

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

### **Anonymous Referee #2**

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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 determine whether the dominant TF peak 242 bp is *Methylomicrobium* or *Methylomonas*. The main T-RF peaks in Figure 4(b), such as 385 bp and 228 bp, had no relevant infor-

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mation on the methanotrophic taxonomy. Compared with T-TF and clone sequencing, Miseq sequencing of pmoA gene would be a better choice for this study.

Minor comments:

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

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

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 14days incubation. So how could you explain the increased or stable methane oxidation by reducing pmoA transcription?

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