

Interactive comment on “Linking phosphorus and potassium deficiency to microbial methane cycling in rice paddies” by Rong Sheng et al.

Anonymous Referee #2

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General comments This is a very interesting study. The authors are trying to elucidate field-scale methane emission flux from microbial ecology perspective, for a better understanding of how anthropogenic activity of fertilizer applications may affect methanocycling microbes and methane emission in the field. The long-term agricultural field experiment with nutrient deficiency was exploited including -P, -K, and -PK and the balanced fertilization treatments (i.e. NPK). Methane emission fluxes were determined in the field at ripening and tillering stages, the transcriptional activity of key functional genes for methanotrophs (*pmoA*) and methanogens (*mcrA*) were determined along with the compositions of these methane-cycling organisms by T-RFLP fingerprinting, plant biomass (above ground and belowground) and soil properties were analyzed. The results showed that that a large amount of CH₄ emitted from paddy soil at rice tillering stage (flooding) while CH₄ flux was negligible at ripening stage (drying). Compared

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to NPK treatment, significantly lower methane flux was observed from P-deficient but not K-deficient fields. Methanotrophic transcript copy number significantly increased in tandem with a decrease in methanogen transcript abundance in P-deficient soils. These results provide important insights on methane-cycling microorganisms in the field thereby contributing to a better understanding of optimization strategy for mitigating methane emission while maintaining crop yield. However, the key message needs to be refined and the focused discussion should be made to establish a correlative link between nutrient constraint and methane emission via plant growth. The major comments are following: (1) Please add a figure showing the correlative relationship between soil phosphorus availability, SOC contents, mRNA and plant biomass and CH₄ emission. This would be the key to understand why nutrient-deficiency constrains the growth of rice plant, which may directly or indirectly affect methane-cycling microorganisms, leading to flux variations of methane emission flux in the field. (2) Please convert the plant biomass table as a figure and place it along with methane flux (3) In the text, please discuss the important role of irrigation regime. For example, midseason drainage and the decline of water table at ripening state may lead to significant decline in methane emission flux. Specific comments: (1) L14. It may be more important for plant rather than for the resident microorganisms (2) L15-17. These sentence may be better placed in the text rather than the abstract. (3) L18. It is difficult establish direct link of P and K deficiency to methanogens and methanotrophs. It might be rephrased as plant productivity or crop productivity (4) L20-25. Again, I do not think there is strong evidence in support of conclusion that P deficiency reduced methane emissions via reduced methane production. It may be more appropriate to say that P deficiency constrains the growth of rice plant, leading to lower biomass and methane production. The reason is that the crop biomass may correlate positively with precursors of methanogens. (5) L47. Replace metabolic genes with functional genes (6) L112. Gas sampling means static chamber measurement of CH₄ flux in the field? (7) L116. Methane emission measurement might be merged with soil sampling. static chamber technique can be first described, then soil sampling was conducted in order

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to explain the dynamic changes of methane flux in the field. The first section can be the site description only. (8) L121. How were samples kept for transportation before measurement. How to avoid leakage? (9) L124. With slight modification (10) L139. T-RFLP fingerprinting (11) L166. Real-time quantitative PCR (12) The materials and methods can be organized as following. 2.1. site description of long-term field experiment; 2.2. Plant biomass and soil properties; 2.3. Methane emission flux measurement; 2.4. Soil microbial DNA and mRNA extractions; 2.5. Composition and abundance of soil methane-cycling communities (including T-RFLP fingerprinting and Real-time quantitative of soil methane-cycling communities). 2.6. Statistical analysis (13) L202. Please describe the management of rice cultivation. For example, basal fertilizers, top dressing of fertilizers, irrigation regime such as mid-season drainage and so on (14) L235. MOB population size (15) L270-273. The major conclusion of this study here falls short of a reasonable story. For example, the author may come up with few sentence explaining why phosphorus deficiency led to reduction in CH₄ emission, while potassium deficiency did not affect net methane emission flux. (16) L298. These different environment conditions should have been in close association with growth status of rice plants under different nutrient regimes. (17) L308-309. Are these organisms methanotrophs, being capable of producing P-liberating enzymes. In addition, if so, it means that there exists the insoluble soil P which can be mineralized by methanotrophs? (18) L309-311. If it is not applied to methanotrophs, please add one or two sentence stating "it should be emphasized that such mechanisms remain unclear in methanotrophs and warrant further study" (19) L323-328. The conclusion should reiterate the key finding of this study. Provide the solid evidence and manage to conclude with a plausible reasoning. For example, the solid evidence is: P deficiency may significantly decrease CH₄ flux rate via reducing the activity of methanogens and enhancing the activity of methanotrophs. It may be more appropriate to say that P-deficient soils showed significantly lower CH₄ flux. This might be attributed to the reduction of methanogens and the stimulation of methanotrophs that could have adapted to changes in soil physiochemical properties in association with rice plant growth under chronic nutrient constraints.

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