Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2019-482-RC3, 2020

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

Anonymous Referee #3

Received and published: 9 March 2020

Dear Authors,

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.

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-

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simplifications. Most importantly, it's unclear how the central conclusion, that well-adapted methanotrophs inhabit niches depending on methane availability (in hypo- and epilimnion), is reached. Wouldn't a match between in situ CH4 concentration and Km (not normalized per cell) be a stronger indication of such an adaptation?

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.

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.

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.

Please also consider specific comments below:

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

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

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

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 ∼20)

L 25 . . .90% of what?

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

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

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

L 58 . . . Lake Rotsee. . .

L 63 ex situ consider replacing with “laboratory incubations”

L 73 four or five campaigns?

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

L 91 how were Schott bottles sealed air-tight?

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

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

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 stoichiom-

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etry of MOX be used to support this speculation?

L 228 critical phase – critical for what?

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

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)

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

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

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.

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