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.

We added appropriate notes on the incubation times in Table 3 and 4. Additionally, we added a note in the materials and methods for the ice “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 and frequent mixing at room temperature. “; and incubations “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 to one bag.”

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 (IMB1 on April 7 and IMB2 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 IMB3, 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 handheld YSI indicates, that IMB3 was sampled from a warmer water mass than the other IMB stations (we suggest indicating both sampling events in Supplementary Figures 1 and 2). However, the NMDS plot (Figure 4) does not show differences between in situ samples taken at IMB1, IMB3 and IMB4. IMB2 is more distant to the other stations, however, temperature profiles (Figure 1) do not indicate different water masses during IMB1, IMB2 and IMB4. We thus treated all sampling days as parallels. We added a respective note in section 3.4

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. "

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

Reply: We suggest restructuring this section and including methane oxidation in the possible microbial processes: "…they differ in concentration and isotope signature. The sediment present at 30–46 cm depth in IC1, which was not observed in IC2, indicates that both ice 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. Subsequent 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). Both oxidation and methanogenesis could either have taken place in situ in the ice or during sample processing and storage. We thus cannot conclude if spatial variability or dissimilar storage conditions led to the differences between both ice cores. "

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 suggest to remove the misleading correlation lines in the revised plot as included below. The legend and text referring to the correlation was modified accordingly.

Other concerns:

Page1

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

L22: methLylotrophs Reply: corrected as suggested.

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

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

Reply: 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: The time span was specified to 100 years.

Page2

L9: what about dilution effects?

Reply: We suggest to modify accordingly: "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 with the surrounding water column (e.g. Gentz et al., 2014). As a result of these biological and physical processes …"

L23: development OF communities Reply: 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.

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 will clarify in the revised manuscript that at here we are referring particularly to non-methanotrophic methylotrophs and other bacteria, which cross-feed on methane derived carbon. See also the next comment.

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

Reply: In the revised manuscript we will rephrased this sentence to: "In those studies the 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 will implement a consistent order first mentioning EL then IMB.

Page4

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

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

Reply: In the revised manuscript we will rephrase 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 and frequent mixing 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: We suggest to add the following explanation "approximately 0.2x (without methane addition, resulting in degassing of in situ methane to the headspace)…" on page 5

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: "and spiked treatments" will be 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 will remove "spiked" or replace 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 will check 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.

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 suggest adding an explanatory note at Table 2. Also, we note how prolonged incubations do appear to affect community structure as noted in section 3.4

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 indicates.

L29: slightly more abundant..compared to what?

Reply: Will be modified to "…only slightly more abundant in the short incubated 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: Will be 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: 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.

Page12

L11: change the position of "also" to " might also have taken place Reply: corrected as suggested

L11: remove the comma after study Reply: corrected as suggested

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 an according explanation here.

L19: "fall into the middle"? mid-range might be clearer Reply: 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: We specified the occurrences accordingly in Materials and Methods, Results and the Discussion

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

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.

Page14

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

L15: remove the "the" before kox Reply: corrected as suggested.

Page15

L1: correlation between what and what?

Reply: 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: We suggest changing 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: we suggest to change this sentence to "Relative abundances of MOB in the inner and bottom sections of the ice cores were even lower with 0 to 0.02% only."

Page16

L7-10: very long sentence. shorten? Reply: we removed suggest changing to: "A tight correlation between the rate constant of methane oxidation and relative abundance of MOB and as well 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. "

L11: comma is wrong before suggests Reply: 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.

Comment to tables and figures: we provided suggestions for revised tables and figures at the end of this reply.

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 will introduce 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 add the 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 Mahoney, 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. 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 melting sea ice with higher per volume methane concentration, 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, which is included below.

Figure 3: the blue dot is not visible for “IMB”

Reply: Thanks for the note. The blue dot is now visible for IMB. The revised figure is included below.

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.

Revised Tables and Figures:

Figure 2:

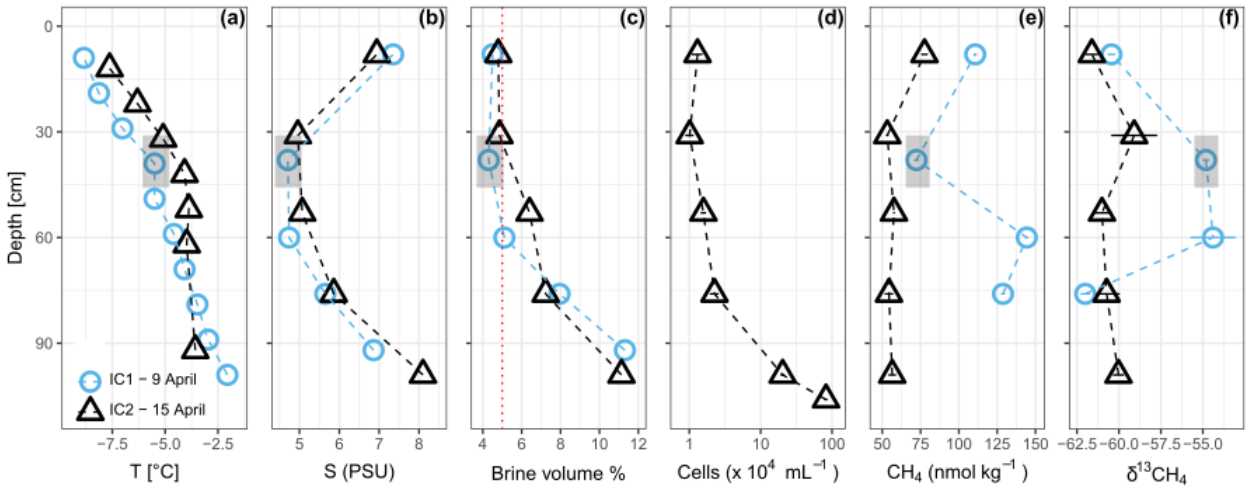

Figure 2: Sea ice temperature (a), bulk salinity (b), brine volume fraction (c), prokaryotic cells mL^{-1} sea ice (for IC2 only) (d), methane concentration (e) and stable isotope ratios (f). The vertical red dotted line in (c) shows a brine volume fraction of 5%, the threshold for permeability (Golden et al., 1998). IC1 had sediment included into the ice matrix at depth 30–46 cm, indicated by the gray box.

Figure 3:

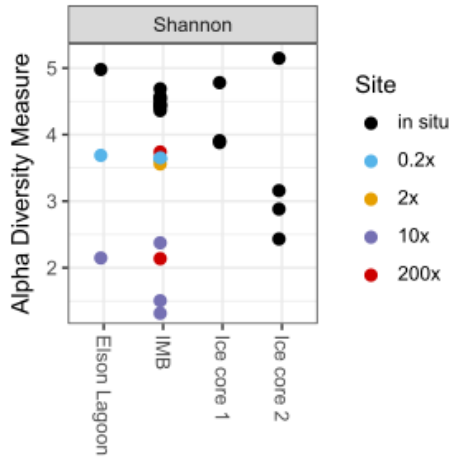

Figure 3: Shannon indices of alpha diversity for V4V5 amplicons.

Figure 6

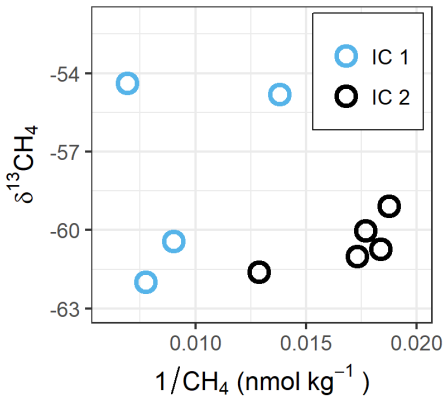

Figure 6: $\delta^{13}CH_4$ vs. reciprocal of CH_4 concentration (Keeling type plot) of ice cores. Within each ice core a shift to more positive $\delta^{13}CH_4$ values in combination with a decrease in CH_4 concentration indicates microbial oxidation. Comparing IC2 to IC1, the shift towards higher 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.

Table 1: Station and sample list

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

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

[2]Paramters: in situ concentration and $\delta^{13}CH_4$ (in situ CH_4), oxidation 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 V4V5 and pmoA

Treatment	**Station**	**V4V5 # 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

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

Treatment	N	Incubation [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[1]	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[2]	0.994 ± 0.0113
2x IMB 2	4	5	37.9 ± 1.8	36.5 ± 1.4	0[1]	0[1]	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[1]	0[1]	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[1]	0[1]	0.9998

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

[2]Negative oxidation rate constant indicating methane production

In addition to adding methane concentrations, we corrected a minor calculation error in r_{ox}

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