Reply from van Grinsven et al. to Community Comment on bg-2021-3

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Community comment on "Methane oxidation in the waters of a humics-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-CC1, 2021

It was really interesting to read a study on methane oxidation conducted in the same study lake as our recent study (Rissanen et al. FEMS Microb Ecol, Volume 97, Issue 2, February 2021, fiaa252). While our study was focused on the genetic potential of methanotrophs, this study provides very valuable novel information on the various factors affecting the activity of methanotrophs in boreal lakes. Here are some minor suggestions, which I think could further improve the manuscript:

Re: We very much appreciate your time and effort in reading and commenting on our study. We also highly value the paper suggestions you delivered. We have replied to your specific comments below. Some of these refer to comments in reply to the reviewers, which can be viewed in separate replies in the Biogeosciences discussion forum.

Line 49-52. It is perhaps worth to mention here study by Zheng et al. 2020 on extracellular electron transfer from methane to Fe-mineral by Methylomonas in hypoxic conditions.

https://pubs.acs.org/doi/abs/10.1021/acs.estlett.0c00436

Re: Reference added.

Line 69-70. Either “can play” or “are likely to play” Re: adapted

Line 73-74 and in especially discussion. Maybe it would be relevant to mention and discuss your results also in the light of our previous study from 2018, which was conducted in the nearby humic boreal lakes, where we also studied lake water methane oxidation with amendments of various EAs (incl. NO3, metals, organic EAs) and in different light conditions, and also studied the community structure and genetic potential of methanotroph communities (Rissanen et al. 2018):

https://www.int-res.com/abstracts/ame/v81/n3/p257-276/

Furthermore, study by Kallistova et al. (2018) might be also relevant to mention and discuss. They also studied methane oxidation and MOB communities in water column of a boreal lake.

https://www.int-res.com/abstracts/ame/v82/n1/p1-18/

Re: Both papers were added to the manuscript.

Line 87-. Study site. It is maybe worth to mention the historical anthropogenic effects, the soaking of flax and hemp, which potentially have contributed to the (chemical) stratification in the lake. In the Finnish publication (Tolonen et al. 1976), it is mentioned in Finnish that “Hampun ja myöhemmin myös pellavan liotus nopeuttivat läheisen Lovojärven pilaantumista jo rautakaudella (Huttunen & Tolonen 1975).” = Soaking of hemp and later also soaking of flax accelerated the pollution of nearby Lake Lovojärvi already during Iron Age”.

Tolonen K, Tolonen M, Honkasalo L et al. Esihistoriallisen ja historiallisen maankäytön vaikutuksesta Lammin Lampellonjärven kehitykseen. Luonnon Tutkija. 1976;80:1–15 (in Finnish with English abstract):

https://www.researchgate.net/publication/311665698_Esihistoriallisen_ja_historiallisen_maankayton_vaikutus_Lammin_Lampellonjarven_kehitykseen_The_influence_of_of_prehistoric_and_historic_land_use_on_Lake_Lampellonjarvi_South_Finland

Unfortunately, I could not find the original reference of Huttunen & Tolonen 1975.

Re: Thank you, for directing us to these interesting references. We have now added this information to the "Study site" section of the "Materials and methods".

Line 118. were fixed Re: corrected
Line 382- What about archaea-driven methanogenesis in anoxic micro-niches?

Re: This is indeed an interesting possibility, but as we can only speculate about it, without any supporting data regarding anoxic micro-niches, we prefer not elaborate on this in the discussion.

Also more generally, there are recent studies suggesting that also methanogenic archaea can oxidize methane anaerobically, e.g. via extracellular electron transfer to solid EAs (iron minerals, organic EAs, anode in bioelectrochemical systems). Maybe worth to mention and discuss. See, e.g.

https://www.sciencedirect.com/science/article/abs/pii/S1385894720328199
https://pubmed.ncbi.nlm.nih.gov/28965392/

Re: Although the potential involvement of methanogens in methanotrophy is really fascinating, the potential contribution of methanogens here would be very low, as they are detected only at certain depths, and at much lower relative abundances than bacterial methanotrophs ("Archaeal methanogens of the genus *Methanoregula* were detected in the water column, but only at 9, 11 and 17 m depth (0.1, 0.1 and 0.3 %)." and Fig. 3). We therefore choose not to add this aspect to the discussion.

Line 468-470. Our study in the same study lake (which has been cited but not in this context) detected genetic potential for nitrate/nitrite/NO – reduction as well as for extracellular electron transfer (to metal minerals and organic EAs) in metagenome assembled genomes of Methylococcales (incl. Methylobacter sp. and the Crenothrix-type MOB), which supports the results of this study on enhancement of methane oxidation by these various EAs. See:

https://academic.oup.com/femsec/article/97/2/fiaa252/6034011

Re: Thank you for pointing this out. We have now included these findings, which wonderfully support our results, into the discussion (*"These patterns are in line with a recent 16S rRNA gene and metagenomic sequencing study in Lake Lovojärvi (Rissanen et al., 2020), which also showed the presence of nitrite reduction genes in Methylococcales metagenome assemblies of the water column, as well as genes related to extracellular electron transfer")*

Line 473-481. Microbial interactions. Maybe worth to include and discuss also the results by Cabrol et al. 2020. They studied anaerobic methane oxidation (AOM) and MOB communities in water

columns of northern lakes and found correlation between Methylococcales and OTUs within Methylotenera, Geothrix and Geobacter genera which indicated that AOM might occur in an interaction between MOB, denitrifiers and iron- cycling partners.

https://www.sciencedirect.com/science/article/pii/S0048969720331053

Re: There are indeed several studies showing the cooccurrence of MOB with certain species, of which especially Methylotenera is often found cooccurring with MOBs. We have, however, decided to not further elaborate on the aspect of possible microbial interactions, as we have little data to support it, only relative abundance data.

Figure 1. Is there a slight increase in O2 towards the deepest depths (from appr. 15 m to the deepest depth)? If there is, what is causing it?

Re: See above the comment on this, in reply to a comment by reviewer #1. The figures are adapted now, to contain the correct data set, of the trace-sensitive oxygen sensor.

References:

Kortelainen et al. 2000. Numbers as subscripts for CH_4, CO_2 and N_2O

Mutyaba 2012. Maybe it could be mentioned that it is Master of Science thesis. And perhaps provide a link to it. https://jyx.jyu.fi/handle/123456789/40735

Rissanen et al. DOI-link is missing.