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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 #2

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Zhent et al presented an interesting investigation of "competitive interaction" between MOB and AOB in a paddy soil using molecular ecological approaches. The topic is obviously of intense interest to the environmental microbiology community and this reviewer agrees strongly with the authors that the interaction of methane cycle and nitrogen cycle is very poorly understood. Thus the work is topical and important to the field.

I have the following suggestions to improve.

1. it was not very clear to me why 13CO2 should be used in combination with 13C-urea (page 3898 lines 1-4). obviously urea catabolism to ammonia generate CO2. it

is also unclear to me why 13C-labelled urea (and 13CO2) is used in the 13C-methane treatment. it seems to me that the key treatment missing is 13C-methane plus 12C-urea and 12C-methane plus 13C-urea.

2. I am not sure I agree with the authors with regarding to the use of "inhibition" of AOB activity (e.g. figure 1b, 1d) in microcosms where methane is added (e.g. see discussion section Page 3903. What is very likely (also suggested by the authors) is that in the present of both urea and methane, MOB cell numbers are increased. In fact, this should be quantified e.g. by qPCR. the sequencing data only show relative abundance of AOB/MOB in the total microbial community. The relative abundance of AOB was indeed low in the treatment without methane, however, it is very likely that AOB cell numbers still increased in those treatments. Therefore strictly speaking, AOB activity was in fact enhanced in those treatments (by urea of course). Again the AOB cell numbers should be quantified (eg. by QPCR). Therefore, my point is that the use of "inhibition" of AOB by methane is in fact misleading since this implies that methane direct inhibits AOB activity (which is very difficult to perceive). The authors should make it absolutely clear that it is the relative numbers of AOB-to-MOB they refer to, but not the absolute cell counts, therefore either cell numbers need to be presented to justify the use of "inhibition" or rewording is required.

3. in page 3908 when the authors discussed mass balance of N. in general, I felt that many assumptions were made and this section reads rather speculative. for example, it is assumed that methane-carbon is assimilated with N at 4:1 ration. it is assumed that MOB oxidise 70% methane in order to assimilate 30% methane-carbon into biomass. with these assumptions it is calculated that 11% of N from urea is denitrified. whilst these assumptions are perceivable, it does not justify the fact that no efforts were made to quantify the denitrification activity and subsequent gas products (N2O, NO2 etc). My overall impression of the discussion is that it is lengthy and not focused. In my opinion, the authors do not need to discuss every aspect of the results, and discussions such as mentioned above, as it stands, is too speculative. Further experiments should be

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carried out to investigate the unaccounted N in the system.

4. an obvious missing discussion point is the investigation of the genetic potential of urea catabolism to ammonia in/with AOB and MOB. there are two well known systems for urea degradation to ammonia though either urease or urea decaboxylase/allophanate hydrolase. Do sequenced MOB have the genetic potential in urea degradation? How about AOB? How about AOA? was it simply because AOA cannot release ammonia from urea? These data are readily available and should be discussed with respect to the competition between ammonia oxidizers and methane oxidizers.

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

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