

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

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Manuscript "No.: bg-2018-193" describes the effect of ammonia on methane oxidation and methanotrophic community in freshwater sediment. The study is interesting and the topic itself is important, but there is a major drawback. One of the main purposes of this manuscript is to investigate the effect of ammonium on community structure of aerobic methanotrophs. However, due to the methods they use, it is difficult to obtain the classification information for methanotrophs. BciT130 I restriction endonuclease was used to digest purified PCR products in this study. However, the digestive enzyme often used for pmoa T-RFLP analysis is Msp I. In the present study, it was difficult to deter-

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mine whether the dominant TF peak 242 bp is *Methylobacterium* or *Methylobacter*. The main T-RF peaks in Figure 4(b), such as 385 bp and 228 bp, had no relevant information on the methanotrophic taxonomy. Compared with T-TF and clone sequencing, Miseq sequencing of *pmoA* gene would be a better choice for this study.

Response: The authors appreciate the reviewer's valuable suggestions. We have added some discussions to explain the choice of enzyme (part 4.4). In spite of the somewhat unsatisfactory taxonomic resolution, TRFLP is still a very fast and economical approach offering an overview of methanotrophic community composition and diversity. We agree that NGS might be a better choice to get a more comprehensive profile of methanotrophic community, and will consider it in our future methanotrophic community studies.

Minor comments: Line 104 In the Materials and Methods section, methods for measuring the physical properties of sediments should be described.

Response: The authors appreciate the reviewer's suggestion. The methods used have been added in the revised manuscript (in Lines 112-116, in red).

Line 182: Submit the Clone sequences to NCBI and list the accession number.

Response: The authors appreciate the reviewer's suggestions. The accession numbers have been already listed in Figure 3 (together with the predicted T-RF length).

Lines 597-623, in Fig 1 and 2, the methane oxidation potential increased (treatment F) or remained relatively stable (treatments C, D, E) during the incubation, however, the *pmoA* transcription was reduced after 14 days incubation. So how could you explain the increased or stable methane oxidation by reducing *pmoA* transcription?

Response: The authors appreciate the reviewer's comments. However, transcripts were not directly linked to the activity. It is likely that the higher concentration of pMMO protein (resulted from the higher transcription in earlier time) maintained the relatively stable methane oxidation potential.

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