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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 #3

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In this study the authors describe community shifts of methane-oxidizing and ammonia-oxidizing bacteria in SIP-incubations of paddy soil mesocosms under treatments with either methane or urea or both. Based on the results of methane consumption and nitrate production in the mesocosms and the results of pyrosequencing of 16S rRNA, pmoA and amoA genes, they conclude that addition of methane or methane and urea stimulates Methylosarcina-like MOB or Methylosarcina and Methylobacter-like MOB, respectively, within the methanotroph community. Furthermore, they conclude that urea alone stimulates AOB in general and Nitrosomonas-related AOB within the total AOB community but that AOB are inhibited in the methane- or methane plus urea-treated mesocosms. As has often been the case before in similar studies, the authors observed no or only weak growth of AOA in their mesocosms. This manuscript provides interesting data on methantrophs and ammonia oxidizers in paddy soils and comes up with some interesting conclusions about potential interactions between these two groups. My major criticism is that it is not clear to what extent these observations are relevant for mechanisms that can be assumed to take place under field conditions. The composition of the methanotrophic community and also their relative fraction within the total microbial community changed substantially under the conditions of the 19-days-incubation experiment. If one assumes that the original community was already adapted to the field conditions, it remains an open question if the observed changes are really representative of mechanisms taking place under field conditions. Here, the authors should provide more information about to what extent the mesocosm incubation was representative of fertilizer applications and methane availability at the sampling site, or to what extent it may reflect responses of microbial communities to fluctuations in environmental conditions. Another critical aspect is that the discussion focuses mostly on biogeochemical processes and interactions while the largest fraction of the results part is dealing with the pyrosequencing derived community data. Consequently, a large part of the biogeochemical conclusions is based on assumed metabolisms derived from the assigned taxonomies. I wonder if this information is sufficient for some of the conclusions regarding biogeochemical interactions. Another aspect related to this is that as far as I can see, triplicate samples were pooled for pyrosequencing analysis but the error range of the method itself remains unknown. So here, small changes in community composition over time or between treatments should be interpreted with caution. Moreover, in the description and discussion of the pyrosequencing data, the authors should generally point out clearly that these data provide relative abundances of sequence reads but not necessarily relative abundances of microorganims within the total community. The authors should state more precisely the objectives of their work and present a clear hypothesis what kind of interactions they expected to find. Moreover, the discussion is lengthy in some parts and should be shortened. Repetition of results should be avoided.

Specific comments:

p.3896, l. 24-26: "However, the research. . .methan oxidation.“ It is unclear what this sentence means. Please rephrase. p. 3898, l. 9: please write "microbial community composition“ p. 3898, l. 2-8: The authors state that hydrolysis of 13C-urea was used to generate ammonia and 13C-CO2. Why did they then add extra 13C-CO2? Please explain. p. 3901, l. 23-24: Please provide more information about denoising and read length of the pyrosequencing reads and provide a reference for "as described previoulsy“. p. 3904, l.12-13: Do the relative abundances given here refer to the total microbial community or only to the relative fraction within MOB? Please clarify. p. 3905, l. 19: Please write here "16S rRNA gene sequences of methanotrophs“. p. 3907, l. 18: Please describe "nitrogen effects“ in more detail. p. 3912, l. 14-15: Interesting is this link between nitrogen. . .methylotrophs in soil.“ This statement is too general, please specifiy. p. 3912, l. 28-30: As far as I can see, the statement that a large part of the N applied was assimilated by MOB is just an assumption (see p.3908, l. 11-13). This should be stated more clearly here. p. 3913, l. 28-29: Here, the wording with "former“ and "latter“ makes the sentence rather complicated. Please rephrase. p. 3914, l. 17-18: This last sentence remains very general.

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