

***Interactive comment on* “Composition of ammonia-oxidizing archaea and their contribution to nitrification in a high-temperature hot spring” by S. Chen et al.**

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Received and published: 24 January 2016

Anonymous Referee #2 Received and published: 26 November 2015

We greatly appreciate the reviewer’s constructive comments. Below, we address all the comments and questions point-by-point. The original reviewer’s comments are italicized and our responses to the reviewer’s comments follow.

General comment

(1). In this study the authors investigated nitrification activity along with the community composition and abundance of ammonia oxidizing prokaryotes in the sediment of a hot

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spring in China. The authors detected ammonia oxidizing archaea related to *Nitrosocaldus yellowstonii*, and abundances of archaeal *amoA* genes were sufficient to explain the observed nitrification rates while bacterial ammonia oxidizers were not detected. The authors concluded that nitrification in these terrestrial geothermal environments is driven by archaea. The manuscript addresses an interesting topic, however, my major concern is that the amount of data presented here is rather limited. Only two samples were taken and analyzed.

Reply: Thanks for the reviewer's constructive comments. In this study, our research goal is to verify the hypothesis that Archaea rather than Bacteria drive ammonia oxidation in high-temperature hot spring environments (We have rewritten the Introduction section and stated this point more clearly). To test this hypothesis, we selected Gongxiaoshe hot spring in Tengchong Geothermal Field as a representative hot spring in which ammonia oxidation driven by Archaea might be active, based on relatively high concentration of ammonia and widely presence of AOA genes in Gongxiaoshe hot spring. Although just two sediment samples were recovered from the margin and the bottom of the pool for incubation experiments and molecular analyses, we think those samples are representative for the current study for following two reasons:

1) Gongxiaoshe hot spring is a small pool with a diameter of ~300 cm and a depth of ~130 cm. Hot spring water in the pool is well mixed and water chemistry shows no difference in different areas of the pool.

2) Sediments of Gongxiaoshe hot spring are found to be only present at the margin of the pool and at the bottom of the pool, representing two typically sedimentary environments in this pool. Samples recovered from the same sedimentary environment (e.g. bottom of the pool) show no difference in mineralogy and geochemistry.

We have stated this point more clearly in the revised version.

(2). it is not clear from the manuscript if these samples were at least taken in triplicates. In order to confirm the message that ammonia oxidizing archaea dominate nitrification

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in this hot spring environment, results of replicate samples showing the same trend would make the outcome more convincing, including the molecular analyses.

Reply: In this study, 16S rDNA and archaeal amoA genes were determined in triplicate. We set up reactors in duplicate for four ^{15}N stable isotope tracing experiments. The results of replicate samples show the same trend. We have added more information in the materials and methods, in Line 7, Page 16260.

(3). Moreover, it is not clear in what way this study is different from the previous studies targeting ammonia oxidation in hot springs that the authors refer to. Here, the authors should point out more clearly in the introduction what new insight into ammonia oxidation in hot springs they expected to gain from their study, and/or why their experimental approach was going beyond what previous studies already did, especially in light of the fact that the amount of data presented in this manuscript is rather limited. Here, more clear research questions or hypotheses would help to better define the research goals of this study.

Reply: Thanks for the reviewer's valuable suggestion. To our knowledge, previous studies targeting ammonia oxidation in hot springs mainly focused on archaeal amoA gene (AOA) via a variety of molecular approaches (e.g. qPCR, 16S rRNA gene library and CARD-FISH). The results from these studies suggested that ammonia-oxidizing archaea (AOA) may be ubiquitous in high-temperature environments and even more abundant than their bacterial counterparts, which has led to a hypothesis that Archaea rather than Bacteria drive ammonia oxidation in high-temperature terrestrial hot spring environments. This hypothesis, however, still needs to be verified. Currently, our knowledge about the activity of AOA in such high-temperature environments is largely constrained, especially due to the data deficiency of ammonia oxidation rates. In-situ incubation experiments are urgently required to verify the potential activity of AOA and their contribution to ammonia oxidation at such high temperature environments. In this study, we determine not only the community structure of AOA but also their potential contribution to nitrification, in combination of culture-based ^{15}N pool dilu-

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tion techniques and uncultured-based molecular approaches (FISH, qPCR and a 16S rRNA gene library). We have rewritten the Introduction section to state more clearly the research goals of this study.

(4). The discussion also needs to be restructured in order to focus more on the key findings of this work. A substantial part of the discussion deals with the estimated per cell activities, however, I have some concerns regarding the assumptions on which this estimation was based (see specific comment below).

Reply: Thanks for the reviewer's constructive comments. We have restructured the Discussion section. More discussions have been made to focus on the key findings of this work, for examples, the variation of AOA amoA gene and nitrification rates.

Specific comments of the reviewer #2

(1). title: Please add "Community composition" at the beginning.

Reply: We have added it.

(2). p. 16256, l. 12: operational taxonomic units

Reply: We have corrected it.

(3). p. 16256, l. 14: rather write AOA-amoA than just AOA because this only refers to gene abundances

Reply: We have corrected it.

(4). p. 16257, l. 13: ...in the function of their ecosystem. Which ecosystem?

Reply: The terrestrial and marine ecosystem. We have changed it in the revised manuscript.

(5). p. 16257, l. 22: Which temperature was the optimum temperature? please give the number here.

Reply: The optimum temperature is 65-72 °C. We have added it in the revised
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manuscript (Line 22, Page 16257).

(6). p. 16258, l. 24: The last sentence is the conclusion of the whole work and should rather not appear in the introduction.

Reply: We have deleted it.

(7). p. 16259, l. 16-17: Why were the water samples diluted prior to storage?

Reply: Once hot spring water cools, chemical components (e.g. silica) in water would become supersaturated and spontaneously precipitate in the bottles. The way to avoid this is to dilute the water samples prior to storage.

(8). p. 16261, l. 20-21: Please give references for the primers A21F and A958R.

Reply: We have added references for the primers A21F and A958R and primers Eubac27f and Eubac1492r (Line 21, Page 16261).

Reference:

DeLong, E. F. Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA, 89: 5685-5689, 1992.

Lane, D. J. 16S/23S rRNA sequencing, p. 115-175. In E. Stackebrandt and M. Goodfellow (eds.), Nucleic acid techniques in bacterial systematics. Wiley, Chichester, UK, 1991.

(9). p. 16265, l. 13: The differences in nitrate concentrations described here are very small. What was the detection limit of the method?

Reply: The detection limit of this method is 0.2 ppm.

(10). p. 16265, l. 16. What is meant by ammonia rates, ammonia oxidation rates? Please specify.

Reply: Sorry for this mistake. "Ammonia rates" should be "Ammonia oxidation rates". We have corrected it in revised manuscript.

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(11). p. 16266, l. 13: What does "extremely similar" mean, can you give percent sequence identity here?

Reply: We have deleted "extremely". The percent sequence identity information has been added in the revised paper (Page 16266, Line 13).

(12). p. 16266, l. 17-24: The phylogeny of AOA-amoA is not update. Please follow the phylogeny suggested by Pester et al. 2012, Environmental Microbiology.

Reply: We have updated the phylogeny of AOA-amoA according to the reviewer's suggestion (Fig. 5). Please see the details in section 3.4, Page 16266.

(13). p. 16267, l. 17: The differences in gene abundances are not convincing, a factor 3 differences could still be within the error range of the qPCR method. Here, the authors should be careful not to over-interpret the differences.

Reply: Thanks for valuable suggestion. We have rephrased this sentence as follows: The copy numbers of archaeal amoA genes in the surface and bottom sediments are 2.75×10^5 and 9.80×10^5 gene copies g⁻¹ sediment, respectively.

(14). p. 16269, l. 1: ...for archaeal 16S rRNA genes, please add

Reply: We have added it.

(15). p. 16269, l. 20-21: The message here is unclear, how does this sentence go together with the information about AOA-amoA gene abundances in the sentence before?

Reply: We have changed the sentence as follow: The bacterial amoA genes were not detected, indicating that AOB is absent or is a minority in this hot spring ecosystem.

(16). p. 16270, l. 1-2: The method section only describes DNA-based work. By which approach did the authors measure archaeal amoA transcripts?

Reply: We didn't measure archaeal amoA transcripts in this study. We are sorry for

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this mistake.

(17). p. 16270, l. 22: This study giving the average amoA gene copy number per cell was published in 1997, long before ammonia oxidizing archaea were first described. I wonder if the authors can really use this number for their estimations of per cell activity.

Reply: Bernander and Poplawski (1997) demonstrated that single cell of thermophilic archaea contained two genomes in stationary phase. Although it is long before ammonia oxidizing archaea (AOA) were first described, we assume that this number can be also used to estimate the per cell activity in this study, due to thermophilic and archaeal nature of AOA harbored in high-temperature Gongxiaoshe hot spring. This method was also adopted by Dodsworth et al. (2011) to estimate the per cell nitrification activity of AOA in two US Great Basin hot springs. However, as suggested by reviewer, some uncertainties of this method may still exist, with respect to the stage of cell cycle and the diversity of archaea. We have added a sentence to state these uncertainties in the revised paper.

Reference: Bernander, R. and Poplawski, A.: Cell cycle characteristics of thermophilic archaea, *J. Bacteriol.*, 179, 4963-4969, 1997. Dodsworth, J. A., Hungate, B. A., and Hedlund, B. P.: Ammonia oxidation, denitrification and dissimilatory nitrate reduction to ammonium in two US Great Basin hot springs with abundant ammonia-oxidizing archaea, *Environ. Microbiol.*, 13, 2371–2386, 2011.

Interactive comment on Biogeosciences Discuss., 12, 16255, 2015.

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