Biogeosciences Discuss., 11, C2807–C2810, 2014
www.biogeosciences-discuss.net/11/C2807/2014/

Biogeosciences
Discussions
Open Access

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

X. S. Tai et al.

leafturnred@163.com

Received and published: 25 June 2014

Dear Dr. Jia,

Thank you very much for handling of our manuscript and thanks a lot to the anonymous referee #2 for his/her wonderful comment. Our replies summarized from the discussions of all the authors are shown as follows.

1. Large pieces and whole sentences have been plagiarized from published papers 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.

Thanks a lot for your kindly reminding! We have taken extra care to organize the manuscript and tried our best to avoid plagiarizing. If there are still some left, it must be coincidence. After final revising, the MS will be delivered for language polishing.

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 sum 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 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.

Done.

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!

Significance assessment of the assignment of AOA/AOB to the different meadow types have been done and the confidence data have been added in the revised MS.

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!

Thank you very much for your good suggestion. To our opinions, Pester et al. (2012, Environ. Microbiol.) suggested an inferred species threshold of 85% amoA identity, however, presence or absence of amoA OTUs could be 97% identity level and the data could be used for making correlation with environmental conditions. Besides, previous

studies have applied a cutoff of 0.03 for assigning of amoA OTUs (Francis et al., 2005, PNAS; Zhang et al., 2009, FEMS Microbiol. Ecol.; Huang et al., 2011, Extremophiles; Hu et al., 2013, J Soils Sediments).

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!

Significance of Q-PCR results were assessed by the program of multiple comparisons with SPSS (Version 16.0). Besides, significant differences of diversity and abundance between AOA and AOB have been done and all of these information have been added in the revised MS.

g. How many sequences were used to generate the phylogenetic tree? 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.

The amoA sequences of those typical cultured ammonia oxidizers have been applied to construct the phylogenetic trees. In the revised MS, both of the phylogenetic trees have been replaced by the ones in the supplemental materials adding with the access numbers.

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!

All Q-PCR reactions were performed in duplicate. The amplification yielded reliable exponential patterns with a template amount in the range of 101 to 108 amoA gene copies. This information has been added in section 2.3 of the revised MS.

4. The discussions reads rather boring and more like an introduction. It would make much more sense to condense the knowledge form literature and relate it to the find-

ings.

Thank you very much for your helpful suggestion. We have rewritten the discussions according to your suggestion in the revised MS.

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

Yours sincerely, Guangxiu Liu

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