Reply to associate editors comments:

Dear Helge,

Thank you for the positive feedback on our manuscript. We addressed all of your suggestions for edits. The answers can be found in the point-by-point reply. In addition, we replaced Figure 7 with a corrected y-axis label. The same correction was performed in tables 3 and 5 ($k_{ox,ppm}$ was replaced by k_{ox}). Small language and formatting edits are indicated in the manuscript via track changes.

Best regards,

Christiane on behalf of all co-authors

Associate Editor Decision: Publish subject to minor revisions (review by editor) (05 Apr 2018) by Helge Niemann
Comments to the Author:
Dear Christiane,

thank you very much again for submitting your work to Biogeosciences. Both reviewers checked the MS and are happy with the modifications. There are only a few minor issues, which I kindly ask you to address:

P1
L8. change phrasing "before it has a chance" reads like active intention of methane to escape. Rather use, "before it can escape.."
Changed as suggested

L24, methane cycling is an erroneous term, which is unfortunately rather widely used in the literature. It implies that methane is transformed into an oxidation product which is then a substrate for MOg. Mostly that is not the case
Changed to "methane dynamics"

L27. Use updated GWP from Etminan et al., 2016
Changed as suggested

P2
L8, there are much earlier discussion on methane dispersal than Gentz et al. 2014 (eg Damm's HMMV work). Also, dispersal/dilution does not hinder CH4 efflux to the atmosphere.
Thanks for this note, we added Damm and Budéus (2003) as reference and rephrased the section to: "Dissolved methane is diluted by the surrounding water column (e.g. Damm and Budéus, 2003; Gentz et al., 2014), in which it is used as a substrate and oxidized by aerobic methanotrophic bacteria (methane-oxidizing bacteria, MOB) (Hanson and Hanson, 1996; Murrell, 2010). As a result oceanic methane concentrations are frequently at low nanomolar levels, leaving only a small fraction of sediment-sourced methane to eventually exchange with the atmosphere (Karl et al., 2008; Reeburgh, 2007)."

L14, no ref given to the rather large seep area west off Svlabard

We added Myhre et al (2016), as reference showing that occasionally supersaturation of methane is also observed at the surface in this seep area.

L17 this is up to 700%

Changed to "up to 7 times", in accordance with next comment; same for P11 L26

L18, 160000% does as a number not make sense. Provide as factor

Changed to "1600 times"

L22, several studies could show that the CH4 content is not the driving force behind activity of MOBs, see refs in James et al., 2016

Rephrased to "Along with factors like oxygen and trace metal availability (Crespo-Medina et al., 2014; Sansone et al., 2001; Semrau et al., 2010), as well as local oceanographic and geologic conditions (Schmale et al., 2015; Steinle et al., 2015), dissolved methane concentration can be a control on the community of MOB and thus methane oxidation rates (Crespo-Medina et al., 2014; Kessler et al., 2011; Mau et al., 2013)."

L30ff, specify which MOBs can be targeted with pmoA

We added "aerobic" in L31 and L33

P4
L5. Rephrase to: temperature and salinity was recorded with…

Rephrased to "temperature and salinity were recorded with"

L12, specify the type of in situ measurement

Changed to "in situ methane concentration and carbon isotope ratios."

P9
L6. 'higher isotope signatures' does not make sense and I presume the value in line 7 is -54.4‰?

Changed to "higher methane concentrations and more positive isotope signatures". It is in fact "-54.4‰" the "-" was lost in the previous line.

P13, see above, RE methane cycling. Rather methane dynamics

Changed to "methane dynamics".
We also changed methane cycling to "methane dynamics" on P7 L22

L13, add Leonte et al., 2017

L14 we added Leonte et al, 2017

L11 here and throughout the text. Don't use x as a multiplication sign but rather ×

When x was used as multiplication sign we exchanged by × (e.g. P8 L21 7.7×10^4). For the treatment names "10x", "200x" etc. we introduced a definition (P5 L6).

L19 sample size in statistics is usually abbreviated as 'n' not 'N'

Throughout the entire manuscript, including figures 5 and 8 and table 3, sample size was changed to "n"

L25. …the microbial community possibly shifted towards…

Changed as suggested

P14

L18, the community size dependent methane oxidation (potential) has been verified in a number of studies (see works by Steinle et al. and refs cited in there), which should be cited here.

We added the following references "control on the methane oxidation potential is the number of MOB, as reported in previous studies (Kessler et al. 2011, Crespo-Medina et al. 2014, Steinle 2015)"

P16

L15ff, this is rather a summary, change the title of the subsection accordingly

Changed as suggested

Methane-oxidizing seawater microbial communities from an Arctic shelf

Christiane Uhlig[1]*, John B. Kirkpatrick[1,2], Steven D'Hondt[1], Brice Loose[1]

[1] Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882, USA
5 [2] The Evergreen State College, Olympia, WA, 98505, USA
*current address: Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, 27570, Germany

Correspondence to: Christiane Uhlig (cuhlig@uri.edu)

Abstract. Marine microbial communities can consume dissolved methane before it ~~has a chance to~~can escape to the atmosphere and contribute to greenhouse warming. Seawater over the shallow Arctic shelf is characterized by excess methane

10 compared to atmospheric equilibrium. This methane originates in sediment, permafrost and hydrate. Particularly high concentrations are found beneath sea ice. We studied the structure and methane oxidation potential of the microbial communities from seawater collected close to Utqiagvik, Alaska, in April 2016. The in situ methane concentrations were 16.3 $\pm$ 7.2 nmol L^{-1}, approximately 4.8 times oversaturated relative to atmospheric equilibrium. The group of methane-oxidizing bacteria (MOB) in the natural seawater and incubated seawater was >97% dominated by Methylococcales (γ-Proteobacteria).

15 Incubations of seawater under a range of methane concentrations led to loss of diversity in the bacterial community. The abundance of MOB was low with maximal fractions of 2.5% at 200 times elevated methane concentration, while sequence reads of non-MOB methylotrophs were four times more abundant than MOB in most incubations. The abundances of MOB as well as non-MOB methylotroph sequences correlated tightly with the rate constant (k_{ox}) for methane oxidation, indicating that non-MOB methylotrophs might be coupled to MOB and involved in community methane oxidation. In sea ice, where

20 methane concentrations of 82 $\pm$ 35.8 nmol kg^{-1} were found, *Methylobacterium* (α-Proteobacteria) was the dominant MOB with a relative abundance of 80%. Total MOB abundances were very low in sea ice, with maximal fractions found at the ice-snow interface (0.1%), while non-MOB-methylotrophs were present in abundances similar to natural seawater communities. The dissimilarities in MOB taxa, methane concentrations, and stable isotope ratios between sea ice and water column point toward different methane dynamics~~cycling processes~~ in the two environments.

25 **1 Introduction**

Methane (CH$_4$) is the third most abundant greenhouse gas contributing to climate change (IPCC, 2014) – exceeded only by water vapor and carbon dioxide. Despite much lower concentrations than carbon dioxide, it has a ~~28x~~ 32 times higher accumulative radiative forcing potential (Etminan et al., 2016)~~(IPCC, 2014)~~ over a time span of 100 years. In the ocean, the two major sources of methane are ongoing biogenic production by microbes in anoxic sediment (Formolo, 2010; Reeburgh,

30 2007; Whiticar, 1999) and release of fossil methane from geological storage (summarized by Kvenvolden and Rogers, 2005;

Saunois et al., 2016). Other sources include release from permafrost, river runoff, submarine groundwater discharge (Lecher et al., 2016; Overduin et al., 2012) and production from methylated substrates under aerobic conditions (Damm et al., 2010; Karl et al., 2008; Repeta et al., 2016). More than 90% of the methane sourced in the seabed is oxidized within the sediment by anaerobic and aerobic oxidation (Barnes and Goldberg, 1976; Boetius and Wenzhöfer, 2013; Knittel and Boetius, 2009;

5 Reeburgh, 1976). The remaining methane either diffuses into the water at the sediment surface, or is released as bubbles, which completely or partially dissolve while rising through the water column (Leifer and Patro, 2002). Dissolved methane is ~~or~~ diluted by the surrounding water column (e.g. Damm and Budéus, 2003; Gentz et al., 2014), ~~(e.g. Gentz et al., 2014)~~. ~~Dissolved methane is~~ in which it is used as a substrate and oxidized by aerobic methanotrophic bacteria (methane-oxidizing bacteria, MOB) ~~in the water column~~ (Hanson and Hanson, 1996; Murrell, 2010) ~~or diluted by the surrounding water column (e.g. Gentz~~

10 ~~et al., 2014)~~. As a result ~~of these biological and physical processes,~~ oceanic methane concentrations are frequently at low nanomolar levels, leaving only a small fraction of ~~subseafloor~~sediment-sourced methane to eventually exchange with the atmosphere (Karl et al., 2008; Reeburgh, 2007).

By contrast, in the Subarctic and Arctic shelf areas, shallow water depths and seasonal sea ice cover complicate the picture. High concentrations of methane have been reported from the entire water column up to the surface around Svalbard (Damm

15 et al., 2005; Mau et al., 2013; Myhre et al., 2016) ~~(Damm et al., 2005; Mau et al., 2013)~~, the Siberian Shelf (Shakhova et al., 2010) and the Beaufort Sea (Lorenson et al., 2016). In addition, during periods of near 100% sea ice cover, gas exchange from the water column to the atmosphere is restricted (Loose et al., 2011). ~~While u~~Under ice free conditions, methane concentrations are frequently found in the range of 15 to 30 nmol L^{-1} or ~~around~~ up to 7 times ~~00%~~ supersaturated with regard to atmospheric equilibrium, while winter concentrations are often 10 to 100 times higher. Maximal concentrations of 5000 nmol L^{-1}, or

20 oversaturation of 1600~~00%~~ times, have been reported from the Siberian Shelf (Lorenson et al., 2016; Shakhova et al., 2010; Zhou et al., 2014).

Along with factors like oxygen and trace metal availability (Crespo-Medina et al., 2014; Sansone et al., 2001; Semrau et al., 2010), as well as local oceanographic and geologic conditions (Schmale et al., 2015; Steinle et al., 2015), dissolved methane concentration ~~is~~ can be ~~an important~~ control on the community of MOB and thus methane oxidation rates (Crespo-Medina et

25 al., 2014; Kessler et al., 2011; Mau et al., 2013). Methane hotspots, promoted by limited gas exchange under sea ice, might thus be candidate ~~regions~~ locations for accumulation of methane oxidizers. In addition, sea ice, particular the ice-water interface, is a hotspot for microbial activity. The ice surface, penetration of light, and constant exchange with the underlying water column favor the development of communities composed of small eukaryotic organisms, microalgae, prokaryotes and viruses; the biomass often being several orders of magnitudes denser than in the underlying water column (Thomas and

30 Dieckmann, 2002).

Methane-oxidizing bacteria use methane as their sole carbon and energy source (Hanson and Hanson, 1996). In the first step, methane is oxidized to methanol catalyzed by the enzyme methane monooxygenase. Since methane monooxygenase is characteristic of nearly all aerobic MOB (Knief, 2015), *pmoA*, the gene encoding for a subunit of the membrane-bound particulate methane mono-oxygenase, has been used as a specific molecular marker for detection and characterization of

aerobic MOB (Knief, 2015; Lüke and Frenzel, 2011; reviewed by McDonald et al., 2008; Tavormina et al., 2008). Methanol is further metabolized to formaldehyde, from which it is either mineralized to carbon dioxide (CO_2), or assimilated into organic compounds and finally biomass (reviewed by Hanson and Hanson, 1996; reviewed by Strong et al., 2015). Different types of MOB are distinguished by their phylogeny and assimilation pathways for formaldehyde. While γ-Proteobacteria or Type I MOB assimilate formaldehyde via the ribulose monophosphate pathway (RuMP), α-Proteobacteria or Type II MOB use the serine pathway (Hanson and Hanson, 1996). Besides these two proteobacterial groups, MOB also occur in the phylum Verrucomicrobia (e.g. Dunfield et al., 2007; Pol et al., 2007).

Methane-derived carbon is also assimilated in non-methane utilizing methylotrophs (non-MOB methylotrophs) or other bacteria in freshwater and temperate marine environments. These non-methane oxidizers are suggested to cross-feed on metabolites produced by the MOB (Hutchens et al., 2003; Jensen et al., 2008; Saidi-Mehrabad et al., 2013).

Knowledge of the microbial communities responsible for methane oxidation in the Arctic and Subarctic is still sparse. During the last years, the first few studies have determined methane oxidation rates from seawater in these regions to cover a range from 10^{-4} up to 3.2 nmol L^{-1} d^{-1} (Gentz et al., 2014; Lorenson et al., 2016; Mau et al., 2013, 2017; Steinle et al., 2015). In only two of these studies, both performed off Svalbard, oxidation rate measurements were combined with analysis of the microbial community. Steinle et al. (2015) quantified MOB by fluorescence in situ hybridization and microscopy. Low but relatively constant cell-specific oxidation rates were determined from the oxidation rates and MOB abundance, indicating that MOB community size is an important control on the total methane oxidation rate in the system. Mau et al. (2013) analyzed the bacterial community with denaturing gradient gel electrophoresis (DGGE) of the 16S gene and compared patterns of PCR products for *pmoA*. Different MOB communities were observed in the meltwater layer and deep water in this stratified system, also reflecting the observed differences in methane oxidation rates. Only one of the eleven analyzed DGGE bands was identified as methanotroph (from the genus *Methylosphaera*) from the deep water in this study, while none were detected in the meltwater, possibly due to the limitations of the method. To our knowledge, no high-throughput sequencing studies of methane-oxidizing bacteria in the Arctic have been published in peer-reviewed literature to date.

We studied methane-oxidizing communities from seawater sampled on the Beaufort Sea shelf close to Utqiagvik, Alaska. Incubation experiments were performed under different methane concentrations to directly compare the bacterial community structure with methane oxidation rates. Seawater incubations, freshly sampled sea water, and sea ice were analyzed for their entire community diversity (16S rDNA) and the presence of MOB (16S rDNA and *pmoA*) using high-throughput Illumina MiSeq sequencing. The aim of this study was to (1) investigate the response of the entire microbial community to an increase in methane abundance, (2) identify types of MOB involved in the oxidation of methane, (3) test for the presence of MOB in natural seawater and sea ice communities and (4) relate these community features to methane oxidation rates.

2 Methods

2.1 Study site

Samples were collected at two sites between 7 April 2016 and 15 April 2016 in the Beaufort Sea (Table 1). Site "Elson Lagoon" (EL) is located north of Utqiagvik, Alaska, (7 April .4.2016, 71.334° N, -156.363° W). At the time of sampling EL was covered
5 with 1.5 m thick sea ice; at approx. 1.5 m water depth, this left only a narrow layer of water between the sea ice and the sediment. Site "Ice Mass Balance Buoy" (IMB) is located 1 km offshore of Utqiagvik, close to the ice mass balance buoy of the sea ice physics group of University of Alaska, Fairbanks (7 April. 4.2016, 71.373° N, -156.548° W, and 9 Apr.il 4.2016, 71.372° N, -156.540° W). This site was characterized by 1 m thick fast ice cover and a water depth of approximately 7 m.

2.2 Sampling and instrument deployment

10 Seawater temperature and salinity records were takenwere recorded with an YSI Professional Plus probe (YSI, Ohio, USA) and a YSI 600 OMS V2 sonde (YSI, Ohio, USA). Water was collected using either a peristaltic pump (Masterflex Environmental Sampler, Cole Parmer, Illinois, USA) or submersible pump (Cyclone, Proactive Environmental Products, Florida, USA) from different water depths. For determination of methane concentration and isotope ratios, water samples were collected as described in Uhlig and Loose (2017). Briefly, in the field, 0.7 to -0.9 L seawater was transferred bubble-free
15 directly into in foil sample bags (# 22950, Restek, Pennsylvania, USA). On return to the laboratory, a 0.1 L headspace of Ultra-High Purity nitrogen (Air Liquide, Anchorage, AK) was introduced into the bags through the septa, and the samples were equilibrated at 30°C at least 6 h for to measure in situ measurements methane concentration and carbon isotope ratiosfor at least 6 h.

For DNA extractions, between 1 to -2 L of seawater were filtered onto Sterivex® filter cartridges (Millipore) with 0.2 µM
20 PES filter membranes directly in the field, or were filled into foldable polypropylene containers and filtered upon return to the laboratory. For nutrient analysis an aliquot of the flow-through of the Sterivex® filters was collected in 15 mL polypropylene tubes (Falcon Brand, Corning, New York, USA) and frozen at -80°C. Seawater was fixed with 2% final concentration formaldehyde (Mallinckrodt Chemicals, Surrey, UK) and stored at 5°C to for later determination of the cell abundance.

Additionally, at site IMB, seawater temperature, salinity and velocities were recorded with an Aquadopp Profiler (Nortek AS,
25 Norway), and a salinity temperature recorder (SBE37SMP, Sea-Bird Scientific, Washington, USA). These were deployed at about 7 m depth on the seafloor between 9 and 15 April.

Sea ice was collected at site IMB only, using a Kovacs Mark II ice corer (Kovacs, Roseburg, Oregon, USA). The ice cores were sectioned into 15 cm and split lengthwise. The outside was cleaned with a sterilized knife to remove microbes possibly transferred from the sampling equipment. The core sections were sealed into custom-made gas-tight tubes (Loose et al., 2011)
30 for determination of methane concentration and isotope ratios. In the laboratory, the gas-tight tubes were flushed with ultrapure nitrogen for several gas volumes (Lorenson and Kvenvolden, 1995). Due to technical limitations, ice core 1 (IC1) was melted within a week at 5°C, while ice core 2 (IC2) was melted within a day, while frequently being mixed, at room temperature.

Samples for molecular biology and cell counts were collected from the melted sea ice similar to the procedure described for seawater. In addition, the bottom 2 cm of one ice core was sampled into a sterile sample bag (Whirlpak, Nasco, Fort Atkinson, WI, USA) for molecular biology processing only. Sea ice brine volume fractions were calculated according to Cox and Weeks (1983).

5 **2.3 Net methane oxidation/production and determination of isotope fractionation factors**

Rates for net methane oxidation/production were determined from the methane mass balance according to Uhlig and Loose (2017). In short, seawater was sampled into multi-layer foil bags. In addition to a headspace of hydrocarbon-free air (Air Liquide, Anchorage, AK), some sampling bags were supplied with a spike of methane. Final dissolved methane concentrations ranged between 3.0 and 4000 nmol L^{-1}, representing approximately 0.2 times* (no methane addition, resulting in degassing of

10 in situ methane to the headspace, 0.2x), 2 times (2x)*, 10 times (10x)* and 200 times (200x)* of the in situ methane concentration. Samples were incubated at 0 to -1°C for 5 to 46 days. Some variation in the incubation period was introduced by logistical constraints. To account for potential diffusive loss of methane, a killed control was prepared for the 200x treatment by adding 0.1 M NaOH.

Assuming first order kinetics for oxidation of methane (Reeburgh et al., 1991; Valentine et al., 2001), net oxidation/production

15 rate constants (k_{ox}) were determined from the methane mass balance in the incubations (Uhlig and Loose, 2017) as

$$\ln\left(\frac{n(CH_4)_{total,t_i}}{n(CH_4)_{total,t_{i-1}}}\right) = -k_{ox,ppm} \times t_{i-(i-1)} \qquad (1)$$

with $n(CH_4)_{total,t_i}$ being the total molar mass of methane in the bag at time t_i.

The net oxidation/production rate (r_{ox}) was calculated from the first order constant and the in situ concentration of methane in the water:

20 $r_{ox} = k_{ox} \times c(CH_4)_{w,in\ situ} \qquad (2)$

Isotopic fractionation factors of methane oxidation ($\alpha_{ox} = \frac{k_{12}}{k_{13}}$) were determined as described in Preuss et al. (2013), using the isotope fractionation approach (Coleman et al., 1981).

$$\ln\left(\frac{c(CH_{4ti})}{c(CH_{4t0})}\right)\left(\frac{1}{\alpha_{ox}} - 1\right) = \ln\left(\frac{1000 + \delta^{13}CH_{4ti}}{1000 + \delta^{13}CH_{4t0}}\right) \qquad (3)$$

where the isotope ratios are described in δ-notation $\delta^{13}C = \frac{R_{sample}}{R_{standard}} - 1$, and R is the isotope ratio of $^{13}CH_4/^{12}CH_4$ in the

25 sample and standard (VPDB, Vienna Peedee Belemnite, McKinney et al., 1950), respectively.

Alpha can be determined as $\alpha_{ox} = \frac{1}{m+1}$ from the slope (m) of the linear regression between $ln\left(\frac{c(CH_{4ti})}{c(CH_{4t0})}\right)$ and $\ln\left(\frac{1000 + \delta^{13}CH_{4ti}}{1000 + \delta^{13}CH_{4t0}}\right)$.

2.3 Analytical procedures

2.3.1 Methane concentration and stable isotope ratios

Methane concentrations and stable isotope ratios were determined with a Picarro G2201-*i* cavity ring-down spectrometer (Picarro, Santa Clara, California, USA) coupled to a Small Sample Isotope Module (SSIM) as described by Uhlig and Loose (2017). After equilibration, the headspace above the seawater or melted ice was subsampled with a gas tight syringe and 1 to 15 mL was injected into the SSIM. Measurements were performed in fast measurement mode. Dissolved methane concentrations were calculated as described in Magen et al. (2014), with the equilibrium constant according to Yamamoto et al. (1976).

2.3.2 Nutrient analysis and flow cytometry

Phosphate, nitrate and nitrite concentrations were determined using a QuickChem QC8500 automated ion analyzer (Lachat, Loveland, Colorado, USA). The total number of prokaryotic cells was counted on a BD Influx[TM] flow cytometer with BD FACS[TM] software. Formol-fixed samples were stained with a final concentration of $1\times$ SYBR Green I (Invitrogen, Molecular Probes, Eugene, Oregon, USA) for 20 to 45 minutes at room temperature in the dark before analysis.

2.4 Nucleic acid extraction and sequencing

DNA was extracted with the PowerWater® DNA extraction kit (MoBio, Carlsbad, California, USA). To remove the filter membrane, the Sterivex® cartridge was opened with a pair of sterilized pliers. The filter membrane was cut out along the edge with a scalpel, transferred into the bead tube, and DNA subsequently extracted according to the manufacturer's protocol. A minor modification was made: the tube was vortexed once for 3 minutes, rotated 180°, and then vortexed for another 3 minutes. The DNA was eluted in 80 µL buffer PW6, after incubating the buffer for 1 minute on the membrane. Quantification was conducted with a Qubit®2.0 Fluorometer (Invitrogen, Carlsbad, California, USA).

The V4-V5 region of the 16S rRNA gene was amplified with forward primer 518F (5´-xx-CCAGCAGCYGCGGTAAN-3´), and an 8:1:1 mix of the reverse primers 926R1 (5´-yy-CCGTCAATTCNTTTRAGT-3´), R2 (5´-yy-CCGTCAATTTCTTTGAGT-3´) and R3 (5´-yy-CCGTCTATTCCTTTGANT-3´) (Nelson et al., 2014). Primers included 33 base pair (bp) adapters (xx, yy) at the 5´end. The final volume of 20 µL PCR reaction contained 0.2 µL PfuUltra II fusion HS DNA polymerase (Agilent Technologies, Santa Clara, California, USA), 50 µM each forward and reverse primer, 25 µM each dNTPs (Thermo Scientific, Waltham, Massachusetts, USA), 10 µg mL^{-1} BSA (Thermo Scientific, Waltham, Massachusetts, USA) and 1 ng template DNA. After initial denaturation for 2 minutes at 95°C, DNA was amplified in 30 cycles of 30 seconds 95°C denaturation, 30 seconds 55°C annealing and 30 seconds at 72°C for extension, with a final extension of 2 minutes at 72°C. The *pmoA* subunit of the particulate monooxygenase (pMMO) was amplified with primer pair 189f (5´-xx-GGNGACTGGGACTTCTGG-3´) and mb661r (5´-yy-CCGGMGCAACGTCYTTACC-3´) (Holmes et al., 1999; Lyew and Guiot, 2003). The PCR conditions were the same as described for the V4-V5 amplicon. All amplicons were purified with

Agencourt® AMPure® XP magnetic beads (BeckmanCoulter, Indianapolis, Indiana, USA) at a ratio of 0.7x~~x~~ bead solution per PCR reaction volume and washed with 80% ethanol.

The primer sequences specified above included adapter sequences (xx, yy) to attach Nextera indices and adapters in a second PCR reaction of 6 cycles with 50 ng template DNA (http://web.uri.edu/gsc/next-generation-sequencing/). Amplicons were

5   sequenced with Illumina MiSeq at 2 $\times$ ~~x~~250 bp read length.

2.5 Sequence analysis

2.5.1 V4-V5 region of 16S rRNA gene

Demultiplexing and adapter removal was performed with Illumina software. V4-V5 sequence quality control and clustering was performed in mothur (Schloss et al., 2009) as follows. Contigs were prepared from forward and reverse reads and culled

10   if they contained ambiguous bases or homopolymers longer than 6 bases. Contaminating sequences observed in kit and filter blanks accounted for 1.4% of all sequences and were removed from all samples. After alignment to the Silva small subunit reference database (v123; Quast et al., 2013), the 408 bp long sequences were preclustered (1% variability allowed) and filtered for chimeras (de novo algorithm) with the UCHIME (Edgar et al., 2011) wrapper in mothur. Sequences identified as chloroplast, mitochondria, Archaea, Eukaryota or unknown were removed and operational taxonomical unit (OTUs were built

15   at a 3% distance level with the opticlust algorithm. OTUs with fewer than 2 reads were removed from further analysis. Visualization and further analysis of sequencing data was performed in R version 3.2.3 (R CoreTeam, 2015) in RStudio Version 0.99.903. Species diversity was analyzed using the phyloseq package (McMurdie and Holmes, 2013) to determine richness (Shannon and Simpson indices) and differences in community structures (Bray-Curtis dissimilarities). Differences in community structure associated with different methane spike concentrations were determined via analysis of similarity

20   (ANOSIM) in the package vegan (Oksanen et al., 2017) on three predefined groups: in situ (n~~N~~=9), 0.2x (n~~N~~=2) and 10x (n~~N~~=3). Groups 1x, 200x short, 200x long with n~~N~~=1 (Table 2), were excluded from the analysis.

2.5.2 Identifying potential methane-oxidizing bacteria

To select groups representing methylotrophs and methanotrophs, 16S OTUs were filtered according to their phylogenetic annotation assigned by mothur for containing the string "meth" on family, order and genus level. This filter is expected to find

25   97% of taxonomically annotated methanotrophs, according to a current review on the diversity of methanotrophs (Knief, 2015). Further, phylogenetic groups potentially involved into methane ~~cycling~~ dynamics were identified as differentially more abundant 16S OTUs between incubations (0.2x, 10x, 200x) and in situ samples using DESeq2 (Love et al., 2014). Only OTUs with an adjusted p-value in DESeq2 <~~-~~0.05 were kept for further analysis. OTUs identified from spike 0.2x were considered to represent groups favored due to the incubations (the "bottle effect") rather than addition of methane, and removed from

30   further analysis. Treatments EL 0.2x, EL 10x and IMB 2x (Table 2) were not included in this analysis, since no replicate

samples were available. The abundance of all candidate 16S OTUs, identified as described above, was determined within every in situ or incubated sample.

Absolute numbers of methanotrophs and methylotrophs were calculated by multiplying the relative 16S sequence abundance with flow cytometric cell counts. The absolute numbers were further corrected for the mean of the 16S gene copy number for the lowest taxonomic rank (class to genus) available in the rrnDB-database (Stoddard et al., 2015).

2.5.3 Particulate methane monooxygenase: *pmoA*

In addition to 16S genes, the alpha subunit of the particulate methane monooxygenase (*pmoA*) was used as molecular marker for MOB. Only *pmoA* forward reads were analyzed. High-quality *pmoA* reads were retrieved according to the following protocol. Using mothur (Schloss et al., 2009), all reads were trimmed to a length of 225 bp to remove sequence fractions with a mean quality score below 30 (fastqc; Andrews, 2010). In addition, reads were clipped whenever the average quality score over a 50 bp window dropped below 30. Sequences with ambiguous bases and homopolymers larger than 6 bp were culled. Only sequences that translated into uninterrupted protein reading frames (Emboss 6.60 / transseq; Rice et al., 2000) were kept for further analysis. Nucleic acid sequences were aligned to a reference dataset of *pmoA* sequences (fungene; Fish et al., 2013) and sequences of a length of at least 220 bp were preclustered (1% variability allowed). De novo chimera filtration was run with the UCHIME (Edgar et al., 2011) wrapper in mothur. A similarity of 93% between *pmoA* sequences was defined to match the 97% cutoff as species definition for the 16S gene (Lüke and Frenzel, 2011). *PmoA* OTUs were built at a maximal distance of 7% between the furthest neighbors to maximize resolution between OTUs due to the short read length and limited number of unique sequences (Supplementary Table 1). To determine the phylogenetic relationship of *pmoA* sequences, nucleotide sequences were aligned against selected reference sequences in Mafft 7.017 (Katoh and Standley, 2013) and a neighbor joining tree calculated in Clustal 2.1 (Larkin et al., 2007) with 1000 replications.

3 Results

3.1 Water column properties

On 7 April 2016, the narrow layer of water between the sediment and ice in Elson Lagoon (nN=1) had a salinity of 21 and a temperature of -1.5°C. Phosphate and nitrate concentrations were 0.74 µM and 4.87 µM, respectively. Methane concentration for Elson Lagoon (nN=1) was 53.2 nmol L^{-1} with a stable isotope ratio of -73.8-‰ (Figure 1) and cell density 7.7 ×× 10^4 cells mL^{-1}. For most days during the sampling period, the water column at station IMB was characterized by temperatures around -1.8°C and salinities of 33.9 to 36.4 (Figure 1, Supplementary Figure 1, 2). Between 11 April and 13 April warmer water (max. temperature observed -0.9°C) was advected, coinciding with a change in current direction. A lower salinity of 27.5 at the ice-water interface indicates melting of the sea ice. Phosphate concentrations at station IMB were 0.99 ± 0.33 µM (nN=9) and nitrate 6.59 ± 4.04 µM (nN=9), with neither showing any trends in the depth profiles (data not shown). Nitrite concentrations were below detection (0.3 µM based on technical replicates). Water column methane concentrations at station IMB ranged

between 9.2 and 25.3 nmol L^{-1} (16.3 ± 7.2 nmol L^{-1}, n=5) (Figure 1), with stable isotope signatures between -55.4‰ and -70.5‰ (-60.6‰ ± 6.3‰, n=5)). Total prokaryotic cell densities, determined as SYBR Green stained cells with flow cytometry, were 6.9 × 10^4 ± 5.7 × 10^3 cells mL^{-1} (n=16).

3.2 Ice cores

Temperature and salinity profiles of the two sea ice cores sampled at 9 and 15 April are shown in Figure 2. Brine volume fractions above 5% indicate that the ice was permeable for water and gases (Golden et al., 1998) in the bottom 50–100 cm, while the upper part of the ice was impermeable. Methane concentrations in the ice were higher than in the water (83.9 nmol kg^{-1} ± 35.0 nmol kg^{-1}, n=9) while the isotope signatures were close to seawater (-60.4‰ ± 3.5‰, n=9). Ice core 1 (IC1), sampled on 7 April, had generally higher methane concentrations and more positive isotope signatures (72.3 nmol kg^{-1} to 144.3 nmol kg^{-1}, -54.4‰ to -62.0‰) than ice core 2 (IC2), sampled on 15 April (53.3 nmol kg^{-1} to 77.6 nmol kg^{-1}, -59.0‰ to -61.6‰). Microbial activity during storage of IC1 at 5°C for one week before analysis might have led to the differences in methane concentrations and isotope ratios. For ice samples, cell counts were performed on IC2 only; they show an increase from 1.0 × 10^4 cells mL^{-1} in the top layers to 8.2 × 10^5 cells mL^{-1} in the bottom two cm of the ice core.

3.3 Net methane oxidation/production and isotope fractionation

The methane oxidation potential of microbial seawater communities at stations EL and IMB was determined from the methane mass balance in incubation experiments (Table 3; Uhlig and Loose, 2017). Final dissolved methane concentrations ranged between 3.0 nmol L^{-1} and 4000 nmol L^{-1}, representing approximately 0.2 times (0.2x) to 200 times (200x) the in situ concentration. Oxygen concentrations at the end of the long incubations ranged between 116% and 126% saturation, while oxygen concentrations at the end of the short incubations were not determined.

Net oxidation rates discussed here were published in Uhlig and Loose (2017) and are summarized for comparison with the microbial community structure. Short incubations (≤10 days) did not show significant oxidation, while long-term incubations (41–46 days) did. Surprisingly, four out of five replicates of treatment 0.2x IMB showed a statistically significant increase in methane of about 0.62 ± 0.21 nmol L^{-1} (n=5) within 10 days (Supplementary Figure 3). In long-incubation samples with significant methane oxidation (10x and 200x spikes), the isotopic signature of the residual methane increased toward heavier (more positive) signatures with fractionation factors α of 1.0230 (10x EL), 1.0225 (10x IMB) and 1.0103 (200x IMB).

3.4 Bacterial community structure

The V4-V5 region of the 16S rRNA gene was sequenced from a total of 10 seawater samples and 7 ice samples (Table 2). Non-metric multidimensional scaling analysis of the Bray-Curtis diversity revealed high similarity across the in situ water samples analyzed for 16S diversity (Figure 4). Samples from site IMB clustered together repeatedly, and we did not observe any differences in community structure coinciding with water depth or temperature (Figure 4). For the IMB samples, IMB 2 was the only sample slightly different from the other in situ samples, though IMB 1, IMB 2, IMB 4 are all characterized by a

colder water mass. Only IMB_3 showed some influence of an incoming warm water mass in the YSI profile (Figure 1), though not yet reaching the bottom (Supplementary Figure 1), but this shift is not seen in the community structure. In contrast to the in situ water samples, the community structure of incubated samples is driven by incubation time. While communities in the short-incubation treatments (5–10 days; 0.2x, 2x, 200x short) were similar to the in situ samples, the long incubations (41–46

5 days; 10x, 200x long) clearly deviated from the in situ samples. In both the long and short incubated clusters samples originating from IMB 1 (0.2x, 10x) and IMB 2 (2x, 200x) are present (Figure 4, Table 2). Microbial communities in ice cores were clearly distinct from those in the water samples and were more distant to each other than were the communities in water samples.

In the in situ seawater communities, Proteobacteria were dominant with relative sequence abundances of 59.5% and 65.5% ±

10 2.5-% for EL (nN=1) and IMB (nN=9), respectively (Supplementary Figure 4). Within the phylum of Proteobaceteria, α- and γ-Proteobacteria made up the majority. The second most abundant phylum was Bacteriodetes with 23% and 19.6% ± 1.4% for EL and IMB, respectively.

Similar to the seawater, sea ice (nN=7) showed a dominance in Proteobacteria (58.9% ± 9.8%), but Bacteriodetes sequences (29.1% ± 11.7%) were slightly more abundant in the ice than in the water. γ-Proteobacteria dominated in all but one sample

15 (IC2 30–46 cm). This one sample, which had clearly visible sediment included into the sea ice structure, was dominated by α-Proteobacteria.

In all incubated samples that were sequenced (nN=10), species richness decreased (Figure 3) and the communities shifted toward higher fractions of γ-Proteobacteria over time. In short incubations (5–10 days; nN=5) γ-Proteobacteria dominated with 61.8% ± 2.9% of sequences, while reaching 81.0% ± 11.1% in long-incubation samples (41–46 days; nN=4). In particular, one

20 operational taxonomical unit (OTU), from the genus *Oleispira*, was very abundant in the long-incubation samples, with 50.1 to 76.3%, compared to abundances <0.04% in the in situ samples. The same OTU was only slightly more abundant in the short-incubation treatments (0.5% to −1.6%) compared to in situ abundances. In addition to the shift in community structure, total cell densities increased to 1.9 $\times$ 10^5 and 3.3 $\times$ 10^6 cells mL^{-1} for short and long incubations, respectively, based on flow cytometric cell counts.

25 **3.5 Methanotrophs, methylotrophs and differentially abundant OTUs**

Using their 16S taxonomic annotation, we identified six groups of aerobic methanotrophs (MOB) (Figure 5). With a maximum of 1.76% ± 0.73%, the relative abundance of MOB was low in all samples (Table 4). Four MOB grouped in the Methylococcales (γ-Proteobacteria), specifically Marine Methylotrophic Group 1 and 2 (MMG1, MMG2), unclassified Methylococcales and the Milano-WF1B-03 family. The three remaining MOB OTUs belonged to the genera *Methylobacterium*

30 and *Methyloceanibacter* (α-Proteobacteria) and *Candidatus "Methylacidiphilum"* (Verrucomicrobia). MOB OTUs were more abundant in natural seawater samples than in sea ice (maximal 0.11% in IC1 0–16 cm), but in contrast to the seawater, α-Proteobacteria MOB dominated in the sea ice.

Furthermore, four clades of non-methane utilizing methylotrophs (non-MOB methylotrophs) were identified, grouping into γ-Proteobacteria Marine Methylotrophic Group 3 (MMG3) and *Methylophaga*, and to the β-Proteobacteria Methylophilaceae (*Methylotenera*, OM43 clade). Non-MOB methylotroph OTUs were more abundant than MOB OTUs with exception of the 200x incubation treatments (Figure 5, Table 4). Ice samples showed the largest difference in abundance between non-MOB methylotrophs and MOB, with a ratio of 21:1 between the two groups. Ice samples also had the highest overall relative abundance of methylotrophs (MOB and non-MOB) of all in situ samples (max: 1.63%, IC1 0–16 cm). Only the 200x long incubations had a higher total number of methylotrophs (3.3%), while this sample was in addition dominated by MOB (2.49%). The second highest relative abundance of MOB was found for in situ EL and IMB with 0.24% ± 0.09% (n=10).

Taking into account the total cell number, a strong increase of MOB groups MMG1 (2 to 700 times) and Milano-WF1B-03 (25 to 75 times) was observed for the 10x and 200x long-incubation samples compared to in situ conditions (Figure 5b).

Taxonomic groups that became differentially more abundant in the incubated samples than in natural communities were the γ-Proteobacteria *Oleispira*, *Colwellia* and *Glaciecola,* as well as Rhodobacteracea (α-Proteobacteria). Except for *Oleispira*, which became dominant, the other taxa had relative sequence read abundances from 1.1% to 12.6% after the oxidation experiments, compared to abundances <0.25% for in situ samples (Supplementary Figure 5).

3.6 Particulate methane monooxygenase (*pmoA*) sequences

A 225 base pair section of the particulate methane monooxgenase gene (*pmoA*) was sequenced in a total of 15 samples (Table 2). The absolute abundance of *pmoA* fragments obtained in sequences ranged from 9331 (IMB in situ, 6.5 m depth) to 72781 (IMB 200x long) reads. In general, incubations with higher methane concentration had more *pmoA* reads than incubations with lower methane concentration or in situ samples. About three times more reads were filtered from the Elson Lagoon in situ sample (33844 reads, n=1) than the IMB in situ samples (11700 ± 1833, n=4).

Two of the 59 *pmoA* OTUs made up 96.8% of all sequences, while all other OTUs individually represented ≤1% of the *pmoA* sequences. The most abundant OTU (71.0% of all sequences) clustered with two uncultured isolates from methane seeps (NCBI accession: HQ738559, EU444875) in the deep sea-3/OPU3 subgroup of γ-Proteobacteria Type I MOB (Hansman et al., 2017; Knief, 2015; Lüke and Frenzel, 2011). The second most abundant (25.8%) OTU was related to *Methyloprofundus sedimentii*, another Type I MOB. Most of the low-abundance OTUs also clustered within the Type I MOB, while only three OTUs (0.07% of all *pmoA* sequences) clustered with Type II α-Proteobacteria MOB *pmoA* sequences (*Methylocystis*, *Methylosinus*).

4 Discussion

4.1 Methane concentration and stable isotope ratios in seawater and ice

Seawater methane concentrations in April 2016 close to Utqiagvik Alaska were supersaturated 2.5 times to 7 times compared to atmospheric equilibrium (3.6 nmol L^{-1}). The concentration at site EL (52.90 nmol L^{-1}, n=1, 7 Apr. 2016) was

in the range of a study by Lecher et al. (2016) in Elson Lagoon under ice free conditions (3.3–124.0 nmol L^{-1}). At site IMB, concentrations were slightly lower (9.5 nmol L^{-1} –25.2 nmol L^{-1}: nN=5, 15 Apr. 4 2018) than previously reported from the same area for ice free (Lecher et al., 2016; mean: 40.6 nmol L^{-1}), and ice covered conditions (Zhou et al., 2014; March/April: 37.5 ± 6 nmol L^{-1}). Shallower depths at IMB exhibit lower methane concentrations (Figure 2), and the isotopic signature
5 mirrors this pattern with more positive values toward the surface. This indicates that methane might be biologically oxidized on the way through the water column, after being released from the sediment.

The sea ice bulk methane concentrations observed in this study (53–144 nmol kg^{-1}) are significantly higher than in a study from the same area (Zhou et al., 2014), but fall within values reported for the Beaufort Sea (5 –1260 nM, Lorenson and Kvenvolden, 1995). Methane carbon isotopic signatures (-54.4‰ to -63.8 ‰) are comparable to the higher end of previous
10 studies for bulk sea ice (-52.1‰ to -83.4‰, Lorenson and Kvenvolden, 1995) and sea ice brine (-75‰, Damm et al., 2015). Although both ice cores were sampled within 300 m distance from each other at site IMB, they differ in concentration and isotope signature. These differences could either be caused by spatial variability between the two ice cores or differences in the processing procedure described in section 2.2. Spatial variability as driving difference between the two ice cores is corroborated by the sediment present at 30–46 cm depth in IC1, which was not observed in IC2, indicating that both ice cores
15 have different freezing histories. The same event that led to inclusion of the sediment into IC1 possibly resulted in inclusion of higher methane concentrations into IC1 compared to IC2 during freeze-up. In addition, microbial processes like oxidation of methane or methanogenesis could have taken place in situ or during sample processing and storage. Microbial oxidation of methane, particularly in the two middle sections (30 –46 cm and 52 –86 cm depth), might have led to the observed shift toward more positive carbon isotope ratios (Figure 2). The different bacterial community introduced through the sediment
20 (Supplementary Figure 4) might have favored oxidation in those two sections compared to the top and bottom sections. MOB identified by our approach were, however, neither more abundant nor phylogenetically distinct in the sediment-loaded section compared to the other sections (Figure 4a). Another microbial process that may have led to the discrepancies between IC1 and IC2 could be methane production from ice algae-derived organic carbon in IC1. With typical carbon isotopic signatures of -20‰ to -30‰ for ice-derived carbon (e.g. Wang et al., 2014), methane produced from this substrate would be enriched in ^{13}C
25 (more positive) compared to the initial pool of methane (about -60‰, Figure 2, Figure 6). Yet, sequences of bacterial taxa that might indicate anoxic conditions (Eronen-Rasimus et al., 2017), which would favor anaerobic methane production, were not significantly more abundant in IC1 than in IC2 (Supplementary Table 2).

Compared to the underlying water column, methane concentrations in the sea ice were two to five times higher. Further, the isotope signatures indicate less oxidized methane (-60.4‰ to -63.8 ‰) in most of the ice sections compared to the upper water
30 column (-55‰ %). Lorenson and Kvenvolden (1995) report higher methane concentrations in sea ice than in the water column for the Beaufort Sea. They attributed the high methane concentrations in the fast ice to inclusion of sediment-sourced methane during the initial freeze-up over the shallow shelf at <10 m water depth (Lorenson et al., 2016). Methane concentrations in IC2, which are close to water column concentrations reported in previous studies for the Barrow shelf (Lecher et al., 2016; Zhou et al., 2014), suggest the same process for our ice cores. Further, in our study, the lower methane concentrations together

with more positive (heavier) isotopic signature in sea water compared to ice, might indicate that the microbial community in the water column is oxidizing more methane during the ice covered period than in the freeze-up period. Higher oxidation rates during ice covered periods compared to ice free conditions were previously reported for the Beaufort Sea. Due to reduced sea-air gas exchange, higher methane concentrations can build up under sea ice cover, which might lead to higher oxidation rates

5 (Lorenson and Kvenvolden, 1995).

4.2 Methane ~~cycling~~ dynamics at different methane concentrations

Net methane oxidation/production rates were determined from water sampled at station IMB 1 and IMB 2 on 7 and 9 April 2016. Both days were characterized by the cold water temperatures ($\leq$-1.8°C; Fig. 1). Different water masses have previously been reported to influence the methane oxidation potential of water column microbial communities off Svalbard (Steinle et al.,

10 2015). In this study, we observed a change in current direction and water temperature consistent with advection of a different water mass into the study area (Supplementary Fig. 1). However, this change occurred on 12 April subsequent to sampling IMB 3, and thus this event would not have influenced the net oxidation potential determined in this study.

Net oxidation rates of the long-incubation treatments at 10x (46 days) and 200x (41 days) methane concentration fall into the mid-range of rates published for Arctic and subarctic environments (Damm et al., 2015; Gentz et al., 2014; Lorenson et al.,

15 2016; Mau et al., 2013, 2017; Steinle et al., 2015) or marine sites with high oxidation rates at oil spills or gas flares (Leonte et al., 2017; Redmond et al., 2010; Valentine et al., 2010), as discussed in Uhlig and Loose (2017). The fractionation factors (α_{ox}) that we observed are higher than previously reported from cold marine environments with a range of α_{ox} from 1.002 to 1.017 (Cowen et al., 2002; Damm et al., 2008; Grant and Whiticar, 2002; Heeschen et al., 2004; Keir et al., 2009; Tsunogai et al., 2000). Some of these fractionation factors, which were calculated from in situ data, might however be underestimates due

20 to mixing effects in the water column (Grant and Whiticar, 2002). The fractionation factors in our study seem to be inversely dependent on the methane spike concentration, with higher fractionation in the 50x (1.023, n~~N~~=6) treatments than in the 200x (1.010, n~~N~~=2) treatments. The relative and absolute abundances of MOB, as well as the dominant MOB types, differed between both treatments, possibly providing explanations of the differences in fractionation rates. Logistical constraints forced us to stop several incubations already after 5 to 6 days. These short-incubation 2x and 200x treatments did not resolve oxidation of

25 methane. While the 2x treatments did not meet the sensitivity threshold for the method (Uhlig and Loose, 2017), the 200x short treatments were likely just about to leave the lag phase when the experiments were stopped. A lag phase of 6 days was observed for the long-incubation 200x samples, in which the microbial community possibly to shifted towards an abundance of MOB that was large enough to cause detectable methane oxidation. To facilitate comparisons between treatments, incubation duration should be kept constant in future studies.

30 The increase in methane concentration in treatment IMB 0.2x (10 days incubation) is surprising since experiments were performed under aerobic conditions. Since the seawater was not pre-filtered through a larger pore-size filter, which would exclude larger particles but allow bacterial cells to pass, production of methane in microanoxic zones (de Angelis and Lee, 1994; Oremland, 1979) should be considered. Furthermore, several studies suggested pathways for methane production in

oxygenated marine systems from methylated compounds or dissolved organic matter (Damm et al., 2010; Florez-Leiva et al., 2010; Karl et al., 2008; Repeta et al., 2016). The methane production rate of 0.06 nmol L^{-1} day^{-1} observed in our study is two to six orders of magnitude lower than previously published methane production rates under aerobic conditions (Damm et al., 2010; Karl et al., 2008). In addition to biological processes, we cannot rule out an abiotic effect leading to the increased

5 methane concentrations, since our experimental setup did not include a killed control at the same methane concentration.

4.3 Abundances of MOB and non-MOB methylotrophs control the methane oxidation potential

We found a strong linear correlation between the net oxidation rate constant (k_{ox}) and the relative abundance of 16S MOB sequences (Spearman rank order coefficient $\rho_s = 0.79$, p=0.006) (Figure 7a, Table 5). This strong correlation is confirmed when correlating against the total abundance or DESeq2 normalized abundance of 16S MOB sequences (Table 5). The correlation

10 to k_{ox} is even stronger for the absolute abundance of *pmoA* sequences retrieved from the respective datasets ($\rho_s = 0.86$, p=0.006) (Figure 7b). This presentation of a direct and statistically significant linear relationship is the first to our knowledge. It agrees with other qualitative reports of positive correlations between methane oxidation rates and abundance of *pmoA* or MOB 16S rRNA genes determined using a variety of methods – quantitative PCR, FISH, or sequencing – for marine water column and lake sediments (Crespo-Medina et al., 2014; Deutzmann et al., 2011; e.g. Rahalkar et al., 2009; Steinle et al., 2015). Future

15 application of marine-specific *pmoA* primers may further improve this correlation (Tavormina et al., 2008).

Cell-specific net oxidation rates in our study (3.2–7.5 fmol $cell^{-1}$ h^{-1}) were relatively constant between treatments. They are two orders of magnitude higher than reported for subarctic sea water (Steinle et al., 2016). Since the cell-specific rates only span a narrow range, the ultimate control on the methane oxidation potential is the number of MOB, as reported in previous studies (Crespo-Medina et al., 2014; Kessler et al., 2011; Steinle et al., 2015).

20 Despite the long incubation time in our experiments and the fact that methane was the only added source of carbon, the relative abundance of MOB determined from 16S reads was low (<2.5%, Table 4). Other studies of natural or man-made gas or oil spills, with dissolved methane concentrations comparably high to our 10x and 200x treatments, reported maximal values of 8 to 34% of MOB (Crespo-Medina et al., 2014; Kessler et al., 2011; Steinle et al., 2015, 2016). Surprisingly, relative sequence abundances of MOB in the natural seawater communities were higher than in the incubations except for the 200x treatment

25 (Table 4). Inferred absolute MOB numbers were higher in 10x and 200x incubations than in situ (Figure 5b). In contrast, absolute MOB numbers in 0.2x and 2x incubations were very similar to in situ abundances, indicating that either the provided methane concentration was too low or the incubation time too short to stimulate MOB growth.

It is puzzling why the fraction of methane oxidizers in the bacterial community did not increase above the observed low percentages although the cell-specific oxidation rates were high and sufficient methane was available, particularly in the 10x

30 and 200x treatments. Oxygen and methane can be ruled out as limiting factors, since both were abundant. Copper, which is essential for expression of particulate methane monooxygenase, can restrict MOB growth (Avdeeva and Gvozdev, 2017; Zhivotchenko et al., 1995). In the absence of copper, many MOB express a copper-independent soluble methane monooxygenase (Hakemian and Rosenzweig, 2007). Since we did neither determine copper concentrations, nor the expression

of particulate and soluble methane monooxygenase, we cannot exclude that copper was limiting in our study. Further, the low relative abundance of MOB sequences could be due to competition with other bacterial taxa for other macro- or micronutrients. In the absence of other added C substrates, these other taxa could have utilized the initial pool of dissolved organic carbon (DOC). DOC concentration is about 68 µM carbon in the Southern Chukchi Sea (Tanaka et al., 2016), which is in the same

5 range as the amount of consumed methane carbon in the 200x treatments and two orders of magnitude higher than the consumed carbon in the 10x treatments.

As a result of the low MOB abundances, the potential of the microbial community to mitigate release of dissolved methane to the atmosphere by oxidation is small. For example, for methane concentrations in the Laptev Sea area, the rates observed in this study would result in 0.2% consumption during the ice covered period. This supports the results from a previous study for

10 the Beaufort Sea, where 1% to 2% of dissolved methane was calculated to be oxidized (Lorenson et al., 2016).

4.4 Structure of the methane degrading microbial community

This first 16S MiSeq sequencing based study on methane-oxidizing sea water communities in the Arctic provides a broader view on the community structure than approaches with FISH and DGGE. The dominance of γ-Proteobacteria MOB in our natural and incubated seawater samples agrees with previous records of MOB diversity for polar and subpolar waters (Mau et

15 al., 2013; Steinle et al., 2015; Verdugo et al., 2016). In addition, non-methane-utilizing methylotrophs were present in all of our samples. The relative read abundance of non-MOB methylotrophs were, similar to MOB, tightly correlated to k_{ox}, and the same correlation holds for the relative abundance of total methylotrophs (MOB plus non-MOB). In contrast, the correlation between OTUs that were differentially more abundant in the incubated samples and k_{ox} was weak (Table 5). This points toward a possible link between the MOB and non-MOB in this methane-oxidizing microbial community, in which non-MOB

20 methylotrophs might play a role for community methane oxidation, whereas the OTUs that were differentially more abundant, are not directly linked to methane oxidation.

Methylophilacaea, the most abundant non-MOB methylotroph in our experiments, have been found to be abundant in sediment methane-oxidizing communities in lakes and marine systems (Beck et al., 2013; Redmond et al., 2010). Possible cooperative behavior between methanotrophs (Methylococcacaea) and non-MOB methylotrophs (Methylophilaceae) was suggested (Beck

25 et al., 2013), in which the latter cross-feeds on intermediate metabolic products of the MOB, i.e. methanol, and can even positively alter the metabolism of the MOB toward methane assimilation (Krause et al., 2017).

To test if the non-methane MOB could be supported by the intermediate substrates produced by MOB, we calculated a budget between the methane carbon assimilated by the growing microbial population ($C_{CH_4,assim}$), and the cell carbon gained during growth ($C_{cell-growth}$) (Figure 8). We assumed (i) a cellular carbon content of 150 fg for exponentially growing bacterial cells

30 (Vrede et al., 2002) and (ii) that about 1/3 of consumed CH_4-carbon is assimilated, with the remaining 2/3 respired to CO_2 (Bastviken et al., 2003; Roslev et al., 1997). $C_{CH_4,assim}$ exceeds MOB-$C_{cell-growth}$ by a factor of 9 to 17, indicating that some of the $C_{CH_4,assim}$ was available for secondary consumption by non-MOB. The entire methylotrophic community (MOB + non-

MOB-methylotroph) growth can also be explained solely by $C_{CH_4,assim}$, supporting the possible link of non-MOB methylotrophs to methane consumption. In contrast, only about 0.1% of the total community growth could be supported by $C_{CH_4,assim}$ in the 10x treatment and 15% in the 200x treatment. The remaining cell growth, e.g. of the differentially more abundant OTUs, must have been supported by other carbon sources, such as initially available DOC.

4.5 MOB and methylotrophs in sea ice

The two sea ice cores analyzed in this study give a first insight into the possible role of methane oxidizers in sea ice. In contrast to seawater samples, MOB found in sea ice samples were mostly α-Proteobacteria. The relative sequence read abundance of MOB in the ice was very low (maximal 0.1%), pointing to an overall low contribution of methane oxidation inside sea ice.

10 The highest relative abundances of MOB were found in the top-most ice sections in both ice cores (Figure 5a). This coincided with the highest methane concentration in case of IC2, whereas the top-most section of IC1 had the second smallest concentration of methane in this ice core (Figure 2e). Relative abundances of MOB in the inner and bottom sections of the ice cores were even lower, with 0 to 0.02% only.

The top-most section of IC1 and the biologically rich bottom section of IC2 had the highest relative abundances of β-

15 Proteobacteria Methylophilaceae, a non-MOB methylotroph. Recently identified as DMS degraders (Eyice et al., 2015), Methylophilaceae might use DMS, a methylated compound abundant in sea ice, as substrate (Kirst et al., 1991).

5 ~~Conclusions~~Summary

We studied the structure and methane oxidation potential of microbial communities from Arctic seawater and sea ice. The natural seawater community had relative sequence abundances of MOB of 0.24% ± 0.09% and was dominated by γ-

20 Proteobacteria MOB, while α-Proteobacteria MOB dominated in sea ice with maximal fractions of ≤0.1% in the surface of the sea ice. In seawater incubations under different methane concentrations, the overall relative abundance of methane oxidizers (MOB) was low, with a maximum of 2.5% and the dominant MOB types were γ-Proteobacteria. A tight correlation between the rate constant of methane oxidation and relative abundances of MOB and non-MOB methylotrophs (Figure 7, Table 5) suggests that the abundance of MOB is a control on the magnitude of methane oxidation. It also suggests that non-MOB

25 methylotrophs might play a role in methane oxidation. The reasons for low MOB abundance, despite ample methane availability, along with the role of methylotrophs in methane oxidation are both open questions.

Higher methane concentrations in the sea ice compared to the underlying water and an offset in stable isotope ratios suggest that either fractionation and solute concentration occurred during freeze-up, or different microbial processes took place within the ice and water. Possible causes explaining this observation include (i) microbial production of methane, even within the

30 ice (Damm et al., 2015), and (ii) microbial oxidation in the water column and at lower rates in sea ice. To address these

hypotheses, future studies should directly compare both sea ice and water, particularly during ice freeze-up, and involve investigation of the microbial processes.

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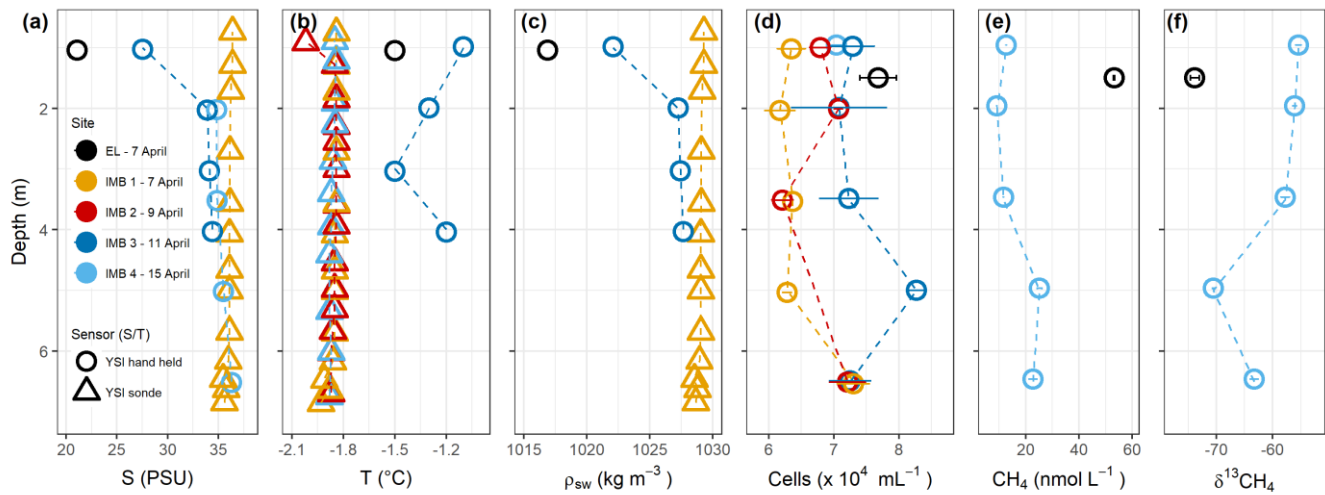
Figure 1: Water column properties during the time series near Utqiagvik. Salinity (a), temperature (b), density (c), cell abundance (d), methane concentration (a) and stable isotope ratios (b). Error bars on cell numbers (d) represent the standard deviation on two technical replicates. Temperature and salinity were determined with an YSI hand held (circle) and YSI sonde (triangle). Salinity for the YSI hand held on 15 April was determined with in the laboratory, thus in situ temperature is missing. Salinity for the YSI sonde on 9 and 15 April is missing due to freezing of the sensor. Methane data is only available for EL at 7 April and for IMB 4 at 15 April.
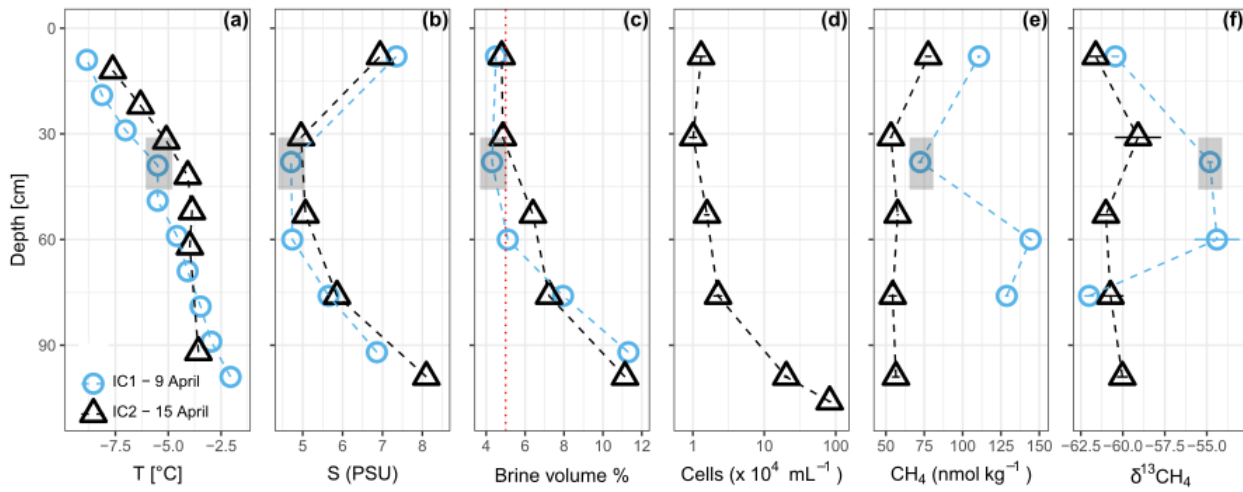
Figure 2: Sea ice temperature (a), bulk salinity (b), brine volume fraction (c), prokaryotic cells mL^{-1} sea ice (for IC2 only) (d), methane concentration (e) and stable isotope ratios (f). The vertical red dotted line in (c) shows a brine volume fraction of 5%, the threshold for permeability (Golden et al., 1998). IC1 had sediment included into the ice matrix at depth 30–46 cm, indicated by the gray box.
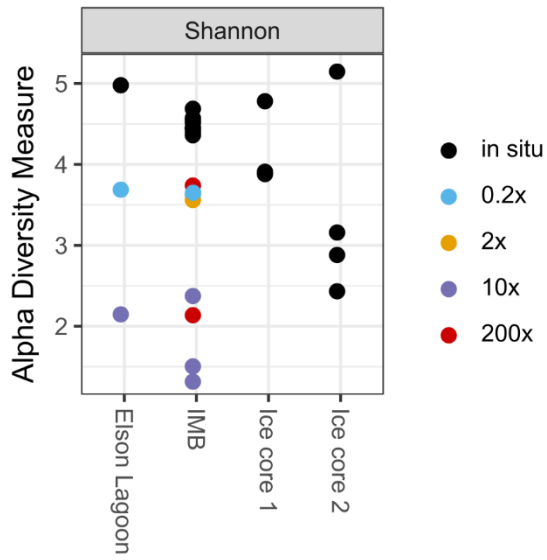
Figure 3: Shannon indices of alpha diversity for V4-V5 amplicons.
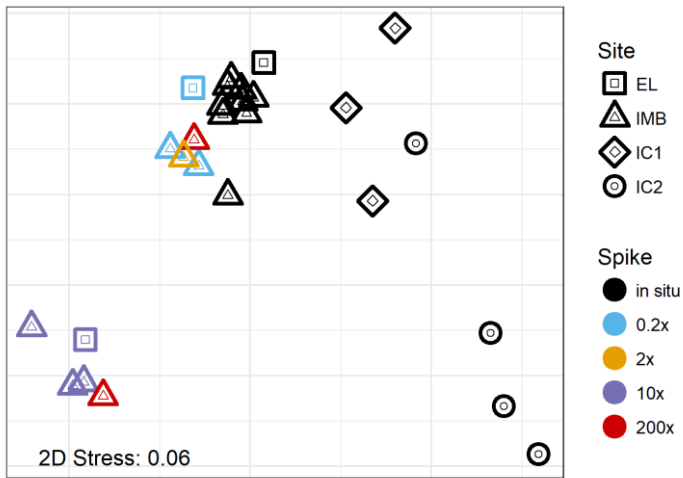
Figure 4: Non-metric multidimensional scaling analysis (unitless) of Bray-Curtis dissimilarities of the 16S read data. The low 2D stress of 0.06 indicates a good two-dimensional representation of the multidimensional dataset with very low prospect of misinterpretation.
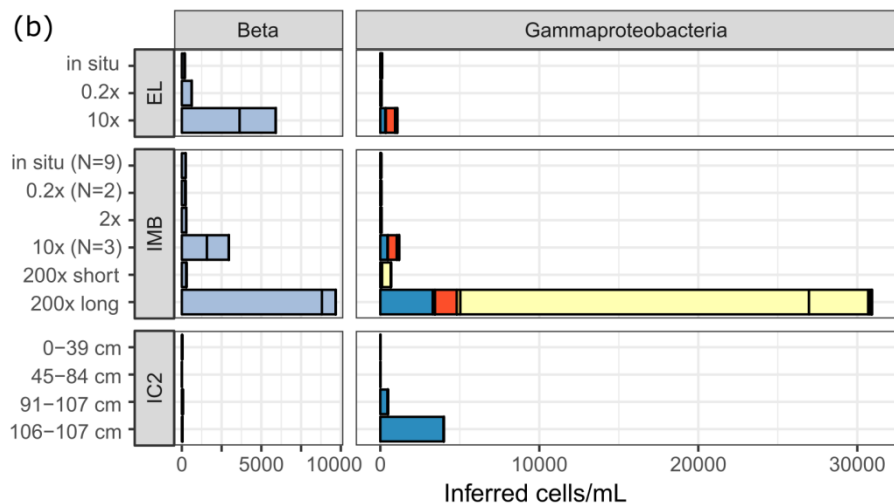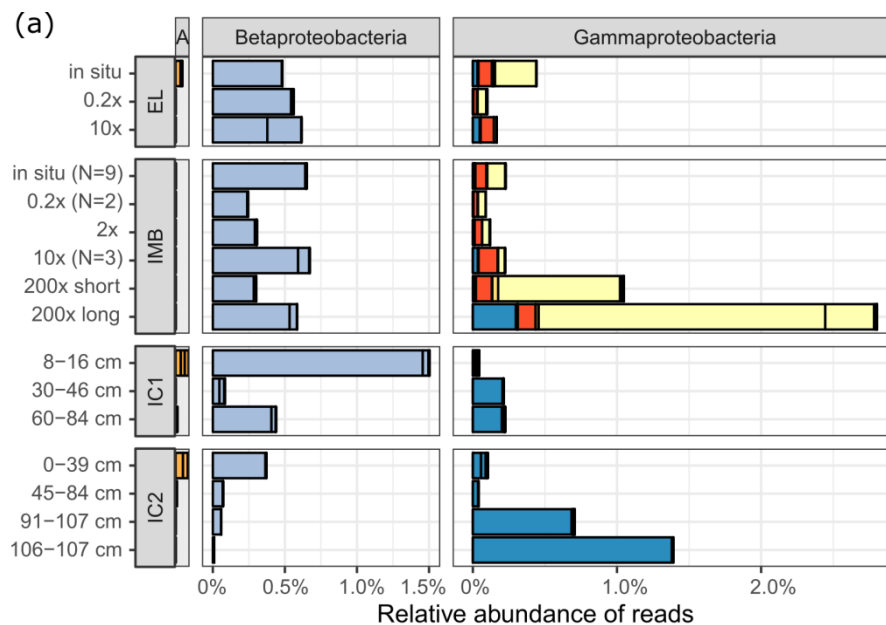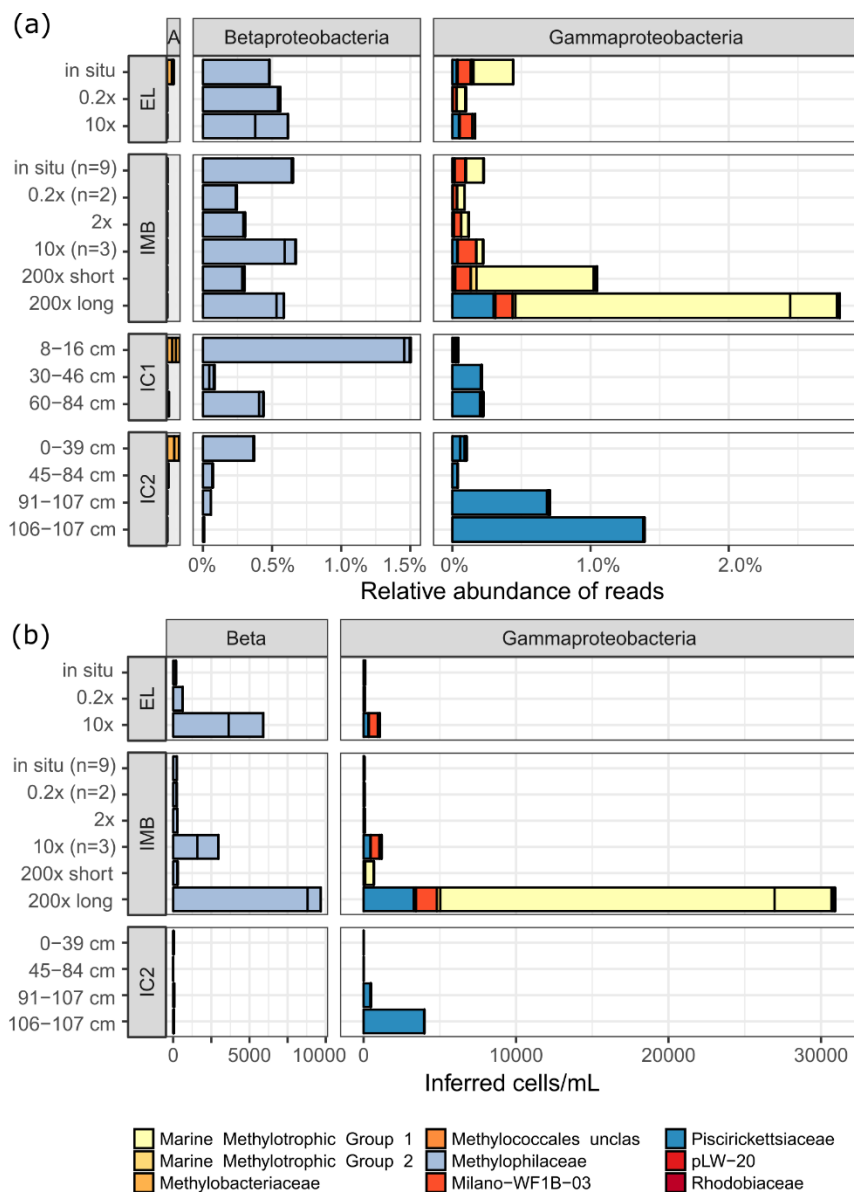
Figure 5: Relative abundances (a) and inferred cell numbers (b) of methylotroph OTUs by family. Sampling sites for water samples are Elson Lagoon (EL) and mass balance buoy (IMB). Ice cores (IC1 and IC2) were collected at site IMB on 9 and 15 April, respectively. The sample name indicates the methane spike concentration compared to in situ methane concentration for IMB and EL, and the ice core section in cm from top (0 cm, ice-snow interface) to bottom (ice-water interface). IMB in situ, 0.2x and 10x are averages of the respective number (~~Nn~~) of samples, all other samples were ~~n~~N=1. Red and yellow shades indicate MOB, while blue shades indicate non-MOB-methylotrophs. (a) α-Proteobacteria (A), β-Proteobacteria (Beta) and γ-Proteobacteria are shown; Verrucomicrobia Incertae Sedis were <0.003% in (a). Scale for α-Proteobacteria is the same as for β- and γ-Proteobacteria. (b) Cell numbers were calculated from the relative abundances shown in (a) with the cell counts from flow cytometry and corrected for the 16S copy number per cell. Verrucomicrobia Incertae Sedis and α-Proteobacteria were < 8 cells mL^{-1}.
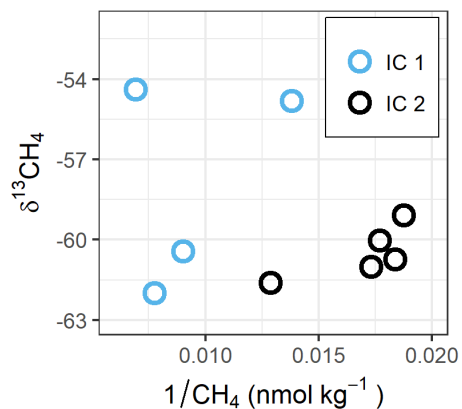
Figure 6: δ¹³CH₄ vs. reciprocal of CH₄ concentration (Keeling type plot) of ice cores. Within each ice core a shift to more positive δ¹³CH₄ values in combination with a decrease in CH₄ concentration indicates microbial oxidation. Comparing IC2 to IC1, the shift toward higher concentrations and more positive δ¹³CH₄ (see also Fig. 2) in IC1 might indicate CH₄ production from a substrate with heavier isotope signature, compared to the values in IC2.
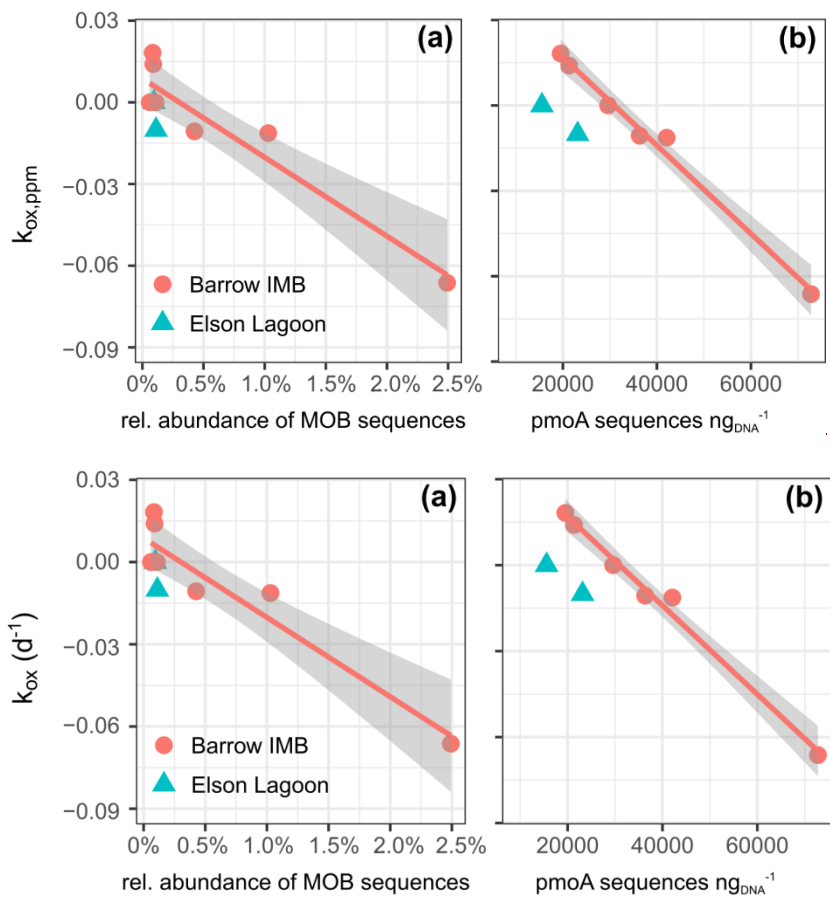
Figure 7: Correlation between net oxidation rate constant ($k_{ox,ppm}$) and the relative abundance of sequences in 16S-´MOB´-OTUs, $R^2_{(MOB-OTUs\sim kox)} = 0.84$ (a) and number of pmoA sequences with $R^2_{(pmoA\sim kox)} = 0.85$ (b). For correlation to the number of total methylotroph OTUs (which includes MOB and non-MOB-methylotrophs in total 16S) $R^2_{(Meth´-OTUs\sim kox)} = 0.81$. The gray shaded area shows the 95% confidence interval of the correlation.
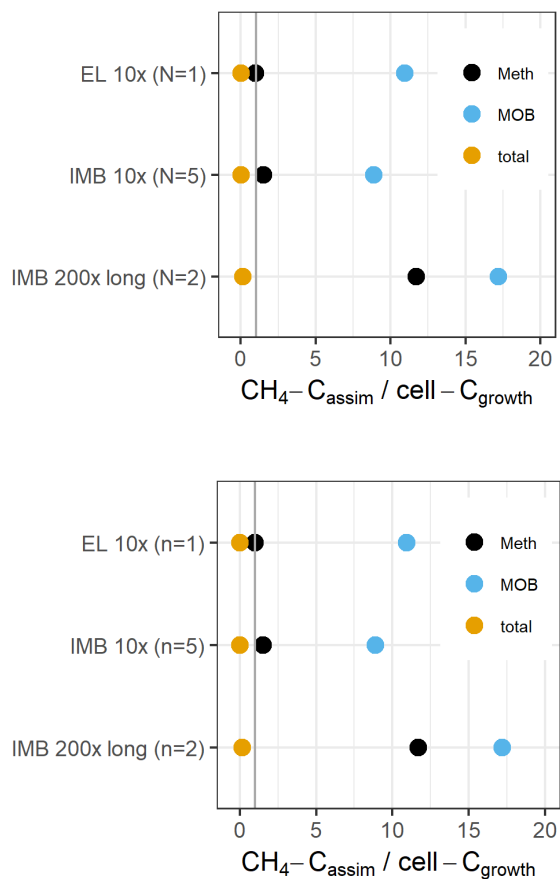
Figure 8: Ratio of methane-carbon assimilated (CH₄-C_assim) to cell-C gained during growth (cell-C_growth), based on flow cytometric cell counts (total) or inferred cell numbers (Meth, MOB). The standard deviation between replicates was 10% to –20%. The vertical line indicates a ratio of 1. Above 1, the entire cell gain can be explained by the assimilated CH₄.

Table 1: Station and sample list

Name[1]	Date	Position	Samples	Parameters[2]
EL	07.04.2016	71.334° N, -156.363° W	water	in situ CH_4, ox rate, T/S, DNA, cell counts, nutrients
IMB 1	07.04.2016	71.373° N, -156.548° W	water	ox rate, DNA[3], cell counts, nutrients[3]
IMB 2	09.04.2017	71.372° N, -156.540° W	water	ox rate, T, DNA[3], cell counts, nutrients[3]
			ice core 1	in situ CH_4, T/S, DNA
IMB 3	11.04.2015	71.372° N, -156.540° W	water	T/S[3], DNA, nutrients, cell counts
IMB 4	15.04.2017	71.372° N, -156.540° W	water	in situ CH_4, T/S, DNA[3]
			ice core 2	in situ CH_4, T/S, DNA, cell counts

[1]Station abbreviations are Elson Lagoon (EL) and ice mass balance buoy (IMB)

[2]Parameters: in situ concentration and $\delta^{13}CH_4$ (in situ CH_4), net oxidation/production rate (ox rate), temperature and salinity (T/S), collection of biomass for DNA extraction (DNA), cell counts, nutrients

5 [3]No complete depth profile available

Table 2: Samples sequenced for V4-V5 and pmoA

Treatment[1]	Station	V4-V5 # of samples	pmoA # of samples
in situ	IMB	9	4
	EL	1	1
	sea ice	7	0
0.2x, 10 days	IMB 1	2	3
	EL	1	1
2x, 5 days	IMB 2	1	1
10x, 46 days	IMB 1	3	2
	EL	1	1
200x, 6 days	IMB 2	1	1
200x, 41 days	IMB 2	1	1

[1]The different incubation times resulted from logistical constraints

Table 3: Methane oxidation parameters during long-term incubation experiments. nN: number of replicates, $c(CH_4)_{initial}$: approximate initial methane concentration, $k_{ox,ppm}$**: net oxidation/production rate constant,** $r_{ox,ppm}$**: net oxidation/production rate at in situ concentration,** α_{ox}**: isotopic fractionation factor during oxidation. Oxidation rates and rate constants are replicated from Uhlig and Loose (2017).**

Treatment	nN[1]	Incubation[2] [days]	$c(CH_4)_{initial}$ [nmol L^{-1}]	$c(CH_4)_{final}$ [nmol L^{-1}]	$k_{ox,ppm}$ [d^{-1}]	$r_{ox,ppm}$ [nmol L^{-1} d^{-1}]	α_{ox}
0.2x EL	1	10	12.7	12.9	0[3]	0[1]	0.9591
10x EL	1	46	132.3	67.7	1.01×10^{-2}	0.54	1.0230
0.2x IMB 1	5	10	4.4 ± 0.5	5.0 ± 0.4	-1.05×10^{-2}	Negative[4]	0.994 ± 0.0113
2x IMB 2	4	5	37.9 ± 1.8	36.5 ± 1.4	0[3]	0[3]	0.9898 ± 0.0104
10x IMB 1	5	46	123.0 ± 5.5	69.4 ± 36.5	9.18×10^{-3}	0.15 ± 0.02	1.0225 ± 0.0005
200x IMB 2 short	7	6	3937.9 ± 148.7	3427.6 ± 160.4	0[3]	0[3]	1.0005 ± 0.0005
200x IMB 2 long	2	41	4089.5 ± 26.1	129.6 ± 95.5	6.62×10^{-2}	1.08 ± 0.17	1.0103 ± 0.0002
200x IMB 2 NaOH	1	41	3953.7	3620.7	0[3]	0[3]	0.9998

[1]Replicates are from different water depth

[2]The different incubation times resulted from logistical constraints

[3]Oxidation rate constants were not significantly different from 0 at a 95% confidence level

[4]Negative net oxidation rate constant indicating methane production

Table 4: Relative abundance of Methylotroph-OTUs in situ, split into methanotrophs (MOB) and non-MOB-methylotrophs ("Methy")

		in situ sea ice	in situ sea water	0.2x, 2x (short)	10x (long)	200x (long+short)
N		7	10	4	4	2
Mean ± sd	MOB	0.04% ± 0.04%	0.24% ± 0.09%	0.09% ± 0.01%	0.17% ± 0.15%	1.76% ± 0.73%
	Methy	0.74% ± 0.50%	0.65% ± 0.12%	0.34% ± 0.13%	0.70% ± 0.62%	0.61% ± 0.29%
min	MOB	0.00%	0.06%	0.08%	0.06%	1.03%
	Methy	0.11%	0.51%	0.23%	0.20%	0.32%
max	MOB	0.11%	0.45%	0.11%	0.43%	2.49%
	Methy	1.53%	0.83%	0.56%	1.72%	0.90%

Table 5: Spearman rank order correlations coefficients (ρ_S) of $k_{ox,ppm}$ vs. the number of sequences of pmoA MOB and non-MOB methylotrophs, and candidate OTUs

	Total	Normalized[2]	Relative abundance	Inferred cell density[3]
pmoA	-0.86**[3]	n.d.	n.d.	n.d.
methylotrophs	-0.81**	-0.97***	-0.79**	-0.63.
MOB	-0.82**	-0.66*	-0.82**	-0.61.
non-MOB	-0.71*	-0.80**	-0.69*	-0.58.
candidate OTUs[4]	-0.07 ns	-0.23 ns	-0.03ns	n.d.

[1]Levels: ρ_S < 0.8 very strong, 0.6 < ρ_S < 0.8 strong

[2]normalized to total abundance of reads using the DESeq2 package

5 [3]MOB cell density was calculated from relative abundance and flow cytometry cell counts, weighted for copy number of 16S for respective OTUs

[4]Significance levels: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns ' 1

[5]Candidate OTUs are OTUs that were differentially more abundant in 10x and 200x incubated samples