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

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Dear Madam/Sir,

Thank you for your comments and suggestions on our manuscript.

We have revised the manuscript accordingly, and the detailed corrections are listed below point by point:

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 methan-cycling microbes and methane emission in the field. The long-term agricultural field experiment with nutri-

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ent 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 compostions 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 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:

#Comment (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.

Answer: We have added the correlation analysis between methanogenic, methanotrophic populations, soil properties and CH4 flux as requested in lines 215-224, line 304-312 and Fig. 5. The details are as below:

Line 215-224: Soil properties such as pH, soil organic carbon and total nitrogen together with gene abundance between the treatments were compared by ANOVA anal-

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ysis using the Statistical Package for Social Science (SPSS 13, SPSS Inc., Chicago, IL, USA). Significance among means was identified using least significant differences. Pearson correlation analysis between CH4 flux, soil properties, plant biomass and population size of resident and active methanogens and methanotrophs was also performed using SPSS. Redundancy analysis (RDA) was used to characterize the relationship between soil properties, plant biomass and the community structures of methanogens and methanotrophs using CANOCO statistical package for Windows 4.5 (Biometris, Wageningen, Netherlands). A Mantel test based on 499 random permutations was used to examine the significant correlations between the differences in soil properties plant biomass and microbial communities.

Line 304-312: Correlation analysis indicated that the CH4 fluxes from field and soil incubation were significantly correlated to the transcript ratio of mcrA/pmoA ($P < 0.05$ and $P < 0.01$, respectively, Table 3) at the tillering stage. In addition, CH4 flux from soil incubation was also significantly correlated with the contents of both total and available phosphorus (TP and AP, $P < 0.01$), SOC ($P < 0.05$) and plant biomass ($P < 0.05$). Redundancy analysis (RDA) indicated that P-deficient induced changes in soil physiochemical properties, such as SOC, TP, AP contents in tandem with plant biomass, were important factors driving community structure shifts of active (mRNA based) methanogens and methanotrophs (Fig. 5).

#Comment (2): Please convert the plant biomass table as a figure and place it along with methane flux

Answer: We have convert plant biomass table and CH4 flux as Fig. 1

#Comment (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.

Answer: We have added discussion in line 382-388:

"Besides, soil water management has been widely known to play an important role in regulating CH4 emission (Cai et al., 1997; Nishimura et al., 2004; Towprayoon et al., 2005). We observed that the CH4 flux were much lower at rice ripening stage when soil was drying than that at tillering stage when soil was under flooding. These phenomena were also reported by previous studies that showing midseason drainage and the disappearance of the water layer induced significant decline in methane emission flux, which might associated with the reduction in methane production and increase in the oxidation of CH4 under drying soil environment (Nishimura et al., 2004; Towprayoon et al., 2005)."

Specific comments:

#Comment (1): L14. It may be more important for plant rather than for the resident microorganisms

Answer: It has been rewrite as below:

"Nutrient status in soil is crucial for the growth and development of plant and resident soil microorganisms. Soil methanogens and methanotrophs can be affected by soil nutrient availabilities and plant growth, which in turn modulate methane (CH4) emissions."

#Comment (2): L15-17. These sentence may be better placed in the text rather than the abstract.

Answer: We have deleted this sentence.

#Comment (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

Answer: It has been rewrite as below:

"Nutrient deficient has been shown to constrain plant growth, however, whether nutrient limitation for plant can also influence the methanogenic and methanotrophic

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communities and their functions are remained unclear. Here, we assessed whether deficits in soil available phosphorus (P) and potassium (K) modulated the activities of methanogens, methanotrophs in a long term (20 y) experimental system undergoing limitation in either one or both nutrients."

#Comment (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.

Answer: It has been rewrite as below:

"Results showed that a large amount of CH4 emitted from paddy soil at rice tillering stage (flooding) while CH4 flux was minimum at ripening stage (drying). Compared to NPK treatment, the soils without P input significantly reduced methane flux rates, whereas without K input did not. Under P limitation, methanotroph transcript copy number significantly increased in tandem with a decrease in methanogen transcript abundance, suggesting that P-deficient induced changes in soil physiochemical properties in tandem with rice plant growth might constrain the activity of methanogens, whereas the methanotrophs might be adaptive to this soil environment. In contrast, lower transcript abundance of both methanogen and methanotrophs were observed in K-deficient soils. Assessments of community structures based upon transcript indicated that soils deficits in P induced greater shifts in the active methanotrophic community than K-deficient soils while similar community structures of active methanogens were observed in both treatments."

#Comment (5): L47. Replace metabolic genes with functional genes

Answer: We have revised as requested.

#Comment (6): L112. Gas sampling means static chamber measurement of CH4 flux

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Answer: Yes, we also supplement some details of the measurement of CH4 flux rate using soil incubation experiment in line 128-142 in the revised version.

#Comment (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 to explain the dynamic changes of methane flux in the field. The first section can be the site description only.

Answer: We have revised as requested.

#Comment (8): L121. How were samples kept for transportation before measurement. How to avoid leakage?

Answer: Gas samples were stored in pre-evacuated vials (Labcolimited high Wycombe UK), which can prevent gas leakage.

#Comment (9): L124. With slight modification

Answer: We have revised as required.

#Comment (10): L139. T-RFLP fingerprinting

Answer: We have revised as required.

#Comment (11): L166. Real-time quantitative PCR

Answer: We have revised as required.

#Comment (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

Answer: We have revised as required.

#Comment (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

Answer: We have added some information regarding the management of rice cultivation, including fertilization and water management in line 105- 112, the details are as below: “For the late rice-cropping season when we sampling, urea was applied with three splits, 40% as basal fertilizer, 50% as tillering fertilizer and 10% as panicle fertilizer. The P and K fertilizers were applied as basal fertilizers before rice transplanting. The basal fertilizers were well incorporated into the soil by plowing to 10-20 cm depth 2 days before rice planting, and the top-dressing was surface broadcasted. Consistent with the water management in local late rice-cropping system, flooding was initiated after early rice harvest before late rice transplanting, and maintained until 10 days before rice harvesting. During this period, a 7 days drainage episode was implemented at late tillering stage.”

#Comment (14): L235. MOB population size

Answer: In this section, we presented both size and community structure of MOB population, hence we use MOB population here.

#Comment (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.

Answer: We describe this in line 323-381.

#Comment (16): L298. These different environment conditions should have been in close association with growth status of rice plants under different nutrient regimes.

Answer: We have deleted this sentence.

#Comment (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?

Answer: We do not have solid evidence to verify that methanotrophs can produce P-liberating enzyme now, here we just speculated that some methanotrophs may produce P-liberating enzymes based on the studies carried on other microbes, further studies based on methanotrophic strains still need to be investigated.

#Comment (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"

Answer: We have added "Although we do not know the real mechanisms about the enrichment of Methylococcus/Methylocaldum under such a poor soil P nutritional status, it could be speculated that the possible adaptations of these MOB groups to a P deficient environment might be attributed to one or more adaptive strategies." in line 363-366.

#Comment (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 flx 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 adapated to changes in soil physiochemical properties in association with rice plant growth under chronic nutrient constraints.

Answer: Conclusion now appears as follows:

P-deficient soils showed significantly lower CH4 flux. This might be attributed to the restriction 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. In contrast, K-deficient did not affect the CH4 flux, which might be caused by the reductions of both methanogenic and methanotrophic activities. Comparatively, more variations within community composition of the active methanotrophs were observed in P-deficient soils than that in K-deficient soils, whereas both P- and K-deficient soils shared similar active methanogenic community structures. We have observed these effects in our quaternary red soils, but to what extent it is transferrable to other soils remains to be established.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-213/bg-2016-213-AC3-supplement.pdf

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