0.14 mg m\(^{-2}\) hr\(^{-1}\)) than at the M. japonicus burrow (0.04 mg m\(^{-2}\) hr\(^{-1}\)) and reference site (0.04 mg m\(^{-2}\) hr\(^{-1}\)). The higher CH4 concentration in headspace at the L. astacina burrow can be attributed to the higher CH4 emissions. Note that the CH4 production is associated with methanogenesis in the sediments because the residents do not generate CH4, as shown in mangrove sediments (Kristensen et al., 2008). We found that methanogenic bacteria were relatively abundant in the deep layers of L. astacina burrows (Table 1). Therefore, L. astacina burrows seem to serve as conduits, where CH4 from the deep sediment layer can avoid oxidation at the wall surface and rapidly diffuse into the atmosphere (e.g., Kristensen et al., 2008). But, the differences of CO2 levels between the decapods and reference sites show that the increased CO2 concentrations over the burrow sites are possibly influenced by the residents, and it also indicates that the primary production (CO2 uptake) is overwhelming at the M. japonicus site compared to at the L. astacina site (Fig. 3).

3.2 Carbon isotope ratios and CH4 oxidation

At both the L. astacina and M. japonicus burrow sites, the CH4 concentrations decreased remarkably from 2.4 to 1.9 ppm and from 2.1 to 1.6 ppm, respectively, commencing 1.5 h into the experiment (Fig. 2a). We believe that this reflects CH4 oxidation (i.e., gas uptake by sediment), which is supported by the observed changes in the δ\(^{13}\)C values of CO2 and CH4 in the chamber. During the chamber experiment at the L. astacina burrow site, the δ\(^{13}\)C of CO2 decreased asymptotically over time for the first two samples, from −17.26 ± 0.62‰ and −17.91 ± 0.47‰ to −20.18 ± 0.39‰ and −20.63 ± 0.21‰, respectively. The importance of the last two δ\(^{13}\)C values is that they equate to the CO2 produced in the sediments (Mora and Raich, 2007). In other words, the origins of chamber CO2 may differ.

To understand the origins of CO2 within the chamber, we investigated the relationship between the δ\(^{13}\)C values of the four CO2 samples and the inverse CO2 concentration (e.g., Widory et al., 2012), together with those of atmospheric CO2 (Fig. 4). Figure 4 shows that the samples could be plotted as a ternary mixing triangle. Three factors may influence the CO2 concentration: 1) the initial headspace CO2, which is recognized as a local atmospheric CO2 characterized by a concentration of 377 ppm and an average δ\(^{13}\)C of −8.3‰ based on long-term measurements for 1991–2011 (Kim, 2014); 2) the trace gas CO2 produced in sediments by microorganisms (in here mud shrimp), which is characterized by a higher CO2 concentration (452 ppm) and a δ\(^{13}\)C of −17.9‰; and 3) the CO2 resulting from the oxidation of CH4, which was heavily depleted of \(^{13}\)C (δ\(^{13}\)C = −20.6‰) with a high CO2 concentration (435 ppm). Therefore, the CO2 associated with CH4 oxidation could result in high CO2 levels in the chamber headspace, indicating that the two end-member origins, respired and CH4 oxidation-driven CO2, are mixed. In comparison, the δ\(^{13}\)C values of CH4 increased somewhat as the CH4 was oxidized (from −60.49 ± 0.46‰ to −58.59 ± 0.34‰), suggesting \(^{13}\)C enrichment of the CH4 remaining in the headspace.
Consequently, from the Figs. 3 and 4, the ternary mixing plot of CO₂ origins could be expected at the burrow sites, as only consider the CO₂ productions by respiration and CH₄ oxidation together with both have a different δ¹³C-CO₂ value.

3.3 CH₄ oxidation potential in burrows of tidal flats

At both burrow sites, the calculated CH₄-oxidation potential (i.e., the uptake rate) ranged from 0.11 ~ 0.16 mg m⁻² h⁻¹; the higher value was that of the M. japonicus burrow site. Thus, the CH₄-oxidation capacity of the burrow sites is likely associated with the activities of methanotrophic bacteria.

Table 1 summarizes the data on methanotrophic bacteria. In the L. astacina burrow wall, the total methanotroph levels did not vary with depth (overall average 0.92%), but the levels of aerophilic Proteobacteria decreased with depth, whereas the levels of the Candidatus methylomirabilis group that consumes CH₄ via nitrite reduction increased from ~ 4% at the surface to ~ 74% at a depth of 25 cm. At a depth of 10 cm in the M. japonicus burrow wall used in the chamber experiment, the overall average proportion of methanotrophs was 1.18%, of which the C. methylomirabilis group constituted approximately 70%, somewhat different from the figures from another M. japonicus burrow site (0.91% overall proportion; ~ 50% C. methylomirabilis) that was not used in the chamber experiment. In comparison, the overall proportions of methanotrophs and that of C. methylomirabilis were 0.36 and 15%, respectively, at the surface of the reference site, indicating that CH₄ oxidation via oxygen consumption may be in play. As mentioned in section 3.2 above, the headspace of the closed chamber had attained equilibrium when CH₄ oxidation commenced at the L. astacina burrow site. Thus, the sites of CH₄ oxidation include the burrow walls, where methanotrophs are abundant.

In the experiment at the reference site, the sediment temperatures at depths of 1 ~ 2 cm ranged from 18.8 ~ 19.4°C, but gradually increased with time to 20.2 ~ 25.7°C and 20.1 ~ 24.9°C at the L. astacina and M. japonicus burrows, respectively. These results indicate that CH₄ oxidation may be enhanced by increased sediment temperatures, which could affect the activity of methanotrophic bacteria (Ishizuka et al., 2000; Ueyama et al., 2015).

4. Conclusions

The decapods (L. astacina and M. japonicus) burrow in tidal flat appears to be a potential sink of CH₄ which can be identified by the variability of chamber-headspace δ¹³C of CO₂ over time in the L. astacina burrow site. Under this circumstance the CO₂ is explained by two types: 1) a respired CO₂ and 2) a mixture of the respired CO₂ with the CO₂ resulting from the CH₄ oxidation, due to decrease of the δ¹³CO₂ in static closed chamber over the duration of the experiment. The step–like CH₄ curve reflects a typical low–level CH₄ flux. It indicates also a slow circulation of the CH₄, by which the contact time with the methanotrophic bacteria is thus sufficient for oxidation to occur. We found that methanotrophs, especially Candidatus methylomirabilis group, are relatively abundant in the burrow wall sediments, indicating possibly occurrence of nitrite–driven AOM within burrows.

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References


