

Point-by-point response to Editor

Second revision for Manuscript ID bg-2014-97

General comments

For final publication in *Biogeosciences*, the referees suggested that your manuscript should be accepted subject to some revisions.

In your very detailed response letter you have commented on their concerns in the Interactive Discussion. In general, you followed the suggestions of the referees. Thus, the revised version of your manuscript could be published in *Biogeosciences* without further delay.

Reply: We are thankful to editor for the positive decision.

Minor Corrections

However, I have to ask you to make two little changes:

1. Line 53: I think you wanted to say: the interactions are more complicated than previously appreciated. Please add the word "more" if I was correct.

Reply: Corrected as follows:

‘These results suggest that type I methanotrophs can outcompete type II methane oxidizers in nitrogen-rich environments rendering the interactions among methane and ammonia oxidizers **more** complicated than previously appreciated.’

2. Line 545: It should read: which possesses nitrogenase and are capable of nitrogen fixing. Please add the word "are".

Reply: Corrected. Thanks!

Other technical corrections by co-authors

1. Page 2 Line 49-50 in revised text, rephrased as follows:

‘Methane addition partially inhibited the growth of *Nitrosospira* and *Nitrosomonas* in urea-amended microcosms, as well as growth of nitrite-oxidizing bacteria.’

Original one as: ‘Methane addition partially inhibited the growth of *Nitrosospira* and *Nitrosomonas* in urea-amended microcosms, in addition of nitrite-oxidizing bacteria.’.

2. Page 3 Line 60-63 in revised text, rephrased as follows:

‘The tight *coupling* between nitrogen fertilization and methane emission from rice paddy ecosystems in combination with the significant contribution of these systems to the global methane emission 15 to 25% (Bodelier, 2011).’

Original one as: ‘The *tight* coupling between nitrogen fertilization and methane emission from rice paddy ecosystems in combination with the significant contribution of these system to the global methane emission 15 to 45% (Bodelier, 2011).’has evoked numerous studies focusing on this topic.’

3. Page 4 Line 90-92 in revised text, rephrased as follows:

‘while sMMO is *only* found in a subset of MOB (Hanson and Hanson, 1996; Lipscomb, 1994; Stein et al., 2012).’

Original one as: ‘while sMMO is found only in a few species (Hanson and Hanson, 1996; Lipscomb, 1994).’

4. Page 4 Line 93-94 in revised text, rephrased as follows:

‘The known soil-retrieved methanol-oxidizing bacteria are quite diverse,’

Original one as: ‘The known soil-retrieved methanol-oxidizing bacteria was with high diversity,’

5. Page 5 Line 129-131 in revised text, rephrased as follows:

‘However, the research efforts focusing on methane effects on nitrification in natural complex ecosystems is very limited,’

Original one as: ‘However, the research focus of methane effect on nitrification in natural complex ecosystems is poor,’

6. Page 6 Line 145-147 in revised text, rephrased as follows:

‘Elucidating these interactions may offer solutions for the effects of nitrogen on methane oxidation which are complicated and often contradictory.’

Original one as: ‘However, the effects of nitrogen on methane oxidation are complicated and contradictory results are often reported.’

7. Page 11 Line 294-296 in revised text, rephrased as follows:

‘Methane oxidation activity was assessed by determining the amount of methane consumed in soil microcosms over the incubation **time** of 19 days and displayed a strong capacity of methane consumption in the paddy soil tested (Fig. S1).’

Original one as: ‘Methane oxidation activity was assessed by determining the amount of methane consumed in soil microcosms over the incubation **course** of 19 days, and the strong capacity of methane oxidation was observed in the paddy soil tested (Fig. S1).’

8. Page 11 Line 304-306 in revised text, rephrased as follows:

‘The presence of CH₄ in the headspace of urea-amended microcosms significantly **suppressed** production of soil nitrate at day 19, although statistically significant **differences were** not observed at day 5 (Fig. 1b, Fig.S2).’

Original one as: ‘The presence of CH₄ in the headspace of urea-amended microcosms significantly **inhibited** production of soil nitrate at day 19, although statistically significant **inhibition was** not observed at day 5 (Fig. 1b, Fig.S2).’

9. Page 13 Line 361-362 in revised text, rephrased as follows:

‘**Although** type II methanotrophs dominate the MOB community in **original soil** at day 0,’

Original one as: ‘**Though** type II methanotrophs dominate MOB communities in **background soil** at day 0,’

10. Page 19 Line 515-516 in revised text, rephrased as follows:

‘However, the fate of unaccounted remaining nitrogen (11%) **needs to be verified by further experimentation.**’

Original one as: ‘However, the fate of unaccounted remaining nitrogen (11%) **need further experiment to investigate.**’

11. Page 19 Line 532-536 in revised text, rephrased as follows:

‘there is a niche for methanotrophy **outcompeting** nitrifying communities. Nitrifiers can operate in the absence of competition with MOB **when the latter are inactive** due to energy-depletion as the result of N₂-fixation.’

Original one as: ‘there is a niche for methanotrophy **where they seem to overwhelmingly outcompete** nitrifying communities. Nitrifiers can operate in

the absence of competition with MOB, which may be inactivated due to energy-depletion as the result of N₂-fixation.'

12. Page 20 Line 550-552 in revised text, rephrased as follows:

'However, significant growth of type II MOB only occurs after 19 days of incubation suggesting that they either have lower growth rates than type I or type II MOB depend on the activity of type I.'

Original one as: 'However, significant growth of type II MOB only occurs after 19 days of incubation suggesting that either lower growth rates as compared to type I or dependency of type II MOB on the activity of type I.'

13. Page 22 Line 616-617 in revised text, rephrased as follows:

'In the meantime, the toxic effect of metabolic intermediates of methane oxidation on nitrifying communities remains elusive.'

Original one as: 'In the meantime, it remains elusive about the toxic effect of intermediates substance during methane oxidation on nitrifying communities.'

14. Page 24 Line 668-670 in revised text, rephrased as follows:

'Assimilation of N by MOB is the most likely mechanism for inhibition of ammonia oxidizers by methane addition.'

Original one as: 'Assimilation of N possibly might provide mechanistic mechanisms for inhibition of ammonia oxidizers by methane addition.'

In addition, we update some references for a better relevance

15. Page 4 Line 97 in revised text, new references added in blue:

The family *Methylophilaceae* is the known obligate methylotrophs that use methanol as the sole source of carbon and energy (Bratina et al, 1992; Devries et al., 1990; Kolb, 2009)

16. Page 4 Line 102 in revised text, new references added in blue:

the discovery of ammonia-oxidizing archaea (AOA) has suggested an important role of archaeal nitrification in the global nitrogen cycle (Könneke et al, 2005; Prosser and Nicol, 2012).

17. Page 4 Line 104 in revised text, new references added in blue:

However, until now the relative contribution of AOB and AOA to ammonia oxidation in agricultural soil is still unclear (Jia and Conrad, 2009; Pratscher et al, 2011; Zhang et al, 2010).

18. Page 5 Line 137 in revised text, new references added in blue:

DNA-SIP has been employed to identify the active methanotrophs (Morris et al, 2002) and ammonia oxidizers in soils (Jia and Conrad, 2009).

Reference

- Bratina, B. J., Brusseau, G. A., and Hanson, R. S: Use of 16S ribosomal-RNA analysis to investigate phylogeny of methylotrophic bacteria, *Int. J. Syst. Bacteriol.*, 42, 645-648, 1992.
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- Jia, Z. and Conrad, R.: Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil, *Environ. Microbiol.*, 11, 1658-1671, 2009.
- Kolb, S.: Aerobic methanol-oxidizing bacteria in soil, *FEMS Microbiol. Lett.*, 300, 1-10, 2009.
- Könneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B., and Stahl, D. A.: Isolation of an autotrophic ammonia-oxidizing marine archaeon, *Nature*, 437, 543-546, 2005.
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- Prosser, J. I. and Nicol, G. W.: Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation, *Trends Microbiol.*, 20, 523-531, 2012.
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- Zhang, L. M., Offre, P. R., He, J. Z., Verhamme, D. T., Nicol, G. W., and Prosser, J. I.: Autotrophic ammonia oxidation by soil thaumarchaea, *P. Natl. Acad. Sci. USA*, 107, 17240-17245, 2010.