

Point-by-point response to Reviewer #2's comments:

Zhao et al. investigated the active aerobic methanotrophs in six different paddy soils. They do find the key roles of pH in mediating the niche differentiation of aerobic methanotrophs. Methylocystis-affiliated type II methanotrophs were most active in soils with pH (5.44-6.10), while Methylobacter/Methylosarcina-affiliated type I methanotrophs were most active in soils with pH (7.02-8.02). In addition, no significant changes in ¹³C-labelled methanotrophic community compositions was detected with the fertilization of ammonium nitrate. The study is interesting and the manuscript is well written. The questions are appropriately introduced and explored and the results are well presented. Therefore, I would recommend the manuscript for publication.

Reply: We thank the reviewer for general positive comments and recommendation for publication of this study.

However, it should be noted these results were obtained from rice paddy soils. The consistency in other soils should be further studied. For example, Deng et al. 2016 (Ref: Identification of active aerobic methanotrophs in plateau wetlands using DNA stable isotope probing) found that both Methylocystis and Methylobacter methanotrophs were dominated in methane consumption in Dangxiong peatland soils with a pH of 6.1. Therefore, the authors should consider more comprehensively when interpreting the results.

Reply: Thanks for the advice. The present study was designed to reveal the potential niche specialization of methane oxidizers influenced by soil pH in the rice paddy fields, which represent one of the most typical anthropogenic wetland ecosystems. We fully acknowledge that the results from this study might not reflect the situation of methanotrophic assembly in soils and sediments from other ecosystems including natural peatlands, in which other factor(s) might be equally or more critical. To prevent overstatement of the role of pH in methanotrophic activity and distribution, in the revised manuscript, (i) we will present our conclusions more carefully and restricted our results, conclusions and discussions "in rice paddy soils". (ii) We will also include the reviewer's advice in the revised discussion as following: "However, the consistence of our conclusion of the pH-based ecological coherence of methanotrophs in other environments should be further studied, since previous studies also revealed co-dominance of type I and II methanotrophs in low-pH natural peatlands (Deng et al., 2016;Esson et al., 2016)"

In line 217, why did the authors cluster the pmoA genes at a nucleotide level of 93/ similarity. In fact, we usually define 93/ similarity at amino acid level and corresponding to methanotrophic species (Reference: Lüke C. and Frenzel P., 2011 Potential of pmoA Amplicon Pyrosequencing for Methanotroph Diversity Studies). If

the authors want to cluster the *pmoA* sequences at a nucleotide level, Wen et al., updated the *pmoA* gene cutoff at nucleotide level to 86/ corresponding to 97/ similarity of 16S rRNA gene (Reference: Wen et al., 2016 Evaluation and update of cutoff values for methanotrophic *pmoA* gene sequences).

Reply: Thanks for this informative comment. Our basis for choosing 93% similarity is from a previous literature describing use of similarity cutoff at the range of 87-95% for *pmoA* genes (van de Kamp et al., 2019). After carefully reading the literatures from the reviewer' comment, we must admit that clustering *pmoA* OTUs at 87% or 86% similarity is theoretically well-founded and was more commonly used. However, in this study, we chose higher similarity (93%) to cluster *pmoA* genes considering that higher resolution clustering of functional markers is useful to detect spatial patterning in microbial assembly as suggested by van de Kamp et al. (2019). Clustering at 93% similarity would result in more observed methanotrophic genotypes (OTUs) than at 86-87% cutoffs, thus allowing detection of potentially fine community composition difference between different soils. Additionally, this similarity cutoff should not change our main conclusion here that either type I or type II methanotrophs dominating active methane oxidation. We will add the following information in the revised manuscript to support our methodology: “Clustering *pmoA* genes at 93% similarity rather than commonly used 86-87% similarity (Wen et al., 2016; Degelmann et al., 2010) is useful to detect spatial patterning in microbial assemblage as suggested previously (van de Kamp et al., 2019)”. We fully respect the reviewer's suggestion and concern here, and hope our explanation is adequate to justify our results.

Reference:

- Degelmann, D. M., Borken, W., Drake, H. L., and Kolb, S.: Different atmospheric methane-oxidizing communities in European beech and Norway spruce soils, *Appl Environ Microbiol*, 76, 3228-3235, 10.1128/AEM.02730-09, 2010.
- Deng, Y., Cui, X., and Dumont, M. G.: Identification of active aerobic methanotrophs in plateau wetlands using DNA stable isotope probing, *FEMS Microbiol Lett*, 363, 10.1093/femsle/fnw168, 2016.
- Esson, K. C., Lin, X., Kumaresan, D., Chanton, J. P., Murrell, J. C., and Kostka, J. E.: Alpha- and Gammaproteobacterial Methanotrophs Codominate the Active Methane-Oxidizing Communities in an Acidic Boreal Peat Bog, *Appl Environ Microbiol*, 82, 2363-2371, 10.1128/AEM.03640-15, 2016.
- van de Kamp, J., Hook, S. E., Williams, A., Tanner, J. E., and Bodrossy, L.: Baseline characterization of aerobic hydrocarbon degrading microbial communities in deep-sea sediments of the Great Australian Bight, Australia, *Environ Microbiol*, 21, 1782-1797, 10.1111/1462-2920.14559, 2019.
- Wen, X., Yang, S., and Liebner, S.: Evaluation and update of cutoff values for methanotrophic *pmoA* gene sequences, *Arch Microbiol*, 198, 629-636, 10.1007/s00203-016-1222-8, 2016.