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

Alexander Savvichev, Igor Rusanov, Yury Dvornikov, Vitaly Kadnikov, Anna Kallistova, Elena Veslopolova, Antonina Chetverova, Marina Leibman, Pavel Sigalevich, Nikolay Pimenov, Nikolai Ravin, and Artem Khomutov

Overview

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 ($NaH^{14}CO_3$ and $^{14}CH_4$). 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 CH_4 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.

Specific comments:

<u>Introduction</u>

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).

Lines 48-50 could be removed since they do not provide necessary information to introduce the study.

Lines 55-61 can be summarized in fewer sentences. I agree that it is good to explain well the reasoning, but I would skip some of the detailed explanations, assuming that the readers are familiar with methane and carbon cycling in lakes.

Lines 66-67: please reformulate this sentence because in the way it is structured, it seems that you mean that methanogens also oxidize methane.

Lines 73-79: I suggest you summarize these sentences into one or two. You can assume that the readers are familiar with the stable isotopic fractionation of methane during microbial methane oxidation.

I think the Introduction should be restructured. Some parts should be removed to avoid excessive information that is not essential for the study question (e.g. excessive details on the origin of lakes, isotopic fractionation of methane during methane oxidation, origin of C for methane production). I also think that you should give more emphasis on the differences between the shallow and deep lakes that could lead to expected differences in methane cycling.

Caption of Fig 1 needs reformulation. Please break down the description of B into two or three sentences or use semicolons to clarify.

Methods

Table 1:

- Please indicate what EC stands for in a footnote of the table. What is 'sm^{-1}' in EC? Should it be $\mu S\ m^{-1}$?
- Typo in 'Secci', should be Secchi.
- What does N and T mean in the Type of lake? Please clarify.
- Same for IV and V in the Basin embedded in. Please clarify.
- Please add maximum depth of lakes in the table.
- Please replace 'Sampling horizons' for 'Sampling depths' since these are water samples (right?).

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

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

Line 129: what is '(C)'?

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

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

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.

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?

Lines 166-167: How did you separate CO_2, biomass and soluble organic matter in the methane oxidation experiments? Please clarify.

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).

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.

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

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

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.

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

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.

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

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

Line 269: where is this sample in Table 3?

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

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

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.

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

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.

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

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

Line 361 should be in the previous paragraph.

Line 367: typo, should be 'acetoclastic'

Line 387, 389: typos, should be 'acetoclastic'

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

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

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

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

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.

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.