Biogeosciences
Discussions

Interactive comment on "Anaerobic methane oxidation in an East African great lake (Lake Kivu)" *by* Fleur A. E. Roland et al.

J. Deutzmann (Referee)

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General comments:

In the manuscript "Anaerobic methane oxidation in an East African great lake (Lake Kivu)" the authors describe the vertical distribution of Methane, NOx, N2O, sulfate, sulfide, Mn and Fe (Fe data are not discussed) in Lake Kivu. In addition, the authors performed incubation experiments to determine aerobic as well as anaerobic methane oxidation rates at selected depths. The authors conclude aerobic and anaerobic methane oxidation takes place in Lake Kivu and that anaerobic methane oxidation rates might exceed aerobic methane oxidation rates during certain times in Lake Kivu. The manuscript reads well and the amount of geochemical data collected over several years is certainly impressive and worth publication. However, several aspects of the

study need to be clarified and evidence for AOM may not be as strong as indicated in this manuscript. Maybe data obtained by other studies can be used to substantiate the claims made by the authors. For example, molecular data on the presence and abundance of known anaerobic methane oxidizers would be helpful and maybe could be found elsewhere if no DNA samples were taken or no frozen material exists e.g. (Ärnceoğlu et al. 2015). I agree with referees 1 and 2 that a clear distinction to other publications by the authors is needed and that data shown in this study should be compared to other studies and the differences or agreements among the studies should be discussed in greater detail.

Specific comments: Interpretation of gradient / profile measurements: To me, the chemocline does not seem to be a very stable layer because the gradients are not very steep there might be convective mixing within the chemocline. Why do you emphasize the stable chemocline at -50 m? At what depth does the chemocline end? You suggest that oxygen might be sometimes penetrating through or into the chemocline (anoxia starting at 60 m water depth). How does that occur without convective mixing that would destroy the chemocline at that depth layer? Is oxygen transported 10 m purely by diffusive transport? How stable is the thermocline, if the temperature difference above and below the thermocline is only 1-2 degrees? Is the difference in density enough to generate a stable zonation? It seems that except for June 2011 and August 2014, all thermoclines are not very steep or at least show steps. This indicates that vertical mixing across the thermocline might take place at least in a spatially restricted zone of a few meters. Could that introduce oxygen into deeper layers? Convective mixing between 45 m and 55 m depth could also explain why the Feb. 2012 profiles do not indicate a decrease of sulfate or NOx below the determined oxycline at 45 m and why sulfide is only detectable below 55 m water depth. In addition, the N2O peak at 50 m might indicate oxygen at that depth as nitrification (requiring O2) as well as denitrification at low O2 concentrations was mentioned as a possible N2O source (Roland et al. Aquat Sci (2016)). Also, in June 2011 oxygen becomes undetectable only below 50 m (fig 2, magnified) while the dashed line indicating the beginning of the anoxic zone

in Fig 3 is drawn at about 47 m water depth. Which depth or oxygen concentration did you choose as the beginning of the "anoxic" zone? What was the detection limit of the oxygen sensor? Could oxygen be present at low concentrations deeper than your measurements indicate and still sustain aerobic methanotrophy? To claim AOM, there has to be unambiguous evidence that all the samples were completely oxygen free. This might not be the case with all the samples based on the profile data shown here. Do you have other data that could help to prove complete anoxia? Was there methanogenesis taking place in some samples? You seem to have enough data points on the concentrations of methane and other compounds to fit a nice curve through the data. It seems that below the thermocline, only diffusive transport occurs. Thus, you should be able to calculate formation or consumption rates of methane and the other reactants from the gradients you obtained and compare them to the rates you obtained in the incubations.

Overall, the shapes of the methane profiles do not show high rates of methane oxidation (which should be visible as curvature in the profiles when assuming diffusive flux through the water column). Methane oxidation should be visible in the profiles if the rates determined in the incubation experiments are comparable to the in situ rates. If AOM is the main methane sink, a clear convex shape of the methane profile should be visible at the depth where methane oxidation was measured in incubations! E.g. some consumption of methane should be visible in the August 2014 methane profile if AOM is consuming methane at considerable rates between -65 and -75 m. In other cases, the absence of oxygen (as shown in the oxygen profile) might not be complete, because the gradients of other species (sulfate, sulfide, N2O,…) do not indicate complete oxygen depletion (Feb. 2012 data) and, thus, methane oxidation might be oxygen dependent. I would assume that some sulfate or NOx is consumed if oxygen is absent and the stratification is stable.

How did you determine standard deviations? If I understand the methods correctly, you took 10 bottles, added molybdate to half of them, and had to kill activity to measure

each of the 5 time points. Thus, the measurements were performed without biological replicates! Could you show the time course data for the methane oxidation rate determinations (maybe in the supplements)? The lack of replicates in AOM incubations (that are very sensitive to disturbances and hard to carry out) paired with the inconsistencies in the results of some incubations (denitrification without nitrate consumption, very "spiky" depth profiles of rates) provide only weak evidence for AOM in Lake Kivu. Data on the organisms involved or higher resolution of sampling (to avoid the spiky depth profiles of the determined rates) would help to alleviate these shortcomings.

How were depth integrated methane oxidation rates calculated? If you don't have data for the upper 40 m of the water column, why do you integrate starting at the air water interface? How did you integrate e.g. the October 2012 data if there is only one sample actually showing significant methane oxidation? What depth layer did you assume to exhibit the measured methane oxidation activities at each point?

It has been shown that aerobic methanotrophs can use traces of oxygen to activate methane and then ferment methanol or use other electron acceptors for respiration (Kits et al. 2015). Could this play a role in Lake Kivu and explain some of your results?

How do you explain natural denitrification in 2013 if you don't observe nitrate consumption in samples from the same site at the same depth? Is nitrate consumption not necessary for denitrification? To observe denitrification of 0.5 μmol L-1 d-1 I would expect at least 1 μmol L-1 d-1 nitrate consumption.

How do you explain the impact of molybdate addition? In Figure 5, it looks as if the data are "hugging the axes". Most of the values are high in one and (close to) zero in the other treatment. Molybdate should not impact denitrification dependent AOM at all and metal dependent AOM should also not be impacted. However, you state that Feb. 2012, AOM coincides with the max NO3 consumption rate suggesting denitrification dependent AOM (line 278) but exactly in this treatment you find that molybdate is decreasing AOM rates. Can you provide evidence that your molybdate data are not just

random scatter around the true AOM rate?

How did you prevent to get an air bubble into the serum vials when closing them with a rubber stopper? In my experience that works in maybe 1 out of 10 cases and in the others there are a few microliter sized air bubbles.

Impact of Fe on AOM is not discussed but mentioned in the beginning!

Why do you show N2O data? N2O has not been shown to be connected to methane oxidation.

technical corrections: L196: "in the anoxic zone" instead of "at the oxic-anoxic interface"

L205: 0.2% of methane on AOM or in methane oxidation in general?

L209: The high sulfate reduction rate and the high AOM rate were not in the same sample (70 m vs. 75 m)? Then how is this "accompanied"? L256ff: add data from other lakes or freshwater sites in the discussion Figure 3: Oxyclines do not strictly represent the depth where oxygen became undetectable according to fig 2. Fig 4 is present twice Fig 4 (2): What information does this figure add to the MS? I would omit it. Fig 5: What crucial and necessary information does this figure add to the MS? I would omit it. Fig 6: No Mn or Fe data. Label the data points to show what date/depth the data come from.

I am looking forward to a fruitful scientific discussion!

References: ÄřnceoÄ§lu, Ö., M. Llirós, T. García-Armisen, S. A. Crowe, C. Michiels, F. Darchambeau, J. P. Descy and P. Servais (2015). "Distribution of bacteria and archaea in meromictic tropical lake kivu (africa)." Aquatic Microbial Ecology 74(3): 215-233. Kits, K. D., M. G. Klotz and L. Y. Stein (2015). "Methane oxidation coupled to nitrate reduction under hypoxia by the gammaproteobacterium methylomonas denitrificans, sp. Nov. Type strain fjg1." Environmental Microbiology 17(9): 3219-3232.

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