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Biogeosciences Discussions

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

Fleur A. E. Roland et al.

froland@doct.ulg.ac.be

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Comment 1: 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. (ÄËĞrnceoÄet 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.

Response 1: We agree with all the reviewers that we did not put enough our work in perspective with the literature. Modifications have been done, as developed hereafter: - Lines 50-52: "Comparatively, in situ AOM has been less clearly measured in freshwaters environments (e.g. in Lake Rotsee; Schubert et al., 2010), and is often considered as negligible compared to aerobic CH4 oxidation due to lower SO42- concentrations than in seawater (Rudd et al., 1974)." - Lines 54-59: "AOM coupled to NO3- reduction (NDMO) has been exclusively observed in laboratory environments (e.g. Raghoebarsing et al., 2006;Ettwig et al., 2010;Hu et al., 2011;Haroon et al., 2013;á NorÃři and Thamdrup, 2014), and its natural significance is still unknown. Also, AOM coupled to Fe and Mn reduction has been proposed to occur in some freshwater environments (e.g. in lakes Matano and Kinneret; Crowe et al., 2011;Sivan et al., 2011;á NorÃři et al., 2013) and marine sediments (Beal et al., 2009), but at our best knowledge, any in situ measurements has been presently reported in the literature." - Lines 284-298: "It was presently assumed that all the CH4 present in the water column of Lake Kivu was produced in anoxic waters, by acetoclastic and hydrogen reduction methanogenesis (Pasche et al., 2011). However, we demonstrate here that a part of CH4 present in oxic waters can come from aerobic CH4 production. Aerobic CH4 production has been recently studied (Bogard et al., 2014;Grossart et al., 2011;Tang et al., 2014;Tang et al., 2016), and different mechanisms have been proposed to explain it, among which a link with phytoplankton activity. This one produces methylated compounds (e.g. dimethylsulfoniopropionate (DMSP)), H2 or acetate, which could then be used by oxygen tolerant methanogenic bacteria to produce CH4 (Jarrell, 1985;Angel et

al., 2011;Grossart et al., 2011). Alternatively, phytoplankton could produce CH4 itself (Lenhart et al., 2016). During our study, the aerobic CH4 production peaks were always located at the basis of the zones of higher chla content. This location may be due to a spatial coupling between the presence of substrates produced by phytoplankton and the presence of oxygen tolerant methanogenic archaea. ÄřnceoÄ§lu et al. (2015) revealed the presence of methanogenic archaea in the anoxic waters and at the oxic-anoxic interface of Lake Kivu, among which Methanosarcinales. It has been shown by Angel et al. (2011) that some archaea belonging to Methanosarcinales are capable to perform methanogenesis under oxic conditions, at lower rates than in anoxic conditions." - Lines 301-303: "Pasche et al. (2011) reported lower aerobic and anaerobic CH4 oxidation rates than those we measured, but their CH4 oxidation measurements were only made during one field campaign, what is not really representative, since as demonstrated during this study, a great seasonal variability can be observed." - Lines 409-421: "In conclusion, we put in evidence a diversified CH4 cycle, with the occurrence of AOM and aerobic CH4 production, in the water column of a meromictic tropical lake. Presently, CH4 oxidation in Lake Kivu was superficially measured by Jannasch (1975), and was estimated on the base on mass balance and comparison to fluxes (Pasche et al., 2011;Borges et al., 2011). It was also supposed to occur based on pyrosequencing results (ÄřnceoÄ§lu et al., 2015;Zigah et al., 2015), which put in evidence the presence of sulfate-reducing bacteria and methanotrophic archaea in the water column and suggested that AOM could be coupled to SO42- reduction. Later, Morana et al. (2015a) made isotopic analysis which revealed the occurrence of aerobic and anaerobic CH4 oxidation in the water column of Lake Kivu, and concluded that aerobic CH4 oxidation was probably the main pathway of CH4 removal. Finally, important CH4 oxidation was also supposed to be responsible for small CH4 fluxes to the atmosphere observed throughout the year (Roland et al., 2016a). However, any of these studies directly put in evidence and measured aerobic and anaerobic oxidation rates and, nothing was known about seasonal and spatial variability of CH4 oxidation in Lake Kivu. Also, any study directly focused on the different potential electron acceptors

for AOM present in the water column, contrary to what we did during this study."

Comment 2: 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?

Response 2: Chemocline in Lake Kivu is not stable. The goal was not to emphasize the stability of the chemocline during our sampling. We noted "was relatively stable" and "started at $\sim$50m" to summarize and in order not to overload the manuscript. You

can see in Figure 2 that the chemoclines (specific conductivity and pH) were not all exactly located at 50 m depth, but were located near 50 m depth. However, we agree with the reviewer that it was not clear and it now reads: "At each date, the thermocline and chemocline (based on specific conductivity and pH) mirrored the oxycline and temperature at the oxic-anoxic interface averaged 23.5 $\pm$ 0.2°C (mean $\pm$ standard deviation)." In Lake Kivu, the stratification of the monimolimnion varies all along the year, depending on the season. During the dry season, oxycline, thermocline and chemocline are located deeper because of the cooling of the waters, what re-oxygenates deeper waters and allow the occurrence of aerobic processes deeper in the water column. Higher external temperatures and oxygen depletion by aerobic processes (according to which nitrification) allow the reestablishment of the stratification during the rainy season. See responses 8 and 12 concerning the oxygen in the incubations. When oxygen is present, the look of the decrease of methane concentrations in the incubations is usually different (see example graphs below). In June 2011, the water column was well anoxic from 47.5m. It seems that an error occurred when making the Figure 2. It is now corrected.

Comment 3: 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.

Response 3: See responses 8 and 12 concerning the oxygen in the incubations. Methanogenesis has been measured in oxic waters by the addition of picolinic acid, which inhibits aerobic methane oxidation (data now added to the manuscript). We

did not directly measure anaerobic methanogenesis (with an inhibitor of anaerobic methane oxidation) but methanogenesis was observed in some incubations where no oxidation was measured. Also data of microbial communities reported by ÄřnceoÄ§lu et al. 2015 suggest that it could occur in the anoxic water column.

Comment 4: 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.

Response 4: See responses 8 and 12 concerning the oxygen in the incubations. We must note that we do not consider AOM as the main methane sink in the water column of Lake Kivu. The main methane sink is aerobic oxidation. We do not understand the reviewer's comment concerning methane consumptions visible with methane vertical profiles. Methane concentrations decrease all along the vertical profiles. In August 2014, CH4 concentration at 75 m depth was equal to 689 μmol L-1, while at 65 m, it was equal to 358 μmol L-1.

Comment 5: 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!

Response 5: The standard deviations were calculated based on both T0. One linear regression of CH4 concentrations was calculated for the first T0, and one for the second T0. The standard deviations are the differences between these two regressions.

Comment 6: 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.

Response 6: There is too much data to show the time courses, even in the supplements. There are more than 60 graphs. We agree with the reviewer that it would have been better to have replicates for each point of the incubations but it was impossible to bring back so much bottles from Africa to Belgium. Without replicates, we usually brought back around 700 bottles. However, the T0 were taken in duplicates, what allow to test the sampling method, which is reliable. The AOM rates were thus calculated based on these two T0 (see response 5). We disagree with the reviewer that we only provide a weak evidence for AOM in Lake Kivu. At depths where an AOM rate is reported, the decrease of CH4 concentrations was flagrant, with both T0, and had never the "look" of aerobic oxidation (i.e. fast decrease at the beginning of the incubation and almost all CH4 consumed in 24h; see example graphs attached; fig.1). We only report AOM rates where no doubt is possible. The strong decreases of CH4 concentrations observed at anoxic depths can only be due to AOM.

Comment 7: 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?

Response 7: The oxidation is not present at all depths in the oxic compartment, since CH4 concentrations are not high enough. The aerobic oxidation rates are always located in a narrow zone near the oxic-anoxic interface (where CH4 is present). For example, in June 2011, the oxic-anoxic interface was located at 47.5 m, and aerobic oxidation was observed from 42.5 m depth. Oxidation was also measured at 35, 40 and 41.5 m depth, but was equal to zero. Aerobic oxidation was thus considered as 0 from the surface to 42 m depth. The same logic has been applied for all the campaigns. In October 2012, we did not observe only one oxidation rate. Methane oxidation was measured at 40, 50, 53, 55, 57.5, 60, 70 and 80 m depth, but was only observed at 53, 55 and 57.5 m depth. So, oxidation rates were considered as equal to zero from the surface to 52 m depth, and from 59 to 80 m depth. It seems that the paragraph was not clear. It now reads: "In October 2012, the CH4 oxidation rate (0.2 μmol L-1 d-1) observed in anoxic waters was negligible compared with the high rate of 10.2 $\pm$ 0.4 μmol L-1 d-1 observed in oxic waters."

Comment 8: 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?

Response 8: Different parameters were used to determine the anoxic depths: 1) Vertical profile of oxygen. 2) Vertical profile of conductivity: the conductivity increases in anoxic waters. 3) The increase of methane, ammonium and sulfide concentrations: in anoxic waters, methane concentrations sharply increase, and ammonium and sulfide appear. 4) The "look" of methane consumption in the incubations: when aerobic oxidation occurs, the decrease of methane concentrations is very fast, and almost all the methane is consumed in 24h, while when AOM occurs, methane consumption is slower. We only consider that the depths are anoxic when all these criteria are encountered.

Comment 9: 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.

Response 9: Denitrification has not been measured in the same samples than nitrate consumption. Nitrate consumption has been measured in methane incubations, while denitrification has been measured in specific incubations. We do not show nitrate consumption in denitrification incubations because this manuscript does not focus on denitrification, but at depths where denitrification was measured, nitrate consumption was also measured of course. Samples for methane and samples for denitrification do not come from the same Niskin bottle, what can explain the differences between the depths. An error maybe occurred during the sampling.

Comment 10: How do you explain the impact of molybdate addition?

Response 10: As widely described in the discussion, this aspect is difficult to explain. We cannot clearly explain why the addition of molybdate increases AOM rates in half of the measurements and decreases them in the other half. Further studies are required to really elucidate the electron acceptors for AOM in Lake Kivu. We think that we cannot always give all the responses with the same experiment. This study illustrates the complexity of AOM and/or of tropical environments. This study thus opens interesting perspectives.

Comment 11: 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?

Response 11: Figure 5 shows that the addition of molybdate decreases AOM rates in half of the measurements and increases them in the other half. In February 2012, AOM coincides with NO3- consumption peak, suggesting the occurrence of AOM coupled to denitrification, but as detailed in the discussion and in table 5, NO3- concentrations can only explain small AOM rates. So, our hypothesis is that AOM may firstly occur with NO3- as electron acceptor when available (since AOM coupled to denitrification is thermodynamically highly favorable) and as NO3- concentrations are low, AOM then occurs with SO42-. So the fact that AOM maybe occurs with NO3- does not prevent the occurrence of AOM coupled to SO42- reduction.

Comment 12: 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.

Response 12: We always overflow the bottles three times the bottle's volume during the filling. The bottles are overfilled, with a drop on the top of the bottle. We used two-leg butyl stoppers (Wheaton 224100-192) allowing the air and excess water to escape from the side of the septum. When the septum is promptly pressed, the excess water in the bottle is removed and the bottle is perfectly filled, without any air bubble. With these three precautions, we never had any external air bubbles in the bottles. .

Comment 13: Impact of Fe on AOM is not discussed but mentioned in the beginning!

Response 13: Fe data and discussion have been added to the manuscript.

Comment 14: Why do you show N2O data? N2O has not been shown to be connected to methane oxidation.

Response 14: N2O can be a product of denitrification, and showing N2O vertical profiles allow to identify the potential occurrence of denitrification, which can be linked to methane oxidation.

Comment 15: L196: “in the anoxic zone” instead of “at the oxic-anoxic interface”

Response 15: Corrected.

Comment 16: L205: 0.2% of methane on AOM or in methane oxidation in general?

Response 16: We are not sure to well understand the reviewer’s question. This sentence means that 0.2 % of the initial concentrations of methane are consumed per hour by AOM.

Comment 17: 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”?

Response 17: Bad wording. The sentence now reads: "A high SO42- consumption rate of 7.5 $\pm$ 0.0 μmol L-1 d-1 was observed near this high oxidation rate, at 70 m depth."

Comment 18: L256ff: add data from other lakes or freshwater sites in the discussion

Response 18: Table 4 shows data from other lakes. We think that this table and the discussion is enough detailed for this manuscript. However, see response 1 for the modifications related to the literature.

Comment 19: Figure 3: Oxyclines do not strictly represent the depth where oxygen became undetectable according to fig 2.

Response 19: The dashed lines well represent the depth at which the water column is completely anoxic. It seems that an error occurred when making Figure 2 but it is now corrected.

Comment 20: Fig 4 is present twice

Response 20: Corrected

Comment 21: Fig 4 (2): What information does this figure add to the MS? I would omit it.

Response 21: This figure is interesting because it allows to confirm the hypothesis made by Roland et al. (2016) according to which a deeper stratification favors aerobic methane oxidation, while a shallower stratification favors AOM. These results thus show seasonal variations of AOM in a tropical lake.

Comment 22: Fig 5: What crucial and necessary information does this figure add to the MS? I would omit it.

Response 22: This figure visually shows that half of the rates is higher with Mo and the other half is lower. It allows to clearly illustrate the results.

Comment 23: Fig 6: No Mn or Fe data. Label the data points to show what date/depth the data come from.

Response 23: No Mn or Fe data because we did not measure Fe or Mn consumption in the incubations. The data come from all field campaigns. This information has been added in the figure caption. We do not think it is required to label all the points, because it would overload the figure.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-300/bg-2016-300-AC3-supplement.pdf

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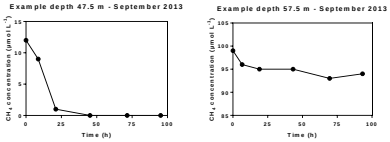

Fig. 1. Example graphs response 6