

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

### **Anonymous Referee #1**

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#### General comments

The authors performed an interesting study on effects of P and K availability in rice paddy soils on methane emission and methanogen and methanotroph presence and community composition. They find reduced CH<sub>4</sub> emission in low P plots, which they attribute to higher methanotroph activity and lower methanogen activity. Effects of potassium on CH<sub>4</sub> emission are less pronounced. Copy numbers of *mcrA* and *pmoA* genes are not linked to CH<sub>4</sub> emission and hardly show a response to fertilization treatments. Transcripts of *pmoA* showed differences in the active MOB community between treatments, also dependent on the rice growth stage.

Strong points of the manuscript are the inclusion of *mcrA* and *pmoA* transcript analysis in combination with CH<sub>4</sub>-flux data, and the use of long-term fertilization plots. However,

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I would like to see improvement on the following issues:

#### Introduction/Discussion

To my understanding, it is quite clear when P or K are limiting plants, but concentrations at which they become limiting to microbes are far less understood. Therefore, I recommend to use terms like 'P/K deficient' with care. For example, your results point at an increase in MOB activity in low P plots, perhaps indicating not so much P-limitation, but an alleviation of excess P? Also, effects may arise from altered (C):N:P:K stoichiometry, rather than concentrations in itself.

Discuss how, because different MOB respond in different ways, results may strongly depend on the initial community composition. Different soils may react in different ways.

#### Methods

The CH<sub>4</sub>-flux method is poorly described. Please provide more detail on the method. Where and how were the samples taken? Did the chambers include rice plants? Did you measure time-series? From how many static chambers per plot? How many replicate gas samples? How many replicates in time? How were total and available P and K determined? Do they reflect availability to plants? / to which extent is P or K unavailable to plants available to microbes?

#### Results

The figures can be improved. It would be helpful to show hierarchical clustering of the samples based on their T-RFLP profiles, to show which samples/treatments are most similar (per sample class). Add gene names and DNA vs mRNA copies inside the panels or on the y-axes of the graphs.

Show correlations between CH<sub>4</sub>-flux and DNA and mRNA copies, and also present the relation between CH<sub>4</sub> flux and mcrA/pmoA transcripts here. You refer to these in the discussion but they are missing in the results section.

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## Discussion

Please also better explain why one would expect DNA copy numbers to be less indicative of community functioning than transcripts.

I am missing some discussion on what these results mean in terms of CH<sub>4</sub>-mitigation potential? Low emissions seem to come at the expense of plant biomass (and possibly nutritional value?).

Specific comments Line 31: I would end this sentence at 'transcriptional level', as the relation between 'population size' and DNA copies is debatable. Line 125, why? Line 254 This seems to conflict with the previous sentence, where members of methylococcus increased. Are these T-RFs representing different species, meaning some methylococcus species increase whereas others decrease? Line 280 describe how they were influenced by the fertilizer regime Line 286 the 'size' of the resident communities is hardly affected. It would be interesting to also discuss the effect of the growth stage of rice on CH<sub>4</sub> flux and methanogen and MOB communities. Line 303. How can you be sure that they were P deficient? Line 325: Add that effects are species specific, different soils may show different effects

Technical comments Line 78, round to whole numbers Line 87, key nutrients -> phosphorus and potassium Line 113, after washing off Line 204 different

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