



Vertical distribution of methane oxidation and methanotrophic response to elevated methane concentrations in stratified waters of the Arctic fjord Storfjorden (Svalbard, Norway)

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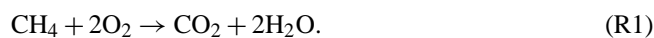
Abstract. The bacterially mediated aerobic methane oxidation (MO_x) is a key mechanism in controlling methane (CH_4) emissions from the world's oceans to the atmosphere. In this study, we investigated MO_x in the Arctic fjord Storfjorden (Svalbard) by applying a combination of radio-tracer-based incubation assays ($^3\text{H-CH}_4$ and $^{14}\text{C-CH}_4$), stable C- CH_4 isotope measurements, and molecular tools (16S rRNA gene Denaturing Gradient Gel Electrophoresis (DGGE) fingerprinting, *pmoA*- and *mxoF* gene analyses). Storfjorden is stratified in the summertime with melt water (MW) in the upper 60 m of the water column, Arctic water (ArW) between 60 and 100 m, and brine-enriched shelf water (BSW) down to 140 m. CH_4 concentrations were supersaturated with respect to the atmospheric equilibrium (about 3–4 nM) throughout the water column, increasing from ~ 20 nM at the surface to a maximum of 72 nM at 60 m and decreasing below. MO_x rate measurements at near in situ CH_4 concentrations (here measured with $^3\text{H-CH}_4$ raising the ambient CH_4 pool by < 2 nM) showed a similar trend: low rates at the sea surface, increasing to a maximum of ~ 2.3 nM day⁻¹ at 60 m, followed by a decrease in the deeper ArW/BSW. In contrast, rate measurements with $^{14}\text{C-CH}_4$ (incubations were spiked with ~ 450 nM of $^{14}\text{C-CH}_4$, providing an estimate of the CH_4 oxidation at elevated concentration) showed comparably low turnover rates (< 1 nM day⁻¹) at 60 m, and peak rates were found in ArW/BSW at ~ 100 m water depth, concomitant with increasing ^{13}C values in the residual CH_4 pool. Our results indicate that the MO_x community in the surface MW is adapted to relatively low CH_4 concentrations. In con-

trast, the activity of the deep-water MO_x community is relatively low at the ambient, summertime CH_4 concentrations but has the potential to increase rapidly in response to CH_4 availability. A similar distinction between surface and deep-water MO_x is also suggested by our molecular analyses. The DGGE banding patterns of 16S rRNA gene fragments of the surface MW and deep water were clearly different. A DGGE band related to the known type I MO_x bacterium *Methylosphaera* was observed in deep BWS, but absent in surface MW. Furthermore, the Polymerase Chain Reaction (PCR) amplicons of the deep water with the two functional primers sets *pmoA* and *mxoF* showed, in contrast to those of the surface MW, additional products besides the expected one of 530 base pairs (bp). Apparently, different MO_x communities have developed in the stratified water masses in Storfjorden, which is possibly related to the spatiotemporal variability in CH_4 supply to the distinct water masses.

1 Introduction

Methane (CH_4) is a potent greenhouse gas with a global warming potential that exceeds carbon dioxide (CO_2) 23-fold over a 100 yr timescale and is, after water vapor and CO_2 , the most important greenhouse gas (IPCC, 2007). Substantial research efforts have consequently been made to understand its sources and sinks. A large part of oceanic CH_4 is generated under reduced conditions in anoxic marine sediments, predominantly through microbially mediated

CO₂ reduction and disproportionation of methylated substrates (Whiticar, 1999; Hinrichs and Boetius, 2002; Formolo, 2010). Sedimentary CH₄ is also formed by thermal breakdown of organic matter and, although of lesser importance, serpentinization and Fischer–Tropsch reaction, both occurring at high temperature and pressure. In addition, conspicuous CH₄ concentration maxima in oxic water layers provided indications for CH₄ production under oxic conditions, possibly mediated by yet unknown microbes using dimethylsulfoniopropionate (DMSP) (Damm et al., 2010) or methylphosphonic acid (MPn) (Karl et al., 2008; Metcalf et al., 2012) as substrate. However, despite the apparent ubiquity of methanogenesis in marine systems and the large area covered by oceans, comparably little CH₄ is liberated from the oceans into the atmosphere because of microbial consumption (Reeburgh, 2007; IPCC, 2007). About 80% of sedimentary CH₄ is consumed in reduced sediments as a result of the anaerobic oxidation of methane (AOM) with sulfate as the terminal electron acceptor (Barnes and Goldberg, 1976; Reeburgh, 1976; Martens and Berner, 1977; Reeburgh, 2007; Knittel and Boetius, 2009). Finally, aerobic CH₄-oxidizing bacteria at the sediment surface and/or in the water column (belonging to the *Alpha*- (type II) or *Gammaproteobacteria* (type I and type X)) consume CH₄ that has bypassed the anaerobic microbial filter according to the following reaction (Hanson and Hanson, 1996; Murrell, 2010):



Several techniques have been used to quantify aerobic methane oxidation (MO_x) rates (Reeburgh, 2007). A common method is to incubate water column or sediment samples with radio-labeled tracers such as ¹⁴C-CH₄ or ³H-CH₄ (Reeburgh et al., 1991; Valentine et al., 2001; Niemann et al., 2006; Mau et al., 2012), which has proven to be highly sensitive. During the incubation, ¹⁴C-CH₄ or ³H-CH₄ is converted at the same rate as the natural, non-labeled CH₄ to ¹⁴CO₂ and ¹⁴C-biomass or ³H₂O. Despite the importance of water column MO_x controlling oceanic CH₄ emission to the atmosphere, only a small number of water column MO_x rate measurements exist, which is particularly true for high-latitude environments (Ward and Kilpatrick, 1990; Griffiths et al., 1982). The available data show a large scatter of rates over several orders of magnitude (Fig. 1), but factors controlling MO_x activity such as temporal variations in CH₄ availability (e.g., Mau et al., 2007a, b; Damm et al., 2007) and the activity of the present MO_x community during time periods with elevated CH₄ availability are not well constrained. Our aims were to investigate MO_x rates at ambient and at elevated CH₄ concentrations as well as to determine differences between MO_x communities thriving at different CH₄ concentrations in a natural marine environment. As a model system, we chose the fjord Storfjorden (Svalbard), which is characterized by seasonal stratification, separating distinct water masses with different CH₄ sources during summer time.

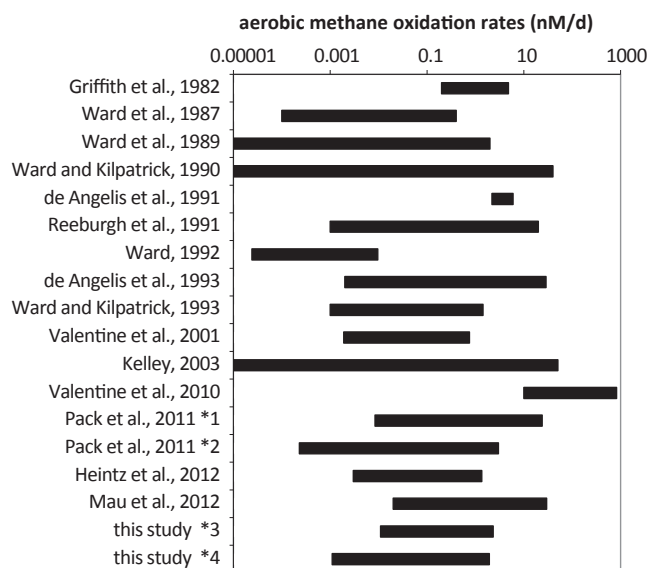


Fig. 1. Range of methane oxidation rates measured at different locations in the ocean water column derived from tracer incubations using ³H-CH₄ (Reeburgh et al., 1991; Valentine et al., 2001, 2010; Heintz et al., 2012, Mau et al., 2012) or ¹⁴C-CH₄ (all others). Pack et al. (2011) compared incubations with ³H-CH₄ (*1) and incubations with low-level ¹⁴C-CH₄ (*2) that were measured with accelerator mass spectrometry. In this study we compared incubations with ³H-CH₄ (*3) and incubations with ¹⁴C-CH₄ (*4).

2 Material and methods

2.1 Study site

Storfjorden is located in the Svalbard Archipelago between the islands Spitsbergen, Barentsøya, and Edgeøya (Fig. 2). CH₄ concentrations in the fjord water exceed atmospheric equilibrium concentration throughout the water column by a factor of 2–16, although surface water CH₄ is of a different origin compared to the CH₄ in subsurface waters (Damm et al., 2008). Surface waters contain recently produced, ¹³C-depleted CH₄, which was proposed to result from a summer phytoplankton bloom producing methylated compounds such as DMSP, which is a potential substrate for methylotrophic methanogenesis. Other potential methanogenic substrates such as methylphosphonates were not investigated in the study area. A CH₄ production–removal cycle appears to be established in the surface water as reflected by varying CH₄ concentrations and ¹³C-CH₄ values (Damm et al., 2008). In contrast, deeper water contains CH₄ that is mixed into the bottom water as a result of brine-enriched shelf water (BSW) formation during wintertime causing enhanced turbulence and repeatedly occurring resuspension of sediments releasing CH₄ (Damm et al., 2007). The winter-released CH₄ is then trapped by increasing water stratification during warmer seasons, and ongoing CH₄ consumption leads to a ¹³C-enriched isotopic signature

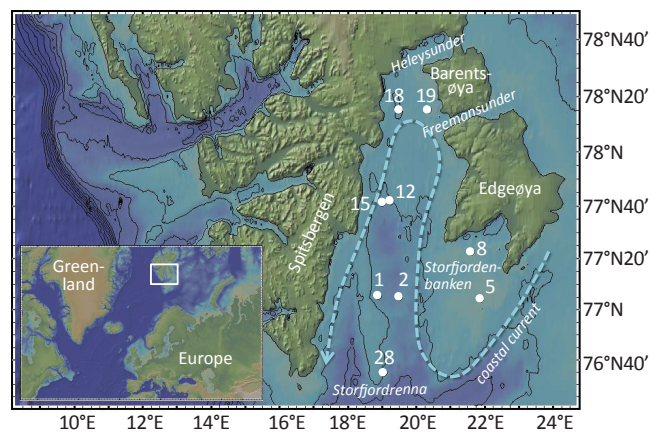


Fig. 2. Locations of the stations in Storfjorden. Stations are marked by white dots and station numbers. The coastal current is indicated by a dashed blue arrow. Contours are drawn every 100 m until 1000 m water depth

of the residual CH_4 . During summertime, the water column is stratified, with surface melt water (MW) and intermediate Arctic water (ArW) constituting the upper water column, while denser BSW is restricted to deep basins (Loeng, 1991). The residence time of the high-salinity water in deeper layers (90–246 days) is longer than the fjord's surface waters (51–141 days) (Geyer et al., 2009).

2.2 Sampling

Water samples were collected from nine stations in Storfjorden and at one open-ocean station ($70^\circ 35.913' \text{ N}$, $10^\circ 51.591' \text{ E}$) during a cruise with RV *Heincke* in August 2010 (Fig. 2, Table 1). The Storfjorden stations were aligned along the cyclonic coastal current flowing into Storfjorden along Edgeøya and out along Spitsbergen (Loeng, 1991; Skogseth et al., 2005) (Fig. 2). We intended to sample and compare the fjord's upper and lower water column because of the different CH_4 sources and water residence times. We sampled vertical profiles throughout the water column, thus recovering samples from MW, ArW, and BSW. All water masses were subsampled for chemical/biogeochemical analyses (method 2.3 and 2.4), but we focused on the MW and BSW for molecular analyses (method 2.5). Specific water depths were sampled with a CTD/rosette sampler equipped with twelve 5 L Niskin bottles, a Sea-Bird SBE 911 plus CTD and an SBE 43 oxygen sensor for online monitoring of salinity, temperature, pressure, and dissolved oxygen.

2.3 CH_4 concentrations and stable isotope composition

Aliquots of seawater were immediately subsampled from the Niskin bottles using 1 L glass bottles for measurements of in situ CH_4 concentrations. CH_4 was extracted from the water by vacuum-ultrasonic treatment within a few hours af-

Table 1. Locations of stations and performed analyses.

Station	Latitude	Longitude	Analysis
1	$77^\circ 05.64' \text{ N}$	$18^\circ 52.67' \text{ E}$	$[\text{CH}_4]$, MO_x -rates, $\delta^{13}\text{C-CH}_4$
2	$77^\circ 05.23' \text{ N}$	$19^\circ 29.69' \text{ E}$	$[\text{CH}_4]$, MO_x -rates time series, DGGE, <i>pmoA</i> , <i>mxhF</i>
5	$77^\circ 04.54' \text{ N}$	$21^\circ 52.25' \text{ E}$	$[\text{CH}_4]$, MO_x -rates, $\delta^{13}\text{C-CH}_4$, DGGE, <i>pmoA</i> , <i>mxhF</i>
8	$77^\circ 22.80' \text{ N}$	$21^\circ 35.43' \text{ E}$	$[\text{CH}_4]$, MO_x -rates, $\delta^{13}\text{C-CH}_4$
12	$77^\circ 41.91' \text{ N}$	$19^\circ 14.49' \text{ E}$	$[\text{CH}_4]$, MO_x -rates, $\delta^{13}\text{C-CH}_4$, DGGE, <i>pmoA</i> , <i>mxhF</i>
15	$77^\circ 41.45' \text{ N}$	$19^\circ 00.16' \text{ E}$	$[\text{CH}_4]$, MO_x -rates, $\delta^{13}\text{C-CH}_4$
18	$78^\circ 15.29' \text{ N}$	$19^\circ 29.07' \text{ E}$	$[\text{CH}_4]$, MO_x -rates, MO_x -rates time series, $^{13}\text{CH}_4$, DGGE, <i>pmoA</i> , <i>mxhF</i>
19	$78^\circ 15.41' \text{ N}$	$20^\circ 20.14' \text{ E}$	DGGE, <i>pmoA</i> , <i>mxhF</i>
28	$76^\circ 34.95' \text{ N}$	$19^\circ 02.41' \text{ E}$	DGGE, <i>pmoA</i> , <i>mxhF</i>
RS	$70^\circ 35.91' \text{ N}$	$10^\circ 51.59' \text{ E}$	$[\text{CH}_4]$, MO_x -rates time series

ter sampling (Schmitt et al., 1991). Hydrocarbon concentrations were measured with a Chrompack 9003 gas chromatograph (GC) equipped with a flame ionization detector (FID). Duplicate measurements indicate an error of 5–10 % (Lammers and Suess, 1994). After GC analyses, an aliquot of the extracted CH_4 gas was transferred into pre-evacuated glass containers for stable carbon isotope analysis performed with an isotope ratio mass spectrometer (IRMS; Finnigan Delta XP plus) in our onshore laboratories. The extracted gas was purged and trapped with the PreCon equipment (Finnigan) to pre-concentrate the sample. All isotopic ratios have an analytical error < 1 ‰ and are presented in the δ notation against the Vienna Pee Dee Belemnite (VPDB) standard.

2.4 Methane oxidation rates

MO_x rates were determined from ex situ incubations of water samples in 100 mL serum vials. The vials were filled bubble-free from Niskin bottles and crimped with rubber stoppers (halogenated butyl elastomer). One set of samples was then incubated with 50 μL of ^3H -labeled CH_4 (160–210 kBq) in N_2 , and a second set was incubated with 10 μL of ^{14}C -labeled CH_4 (12–15 kBq). $^3\text{H-CH}_4$ tracer addition raised ambient CH_4 concentrations by 1–2 nM and $^{14}\text{C-CH}_4$ addition by 440–540 nM. The samples were subsequently shaken for ~ 10 min on an orbital shaker to facilitate tracer dissolution and then incubated in the dark at 2°C . CH_4 oxidation rates (r_{ox}) were calculated assuming first-order kinetics (Reeburgh et al., 1991; Valentine et al., 2001):

$$r_{\text{ox}} = k'[\text{CH}_4], \quad (1)$$

where k' is the effective first-order rate constant calculated as the fraction of labeled CH_4 oxidized per unit time, and $[\text{CH}_4]$ is the in situ CH_4 concentration. In order to determine a suitable incubation time period, we performed parallel time series incubations with samples collected from the fjord (stations 2 and 18) and from an open-water station (reference

station – RS). During each incubation series, tracer consumption was measured in duplicates after a time period of 0.5, 1, 2, 3, 4 and 5 days. In the CH₄-rich waters of the fjord, our results showed a linear tracer consumption of about 5–15 % over the first three days of incubation (Fig. 3). A potential bias due to substrate limitation and/or variations in reaction velocity thus seems negligible, at least over a time period of 3 days, which we chose for our ex situ incubations. Just as the time series incubations, vertical distribution of MO_x was determined in duplicates.

Incubations with ³H-CH₄ and measurements of ³H-CH₄ and ³H-H₂O were carried out according to Valentine et al. (2001) and Mau et al. (2012). Briefly, total activity (³H-CH₄ + ³H-H₂O) was measured in 1 mL of sample aliquot by wet scintillation counting, and activity of ³H-H₂O was measured after sparging the sample for > 30 min with N₂ to remove remaining ³H-CH₄.

Incubations with ¹⁴C-CH₄ were terminated by injecting 0.5 mL of 10 M NaOH and adding a 5 mL headspace so that the remaining ¹⁴C-CH₄ accumulated in the headspace and the produced ¹⁴C-CO₂ and ¹⁴C biomass was trapped in the aqueous NaOH solution. Separation and activity measurement of ¹⁴C-CH₄ and ¹⁴C-CO₂ were carried out analogously to previous measurements of CH₄ turnover in sediments (Treude et al., 2003; Niemann et al., 2005). In short, ¹⁴C-CH₄ in the headspace was combusted to ¹⁴C-CO₂, while ¹⁴C-CO₃²⁻ was converted to ¹⁴CO₂ through acidification with HCl. In either case, ¹⁴C-CO₂ was trapped in a solution of methoxyethanol and phenylethylamine, and the radioactivity was measured by wet scintillation counting. We also measured remaining radioactivity in the sample after ¹⁴C-CH₄ and ¹⁴CO₃²⁻ removal (probably ¹⁴C incorporated into biomass), which ranged between 4 and 84 % (average 35 %) of the total product.

2.5 Diversity of MO_x community

The diversity of the natural bacterioplankton assemblages was examined by denaturing gradient gel electrophoresis (DGGE) based on the 16S rRNA gene. Immediately after sampling, bacterial cells were concentrated on Nuclepore filters (0.2 μm pore size) and the filters were stored frozen at –20 °C until DNA extraction. Total community DNA was extracted using the UltraClean Soil DNA Kit (MoBio Laboratories, USA). One to five microliters of DNA extract was applied as the template in the 16S rRNA gene specific PCR, with GM5 plus GC-clamp as the forward primer and 907RM as the reverse primer (Muyzer et al., 1993). PCR conditions were as described by Gerdes et al. (2005). PCR products (ca. 500 bp) were analyzed by DGGE based on the protocol of Muyzer et al. (1993) using a gradient chamber. Clearly visible bands of the DGGE pattern were excised from the gel and reamplified by PCR (Gerdes et al., 2005) and sequenced. The 16S rRNA gene sequences were then assigned to the new higher-order taxonomy proposed in Bergey's taxonomic

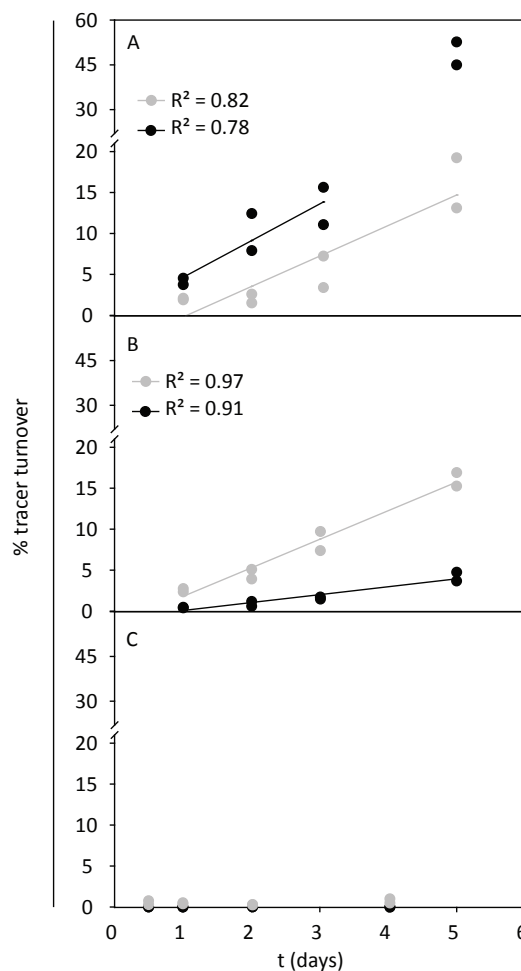


Fig. 3. Time series incubation of water samples collected at (A) station 2 (77°5.226' N and 19°29.694' E) at 135 m water depth, at (B) station 18 (78°15.288' N and 19°29.070' E) at 50 m water depth, and at (C) a reference station (RS, 70°35.91' N and 10°51.59' E) at 101 m water depth. ¹⁴C-CH₄ and ³H-CH₄ results are shown as black and gray circles, respectively.

outline of the “Prokaryotes” by the “Ribosomal Database Project (RDP) Classifier” (Wang et al., 2007). The sequences were further compared with those deposited in GenBank using the BLAST algorithm.

The presence of CH₄-oxidizing bacteria in the communities was screened by the two functional primer sets “pmoA” and “mxaF”, targeting the genes encoding subunits of the particulate methane monooxygenase (pMMO) and the methanol dehydrogenase (MDH), respectively. Both enzymes are key enzymes for methanotrophs (e.g., McDonald et al., 2008). However, the *mxaF* gene is also present in almost all other methylotrophic bacteria. The primer sets and amplification conditions employed in the gene-specific PCR reaction are described in Holmes et al. (1995) and McDonald and Murrell (1997), respectively.

3 Results

3.1 Water column biogeochemistry

According to Skogseth et al. (2005), we could identify three distinct water masses: melt water, MW ($T: > 0.0^{\circ}\text{C}$, $S: < 34.2$); Arctic water, ArW ($T: < 0.0^{\circ}\text{C}$, $S: 34.3\text{--}34.8$); and brine-enriched shelf water, BSW ($T: < -1.5^{\circ}\text{C}$, $S: > 4.8$) (Fig. 4d).

The MW extended from the surface to ~ 60 m water depth; this is the depth range where the thermocline is located and temperature decreased by $\sim 4^{\circ}\text{C}$ (Fig. 4a). In the MW, CH_4 concentrations increased from ~ 20 nM at the surface to 72.3 nM at 60 m water depth (Fig. 5a). All concentrations were high and oversaturated with respect to the atmospheric equilibrium concentration of 3.3–3.9 nM (at the relevant T/S conditions, Wiesenburg and Guinasso, 1979). Similar to concentrations, microbial oxidation rates determined with ^3H - and ^{14}C tracer increased with depth to 2.3 nM day^{-1} and 0.77 nM day^{-1} , respectively, at 60 m (Fig. 5b and c). In the MW, rates measured with ^{14}C tracer (440–540 nM ^{14}C - CH_4 added) were consistently lower than those determined with ^3H tracer (1–2 nM ^3H - CH_4 added). $\delta^{13}\text{C}$ - CH_4 values in this water mass ranged between -43.5 and -53.6 ‰ (Fig. 5d).

In the ArW, (60 to ~ 100 m water depth) oxygen concentrations decreased with depth from 350 to 320 μM (Fig. 4c) and CH_4 concentrations from 42 to 6.5 nM (Fig. 5a). Both MO_x rates determined with ^3H and ^{14}C tracer showed a maximum at ~ 80 m in this water mass (Fig. 5b and c). The stable carbon isotopic signature of CH_4 showed a strong shift from -46 to about -32 ‰ at ~ 80 m (Fig. 5d).

The BSW (> 100 m water depth) was characterized by oxygen concentrations below 320 μM (Fig. 4c). CH_4 concentrations decreased slightly with depth, but were stable below 120 m (8–9 nM, Fig. 5a). MO_x rates determined with ^3H -labeled CH_4 showed a similar trend to the CH_4 concentrations. However, while ^3H - MO_x rates (approximately in situ rates) were low, rates determined with ^{14}C -labeled CH_4 (rates determined at elevated CH_4 concentrations) were comparably high, with a maximum of 1.9 nM day^{-1} at ~ 100 m water depth (Fig. 5b and c). The carbon isotopic signature of the CH_4 decreased steadily from its maximum of -30 ‰ at 100 m to -39 ‰ in the lowermost sample (136 m, Fig. 5d).

3.2 Microbial communities

3.2.1 DGGE of 16S rDNA

Similar to the biogeochemical results, the MW and BSW at the studied stations (stations. 2, 5, 12, 18, 19) showed distinct DGGE banding patterns (Fig. 6, Table 2) indicating that surface MW and deep waters were populated by different microbial communities.

The MW samples showed strong DGGE bands that we could assign to eukaryotic-chloroplast DNA (#3, #4) and to

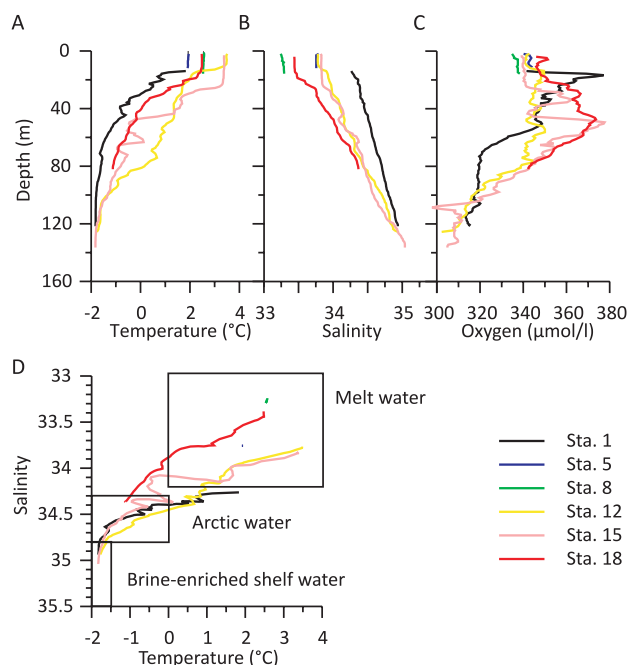


Fig. 4. Depth profiles of temperature (A), salinity (B), and oxygen concentrations (C), as well as a temperature–salinity graph with temperature–salinity ranges of the dominant water masses in Storfjorden (D). Stations 5 and 8 are less than 20 m deep and appear as dots in the temperature–salinity graph.

Alphaproteobacteria of the genera *Phaeobacter* and *Sulfitobacter* (#7, #8). The affiliation to the genus *Phaeobacter* was, however, relatively weak (0.51 confidence value, Table 2), indicating a possibly yet-undescribed bacteria type. Additional bands (#5, #9, and #11) could be assigned to the genera *Fluviicola* within the phylum *Bacteroidetes*, *Haliea* within the phylum *Proteobacteria*, and *llumatobacter* within the phylum *Actinobacteria*. Although we could measure CH_4 oxidation in the surface waters, the DGGE based on the 16S rRNA gene did not reveal known methanotrophs.

In contrast to the diverse MW community, all deep-water samples (station 12, 127 m; station 2, 138 m; station 18, 136 m) showed a quite low diversity with only two strong (#6 and #7) and one weaker DGGE band (#10) (Fig. 6). Band #7 was also common in the upper water masses, while band #6 was only found in the BSW samples. This band could be affiliated with *Methylosphaera*, which is a known type I MO_x bacterium (Bowman et al., 1997). However, the confidence value of 0.38 was relatively low (Table 2). The deep-water-specific band #10 could be assigned to the sulphate reducer *Desulfobacca*, also with a relatively low confidence level (0.19, Table 2).

3.2.2 Molecular marker genes of methanotrophs

The *pmoA* gene that encodes the alpha subunit of the particulate methane monooxygenase is a molecular marker gene

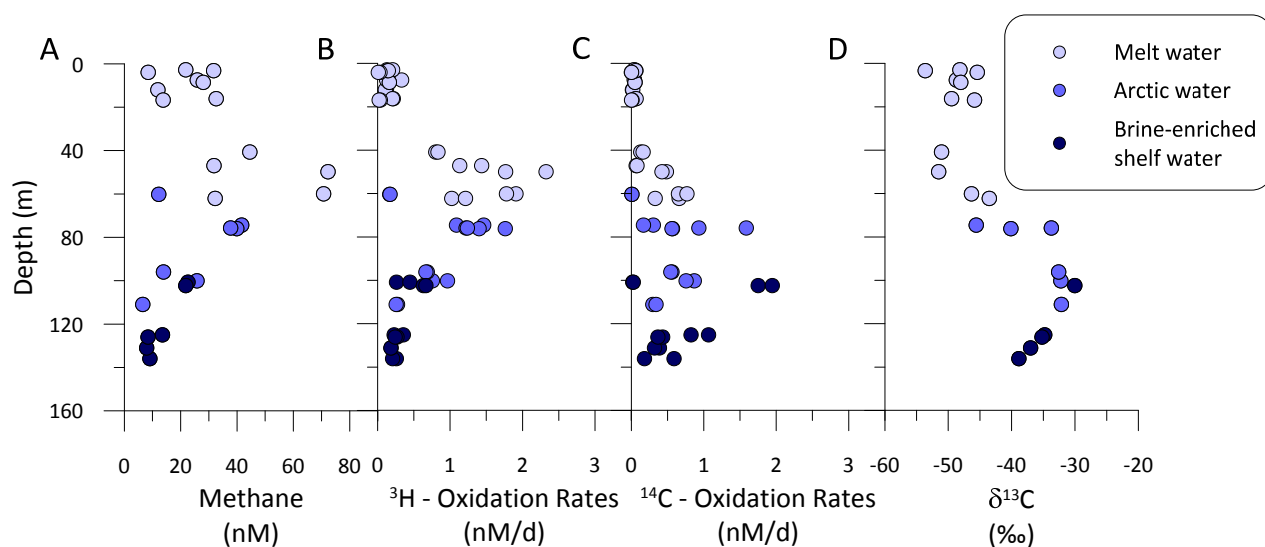


Fig. 5. Depth profiles of CH_4 concentrations (A), oxidation rates derived by $^3\text{H}\text{-CH}_4$ - (B) and $^{14}\text{C}\text{-CH}_4$ tracer (C), and CH_4 stable carbon isotope composition ($\delta^{13}\text{C}$ values, D). Samples are color-coded according to the water masses (Fig. 4).

Table 2. Classification of partial 16S rRNA sequences to bacterial taxa performed with the RDP Classifier (Wang et al., 2007). The confidence value (0–1) for assignment at the level of class and genus is given in parentheses.

No.	Class	Genus
1	Alphaproteobacteria (1)	<i>Cand. Pelagibacter</i> (1)
2	Flavobacteria (1)	<i>Polaribacter</i> (1)
3	Cyanobacteria (1)	Chlorophyta (0.98)
4	Cyanobacteria (1)	Chlorophyta (1)
5	Flavobacteria (1)	<i>Fluviicola</i> (0.81)
6	Gammaproteobacteria (1)	<i>Methylosphaera</i> (0.38)
7	Alphaproteobacteria (1)	<i>Phaeobacter</i> (0.51)
8	Alphaproteobacteria (1)	<i>Sulfitobacter</i> (0.97)
9	Gammaproteobacteria (1)	<i>Haliea</i> (1)
10	Deltaproteobacteria (0.27)	<i>Desulfobacca</i> (0.19)
11	Actinobacteria (1)	<i>Ilumatobacter</i> (1)

of methanotrophs (McDonald et al., 2008). In contrast to the 16S rRNA-based survey, the *pmoA*-based PCR yielded amplicons within all surface- and deep-water samples (Fig. 7) attesting to the ubiquitous presence of MO_x communities in waters of Storfjorden. However, besides the expected product of 530 bp, all deep-water samples showed a further, longer amplicon. Nevertheless, none of the > 530 bp amplicons could be affiliated with known *pmoA* genes, which suggests either novel *pmoA* types or unspecific PCR products. A similar distinction of the water masses was also apparent from the distribution of the *mxoF* gene (Fig. 7) that encodes the enzyme methanol dehydrogenase, which catalyzes the second step in CH_4 oxidation. The *mxoF* gene was also found

in all samples, but deep-water samples showed several additional, weak, and shorter *mxoF* bands.

4 Discussion

4.1 Water column stratification and methane sources

Storfjorden water column mixing regimes were the subject of several previous publications (e.g., Haarpaintner et al., 2001; Skogseth et al., 2005; Fer, 2006). The fjord is a deep semi-enclosed basin in the Svalbard Archipelago characterized by brine formation as a result of ice formation in latent heat polynyas during wintertime (Haarpaintner et al., 2001). Descending brines induce strong vertical mixing (Jardon et al., 2011) and turbulence at the sediment–water interface. However, accumulation of brine in bottom waters also leads to a stabilization of the water column, which is further enhanced through a ~ 60 m thick surface layer of relatively salt-depleted MW in summertime (Fig. 4). The residence time of the deep BSW is 90–246 days, relatively long compared to the 51–141 days of the surface water (Geyer et al., 2009), so that ongoing oxygen consumption leads to the comparably low oxygen levels that were detected previously (Anderson et al., 1988) and in this study.

CH_4 concentrations in Storfjorden are generally high, at 6–72 nM. These elevated concentrations originate from microbial methanogenesis in the sediments and enhanced transport from sediments into the water column as a result of the descending brines inducing turbulence at the sediment–water interface (Damm et al., 2007). However, CH_4 concentrations indicate a second CH_4 source at 40–60 m water depth (Fig. 5a). Here O_2 concentrations were high as well (Fig. 4c),

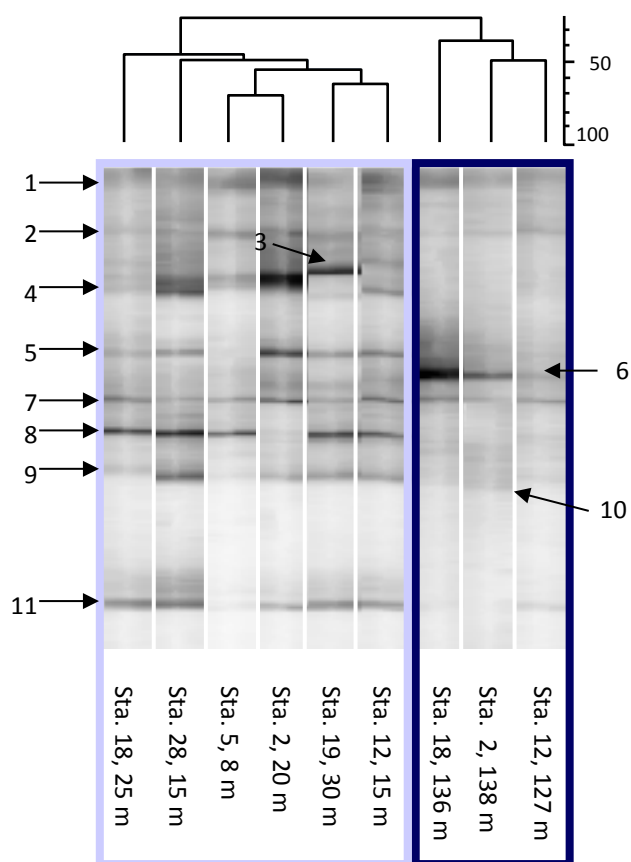


Fig. 6. DGGE profile of 16S rRNA gene fragments of MW and BSW samples from different stations in Storfjorden. Numbers on the left-hand side of the lanes indicate excised and successfully sequenced DGGE bands, whose phylogenetic assignment is listed in Table 2. MW and BSW samples are framed by a light-blue and dark-blue rectangle, respectively. Dendrogram derived from UP-GMA cluster analysis with the similarity coefficient of Jaccard.

possibly indicating a maximum of phytoplankton. The second CH_4 source could thus be related to water column in situ production by yet unidentified microorganisms utilizing the phytoplankton metabolite DMSP as a carbon source (Damm et al., 2008) or microbially produced MPn as a potential phosphorus source (Karl et al., 2008; Metcalf et al., 2012). However, further investigations are required to determine the role of these compounds as potential CH_4 precursors at Storfjorden.

While a significant fraction of the CH_4 is consumed (see Sect. 4.2), Storfjorden is apparently a CH_4 source to the atmosphere (Damm et al., 2007), as indicated by CH_4 concentrations of up to 30 nM in the well-mixed surface layer. These concentrations are highly supersaturated with respect to the atmospheric equilibrium (3.3–3.9 nM, Wiesenburg and Guinasso, 1979).

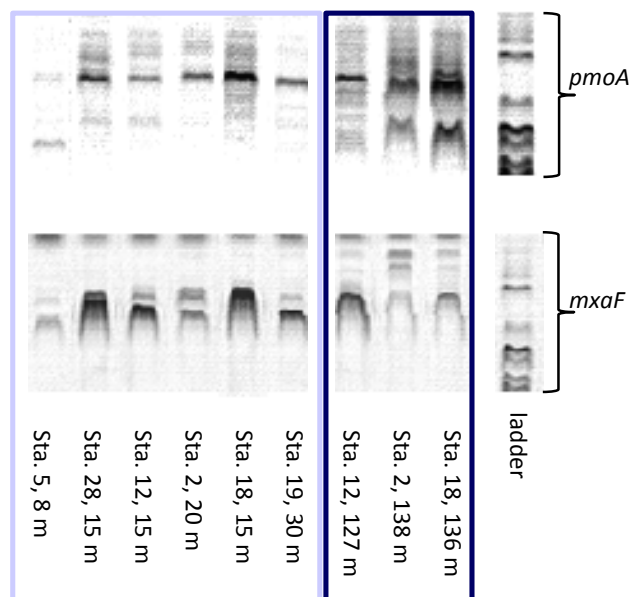


Fig. 7. Agarose-electrophoresis gels of PCR products of the *pmoA* and *mxoF* genes obtained from surface MW and deep BSW water samples of different stations in Storfjorden. MW and BSW samples are framed by a light-blue and dark-blue rectangle, respectively.

4.2 Vertical distribution of methane oxidation

Maximum MO_x rates in the water column of Storfjorden were $\sim 2 \text{ nM day}^{-1}$, and are thus very similar to MO_x rates measured in the Santa Barbara Basin (Pack et al., 2011; Mau et al., 2012) and the Black Sea (Reeburgh et al., 1991), which are both well-known areas of large methane input (Fig. 1). However, our rates are 3 orders of magnitude lower compared to the measurements conducted after the Deep Water Horizon accident in the Gulf of Mexico, during which catastrophic amounts of hydrocarbons were released into the water column, triggering a rapid response in MO_x activity (Valentine et al., 2010; Kessler et al., 2011). Other MO_x rate measurements were conducted in Bristol Bay and the southeast Bering Sea (Griffith et al., 1982), the Cariaco Basin (Ward et al., 1987), Saanich Inlet (Ward, 1989 and Ward and Kilpatrick, 1990), in Southern California Bight (Ward, 1992; Pack et al., 2011; Heintz et al., 2012; Ward and Kilpatrick, 1993), in the Eel River basin (Valentine et al., 2001), the Gulf of Mexico (Kelley, 2003), and at hydrothermal vents at Juan de Fuca Ridge (de Angelis et al., 1991, 1993). It appears that most of these MO_x rates fall into the range between 0.001 and 10 nM day^{-1} and that MO_x activity is elevated in ocean environments with high CH_4 concentrations. However, MO_x activity in the surface- (Ward, 1992; Kelley, 2003, this study) and open ocean (Sauter et al., 2006) seems to be rather low.

In addition to these larger-scale trends, our results from Storfjorden indicate distinct and vertically separated regimes of CH_4 oxidation. This distinction is apparent when

comparing MO_x rates in deep BSW (> 100 m) with surface MW (< 60 m). The ArW (60–100 m) appears to be an intermediate between the two regimes (Fig. 5b and c). We incubated parallel samples with ^3H - and ^{14}C -labeled CH_4 . While absolute rate measurements with ^3H - CH_4 were moderate in ArW and BSW, rates with ^{14}C - CH_4 were elevated in these water masses. We suggest that this is related to the different amounts of CH_4 that were added as a result of ^3H - CH_4 compared to ^{14}C - CH_4 application. While in incubations with ^3H - CH_4 , the final CH_4 concentrations were only raised by < 2 nM, ^{14}C - CH_4 amendments lead to a CH_4 increase of ~ 450 nM. It is therefore reasonable to assume that the activity of the deep-water MO_x community was stimulated as a result of elevated CH_4 concentrations (Pack et al., 2011). This is most likely related to enzyme kinetics (Ward and Kilpatrick, 1990; Bender and Conrad, 1993; Smith et al., 1997), which can be described with the Michaelis–Menten model (Button, 1985; Johnson and Goody, 2011, translation of the 1913 Michaelis–Menten paper). The Michaelis–Menten relation shows that enzyme activity, expressed by the reaction rate, increases hyperbolically with substrate concentration but levels off once the enzymatic machinery involved in the metabolic pathway is saturated with substrate. Similar relations were found between cell- or community-specific rates and substrate concentrations (Button, 2010, and references therein). For a stable community, a maximum rate thus exists, which may only increase as a result of elevated enzyme concentrations (e.g., population growth) and/or optimization of cytoarchitectural components relevant for substrate metabolism (e.g., transporter system). We could show that substrate turnover rates were linear over the incubation time period of three days (Fig. 3). At least for our incubation experiments, it thus seems unlikely that the CH_4 amendments induced an increase in enzyme concentration or optimization of other parameters relevant for substrate metabolism.

The derivative of the Michaelis–Menten function for low substrate concentrations (CH_4 concentrations lower than half saturation constant, k_m) yields the first-order rate constant (k'), which, multiplied with the substrate concentration, defines the actual rate (r_{ox} ; see Eq. 1). Consequently, under substrate-limiting conditions, k' values are high but decrease if substrate concentrations approach enzyme saturation level. This relationship is depicted in Fig. 8. In MW (the fjord's surface layers), k' values were high during ^3H - CH_4 incubations, i.e., without substantial CH_4 amendments, but the addition of CH_4 in the ^{14}C - CH_4 incubations led to a substantial decrease (5–10 fold) in k' , which suggests enzyme saturation. On the other hand, the deep-water community in ArW and particularly in BSW appeared to operate at CH_4 concentrations below saturation because the addition of CH_4 through ^{14}C - CH_4 tracer application led to an increase in k' compared to parallel incubations with ^3H - CH_4 .

It should be noted that rate measurements with two different substrate concentrations (we added 2 nM and 450 nM) are not useful for a kinetic study yielding k_m and/or the max-

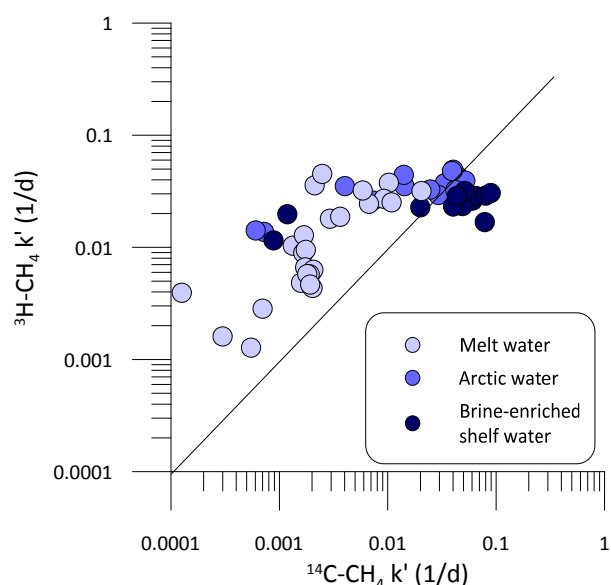


Fig. 8. Comparison of rate constants (k') determined with ^3H - CH_4 - and ^{14}C - CH_4 tracers. Straight line shows the 1:1 fit; that is, if k' derived from both tracers were equal. Samples from surface melt water fall above this line (k' determined by ^3H - CH_4 is higher than k' derived by ^{14}C - CH_4) and samples from the deep brine-enriched shelf water mainly fall below this line (k' determined by ^{14}C - CH_4 is higher than k' derived by ^3H - CH_4).

imum reaction velocity (v_{max}). Nevertheless, adaptation to different substrate concentrations, as indicated by our results, can (at least in parts) be explained by the Michaelis–Menten concept. The MO_x community inhabiting the deeper water masses, which are periodically exposed to high CH_4 concentrations, seems to be adapted to high CH_4 substrate levels. In contrast, the community inhabiting surface waters with generally low CH_4 concentrations seems not to be adapted to metabolize additional CH_4 , which could be related to a low enzymatic k_m . Low k_m values, which were found to range between 10 nM and 10 μM (e.g., Bender and Conrad, 1993; Baani and Liesack, 2008), could indeed explain this phenomenon. However, the available k_m values from the literature were determined from organisms found in terrestrial or freshwater environments or from cultured bacteria, which most likely do not represent the rather unknown marine communities. Furthermore, the enzymatic k_m may not be the same as the apparent cell/community-based k_m (see discussion by Button, 2010).

The question remains as to why the MO_x communities in deep and surface waters were apparently adapted to high and low CH_4 concentrations, respectively. Relatively low CH_4 concentrations in deeper water layers seem to be a regular feature of Storfjorden, at least during summertime (Damm et al., 2008). However, during wintertime, CH_4 export from the sediments is enhanced, leading to elevated CH_4 concentration of up to ~ 60 nM with a $\delta^{13}\text{C}$ -signature of -40 to

–50‰ in deeper water layers of Storfjorden (Damm et al., 2007). It thus appears reasonable to assume that the deep-water community is adapted to comparably high wintertime CH₄ concentrations. In summertime, ongoing CH₄ oxidation leads to decreasing CH₄ concentrations and an increase in ¹³C in the residual CH₄ (Fig. 5). In contrast, surface CH₄ seems only to increase strongly during summer (to ~ 50 nM), potentially as a result of CH₄ production in the oxic water column. However, we cannot explain why surface-water methanotrophs appear not to have adapted to the high summertime CH₄ concentrations or possibly lack the ability to adapt.

4.3 Microbial community

Similar to the MO_x regimes, the diversity of the bacterial assemblage was different when comparing surface MW to the deep BSW. Our DGGE analyses indicate a higher microbial diversity in surface- compared to the deep water (Fig. 6, Table 2). Nevertheless, we only found one band in the surface water (#9) and one band in the deep water (#6) that might be related to CH₄ oxidizers. Band #9 could be affiliated to the genus *Haliaea* of which novel isolates were found to oxidize ethylene and to possess genes similar to those encoding pMMO (Suzuki et al., 2012). Band #6 could be assigned to a known aerobic methanotroph of the genus *Methylosphaera* (yet with a relatively low confidence value of 0.38). Species of the order *Methylosphaera* were previously found in Antarctic marine salinity, meromictic lakes (Bowman et al., 1997). The different patterns of MO_x-related bands in surface- and deep water thus indicate the presence of different MO_x communities in these water masses.

Similar to the 16S gene rRNA-based survey, the *pmoA* and *mxoF* gene analyses indicated differences between surface- and deep-water masses (Fig. 7). All samples analyzed generated amplicons with both primer sets; the deep-water samples, however, showed an additional, longer *pmoA* band and several weak, shorter *mxoF* bands suggesting the presence of different, and possibly novel, *pmoA*- and *mxoF*-related gene sequences or unspecific PCR products. Similar suggestions (novel sequences or unspecific PCR products) were given in other studies using the *pmoA* primers, which we used, resulting in a limited coverage of the methanotrophic diversity and yielding PCR products that could also not be assigned to known *pmoA* types (Bourne et al., 2001; Henckel et al., 2000; Tavormina et al., 2008). Nevertheless, in addition to the 16S banding pattern and MO_x rates at elevated CH₄ concentrations, this further indicates that surface- and deep waters comprise different MO_x communities.

The question remains as to what are the driving mechanisms for the development of the MO_x communities in the different water masses. Here, we suggest that resuspension of sediments as a result of turbulent mixing during wintertime could have inoculated the deeper water masses with sediment microbes including benthic MO_x communities. These are of-

ten distinct from planktonic communities (Bowman et al., 1997; He et al., 2012; Tavormina et al., 2008), and probably adapted to higher CH₄ concentrations. This scenario would also explain the presence of the sulfate reducer *Desulfobacca* in the oxic deep waters. Sulfate-reducing bacteria are usually adapted to an anoxic environment (e.g., sediments), and may tolerate only low O₂ levels, yet resting cells of sulfate reducers were also found in fully oxygenated waters (Hastings and Emerson, 1988; Teske et al., 1996). In contrast to the deep waters, the comparably short residence time of surface waters and the rather rapid exchange with the Barents Sea argues for a planktonic source of MO_x communities in this water mass.

5 Conclusions

Incubation experiments with different substrate levels (here we used different tracers) are useful to identify distinct methanotrophic responses in different water masses. With respect to the natural CH₄ concentrations of our study site (< 80 nM, Fig. 5), we used ³H-CH₄ amendments, which altered absolute CH₄ concentrations by 1–2 nM. The ¹⁴C-CH₄ amendments, in contrast, increased CH₄ concentrations by 440–540 nM. The ³H-CH₄ ex situ tracer incubations thus yield rates that may be similar to in situ rates. ¹⁴C-CH₄ ex situ rates were within the same order of magnitude as those determined with ³H-CH₄. Yet, because of the high CH₄ concentration increase during our incubations with ¹⁴C-CH₄, these ex situ rates rather provide an indication for the rate potential of the MO_x community. Rate measurements typically provide a temporal snapshot, which is difficult to upscale, particularly in environments with spatiotemporally varying CH₄ fluxes. Knowledge on the MO_x rate at elevated CH₄ concentrations, on the other hand, provides a means to estimate the response in MO_x activity in relation to changing CH₄ fluxes.

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