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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.

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First of all, we would like to thank the reviewers for their positive and very constructive comments. In the section below, we will provide a point-by-point reply to the suggestions and comments provided by the reviewers.

Reviewer comment 1: The paper is well written and the study well executed. My only major scientific criticism is that the authors focus solely on sulphate-AOM but do not present concentrations of alternative electron acceptors. It's possible that AOM in lake coupled to another electron acceptor such as, nitrate, nitrite, Fe(III), or Mn(IV): Why do the authors assume (e.g. on page 15680 line 6) that sulphate-AOM is occurring and not another form of AOM ? Did they measure concentrations of sulphate, nitrate, nitrite, Fe (III), Mn (IV) ?



> Reply : This is an interesting comment. It has been reported frequently in the literature that 10Me16:0 and C17 MUFA are especially abundant in sulphate reducing bacteria, as mentioned in the text. However, it is true that the phylogenetic resolution of PLFA analysis is rather low, and hence it is difficult to unambiguously identify the organisms involved in this anaerobic oxidation process. Recent studies have revealed that marine anaerobic oxidation of CH4 (AOM) is indeed coupled to a larger variety of electron acceptors than previously thought. For instance, it has been shown that the sulphate-reducing bacterial partners of methanotrophic archaea could also reduce iron (Coleman et al. 1993). Moreover, anaerobic oxidation of methane could also be carried out syntrophically by a consortium between methanotrophic archaea and denitrifying bacteria (Raghoebarsing et al. 2006), or between methanotrophic archaea and manganese reducing bacteria (Beal et al. 2009). The "discussion" section of our manuscript has been modified to take these studies into account. However, the major aims of our study were (i) to quantify the contribution of CH4-derived carbon to the biomass, (ii) to quantify methanotrophic bacterial production, (iii) to quantify methanotrophic bacterial growth efficiency, and (iv) to identify which were the aerobic methanotrophs involved in CH4 oxidation based on PLFA analyses. Our experiments were not designed to identify which electron acceptors were linked to anaerobic methane oxidation (although this is an interesting research topic), and hence we did not measure the concentrations of Fe(III), Mn (IV) and SO42- (in September 2012) in the water column of Lake Kivu. Further investigations, with a special focus on the coupling between anaerobic CH4 oxidation and other processes, would be needed to shed more light on this. The revised text now reads : "A significant MBP rate (1.3 $\mu$mol L-1 d-1) was measured under low-oxygen conditions (< 3 $\mu$mol L-1) at 60 m during the rainy season (February 2012). Moreover, the PLFA labelling pattern was drastically different, with a more important specific 13C incorporation into 10Me16:0 and C17 MUFA instead of the C16 MUFA, relative to their concentrations. This different labelling pattern suggests that a different population of methanotrophs was active in CH4 oxidation deeper in the water column. Archaea lack ester-linked fatty acids in their membrane and are therefore

undetectable in PLFA analysis. However 10Me16:0 and C17 MUFA are known to be especially abundant in sulphate-reducing bacteria (Macalady et al. 2000, Boschker and Middelburg 2002), one of the syntrophic partner of anaerobic CH4 oxidizing archaea (Knittel & Boetius 2009). Hence, the specific labelling of 10Me16:0 and C17 MUFA under low-oxygen conditions could indicates that a fraction of the upward flux of CH4 was oxidized syntrophically by an archaea/bacteria consortium, and might support the hypothesis that the bacterial partner grow on CH4-derived carbon source supplied by anaerobic methane oxidizers within the consortium, as already suggested by the results of an in vitro labelling (13CH4) study (Blumenberg et al. 2005). However, our data does not necessary imply that anaerobic methane oxidation would be coupled with SO42- reduction, as some sulphate-reducing bacteria have been also found to be able to reduce iron (Coleman et al. 1993). Furthermore, the phylogenetic resolution of SIP-PLFA analyses in rather low (Uhlík et al. 2009), and recent studies showed that anaerobic methane oxidation could be carried out syntrophically by consortium between methanotrophic archaea and denitrifying bacteria (Raghoebarsing et al. 2006), or between methanotrophic archaea and manganese reducing bacteria (Beal et al. 2009). Further investigations would be needed to address more accurately which is the electron acceptors coupled to anaerobic CH4 oxidation".

Reviewer specific comments :

> Reply : The text was corrected for grammatical errors following the suggestions provided by the reviewer.

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