

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

Anonymous Referee #2

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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),

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all conclusions drawn in the last section are essentially the same as in their previous study. 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. 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. 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 limited.

Specific comments:

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

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.

Ln 31: in “the” relative importance?

Ln 32: in “the” dry season?

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

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different electron acceptors in marine, brackish and freshwater environments? What is the current knowledge of water column AOM?

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

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⁺?

Ln 125: What about Fe²⁺ production rates?

Ln 128: Change “standards deviations” to “standard deviations”, i.e. remove “s” after standard.

Ln 137: Just a suggestions: I would rewrite this equation to $R_0 = R_m * O_{2a}/O_{2r}$. In my opinion, this is more intuitive. However, I leave it up to the authors to decide.

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

Ln 192: Data for NH₄⁺ is not shown.

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. 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

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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.

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

Lns 229-231: What about Fe?

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.*).

Ln 252: Please change “contribute to differences” to “contribute to the differences”.

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).

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

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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 NO_x concentrations.

Ln 322: "estimated by a parallel study"

Ln 324: "Considering the very high"

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

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

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

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.

Figures:

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

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

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

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

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

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