Evaluating radioisotope-based approaches to measure anaerobic methane oxidation rates in lacustrine sediments

Guangyi Su,1* Helge Niemann,1,2 Lea Steinle,1 Jakob Zopfi,1 Moritz F. Lehmann1
1Department of Environmental Sciences, University of Basel, Basel, Switzerland
2Department of Marine Microbiology and Biogeochemistry, NIOZ Royal Institute for Sea Research, and Utrecht University, Texel, The Netherlands

Abstract

The microbial anaerobic oxidation of methane (AOM) is the dominant sink for methane in anoxic sediments. AOM rate measurements are essential for assessing the efficacy of the benthic methane filter to mitigate the evasion of this potent greenhouse gas to the atmosphere. Incubation techniques with trace amounts of radiolabeled substrate (typically 14CH4) represent the most sensitive approach for methane oxidation rate measurements. Yet, radiotracer application can be performed in different ways, rendering the comparability of AOM rate measurements in field and laboratory investigations problematic. We compared four different 14CH4-based short-term incubation approaches to quantify methane turnover rates in lake sediments. Three of the applied methods yielded similar and reliable downcore rate profiles. They provided clear evidence for AOM with maximum rates of 15 nmol cm−3 d−1 at ~17 cm sediment depth. Using the short-term slurry incubation (SL) method, however, we were unable to detect the AOM activity maximum that we observed with the other approaches. We hypothesize that changes in the microbial structure and disruption of physical interactions due to mixing of sediments negatively affected the activity of AOM communities and longer incubation times are necessary to enhance the sensitivity of this approach. Minor variabilities in rate measurement that we found in the non-SL incubations may be related to small-scale sediment heterogeneity, differential partial methane loss during sample handling, and/or an uneven application of the radiotracer. Whole-core incubations interfere the least with the in situ conditions, but the ultimate choice of the AOM rate measurement method will depend on the individual sampling requirements.

Methane (CH4) is an important contributor to greenhouse forcing (Lelieveld et al. 1998), providing a strong rationale for characterizing global fluxes of CH4. Lakes represent an important source of CH4 to the atmosphere (Bastviken et al. 2004; Borrel et al. 2011) and most of the lacustrine methane is produced by methanogenic microorganisms within anoxic sediments. Yet, a large fraction (57–100%) of the biogenic CH4 produced is oxidized by methanotrophic microorganisms within anoxic or oxic sediments, or in the water column (Bastviken et al. 2002; Schubert et al. 2011; Blees et al. 2014a,b). The anaerobic oxidation of methane (AOM) has mostly been studied in benthic marine environments and it is typically coupled to sulfate reduction (Boetius et al. 2000; Knittel and Boetius 2009), but other electron acceptors are possible (Ettwig et al. 2010, 2016; Sivan et al. 2011; Haroon et al. 2013; Deutzmann et al. 2014; Cai et al. 2018). Evidence for AOM in freshwater environments is rare and not always conclusive (Crowe et al. 2011; Schubert et al. 2011; Bray et al. 2017). In contrast, there is multiple evidence for aerobic methane oxidation (MOx) in lakes, and a great diversity of bacteria and niches of the different aerobic methanotrophs were described (Hanson and Hanson 1996; Blees et al. 2014a,b; Oswald et al. 2016a,b). Independent of the mode of methane oxidation, microbes play a pivotal role in modulating lacustrine methane fluxes and mitigating CH4 emissions to the atmosphere, with important implications for the global CH4 budget (Reeburgh 2007).

AOM and MOx rate measurements are essential for evaluating the efficacy of the sequential biological methane filter described above, and to better understand the controls on the balance between CH4 production and consumption. Environmentally realistic methane oxidation rates (MORs) also allow us to better understand the observed methane concentrations and the
discrepancy between high production of methane in the sediments and relatively low fluxes of methane to the atmosphere (Bastviken et al. 2004; Reeburgh 2007). Despite a plethora of methane dynamics-related studies in lakes focusing on net fluxes of methane in the water column or to the atmosphere (e.g., Bogard et al. 2014; Blees et al. 2015; Wik et al. 2016), we still only know little about absolute rates and their controls on anaerobic methane oxidation in lakes, particularly in anoxic lake sediments. Similarly, our knowledge about the electron acceptors involved in AOM in freshwater environments, where sulfate-based AOM is likely to be less important than that in marine environments, is still rudimentary. Ongoing and future work to address this knowledge gap relies on the capacity to provide accurate and reliable quantitative assessments of the location and magnitude of methane oxidation in lacustrine sediments.

Multiple geochemical and microbiological approaches exist to elucidate methane oxidation pathways in marine and freshwater environments. Potential sites of methane oxidation, both in the water column and within sediments, may be pinpointed from CH4 concentration profiles, characteristic stable carbon isotope signatures of CH4, or through phylogenetic analyses of the microbial community and specific microbial biomarkers. Approaches to actually quantify MORs in sediments include the application of one-dimensional numerical reaction-diffusion models (Jørgensen et al. 2001), flux measurements combined with the analysis of methane concentration gradients (Reeburgh 1976), monitoring of CH4 concentration changes in sediment slurry incubations (SLs) (Nauhaus et al. 2002; Hershey et al. 2015), as well as incubations with 13CH4 (Moran et al. 2008; Beal et al. 2009; Egger et al. 2015), or with a radioactive tracer such as 14CH4 (Reeburgh 1980; Iversen and Blackburn 1981; Boetius et al. 2000; Norði et al. 2013; Segarra et al. 2013; Steinle et al. 2016).

For 13CH4 and 14CH4 assays, the incorporation of labeled methane into metabolic products is quantified by stable isotope ratio mass spectrometry and liquid scintillation counting, or less common, by accelerator mass spectrometry (Pack et al. 2011). Tracer-based approaches are generally most direct, leaving little doubt as to which process is quantified. Yet, stable isotope (i.e., 13CH4) derived rate measurements can be problematic in carbonate-rich sediments, where the dissolved inorganic carbon (DIC) concentration in pore water (i.e., the natural 13C background) is very high, so that excess 13C-DIC from methane turnover is difficult to quantify. The radiolabel 14CH4 incubation approaches are more sensitive than 13CH4-based methods, and the measured rates tend to be more precise because high background levels are not an issue (Reeburgh 1980; Iversen and Blackburn 1981; Iversen and Jørgensen 1985; Treude et al. 2003). Thus, despite the strict safety regulations for handling radiotracer materials in the field and in the laboratory, the 14CH4 incubation (with standard decay counting techniques) is the most commonly used method to measure rates of anaerobic methane oxidation in marine settings. In contrast, radiolabel AOM rate measurements in lake sediments are still rather sparse (Deutzmann and Schink 2011; Norði et al. 2013).

To obtain depth-specific rate profiles in marine or lacustrine sediments, radiotracer application can be performed in different ways. 14CH4 can be applied by the whole-core-injection method (Jørgensen 1978), where the dissolved tracer is injected at various sediment depths, either into the sediment cores directly (Iversen and Blackburn 1981) or in subsampled push-cores (Jørgensen 1977; Treude et al. 2003; Niemann et al. 2006; Steinle et al. 2016). Radiotracer can also be applied to discrete samples after downcore subsampling with cut-off glass syringes, either directly to the syringe (e.g., Treude et al. 2003; Niemann et al. 2005; Norði et al. 2013) or after transfer of the sediment slurries to separate incubation vials. Moreover, depending on the approach, the 14CH4 can be applied as gas bubble or injected in dissolved form. The different approaches are likely to have different strengths and limitations. The best choice of application for a given environment will represent a compromise between minimizing the use of radioactive material/costs and maximizing the sensitivity of rate measurements, with minimum interference with the natural conditions. To the best of our knowledge, there has been no comprehensive study to compare different 14CH4 incubation methods for measuring depth-specific MORs, neither in marine nor in lacustrine sediments.

In this study, our objective was to compare and evaluate different radioactive 14CH4 tracer incubation methods to quantify anaerobic methane oxidation in lake sediments. We demonstrate that despite minor variations in the absolute rates measured, which may be related to the differences in subsample handling, all but one of the methods tested (i.e., subcore [SC], glass syringe [GS], and whole-core incubations [WC]) allowed us to detect AOM in the studied lake sediments, providing a coherent picture as to where in the sediment column AOM occurs.

**Materials and procedures**

**Study site**

Lake Cadagno is an alpine meromictic lake located in the southern Alps of Switzerland (46°33’03”N 8°42’42”E). A chemocline located between 9 and 14 m depth separates the oxic mixolimnion, the upper layer in a meromictic lake, from the sulfidic monimolimnion (the lower layer). Due to water infiltration from high-ionic strength subaquatic springs, Lake Cadagno features relatively high concentrations of sulfate (＞1 mmol L⁻1) (Dahl et al. 2010). Previous work has provided geochemical evidence for AOM in the sediments of Lake Cadagno (Schubert et al. 2011), but no rate measurements were performed. Sediment samples were collected from a sampling platform in the deepest part of the lake, at 21 m depth.

**Sample processing**

Using a gravity corer, a total of six undisturbed sediment cores (inner diameter 62 mm, PVC) were recovered in October 2016. For the analysis of dissolved CH4 concentrations and δ13CH4 in
the pore water, 3 mL of wet sediment was taken at different depths immediately after core recovery. Sediment samples were collected through predrilled holes at a 2 cm resolution using cut-off 3 mL syringes, immediately transferred into a 20 mL glass serum vial containing 7 mL 10% NaOH, and sealed with black bromobutyl stoppers (Rubber B. V., The Netherlands, part no.: 7395; Niemann et al. 2015). One sediment core for pore-water extraction and four additional cores for rate measurement were taken back to the home laboratory and stored at 4°C in the cold room until further processing on the next day.

For AOM rate measurements, we used four different approaches (for details, see the Supporting Information): (1) SC, (2) GS, (3) WC, and (4) SL. For the SC treatment, one of the intact gravity cores was subsampled vertically by inserting three smaller push-core liners (inner diameter of 16 mm and 29 cm long, PVC) (Jøgensen 1977; Treude et al. 2003; Niemann et al. 2006; Steinle et al. 2016). Care was taken that the water-sediment interfaces in both the original core and the push-cores were approximately at the same level. Subcores were then closed with gray thick rubber stoppers without headspace. Twenty microliter 14CH4 tracer dissolved in anoxic water (activity ~ 0.23 kBq) was injected into each subcore through the side-holes sealed with silicon gel at a depth interval of 1.5 cm. For the GS treatment, triplicate samples were taken every 2 cm from a gravity core through predrilled side ports using cut-off glass syringes, which were then sealed with black butyl stoppers. The same 14CH4 solution as for the SC incubations was injected (20 μL) directly into each of the glass syringes containing 4 mL sediment. For the WC treatment (Jorgensen 1978), similar to the SC treatment, the entire gravity core was spiked with 14CH4 tracer through predrilled side-holes sealed with silicon gel at 2 cm intervals. Given the much larger sediment volume in the core, we had to introduce a substantially higher amount of 14C-CH4 to yield consistent relative activities (14C-CH4 per volume of incubated sediment) across the different approaches applied here. To account for this, the radiotracer was always injected as a gas bubble (20 μL) for WC treatments. Finally, for the SL treatment, 4 mL wet sediment samples were taken from a fourth sediment core through predrilled side ports every 2 cm by using 5 mL cut-off syringes. Samples were then transferred into 20 mL glass serum vials containing autoclaved and anoxic sulfate-amended water, at sulfate levels that are representative for the sediment-water interface in Lake Cadagno (i.e., ~ 1 mmol L−1). Sediment samples were gently pushed into the vials and then closed headspace-free with polytetrafluoroethylene coated gray chlorobutyl stoppers (Wheaton, U.S.A., part no.: 444704) and shaken thoroughly to equilibrate the pore-water methane between the aqueous and the gas phase. Biological activity of sediments in glass syringes, and slurries in incubation vials, was stopped in the same way, i.e., samples were transferred into 100 mL bottles and fixed with 20 mL NaOH (5% wt/wt). For the termination of the WC, the core was extruded, and triplicate samples (~ 4 mL) were collected from 2 cm slabs from the sediment, using 20 mL cut-off syringes, and quickly transferred into bottles with NaOH solution.

Pore-water and sediment geochemical analyses

Methane concentrations in the headspace were measured with a gas chromatograph (Agilent 6890N) linked to a flame ionization detector, using hydrogen as a carrier gas (e.g., Blees et al. 2014a). The carbon isotope composition of CH4 from the headspace was determined using a mass spectrometer (T/GAS PRE-CON, Micromass UK), connected to a preconcentration unit (e.g., Oswald et al. 2015). Stable carbon isotope ratios are reported in the conventional δ-notation (in ‰) relative to the Vienna Pee Dee Belemnite standard (V-PDB). The δ13C values of methane have an analytical error of ± 1‰.

Pore water was extracted by centrifugation of the sediment and the supernatant was filtered through 0.45 μm membrane filters (PES, Sarstedt, Germany). Pore-water concentrations of sulfate were analyzed by ion chromatography (882 Compact IC plus, Metrohm, Switzerland) (Oswald et al. 2015). Stable carbon isotope ratios were determined spectrophotometrically using the Cline method (Cline 1969). DIC concentrations were quantified using a total carbon analyzer (TOC-5050A, Shimadzu, Kyoto, Japan; Sullivan et al. 2013).

MOR measurements

14CH4 activity measurement

Following the procedure of Treude et al. (2003), samples from all the different incubation experiments were treated the same way after the incubations were stopped (Treude et al. 2003; Mau et al. 2013; Blees et al. 2014a,b). In brief, the headspace of a fixed sample was purged with air (30 mL min−1 for 30 min) through a heated (850°C) quartz tube filled with copper oxide, where the residual 14CH4 was combusted to 14CO2. The produced 14CO2 was then captured in two scintillation vials (20 mL) containing 8 mL of CO2-absorbant (a mixture of phenylethylamine and methoxyethanol with a volume ratio of 1/7). The cumulative sample radioactivity was then determined by liquid scintillation counting (2200CA Tri-Carb Liquid Scintillation Analyzer) after the addition of 8 mL of a scintillation cocktail (Ultima Gold, PerkinElmer) to each vial and thorough mixing using a vortex-mixer.
**14CO₂ analysis**

For the quantification of microbially produced 14CO₂, we proceeded as previously described (Treude et al. 2003; Mau et al. 2013), with some modifications. In brief, product of the 14CH₄ oxidation was released from the alkaline liquid phase (i.e., from the carbonate pool of the NaOH-fixed sample) by adding 3 mL of concentrated HCl (37%), shifting the carbonate equilibrium toward CO₂. The 14CO₂ was subsequently purged and trapped as described above. Measured 14CO₂ radioactivities were blank-corrected by subtraction of the average amount of 14CO₂ recovered in killed controls (Treude et al. 2005). Excess 14CO₂ activity was considered to be zero if the corrected value was lower than three times the standard deviation (SD) of the control mean values.

**Remaining activity**

A minor fraction of the labeled methane may be assimilated into biomass (Blees et al. 2014b). Therefore, after the 14CO₂ activity measurement, we determined the remaining radioactivity in a 2 mL aliquot of the acidified mixture (amended with 4 mL Ultima Gold) by liquid scintillation counting (Blees et al. 2014a,b).

**AOM rate calculation**

First-order rate constants (k) were calculated using the activity of the three different carbon pools (Eq. 1).

\[
 k = \frac{A_{\text{CO}_2} + A_R}{A_{\text{CH}_4} + A_{\text{CO}_2} + A_R} \times t^{-1}
\]

where \(A_{\text{CH}_4}\), \(A_{\text{CO}_2}\), and \(A_R\) represent the radioactivity of CH₄, CO₂, and the residual radioactivity (which includes biomass and metabolic intermediates), respectively, and \(t\) represents the incubation time in days. MORs were then calculated using the values for \(k\) and the methane concentration at the start of the incubation (Eq. 2).

\[
 \text{MOR} = k \times [\text{CH}_4], \text{ in } \mu\text{mol cm}^{-3} \text{d}^{-1}
\]

For [CH₄], we used the in situ methane concentration determined separately in the core dedicated to geochemical analyses. Given that the tracer addition did not increase the total methane concentration in a sample much over in situ levels, calculated MOR values can be considered to be absolute rates. As all incubations were conducted under anaerobic conditions, with sediments/slurries containing substantial amounts of sulfide as an oxygen buffer, MORs determined here represent rates of AOM rather than MOx.

**Assessment and discussion**

**Geochemical profiles**

Methane concentrations in the anoxic Lake Cadagno sediments displayed maximum values between 3 and 4 mmol L⁻¹ in the lower parts of the sediment core (Fig. 1a) and decreased toward the sediment-water interface to < 0.2 mmol L⁻¹. The methane depth distribution thus suggests that methane is oxidized within the sediments. The sulfate profile showed an inverse pattern with pore-water concentrations decreasing from 0.9 mmol L⁻¹ at the sediment surface to 0.01 mmol L⁻¹ at 30 cm depth. Considerable amounts of sulfide (~ 1 mmol L⁻¹) were detected between 3 and 15 cm, providing clear evidence for sulfide production from microbial sulfate respiration. However, in contrast to most diffusive marine settings, a clear sulfate-methane transition zone that would allow us to pinpoint the location where in the sediments sulfate-dependent AOM rates are highest was not observed. Relatively high concentrations of

![Fig. 1](image-url)

**Fig. 1.** (a) Pore-water concentrations of methane (CH₄, filled circles), DIC (open circles), sulfate (SO₄²⁻, filled triangles), and total sulfide (H₂S, open triangles) in Lake Cadagno sediments (October 2016). (b) Stable carbon isotope ratios (δ¹³CH₄) of dissolved methane in the pore water. The solubility of CH₄ in water at in situ temperature (4°C) is 2.25 mmol L⁻¹.
both methane and sulfate coexisted in the surface sediments, indicating favorable conditions for AOM down to 30 cm sediment depth.

Previous work has provided putative evidence that AOM is constrained to the uppermost sediment layers, with relatively strong positive shifts in the methane $\delta^{13}C$ toward the sediment-water interface (Schubert et al. 2011). AOM tends to increase the pore-water carbonate alkalinity through production of bicarbonate, yet a clear sediment horizon where AOM takes place was not evident from the DIC concentration profile (Fig. 1a). Moreover, a typical methane concentration-to-carbon-isotope relationship, with strongly increasing methane $\delta^{13}C$ values with decreasing methane concentrations right underneath the sediment surface, in contrast to previous work by Schubert et al. (2011), was not observed (Fig. 1b). In general, $\delta^{13}$CH$_4$ profiles alone may be misleading with respect to assessing methane oxidation, because multiple microbial processes (including hydrogenotrophic and acetoclastic methanogenesis) can be involved, co-occurring at corresponding sediment depths and producing interfering isotope signatures. The methane $\delta^{13}$C in this study showed only a relatively subtle increase from $-78.7‰$ to $-74.8‰$ below the sediment-water interface. Thus, both the pore-water biogeochemical as well as the $\delta^{13}$CH$_4$ profiles in this study are clearly more ambiguous with respect to where exactly methane oxidation within the sediments may take place primarily, highlighting the necessity to measure AOM rates as a function of sediment depth.

**AOM rates**

The vertical AOM rate profiles obtained from three of the different incubation methods (SC, GS, and WC) displayed very similar patterns (Fig. 2a–c). Generally, no or very low AOM activities were detected in the upper sediment layers. MORs started to increase below ~9 cm and highest rates were consistently observed at 17 ± 1 cm below the sediment-water interface (i.e., 15.9 ± 2.1 nmol cm$^{-3}$ d$^{-1}$, 15.2 ± 8.8 nmol cm$^{-3}$ d$^{-1}$, and 14.7 ± 2.6 nmol cm$^{-3}$ d$^{-1}$ when measured with the SC, GS, and WC approach, respectively). Below the maximum, methanotrophic activity decreased rapidly, with AOM rates of 2.1 ± 1.2 nmol cm$^{-3}$ d$^{-1}$, 1.6 ± 0.8 nmol cm$^{-3}$ d$^{-1}$, and 1.4 ± 0.7 nmol cm$^{-3}$ d$^{-1}$ at 23 cm sediment depth, respectively. Thus, all three approaches provided clear and consistent geochemical evidence for AOM in the sediments of Lake Cadagno, and the
results agreed well with regards to the location of, and maximum rates within, the AOM zone. It is beyond the scope of this study to discuss in detail the biogeochemical and ecological context of the observed AOM rate profiles (i.e., potential electron acceptors and microbial players involved). But what becomes clear from the data set presented here is that all three approaches (SC, GS, and WC) are useful to reliably estimate the biologic methane filtering capacity within the sediments (Figs. 2, 3), and our results imply that ecosystem methane flux comparisons remain unbiased in the event that more than one of the three different AOM rate measurements techniques are involved. Generally, the AOM rates in Lake Cadagno sediments as obtained from the non-SL incubations (SC, GS and WC) were comparable to those reported for other freshwater lake sediments (Norði et al. 2013) and wetlands (Segarra et al. 2013, 2015). Compared to marine sediments, they fell within the lower range of reported AOM rates (Iversen and Blackburn 1981; Iversen and Jørgensen 1985; Treude et al. 2003; Niemann et al. 2006, 2009; Steinle et al. 2016).

Although the patterns of rate profiles in the sediments were similar among the non-SL approaches, discrepancies were observed for single depths above and below the AOM rate peak, which resulted in considerable differences in the integrated rates within the top 23 cm of sediment (SC: 122.5 ± 19.5 nmol cm⁻² d⁻¹; GS: 58.2 ± 19.9 nmol cm⁻² d⁻¹; WC: 83.3 ± 8.6 nmol cm⁻² d⁻¹). Moreover, between different approaches, the reproducibility was quite variable. For example, a significantly higher SD was observed for the rate maximum determined by SC compared to WC (e.g., at 15 cm 13.6 ± 10.5 for SC and 14.1 ± 1.9 for WC). We cannot fully exclude that part of the data scatter is due to true natural variability. Yet, the relatively large error for the maximum AOM rates in the SC treatment can be easily explained, when considering the single replicate k profiles (Fig. 3). While maximum k values for both GS and WC were found at 17 cm in all triplicates, depth-dependent k values for the three different subcores were located at 15 cm, 17 cm, and 19 cm depth, respectively, indicating a shift of the sediment horizons due to differential sediment compression/displacement when pushing the subcores into the gravity core (this effect can be observed visually during subsampling). To account for this effect, we aligned the profiles of the replicate MOR measurements

![Fig. 3. Depth-dependent first-order rate coefficients (k values) for AOM based on different incubation approaches: (a) SC, (b) GS, (c) WC, and (d) SL. Mean (black filled circles) and SD of the three replicate measurements (gray lines) are shown.](image)

![Fig. 4. AOM rate profile of SC incubation (a) before and (b) after depth correction (± 1 cm offset) for two subcores. Mean (black filled circles) and SD of the three replicate measurements (gray filled circles) are shown. Lower SDs for the peak-rates were achieved after alignment.](image)
(correction for the ± 1 cm offset), yielding more consistent depth profiles, and the ± SDs for the peak-rates are much lower (Fig. 4). In addition to displacement, smearing of sediments during core introduction may result in a further shift of “sediment depths” in the subcores. Finally, sediment heterogeneity or minor tilting of the sediment layers in the parent core may also contribute to the observed discrepancies (Treude et al. 2003). For future studies, we recommend that the degree of core compaction may be verified, for example, through comparative measurements of pore-water sulfate concentrations in both the parent and subcores.

To some extent, the rate differences observed between methods and replicates may also be attributed to differential methane loss, which will result in a lower total radioactivity and can have a biasing effect on the final AOM rates. Some approaches may be more susceptible to methane loss than others during handling. In order to assess whether parts of methane were lost, total radioactivity in the AOM samples can be compared with the radioactivity of injected tracer. It is important to note, however, that variation in the total radioactivity will not affect tracer turnover, and in turn the calculation of k values. Only when there is methane loss after the AOM incubation, the final absolute rate will be overestimated because the residual 14CH4 radioactivity is underestimated, leading to increased k values (see Eq. 1).

Radioactivity yields can best be assessed in the two approaches where the 14C tracer is injected into a defined and closed volume (i.e., SL and GS). Total radioactivities for SL samples (mean ± SD, 227 ± 23 Bq) were very similar to the directly measured value (20 μL, ~ 233 Bq) (Fig. 5), indicating no significant methane loss. In contrast, total radioactivities in the GS incubation samples were much lower (mean ± SD, 162 ± 22 Bq), although the same amount of 14CH4 was injected. The lower total radioactivity can possibly be attributed to diffusive methane loss at the plunger side of the glass syringe during the incubation. Theoretically, only 14CH4 loss occurring at the end of incubation or during AOM sample transfer would result in overestimated rates (Treude et al. 2005; Knab et al. 2009). At least at some depths, GS-based rates were, if at all, lower when compared to rates determined using SC and WC. This suggests that the apparent radiogenic methane loss occurred right at the beginning of the incubation, when the tracer was applied or 14CH4 was lost continuously during incubation. Overall, the different non-SL approaches provided a reliable quantification of actual AOM rates in the studied sediment, and the relatively good agreement between these approaches suggests that the 14CH4 loss was not significant enough to markedly affect the calculated k values. At least, our data do not provide any clear evidence for one approach being more, or less, susceptible to 14CH4 loss-driven AOM rate overestimation than the other (except for SL).

Most strikingly, the AOM rate profiles obtained from the SLs were completely different compared to the other approaches (Fig. 2d). They did not reveal the clear pattern of AOM rates that we observed with the other methods within the subsurface sediments, as absolute rates in the methane oxidation zone were barely above zero (i.e., 1.8 ± 3.0 nmol cm−3 d−1 at 3 cm, 1.3 ± 0.2 nmol cm−3 d−1 at 19 cm, and 3.1 ± 2.2 nmol cm−3 d−1 at 21 cm). Hence, information as to where the main AOM zone is located could not be gained from the SL-incubation based rate profiles. In contrast to SC, GS, and WC incubations, the samples used for SL-AOM incubations were mixed with anoxic sulfate-containing water, disrupting the sediment texture and changing the microhabitat completely. Anaerobic methanotrophs in marine sediments usually depend on syntrophic partners, e.g., sulfate-reducing bacteria, and AOM is often carried out by microbial consortia (Boetius et al. 2000; Orphan et al. 2002). If this was the case also in the lake sediments studied here, the perturbation during slurry preparation may have affected the structure of AOM-hosting aggregates and thereby compromised the methanotrophic activity in the SL incubations. The very low methane turnover (i.e., k values) also at depths where the other approaches revealed maximum AOM rates (Fig. 3) supports the idea that low/absent AOM may be due to changes of the microenvironmental conditions and/or disruption of the interactions between consort ing organisms. The effect, however, seems to be transient as SLs have been used successfully to detect AOM in marine sediments, albeit with much longer incubation times than used in the present study here (Beal et al. 2009; Egger et al. 2015). Thus, we recommend that subsamples for MOR measurement are preincubated for anaerobic conditioning of the microbial communities (Segarra et al. 2015), and that the main slurry experiments are performed over longer periods of time (i.e. > 2 weeks).
Concluding remarks

Here, we compared four different $^{14}$CH$_4$-tracer-based techniques to assess depth-specific in situ AOM rate measurements in lake sediments. Overall, our data demonstrate that these approaches, which involved incubations of the undiluted sediments (i.e., not mixed to slurries), yielded consistent and reliable results, allowing us to pinpoint the zone of AOM and to quantitatively assess MORs. Obviously, WCs induce minimal interference with in situ conditions, which may be particularly important where metabolic or physical interaction between the methane oxidizing microorganisms and partner bacteria are expected. The WC approach produced the best reproducibility among the tested techniques, yet it requires larger quantities of $^{14}$CH$_4$, which implies higher costs and more radioactive waste. Moreover, in some situations, WCs may not be feasible. For example, when using long piston/gravity cores, commonly only one core over, in some situations, WCs may not be feasible. For example, to investigate AOM modes by testing the involvement, they are viable in longer-term experimental studies seeking, for example, to investigate AOM modes by testing the involvement of different electron acceptors.

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Conflict of Interest

None declared.