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Interactive comment on "Methanotrophy within the water column of a large meromictic tropical lake (Lake Kivu, East Africa)" *by* C. Morana et al.

Anonymous Referee #2

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General comment

This manuscript examines methane oxidation and microbial communities involved in this process by using the natural abundance stable carbon isotopic compositions of methane, particulate organic carbon and phospholipid fatty acids. They also used 13C-labelled methane in lake-water incubations to evaluate methanotrophic bacterial production and methanotrophic bacterial growth efficiency. The manuscript is within the scope of Biogeosciences and would considerably advance our knowledge of methane oxidation dynamics in tropical lakes in general. However, the manuscript could be considerably improved by a better motivation of the need for this particular study in the context of previous studies in the lake, and a more careful and extended/broader discussion of the dataset. Some of the conclusions (eg. that type I methanotrophs oxidize

methane within the oxycline) appear not to be supported by the dataset and should be carefully considered during revision of the manuscript. The manuscript should also be edited for type-o errors and grammar.

Specific comments

Pg 15664

Line 5: change to 'natural abundance stable carbon'. . ...

Line 7: List the specific inorganic and organic carbon pools (e.g. POC, CH4, PLFA)

Line 13: Change to 'Our data revealed'. . .. .

Line 22: what about type X? Did you find evidence for or against the involvement of type X MOB in aerobic methane oxidation?

Introduction

The introduction is too pedestrian. I find no motivation for this particular study in the introduction, neither are new, more recent general and lake-specific knowledge introduced and/or discussed. For instance, the specific types of methanotrophs and their identifying characteristics are not explored at all and the AOM coupled to sulfate reduction is presented as though it was the ONLY process of AOM. Recent studies show ANME are able to oxidize methane without the SRB partners. This should be noted in the introduction. Also, the authors should thoroughly discuss previous methane-based studies in Lake Kivu. They should then discuss gaps in research that their study sought to address.

Pg 15666

Line 14 – 'bur' should be 'but'

Pg 15667

Line 3: which inherent characteristics? Please list/discuss them.

Materials and methods

Pg 15667

Line 23: is 'wanve' supposed to be 'wave'?

Pg 15668

Line 19: what exact volume of headspace was created?

Pg 15670

Line 1: Estimation of reproducibility was based on what? Please be specific.

Line 17: Here and elsewhere, change 'tube' to 'tubing'. Specify if PE, PP, PC, PTFE etc

Results

Pg 15672

Line 15: Oxycline seem to start at 40 m rather than 50 m. Please check.

Line 17: Here and elsewhere, how could you tell where the 'oxic-ANOXIC transition' was when the detection limit of your oxygen sensor was as high as 3 umol L-1? This means the depths you consider anoxic could actually contain as much as 3 uM oxygen. . .meaning a much deeper depths could be actually oxic. Are you sure the detection limit was not lower than this? If it was as high as reported, it would be advisable to avoid using the term 'oxic-anoxic' or at the least, you should use literature references to support your data in establishing this transitional layer.

Pg 15674

Lines 13-15: The use of 'oxic-anoxic condition' and 'low-oxygen conditions' is confusing. How can you have transition from oxic to anoxic condition, and then below the anoxic condition, you somehow also have a low-oxygen condition? This is an issue because of the detection limit of the oxygen sensor used. However, you can work around

this by avoiding using the term 'oxic-anoxic transition'.

Pg 15675

Lines 19 & 22: 105% and 142% are confusing. Please recheck your computations. The microbes cannot oxidize any more methane than what is available. I suggest to either report the average values or use fractionation factors that give reasonable estimates of the fraction of methane that is oxidized.

Pg 15676

Lines 4-18: I have issues with the end-members used in the mixing model. The bulk POC should already contain some methanotrophic biomass so it should not be used as the end member of the sedimenting organic matter. Either completely remove these estimates from the paper or you should use C13 of diagnostic biomarkers or better still, use C13 of CO2/DIC (and correct for photosynthetic fractionation) as the sedimenting OM end-member.

Pg 15679

Line 4: 13C-depletion of C16 MUFA was not within the oxycline (Fig 2d). The 13C depletion rather appears to start right below (or at) the transition between the 'oxycline' and the 'low-oxygen waters', extending way deeper into the 'the low-oxygen' depths which could potentially be anoxic depths. This observation should be discussed. Why are the type I methanotrophs active within these depths?

Also, in the 13C-labelled methane tracer studies that the authors performed, all the samples that were used for the incubations were taken from depths below the oxycline (except 40 m in Feb 2012) (Fig. 4a &b). In September 2012, the 'oxycline' was above 55 m and the 'low-oxygen waters' below this depth. The incubated samples were from 62.5 -70 m (Fig 4a). Similarly, in February 2012, the 'oxycline' was above 45 m with low-oxygen waters below this depth. The samples used for the incubations were from 40m, 50m and 60m (Fig 4b). The incubations that showed labelled 13C-CH4 incorporation

by C16 MUFA (type I methanotrophs) were all from depths below the oxycline, that is, 65 m in September and 50 m in February.

So both the in situ PLFA and tracer PLFA 13C data show that the type I methanotrophs are active way below the oxycline, in the ‘low-oxygen’ and potentially anoxic depths. The authors should discuss these observations. Based on the data, I am not convinced that the type I methanotrophs are active in methane oxidation in the oxycline or oxic zone as suggested by the authors.

Pg 15680

Line 2: While previous studies have also noted the involvement of sulfate reducing bacteria in the deep waters of the lake, as was observed in this study, 10 Me 16:0 and C17 MUFA are not typically used as biomarkers for sulfate-reducing bacteria. The use of these biomarkers should be thoroughly and convincingly discussed in the context of wider literature.

Figures

Please include a map of the Lake showing the study sites.

Fig 1 (a,b,c) : Please use different scales for the δ13C of CH4 and δ13C of POC. Also, the CH4 concentrations should be reported in mmol L-1 . I reckon that using the same unit as oxygen allows for easy comparison, but in this particular instance, it is better to keep the methane concentration in mmol L-1. With this figure and all others, it would be informative to the reader to indicate the precision of the measurements by way of error bars.

Fig 3. It will help the reader if you include the time (hours) on each data point.

Fig 4. You should note in the figure title that all the samples in Fig 4a that were incubated were from depths below the oxycline and within the low-oxygen region. Similarly, include in the title that 50 m and 60 m are below the oxycline, and are within the low-oxygen depths.

Interactive comment on Biogeosciences Discuss., 11, 15663, 2014.