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# *Interactive comment on* "The water column of the Yamal tundra lakes as a microbial filter preventing methane emission" *by* Alexander Savvichev et al.

**Alexander Savvichev et al.**

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Dear reviewer, we are grateful for the great work you have done. We tried to take into account all comments and answer all questions. 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



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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 CO2 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 his comment concerning the temperature profiles. These data will be 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.

66-67 Reformulated based on the reviewer's comment. Caption of Fig 1 Reformulated based on the reviewer's comment.

Methods Table 1: âĂć Please indicate what EC stands for in a footnote of the table. What is 'sm-1 ' in EC? Should it be $\mu$S m-1 ? Corrected: Electrical Conductivity (EC), $\mu$S cm-1

âĂć Typo in 'Secci', should be Secchi. Corrected.

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

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

âĂć Please add maximum depth of lakes in the table. Added in the first column of the table 1.

âĂć 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 SO42- 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,





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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





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and assuming the density of wet biomass equal to 1.0 mg mm-3. Specific biomass of microbial cells (B) is therefore presented in $\mu$g 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 caption 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.





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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 ($\mu$mol CH4 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 $\mu$mol CH4 m$-$2 day$-$1) was much lower than the rate of methane oxidation (300–1350 $\mu$mol CH4 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





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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.

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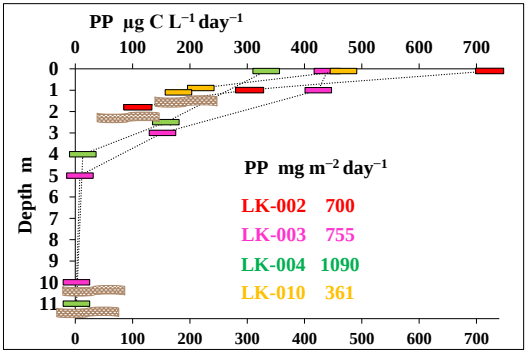


**Fig. 1.** Fig. 2. Primary production in four lakes of the Yamal Peninsula (August 2019): in the water horizons, $\mu$g C L−1 day−1, and calculated integral values PP, mg C m−2 day−1 (in the center of the picture fi



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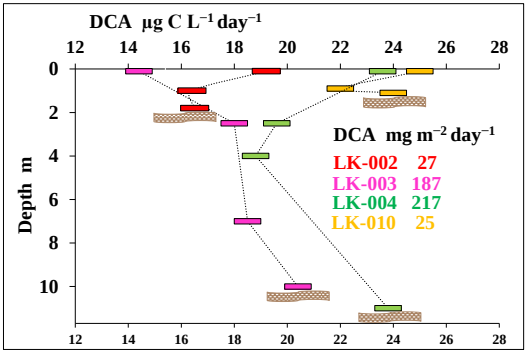


**Fig. 2.** Fig. 4. Dark CO2 assimilation (DCA) in four lakes of the Yamal Peninsula (August 2019): in the water horizons, $\mu$g C L−1 day−1, and calculated integral values DCA, mg C m−2 day−1 (in the center of the



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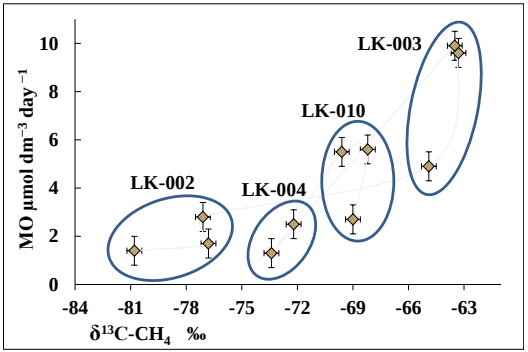


**Fig. 3.** Fig. 8. Isotopic composition of methane carbon ($\delta$ 13 C-CH 4 , ‰ in the bottom sediments of four Yamal lakes and the rates of methane oxidation (MO, $\mu$mol dm $-3$ day $-1$ ).



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