Biogeosciences Discussions

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Comment on bg-2021-3

Anonymous Referee #1

Referee comment on "Methane oxidation in the waters of a humic-rich boreal lake stimulated by photosynthesis, nitrite, Fe(III) and humics" by Sigrid van Grinsven et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-3-RC1, 2021

This interesting study investigates the biogeochemical methane cycle in a relatively shallow, eutrophic boreal lake using a wide range of chemical, microbiological and molecular techniques. The authors show that the lake Lovojärvi has a very active methane cycle mostly driven by upwards diffusing methane from the sediment (produced by methanogenesis) but also provide evidence for an additional source of methane within the water column near the Chla maxium. Methane seems to be efficiently consumed by the microbial community, in particular at the oxic-anoxic interface as well as in the anoxic hypolimnion. Using 13C-methane incubations, the authors show that methane oxidation in the anoxic hypolimnion seems to be coupled to in situ production of oxygen at shallower depths, while some of the tested electron acceptors appear to stimulate methane oxidation in the dark anoxic hypolimnion. Furthermore, FISH and 16S rRNA amplicon analyses indicate that alpha- and gammaproteobacterial methanotrophs appear to be the dominant methanotrophs.

Overall I find this study very interesting. Considering that boreal lakes are quite poorly characterized in respect to methane cycling, I believe that this study is a valuable addition to the current literature. The manuscript nicely highlights that light-driven methane oxidation as well as AOM coupled to other electron acceptors can be important processes in the anoxic hypolimnion of shallow boreal lakes. The evidence for photosynthesis-fueled methanotrophy appears robust und the authors do a good job discussing some of the observed anomalies (e.g. +O2 vs. light, 3 m vs. 4 m). However, I'm more skeptical about proposed stimulation of MOR by some of the amended substrates, in particular AQDS, and I feel that the authors should be more careful not to overstate the results of their incubation experiments (see point #1). Other than that, I have only minor suggestions. The manuscript is generally well written and understandable, and the Methods are rather brief but for the most part adequately described. The introduction could be more focused on methane cycling in boreal lakes in general (see point #2) and it would be helpful if the authors could provide some context around why Lovojärvi was studied (see point #3).

Specific points:

#1 Stimulation of MOR in incubations

For some incubations, there is a clear increase in MOR (e.g. light) and the data looks robust to me. However, for other incubations the stimulation is much less pronounced (e.g. Fe, humic acid) or even so small that the difference is in my opinion within the margin of error (for AQDS). Without independent biological replicate incubations, which I don't think the authors did (please correct me if I'm wrong), I am not entirely convinced that the presented data for AQDS (and possibly Fe and humic acid) conclusively show a stimulation of MOR. As it is an interesting and important conclusion, I encourage the authors to provide some additional data (e.g. statistical tests) to support their claims.

#2 Introduction could be more focused on boreal lakes

In its current form, the introduction is very general. While I agree that boreal lakes are not excessively studied, I believe that more can be said in the introduction than that "studies […] are relatively scant". I encourage the authors to expand their introduction with more information about biological methane oxidation in boreal lakes (e.g. what is known about humic substances and why are they important, availability of other TEA, are they often Fe- and Mn-rich?).

#3 Boreal lakes and Lovojärvi

Lovojärvi strikes me as a quite unique lake (presence of halocline, extreme CH4 concentration above the sediment, meromixis). Is this a typical boreal lake with typical physico-chemical features? Since the authors use their findings to make general conclusions regarding the biological methane filter and the emission potential in boreal lakes (e.g. lines 14-18), it would be important to include some discussion/description on how representative Lovojärvi is for boreal lakes in general.

Minor points:

Line 64: “aerobic MOB” sounds counterintuitive in this context. Please rephrase.

Line 73: There is definitely more literature available on methane oxidation in boreal lakes (e.g. Rissanen et al. 2017, https://doi.org/10.1093/femsec/fix078)

Line 164: How much water was typically filtered?

Line 174: How many cells were counted?

Line 191: Include some information regarding sequencing depth (either here or as a table)

Line 233: The NOx profiles are quite stunning. I assume that the nitrate and nitrite peak close to the base of the oxycline are due to microbial ammonia oxidation. But what could be the source of nitrite in the bottom water?

lines 292: The meaning of 'other Methylococcaceae' is unclear to me. Please specify.

lines 292-296: I'm confused. Methylocystaceae abundance seems low but this sentence suggests to me that they might be high since you detected unknown Rhizobiales bacteria? Please clarify.

lines 283-301: It's not clear to me according to what logic the abundances of different methanotrophs, methylotrophs and some seemingly random taxa (Acidoferax, Planctomycetaceae, Rhizobiales) are listed one after the other. Please restructure. Also, some of these groups are never discussed and it's not clear why they are specifically mentioned here.

Line 290: Were you able to observe any filamentous gamma-MOB using FISH?

line 311: "natural conditions" suggests that different light intensities were used for incubations from 3m and 7, please clarify.

Line 319: Given the uncertainties in Table S3, AQDS 5m MOR increase does not look significant.

line 348: What is meant by a concentration of +/- 0.5 uM ?

line 392-401: It would be interesting if the authors could slightly expand on methanogenesis by phototrophs by including some brief speculation what cyanobacterial groups could be responsible for this (using the amplicon data).

Lines 486-488: The contribution of methanotrophs is indeed important, however, I suppose the halocline also plays an important role?

Fig 1: This is quite a busy figure that could use some improvement. I suggest that change the scale of the x-axis for oxygen to highlight the O2 dynamics the lower concentration range (as shown in Fig. S1). In panel A, it looks as if oxygen concentration increases slightly in the hypolimnion, please comment (also in Fig. 2). In panel C, value for MOR – NO2 at 7 m is clearly <1.5 while table S3 shows a value of 1.54. Please explain error bars in legend.

Overall I suggest that the authors revise it to improve clarity. For example: i) not all x-axis same length (panesl C and D) or ii) error bars sometimes not visible.

Fig 2: In my opinion, the y-axis could be limited to 10 m in order to focus on the upper water column.

Fig 3. Only cosmetic, but there is an offset between lines and symbols.