

Interactive comment on “Ammonia impacts methane oxidation and methanotrophic community in freshwater sediment” by Yuyin Yang et al.

Yuyin Yang et al.

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The manuscript by Yang et al. describes the effect of different amendments of ammonium salt in sediments of a Lacustrine lake for two weeks and monitoring of gene and transcript abundance of *pmoA* and measurement of methane oxidation potential. Some specific comments regarding the manuscript are listed below: 1. The statement made by the authors that the impact of ammonia on aerobic methanotrophs is unclear is not true. In fact a recent study performed by Liesack group have shown that ammonia specifically inhibits high affinity methane oxidation using *Methylocystis* sp. strain SC2 as the model system (Dam et. al 2014). They should refer to the study and make

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a discussion on the high affinity methanotrophs in such environments. The authors have totally neglected the high affinity methanotrophs in their discussions. Response: The authors appreciate the reviewer's suggestions. Essential information about high affinity methanotrophs has been added to the manuscript. In eutrophic lake sediment, methanogenesis was quite active, and methane was abundant. Therefore, low-affinity methanotrophs might be of greater importance in the studied lake ecosystem.

2. The use of BciT130 for T-RFLP is also unusual. The authors must clarify the use of such an unusual restriction enzyme for generation of T-RF cuts, instead of Msp I, which have been widely used for methanotrophs. Response: The authors appreciate the reviewer's suggestions. Essential discussion has been added to the manuscript. In brief, we tested all the enzymes on <http://www.restrictionmapper.org> with some previously retrieved pmoA NGS sequences from the same lake. In silico analysis indicates that BciT130 could generate more T-RFs, and also present a better phylogenetic resolution than MspI.

3. In the introduction and discussion sections the authors need to mention the importance of ammonium and methane oxidation in Lacustrine environments. What physiochemical or biogeochemical evidences are there that prove the studied lake is Lacustrine in nature. In fact the term "Lacustrine" have only be used twice in the abstract and nowhere else. Response: The authors appreciate the reviewer's suggestions. Revisions have been made as suggested.

4. What impact does the authors think will this study have on such lake ecosystems? Do they want to mimic some future possibilities or so? A proper objective must be developed at the beginning and the experimental design should be in sync to the objective. The different physiochemical data of the sediments must be mentioned in the result and those should be discussed in relation to the methanotroph community. Response: The authors appreciate the reviewer's suggestions. Revisions have been made in the manuscript. Eutrophication is one of the major concerns in most of freshwater lakes in China. And excess ammonium input could always be observed in these

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environments. Therefore, the current study is aim to investigate how methanotrophic community response to high concentration of ammonium input in lakes experiencing eutrophication process.

5. Use of terminologies like treatment A, B, C etc throughout the text is making the manuscript difficult to follow at times. Response: The authors appreciate the reviewer's suggestions. Revisions have been made to make the manuscript more readable.

6. How many clone library sequences were performed? This needs to be mentioned. Response: The authors appreciate the reviewer's suggestions. A total of 96 clones was subjected to sequencing, and 93 sequences were retrieved. This has been added to the manuscript.

7. Fig. 4: Is it a 100% graph? Response: No. Some less abundant T-RFs were not shown in the graph.

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