**Biogeosciences
Discussions**

Interactive comment on "Ecosystem-specific selection of microbial ammonia oxidizers in an acid soil" *by* M. Saiful Alam et al.

Anonymous Referee #2

Received and published: 27 March 2013

General comments: The authors studied abundance and population structures of AOA and AOB in the soil samples collected from the ecological experimental station. To investigate community diversification and population shifts of AOA and AOB in acidic soils, the authors selected maize and paddy rice fields receiving long-term experimental fertilization loadings as model ecosystems. The authors revealed that the fertilization processes can increase the numbers of AOA and AOB than the non-fertilized sites. The group 1.1a AOA dominated in paddy fields while the group 1.1b AOA were mainly found in upland soils. On the other hand, AOB populations were monotone and mainly occupied by the cluster 3 Nitrosospira. Although some aspects of the study might be of potential interest for readers, I have major concerns about the goal of this research, experimental designs, interpretation of data sets and discussions.

Title: ecosystem-specific selection in an acidic soil. The title of this manuscript is quite inscrutable. I think this title does not reflect the work performed in this study.

I see the mismatch between the research goals and actual experimental approaches. In other word, the experimental sets used in this study were not suitable to answer author's ecological questions. For example, the last sentence of the introduction, the authors say "Conversion of upland acid soil to paddy field can result in the depletion of oxygen in soil, leading to ecological pressure for the evolution of obligate aerobic AOA and AOB". I believe the controlled model experiments are necessary if the author's major interest is the shift (or evolution?) of AOA and AOB populations with the gradient of oxygen. Not only oxygen level but also various factors were different between maize and rice fields. Thus, it is almost impossible to conclude the difference of AOA communities found in maize and rice fields were actually created by the difference of oxygen level. First, I must point out that the authors did not mention how the difference of two crops could influence the AOA and AOB populations. Second, the sampling time was different between these two sites. I believe the seasonal population changes must be considered. Moreover, paddy field sampling was done after harvesting of late rice. It indicates that the field was already dry and not anoxic. Third, the pH levels of upland and paddy fields were different each other (Table 1). Can the authors eliminate the possibility that the shift of AOA populations was mainly caused by the increase of pH? Forth, the interactions between ammonia oxidizers and other competitive and corporative microorganisms were unclear. I think paddy rice fields are more likely eutrophic freshwater lake sediments while maize soils are typical soil environments. In general, group 1.1a AOA are common in aquatic environments while group 1.1b AOA are common in soil environments. Without thinking the level of oxygen, the shift of AOA population might be explained by other environmental factors since these two soils are so different in many ways. Moreover, the surface of paddy field sediment is occasionally saturated by oxygen because of the photosynthesis of benthic eukaryotic algae and cyanobacteria. The authors collected the soil samples with the depth of 15 cm. I am afraid this rough soil sampling ruined the real depth profiles of microbial populations.

I could not find the accession numbers of DNA sequences determined in this study.

Specific comments: P1718, lines 2-3. Please delete "and evolutions". I believe it is hard to discuss about the evolution of ammonia-oxidizing microorganisms with the experimental design of this manuscript. Line 9. Please spell out amoA. Line 21. ...the marine group 1.1a AOA could be better adapted to low-oxygen environment .... As mentioned above, the experimental observation in this study cannot specify the influence of oxygen due to the inappropriate experimental settings.

P1721, line 20. Nine different treatments. But 10 different treatments are seen in upland soils in Fig. 1.

P1723, line 4. Please remove "fresh". Line 6. "at speed 6.0 for 40s" is better. Line 18. Please specify the types of plasmid used, hopefully with accession numbers.

P1724, lines 16-19. I believe the authors were at least able to use the nested approach including the first amplification with no GC clamp bacterial amoA primers then with GC clamp bacterial amoA primers. I believe it is almost impossible to compare AOA and AOB communities by using completely different two molecular techniques. The authors were able to analyze AOA communities by using clone library approaches as well as bacterial case.

P1728, line 11. Which statistical analysis was used? Lines 24-26. Was that dry or wet when the sampling was done?

P1732, 19-21. Not strong! Physiology studies using pure cultures or enrichment cultures are ideal to see the oxygen responses.

P1741. Table 1. Why we do not see the difference of nitrogen and carbon loadings between NPK and 2NPK treatments?

P1743. Fig. 2. Since the authors obtained beautiful DGGE profiles, the authors may apply clustering methods and diversity index calculation.

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P1745. Fig. 4. I think the authors should mention the original sources of AOB clustering.

End

Interactive comment on Biogeosciences Discuss., 10, 1717, 2013.