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Interactive comment on "Competitive interactions between methane- and ammonia-oxidizing bacteria modulate carbon and nitrogen cycling in paddy soil" *by* Y. Zheng et al.

Anonymous Referee #1

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The authors present an intruguing experiment that demonstrates that urea fertilization combined with a high methane concentration (~10,000 ppm) may inhibit ammonia oxidizers and ammonia oxidation. The authors provide hints that type I MOB were N-limited and outcompeted the obvioulsly the much slower responding AOB when utilizing urea.

Used methods (amplicon pyrosequencing, DNA stable isotope probing) are state of the art methods and all experiments were well conducted. The english is largely of good quality.

The reviewer has some major concerns:

1) It would be extremely helpful to present in figure 5 not only MOB identities based on the old fashioned classification system (typeI or II), but name genera, as the authors do then finally in the discussion section and Fig. 3.

2) Type II methanotrophs did not rapidly respond to added methane or urea. Are the detected organisms known to be diazotrophic, i.e., are these specialists that respond under N-limited conditions? Please, discuss this issue in the revised manuscript version.

3)Before the experiment soil was pre-incubated. The reviewer did not see any data that documents, which changes in the methanotrophic and ammonia-oxidizing communties occurred during this pre-incubation period. This lack of informtion make the relevance for the in situ situation less likely. Please, discuss this issue in the revised manuscript.

4) Methanol-oxidizers: The authors do not explain how they decided, which of the detected taxa were methanol-utilizers (this is also not documented for ammonia-oxizers, nitrite oxidzers, and methanotrophs). There a lot of methanol-oxidizers known that occur in soil and were likely overlooked when defining this functional group (for reference Kolb 2009 FEMS Letters, Stacheter & Kolb 2013 FrontMic).

5) Fig. 1, The reviewer thinks, that it would improve understanding of the complex experiment, when nitrate and ammonia data would be presented as line graphs in a separate figure.

6) Fig. 5, Is the sequence coverage high enough to allowed for statistical comparison of single datasets? Please, provide coverages and rafaction analyses. Please, correct in the label of the y-axis '...on genus level...'

7) Please define in the beginning of the text once the abbreviation 'd.w.s'. It means 'dry weight of soil'?

8) Do the authors also consider 16S rRNA phylotypes of the genus Nitrosococcus as AOB? Where these AOB detected?

9) Discussion. The authors state that denitrfication took place suggesting an reduced oxygen availability (3908, ln 24-26). a) The authors did not provide any data on this. b) Denitrification can be very active at slightly lowered oxygen levels. The whole issue is pure speculation.

10)Discussion: The authors stated that MOB have a 'memory' for optimal growth conditions. The whole concept sounds arkward. Such a memory might occur somehow on community level or might just be a misinterpretation because the phylogenetic resolution of such studies are too imprecise and the found identical taxa were not identical on phenotypic level. Please, remove it or extend this point with more details.

minor comments

abstract: The final conclusion (last sentence)is not very concise and convincing. Please, provide a more conclusive statement what can be learned on competition between AOB and MOB in rice field soil. This statement is the take home message.

3895 ln25-27, What do you intend to state here. Please, find a more concise wording.

3896 ln 21, correct '...methanotrophs might...'

3897 ln9-14, Please provide a more sharpened rational why the study is important.

3898,ln2-12, Why was no additional control with only 12CH4 being used.

3899-3900, Please, put in references for the used SIP protocol.

3901, ln6 correct '...high-quality...'

3901, ln 25, it does make any sense to cluster pmoA sequences at a level of 97% similarity. It has been suggested that an average similarity of 87% is species-indicative. Of course any threshold can be used, but then a rational is mandatory.

3908, ln9-10 correct 'The ratio of N to CH4 is approximately 0.11 ...'

3908, ln 16 correct '...mineral N,...'

3911, ln 12, correct '...low methane habitats.'

3911, ln 15 correct '...in the pmoA gene...'

3911, ln 13-27, Can you exclude that the pmoA primers and 16S rRNA primers did not cover the same diversity of organisms. If not, please, note also this as another technical challenge when comparing 16S rRNA gene with pmoA datasets.

3913, ln 5 correct '...three species...'

3913, ln 8-10, the reviewer is not convinced that substantial amounts of formaldehyde would be relased. Normally formaldehyde is to its largest amount bound to cofactors to keep the cell-internal concentrations as low as possible. This system is highly efficient and works as well at high millimolar CH4 concentrations. Methanol is a completely different issue since the reaction rate of the MeOH dehydrogenase is usually such low that methanol production at high methane concentrations exceeds its consumption. This process is located in periplasm and thus, substantial amounts of an metabolic intermediate can be released. Please, remove formaldehyde from the statement or provide literature evidence that it might have happened.

3914, correct '...communities...'

3914, ln 13-15. This is very speculative based on the presented data. The authors did not provide any evidence for oxygen depletion.

3914, The study did not provide any direct evidence that methanol or any other metabolite was assimilated by other methylotrophs. Thus, the sentence is overstated. Please, down tone it a bit.

Fig S3, correct in figure legend '..affiliation...' and NOT '...designation...'

Interactive comment on Biogeosciences Discuss., 11, 3893, 2014.