

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

Anonymous Referee #2

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General comments

This manuscript describes the application of an *amoA* gene survey in soils of five meadow types in a mountain range of northwestern China with quantitative PCR, cloning and phylogenetic analysis. The authors report on the effects of environmental properties on the abundance and community structure of archaeal and bacterial ammonia-oxidizers. Due to technical and analytic flaws (see below) I have doubts about the justification of most of the conclusions.

Specific comments

1. Large pieces and whole sentences have been plagiarized from published papers
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without any change. This is not good scientific practice and needs to be changed! Additionally, the manuscript shows poor English in several places and needs to be corrected by a native speaker.

2. Statistical analysis have not been applied correctly or misinterpreted: a. An RDA analysis only shows the variance that is explained by the factors included in the model and therefore always sums up to 100% over all axes. Therefore, RDA conducted here did not show that everything is explained! b. Furthermore, factors that go into the RDA need to be centered (z-transformed) in order to make them comparable to each other. Otherwise a change of 1 unit in pH is treated equal to a change of 1 unit of concentration of nitrate for example, which leads to strong underestimation of the influence of pH in this example. c. Finally, AOA and AOB communities should be analyzed separately by RDA. d. In PCA analysis no assessment of significantly different groups of AOA or AOB has been made. Without confidence intervals around supposedly separated groups of phylotypes conclusions cannot be drawn! e. Assigning *amoA* OTUs at a level of 0.03% sequence divergence is wrong! It should rather be 0.15%! Check Pester et al 2011, EMI for reference. Binning at such a high level of similarity as done here leads to severe overestimation of diversity and hence all diversity results and conclusions are flawed! f. How were significant differences of qPCR results assessed? Figure 2b gives indications for sig. differences. . . Also check for significant differences of diversity and abundance between AOA and AOB before making conclusions on who is more diverse! g. How many sequences were used to generate the phylogenetic trees? Especially for AOA it looks like only six sequences of well-known AOA have been incorporated into the analysis. This is well below what is needed and does not suffice to postulate the finding of a new phylogenetic group see Pester et al 2011, EMI for a good tree.

3. Q-PCR products should be analyzed by gel to see if the amplicons actually represent AOA and AOB, as numbers found are very low and could also be false positives. What was the limit of detection for the qPCR assays? Please report!

4. The discussion reads rather boring and more like an introduction. It would make

much more sense to condense the knowledge from literature and relate it to the findings

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