Reply to associate editor:

Dear Christiane,

thank you very much for handing in your replies to the reports of two reviewers that assessed your MS. These raised substantial concern to which I agree. In accordance with your replies, which seem sound, I would hereby like to invite you to prepare a revised version of your MS. Please pay particular attention to the inconsistencies with respect to sample treatment (thawing, incubation time etc), and add discussion on this matter so that it becomes clearer in how far the different treatments can be compared. Reviewer 2 provided important and helpful comments regarding this matter. I am aware that this is a field study and that sampling and replication is not always textbook-like. Nevertheless, make sure to clearly note and discuss when other inconsistencies occurred (eg differences in water masses) and in how far this limits/affects the significance of your MS. Water mass transport, eg, can severely change microbial community composition, abundance and activity (see eg Steinle et al., 2015); thus counting samples from differential water masses as replicates is a bit complicated.

Best wishes, Helge

Dear Helge,

Thank you for your invitation to resubmit a revised version of our paper. We have addressed all reviewer comments and our replies can be found in the point-to-point replies. Changes in the manuscript are indicated with lines numbers for the edited manuscript.

We added information to clarify that all samples in our oxidation rate incubations were taken before the current reversal and were thus not influenced by the different water mass (4.2; P13 L4-10). In addition, we highlighted how DNA sampling of both water masses shows that community structure does not change with the inflow of the warm water (3.4; P9 L25-30).

We particularly also addressed the problem of different experimental procedures. Ice melting procedures and the influence of microbial activity during prolonged melting were indicated in Section 2.2 (Material and Methods, P4 L26-28), 3.2 (Results, P9 L8-9), and discussed in 4.1 (P12 L8-23).

We added explanation and discussion on the different incubation durations in section 3.4 (P9 L30 - P10 L3) and 4.2 (P13, L21-27), respectively. These point out, that the incubation time has a major influence on the community composition, and that the short incubations were too short to resolve oxidation rates, either because of very small changes in concentration (2x) or due to a long lag phase (200x treatments).

In addition to the edits according to the reviewers comments and editing language, a calculation error on the oxidation rates (r_{ox}) in Table 3 was corrected, which leads to a change in numbers of about 5-12%.

We believe that the updated manuscript addresses and clarifies all of the questions and comments raised by the reviewers and yourself, and has been improved with everyone's contributions. Thank you and the reviewers for your time and effort.

Best regards,

Christiane, on behalf of all coauthors

Reply to comments of Anonymous Referee #1:

This is the first study to use next generation sequencing techniques to study methane oxidizing bacteria in Arctic seawater and sea ice, combining community analysis with measurement of methane oxidation rates and incubation experiments with varying methane concentrations and incubation time. Methane oxidizing bacteria can play an important role in reducing methane flux to the atmosphere, but relatively little is known about what controls their abundance and activity, so this paper makes a nice contribution to the literature. The methane-oxidizing microbial communities in seawater and sea ice are different, but in both environments their relative abundance is fairly low. Relative abundance remained low even after incubation at elevated methane concentrations, though total abundance increased. I have no major criticisms of this paper.

Reply: We would like to thank Referee #1 for the encouraging and helpful review. Please find our replies to the specific comments below.

Specific comments: P6, L25: how many contaminant sequences were removed, relative to total sequences?

Changed to: Contaminating sequences observed in kit and filter blanks accounted for 1.4% of total reads and were removed from all samples (P7 L5)

P9, L16: 16S rRNA gene

Changed to: "16S rRNA" corrected to "16S rRNA gene" (P9 L24)

P14:I'm not sure this means copper couldn't be limiting methane oxidation

Reply: Thanks for this comment. It is true, that we cannot rule out that methanotrophs containing particulate MMO and soluble MMO were growing on limiting copper (resulting in the presence of pmoA genes) while the actual oxidation was performed by sMMO. We suggest to modify the statement to "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. " (P14, L28-32)

Figure S5: Is there a list of candidate OTUs somewhere? Figure S5 doesn't show many of these very clearly- I can't even tell what group of proteobacteria is shown in the first panel. Any theories on what's going on with these organisms if they're not oxidizing methane?

Reply: Thanks for the note. The labels of the graph were fixed and provided in the figure legend. A list of the candidate OTUs was prepared for the revised version of the Supplementary Material.

We hypothesize that those groups might be involved in cross-feeding on intermediate metabolites produced by the methanotrophs, which was for example observed for *Cowellia* (Jensen et al., 2008). But not the entire cell gain can be explained by methane as C-source. We thus suggest that the candidate OTUs might feed on available DOM, *Oleispira* for example is known to prefer complex organic substances (Yakimov et al. 2003). Although we aimed to remove OTUs favored by the "bottle effect", by removing types that were also abundant in 0.2x incubations, it is possible that the candidate OTUs are favored by

the long incubations since the 10x and 200x incubations were incubated for about 41 to 45 days compared to 10 days for the 0.2x treatment.

Reply to comments of Anonymous Referee #2:

Uhlig et al. present novel data of the methane oxidizing community structure from in situ samples and incubation experiments from ice and water at an Arctic shelf. They sequenced both the pmoA gene and the v4/v5 region of the 16S rRNA gene, which has not been done often. They compare these results to hydrophysicochemical data and net methane oxidation rates. (Part of?) The methane oxidation rates have been presented in Uhlig 2017 (L&O methods). Although the data is interesting and is a valuable addition to the presently available pool of research the MS needs more work and several points have to be clarified before it can be published.

Reply: We would like to thank Referee #2 for the very detailed and helpful comments to improve our manuscript. We addressed all concerns and provide suggestions how to edit the manuscript. Please find our detailed answers below.

Major concerns

Sampling and incubations: I think it is difficult to compare two ice cores when one of them was melted at 5 degC for 1 week, the other at room temperature for 1 day. The authors mention it but also for further experiments experimental setups should be kept consistent between samples. The same is true for the incubation time of short and long incubations. They are not consistent between treatments. Why? Was it due to a limitation of the research station? Why where there not always replicates and dead controls taken? That should not be a problem if sampling takes place with a pump.

Reply: We agree with reviewer 2, that in our study unfortunately treatments (melting temperature and incubation times) for samples were not always the same, which makes comparison less straight forward. Fieldwork for the present study both served to develop the method to use stable isotopes to determine methane oxidation rates (Uhlig and Loose, 2017) and to attempt gaining basic insights into the methanotrophic community. Harsh conditions while sampling on the ice as well as the limited time period and resources in the field laboratory as well as logistic limitations caused setbacks and required us to make tradeoffs in the number of killed controls, replicates, incubation period and ice melting procedures. For example, not all incubations could be transported back to home laboratory to continue analysis as presented for the long incubations. The negative control for incubations taken at station IMB 1 was unfortunately lost during the experiment. Other experiments with water from non-polar locations (e.g. Narragansett Bay presented in Uhlig & Loose 2017), did not indicate diffusive loss or isotopic fractionation in the killed treatments. We agree that these conditions should be kept more consistent in future studies and more negative controls should be taken.

In the revised manuscript we added further explanation addressing the problem of the different experimental procedures and possible consequences for interpretation of the results.

Ice melting procedures and the influence of microbial activity during prolonged melting were indicated in

- Material and Methods (2.2; P4 L26-28) "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.";
- Results (3.2; P9 L8-9) "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."; and

- Discussion (4.1; P12 L8-9, L12-13) "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.", "In addition, microbial processes like oxidation of methane or methanogenesis could have taken place in situ or during sample processing and storage."

Regarding the different incubation durations we added a general explanation in the Materials and Method section (2.3, P5 L7-8): "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 0.1M NaOH."
Further explanation and discussion in section 3.4 (P9 L30 - P10 L3) and 4.2 (P13, L21-27) point out, that the incubation time has a major influence on the community composition, and that the short incubations were too short to resolve oxidation rates, either because of very small changes in concentration (2x) or due to a long lag phase (200x treatments).

- P9 L30 - P10 L3 "For incubated samples, the final community structure is driven 30 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 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)."
- P13, L21-27 "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 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."

Are the IMB samples all from the same day and did you do the replicates all from one depth so that the initial community composition and methane concentrations were the same? What about the water mass that was different during several days? Did you compare the community composition during the two different 'conditions'? I could expect a different community since temperature and oxygen, as well as ADCP data both show the presence of a different water mass.

Reply: Samples for incubations were taken at site IMB on two different days (IMB 1 on April 7 and IMB 2 on April 9) as given in Table 1 and Table 3. Samples were taken at 5 depths between 1 m and 6.5 m. We treated those samples as replicates since the phylogenetic analysis did not indicate differences in species composition in the depth profile. All samples from the depth profile, which was taken at IMB 3, cluster close together in the NMDS analysis (Figure 4). In accordance with this, the more detailed plot on V4V5 diversity in Supplementary Figure 4 does not show differences with depth. The same holds true for the 1 m and 6.5 m samples taken at IMB 1.

The handheld YSI indicates that IMB 3 was sampled from warmer water than the other IMB stations, which however does not hold true in the ADCP data (we indicated both sampling events in the revised Supplementary Figures 1 and 2). The NMDS plot (Figure 4) does not show differences between the in situ communities sampled at IMB 1, IMB 3 and IMB 4. IMB 2 is more distant to the other stations; however, temperature profiles (Figure 1) indicate cold water during all of IMB 1, IMB 2 and IMB 4.

In the revised manuscript we added information to clarify that all samples in our oxidation rate incubations were taken before the current reversal and were thus not influenced by the different water mass (4.2; P13 L4-10). In addition, we highlighted how DNA sampling of both water masses shows that community structure does not change with the inflow of the warm water (3.4; P9 L25-30).

Data: Are you presenting the same net oxidation rates as in your paper in L&O methods? If yes, this should be mentioned more clearly.

Yes, the net oxidation rates that we compare the microbial sequence data to here were published in Uhlig & Loose, 2017 – this was referenced accordingly in Table 3 and in the results section. In order to present this more clearly, we also made multiple modifications to the revised version, including the results and discussion. As stated in the revised results section, "Net oxidation rates discussed here were published in Uhlig and Loose (2017) and are summarized for comparison with the microbial community structure. " (P9 L17). Caption of Table 3 was modified to "Oxidation rates and rate constants are replicated from Uhlig and Loose 2017"

Discussion 4.1.: The discussion about CH4-isotopic composition in the ice is incomplete and not very clear. This should be changed.

Reply: We restructured this section and included methane oxidation in the possible microbial processes: "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 10 cores 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 15 sediment (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 20 substrate would be enriched in 13C (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)."

Figure 6: Did you exclude one point for the correlation of IC1? If yes, why? If no, how can the correlation look like this? For IC2, you make a linear correlation mainly dominated by one point. Do you think that is ok?

Thanks for this helpful comment. We removed the misleading correlation lines in the revised plot. The legend and text referring to the correlation was modified accordingly.

Other concerns:

Page1

L21: Please write "…80%. Total MOB abundances.. Reply: L21 corrected as suggested.

L22: methLylotrophs Reply: L22 corrected as suggested.

L22: "present in abundances compared to .."; present in SIMILAR abundances? Reply: L22 changed to "..were present in abundances similar to natural.."

L23/24: (last sentence of abstract) not very clear sentence. please change

Reply: L23-25 we suggest changing the sentence to "The dissimilarities in MOB taxa, methane concentrations and stable isotope ratios between sea ice and water column point toward different methane cycling processes in the two environments."

L27: 25x higher radiative forcing is only true if you consider a certain time period. please specify

Reply L 28: the time span was specified to 100 years.

Page2

L9: what about dilution effects?

Reply L6-8: We modified to: "Dissolved methane 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 et al., 2014).

L23: development OF communities Reply: L25 corrected as suggested.

L29: what about the primers developed by tavormina et al. 2008? why did you not use them, especially in a marine environment?

Reply: We agree that application and comparison of this primer set could have been useful. However, we had to select one primer set and we made that choice in order to maximize comparison to other reports. In the revised manuscript, while we are satisfied by the tight correlation between methane oxidation rates and the data produced with the primers we used, we suggest that the Tavormina et al. primer set in future work may be one way to improve upon these studies. "Future application of marine-specific pmoA primers may further improve this correlation (Tavormina et al., 2008)." (P14 L13/14 & P2 L32)

L3: are methanotrophs not methylotrophic? please specify

Reply: We thank reviewer #1 for this comment. Methanotrophs are included in the larger groups of methylotrophs. We clarified in the revised manuscript that here we are referring particularly to "non-methane utilizing methylotrophs" and other bacteria, which cross-feed on methane derived carbon. See also the next comment. (P3 L5)

L4: what do you mean by: "THIS is attributed..

Reply P3 L5-7: In the revised manuscript we rephrased this sentence to: "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)."

L29: afterwards, you talk about the two sites in a different order. first IMB, then EL. Check for consistency throughout the MS since it is much easier to follow

Reply: We implemented a consistent order first mentioning EL then IMB.

Page4

L5: add YSI, Ohio, USA to the sonde Reply: P4 L6 corrected as suggested.

L26: What is IC1 and 2. First, talk about 1, then 2 and add abbreviation

Reply P4 L24-26: In the revised manuscript we rephrased to "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."

Page5

L2: how do you get 0.2x of in situ concentrations? did you remove methane? (ok, explained on page 13, add explanation on page 5)

Reply P5 L5/6: We suggest adding the following explanation "approximately 0.2x (without methane addition, resulting in degassing of in situ methane to the headspace)…"

Page6

L8: is it not common to write v4-v5 region or v4/v5?

Reply: Thanks for the note. We changed all occurrences in the manuscript to "V4-V5"

Page7

L15: between and spiked? I don't understand

Reply: P7 L23 "and spiked treatments" substituted by "incubations"

L15 and bellow: why do you call the 0.2x treatment spiked? you did not add methane to it as far as I understood

Reply: In the revised manuscript, we removed "spiked" or replaced it by "incubated" to correct for the incorrect wording regarding the 0.2x treatments.

Page8

L10: in supp. fig.1 the maximum seems to be -0.9 degC

That's correct, the "-" sign was shown in line 9.

L18: here and throughout the MS, check 103 etc.

Reply: Thanks, we checked and corrected all occurrences.

Page9

L1: why no cell counts for IC1

Reply: Unfortunately, we did not collect samples for cell counts for IC1. The description was modified accordingly in P9 L9.

L10/table2: why did you not incubate all different treatments the same amount of days? it makes it more difficult do compare the data

Reply: The different incubation times were caused by logistical constraints in the field and for sample transport. We acknowledge that this is not ideal and should be improved in further studies. We added an

explanatory note at Table 2. Also, we note how prolonged incubations do appear to affect community structure as noted in section 3.4 (P9 L30 – P10 L3)

L28: do you have an idea in what processes members of oleispera could be involved?

Since *Oleispira sp.* are only found in high abundances in the long incubations and are typically degrading hydrocarbons or other complex organic substances (Yakimov, 2003), we speculate that they might consume complex DOM compounds and at a later stage of the incubation, after the more easily degradable DOM compounds are depleted. Growth cannot be supported by CH_4 compound cross-feeding only, as Figure 8 and the respective calculation indicate.

L29: slightly more abundant..compared to what?

Reply P10 L18/19: Will be modified to "…only slightly more abundant in the short-incubation treatments (0.5%–1.6%) compared to in situ abundances."

Page10

L3: "deviated further" further than what if short incubations were similar to in situ samples?

Reply: P10 L2 was modified to "…clearly deviated from the in situ samples"

Page11

L15: add "depths AT IMB exhibit.." for better understanding. it is confusing when you're talking about which site Reply: P11 L31 corrected as suggested

L24-L30: you only talk about methanogenesis. what about methane oxidation? it would also alter the isotopic composition of the remaining methane..
Reply: We would like to thank reviewer 2 for this advice. Please refer to our reply to the comment on Discussion 4.1 above. We restructured the discussion and added discussion on oxidation in P12 L12-23.

Page12

L11: change the position of "also" to " might also have taken place Reply: The paragraph was rephrased 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." (P12 L27-30)

L11: remove the comma after study Reply: see answer to previous comment.

L15/16: what is a possible explanation of higher oxidation rates during ice covered conditions?

Reply: In the study by Lorensen and Kvenvolden (2016), higher oxidation rates coincided with higher methane concentrations. Methane concentrations in this study were often found to be elevated under the sea ice cover due to reduced sea air exchange. We suggest to add "Due to reduced sea-air gas exchange, higher methane concentrations can build up under sea ice cover, which might lead to higher oxidation rates (Lorenson and Kvenvolden, 1995)." (L13 L1/2)

L19: "fall into the middle"? mid-range might be clearer Reply: P13 L12 corrected as suggested

L22: how many points did you have to calculate the fractionation factors?

Reply: The fractionation factors were calculated from the slope of the linear regression of 5 to 6 data points for each replicate. The number for replicates for each treatment is given in Table 3.

Page13

L3: please make sure that you're always talking about net methane oxidation/production as you're not directly measuring rates. here, and throughout the ms

Reply: Thank you. We specified the occurrences accordingly in Materials and Methods, Results and the Discussion

L9: why make a new paragraph? Reply: we removed the paragraph P30 L31

L11: do you mean "phosphonates" or "methylated phosphonates"?

Reply: removed, when discussion was shortened (refer to next comment)

L3-L22: a lot of discussion for data, for which you do not have a killed control..please shorten
Reply: Thanks for this note. We have shortened the discussion to about half length P13 L28 – P14 L3

Page14

L9: delete the in before in situ Reply: P14 L23 corrected as suggested.

L15: remove the "the" before kox Removed when discussion on copper limitation was edited according to the suggestion by reviewer 1.

Page15

L1: correlation between what and what?

Reply: P15 L15 changed to: "In contrast, the correlation between OTUs that were differentially more abundant in the incubated samples and k_{ox} was weak (Table 5)"

L25: what about IC1?

Reply: P16 L8-11 was changed to "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 IC2, whereas the top-most section of IC1had the second smallest concentration of methane in this ice core (Figure 2e)."

L25: abundance OF MOB Reply: see next comment

L25-27: I do not understand this sentence

Reply P16 L10/11: We change this sentence to "Relative abundances of MOB in the inner and bottom sections 10 of the ice cores were even lower, with 0 to 0.02% only."

Page16
L7-10: very long sentence. shorten? Reply: we restructured to: "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 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." (P16 L20-24)

L11: comma is wrong before suggests Reply: P16 L25 corrected as suggested.

L13/14: if you consider possible methane production within the ice, why not methane oxidation?

Reply: We aim to highlight the differences of methane concentrations and isotope ratios between the sea ice and sea water. We included the oxidation in sea ice but noted that this would be at lower rates, to explain the observed differences. "Possible causes explaining this observation include (i) microbial production of methane, even within the ice (Damm et al., 2015), and (ii) microbial oxidation in the water column and at lower rates in sea ice." (P16 L28)

Comments to tables and figures

Table 1: What is the difference between nutrients and nutrients depth profile (IMB2/3)? you also did cell counts for IC2, right?

Reply: Complete depth profiles in the water for DNA, nutrients and in situ CH_4 are only available for the stations explicitly mentioned in the parameters list in Table 1. We introduced another superscript explanation, to identify the parameters that do not have a complete profile and remove the "depth profile" from the parameters list.

We will added cell counts for IC2. Thanks for noting!

Table 2: why did you not take any samples for pmoA of the sea ice?

Reply: To our knowledge this is the first study to use this set of *pmoA* primers with Illumina MiSeq technology. For the pilot run in our study, we focused on water samples only. Due to the amplicon length causing a gap in sequencing overlap, we were only able to use the forward read for analysis. This reduces the possibilities to compare the *pmoA* sequences to existing Sanger or 454-sequencing studies with longer read length. We thus decided not to analyze sea ice samples for *pmoA* diversity. In addition, the quantification of *pmoA* reads was related to oxidation rate measurements, which were only determined from water samples.

Table 3: please indicate the initial and final dissolved methane concentrations. Maybe add a line between EL and IMB.

Reply: We added the initial and final dissolved methane concentrations and the line between EL and IMB.

Figure1: why does the hand held YSI indicate warmer temperatures but the adcp not? how do you think would a regularly melting ice (due to warmer water from the south) influence methane concentrations and the community structure?

Note on current directions in Supplementary Figure 1: The more frequent current direction along Barrow Canyon is northeastward transporting cold Chuckchi Sea resident water (Aagaard and Roach, 1990; Woodgate et al., 2005). The current reversal leads to a southwest direction combined with upwelling of warmer Arctic Intermediate water (Atlantic Layer). The current directions indicated in Supplementary Figure 1 are very likely influenced by the local conditions and may not reflect the general transport through Barrow Canyon (pers. comm. Andy Mahony, University Fairbanks, AK). Factors that might be

causing this disturbance are for example (i) seawater swirling around Point Barrow or (ii) grounded fast ice at the shallow deployment site beneath the sea ice local. It is thus likely that the current direction indicated by the ADCP does not reflect the general flow along Barrow Canyon. We thus interpreted the temperature signature with given literature to determine the general source region of the advected water. We added this explanation to Supplementary Figure 1.

Reply: The temperature difference between the water column (handheld YSI) and bottom signal (moored ADCP and CTD), might be caused by incomplete mixed of the water column. The current was just changing direction according to the ADCP data, when we sampled on 11 April. Warmer water might thus be dominating the upper water layers causing the melt signal with lower salinity/density, while the bottom water was still cold (pers. comm. Hajo Eiken, University Fairbanks, AK). We do, however, expect that these stratifications would not be stable over extended time periods due to the shallow water depth and tidal influence (Figure 1).

Given the clear signal in a change in current direction and the temperature signature, we derive that the warmer water mass is likely upwelled Arctic Intermediate water (Atlantic Layer). These waters are probably low in methane oxidizers since methane concentrations in intermediate water depths are usually low. When this warmer water leads to melting of sea ice that has a higher methane concentration per volume; methane concentration in the upper water column might increase. Additionally, the warmer water might lead to short periods of increased oxidation due to higher microbial turnover at higher temperatures.

Figure 2: grey box is missing

Reply: Thanks for the note. The grey box was accidentally lost in the figure and is now added in the revised Figure 2.

Figure 3: the blue dot is not visible for "IMB"

Reply: Thanks for the note. The blue dot is now visible for IMB.

Figure 8: "..Above the entIRE cell gain.."

Reply: Will be changed to "… the entire cell gain.."

Please check for double-spaces in between words.

Reply: Thanks for the note. We checked the entire manuscript for double spaces and removed them.

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~~Methane~~ Methane-oxidizing seawater microbial communities from an Arctic shelf

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Abstract. M~~arine m~~icrobial communities ~~of the ocean~~ can consume dissolved methane ~~dissolved in seawater~~ before it has a chance to escape to the atmosphere and contribute to greenhouse warming. Seawater over the shallow Arctic shelf is characterized by excess methane compared to ~~the~~ atmospheric equilibrium. This methane ~~originating~~ originates in sediment~~s~~, permafrost and hydrate~~s~~. 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 ± 7.2 nmol L^{-1}, approximately 4.8 times oversaturated ~~compared~~ relative to ~~the~~ atmospheric equilibrium. The group of methane-oxidizing bacteria (MOB) in the natural seawater and incubated seawater ~~incubations~~ was >97% dominated by Methylococcales (γ-Proteobacteria). Incubations of seawater under a range of methane concentrations led to ~~a~~ 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 methane concentrations of 82 ± 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-meth~~l~~ylotrophs were present in abundances ~~compared~~ similar to natural seawater communities. The ~~differences~~ dissimilarities in MOB taxa~~,~~, ~~and an offset in~~ methane concentration~~s~~ and stable isotope ratios between ~~the~~ sea ice and ~~the~~ water column point toward different methane cycling processes in ~~both habitats~~ the two environments.

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 2~~8~~5x higher accumulative radiative forcing potential (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~~s~~ (Formolo, 2010; Reeburgh, 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 ~~inside~~ within the sediment by anaerobic and

5   aerobic oxidation (Barnes and Goldberg, 1976; Boetius and Wenzhöfer, 2013; Knittel and Boetius, 2009; Reeburgh, 1976). The remaining ~~fraction of~~ methane either diffuses into the water at the sediment surface, or ~~can be~~is released as bubbles, which completely or partially dissolve while rising through the water column (Leifer and Patro, 2002). Dissolved methane 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 et al., 2014). As

10   a result of these biological and physical processes, oceanic methane concentrations are frequently ~~found~~ at low nanomolar levels, leaving only a small fraction of subseafloor-~~-~~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), 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 under ice free conditions, methane concentrations are frequently found in the range of 15 to 30 nmol L^{-1} or around 700% supersaturated with regard to ~~the~~ atmospheric equilibrium, winter concentrations are often 10 to 100 times higher. Maximal concentrations of 5000 nmol L^{-1}, ~~or an~~ oversaturation of 160000%, have been reported from the

20   Siberian Shelf (Lorenson et al., 2016; Shakhova et al., 2010; Zhou et al., 2014).

~~Besides others~~Along with factors like oxygen and trace metal availability (Crespo-Medina et al., 2014; Sansone et al., 2001; Semrau et al., 2010), dissolved methane concentration is an important control on the community of MOB and thus methane oxidation rates (Crespo-Medina et 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 for accumulation of methane oxidizers. In addition, sea ice, ~~and~~

25   ~~in~~ particular the ice-~~-~~water interface, is a hotspot for microbial activity. The ice surface, penetration of light, and ~~the~~ 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 Dieckmann, 2002).

Methane-~~-~~oxidizing bacteria use methane as their sole carbon and energy source (Hanson and Hanson, 1996). In the first

30   step, methane is oxidized to methanol catalyzed by the enzyme methane mono-oxygenase. Since methane mono-oxygenase is characteristic ~~to~~ of nearly all MOB (Knief, 2015), *pmoA*, the gene encoding for a subunit of the ~~membrane~~ membrane-bound particulate methane-~~-~~mono-oxygenase, has been used as a specific molecular marker for detection and characterization of MOB (Knief, 2015; Lüke and Frenzel, 2011; reviewed by McDonald et al., 2008; Tavormina et al., 2008). Methanol is further metabolized to formaldehyde, from ~~where~~ which it is either mineralized to carbon dioxide (CO_2), or assimilated into

organic ~~carbon~~ 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 pathway~~s~~ 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 ~~were recently~~ also ~~identified~~ occur in the phylum Verrucomicrobia (e.g. Dunfield et al., 2007; Pol et al., 2007).

Methane-derived carbon ~~has further been shown to be~~is also assimilated in non-methane utilizing methylotrophs (non-MOB methylotrophs) ~~methylotrophic~~ or other bacteria in freshwater and temperate marine environments. ~~This is attributed to cross feeding by non~~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~~ 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, ~~as well as~~ 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) ~~was~~ is located north of Utqiagvik, Alaska, (7.4.2016, 71.334° N, -156.363° W~~), .~~). At the time of sampling EL was covered with 1.5 m thick sea ice~~;~~, at approx. 1.5 m water depth, this left~~leaving~~ only a narrow layer of water between the sea ice and the sediment.~~–~~ Site "Ice Mass Balance Buoy" (IMB) ~~was~~ 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 (7.4.2016, 71.373° N, -156.548° W, and 9.4.2016, 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

Seawater temperature and salinity records were taken 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)~~(2017)~~. Briefly, in the field, 0.7-0.9 L seawater was transferred ~~bubble~~ bubble-free directly into in foil sample bags (# 22950, Restek, Pennsylvania, USA). ~~Upon~~ 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 for in situ measurements for at least 6 h.

For DNA extractions, between 1–2 L of seawater were filtered onto Sterivex® filter cartridges (Millipore) with 0.2 µM 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~~ 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~~number~~.

Additionally, at site IMB, seawater temperature, salinity and velocities were recorded with an Aquadopp Profiler (Nortek AS, 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. ~~and t~~The outside was cleaned with a sterilized knife to remove microbes possibly transferred from the sampling equipment. The core sections were sealed into ~~custom~~ custom-made ~~gas~~ gas-tight tubes (Loose et al., 2011) for determination of methane concentration and isotope ratios. In the laboratory, the ~~gas~~ gas-tight tubes were flushed with ultrapure nitrogen for several gas volumes (Lorenson and Kvenvolden, 1995). ~~The ice samples were~~

Due to technical limitations, ice core 1 (IC1) was melted within a week at 5°C, while ice core 2 (IC2)~~at~~ was melted within a day, while frequently being mixed, at room temperature~~, with frequent mixing within a day (IC2), or within a week at 5°C (IC1)~~. 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).

2.3 Net ~~O~~methane oxidation/production ~~rates~~ and determination of isotope fractionation factors

~~Methane~~ Rates for net methane oxidation/production ~~rates~~ 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), ~~the~~ 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 0.2x (~~without~~ no methane addition, resulting in degassing of in situ methane to the headspace), 2x, 10x and 200x of the in situ methane concentration. Samples were incubated at 0-1°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 0.1M NaOH.

Assuming first order kinetics for ~~the~~ oxidation of methane (Reeburgh et al., 1991; Valentine et al., 2001), net oxidation/production rate constants (k_{ox}) were determined from the methane mass balance in the incubations (Uhlig and Loose, 2017) as

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

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:

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

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).

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

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 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

5   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
10   al. (1976).

2.3.2 Nutrient analysis and flow cytometry

Phosphate, nitrate and nitrite concentrations were determined ~~on~~ 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~~ Formol-fixed samples were stained with a final concentration of 1x SYBR Green I
15   (Invitrogen, Molecular Probes, Eugene, Oregon, USA) for 20 to 45 min 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
20   protocol. A minor modification was made: the tube was vortexed ~~two times~~ once for 3 minutes~~,~~ ~~and~~ rotated 180°, ~~in between~~ and then vortexed for another 3 minutes. The DNA was eluted in 80 µL buffer PW6, after incubating the buffer for 1 min 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-
25   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 min 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 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 sequenced with Illumina MiSeq at 2x250 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 if they ~~containing~~ contained ambiguous bases or homopolymers longer than 6 bases. Contaminating s~~S~~equences 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~~chloroplast, ~~Mitochondria~~mitochondria, Archaea, Eukaryota or unknown were removed and operational taxonomical unit (OTUs were built 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) ~~testing for the~~ to determine richness (Shannon and Simpson indices) and differences in community structures (Bray-Curtis dissimilarities). Differences ~~of the~~in community structure ~~between~~associated with different methane spike concentrations were determined via analysis of similarity (ANOSIM) in the package vegan (Oksanen et al., 2017) on three predefined groups: in situ (N=9), 0.2x (N=2) and 10x (N=3). Groups 1x, 200x short, 200x long with 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 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 were identified as differentially more abundant 16S OTUs between ~~and spiked treatments~~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

5 removed from further analysis. Treatment~~s~~ ~~IMB 2x,~~ EL 0.2x~~, and~~ EL 10x and IMB 2x (Table 2) were not included in~~to the~~ 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 ~~spiked~~ 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

10 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 ~~for *pmoA*~~. ~~High~~ 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

15 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

20 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 ~~on~~ 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

25 (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

~~During the sampling in~~ On 7 April 2016~~,~~ ~~T~~the narrow layer of water between the sediment and ice in Elson Lagoon (N=1) had a salinity of 21 and a temperature ~~at the seawater freezing point~~of -1.5°C. Phosphate and nitrate concentrations were

30 0.74 µM and 4.87 µM, respectively. Methane concentration for Elson Lagoon (N=1) was 53.2 nmol L^{-1} with a stable isotope ratio of -73.8 ‰ (Figure 1) and cell density 7.7 x 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 northeastward directed Barrow Current, with water temperatures around -1.8°C and salinities of 33.9 to 36.4 (Figure 1, Supplementary Figure 1, 2) for most days. Between 11 April and 13 April the current changed southward bringing warmer water (max. temperature observed 0.9°C), reflected in the temperature profile for 11 April.~~ 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 (N=9) and nitrate 6.59 ± 4.04 µM (N=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, 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 $6.9 \times 10^4 \pm 5.7 \times 10^3$ cells mL^{-1} (N=16).

~~The narrow layer of water between the sediment and ice in Elson Lagoon (N=1) had a salinity of 21 and a temperature at the seawater freezing point. Phosphate and nitrate concentrations were 0.74 µM and 4.87 µM, respectively. Methane concentration for Elson Lagoon (N=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}.~~

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 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 showed an increase ~~versus ice depth~~ 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 ~~O~~methane oxidation ~~rates~~/production and isotope fractionation ~~during oxidation~~

The methane oxidation potential of microbial seawater communities at stations EL and IMB was determined from the methane mass balance in ~~spiked~~ incubation experiments (~~Table 3~~(Table 3; Uhlig and Loose, 2017)~~)~~. Final dissolved methane concentrations ranged between 3.0 and 4000 nmol L^{-1}, representing approximately 0.2x to 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 ~~was~~ were not determined.

~~Oxidation~~ Net oxidation rates discussed here were published in Uhlig and Loose (2017) ~~but~~ and are summarized ~~here~~ for comparison with the microbial community structure. Short incubations (≤10 days) did not show significant oxidation, while ~~long term~~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 IMB3 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 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.

~~The~~ In the in situ seawater communities, ~~did not show gradients between sample dates or water depths. P~~Proteobacteria were dominant with relative sequence abundances of 59.5% and 65.5% ± 2.5 % ~~and 59.5%~~ for EL (N=1) and IMB (N=9)~~ and EL (N=1)~~, 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% ~~and 23%,~~ for EL and IMB ~~and EL~~, respectively.

Similar to the seawater, sea ice (N=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 (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 (N=10), species richness decreased (Figure 3) and the communities shifted toward higher fractions of γ-Proteobacteria over time. In short incubations (5–10 days; N=5) γ-Proteobacteria dominated with 61.8% ± 2.9% of sequences, while reaching 81.0% ± 11.1% in long-~~incubated~~incubation samples (41–46 days; N=4).

In particular, one operational taxonomical unit (OTU), from the genus *Oleispira*, was very abundant in the long-~~time~~ 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-~~incubated~~ incubation treatments (0.5%–1.6%) compared to in situ abundances. In addition to the shift in community structure, total cell densities increased to 1.9 x 10^5 and 3.3 x 10^6 cells mL^{-1} for short and long incubations, respectively, based on flow cytometric cell counts.

~~NMDS of the Bray-Curtis diversity revealed a high similarity across all of the in situ water samples that were analyzed for 16S diversity (Figure 4). Communities in the short incubation treatments (5–10 days; 0.2x, 2x, 200x short) were similar to the in situ samples, while the long incubations (41–46 days; 10x, 200x long) deviated further. Microbial communities in ice cores clearly separated from the water samples and were more distant to each other than the water samples.~~

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* 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 ~~OTUs~~ 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 ~~and EL~~ with 0.24% ± 0.09% (N=10).

Taking into account the total cell number, a strong increase of MOB groups MMG1 (2 to 7~~100~~ times) and Milano-WF1B-03 (~~10~~ 25 to 75 times) was observed for the 10x and 200x ~~long~~ long-~~incubated~~ incubation samples compared to in situ conditions (Figure 5b).

Taxonomic groups that became differentially more abundant in the ~~spiked~~ 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% ~~in~~ 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 and 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 each 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 seae-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 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 250% to 700% compared to levels at atmospheric equilibrium (3.6 nmol L^{-1}). The concentration at site EL (52.90 nmol L^{-1}, N=1, 7.4.2016) 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}: N=5, 15.4.2018) 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 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 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 tThe sediment present at 30–46 cm depth in IC1, which was not observed in IC2, indicates indicating that

both ice cores have ~~a~~ different freezing ~~history~~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 (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 (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).

~~Differences in methane concentration and isotope ratios could further have been caused by sample storage, which might have allowed for microbial activity, e.g. anaerobic methanogenesis, resulting in increasing methane concentrations. Ice algae derived organic carbon, which has a carbon isotopic signature of about -20‰ to -30‰ (e.g. Wang et al., 2014), could serve as substrate for methane production. Methane produced from this substrate would be enriched in ^{13}C (more positive) compared to the initial pool of methane (about -60‰ in IC2) and could explain the shift of the bulk methane isotopic signatures towards more positive values for IC1 (Figure 2, Figure 6). Sequences of bacterial taxa that might indicate anoxic conditions in the melted ice according to a study on an anoxic ice core (Eronen-Rasimus et al., 2017), were, however, not significantly more abundant in IC1 than in IC2 (Supplementary Table 2). An analysis of similarity (ANOSIM) between the two ice cores (not including the sediment influenced section), indicates that differences in the community structure between the two ice cores are not significantly higher than within cores (ANOSIM: R=0.214, P=0.2). Thus, we thus cannot conclude if the differences in storage time or spatial variability led to the observed differences. Nevertheless, we assume that different microbial processes were taking place from the time of sea ice formation until measurement, which lead to the differences in methane concentration and stable isotope ratios.~~

Compared to ~~the concentration in~~ the underlying water column, methane concentrations in the sea ice ~~sampled at station IMB~~ were two to five times higher. ~~In addition~~Further, the isotope signatures indicate less oxidized methane (-60.4 to -63.8 ‰) in most of the ice sections compared to the upper water column (-55%). Lorenson and Kvenvolden (1995)~~In the Beaufort Sea,~~ report higher methane concentrations ~~and isotopic signatures~~ in sea ice than ~~were also found to be higher than values generally observed~~ in the water column for the Beaufort Sea ~~(Lorenson and Kvenvolden, 1995)~~. ~~In that region the~~They attributed the high methane concentrations in the ~~fast ice — sea ice which is attached to the shore line —~~ over the shallow shelf ~~(<10 m water depth) were attributed~~ to ~~the~~ inclusion of ~~sedimentary~~ sediment-sourced methane during the initial freeze-up over the shallow shelf at <10 m water depth (Lorenson et al., 2016). ~~m~~Methane concentrations in IC2, which are close to

water column concentrations reported in previous studies for ~~the same region~~the Barrow shelf (Lecher et al., 2016; Zhou et al., 2014)~~(Lecher et al., 2016; Zhou et al., 2014)~~, suggest the same process for our ice cores. ~~That this process also might have taken place in the fast ice sampled in this study, is supported by the fact that methane concentrations in IC2 are close to water column concentrations reported in previous studies for the same region (Lecher et al., 2016; Zhou et al., 2014).~~

5 ~~T~~Further, in our study, t~~he lower ~~sea water~~methane concentrations~~ in our study,~~ together with ~~the ~~more positive (heavier) isotopic signature in sea water compared to ~~the ~~ice, might indicate that the microbial community in the water column is oxidizing more methane during the ice covered period than in the freez~~e~~-up period. Higher oxidation rates during ice covered periods compared to ice free conditions were previously reported for the Beaufort Sea ~~(Lorenson and Kvenvolden, 1995)~~. Due to reduced sea-air gas exchange, higher methane concentrations can build up under sea ice cover, which might lead to

10 higher oxidation rates (Lorenson and Kvenvolden, 1995).

4.2 Methane cycling 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

15 (Steinle et al., 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.

~~Oxidation ~~Net oxidation rates of the long-~~-~~incubation treatments at 10x (46 days) and 200x (41 days) methane concentration

20 fall into the ~~middle~~mid-range of rates published for Arctic and subarctic environments (Damm et al., 2015; Gentz et al., 2014; Lorenson et al., 2016; Mau et al., 2013, 2017; Steinle et al., 2015) or marine sites with high oxidation rates at oil spills or gas flares (Redmond et al., 2010; Valentine et al., 2010), as discussed in Uhlig and Loose (2017). The fractionation factors (α_{ox}) that we observed ~~with these oxidation rates were~~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.,

25 2004; Keir et al., 2009; Tsunogai et al., 2000). Some of these fractionation factors, which were calculated from in situ data, might however be ~~underestimated ~~underestimates due to mixing effects in the water column (Grant and Whiticar, 2002). The fractionation factors ~~observed ~~in our study seem to be inversely dependent on the methane spike concentration, with ~~a ~~higher fractionation in the 50x (1.023, N=6) treatments than in the 200x (1.010, N=2) treatments. The relative and absolute abundances of MOB, as well as the dominant MOB types, differed between both treatments, possibly providing explanations

30 ~~to ~~of the differences in fractionation rates.

Logistical constraints forced us to stop several incubations already after 5 to 6 days. The~~se~~ short-~~-~~incubation 2x and 200x treatments ~~-~~did not ~~show~~resolve oxidation of methane. While the 2x treatments did not meet the sensitivity threshold for the method ~~A longer incubation time would have been required for the 2x treatments to meet the sensitivity threshold for the~~

~~applied method given the observed low oxidation rate constants in other samples~~ (Uhlig and Loose, 2017)~~.~~ ~~T~~the 200x short treatments were likely just about to leave the lag phase when the experiments were stopped ~~after 7 days~~. A lag phase of 6 days was observed for the long-~~-~~incubation 200x samples, in which the microbial community possibly to shifted toward~~s~~ 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.

~~-~~The increase in methane concentration in treatment IMB 0.2x (10 days incubation) is surprising since experiments were performed under aerobic conditions. ~~We were, however, able to amplify the methyl coenzyme M reductase (mcrA), a marker for anaerobic methane production, in all four IMB samples analyzed for it, including this incubation (1 480 sequences detected, data not shown).~~ 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~~, which use~~ from methylated ~~substrates~~ compounds like dimethylsulfonioproprionate (DMSP), dimethylsulfide (DMS), ~~methylphosphonate or other phosphonates in marine~~ or dissolved organic matter (Damm et al., 2010; Florez-Leiva et al., 2010; Karl et al., 2008; Repeta et al., 2016). ~~The rich microalgal and bacterial community found at the underside of sea ice could be a source for DMSP and DMS . Dissolved organic phosphate, which encompasses phosphonates, could originate from river runoff or sediment resuspension (Piper et al., 2016). In addition to biological processes, we cannot entirely rule out an abiotic effect leading to the increased methane concentrations, since our experimental setup did not include a killed control at the same methane concentration.~~ The methane production rate of 0.06 nmol L^{-1} day^{-1} observed in our study ~~is~~ is two to six orders of magnitude lower ~~compared to~~than previously published methane production rates under aerobic conditions~~, which range between 5 and 4000 nmol L^{-1} day^{-1}. The high rates in these studies were, however, obtained with the addition of high amounts of potential substrates DMSP and methylphosphonate~~ (Damm et al., 2010; Karl et al., 2008)~~., while in our study none of the possible substrates were added.~~ In addition to biological processes, we cannot ~~entirely~~ rule out an abiotic effect leading to the increased methane concentrations, since our experimental setup did not include a killed control at the same methane concentration.

~~The 0.2x incubations, without addition of methane, had a dissolved methane concentration five times lower than the in situ methane concentration, as most methane would have degassed into the headspace. We hypothesize that in all other treatments the higher methane concentrations lead to oxidation rates which masked the methane production signal.~~

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

We found a strong linear correlation between the ~~bulk~~ net oxidation rate constant (k_{ox}) and the relative abundance of 16S MOB sequences (Spearman rank order coefficient ρ_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 to k_{ox} ~~was~~ is even stronger for the absolute abundance of *pmoA* sequences ~~(ρ_s = 0.86, p=0.006)~~ retrieved from

the respective datasets ~~(ρ~~$_s$~~ = 0.86, p=0.006) (Figure 7b)~~. This presentation of a direct and statistically significant linear relationship is ~~a~~ 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 application of marine-specific *pmoA* primers may further improve this correlation (Tavormina et al., 2008).

~~Cell~~ 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 ~~the~~ subarctic ~~marine environment~~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.

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 ~~at~~of natural or man-made gas or oil spills, with ~~comparably high~~ dissolved methane concentrations comparably high ~~like in~~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 (Table 4). Inferred absolute MOB numbers were higher in 10x and 200x incubations than ~~in~~ in situ (Figure 5b). In contrast, absolute MOB numbers in 0.2x and 2x incubations were very similar to in situ abundances~~, while the low spiked samples were lower (Figure 5b)~~, indicating that either the provided methane concentration ~~at 0.2x and 2x~~ 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 and 200x treatments. Oxygen and methane can be ruled out as ~~the~~ 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)~~(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, we consider copper as unlikely to be limiting, since *pmoA* is only expressed in the presence of copper, yet correlates with the k~~$_{ox}$~~. Additionally, we would expect an impact on the cell-specific oxidation rate if and when copper restricted oxidation. Potentially~~Further, the low relative abundance of MOB sequences ~~is~~ 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 ~~been utilizing~~ 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 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 the Beaufort Sea, where ~~an amount of~~ 1–2% 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 ~~is in agreement~~ agrees with previous records ~~on~~ of MOB diversity for polar and subpolar waters (Mau et 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, ~~T~~the correlation ~~is, however, weak~~ between~~for the~~ 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, ~~and that~~in which non-MOB 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). ~~A p~~Possible cooperative behavior between methanotrophs (Methylococcacaea) and non-MOB methylotrophs (Methylophilaceae) was suggested (Beck 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~~s~~ 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 (Vrede et al., 2002) and (ii) that about 1/3 of consumed CH_4-carbon is assimilated, ~~while~~ with the remaining 2/3 ~~are~~ respired to CO_2 (Bastviken et al., 2003; Roslev et al., 1997). $C_{CH_4,assim}$ exceeds MOB-$C_{cell-growth}$ by ~~y~~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, ~~like~~ 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 ~~on~~ 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. The highest relative abundances of MOB were found in the top-most ice sections ~~of~~ in both ice core~~s~~ (Figure ~~5b~~5a). This coincided ~~coinciding~~ with the highest methane concentration in case of IC2 ~~(Figure 2e).,~~ whereas the top-most section of IC1 had the second smallest concentration of methane in this ice core (Figure 2e). ~~Abundances~~Relative abundances of MOB in the inner and bottom sections of the ice cores were even lower, with ~~one to two orders of magnitude lower or in case of the biologically rich bottom section of IC2, no typical MOB were identified at all~~ 0 to 0.02% only. The ~~biologically rich bottom section of IC2 and the~~ top-most section of IC1 and the biologically rich bottom section of IC2 had the highest relative abundance~~s~~ of β-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

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-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 abundance~~s~~ of MOB and ~~as well~~ non-MOB methylotrophs (Figure 7, Table 5) suggests that ~~(1)~~ the abundance of MOB is a control on the magnitude of methane oxidation. It also suggests ~~and (2)~~that non-MOB methylotrophs might play a role in methane oxidation~~, possibly by interacting with the MOB~~. The reasons for low MOB abundance ~~of MOB~~, despite ample methane availability, ~~and~~ 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 ~~processes~~ causes explaining this observation ~~could be~~include (i) microbial production of methane, even within the ice (Damm et al., 2015), ~~or~~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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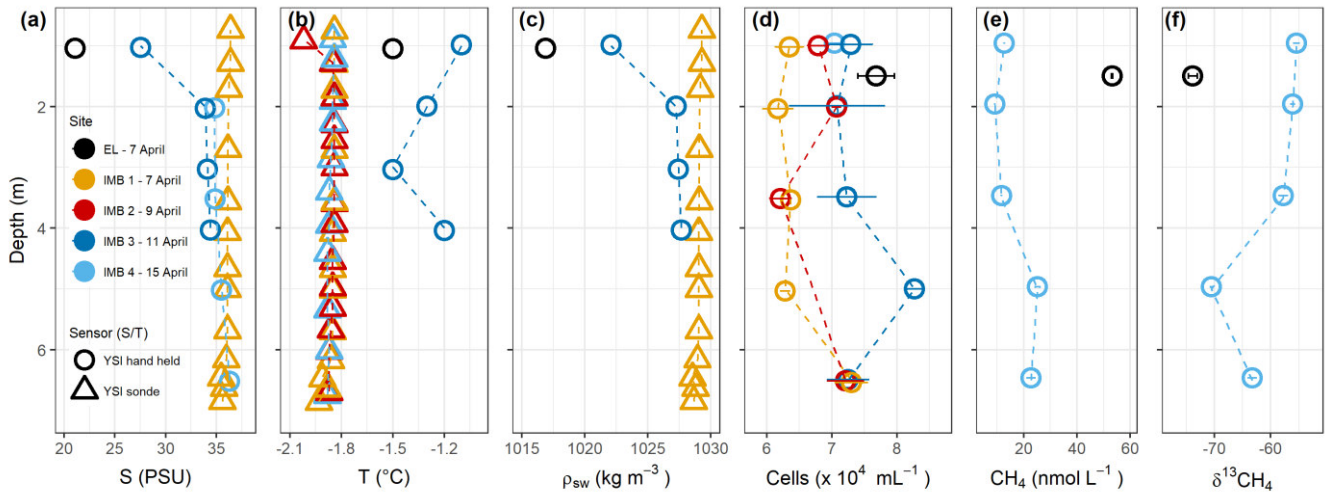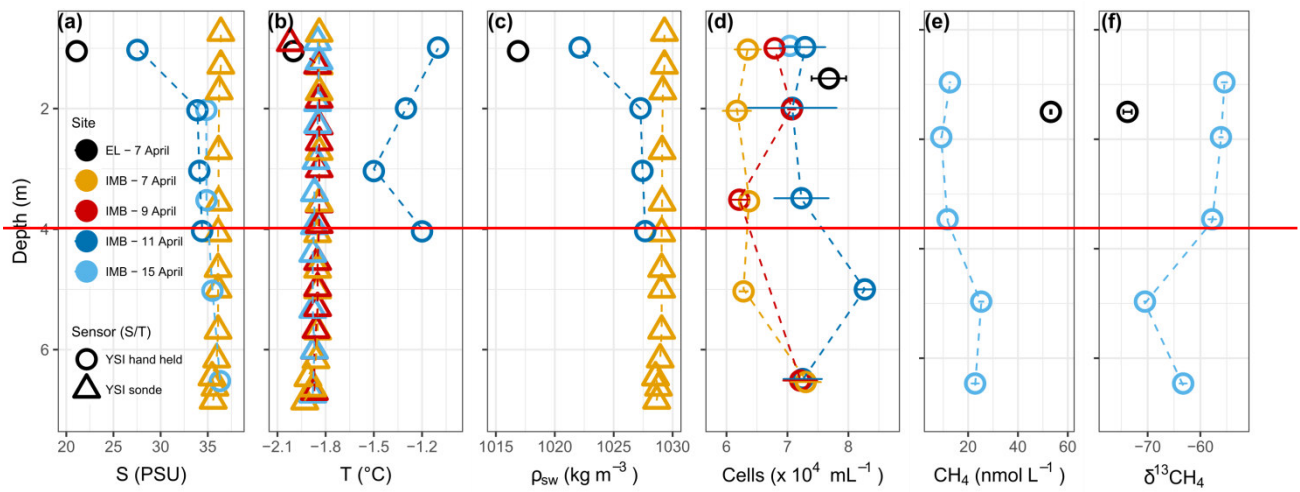
Figure 1: Water column properties during the time series near Utqiagvik. Salinity (a), temperature (b), density (c), cell ~~numbers~~ abundance (d), methane concentration (a) and stable isotope ratios (b). Error bars on cell numbers (d) represent~~is~~ the standard deviation on two technical replicates. 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.
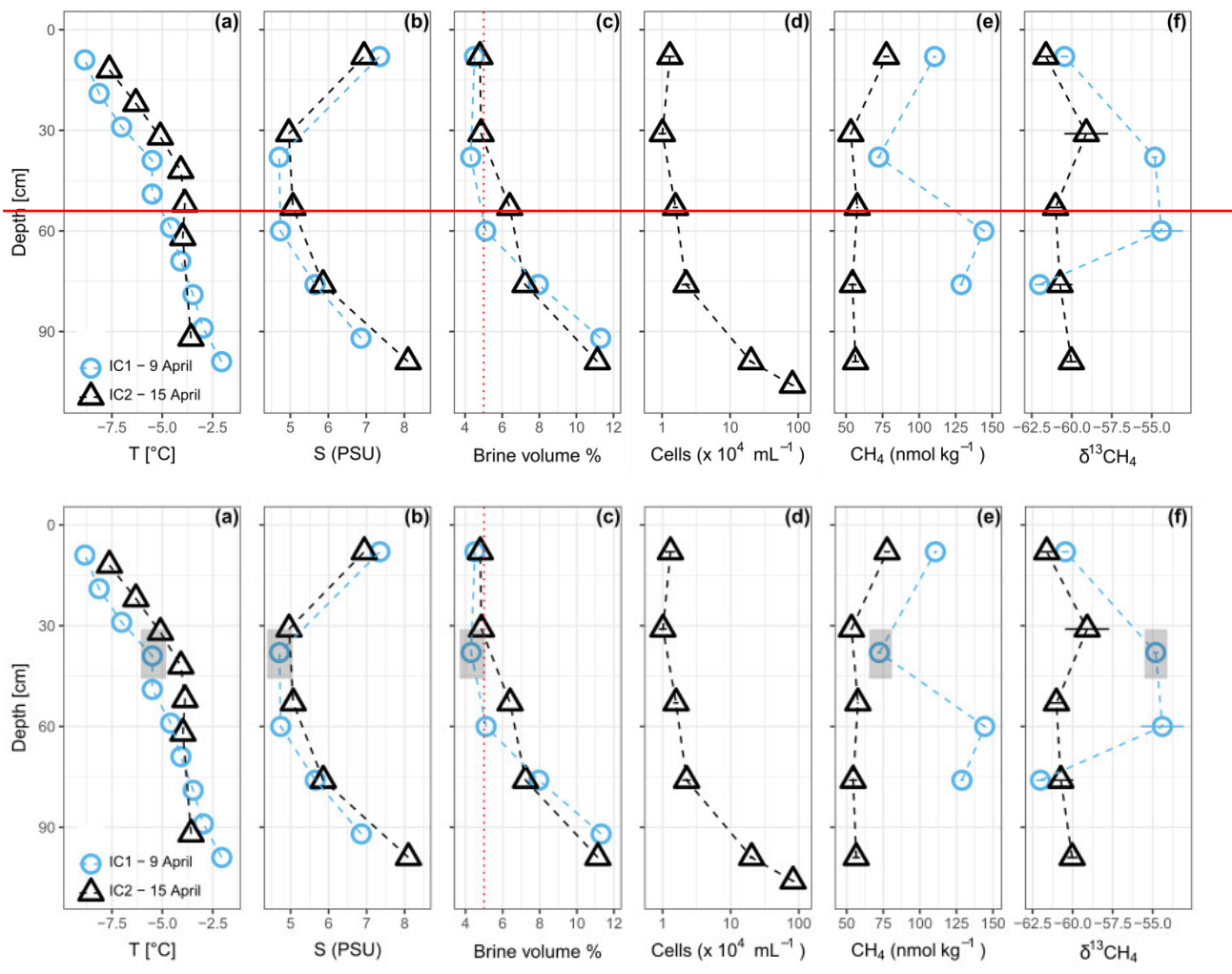
Figure 2: Sea ice temperature (a), bulk salinity (b), brine volume fraction (c), prokaryotic cells mL^{-1} sea ice **(for IC2 only)** (d), methane concentration (e) and stable isotope ratios (f). The vertical red dotted line in (c) shows a brine volume fraction of 5%, the threshold for permeability (Golden et al., 1998). IC1 had sediment included into the ice matrix at depth 30–46 cm, indicated by the gray box.
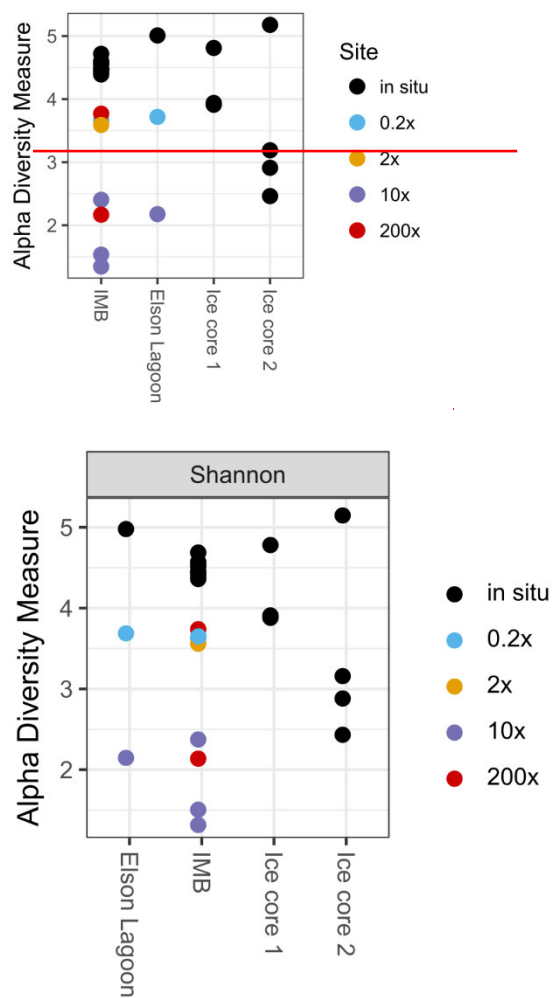
Figure 3: Shannon indices of alpha diversity for V4-V5 amplicons.
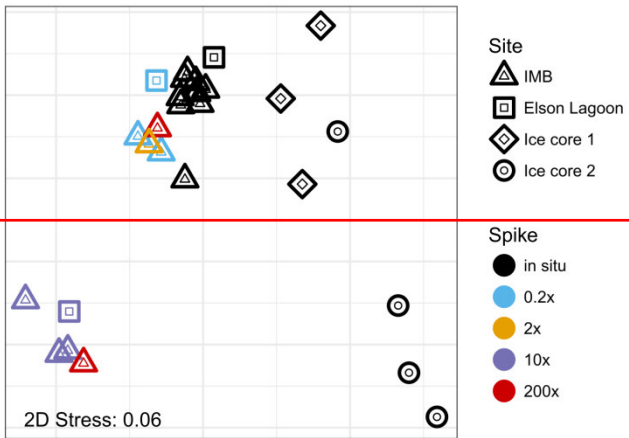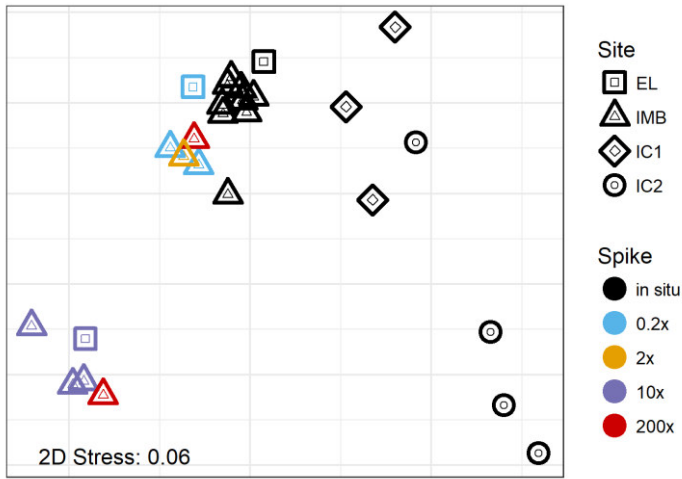
Figure 4: Non-metric multidimensional scaling analysis (unitless) of Bray-Curtis dissimilarities of the ~~original~~ 16S read data. The low 2D stress of 0.06 indicates a good two-dimensional representation of the multidimensional dataset with very low prospect of misinterpretation.
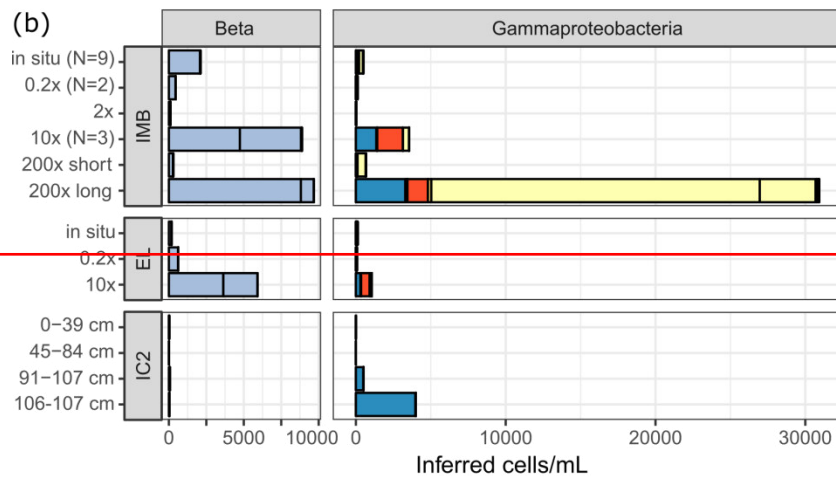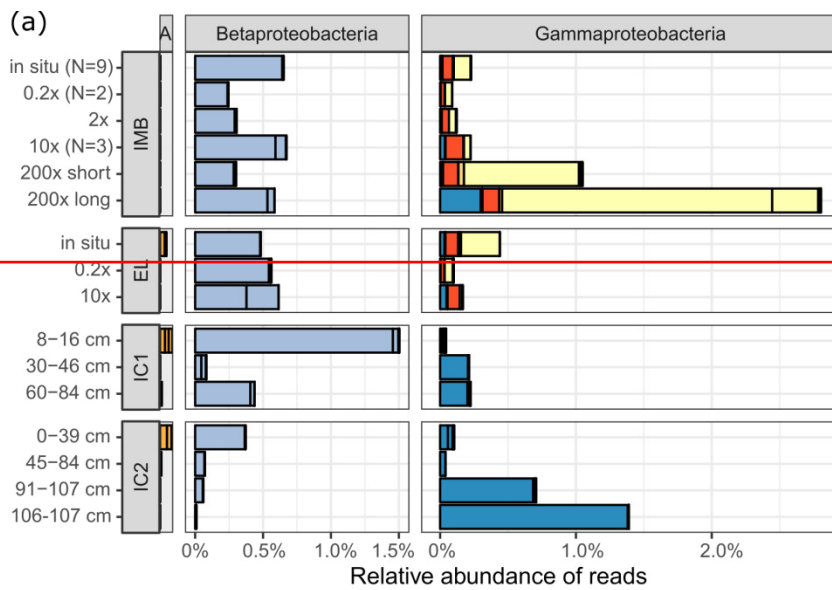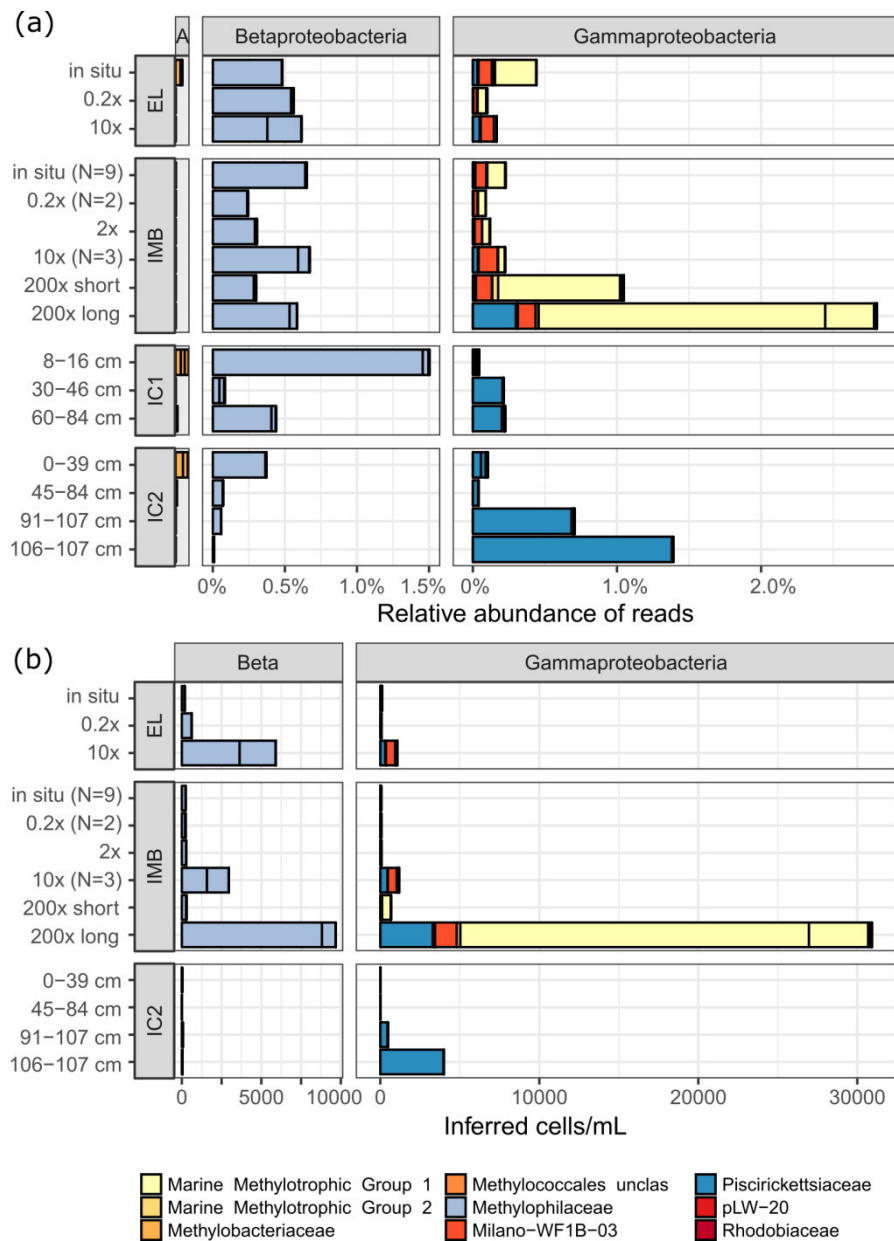
Figure 5: Relative abundances (a) and inferred cell numbers (b) of methylotroph OTUs by family. Sampling sites for water samples are ~~Elson Lagoon (EL) and~~ **mass balance buoy (IMB)** ~~and Elson Lagoon (EL)~~**. Ice cores (IC1 and IC2) were collected at site IMB on 9 and 15 April, respectively. The 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 (N) of samples, all other samples were 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}.
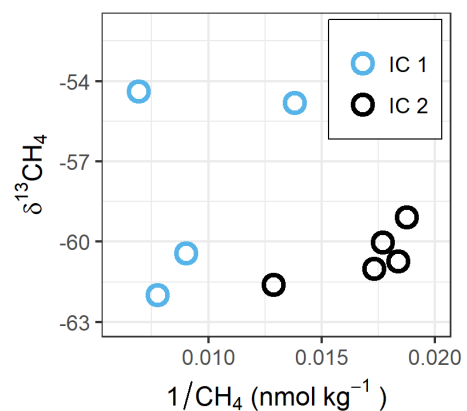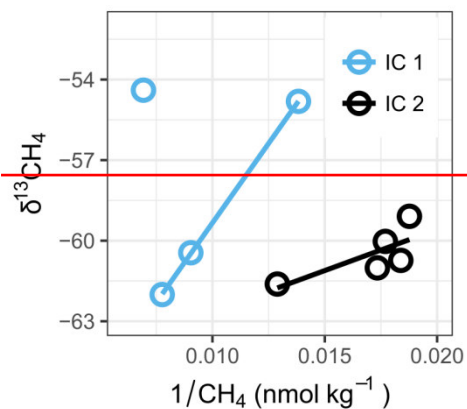
Figure 6: $\delta^{13}CH_4$ vs. reciprocal of CH_4 concentration (Keeling type plot) of ice cores. ~~Within each ice core a~~ ~~The correlation between~~ shift to more positive $\delta^{13}CH_4$ ~~values and a~~in combination with a decrease in CH_4 concentration ~~in each ice core~~ indicates microbial oxidation. Comparing IC2 to IC1, the shift toward higher concentrations and more positive $\delta^{13}CH_4$ (see also Fig. 2) in IC1 might indicate CH_4 production from a substrate with heavier isotope signature, compared to the values in IC2.
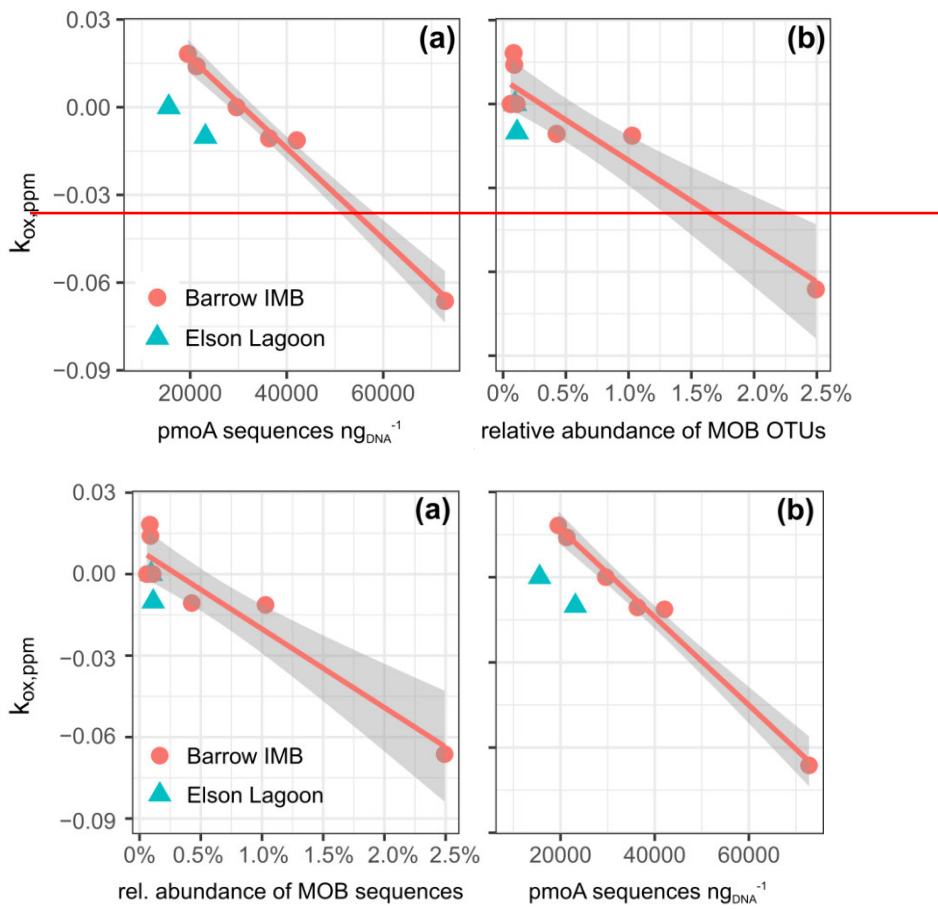
Figure 7: Correlation between ~~the~~ net oxidation rate constant ($k_{ox,ppm}$) and the relative abundance of sequences in 16S-´MOB´-OTUs, $R^2_{(MOB\text{-}OUTs\sim kox)} = 0.84$ ~~and the number of pmoA sequences with $R^2_{(pmoA\sim kox)} = 0.85$~~ (a) and number of pmoA sequences with $R^2_{(pmoA\sim kox)} = 0.85$ ~~and the relative number of sequences in ´MOB´-OTUs $k_{ox}=0.0065$ x MOB% - 3.15, $R^2_{(MOB\text{-}OUTs\sim kox)} = 0.84$~~ (b). For correlation to the number of total methylotroph OTUs (which includes MOB and non-MOB-methylotrophs in total 16S) $R^2_{(´Meth´\text{-}OUTs\sim kox)} = 0.81$. The gray shaded area shows the 95% confidence interval of the correlation.
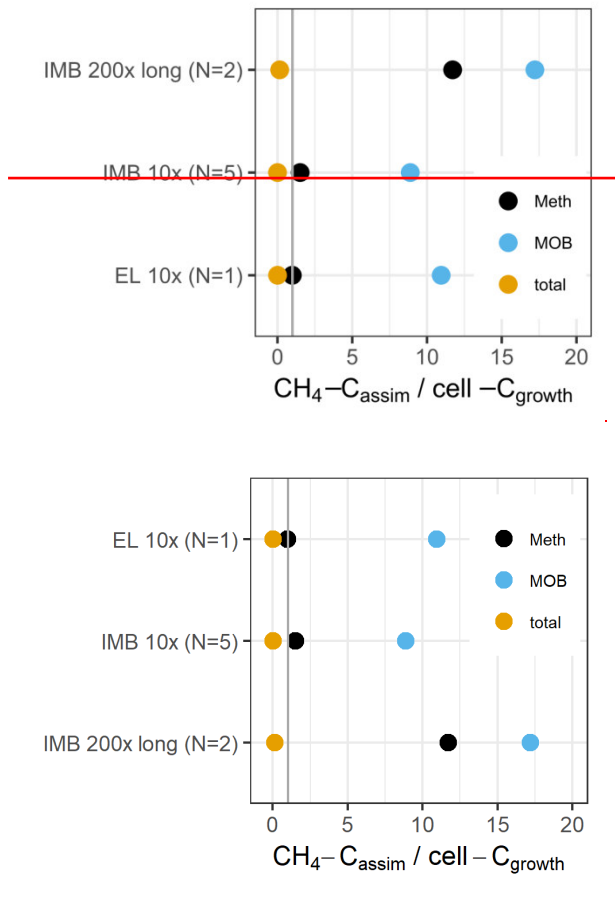
Figure 8: Ratio of methane-carbon assimilated (CH$_4$-C$_{assim}$) to cell-C gained during growth (cell-C$_{growth}$)~~,~~ based on flow cytometric cell counts (total) or inferred cell numbers (Meth, MOB). The standard deviation between replicates ~~for replicates~~ was 10–20%. The vertical line indicates a ratio of 1. Above 1~~,~~ the ~~entrie~~ entire cell gain can be explained by the assimilated CH$_4$.

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~~ ~~depth profile~~, cell counts
IMB 4	15.04.2017	71.372° N, -156.540° W	water	in situ CH$_4$ ~~depth profile~~, 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 δ^{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

[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. N: 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	N[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: $\rho_S < 0.8$ very strong, $0.6 < \rho_S < 0.8$ strong

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

[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