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

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Received and published: 18 November 2016

Comment 1: In general I find this manuscript OK but a little bit superficial. This also concerns the English writing which is not bad but could benefit from a native speaker. This lake is very complicated in respect to biogeochemistry due to its very high gas content in the deeper layers and this is known from a lot of publications that tried to understand e.g. the methane cycle. I think there was a very weak literature study done before writing this ms. e.g.: “In freshwaters environments, AOM has been less studied and is often considered as negligible compared to aerobic CH4 oxidation 45 due to lower SO42- concentrations than in seawater (Rudd et al., 1974). However, other potential electron acceptors for AOM, such as nitrate (NO3-), iron (Fe) and manganese (Mn) (Borrel et al., 2011; Cui et al., 2015): : :: This is not true at all since we see now many more publications in this direction (see below for examples). It would be really good if the authors would read them and include them in their arguing. Especially one about methane oxidation in Lake Kivu or the one on closely located Lake Tanganjika should be interesting and included. It would be good to put your work a little bit more in perspective of what has been done before.

Response 1: We do not consider that AOM in freshwaters environments is not well studied (in this case, we would have said “understudied” or “poorly studied”), but it is a fact that AOM in the water column of freshwaters environments is less studied than in marine environments. Numerous studies suggested below by the reviewer, although they are interesting, are not direct, in situ measurements of AOM in freshwaters, contrary to what we made during this study. For examples, the study of Durisch-Kaiser et al. (2011) did not directly measure AOM in Lake Tanganyika, but suggested that AOM could occur based on a model. Also, the study of Oswald et al. (2016) measured potential CH4 oxidation rates, not in situ rates. However, we agree with the reviewer 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; a Norfi 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; a Norfi 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. dimethylsulphoniopropionate (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. ÄrnceoÅ§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 (ÄrnceoÅ§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.”

We also added results focusing on aerobic CH4 production in the water column, and discuss these results in parallel to pigment results.

Comment 2: I also think that publications nowadays dealing with the understanding of methane oxidation whether it be aerobic or anaerobic are also looking at the organisms involved by all kinds of molecular tools and I find it a little bit sad that here only the geochemistry is looked at.

Response 2: The determination of the bacterial and archaeal communities has been previously done in Lake Kivu (Pasche et al. 2009; Llirós et al. 2010; Llirós et al. 2012; Inceoglu et al. 2015a, b). Moreover, some previous studies (Inceoglu et al. 2015a, b) were made in parallel to this study, during the same field campaigns (February 2012 and October 2012), by a companion team. However, we agree with the reviewer that we did not insist enough on previous studies made in Lake Kivu and in other environments, and we corrected that, as described in response 1.

Comment 3: The sampling strategy is with taking samples at a 5 m (or 2.5 m at best) sampling resolution not of what I think should be expected in a lake where geochemical processes are running on much shorter distances. Of course this is difficult with a Niskin bottle alone and other techniques should have been probably be used.

Response 3: We strongly think that the sampling resolution is precise enough. The sampling depths were determined according to the depth of the oxycline and chemocline (so according to the stratification), with a higher resolution (generally every 2.5 m)
in the interface oxic-anoxic. If we look at oxidation rates measured, for each campaign, we can see that we usually measured low rates followed by high peaks, and ending with low rates. So, we well determined the active zones, for each campaign. We do not think that measuring rates every 1 m in these zones would have brought important additional information.

Comment 4: If you read through the result section 3.2 you can really not see any trends it is just a description of how different the lake was during different seasons, month and years. So I really wonder how representative those measurements are.

Response 4: We agree with the reviewer that this section is descriptive, but it is a result section. This section highlights the different points discussed later in the discussion section. It is structured as followed: results from dry season, results from rainy season and results with molybdate added. Moreover, paragraphs with results from rainy season and dry season both start with a sentence which "summarizes" the trend observed (i.e. "The dry season was characterized by higher maximum CH4 oxidation rates in oxic waters compared to anoxic waters." and "During the rainy season, maximum CH4 oxidation rates in anoxic waters were higher than in oxic waters."). We added a sentence for the last paragraph, which reads: "When molybdate was added, different profiles were observed."

Comment 5: In general methane oxidation rates are also really high compared to other lakes Table 4 and to former measurements from Lake Kivu, are they correct? This is also questioned when reading: "For example, the maximum aerobic CH4 oxidation rate of 27 ± 2 µmol L-1 d-1 observed 235 at 55 m depth in August 2014 occurred at CH4 concentrations of 42 ± 2 µmol L-1." This is unreasonable that the methane is turned over in two days. I think methane oxidation rates in this ms. are much too high.

Response 5: As described in the M&M, rates reported here are maximum rates. They were calculated based on the maximum slope observed during the incubations. It is not possible to show the incubation profiles for all depths measured, for all campaigns (there are more than 60 graphs; Table 1 is a summary), but the decrease of CH4 concentrations through time was not linear for all depths and all campaigns. Sometimes, there was a very strong and fast decrease of CH4 concentrations at the beginning of the incubation, followed by a plateau, probably due to substrates limitation. This type of profile was particularly observed in oxic waters. So, in the example given in the manuscript and cited above by the reviewer, 68% of the initial CH4 present were consumed after ~24h (Table 1), and almost no consumption was observed after ~24h (see the example graph attached (Fig.1)). The rate was calculated based on the slope from time 0 to time ~24h (25h exactly). This means that the oxic bacterial community is capable to rapidly consume the CH4 available, and is limited by CH4 concentrations. We do not see how overestimation of rates could be possible with the method we used. It is a fact, and clearly visible, that ~70% of the initial CH4 was consumed after 24h, which gives a maximum CH4 oxidation rate of ~27 µmol L-1 d-1 without molybdate added (slope of ~1130). The same logic have been applied for all the rates measured. The method we used have been described, tested and approved by many other studies, including the study of Bastviken et al. (2002).

Comment 6: What can we learn from Table 5? It is just the measured concentration of the electron acceptors that might be used during CH4 oxidation but this does not say that this is also the case. This gives no information on what is really happening in the Lake Kivu and hence not very useful.

Response 6: Table 5 allows to show the potentiality of our different hypotheses. For example, it shows that NOx concentrations are generally too small to be responsible for most of AOM rates observed. So, this table allows to reject NOx and particulate Mn as main electron acceptors for AOM in Lake Kivu, and shows that SO42- concentrations are clearly sufficient to explain almost all AOM rates observed. We think that we cannot emit hypotheses on the potential electron acceptors for AOM in Lake Kivu without showing the concentrations of these elements actually present at the same depths (and so the feasibility of the processes).
Comment 7: “SO42- consumption rates were calculated from the change in time of SO42- concentrations measured with the nephelometric method, which might not be precise enough, since the detection limit was 52 µmol L-1.” What does this mean? Does this help to explain methane oxidation rates?

Response 7: We agree with the reviewer that this sentence was not clear. This means that we probably missed or underestimated SO42- consumption rates in our incubations due to the method used, since the detection limit is high. So, at some depths, SO42- consumption maybe occurred (and could thus be potentially linked to AOM), but is reported here as zero because of the detection limit. We added this information to the paragraph, which now reads (Lines 313-318): “SO42- consumption rates were calculated from the change in time of SO42- concentrations measured with the nephelometric method, which might not be precise enough, since the detection limit was 52 µmol L-1. So, due to this high detection limit, we might miss or underestimate some SO42- consumption rates, which could potentially be linked to AOM. Vertical profiles of SO42- concentrations, measured by ion chromatography (detection limit of 0.5 µmol L-1), show that SO42- is present in enough quantity to explain AOM rates observed, for all campaigns (Table 5).”

Comment 8: The discussion between line 325 and 354 leads to nowhere. There is no real explanation why molybdate introduction would in one case enhance methane oxidation and on one hand reduce it. The competition explanation is pulled down also immediately after bringing it up. So what happens with the molybdate?

Response 8: As mentioned in 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 9: There are several wrong participles used which would also make a native English speaker necessary, e.g., “Samples for sulfide (HS-) concentrations were collected in 50 ml plastic vials, after being filtered through a 0.22 µm syringe filter.” Should read filtered through (several times: : :)

Response 9: We thank the reviewer for having highlighted these errors, which are now corrected. The manuscript was read by a native speaker before being submitted, but he probably missed some mistakes.

Comment 10: In general I do not see also after reading Morana et al. 2015 what is so new about this manuscript. It describes methane concentrations and compared them to possible electron acceptors that might oxidize it. Since sulfate is occurring in sufficient amount it is the most likely electron acceptor but this is not proved (I do not judge the incubations due to their very different results in different years and seasons to be of any proof) and also mentioned by Pasche et al. 2011. The only new statement is the difference between aerobic and anaerobic oxidation in dry and rain seasons but if this justifies publication is not on me to judge.

Response 10: We do not only describe CH4 concentrations. We made incubations to measure CH4 oxidation rates, what is completely different from only give CH4 concentrations profiles. Until now, AOM was only supposed to occur in the water column of Lake Kivu, based on PLFA analyses (e.g. Morana et al. 2015a; Zigah et al. 2015),
isotopic analyses (Morana et al. 2015a,b; Zigah et al. 2015) or pyrosequencing analyses (e.g. Inceoglu et al. 2015a,b), but was not quantified. It is the first study to clearly put in evidence and measure AOM in the water column of this tropical lake, and to quantify the oxidation rates. There are two studies of Morana et al. (2015). The study of Morana et al. (2015a) focused on the importance of aerobic methanotrophy for the foodweb in Lake Kivu. The only link with AOM is their PLFA analyses, whose results showed the presence of a sulfate-reducing bacteria community in O2-depleted waters. They thus suggested that AOM could occur with SO42- as electron acceptors but noted that further investigations were required. They also supposed that aerobic methanotrophy is the main pathway of CH4 oxidation in Lake Kivu, and hypothesized that its relative importance could change according to the structure of the water column, what we demonstrate here. The study of Morana et al. (2015b) assessed the general biogeochemistry of Lake Kivu, and CH4 oxidation is only mentioned as being potentially important for the ecology of Lake Kivu. So, we do not understand the comment of the reviewer, since the two previous studies of Morana et al. (2015) are completely different from this manuscript. Moreover, as said by the reviewer, it is also the first study to show the influence of the season on the relative importance of aerobic and anaerobic CH4 oxidation.


Response 11: We thank the reviewer for these suggestions. Some of them, that we judged pertinent for this study, have been added to the manuscript.

**Fig. 1.** Graph response 5