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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 #2

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Mayr, Zimmermann and colleagues studied the methane oxidation kinetics in the epi- and hypolimnion of a eutrophic lake during autumn/winter lake overturn and report changing methane uptake kinetics. Likewise, changes in pmoC, A and B gene expression profiles were observed, indicating adjustments in the active methanotrophic community in dependence on methane availability. I see value in the presented work, but also limitations and open questions that would have to be clarified.

First of all, I request the authors to point out that they measured apparent methane oxidation kinetics. This should be clearly indicated throughout the manuscript.

In this study, all conclusions are derived from 1 - 2 l of water per sample, taken at

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four different time points of the epilimnion and hypolimnion, respectively. However, no replicate samples were taken per layer. I find this hardly acceptable. How representative are the findings of 1 l water for the whole stratification layer of a lake? Can the authors be sure that the differences they see are indeed related to the respective water bodies? Already the molecular analysis of a second filter, which is a methodological replicate that was included for one sample, shows some differences (Fig. 3). Thus, I find it largely impossible to relate differences in the active methanotrophic community to stratification, especially in December and January and especially for pmoB and C (Fig. 3; statements l. 266-267), without knowing anything about the biological variation within a layer. The repeated measurements over time provide some evidence, but do not solve this issue when it comes to minor differences between specific samples. Besides, the reason for including one experimental replicate (January, hypolimnion) or the conclusions derived from this sample are not mentioned anywhere.

How do the authors know that they had a representative sample from the hypolimnion at the last sampling date? There is no change in temperature evident and the decline in oxygen concentrations does not reach oxygen concentrations as low as at the earlier time points. Likewise, methane concentration in this sample is not as high as in the other samples from the hypolimnion. Thus, it appears that the sample was not taken at appropriate depth to be comparable with the others.

The conclusion about the specific enrichment of well-adapted methanotrophs with particular methane oxidation kinetics (l. 23) is conceivable, but should be drawn more carefully, because it remains unclear whether the observed kinetics are indeed adaptations of particular competitive methanotrophs under oligotrophic conditions, especially with regard to affinity. As only apparent parameters could be estimated, it remains unclear whether the methane monooxygenase of the respective organisms has indeed a higher affinity (lower Km) and is thus more competitive. It should be kept in mind in this context that a low apparent Km is not necessarily a specific adaptation to low methane concentrations, but can be the result of starvation (see Dunfield and Conrad

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2000, AEM).

To determine methane uptake kinetics (Fig. 2), the samples were apparently incubated at the temperatures measured in the epilimnion. However, samples from the hypolimnion encounter much lower temperatures in autumn. How does that affect comparability of the obtained results and conclusions about in situ conditions? This should be taken into account.

Related to this point: Considering that altered temperature and oxygen conditions were used to characterize the methane uptake kinetics in vitro, to what extent can the findings be translated to in situ conditions, considering that these factors can affect the measured Km and Vmax (see the study of Thottathil et al 2019, who report that increasing oxygen concentrations in lake water can reduce maximum methane oxidation rates; doi.org/10.1007/s10533-019-00552-x). Is it conceivable that Vmax in the hypolimnion is underestimated when determining oxidation rates at higher oxygen concentrations in vitro?

I find it very unfortunate that the identification of methanotrophs stops at the level "type Ia, type Ib, type II". The sequence information should provide more detailed information about the identity of the methanotrophs. At least for pmoA comprehensive datasets are available covering besides cultivated strains diverse groups of uncultivated taxa, so that more information could have been extracted here to identify conspicuous taxa.

Specific comments: l. 19 and 291: According to the data in table S1, the difference in Km is 20-fold, not 2 orders of magnitude

l. 25: Where in the presented work is it shown or discussed that 90% of the methane are removed? It appears that this is not a conclusion that is derived from the presented work.

l. 65: Metagenomic data were used as a basis for the metatranscriptomic data analysis, but are not presented independently; thus, I would not emphasize the metagenomics

approach here for the analysis of MOB assemblages.

l. 73: Five campaigns in autumn 2017 does not appear correct (three samplings in 2017 and one in 2018 according to the presented results)

l. 74-75: More measured parameters are given here than presented; harmonize.

l. 78: I do not find any helpful information about the radio isotope tracer technique in Steinle et al 2015. While the cited references enabled me to understand how methane oxidation rates were determined, they do not allow me to evaluate whether/how this procedure can be used to survey methane oxidation kinetics.

l. 80: How much methane was in this mixture?

l. 100-106: The authors describe different criteria that were used to identify and eliminate outliers here. Point four states that data points were removed in case less then two replicates remained. According to l. 93, duplicates were prepared. Does that mean that data for a specific methane concentration were lost each time one of the two replicates was identified as outlier? In this context, it is also unclear what Fig. 1 e-h shows. Do the presented data points represent individual measurements or are these mean values of the two replicates? Sometimes, I see two data points at a specific concentration, but sometimes I see only one point. Please clarify.

It would be valuable to know how many high-quality reads the authors generated per sample in the metagenomic and metatranscriptomic analysis, respectively.

l. 157: Why three samples in October; to my understanding there should be one from the epilimnion and one from the hypolimnion per point of time.

l. 163: Can a few words be added to describe this custom database? How was it set up? What type of data does it include?

l. 202-205, l. 295 and perhaps elsewhere: wording: do the authors refer to Km or a0 here when talking about affinity?

l. 204-207: I cannot follow argumentation here. And how do the authors explain that the organisms with the higher Vmax and lower Km disappear in January (Fig. 1h), although they should have a competitive advantage?

l. 241: A range of 1 – 40 is a bit outdated. Atmospheric methane oxidizers in soil are meanwhile known to have a0s values with up to 195 x 10-12 L/cell*h (Tveit et al) and in upland soils, estimates are ranging up to 800 x 10-12 L/cell*h (Kolb et al 2005; doi:10.1111/j.1462-2920.2005.00791.x)

l. 248-250: I find the 25% and 93% values critical here, because huge differences are observed at the individual time points. Especially the 93% value appears to be strongly affected by the huge difference observed in December.

l. 254: What do the authors mean with aggregate properties here? What aggregates do they refer to?

l. 256 – 258: It would be very valuable if the described findings could be seen in Figure 3.

l. 292-293: The transcription of genes does not relate to enzyme affinity or apparent Km values; thus, I cannot follow argumentation here.

l. 301: I do not necessarily agree to the term “entirely” in the context with “kinetic traits”; other environmental conditions may have affected the kinetic parameters. Please keep in mind that you can only measure apparent parameters, not enzyme kinetics.

l. 303-304: Please note that Methylocapsa gorgona does not possess a second pmoA gene for “high-affinity oxidation” despite being able to live on very low methane concentrations (Tveit et al).

References: The reference list does not allow to differentiate publications (e.g. Mayr et al 2019a, b, c). The reference list lacks information about the year the work has been published and the indices a,b,c.

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Figure 1: The axis showing oxygen concentrations should have a more increments.

Figure 2: explain error bars

Figure 3: The distinction by color is difficult in plots a1-c1; why not choosing more distinct colors / a broader range of colors per plot? This is of particular importance, as the relative abundances cannot be taken from Table S2 without additional calculations. It is currently impossible to identify type Ib or type II methanotrophs based on the color code and without further invest. However, as pointed out above, it would be even more valuable if more taxonomic information could be provided.

Table S3: Provide reference for Knief et al 2015.

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