Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2019-482-AC4, 2020

Interactive comment on "Lake mixing regime selects methane-oxidation kinetics of the methanotroph assemblage" *by* Magdalena J. Mayr et al.

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Received and published: 31 March 2020

Reply to Reviewer 3:

This manuscript describes the study of methane oxidation (MOX) during lake overturn in Lake Rotsee in Switzerland. You combine measurements of MOX kinetics with metatranscriptomic analyses of methane monooxygenase genes and report differences between epi-and hypolimnion during stratification and a convergence of MOX kinetics and gene expression during lake water mixing. You conclude that methane oxidizers with well-adapted kinetics occupy distinct niches in stratified lakes.

Answer: Thank you for your valuable comments. In the following, we address all open questions and limitations raised.

While I think the report of kinetic parameter of methane oxidation is of great relevance, however, I found that the manuscript suffers from a lack of clarity and over-simplifications. Most importantly, it's unclear how the central conclusion, that welladapted methanotrophs inhabit niches depending on methane availability (in hypo- and epilimnion), is reached. Wouldn't a match between in situ CH_4 concentration and Km (not normalized per cell) be a stronger indication of such an adaptation?

Answer: Thank you for your positive assessment of the relevance to report kinetic parameters.

We do indeed find a higher affinity (low Km) in the low-methane epilimnion compared to the methane rich hypolimnion as long as stratification is present, and this finding is a key part of our argument (see e.g. abstract). That the Km values do not match the in-situ methane concentrations is however not unexpected. In the mixed layer and under the assumption of a steady-state, the flux of methane from the hypolimnion is balanced with the (non-normalized) methane oxidation rate. Under these conditions, the in-situ methane concentration does depend on the half-saturation constant (Km) but should be lower than Km. Per definition, the half-saturation constant is the substrate concentration where the growth rate is half the maximum growth rate. Even if the growth rate is only half the maximum growth rate, microbial methane oxidation continues, and methane concentrations decrease to values below Km. We added the calculations behind this argument as a supplement to this author reply and we will consider adding this rationale and the calculations to the revised manuscript. In the hypolimnion, oxygen likely becomes limiting and, as a consequence, methane concentrations can be much higher than the half-saturation constant.

In the revised manuscript, we will improve the clarity of our central conclusion and avoid over-simplification. We can incorporate the above points into the discussion for clarity.

I also believe a better use of the metatranscriptomic data could help to strengthen this point. A finer taxonomic resolution based on the pmoCAB genes and a more quantitative characterization of the community turnover should be possible – and could help to make the point that indeed there are distinct populations of MOB that are adapted to in situ CH4 concentration. Accordingly, I think that Figure 3 a1-c1 is not the ideal way to convey this important point. Maybe a combination of SI Fig. 2 (which I think shows quite nicely the convergence towards similar gene expression patterns in January, with a Figure showing the taxonomic composition of MOB during lake overturn would be a better choice.

Answer: Yes, we agree. We will provide a finer taxonomic resolution for pmoA in the revised manuscript. pmoC and pmoB are less indicative for taxonomic classification because they are not usually sequenced and used for this purpose. Yes, we will consider using the SI Fig. 2 instead, which shows the community turnover more clearly in a revised manuscript.

âĂČ Moreover, I was somewhat irritated by the rather vague description of the environmental conditions during lake overturn. The traditional definition of lake stratification and hence the difference between epi- and hypolimnion based on temperature rather than oxygen. And while the manuscript addresses MOX during lake overturn, you refer to the oxycline for sampling. I understand that the temperature profiles shown in SI Fig 1 may not be as clear as the oxygen profiles shown in Fig. 1 – but I would advise to show all profiles (also conductivity which should explain the inverse stratification pattern in December) and to be very clear with the definition of overturn, thermos- and oxycline.

Answer: We will add the temperature and conductivity profiles to Figure 1 in the revised manuscript and define overturn, thermo- and oxycline more clearly.

Finally, given the relatively low number of samples and the fact that the pattern was (only) observed in Lake Rotsee, I think the manuscript should be thoroughly rewritten

to make clear that this may reflect a specific situation in the (relatively eutrophic) Lake Rotsee. Also, there are several cases of speculation or exaggerated extrapolation, which should be avoided.

Answer: The effort of obtaining the time resolved data for two water layers was very substantial (described in the answers to Reviewer 1 and 2). Nevertheless, we agree that we only have a limited number of samples from a single lake. We will highlight this more clearly in a revised manuscript and explicitly state that further investigations in other lakes will be required to confirm our findings.

L 11 In freshwater lakes. . . so, this excludes saline lakes? Consider removing "freshwater"

Answer: We will remove freshwater here and mention it in Line 14: "in a seasonally stratified freshwater lake".

L 14 we tested the hypothesis that methanotroph assemblages in a seasonally stratified lake. . .

Answer: In the revised manuscript we will make sure that we only studied a single lake and will specifically discuss limitations of the transferability of our results.

L 18 consider a brief explanation of the meaning "half-saturation constant" here

Answer: We will add a brief definition of "half-saturation constant" in the revised manuscript.

L19 . . .Km differed by two orders of magnitude – but in the results it seems that they differed between 15 and 0.7 uM (a factor of $\sim$20)

Answer: We will correct this in the revised manuscript.

L 25 . . .90% of what?

Answer: 90% of the methane that is transferred to the epilimnion during lake overturn is oxidized. Since this is a result from an earlier study we will rephrase this sentence in the revised manuscript.

L28 can you talk about a climate IMPACT of lacustrine systems?

Answer: Yes, we think so. According to DelSontro et al (2018), lacustrine systems emit an equivalent of about 20% of the global fossil fuel CO2 emissions, and methane contributes approx. 75% to this. Continuing eutrophication of lacustrine systems will most likely further increase emissions by another 30-90% (DelSontro et al. 2018, Beaulieu et al. 2019) We suggest to clarify: “Methane is a major contributor to the climate impact of greenhouse gas emissions from lakes”.

L 31 anoxic habitats.... In the oxygen-depleted hypolimnion... repetitive

Answer: We suggest to remove “anoxic habitats” in the revised manuscript.

L 47 kinetic traits... Use kinetic parameter instead (see L 48)

Answer: We will harmonize the use of “kinetic traits” and “kinetic parameter” in the revised manuscript.

L 58 ...Lake Rotsee...

Answer: We will remove the “a” written before “Lake Rotsee”.

L 63 ex situ consider replacing with “laboratory incubations”

Answer: Yes, we will replace this in the revised manuscript.

L 73 four or five campaigns?

Answer: Thanks, four campaigns. We will correct this in the revised manuscript.

L 77 and onward. Please provide more detail on this method including how the killed controls were treated.

Answer: We will provide more detail in the revised manuscript. The killed controls were

treated in the exact same way as the samples with the exception that we added 1 mL of ZnCl2 (50% v/w) to stop biological activity right after we filled the serum vial with the sample. The average water fraction radioactivity of the killed controls were used as background radioactivity in the outlier detection procedure.

L 91 how were Schott bottles sealed air-tight?

Answer: The Schott bottles were not sealed air-tight. Since gasses were stripped from the samples anyway for these analyses, this was not necessary. For determinations of methane concentration and “in-situ” rates, we directly filled samples into serum vials and those were sealed air-tight (see sections 2.4, 2.5).

L 110 we determined the in-situ MOX rate... in duplicate ex-situ incubations... Confusing, please rewrite.

Answer: We suggest to remove “in-situ” and change ex-situ incubations to laboratory incubations in the revised manuscript.

L 161 an 167 reads shorter than 400 or 300 bp were removed?

Answer: We first used a general approach to identify genes (prodigal) removing <400bp genes. During targeted gene identification with prokka and diamond again shorter gene pieces of MMO were identified and we removed short gene fragments <300bp. We will consider harmonizing the base pair cut-off in a revised manuscript.

âĂČ L 183 aerobic methane oxidation likely contributed to this oxygen depletion in the epilimnion. This seems very speculative for me. Could a back of the envelope calculation, e.g. knowing the volume and CH4 concentration in the hypolimnion and the stoichiometry of MOX be used to support this speculation?

Answer: We assume that the methane oxidation, leading to the oxygen depletion, mainly occurs in the epilimnion itself. This methane oxidation can occur despite the low methane concentration and is fuelled by the flux of methane into the epilimnion. The stoichiometry of microbial methane oxidation is: ãĂŰCHãĂŮ_4+(2-y) O_2→(1-y) ãĂŰ-

COãĂŮ_2+yãĂŰCH_2 OãĂŮˆBM+(2-y) H_2 O where y is the carbon use efficiency. Based on theoretical considerations and experimental data, a carbon use efficiency of 0.4 has been reported (Leak & Dalton 1986). This means that per mole of methane 1.6 moles of oxygen are used. The mixed layer depths for the four sampling campaigns are roughly 6, 10, 12 and 14m, which results in mixed layer volumes of 2.5, 3.7, 4.1 and 4.3 GL in Lake Rotsee. Multiplying the measured methane oxidation rates in the epilimnion with these volumes results in a total methane oxidation of 600, 11560, 11800 and 200 mol d-1. Integrated over the time period of the four campaigns, this results in a total of 0.66 Mmol of methane that were oxidized during this time. This corresponds to a removal of 1.1 Mmol of oxygen from the epilimnion. In an average volume of the mixed layer of 3.7 GL with an initial concentration of 340 μM (10.9 mg L-1) of oxygen, this would reduce the oxygen concentration by 180 μM to 160 μM or to about 5 mg L-1. Note that possible oxygen production and exchange with the atmosphere are not included here. We will consider adding this rationale to the revised manuscript.

L 228 critical phase – critical for what?

Answer: During this time, methane that has accumulated in the hypolimnion is rapidly transported to the surface and is potentially released to the atmosphere – i.e. critical for potential outgassing to the atmosphere and thus for climate relevance. We'll clarify this in the revised manuscript.

L 233 specific affinity towards methane. . . unclear what is meant here.

Answer: We wanted to specify that we mean the specific affinity for methane and not for any other nutrient and will make this clear in the revised manuscript.

L 235 was the convergence only driven by changes in kinetic parameter in the epilimnion (or also in the hypolimnion as seems apparent from Fig. 2 a)

Answer: The convergence was driven by changes in kinetic parameter in the epilimnion and the hypolimnion. We'll clarify this in a revised version of the manuscript.

L 289 remove "as in many other stratified lakes" – too speculative (or include references, but I would not advise so in the conclusion part)

Answer: We will rephrase this sentence in the revised manuscript and provide a reference. That low methane concentrations are found in the epilimnion and higher methane concentrations in the hypolimnion is very common, especially in small lakes e.g. (Bastviken et al. 2004, Borrel et al. 2011), which can vary seasonally like in Rotsee or in permanently stratified lakes elevated methane concentrations can persist over many decades. Continuing eutrophication of lakes and lack of recovery of eutrophic lakes will likely increase the number of lakes with anoxic methane-rich bottom waters in future (Jenny et al. 2016, Beaulieu et al. 2019).

We will tone down the generalized claims elsewhere in the manuscript in order to put our results into context without overstating generalizability.

L 295 adaptation to oligotrophic conditions – Lake Rotsee can not be considered oligotrophic

Answer: What we mean is adaptation to low methane availability, we will change the term accordingly.

L 298 transport of methane into the epilimnion provided and advantage for fast-growing MOB over slower competitors. This is not shown (at least in this manuscript) and should be removed.

Answer: We have provided evidence for the dynamic adaptation in (Mayr et al. 2020), but the reviewer is correct that the phrasing here is misleading and while our data here is in line with the previous investigation the conclusion cannot be made from the data in this paper. We will remove or rephrase this sentence in the revised manuscript.

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Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-482/bg-2019-482-AC4-supplement.pdf

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2019-482, 2020.