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Discussions

Interactive comment on "Distribution of ammonia oxidizers in relation to vegetation characteristics in the Qilian Mountains, northwestern China" *by* X. S. Tai et al.

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Dear Dr. Jia,

Thank you very much for handling of our manuscript and thanks a lot to the anonymous referee #3 for his/her wonderful comment. Our replies summarized from the discussions of all the authors are shown as follows.

1. The topic is of general interest to readers interested in distribution patterns of ammonia oxidizers in different environments. However, in this merely descriptive inventory of AOA and AOB abundance and community composition, the biogeochemical relevance of the observed community patterns in terms of nitrification activity remains unclear.

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Nitrification rates were not measured, and the soil chemical data are not discussed in this context. Consequently, the contribution to our current knowledge is very limited.

Thanks a lot for the good suggestions. Activities of AOA and AOB in soils of the study region were not detected in situ. Combination of nitrification activity and ammonia-oxidizing communities will be paid more attention to in future. Discussing on the soil chemical data has been added in section 3.1 in the revised MS.

2. With the objectives stated at the end of the introduction, the authors follow an experimental design that has been numerous studies before. They should place their study more clearly in the context of previous research.

It is an excellent suggestion and changes have been done.

3. The sentence that "More studies about the relative contributions of AOA and AOB to ammonia oxidation are necessary" (p.5125, l. 25-26) is very general, and the aspect of ammonia oxidation activity is not addressed in this study. The author should point out more clearly what their expectations or hypotheses were regarding potential effects of meadow types on ammonia oxidizers.

That is a helpful suggestion, in the revised MS, we have talked more about the potential effects of vegetation diversity and coverage on ammonia-oxidizing community characteristics.

4. Besides, the interpretation of data needs revision. As one can see from Fig. 2b, the gene copy numbers of amoA are rather low compared to other studies and differences between meadow types are not large. Here, the authors should compare the observed gene abundance to published data from other soils, and they should discuss the observed differences among sites in the context of the error range of the qPCR method.

Thank you! We have made a re-interpretation of the Q-PCR data according to this good suggestion.

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5. Similarly, I wonder if 50 sequences per clone library are enough to reliably calculate relative fractions of individual phylotypes and to use these data for multivariable statistics. What was the coverage of the clone libraries? Was it the same for all the samples to allow the comparison among samples?

The rarefaction curves and the coverage of the clone libraries have proved that 50 clones of each library is enough for statistics and these information have been added in the supplemental materials.

6. Finally, large parts of the discussion consist of a summary of literature findings, however, the link to the results obtained in this study is not always clear (e.g., p. 5132, l. 1-19, p. 5133, l. 18-26). Here, a thorough revision of the discussion is needed to place the author's findings more clearly in the context of other studies. Especially the observed relationship between AOP community composition and vegetation type and coverage is poorly discussed. What could be the mechanisms by which vegetation has an influence on the community composition and abundance of AOA and AOB? Why should especially vegetation cover play a role in these relationships?

Thanks a lot for your suggestions! Now we realized that it would be much more clear that let the discussion start with listed data or showed in figures. In this revision, the discussion were changed.

Specific comments: 1. p. 5126, l. 24: Please indicate from which depth soil samples were taken.

In p. 5126, l. 20-23, the sentences "Species and numbers of plants were investigated in situ, . . . for a total of three samples from each site." have been replaced by "We placed three quadrats within each study site, species and numbers of plants were investigated in situ, as well as vegetation coverage (Wang et al., 2003, Plant Ecol.; Wu, 2011, Acta Ecologica Sinica). Five soil samples were collected from each quadrat and pooled, for a total of three samples from each site. Each soil sample was taken from the depth of 20 cm of each profile."

2. p. 5128, l. 2: Should the annealing temperature for this primer set not be 53âĎČ instead of 63âĎČ? Please check.

Done. The annealing temperature has been tested by preliminary experiment and referred to Wang et al. (2012, Can. J. Microbiol.).

3. p. 5128, l. 15: Why did the authors use a cutoff of 0.03 for OTU assignment? Please explain.

Pester et al. (2012, Environ. Microbiol.) have demonstrated that presence or absence of amoA OTUs (97% identity level) correlated with geographic location. It is consistent with the experimental designing of our study.

4. p. 5128, l. 25: Please provide the name of the software used for tree calculation.

Mega (Version 4.0) was used for amoA phylogenetic tree calculation. This information has been added in section 2.4 in the revised MS.

5. p. 5129, l. 16: What dose " a high NH4+" mean? Please provide numbers.

In p. 5129, l. 16, "a high NH4+" was changed to " a high NH4+ (86.2 - 91.8 mg âĂć kg-1)" in the revised MS.

6. p. 5129, l. 18: Please provide gene copy numbers in the text. What was the estimated relative fraction of AOA and AOB within the total community? Would this yield the same overall picture of differences among sites?

These information have been added as a table in the revised MS.

7. p. 5130, l. 4-10: Please provide a reference for the nomenclature of AOA phylogeny.

Done.

8. p. 5131, l. 3-8: Why did the authors not include NH4+ in the RDA analysis?

Ammonium (NH4+) with less information load was not applied to make a correlation analysis with the ammonia-oxidizing community characteristics in the RDA analysis

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(Table 2). Otherwise, the RDA analysis has been changed according to the relevant suggestions made by all the referees.

9. p. 5132, l. 23-25: What is the basis of defining a new AOA group? What was the sequence distance to other groups?

Pester et al. (2012, Environ. Microbiol.) suggested a new nomenclature of species-level OTU using an inferred species threshold of 85% amoA identity. The sequence divergence between the new group to other typical groups is more than 15% detected by program of Mothur (v.1.31.2; Last updated: 6/13/2013; Schloss et al., 2009, Appl. Environ. Microbiol.). The suspected new group was remotely related to the cultured members of Nitrososphaera. More culture-dependent researches are needed to prove the existence and activity of the suspected new group in our future works.

10. p. 5132, l. 25: What is so special about the study region? Please explain.

The study region locates at a high-altitude with strong UV-radiation, low temperature and poor nutrient (Wang et al., 2009, Chinese Sci. Bull.). Alpine meadow is a dominant vegetation type and covers most of the study region (Kato et al., 2006, Global Change Biol.). Climate and vegetation of the study region are all unique. As a result, microbial communities in topsoil layers encounter extreme conditions that may lead to unique survival adaptations and differences in the community composition (Zhang et al., 2009, FEMS Microbiol. Ecol.).

11. p. 5133, l. 17-18: A similar sentence already appears in the results section (p. 5130, l. 26-27). This conclusion is too general, please be more precise.

The repeated sentence in p. 5130, l. 26-27 has been omitted in the revised MS and the conclusion in p. 5133, l. 17-18 has been rewritten.

12. Fig. 3: What is the difference between the two graphs shown in this figure?

It is a fault during the first revising, the upper figure should be removed.

13. Fig. 4 and 5: More reference sequences from other studies and cultured species should be included in the tree calculation.

The amoA phylogenetic trees have been recalculated according to the suggestions in the revised MS.

Thanks again for the excellent suggestions provided by the anonymous referee.

Yours sincerely, Guangxiu Liu

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Interactive comment on Biogeosciences Discuss., 11, 5123, 2014.