

## ***Interactive comment on “Intersecting Methane Production and Oxidation Zones in Freshwater Sediments” by Xueping Chen et al.***

**Anonymous Referee #1**

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General comments: The paper of Chen et al investigated the pathways of methane production and oxidation in sediment of a freshwater reservoir. The authors collected several datasets on biogeochemical parameters, incubations and molecular inventory. The data are potentially interesting, but the analysis and interpretation are poor. The authors did not consider including in the analysis several important microorganism groups (e.g. ANME), nitrate concentrations were not measured; the molecular analysis was in general relatively superficial. The discussion section contained only few references and did not put the results into perspective with already available literature. The figures were of poor quality and partly showed false claims (Fig4). The authors should consider asking a native speaker to correct the language of the manuscript. I suggest re-writing the manuscript which should include an in-depth analysis of molecu-

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lar data, stoichiometric calculations (see comment below) and careful integration of all data. The authors should also make themselves familiar with recent literature available of AOM.

Specific comments: Line 76: the authors should cite Haroon et al 2013 for nitrate-dependent AOM Line 131: a subsample of what? Line 137: At an in-situ pH of 8, formic acid/acetic acid/propionic acid would be present as formate/acetate/propionate. Please correct this Lines 152-157: It is unclear to me how and in what form acetate was added to the sediment. Was the sediment slurried with liquid in order to achieve homogenous distribution? Line 155: “thick butyl rubber stoppers”: what kind? Please indicate the manufacturer and type. It is known from previous studies that black rubber stoppers inhibit anaerobic methane oxidation. Since the authors used same stoppers for AOM experiments, the obtained results might be false. Lines 159-160: Instead of indicating what volume was added, indicate the final headspace concentration, headspace volume and headspace pressure. I assume non-labelled methane was added? Lines 184-185: How were the data normalized? How many sequences per sample were used for final analysis? Line 187: Please indicate the settings used in the pipeline. Line 192: Silva108 is an old release dating back to 2011. Why was such an old version used? Lines 193-195: It is not clear what was done. All steps and settings should be specified. The obtained data should be reproducible by other researchers. Lines 201-203: What are these primers? No reference, no information of specificity, no information on binding sites Line 222/Fig1: What is the oxygen diffusion depth in this sediment core? Why was nitrite measured but not nitrate? What form of iron was measured, I suppose Fe(II)? Lines 272-274: What is 100 Lines 291-292: I suppose NC10 includes all sequences assigned to this phylum? I hope the authors are aware that so far only some members from clade a were shown to perform AOM. Other members of this phylum were never shown to perform AOM and are likely to have different metabolisms. Line 293: Nitrate reductase? The method section does not describe any analysis of nitrate reductases. The authors refer to Figure 4D where the relative abundance of NC10 sequences is shown. What does this mean? Figure4:

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What do percentages refer to, total bacteria? Why are some groups analyzed down to genus level and others not? NC10 comprises the whole phylum, what is the relative abundance of *Methylomirabilis* sequences? Line 310: Can this claim be backed up by molecular data on known aerobic methanotrophs? I missed that data in the results section Line 315: Figure 1 does not contain data on nitrate concentrations. I also miss nitrate data for proper interpretation of AOM driven by N oxides Line 316: Authors claim that nitrite-dependent AOM took place in deeper layers. Where does the nitrite come from if oxygen only penetrates within the upper cm of the sediment profile? Lines 314-316: This is very vague Lines 325-328: The authors discuss very high AOM activities based on bottle incubations. The authors did not add any extra electron acceptors into those bottles which means that methane oxidation is sustained by in-situ electron acceptors. It is in my view an essential step to calculate the stoichiometries: how many electron acceptors were present in respective samples and how do concentrations correlate to methane consumption activities? Lines 340-342: The authors should be very careful with such statements. Activity does not directly correspond with the number of genes. Line 348: I don't understand the sentence. Please rewrite Lines 351-354: This is very vague. Stating the metabolism type based on 16S characterization at order level is farfetched. I expect characterization at genus level. Also, there are no references! Line 363: *Methanosarcina* uses acetate, not acetic acid. This is basic physiology. Lines 378-379: I did not see evidence for this claim from the presented data. Fig6: The figure contains several false claims. Not all NC10 perform AOM but so far only one genus, *Methylomirabilis*; *Methylomirabilis* does not use nitrate; how are metal oxides supposed to catalyze sulfate reduction to sulfide? The ANME abbreviation stands for anaerobic methane oxidizing archaea which were not discussed in this study at all. Why are NOB mentioned here? Line 406: "undetected iron minerals". Were any analyzes performed? Line 415: "high abundance of nitrate reductase". What does this supposed to mean?

Please also note the supplement to this comment:

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<https://bg.copernicus.org/preprints/bg-2020-330/bg-2020-330-RC1-supplement.pdf>

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-330>, 2020.

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