

Different  
methanotrophic  
potentials in stratified  
polar fjord waters

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# Different methanotrophic potentials in stratified polar fjord waters (Storfjorden, Spitsbergen) identified by using a combination of methane oxidation techniques

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## Abstract

The bacterially mediated aerobic methane oxidation (MOx) is a key mechanism in controlling methane (CH<sub>4</sub>) emissions from the world's oceans to the atmosphere. In this study, we investigated MOx in the Arctic fjord Storfjorden (Spitsbergen) by applying a combination of radio-tracer based incubation assays (<sup>3</sup>H-CH<sub>4</sub> and <sup>14</sup>C-CH<sub>4</sub>), stable C-CH<sub>4</sub> isotope measurements, and molecular tools (16S rRNA 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 60–100 m and brine-enriched shelf water (BSW) down to 140 m. CH<sub>4</sub> concentrations were supersaturated with respect to the atmospheric equilibrium (~3 nM) throughout the water column, increasing from ~20 nM at the surface to a maximum of 72 nM at 60 m and decreasing below. MOx rate measurements at near in situ CH<sub>4</sub> concentrations (here measured with <sup>3</sup>H-CH<sub>4</sub> raising the ambient CH<sub>4</sub> pool by < 2 nM) showed a similar trend: low rates at the sea surface increasing to a maximum of ~2.3 nMd<sup>-1</sup> at 60 m followed by a decrease in the deeper ArW/BSW. In contrast, rate measurements with <sup>14</sup>C-CH<sub>4</sub> at elevated CH<sub>4</sub> concentrations (incubations were spiked with ~450 nM of <sup>14</sup>C-CH<sub>4</sub>, providing an estimate of the CH<sub>4</sub> oxidation potential) showed comparably low turnover rates (< 1 nMd<sup>-1</sup>) at 60 m, but peaked in ArW/BSW at ~100 m water depth, concomitant with increasing <sup>14</sup>C-values in the residual CH<sub>4</sub> pool. Our results indicate that the MOx community in the surface MW is adapted to relatively low CH<sub>4</sub> concentrations. In contrast, the activity of the deep water MOx community is relatively low at the ambient, summertime CH<sub>4</sub> concentrations but has the potential to increase rapidly in response to CH<sub>4</sub> availability. A similar distinction between surface and deep water MOx is also suggested by our molecular analyses. Although, we found *pmoA* and *mxoF* gene sequences throughout the water column attesting the ubiquitous presence of MOx communities in Storfjorden, deep water amplicons of *pmoA* and *mxoF* were unusually long. Also a DGGE band related to the known Type I MOx *Methylobacter* was observed in deep BSW, but absent in surface MW. Apparently, different

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MOx communities have developed in the stratified water masses in Storfjorden, which is possibly related to the spatiotemporal variability in CH<sub>4</sub> supply to the distinct water masses.

## 1 Introduction

5 Methane (CH<sub>4</sub>) is a potent greenhouse gas with a global warming potential that exceeds carbon dioxide (CO<sub>2</sub>) 23-fold over a 100 yr timescale and is, after water vapour and CO<sub>2</sub>, 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<sub>4</sub> is generated under reduced conditions in anoxic marine sediments, dominantly through microbially mediated carbonate reduction and disproportionation of methylated substrates (Whiticar, 1999; Hinrichs and Boetius, 2002; Formolo, 2010). Sedimentary CH<sub>4</sub> is also formed by thermal breakdown of organic matter and, although of lesser importance, the Fischer–Tropsch reaction, both occurring at high temperature and pressure. In addition, conspicuous CH<sub>4</sub> concentrations maxima in oxic water layers provided indications for CH<sub>4</sub> production under oxic conditions possibly mediated by yet unknown microbes using dimethylsulfoniopropionate (DMSP) (Damm et al., 2010) or methylphosphonic acid (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 CH<sub>4</sub> is liberated from the oceans into the atmosphere as a result of microbial consumption (Reeburgh, 2007; IPCC, 2007). About 80 % of sedimentary CH<sub>4</sub> is consumed in reduced sediments as a result of the anaerobic oxidation of methane (AOM) with sulphate as the terminal electron acceptor (Reeburgh, 2007; Knittel and Boetius, 2009). Finally, aerobic CH<sub>4</sub>-oxidising bacteria at the sediment surface and/or in the water column (belonging to the alpha (Type II) or gamma (Type I and Type X) subdivision of the *Proteobacteria*) consume CH<sub>4</sub> that has

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by-passed the anaerobic microbial filter (Hanson and Hanson, 1996) according to the following reaction:



Several techniques have been used to quantify aerobic methane oxidation (MOx) rates (Reeburgh, 2007). A common method is to incubate water column or sediment samples with radio-labelled tracers such as  $^{14}\text{C-CH}_4$  or  $^3\text{H-CH}_4$  (Reeburgh et al., 1991; Valentine et al., 2001; Niemann et al., 2006; Mau et al., 2012), which has been proven highly sensitive. During the incubation,  $^{14}\text{C-CH}_4$  or  $^3\text{H-CH}_4$  are converted at the same rate as the natural, non-labelled  $\text{CH}_4$  to  $^{14}\text{CO}_2$  and  $^{14}\text{C-biomass}$  or  $^3\text{H}_2\text{O}$ . Despite the importance of water column MOx controlling oceanic  $\text{CH}_4$  emission to the atmosphere, only a small number of water column MOx rate measurements exists, 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 MOx activity such as temporal variations in  $\text{CH}_4$  availability (e.g. Mau et al., 2007a, b; Damm et al., 2007) and the rate potential, i.e. the maximum uptake rate, of the present MOx community are not well constrained.

Our aims for this study were to investigate MOx rates and rate potentials as well as the key MOx community in response to different  $\text{CH}_4$  concentrations in a natural marine environment. As a model system, we choose the fjord Storfjorden (Svalbard), which is characterised by seasonal stratification, separating distinct water masses with different  $\text{CH}_4$  sources during summer time.

## 2 Material and methods

### 2.1 Study site

Storfjorden is located in the Svalbard Archipelago between the islands Spitsbergen, Barentsøya, and Edgeøya (Fig. 2).  $\text{CH}_4$  concentrations in the fjord water exceed

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atmospheric equilibrium concentration throughout the water column by a factor of 2–16, although surface waters CH<sub>4</sub> is of a different origin compared to the CH<sub>4</sub> in subsurface waters (Damm et al., 2008). Surface waters contain recently produced, <sup>14</sup>C-depleted CH<sub>4</sub>, which was proposed to result from a summer phytoplankton bloom producing methylated compounds such as DMSP, which is a potential substrate for methylotrophic methanogenesis. A CH<sub>4</sub> production-removal cycle appears to be established in the surface water as reflected by varying CH<sub>4</sub> concentrations and <sup>13</sup>C-CH<sub>4</sub> values (Damm et al., 2008). In contrast, deeper water contains CH<sub>4</sub> 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 re-suspension of sediments releasing CH<sub>4</sub> (Damm et al., 2007). The winter-released CH<sub>4</sub> is then trapped by increasing water stratification during warmer seasons and on-going CH<sub>4</sub> consumption leads to a <sup>13</sup>C-enriched isotopic signature of the residual CH<sub>4</sub>. During summer time the water column is stratified where surface melt water (MW) and intermediate Arctic water (ArW) occupy the upper water column, whereas denser BSW is restricted to deep basins (Loeng, 1991). The residence time of the high-salinity water is longer in deeper layers (90–246 d) compared to the fjord's surface waters (51–141 d) (Geyer et al., 2009).

## 2.2 Sampling

Water samples were collected from nine stations in Storfjorden and at one open ocean station (70° 35.913' N and 10° 51.591' 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<sub>4</sub> 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

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BSW for molecular analyses (method 2.5). Specific water depths were sampled with a CTD/rosette sampler equipped with 12 five-litre Niskin bottles, a Seabird SBE 911 plus CTD and an SBE 43 oxygen sensor for online monitoring of salinity, temperature, pressure and dissolved oxygen.

### 5 2.3 CH<sub>4</sub> concentrations and stable isotope composition

Aliquots of sea water were immediately subsampled from the Niskin bottles using 1 L glass bottles for CH<sub>4</sub> concentration measurements. CH<sub>4</sub> was extracted from the water by vacuum-ultrasonic treatment within a few hours after sampling (Schmitt et al., 1991). Hydrocarbon concentrations were measured with a Chrompack 9003 gas chromatograph (GC) equipped with a flame ionization detector (FID). Duplicate analyses indicate an error of 5–10 % (Lammers and Suess, 1994). After GC analyses, an aliquot of the extracted CH<sub>4</sub> 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 PreCon equipment (Finnigan) to pre-concentrate the sample. All isotopic ratios have an analytical error  $\leq 1\text{‰}$  and are presented in the  $\delta$ -notation against the Vienna Pee Dee Belemnite (VPDB) standard.

### 2.4 Methane oxidation rates

MOx 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  $\mu$ l gas mixture comprised of <sup>3</sup>H-labelled CH<sub>4</sub> (160–210 kBq) and a second set was incubated with 10  $\mu$ l of <sup>14</sup>C-labelled CH<sub>4</sub> (12–15 kBq). <sup>3</sup>H-CH<sub>4</sub> tracer addition raised ambient CH<sub>4</sub> concentrations by 1–2 nM and <sup>14</sup>C-CH<sub>4</sub> addition by 440–540 nM. The samples were subsequently shaken for  $\sim 10$  min on an orbital shaker to facilitate tracer dissolution and then incubated in the dark at 2 °C. CH<sub>4</sub> oxidation rates ( $r_{ox}$ ) were calculated

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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 labelled  $\text{CH}_4$  oxidised per unit time and  $[\text{CH}_4]$  is the ambient  $\text{CH}_4$  concentration.

5 In order to determine a suitable incubation time period, we performed parallel time series incubations with samples collected from the fjord (Station 2 and 18) and from an open water station (reference station – RS). During each incubation series, tracer consumption was measured in duplicates after 0.5, 1, 2, 3 and 4 or 5 days. In the  $\text{CH}_4$  rich waters of the fjord, our results showed a linear tracer consumption of about  
10 5–15% over the first 3 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  $\text{MOx}$  was determined in duplicates.

Incubations with  $^3\text{H-CH}_4$  and measurements of  $^3\text{H-CH}_4$  and  $^3\text{H-H}_2\text{O}$  was carried out  
15 according to Valentine et al. (2001) and Mau et al. (2012). Briefly, total activity ( $^3\text{H-CH}_4 + ^3\text{H-H}_2\text{O}$ ) was measured in 1 mL of sample aliquot by wet scintillation counting and activity of  $^3\text{H-H}_2\text{O}$  was measured after sparging the sample for  $\geq 30$  min with nitrogen gas to remove remaining  $^3\text{H-CH}_4$ .

Incubations with  $^{14}\text{C-CH}_4$  were terminated by injecting 0.5 mL of 10 M NaOH  
20 and adding a 5 mL headspace so that the remaining  $^{14}\text{C-CH}_4$  accumulated in the headspace and the produced  $^{14}\text{C-CO}_3^{2-}$  and  $^{14}\text{C-biomass}$  was trapped in the aqueous NaOH solution. Separation and activity measurement of  $^{14}\text{C-CH}_4$  and  $^{14}\text{C-CO}_3^{2-}$  were carried out analogous to previous measurements of  $\text{CH}_4$  turnover in sediments (Treude et al., 2003; Niemann et al., 2005). In short,  $^{14}\text{C-CH}_4$  in the  
25 headspace was combusted to  $^{14}\text{C-CO}_2$ , while  $^{14}\text{C-CO}_3^{2-}$  was converted to  $^{14}\text{C-CO}_2$  through acidification with HCl. In either case,  $^{14}\text{C-CO}_2$  was then trapped in a solution of methoxyethanol:penylethylamine and the radioactivity was measured by

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wet scintillation counting. We also measured remaining radioactivity (presumably org.  $^{14}\text{C}$ ) in the sample after  $^{14}\text{C-CH}_4$  and  $^{14}\text{CO}_3^{2-}$  removal.

## 2.5 Diversity of MOx 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  $\mu\text{m}$  pore size) and the filters were stored frozen at  $-20^\circ\text{C}$  until DNA extraction. Total community DNA was extracted using the Ultraclean soil DNA kit (MoBio Laboratories, USA). 1–5  $\mu\text{L}$  DNA extract was applied as template in the 16S rRNA gene specific PCR with GM5 plus GC-clamp as forward primer and 907RM as reverse primer (Muyzer et al., 1993). PCR conditions were as described by Gerdes et al. (2005). PCR-products (ca. 500 bp) were analysed 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 re-amplified by PCR as described by Gerdes et al. (2005) and sequenced. The 16S rDNA gene sequences were then assigned to the new higher-order taxonomy proposed in Bergey's taxonomic 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  $\text{CH}_4$  oxidising bacteria in the communities was screened by 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 methylo-trophic 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.

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## 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 ( $T$ :  $< -1.5^{\circ}\text{C}$ ,  $S$ :  $> 34.8$ ) (Fig. 4d).

The MW extended from the surface to  $\sim 60$  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,  $\text{CH}_4$  concentrations increased from  $\sim 20$  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  $^3\text{H}$ - and  $^{14}\text{C}$ -tracer increased with depth to  $2.3\text{ nM d}^{-1}$  and  $0.77\text{ nM d}^{-1}$ , respectively (Fig. 5b and c). In the MW, rates measured with  $^{14}\text{C}$ -tracer were consistently lower than those determined with  $^3\text{H}$ -tracer.  $^{13}\text{C}\text{-CH}_4$  values in this water mass ranged between  $-43.5$  and  $-53.6\text{‰}$  (Fig. 5d).

In the ArW, (60 to  $\sim 100$  m water depth) oxygen concentrations decreased from  $350$  to  $320\text{ }\mu\text{M}$  (Fig. 4c) and  $\text{CH}_4$  concentrations from  $42$  to  $6.5\text{ nM}$  (Fig. 5a). Both, MOx rates determined with  $^3\text{H}$  and  $^{14}\text{C}$ -tracer show a maximum at  $\sim 80$  m in this water mass (Fig. 5b and c). The stable carbon isotopic signature of  $\text{CH}_4$  showed a strong shift from  $-46\text{‰}$  to about  $-32\text{‰}$  at the MW/ArW interface (80 m, Fig. 5d).

The BSW ( $> 100$  m water depth) was characterised by oxygen concentrations below  $320\text{ }\mu\text{M}$  (Fig. 4c).  $\text{CH}_4$  concentrations decreased slightly with depth, but were stable below  $120$  m ( $8\text{--}9\text{ nM}$ , Fig. 5a). MOx rates determined with  $^3\text{H}$ -labelled  $\text{CH}_4$  show a similar trend as the  $\text{CH}_4$  concentrations. However, while  $^3\text{H}$ -MOx rates were low, rates determined with  $^{14}\text{C}$ -labelled  $\text{CH}_4$  were comparably higher with a maximum of  $1.9\text{ nM d}^{-1}$  at  $\sim 100$  m water depth (Fig. 5b and c). The carbon isotopic signature of

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the CH<sub>4</sub> 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

5 Similar to the biogeochemical results, the MW and BSW at the studied stations (St. 2, 5, 12, 18, 19) showed DGGE banding patterns (Fig. 6, Table 2) indicating that surface- and deep-water 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 *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 *Flavicola* within the phylum *Bacteroidetes*, *Halieta* within the *Gammaproteobacteria*, and *Ilumato-*  
10 *tobacter* within the phylum *Actinobacteria*. Although we could measure CH<sub>4</sub> 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 (Sta. 12, 127 m, Sta. 2, 138 m, Sta.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  
20 upper water masses while band #6 was only found in the BSW samples. This band could be affiliated to *Methylosphaera*, which is a known type I aerobic methanotrophic bacterium (Bowman et al., 1997). However, the confidence value of 0.38 (Table 2) was relatively low. 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).

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## 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 of methanotrophs (McDonald et al., 1997). In contrast to the 16S rRNA based survey, the *pmoA* based PCR yielded amplicons within all surface- and deep-water samples (Fig. 7) attesting the ubiquitous presence of MOx communities in waters of Storfjorden. However, besides the expected product of 530 bp, all deep-water samples showed a further, longer amplicon. Nevertheless, the sequences of all these amplicons could not be affiliated to known *pmoA* genes. 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 catalyses the second step in CH<sub>4</sub> 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 characterised 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 stabilisation of the water column, which is further enhanced through a ~ 60 m thick surface layer of relatively ion-depleted MW in summertime (Fig. 4). The residence time of the deep BSW is with 90–246 d relatively long compared to 51–141 d of the surface water (Geyer et al., 2009), so that on-going oxygen

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consumption leads to the comparably low oxygen levels that were detected previously (Anderson et al., 1988) and in this study.

CH<sub>4</sub> concentrations in Storfjorden are generally high with 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<sub>4</sub> concentrations indicate a second CH<sub>4</sub> source at 40–60 m water depth (Fig. 5a). Here O<sub>2</sub> concentrations were high as well (Fig. 4c), possibly indicating a maximum of phytoplankton. The second CH<sub>4</sub> source is probably related to water column in situ production by yet unidentified microorganisms utilising the phytoplankton metabolite DMSP as a carbon source (Damm et al., 2008). While a significant fraction of the CH<sub>4</sub> is consumed (see Sect. 4.2), Storfjorden is apparently a CH<sub>4</sub> source to the atmosphere (Damm et al., 2007) as indicated by CH<sub>4</sub> 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).

## 4.2 Vertical distribution of methane oxidation

Our results indicate two regimes of CH<sub>4</sub> oxidation when comparing deep BSW (> 100 m) and surface MW (< 60 m). The ArW (60–100 m) appears to be an intermediate between the two regimes. This distinction is apparent from the vertical distribution of MOx rates (Fig. 5b and c). We incubated parallel samples with <sup>3</sup>H- and <sup>14</sup>C-labelled CH<sub>4</sub>. While absolute rate measurements with <sup>3</sup>H-CH<sub>4</sub> were moderate in ArW and BSW, rates with <sup>14</sup>C-CH<sub>4</sub> were elevated in these water masses. We suggest that this is related to the different amounts of CH<sub>4</sub> that were added as a result of <sup>3</sup>H-CH<sub>4</sub> compared to <sup>14</sup>C-CH<sub>4</sub> application. While in incubations with <sup>3</sup>H-CH<sub>4</sub>, the final CH<sub>4</sub> concentrations were only raised by < 2 nM, <sup>14</sup>C-CH<sub>4</sub> amendments lead to a CH<sub>4</sub> increase of ~ 450 nM. It is therefore reasonable to assume that the activity of the deep water MOx community was stimulated as a result of elevated CH<sub>4</sub> concentrations (Pack et al., 2011). This is

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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; Translation of the 1913 Michaelis-Menten paper; Johnson and Goody, 2011). 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 enzyme concentration increase (e.g. population growth) and/or optimisation of cytoarchitectural components relevant for substrate metabolism (e.g. transporter system). We could show that substrate turnover rates were linear over the incubation time of 3 d (Fig. 3), so that it seems unlikely that the CH<sub>4</sub> amendments induced an increase in enzyme concentration or optimisation of other parameters relevant for substrate metabolism, at least over the time period of our incubation experiments.

The derivative of the Michaelis-Menton function (for low substrate concentrations) 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 <sup>3</sup>H-CH<sub>4</sub> incubations, i.e. without substantial CH<sub>4</sub> amendments, but the addition of CH<sub>4</sub> in the <sup>14</sup>C-CH<sub>4</sub> 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<sub>4</sub> concentrations below saturation because the addition of CH<sub>4</sub> through <sup>14</sup>C-CH<sub>4</sub> tracer application led to an increase in  $k'$  compared to parallel incubations with <sup>3</sup>H-CH<sub>4</sub>.

The question remains as to why the MOx communities in deep and surface waters were apparently adapted to high and low CH<sub>4</sub> concentrations, respectively. Relatively low CH<sub>4</sub> concentrations in deeper water layers seem to be a regular feature of

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Storfjorden, at least during summertime (Damm et al., 2008). However, during winter time, CH<sub>4</sub> export from the sediments is enhanced leading to strongly elevated CH<sub>4</sub> concentration of up to ~ 60 nM with a <sup>14</sup>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 CH<sub>4</sub> concentrations. In summertime, on-going CH<sub>4</sub> oxidation leads to decreasing CH<sub>4</sub> concentrations and an increase in <sup>13</sup>C in the residual CH<sub>4</sub> (Fig. 5). In contrast, surface CH<sub>4</sub> seems only to increase strongly during summer (to ~ 50 nM), potentially as a result of phytoplankton induced DMSP production, which fuels methanogenesis in the oxic water column (Damm et al., 2008). However, we cannot explain why surface-water methanotrophs appear not to have adapted to the high summertime CH<sub>4</sub> concentrations or possibly lack the ability to adapt.

### 4.3 Microbial community

Similar to the MOx 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 could be related to CH<sub>4</sub> oxidisers. Band #9 could be affiliated to the genus *Haliea* of which novel isolates were found to oxidize ethylene and to possess genes similar to particulate methane monooxygenases (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 MOx-related bands in surface- and deep water thus indicate the presents of different MOx-communities in these water masses.

Similar to the 16S rRNA based survey, the *pmoA* and *mxoF* gene analyses indicated differences between surface- and deep water masses (Fig. 7). Although, both genes

were detected in all samples analysed (attesting an ubiquitous presence of MOx communities in Strofjorden), the deep water samples showed an additional, longer *pmoA* band and several weak, shorter *mxoF* bands suggesting the presence of different *pmoA* and *mxoF* related gene sequences. In addition to the 16S banding pattern and rate potentials, this further indicates that surface- and deep waters comprise different MOx communities.

The question remains as to what are the driving mechanisms for the development of the MOx 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 MOx communities. These are often distinct from planktonic communities (Bowman et al., 1997; He et al., 2012; Tavormina et al., 2008) and probably adapted to higher CH<sub>4</sub> concentrations. This scenario would also explain the presence of the sulphate reducer *Desulfobacca* in the oxic deep waters. Sulphate reducing bacteria are usually adapted to an anoxic environment (e.g. sediments) and may tolerate only low O<sub>2</sub> levels, yet resting cells of sulphate reducers were also found in fully oxygenated waters (Hastings and Emerson, 1988; Teske et al., 1996). The comparably short residence time of surface waters and the rather rapid exchange with the Barents Sea argues for a planktonic source of MOx communities in this water mass.

## 5 Conclusions

Incubation experiments with different substrate levels (here we used different tracers) are useful to identify distinct methanotrophic potentials in different water masses. With respect to the natural CH<sub>4</sub> concentrations of our study site (< 80 nM, Fig. 5), we used <sup>3</sup>H-CH<sub>4</sub> amendments, which hardly altered absolute CH<sub>4</sub> concentrations, and <sup>14</sup>C-CH<sub>4</sub> amendments, which in contrast increased CH<sub>4</sub> concentrations by ~ 1 order of magnitude. The <sup>3</sup>H-CH<sub>4</sub> ex situ tracer incubations thus yield rates that may be similar to in situ rates. <sup>14</sup>C-CH<sub>4</sub> ex situ rates were within the same order of magnitude as those

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determined with  $^3\text{H-CH}_4$ . Yet, because of the high  $\text{CH}_4$  concentration increase during our incubations with  $^{14}\text{C-CH}_4$ , these ex situ rates rather provide an estimate for the rate potential of the MOx community. Rate measurements typically provide a temporal snapshot, which is difficult to upscale particularly in environments with spatiotemporal varying  $\text{CH}_4$  fluxes. Knowledge on the MOx rate potential, on the other hand, provides a mean to estimate the response in MOx activity in relation to changing  $\text{CH}_4$  fluxes.

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**Table 1.** Locations of stations and performed analyses.

Station	Latitude	Longitude	Analysis
1	77° N 05.64'	18° E 52.67'	[CH <sub>4</sub> ], MOx-rates, <sup>13</sup> C-CH <sub>4</sub>
2	77° N 05.23'	19° E 29.69'	MOx-rates time series, DGGE, <i>pmoA</i> , <i>mxoF</i>
5	77° N 04.54'	21° E 52.25'	[CH <sub>4</sub> ], MOx-rates, <sup>13</sup> C-CH <sub>4</sub> , DGGE, <i>pmoA</i> , <i>mxoF</i>
8	77° N 22.80'	21° E 35.43'	[CH <sub>4</sub> ], MOx-rates, <sup>13</sup> C-CH <sub>4</sub>
12	77° N 41.91'	19° E 14.49'	[CH <sub>4</sub> ], MOx-rates, <sup>13</sup> C-CH <sub>4</sub> , DGGE, <i>pmoA</i> , <i>mxoF</i>
15	77° N 41.45'	19° E 00.16'	[CH <sub>4</sub> ], MOx-rates, <sup>13</sup> C-CH <sub>4</sub>
18	78° N 15.29'	19° E 29.07'	[CH <sub>4</sub> ], MOx-rates, <sup>13</sup> CH <sub>4</sub> MOx-time series, DGGE, <i>pmoA</i> , <i>mxoF</i>
19	78° N 15.41'	20° E 20.14'	DGGE, <i>pmoA</i> , <i>mxoF</i>
28	76° N 34.95'	19° E 02.41'	DGGE, <i>pmoA</i> , <i>mxoF</i>
RS	70° N 35.91'	10° E 51.59'	MOx-rates time series

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**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 brackets.

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

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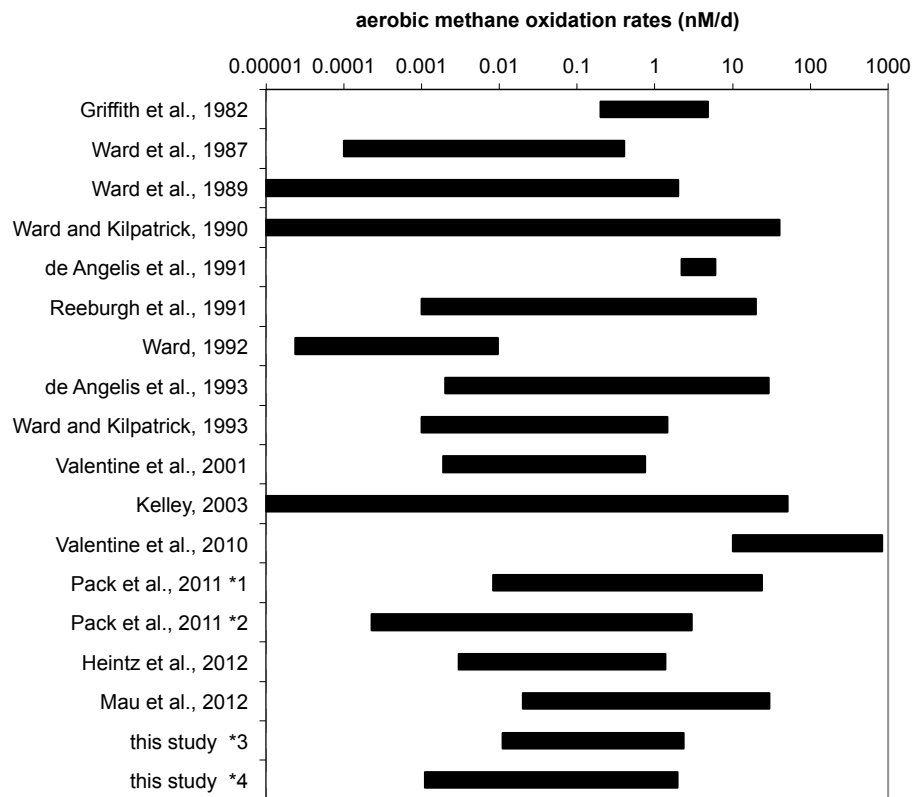
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**Fig. 1.** Range of methane oxidation rates measured at different locations in the ocean water column derived from tracer incubations using  $^3\text{H-CH}_4$  (Reeburgh et al., 1991; Valentine et al., 2001) or  $^{14}\text{C-CH}_4$  (all others). Pack et al., (2011) compared incubations with  $^3\text{H-CH}_4$  (\*<sup>1</sup>) and incubations with low-level  $^{14}\text{C-CH}_4$  (\*<sup>2</sup>) that were measured with accelerator mass spectrometry. In this study we compared incubations with  $^3\text{H-CH}_4$  (\*<sup>3</sup>) and incubations with  $^{14}\text{C-CH}_4$  (\*<sup>4</sup>).

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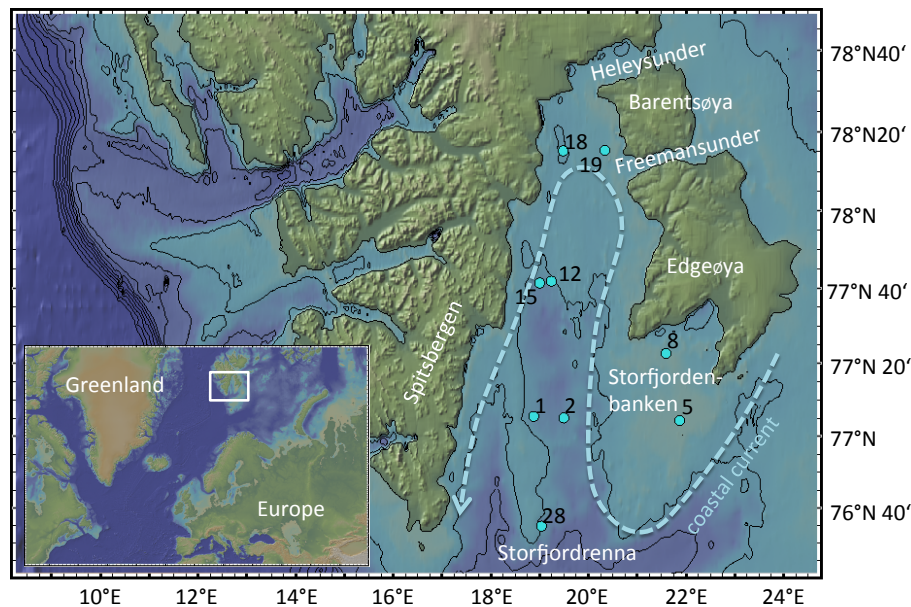
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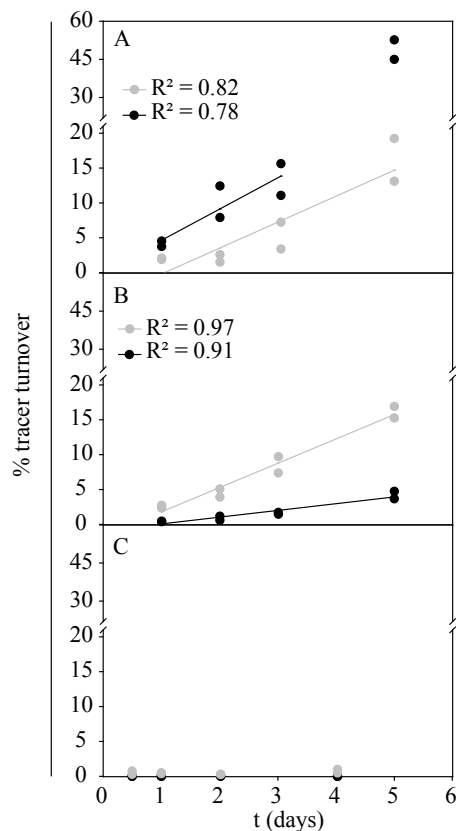


**Fig. 2.** Locations of the stations in Storfjorden. Stations are marked by blue dots and station numbers. Contours are drawn every 100 m until 1000 m water depth.

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**Fig. 3.** Time series collected at station 2 ( $77^{\circ} 5.226' \text{ N}$  and  $19^{\circ} 29.694' \text{ E}$ ) at 135 m water depth, at station 18 ( $78^{\circ} 15.288' \text{ N}$  and  $19^{\circ} 29.070' \text{ E}$ ) at 50 m water depth, and at a reference station (RS,  $70^{\circ} 36.117' \text{ N}$  and  $10^{\circ} 51.454' \text{ E}$ ) at 101 m water depth.  $^{14}\text{C-CH}_4$  and  $^3\text{H-CH}_4$  results are shown as black and gray circles, respectively.

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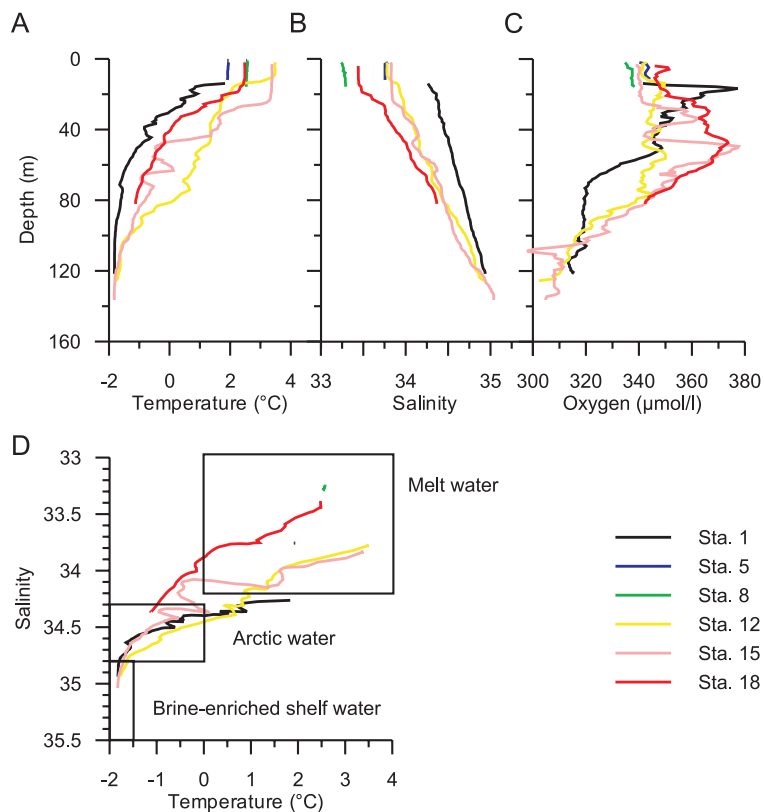
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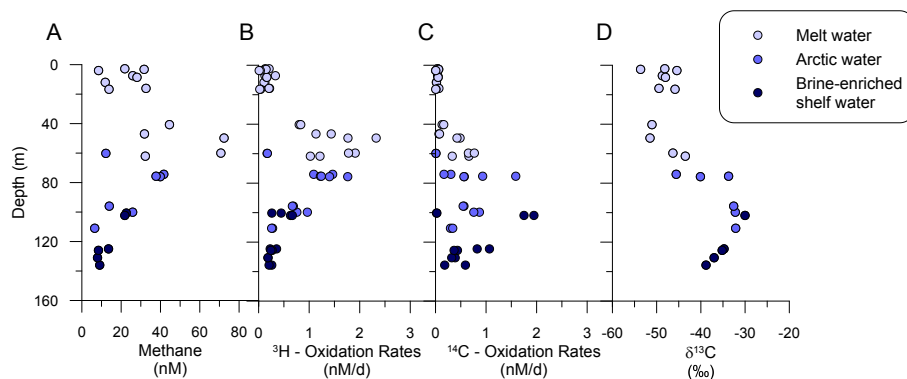


**Fig. 4.** Depth profiles of temperature (A), salinity (B), and oxygen concentrations (C) as well as a temperature–salinity graph with temperature–salinity ranges of the dominant water masses in Storfjorden (D). Stations 5 and 8 are less than 20 m deep and appear as dots in the temperature–salinity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

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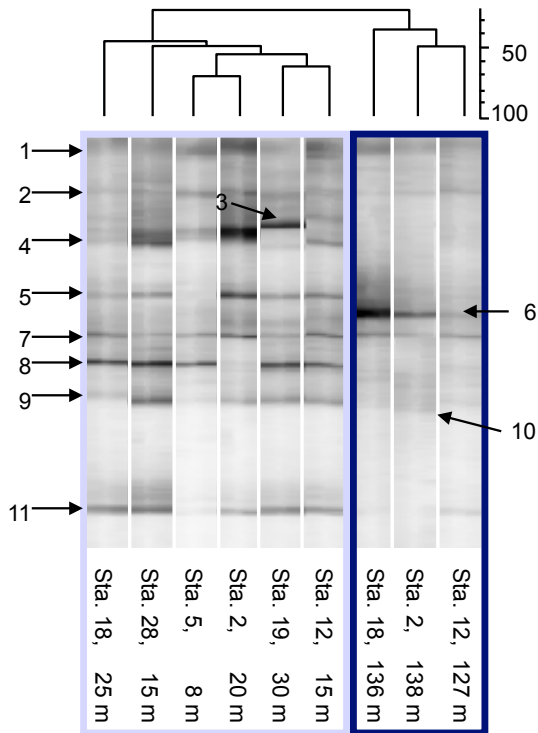
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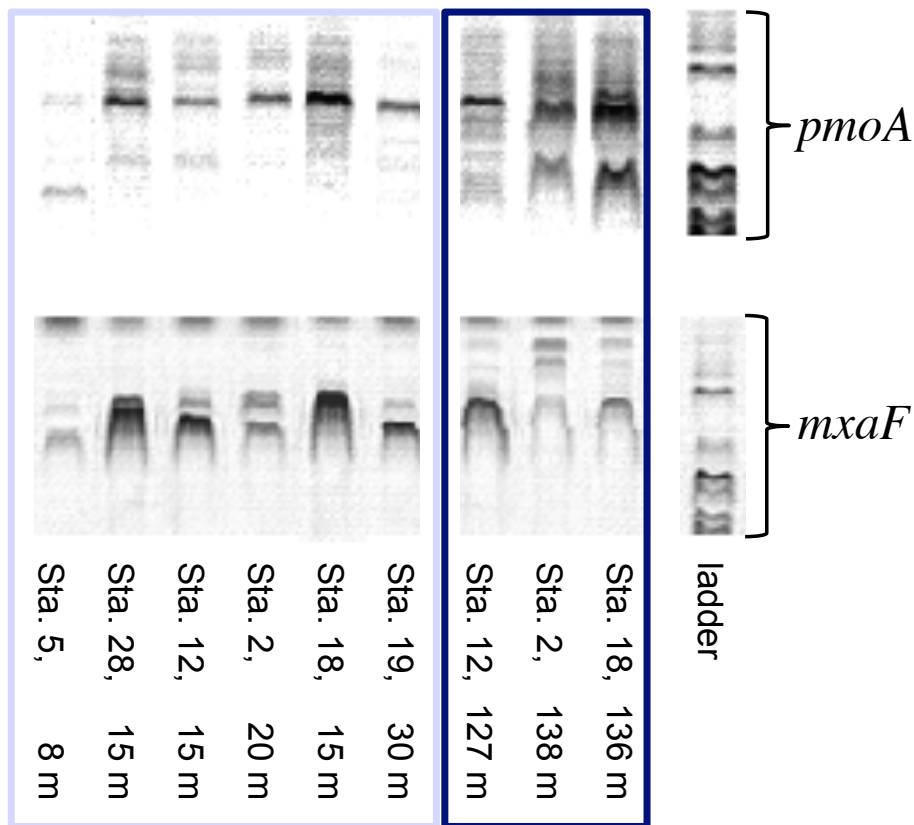


**Fig. 5.** Depth profiles of  $\text{CH}_4$  concentrations (**A**), oxidation rates derived by  $^3\text{H}$ - $\text{CH}_4$ - (**B**) and  $^{14}\text{C}$ - $\text{CH}_4$ -tracer (**C**), and isotopic C- $\text{CH}_4$  ratio ( $^{14}\text{C}$  values, **D**). Samples are color-coded according to the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

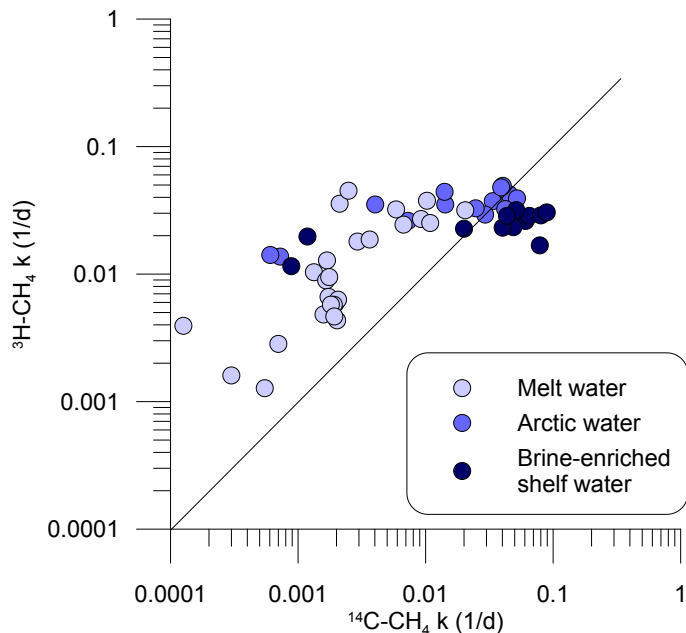
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**Fig. 6.** DGGE profile of 16S rRNA gene fragments of MW and BSW samples from different stations in the Storfjorden. Numbers on the left hand side of the lanes indicates excised and successfully sequenced DGGE bands whose phylogentic assignment is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



**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.



**Fig. 8.** Comparison of rate constants ( $k'$ ) determined with  $^3\text{H-CH}_4$ - and  $^{14}\text{C-CH}_4$ -tracer. Straight line shows the 1 : 1 fit, i.e. if  $k'$  derived from both tracers would be equal. Samples from surface melt water fall above this line ( $k'$  determined by  $^3\text{H-CH}_4$  is higher than  $k'$  derived by  $^{14}\text{C-CH}_4$ ) and samples from the deep brine-enriched shelf water mainly fall below this line ( $k'$  determined by  $^{14}\text{C-CH}_4$  is higher than  $k'$  derived by  $^3\text{H-CH}_4$ ).

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