

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

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Received and published: 13 November 2020

Review- Chen et al. 2020 BG I read carefully read your ms. It seems that a lot of work has been done, however I think that the work is over simplified and needs to be more thorough and precise before it can be considered for publication. Major concerns: 1) The main finding, as presented already in the title, is not novel. It has been shown in the last decade in many studies that there is an overlap between methane production and oxidation in marine and freshwater sediments. There are also studies that described the different layers of different AOM and methanogenesis pathways and overlaps, as you mention yourself (very nicely shown by He et al., 2019). Therefore, there is a problem in the novelty aspect of this study and fitting it to BG, as well as incorrect statements in the text (e.g L 31). Thanks for your comments. Our finding was to

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explain the active and complex methane metabolic cycle in the sediments which might regulate the methane release from the sediment to uploading water. The ecological niche in the freshwater sediment in this study was proposed to help to explain the methane distribution along the sediment profile. And as you suggested below, we would focused on the complex microbial community involved in methane cycle and the controlling parameters. 2) The occurrence of iron reducing bacteria and iron reduction above sulfate reduction, can be interpreted also as classic organoclastic iron reduction and not necessarily as iron-AOM. We totally agree with you. In addition, based on high rates of AOM from the slurry incubation, we believe that there might be iron and sulfate reduction partly coupled to AOM. However, it's a pity that we didn't get the in situ activity rates of AOM, and we could not accurately calculate the relative contribution of AOM coupled with sulfate or iron. We would supplement the organoclastic iron reduction in the discussion.

3) The analytical methods are not detailed, partly explained and without any statistics. Please explain how did you measure exactly  $d_{13}C$  CO<sub>2</sub> (no measurement of DIC as written in Fig. 1?), NO<sub>2</sub>-, TOC? What is the error on the symbols? Im not sure I trust the NO<sub>2</sub> peaks you see in Fig 1 at 12 cm depth below the sulfate decrease. It seems to me like a constant very low value all along the sediment. I have concerns also reg. the methane profiles. Did you have duplicates for the geochemical and the biological parameters? Sorry for the missing information. We indeed examine the DIC content, and we would supplement the data, method and statistics in the text. We have verified that the nitrite should be instead of nitrate with the analytical center. The concentration of methane was measured as capacity in pore water, which has indeed no replicates because of the relatively little amount of pore water. Other parameters have duplicates and we would supplement the statistics in the text. 4) How do you explain that the AOM rates were higher than the production rates? Sorry for the misleading statement. We incubated the slurries after layered cutting for the methane production and oxidation, respectively. When incubated for the oxidation, we external added methane in the headspace (final concentration as 1%), while incubated for the production, we did not

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add any substrate, and the production rates were increased after acetate supplement. The different concentration substrate for AOM and production might result in different oxidation and production potential.

5) Fig. 5 can be explained also by changing the main mechanism for methanogenesis. Thanks for your comments. We would supplement explanation of methanogenesis.

6) There is limited discussion on the microbial community, the types of methanogenesis, the controlling parameters etc. Thanks for your suggestion. We would supplement the microbial community and controlling parameters.

Based on the above I recommend: 1) Showing the data with the statistics and the patterns considering this statistics. Thanks. We would revised the figures and text correspondingly. 2) Changing the focus of the paper from this overlap and AOM to the complex microbial community and methane cycle in general (methanogenesis and AOM) considering the controlling parameters. Fig. 2 is nice (just check the AOM higher rates than meth) and the discussion should be expanded around this topic + the population (Fig. 3 and 4). Thanks for your suggestion. We would focus on the microbial community involved in methane cycle and the controlling parameters.

More comments: 5) Fig. 1, you don't need to put in each graph the same CH<sub>4</sub> profile, just add another graph of CH<sub>4</sub>. We would revised the figure 1. 6) Please edit the English. There are several typos (as in L. 226 –decreases and not decrease) Thanks. We would ask for polish the English. 7) Why do you think you had less methanomicrobiales than methanosarcinales? Please explain, it's not typical in fresh water sediments. Sorry for misleading statement. The relative abundance of methanosarcinales is indeed higher than that of methanomicrobiales in this study.

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

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