

## Point-by-point response to the comments of the reviewer #1

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### General comments

1. To reveal niche specialization of ammonia-oxidizing archaea and bacteria (AOA and AOB, respectively) in acidic soils, the authors studied the distribution and community composition of both groups along a moisture gradient from a paddy soil to an upland maize soil.

Although the authors did good work, there are a number of weaknesses in the manuscript. In the first place, the authors have analyzed the communities of AOA and AOB with different methods, which are DGGE and clone library construction, respectively. It is very likely that both methods screen the respective communities to a different depth.

**Reply:** We would like to thank anonymous referee #1 for the overall positive comment, and for pointing out the weaknesses that have resulted in the misleading in the original manuscript.

Here we acknowledge that the communities of AOA and AOB were analyzed with different methods, which are DGGE and clone library construction, respectively. It is very likely that both methods screen the respective communities to a different depth. However,

This study was performed to relate the community changes of AOA and/or AOB to conversion of upland soil to paddy soil, rather than to make pairwise comparison between AOA and AOB communities. DGGE showed clear shift of AOA communities, while we are not able to get PCR amplicons of AOB for DGGE analysis. Therefore, molecular survey of AOB was done by clone library construction.

To avoid misleading, the title was rephrased as '[Conversion of aerobic upland to flooding paddy field alters community structure of archaeal ammonia oxidizers in an acid soil](#)'.

2. In the second place, the authors have shown that in the creation of paddy fields from upland soil by flooding these soils for more than hundred years, the soil carbon content and the pH increased. Nevertheless, the authors hang their entire argument for the change in communities on the restricted availability of oxygen in flooded soils (that they have not measured), while it is known that both organic

nitrogen (as part of the soil carbon) and pH are selective factors in relation to niche specialization between AOA and AOB.

Hence, I believe that the present manuscript is not acceptable for publication and should be rejected.

**Reply:** We admit that soil organic nitrogen and pH may play an important role, in addition to the flooding management. Therefore, the balanced discussion was made in the revised ms by including the possible effect of soil organic nitrogen and pH on community changes of AOA in acid soil.

After conversion of aerobic upland to paddy field, it appears plausible that the depletion of electron acceptor served as the primary factor influencing community changes of AOA, because long-term field flooding management leads to sequential reduction of electron acceptors such as O<sub>2</sub>, Fe<sup>3+</sup> and Mn<sup>5+</sup>, which in turn results in significant elevation of soil pH and soil organic carbon. Therefore, it is likely that soil pH and organic carbon may be the secondary driving force. This was further supported by the following experimental results.

As for upland soils, positive correlation between pH and AOA community changes was not evidenced (Fig. 5). For example, there are 10 different long-term field treatments using different fertilizer combinations since 1986, and soil pH varied from 3.68 to 5.07. However, we observed no significant changes in DGGE fingerprints of archaeal *amoA* genes between CK (pH 3.98) and P plots (pH 4.21) of upland soils (Fig. 1). Similar results were obtained for the 9 different treatments of paddy soil.

#### **Specific comments of the reviewer #1**

1. Lines 2-3 what about the availability of other nutrients such as phosphate, carbon dioxide and iron. I can imagine that they vary also in acidic soils. The authors may better say "the major energy-generating compounds, i.e. ammonia and oxygen"

**Reply:** Corrected.

2. Line 10 Insert "a" before "slight decline"

**Reply:** done.

3. Line 23 Replace "as" by "is".

**Reply:** done.

4. Lines 28-29 Can this still be stated with the knowledge on the global importance of the Anammox bacteria?

**Reply:** rephrased as ‘Ammonia oxidation is the first and rate-limiting step of nitrification that plays an important role in global nitrogen cycle’.

5. Lines 32-46 Order of the sentences not clear. Say first something on the genomics and metabolism and later something on the mutual distribution and community composition of the AOA and AOB in (acidic) soils

**Reply:** done

6. Line 33 Bacteria and Archaea not italic

**Reply:** Corrected

7. Lines 48-49 Do natural environments include anthropogenically disturbed ecosystems? Please describe what is meant with natural.

**Reply:** Natural environmental was replaced with ‘complex environment’.

8. Line 33 Line 57 Contrasting to what? The former sentence?

**Reply:** rephrased ‘A number of studies have shown that long-term fertilization significantly altered community structure of AOB rather than AOA in alkaline soil (Chu et al., 2007; Shen et al., 2008; Wu et al., 2011), while in acid soils it appears that AOA rather than AOB communities responded positively to field fertilizations (He et al., 2007; Wessén et al., 2010)’.

9. Line 75 Insert “the” before “natural environment”

**Reply:** done

10. Line 76 Change order to “different long-term fertilizations”

**Reply:** done

11. Line 79 Insert “from” before “maize field”

**Reply:** done

12. Line 79 Change “are originated” to “originate”

**Reply:** done

13. Line 81 Insert “the” before “soil”

**Reply:** done

14. Line 81 Replace "ecological" by "environmental"

Reply: done

15. Lines 122-124 Samples from the upland soil were collected from 0-20 cm depth and in April, whereas samples from the paddy soil were collected from 0-15 cm depth and in November? Why these differences and could they have affected the outcome of the analyses?

Reply: This sampling strategy is generally employed for soil physiochemical analysis. The reason is that conversion of aerobic upland to flooding paddy field after hundred years leads to significant accumulation of soil organic matter, while the fertility of upland acid soil is still considerably low. Therefore, soil at 0-20 cm depth is more representative of paddy soil, while 0-15 cm depth reflects the most active site of upland agricultural soil in this study. (Note the acid soil is notoriously infertile in southern China tested in this study). This difference is not expected to affect the outcome of the analyses because the flooding management of >100 years likely play a more important role.

16. Lines 176-178 what could have been the consequences of comparing AOA and AOB communities by to different methods, i.e. DGGE and cone libraries, respectively? Both methods will yield a different insight in the community composition.

Reply: We believe it is not comparable.

17. Lines 200-202 Why that specific for the clone library construction and not for the DGGE?

Reply: DGGE was not performed for bacterial amoA genes because we could not obtained PCR amplicons with GC-clamp, despite using a range of degenerate primers and PCR conditions that have been used to successfully amplify these genes from other soils (Stephen et al., 1999; Nicolaisen and Ramsing, 2002). Therefore, clone library was constructed for AOB analysis for both paddy and upland soils.

18. Line 239 and following. This paragraph is apparently meant to compare the paddy and the upland soil in general. Saying then something on the effect of N amendments makes it confusing as it seems the next paragraph is meant to describe the effects of fertilization sec. Hence, I would suggest to omit the sentence on the N amendments starting in line 246.

Reply: done

19. Line 243 Omit "particularly" as the whole statement deals already with paddy soils.

**Reply:** Corrected

20. Lines 249-250 Are such differences really significant?

**Reply:** They are not statistically significant. Therefore, the term of 'significant' is not used in the text.

21. Lines 288-289 In contrast to the text, Figure 2 presents triplicates.

**Reply:** Corrected

22. Line 292 Figure 2 shows only the DGGE bands in CK, P and K. Why not N? The latter could have been more interesting in relation to the function of the AOA.

**Reply:** The N treatment is actually the starting point for this study. However, as shown in Fig. 5, it appears long-term field fertilization showed little impact on AOA communities regardless of paddy and upland soils. Fortunately we are lucky that significant difference of AOA communities was observed between paddy and upland soils. Therefore, we focused on the flooding effect rather than nitrogen fertilization.

23. Lines 294-295 Could one conclude from the numbers of DGGE bands that the paddy soils are more divers? With DGGE only the dominant genes are shown, while a large part of the diversity can still be hidden. May be, one could say that the paddy soil showed more evenness with respect to the bands of *amoA* genes.

**Reply:** Yes, we believe AOA in paddy soils are more diverse. It has been revised.

24. Lines 301-304 I miss the quantitative data that would underpin statements about dominance or changes in community composition. A phylogenetic tree is hardly the instrument to do so

**Reply:** Done. Most of cloned *amoA* genes are phylogenetically most closely associated with the *Nitrosospira* cluster 3. The quantitative data were included in the revised version for clarity.

25. Lines 308-330. The text is only based on visual inspection of the DGGE patterns. I wonder why the authors did not apply statistical

methods like the ANOSIM method to compare communities of AOA in the different treatments in the two soils.

**Reply:** Done. clustering analysis were performed and diversity index was calculated, and both of which were included in the revised version.

26.Lines 331-338. It would be helpful to include a table with the numbers found in each OTU in each treatment in each soil type.

**Reply:** Done. In the phylogenetic tree, the number of sequences found in each OTU in each treatment in each soil type has been included.

27.Lines 341-343. Please rephrase, as the present sentence is hard to follow

**Reply:** rephrased as 'The ecological force driving the emergence and diversification of microbial communities is poorly understood'

28.Lines 346-348. The authors stress the role of oxygen in the selection of AOA and AOB in flooded soils. However, as we can see from Table 1, also the amount of carbon and the pH changes. Both can also be a selective factor towards the selection of different ammonia oxidizers, especially when the carbon also comprises organic nitrogen.

**Reply:** Yes. We agree with the comments, and the possible roles of soil pH and organic carbon were discussed in the revised version.

29.Line 351 Insert "the" before "field"

**Reply:** done

30.Line 353 Insert "act" after "oxygen and ammonia"

**Reply:** done

31.Line 354 "experiments" instead of "experiment"

**Reply:** done

32.Line 357 Replace "are originated" by "originate"

**Reply:** done

33.Lines 360-361 Transfer "communities" to "structures", i.e. "community structure of obligate aerobic AOA and AOB"

Reply: done

34.Lines 362-363 Again, is it only oxygen that selects the distinct communities?

Reply: Corrected. The tone has been tuned down for a balanced discussion.

35.Line 371-372 Why would oligotrophic lakes be microaerophilic?

Reply: Corrected.

36.Line 385 Change "maintenance and emergence" in "emergence and maintenance"

Reply: done

37.Line 411 Change "growth parameters" to "kinetic parameters" as the experiments had been done in continuous culture at fixed growth parameters.

Reply: done

38.Line 414 Insert "that" before "AOB communities"

Reply: done

39.Lines 414-415 Omit "deprivation" as this is already indicated by "severe starvation"

Reply: done

40.Lines 430-433 Rephrase, as the sentence is hard to understand.

Reply: done. A number of studies have shown that long-term field fertilization altered the community structure of AOB rather than AOA in alkaline agricultural soils such as black soil (Fan et al., 2011), paddy soil (Wu et al., 2011), grassland soil (Shen et al., 2011) and sandy loam soils (Shen et al., 2008).

41. Lines 433-453. Is this conclusion not based on the use of different methods that have been applied to analyze the communities of AOA and AOB?

Reply: Yes. This study is not meant to evaluate the relative importance of AOA and AOB on nitrification in soil. Therefore, the conclusion would not be biased, despite using DGGE and clone library for AOA and AOB respectively.

42.Line 448 Again, only oxygen?

Reply: corrected.