Biogeosciences

Discussions

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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: The manuscript by Roland et al. addresses the interesting topic of methane oxidation in the water column of a deep meromictic tropical lake. To date, little is known about methane oxidation in such systems. The authors show depth profiles of potential electron acceptors for the anaerobic oxidation of methane and performed laboratory incubation studies with water samples from different water depths. This study is within the scope of biogeosciences and should find broad interest among its readers. However, I am questioning the innovative approach and the scientific significance of this work, i.e. its contribution to improve our current understanding of the methane cycle in Lake Kivu and AOM in general. In particular, I recommend that the authors more clearly discuss how the findings presented here advance the results reported in an earlier study by Roland et al. (2016; Aquat. Sci.). With the exception of

the first three lines (stating that sulfate could be the main electron acceptor for AOM in this system), all conclusions drawn in the last section are essentially the same as in their previous study.

Response 1: The study of Roland et al. (2016) focused on the seasonal variability of CH4 and N2O fluxes in the epilimnion of Lake Kivu. During that study, CH4 oxidation was not measured, and was just proposed as a potential explanation for seasonal variability of CH4 fluxes to the atmosphere. No process rates were measured, and the paper only focused on the oxic compartment of Lake Kivu. The occurrence of AOM and the relative importance of aerobic and anaerobic CH4 oxidation according to the season were just two hypothesis. Here, we clearly measured aerobic and anaerobic CH4 oxidation, and we calculated the rates of these processes during different seasons. So during this study, we demonstrate that the hypothesis of Roland et al. (2016), i.e. occurrence of AOM in the water column of Lake Kivu and seasonal variability of the relative importance of aerobic and anaerobic CH4 oxidation, were correct. So, both studies are completely different. However, we agree with the reviewer that we did not highlight enough links that can be made between our study and previous studies, and we thus added a paragraph in the conclusion, which reads: "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: In my opinion, the authors should perform a more thorough literature study and put their work more in perspective of what has been done so far in the field of methane (and particularly in AOM) research.
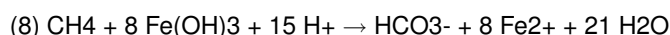
Response 2: We think that the introduction clearly makes the state of the art, by firstly introducing the CH4 cycle, then focusing on AOM and finally on Lake Kivu. We added a small paragraph, which reads: "AOM coupled to NO3- reduction (NDMO) has been exclusively observed in laboratory environments (e.g. 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." In our opinion, this introduction is precise enough and does not require to be extended, since the aim of this study is not to make a review of the literature. However, we agree that our discussion and conclusion did not integrate enough previous studies on Lake Kivu (see response 1).

Comment 3: Furthermore, not all aspects discussed in the abstract are presented in the manuscript: The potential contribution of Fe oxides for AOM and the bacterial abundance are not given.

Response 3: The bacterial abundance was showed in a first version of the manuscript, but we finally decided not to show these data because it did not give any interesting information. We forgot to remove this information from the abstract. It is now corrected. Since dissolved Fe concentrations were very low (<2 μmol L-1) during all the vertical profiles (information reported in the results section), we firstly decided not to show Fe

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results, since Fe cycling in the water column of Lake Kivu seems to be not well developed. Moreover, when dissolved Fe concentrations were measured in the incubations in August 2014 (in the same samples than Mn concentrations; Fig. 4), any production rate was observed. But we agree with the reviewer that all these aspects were not clearly reported in the manuscript. We now present Fe concentrations in Fig. 3, and we added information in the results and discussions sections, which read: - Results: "While particulate Fe concentrations were up to 15 μmol L-1 in oxic waters, in September 2013, dissolved Fe concentrations were very low (less than 2.5 μmol L-1 all along the vertical profiles, during the three field campaigns). On the contrary, particulate Mn concentrations were low (less than 2 μmol L-1), with a maximum concentration peak located just above the oxic-anoxic interface, for the three campaigns, and dissolved Mn concentrations increased with depth, until maximum concentrations of 10 μmol L-1 in anoxic waters." - Discussion: "Also, it seems that Fe cycling in the water column of Lake Kivu is not well developed, since while particulate Fe concentrations were up to 15 μmol L-1, dissolved Fe concentrations were very low (Fig. 3), suggesting that Fe reduction is a limited process. While particulate Fe concentrations were high enough to explain up to 100% of the small AOM rates observed (Table 5), dissolved Fe concentrations can only explain up to 24% of the small AOM rates, and only 1-5 % of higher AOM rates, according to Eq. (8) (Beal et al., 2009):

(8) CH4 + 8 Fe(OH)3 + 15 H+ → HCO3- + 8 Fe2+ + 21 H2O

Moreover, in August 2014, no Fe2+ production rate was observed in the incubations, without and with molybdate added, which tends to support the low occurrence of Fe reduction in the water column of Lake Kivu. It is thus likely that Fe does not play a significant role for AOM."

Comment 4: I am also missing a better discussion about why addition of molybdate resulted in increased methane oxidation rates in half of the incubation bottles. To me, their current explanations are not satisfying enough. The enhanced rates are a quite surprising finding and I think it nicely shows that our understanding of AOM is still very

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limited.

Response 4: As mentioned line 382, the increases of AOM rates with molybdate added are difficult to explain. We cannot clearly give a strong and definitive explanation of what happened, and we thus give hypotheses and show our intellectual approach. So, the discussion firstly rejects the possibility of an experimental error, which is already a good point. Then, we give the hypothesis with the competitive relationships, which seems to be unlikely according to us, due to the low concentrations of the other electron acceptors. But with the dataset presented in this manuscript, we cannot definitively reject this hypothesis. These results thus reflect the complexity of the lake and of AOM and require further studies, since all the answers cannot be always given with a unique experiment. But we agree with the reviewer that these information were not clearly written in the manuscript, and we thus added them at the end of this section, which reads: "However, with the present dataset, this hypothesis cannot be definitively ruled out, and further studies are required to really understand the influence of molybdate on the bacterial communities. The measurement of the bacterial communities' evolution in the incubations, without and with molybdate added, would be really interesting."

Comment 5: Ln 18: Although mentioned in the abstract, iron as a potential electron acceptor for AOM is not discussed in this study.

Response 5: See response 3

Comment 6: Lns 19-20: The authors state that bacterial abundance all along the vertical profiles was also determined during three field campaigns. However, I cannot see where they provide these results nor where they are being discussed. Please clarify.

Response 6: See response 3.

Comment 7: Ln 31: in "the" relative importance?

Response 7: Corrected.

Comment 8: Ln 32: in "the" dry season?

Response 8: Corrected.

Comment 9: Lns 34-49: The introduction section is rather short and could be extended. In particular, I suggest performing a more thorough literature study regarding anaerobic oxidation of methane. Most key papers of recent progress in the field of AOM are missing, in particular with respect to alternative electron acceptors such as nitrate, iron and manganese (see, for example, Raghoebarsing et al., 2006; Ettwig et al., 2008; Beal et al, 2009; Knittel and Boetius, 2009; Crowe et al., 2011; Sivan et al., 2011; Segarra et al., 2013; Riedinger et al., 2014; Egger et al., 2015; McGlynn et al., 2015; Wegener et al., 2015; Scheller et al., 2016). What do we know about the importance of AOM coupled to different electron acceptors in marine, brackish and freshwater environments? What is the current knowledge of water column AOM?

Response 9: See response 2.

Comment 10: Lns 60-61: Although stated here, the potential link to Fe reduction is not discussed further in the manuscript.

Response 10: See response 3.

Comment 11: Lns 112-113: Was the filtration done under oxic conditions? If so, could oxidation artefacts explain the presumably low (and not reported) concentrations of dissolved Fe2+?

Response 11: The filtration was not done under strict anoxic conditions. However, water was directly taken from the Niskin bottle by means of syringes, and filtration was directly and rapidly done in a closed filtration set. The filters and filtrates were then rapidly preserved in HNO3- and kept frozen until analyses. The time required for the filtration was lower than the time required for Fe oxidation, since the filtrations were very fast. Moreover, Fe and Mn concentrations were measured on the same samples, and dissolved Mn concentrations were higher. Our team, who works on Lake Kivu for many years, has always reported low Fe and Mn concentrations in the water column of

Lake Kivu.

Comment 12: Ln 125: What about Fe2+ production rates?

Response 12: Any Fe2+ production rate has been observed. This information is now reported in the manuscript.

Comment 13: Ln 128: Change "standards deviations" to "standard deviations", i.e. remove "s" after standard.

Response 13: Corrected.

Comment 14: Ln 137: Just a suggestions: I would rewrite this equation to R0 = Rm * O2a/O2r. In my opinion, this is more intuitive. However, I leave it up to the authors to decide.

Response 14: We thank the reviewer for his/her suggestion; text was changed accordingly.

Comment 15: Ln 184: Is the standard deviation really 0.5 μmol/L? With mean CH4 concentrations of 0.3 μmol/L this would point towards negative values.

Response 15: The standard deviation is due to the fact that the data is not normally distributed.

Comment 16: Ln 192: Data for NH4+ is not shown.

Response 16: Sentence removed.

Comment 17: Lns 194-195: Without looking at Fig. 3, it is not clear that the 153 umol/L refer to the mean sulfate concentration in the oxic waters and the 42 umol/L to the mean sulfide concentration in the anoxic water column. Please rephrase. I also recommend removing the statement of the mean concentration of sulfide and, as stated in lns 196-197, describing the increase in the profile instead.

Response 17: The sentence has been rephrased and now reads: "The mean of SO42-

concentrations in oxic waters (from 0 to 50 m depth) was 153 $\pm$ 21 μmol L-1, while the mean of H2S concentrations in anoxic waters (at 70 m depth) was 42 $\pm$ 25 μmol L-1."

Comment 18: Ln 198: Could these low concentrations of dissolved Fe2+ be due to oxidation during filtration, i.e. precipitation of Fe oxides? Note that any Fe2+ produced during Fe-AOM would also be quickly scavenged by the high concentrations of sulfide in the deeper water column. Thus, Fe-AOM could be occurring without a measurable accumulation of dissolved Fe2+. It is therefore wise to look at the profile of particulate Fe (which is not shown here, but, according the method section, should have been measured) in order to evaluate if Fe oxides are still present in the anoxic and/or sulfidic waters and thus could be used for AOM. Also, if Fe oxides are present (and not due to an oxidation artefact), they could stimulate sulfate-driven AOM through re-oxidation of sulfide to sulfate (see Sivan et al., 2014). Please discuss.

Response 18: See response 3 and 11. We think that Fe cycling is not well developed in the water column of Lake Kivu (see response 3). Also, as particulate Fe concentrations are very low compared to SO42- concentrations (for example, in May 2013, at 60 m depth, particulate Fe concentrations were 5 μmol L-1, while SO42- concentrations were 150 μmol L-1), we think that the potential re-oxidation of H2S by particulate Fe cannot significantly influences the AOM rates (SO42- is not limiting at all).

Comment 19: Lns 227: I suggest reporting the lower rates with one significant digit (instead of "0"). The same holds for Tables 1 and 2.

Response 19: Corrected.

Comment 20: Lns 229-231: What about Fe?

Response 20: See response 3.

Comment 21: Lns 239-244: I cannot fully understand the author's reasoning in these lines. In particular, I strongly question the use of Fig. 5. The depth-integrated oxic rates are obviously dependent on the oxygenated layer depth, as they are integrated over

the oxic water depth. Thus, if the oxic water depth increases, the depth-integrated rate also increases (the area for oxic methane oxidation increases). However, it does not directly imply that the rates themselves also increase. In fact, oxic methane oxidation rates for October 2012 are lower than for September 2013 and June 2011, although the oxic layer depth for October was deeper compared to these months (see Fig. 4). Hence, the question remains whether the rates in the rainy season are really higher or whether it is just a question of integrated CH4 removal as suggested by the authors in a previous publication (Roland et al., 2016; Aquat. Sci.).

Response 21: We understand the point of view of the reviewer. However, even if the integration is made on a wider oxic compartment, it does not mean that it influences the integration rates. Indeed, 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). So the integrated oxic CH4 oxidation rates will not be necessarily higher in a wider oxic compartment. For example, in June 2011, the oxic-anoxic interface was located at 47.5 m, and aerobic oxidation was observed from 43 m depth, while in August 2014, the oxic-anoxic interface was located at 60 m depth and aerobic oxidation was only observed from 54 m depth. On average, the aerobic CH4 oxidation rates during the dry season were observed on a depth interval of 8 m, while it was observed on an interval of 9.5 m during the rainy season.

Comment 22: Ln 252: Please change "contribute to differences" to "contribute to the differences".

Response 22: Corrected.

Comment 23: Lns 262-264: Thermodynamically, Fe oxides are favorable electron acceptors for AOM, yes. However, recent work suggests that the kinetics of this reaction are likely very slow (see, for example, Beal et al., 2009; Sivan et al., 2011; Segarra et al., 2013; Egger et al., 2015). The reference here refers to a non per-reviewed paper

that has not been revised for publication for the journal biogeosciences. Please refer to key publications (see also comment earlier).

Response 23: It is true that the reference used here (Sturm et al. 2016) has not been yet peer-reviewed, but even after revision the rates reported should not change, since the method of measurement used during their study is a method widely used and accepted in literature. This paper is just cited to compare rates between Lake Matano and Lake Kivu, two meromictic tropical lakes. Although AOM coupled to Fe reduction is a slow process, it is highly more favorable than AOM coupled to SO42- reduction (-572 and -17 kJ/mol CH4, respectively). So, if Fe concentrations are high, like in Lake Matano, it is likely that AOM is mostly coupled to Fe reduction. This aspect, combined to higher CH4 concentrations, can thus explain higher rates observed in Lake Matano.

Comment 24: Lns 274-275: This method may underestimate actual sulfate reduction rates due to potential re-oxidation of sulfide to sulfate. Please discuss.

Response 24: We suppose that the reviewer wanted to say that this method may overestimate SO42- reduction rates, since SO42- concentrations may be higher due to re-oxidation of HS- to SO42-. However, SO42- concentrations measured in the incubations were not higher than those measured for the vertical profiles, except for the depth where the higher peak of SO42- consumption was measured (at 70 m depth). An error probably occurred with the first measurement, and a new measurement was made, but the wrong value was reported here, by mistake. The new value of SO42- consumption for the depth 70 m is now reported in Fig. 4 and Table 2. However, as explained Lines 308-310, SO42- consumption rates may be underestimated due to the low precision of the method used.

Comment 25: Lns 292-293: The statement that nitrate is not an important electron acceptor for AOM appears to be in direct contradiction to ln 295, where the authors suggest that the low AOM rates can be fully explained by NOx concentrations. Response 25: It is not a contradiction. NO3- is not an important electron acceptor for

AOM, since it can only explain very small rates (from 0.2 to 0.5 μmol L-1 d-1). For higher rates, NO3- concentrations are clearly too low. And this without tending into account that NO3- can also be used for heterotrophic denitrification, which is a more favorable process. So, when tending into account these two aspects, we can clearly state that NO3- is not an important electron acceptor for AOM in Lake Kivu.

Comment 26: Ln 322: "estimated by a parallel study"

Response 26: Corrected

Comment 27: Ln 324: "Considering the very high"

Response 27: Corrected.

Comment 28: Lns 333-334: Oxygen supply could also induce Mn- and Fe-oxides. Please add.

Response 28: Added.

Comment 29: Lns 335-344: How can a potential increase in sulfate stimulate SO4-AOM if molybdate inhibits sulfate reduction?

Response 29: Corrected.

Comment 30: Ln 354: Start a new section "5. Conclusions"

Response 30: Added.

Comment 31: Lns 357-365: In my opinion, these are the same conclusions as presented in another work by the authors (Roland et al., 2016; Aquat. Sci.). Thus, the only new result (based on weak evidence) is that sulfate may be the main electron acceptor for AOM in Lake Kiwu.

Response 31: See response 1.

Comment 32: The numbering of the figures is not correct (Fig. 4 is used twice). Please revise.

Response 32: Corrected.

Comment 33: Fig. 2: What about NH4+, particulate Fe and dissolved Fe?

Response 33: Showing NH4+ vertical profiles has no sense in the frame of this study. We firstly decided to not show the vertical profiles of particulate and dissolved Fe because Fe2+ production rates measured in August 2014 were negligible and dissolved Fe concentrations were very low (< 2 μmol L-1), strongly suggesting a limited Fe cycle in the water column of Lake Kivu, and thus a negligible importance for AOM. We now present these results.

Comment 34: Fig. 5: What does this figure add (see comment above)?

Response 34: Now Fig. 6. This figure is important, since it shows that the importance of aerobic CH4 oxidation rates tends to be dependent of the structure of the water column. This figure thus shows the seasonal variations of the importance of aerobic CH4 oxidation in the water column of Lake Kivu. We strongly think that it is a very interesting result. It is a clear evidence of the hypothesis of Roland et al. (2016).

Comment 35: Fig. 6: Perhaps add information (such as depth, months etc.) about the samples?

Response 35: Now Fig. 7. The caption has been completed and now reads: "Comparison between AOM rates (μmol L-1 d-1) measured without and with molybdate (Mo) added, during all field campaigns."

Comment 36: Fig. 7: What are the points referring to, i.e. to which depth, months etc.?

Response 36: Now Fig. 8. The points refer to anoxic depths where: (a) AOM and SO42- consumption co-occurred, (b) AOM and NO3- consumption co-occurred and (c) AOM and natural denitrification co-occurred, during all field campaigns. Note that because of log scales, rates equal to zero are not represented. The caption has been completed and now reads: "Comparison between measured and calculated AOM rates (μmol L-1 d-1) based on (a) SO42- consumption rates, (b) NO3- consumption

rates and (c) Natural denitrification, for all field campaigns. Note the log scales."

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-300/bg-2016-300-AC2-
supplement.pdf

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