Review of: The water column of the Yamal tundra lakes as a microbial filter preventing methane emission

Reviewer 1

General Comments:

This manuscript contains information that appears to be of considerable value in understanding the role of methane production and consumption in both deep and shallow Yamal tundra lakes. It likely has valuable application to understanding these processes in thermokarst lakes across the arctic. However, there are some significant adjustments/edits needed.

The manuscript needs a thorough proof read. There are many grammatic errors/issues, I've outlined a fair number in the technical corrections, but this is by no means comprehensive.

Further, this manuscript needs to be more focused and organized. A more clearly outlined hypothesis/research statement at the end of the introduction would be helpful. There is a wealth of information presented here, but it is not immediately clear how some information relates to the stated goals. E.g. the information presented in section 3.1 seems to be fairly important to the processes involved in methane production, but there is minimal discussion of these results in section 4.

We thank the reviewer for carefully reviewing our manuscript.

Specific Comments:

Line 42: Thermokarst lakes are also widespread in Northwestern Canada and the Hudson Bay Lowlands – eg. Marsh et al. (2009): Marsh, P., Russell, M., Pohl, S., Haywood, H. and Onclin, C.: Changes in thaw lake drainage in the Western Canadian Arctic from 1950 to 2000, Hydrol. Process., 23(1), 145–158, doi:10.1002/hyp.7179, 2009.

Introduced into the text on the advice of the reviewer

Table 1: This is a small sample size of lakes, and characteristics appear to be quite different depending on the lake, especially temperature. I think some discussion of possible reasons for these differences is merited. Also, I have a hard time believing the bottom temperatures for the deep lakes? How is it that LK-004 has a temperature of 14.2 C at a depth of 11m while LK-002 is only 11.7 C at the surface? This seems like a very weak temperature gradient for 11m of depth? Do you have temperatures (and other variables) available for all sample depths? It would be helpful if they were all presented here.

Explanation concerning the absence of stratification in the water column of deep lakes was added to the text.

Fig 2 & Lines 206-208: Define what is considered to be the photic layer. Is this the integral of the entire water column? In line 207, the term photic depth is used instead? Also, it is not apparent from Fig 2 that LK-003 has higher PP than LK-002 as claimed? This requires further explanation.

Fig. 2 was changed according to the Reviewer's recommendations. PP values for all tested water horizons are provided. It may be seen that in shallow lakes photosynthetic production was detected at all depths, including the near-bottom horizon. The terms photic layer and photic depth were removed as hindering the understanding of the experimental procedure. Integral PP values are shown separately.

Line 209 & Fig 3a: LK-004 appears to contradict the claim of < (± 0.5 mg L−1) variability in DOC within the water column?

Corrections made to figure 3A

Line 203 & Fig 4: Should this figure show the DCA values for the near-bottom layer of the lakes as well for better comparison?

Fig. 4 was changed according to the Reviewer's recommendations. DCA values for all tested

water horizons, including the near-bottom ones, are provided.

Fig 2 & 4: The integrated values would be better shown in a separate subplot or with a secondary y-axis to make it more apparent they are in different units.
On Figs. 2 and 4 integral values of PP and DOC are shown separately, and their different units are noted.

Line 315: What are the other mechanisms of formation?
A brief description of other mechanisms of formation has been added to the manuscript.

Lines 384 – 397: I think this point needs further clarification? Why did you only measure hydrogenotrophic methanogenesis if it is likely to be only a small fraction of total methanogenesis? The methane production vs. oxidation figures presented in here make it seem like there should be no methane emissions because rates of oxidation are orders of magnitude higher than production?
We agree that this comment of the reviewer is very significant. Indeed, on the basis of our radioisotope studies, it is impossible to carry out a full-fledged balance calculation of methane production. Therefore, we restrict ourselves to the following conclusion. «*Based on the above calculations, it can be concluded that the contribution of hydrogenotrophic methane to the total methane production in the upper sediment layer does not exceed 5%*».

Line 471-474: From the results presented here is it possible to get some sense of the relative magnitude methane emissions from the surfaces deep vs. shallow lakes? What are the implications of these findings in regards to climate change?
Indeed, the main superconclusion of our studies is that the microbial community of the water column of deep lakes is a fairly effective gas filter. The efficiency of methane utilization in the water column of thermokarst lakes is lower. It can be assumed that climate warming will lead to an increase in the total area of thermokarst lakes, which will enhance the effect of methane release into the atmosphere. To carry out quantitative calculations, it is required to use other methods than we use, namely the use of floating cameras. We believe this is the subject of another study.

Technical Corrections:
Fig 1, Table 1, & throughout text: Would it not be better to refer to LK-010 as LK-001?
The digital names of the lakes are taken from a large database used by Yamal cryogeologists. In this database, a lake named LK-001 already exists.

Line 22: (90−1000 μmol CH4 dm−3) – What timeframe is this over? One day? The whole summer? Clarify the temporal unit.
Changed. The data refer to the concentration of dissolved methane, not to its production.

Line 37: Consider rewording- e.g. These lakes have been classified as thermokarst lakes in continuous ice-rich permafrost (Dubikov, 1982) although other origins have also been proposed (Arctic and Antarctic Research Institute, 1977; Kritsuk, 2010).
Changed
Line 41: Change “in case if the topography of the area is flat” to “in flat areas”.
Changed
Line 47: Change “the increase of total lake area by 12% is observed” to “an increase in total lake are of 12% has been observed”
Changed

Line 70: Change “are usually revealed" to “usually occur”
Changed
Line 76: Define acronym OM
Definition has been provided above (line 55). Used this acronym several times more.
Line 78: Remove the word therefore
Removed
Line 80: Change “widely presented” to widespread
Changed to "common"
Table 1: Define or be clearer with use of acronyms (e.g. NL/WL – Lat/Lon). EC, this acronym isn’t used in the text, but you spell out electrical conductivity on line 134. Be consistent. What is secci depth? Also, LK-004 is missing a “)” after the depth in the left-most column.
Corrected
Line 260: This sentence as is reads like it might be more appropriate in section 2.2. You could reword to say something like “Bottom sediment samples from the bottom surface and to the depth of 14−15 cm are described in Table 3.”
Changed
Line 273-274: Be consistent with acronym usage, you use methane oxidation in one sentence then MO in the next.
Corrected
Line 442: annual methane what? Production? Emission?
Corrected

Reviewer 2

This manuscript deals with the differences in microbial processes, with focus on methane production and oxidation, between shallow and deep lakes of the Yamal tundra. Sampling was performed to measure methane concentration and stable isotopic signature in different depths as well as other environmental variables, and to collect samples for the characterization of microbial communities inhabiting the water column and sediments. Water and sediments samples were also taken for the determination of hydrogenotrophic methane production and methane oxidation rates using 14C labelled substrates (NaH14CO3 and 14CH4). Light and dark CO2 assimilation incubations were also measured. Based on the measured rates of methane production and oxidation, on the stable isotopic signature of in situ methane and on the microbial communities detected in the sediments and water column, this study proposes an overview of the CH4 cycle and microbial players in the two types (dep and shallow) of studied lakes. They conclude, based on the differences in the rates of methane production and consumption, as well as on the profiles of methane concentration, that the water columns of deeper tundra lakes are better methane filters than of the shallower lakes. The study provides a thorough investigation of C processes and microbial communities in the water and sediments and valuable information on these systems, but I believe some information is unnecessary to support the conclusions (ex: Fig 2, Fig 3, Fig 4, Table 2), creating detours in the main message of the manuscript. I think these should be better incorporated in the Discussion and Conclusion sections or removed from the manuscript. For example, Figure 9, which summarizes the main findings of the paper, does not show primary production, DOC, nor dark and light CO2 assimilation results. On the other hand, other information that are important to understand the differences between the systems and would support the conclusion, such as temperature profiles indicating the physical structure of the lakes, are missing. The manuscript also needs a grammar and spelling check. Therefore, I believe the manuscript needs a major revision.

Our manuscript presents the data on the composition and activity of microbial communities, somewhat focused on the methane cycle. The research subjects were four lakes of the continuous permafrost zone. Prior to our studies, we had no idea of the scale of the effect of lake depth on the rates of the methane cycle processes. The composition of microbial communities of the methane cycle and the rates of the relevant microbial processes certainly depend on such parameters as primary production and qualitative and quantitative composition of organic matter. Depth was only one easily discernible factor affecting the rate of microbial methane oxidation. We expected organic matter produced during the summer algal bloom (Fig. 2) to be the first component of the carbon cycle. The concentration of dissolved organic matter in the water and sediments (Fig. 3) is certainly a factor affecting the rates of microbial processes of the methane cycle. Total microbial abundance (Table 2) and the rate of dark CO_2 assimilation (Fig. 4) characterize the state of the microbial community. In our opinion, these parameters are required for assessment of the trophic status of the studied basins, and removal of this information from the manuscript will result in a loss of valuable data.

We thank the Reviewer for comments concerning the temperature profiles. It is, however, not provided, since in summer the lakes are not stratified, and the temperature differences between the surface and near-bottom layers do not exceed 3°C. This information was added to the revised version of the manuscript.

Introduction The introduction can be more fluid and provide only information essential to the problem. For example, I think there are excessive descriptions on the origin of the lakes (e.g. lines 40-43 can be summarized; lines 47 and 48 could be removed).

In our opinion, the Introduction is written in the classical style. The text includes substantiation of the studied problem and a short description of the origin of tundra lakes. The publications reporting wide occurrence of thermokarst lakes are listed. The data on association between climatic changes and formation of new thermokarst lakes are cited. The Introduction section is 60 lines long, which in less than in most *Biogeoscience* publications. In our opinion, removal of lines 40-43, 47-50, 66-61, and 73-79 will not improve the Introduction.
Lines 66-67 Reformulated based on the reviewer's comment.
Caption of Fig 1 Reformulated based on the reviewer's comment

Methods

Table 1:
• Please indicate what EC stands for in a footnote of the table. What is 'sm^{-1} ' in EC?
Should it be µS m^{-1} ?
Corrected: Electrical Conductivity (EC), µS cm^{-1}

• Typo in 'Secci', should be Secchi.

Corrected.

- What does N and T mean in the Type of lake? Please clarify.

Removed.

• Same for IV and V in the Basin embedded in. Please clarify.

Removed.

• Please add maximum depth of lakes in the table.

Added in the first column of the table 1.

• Please replace ‘Sampling horizons’ for ‘Sampling depths’ since these are water samples (right?).

Corrected.

Lines 111-114: is this information on the ions essential to this work? It seems to me that it is not.

In our opinion, the information on the ion composition is important. Ion ratio in fresh waters is known to vary, with higher SO_4^{2-} concentration resulting in shifts in the microbial community composition and intensification of sulfate reduction.

Lines 122-124: refer the reader to Table 1 instead of listing the sampled depth in the text.

Corrected.

Line 129: what is ‘(C)’?

Removed.

Lines 152-155: break the sentence in two. There are two colons (:)

Corrected.

Line 156: sampling depths for incubations were the same described in Table 1? Please specify.

Corrected.

Line 161: “Incubation of water and sediment samples to determine the rates of other processes was also carried out in situ.” Please specify which other processes were measured in incubations in situ.

Added based on the reviewer's comment.

Line 161: Under which conditions did you keep the experiments for determination of MG? Were the sediments anoxic when sampled? How did you keep samples anoxic during incubations?

Sediment samples were collected from intact cores into cut-off syringes sealed with rubber stoppers, avoiding air inflow. The labeled compounds and the fixing agent (KOH) were injected through the rubber stopper. Thus, no contact between the samples and air occurred at any stage of the experiment. The procedure has been described in detail in Pimenov, N.V. and Bonch-Osmolovskaya, E.A., In situ activity

studies in thermal environments, Methods in Microbiology, vol. 35, Extremophiles, Rainey, F.A. and Oren, A., Eds., Amsterdam: Acad. Press, Elsevier, 2006, pp. 29–53.

Lines 166-167: How did you separate CO2, biomass and soluble organic matter in the methane oxidation experiments? Please clarify.

Detailed description of the procedure would have taken too much space. It has been described previously (Pimenov, N.V. and Bonch-Osmolovskaya, E.A., In situ activity studies in thermal environments, Methods in Microbiology, vol. 35, Extremophiles, Rainey, F.A. and Oren, A., Eds., Amsterdam: Acad. Press, Elsevier, 2006, pp. 29–53).

Lines 170-177: Not sure what is the difference between the description that starts in line 171 and the other starting in line 176. Maybe you should start with lines 176-177 and then explain how the isotopic composition was calculated based on the light and heavy isotopes concentrations (lines 171-175).

Reformulated based on the reviewer's comment.

Results

Line 200: This sentence is not clear and needs rephrasing. Why is it the main characteristic of water bodies? Something like “PP is the main process of C fixation in lakes” would make more sense to me.

The Results section indeed begins with our results on the phytoplankton primary production. In our opinion, freshly produced organic matter of phytoplankton origin is the initial substrate for methanogenesis in the sediments of the studied lakes. We have rephrased the sentence according to the Reviewer's comments.

Line 208: …higher PP in **the** two deep mature lakes (Fig. 2)

Corrected to “a little higher PP in the two deep mature lakes.”

Line 208: Please repeat here which lakes are the deep mature ones to help the readers.

Added.

Table 2: what is the unit of biomass? ug of what per L? Carbon? What was the conversion factor to go from cell volume (um3) to biomass? Please add it in the methods section.
Biomass was calculated using the data on the volume of microbial cells and assuming the density of wet biomass equal to 1.0 mg mm^{-3}. Specific biomass of microbial cells (B) is therefore presented in μg L^{-1}. Although carbon content in the biomass can be easily calculated using the known coefficient of 20 fg C per cell (Lee and Fuhrman, 1987), we do not think this is necessary, since the data will not be discussed further.

Line 225: please indicate what are ‘aggregated cells’ and how you measure them in the methods section.

During microscopic examination of the stained preparations, single cells and cells associated with aggregates were enumerated separately. A group of cells with a common outline, in which enumeration of individual cells was difficult, was considered an aggregate.

Figures 2 and 4: it would be better to create a legend for the bars fills (instead of using the numbers 1 and 2). Also, a secondary y axis would be more appropriate than showing both series of data in the same axis.

Figs. 2 and 4 were completely remade in accordance with the Reviewer's comments.

Line 230: please repeat here which one is the thermokarst lake.

Added.

Table 3: what is s1, s2, s3? Please clarify or remove them if not relevant.

S1, s2, and s3 are designations of the sediment samples, which are used also on Fig. 7. Abbreviations are explained in the legend to Table 3.

Line 269: where is this sample in Table 3?

The error is corrected. Sample LK-004 K is shown in Table 3.

Lines 286-287: it should be like that right? Since the primer used targeted the bacterial 16S rRNA gene.

Yes, the primers used in this work were amplified with both bacterial and archaeal groups.

Figure 7: please indicate in the caption what ‘w’ and ‘s’ after that name of the sample mean in the x axis.

W and S are designations for the water and sediment samples, respectively. The figure caption was modified accordingly.

Line293-294: did you use a primer for the bacterial 16S rRNA gene and another for the archaeal 16S rRNA gene? Or does the primer used cover both groups? Please clarify this in the Methods section and provide a reference for the primer’s coverage of both groups if it is the case. It was not clear that you were evaluating both Bacterial and Archaeal communities.

Yes, the primers used in this work were amplified with both bacterial and archaeal groups. The relevant reference was added: Frey B, Rime T, Phillips M, Stierli B, Hajdas I, Widmer F, and Hartmann M. Microbial diversity in European alpine permafrost and active layers. FEMS Microbiol Ecol. 2016; 92(3):fiw018. https://doi.org/10.1093/femsec/fiw018

Line 293: if you use the word ‘significantly’, please provide some statistical verification of the difference.

Corrected.

Line 337: known aerobic methanotrophs are in the Gammaproteobacteria, Alphaproteobacteria and Verrucomicrobia. There are not methanotrophs in the Betaproteobacteria class to my knowledges. Please verify and fix.

Corrected. It was our mistake, since there are no methanotrophs in the Betaproteobacteria class.

line 356-357: could you provide a correlation coefficient of some sort of quantitative estimation of such comparison?

Comparison of the data on activity of methane oxidation and *Methylobacter* relative abundance is an estimate. We are not sure the correlation coefficients will affect our cautious conclusions.

Line 358: What about the C. Methylomrabilis oxyfera within the candidate phyla NC10? Ettwig et al 2015 and others.

No other methanotrophic bacteria and archaea were detected, including the candidate phylum NC10.

Line 361 should be in the previous paragraph.

The paragraphs were merged.

Line 367: typo, should be ‘acetoclastic’

Corrected. Both variants occur in the literature, although "acetoclastic" is indeed more common.

Line 387, 389: typos, should be ‘acetoclastic’

Corrected.

Lines 391-397 should be in the Results section in my opinion.

In lines 391–397, the calculated integral data on methane production and oxidation in the upper sediment layer (μmol CH_4 m^{-2} day^{-1}) are presented. They were calculated for the 0-15 cm sediment layer. Since changing this range in any direction results in different calculated values, we think that the results of our calculations are not experimental results, but rather belong to the Discussion section.

Lines 391-397: How do you explain higher methane production rates than methane oxidation rates? Some discussion on this would be interesting.

In the studied sediments, the rate of methane production (0.6–14.5 μmol CH_4 m^{-2} day^{-1}) was much lower than the rate of methane oxidation (300–1350 μmol CH_4 m^{-2} day^{-1}). This discrepancy may be caused either by low rates of hydrogenotrophic methanogenesis or by methane inflow from deeper sediment layers (below the studied horizons).

Fig 8: the x axis should be inverted (the y axis should cross the x axis at the lowest (more negative) signature values).

The figure was modified according to the Reviewer's request.

Lines 407-410: not needed to repeat the enrichment in 13C-CH4 during methane oxidation here. It was already explained in the Methods section.

Interpretation of the data on carbon isotope fractionation during microbial methane oxidation is not unequivocal. Fractionation depends on a number of parameters and can not be predicted from initial conditions. The text of lines 407–410 belongs to the Discussion section, since it considers the possible reasons for the changes in the carbon isotopic composition due to MO.

Section 4.4: the comparison between summer and winter is very interesting, but in my opinion is out of the scope of the manuscript. There are many interesting results in the ms already – and in my opinion some can be further discussed –, while the seasonal differences add other information that is not in the aim of this study. In addition, Table 4 shows data for all lakes together (if I understood correctly), which makes the reader wonder the differences between lakes since the focus of the study is the differences between deep and shallow lakes.

Research on the rates of microbial processes and the composition of microbial communities in the Yamal polar lakes in summer and winter was carried out by the same team using the same methods. This is probably the first seasonal study of such difficult-to access polar basins. Although comparison of the winter and summer data did not yet yield clear conclusions, it still retains importance.

Figure 9: I cannot read the microbial player responsible for methane oxidation in the deep lakes. This figure is a nice summary of the results, maybe other components, if kept,should be included.

Fig. 9 was intended as a conclusion of the Discussion section. In our opinion, introduction of additional material will hinder its understanding.