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

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Reply to Reviewer 1: The manuscript on "Lake mixing regime selects methane oxidation kinetics of the methanotroph assemblage" by Mayr and Zimmermann et al, is a well written manuscript on the ecophysiology of methanotrophic bacteria.

Answer: Thank you for the positive assessment.

I only have the following remarks: Method section on the kinetics: - As you describe in detail how you eliminated outliers from the calculations I was wondering what the percentage of outliers was? From my (more marine) experience it is difficult to get

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good kinetic data, as many of my “Kinetic incubations” only gave erratic results:

Answer: The criteria eliminated 33% of the data. We prepared incubations in triplicates except for the first field campaign where we only prepared duplicates. Except for the first sampling date, we measured each incubation replicate twice. This resulted in 594 measurements, 72 single measurements and 261 measurement duplicates. We averaged “measurement replicates” resulting in 333 data points. From these 333 data points, we removed outliers as described. In total only 221 datapoints were considered in the final analysis (66% of all datapoints without measurement replicates). For the five individual outlier criteria (line 100 – 106), the percentages of detected outliers are:

(1) water fraction radioactivity that was outside 2σ from the average water fraction radioactivity of all replicates: 4%

(2) water fraction radioactivity that was not above 2σ from the background water fraction radioactivity: 4%

(3) less than two replicates after the removal of outliers: 3%

(4) resulting methane oxidation rate outside 2σ from the average methane oxidation rate of all replicates: 19%

(5) methane oxidation rate that was higher than the methane oxidation rate measured for the replicates with the highest methane concentration: 3%

So indeed, the outlier rate is fairly high and this is why we established a clear set of numeric criteria and a large number of measurements to obtain reliable data. The high percentage of outliers for criteria 4 is related to the fact that methane oxidation rates are associated with a higher error than individual measurements because they are computed from multiple individual measurements. The 2σ approach is one recommended approach for outlier detection (see e.g. Leys et al. 2013) We propose to show the elimination (in %) in the revised manuscript and to further clarify the procedure and its motivation (also with regards to a comment by reviewer 2).

Filtering the samples water on the 0.2 ym filters. How long did it take to filter 1 – 2 liters on such a filter?? To my experience this may last very long: : :. Thus, did you use any prefilters? And could the RNA composition change of this presumably longer time??

Answer: We did not record the exact duration but in our experience filtration takes typically less than 10 and always less than 15 minutes. In order to retrieve enough RNA for metatranscriptomics and at the same time reducing filtration time as much as possible we used large (142 mm) diameter filters. We did not use prefiltration since some filamentous methanotrophs can be quite large (length of up to >100 μm (Oswald et al. 2017). We took the samples with a niskin bottle and we connected the tubing for the filtration device directly to the niskin bottle. The sample was kept shaded (inside the niskin bottle) and did not experience major temperature changes in the cool autumn/winter weather of the sampling season for this experiment. Thus, changes from the in-situ transcriptional profile are expected to be minor. In-situ filtration might still be preferable, but the equipment for this was not available to us at the time of this work. We will note the approximate time and diameter of the filter to a revised manuscript and other detail to clarify the steps that were taken to insure rapid preservation of the transcriptome.

Result /Discussion - Figure 1: I think it is essential also to show the methane concentrations, in situ MOXrates and also the cell numbers in one figure, as the “environmental background information”.

Answer: Methane concentrations are already shown in Fig. 1 (numbers with arrows) we only measured at the sampling depths in this study. The cell numbers that we used in the calculations are shown in Supplementary Table 1, but we will include this data either in the main text or in a figure or table for a revised manuscript.

The second part of fig. 1 the kinetics should go in a separate figure, as this is more an experimental aspect.

Answer: We used this format deliberately to directly connect the core (kinetic) data

of the study with the graphs showing the environmental situation, which we hoped would make it easier for readers to understand how these are linked. We can certainly separate these parts into independent figures in the revision if the reviewer and editor think this would be a better solution.

- Comment on figure 2: to me another way of seeing the data is, that in the epilimnion Km is much higher in October, but is getting more and more similar to the hypolimnion.

Answer: There might be a misunderstanding here? The epilimnion data are shown in orange, and thus are actually lowest in October. (See Fig. legend)

Thus it is not a mixing of two compartments but more of an approximation of the epilimnetic traits to the hypolimnetic ones ??

Answer: Indeed, as we show in work now in press at Communications Biology (Mayr et al. 2020) (https://doi.org/10.1101/707836) there is more going on than simple mixing, i.e. complex dynamics of the community, and species mixed in from the hypolimnion typically do not establish in the mixed layer. This was also reflected in the transcriptomes obtained for this work. By the end of the mixing process however, almost the entire water mass was indeed mixed, and we observed only a small remnant of the hypolimnion. We did briefly outline our understanding of the dynamics (L289-300) but will seek to improve on this in the revision.

References:

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Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-482/bg-2019-482-AC1-supplement.pdf

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