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Discussions

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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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Anonymous Referee #1 Received and published: 13 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). This manuscript aimed at addressing contribution to nitrification in a high-temperature hot spring. The high abundances of ammonia oxidizing archaea (AOA)

were determined by qPCR. The number of the studies on nitrification process and corresponding source microorganisms is still rare. Therefore, this paper is timely and the idea behind is interesting. However, it is just a single site in Tengchong hot spring system and could not represent the whole region. The results just simply show the data and lack logical writing. Some paper close related are not cited or fully discussed in this manuscript (Hou et al. 2013; Xie et al. 2014). The authors could investigate more sites to get a statistic results for the nitrification process in the region, compare with other geothermal systems and get some overall rationales for the similarities or differences among them.

Reply: Thanks for the reviewer's constructive comments. We agree with that more data of AOA activity from different sites will enhance our understanding of the nitrification process in the whole Tengchong Geothermal Field. However, in this study, our research goals primarily focus on verifying the hypothesis that Archaea rather than Bacteria drive the ammonia oxidation in high-temperature territorial hot spring environments (we have pointed it out more clearly in the revised paper). Therefore, we selected a representative site in which the ammonia oxidation driven by Archaea might be active in Tengchong Geothermal Field to test this hypothesis.

There are two reasons for us to choose Gongxiaoshe hot spring as the research site to test the hypothesis in this study: 1) Ammonia concentration in Gongxiaoshe hot spring water is 102.61 μg L-1, thermodynamically favorable to ammonia oxidation; 2) The ammonia-oxidizing archaea "Candidatus Nitrosocaldus yellowstonii" were dominant in hot spring water and no AOB amoA genes were detected in the hot spring.

In the revised paper, we have rewritten the Introduction section to make the research goals more clearly. In addition, we have added more information on the selection of the site and added more discussions on the difference in ammonia oxidation rates among Gongxiaoshe hot spring and other geothermal systems. The papers close related to this work have also been cited and discussed in the revised paper.

Specific comments of the reviewer #1

(1). Page 16258, line 6-14, Hou et al. (2013, Plos one) and Xie et al. (2014, EM) have shown the dominated of AOA in the Gongxiaoshe hot spring. It should be mentioned here.

Reply: We have added it in revised manuscript.

(2). Page 16260, line 20. What is the brand for the GC-IRMS?

Reply: The brand of the GC-IRMS is Thermo Scientific from USA. We have added it in revised manuscript.

(3). Page 16262, line 10-20. How many sequences have you got? What is the representation of those sequences? Hou et al. (2013, Plos one) have already published thousands of 16S rRNA sequences from this site, why do not you cite some to build the phylogenetic tree?

Reply: A total of 152 archaeal clone sequences were obtained in this study. The coverage ranged from 90% to 93%. In addition, we got 141 bacterial clone sequences in this study. The coverage ranged from 76% to 83%. We have randomly selected forty 16S rRNA sequences from the Short Read Archive database at NCBI (Hou et al., 2013) to build new phylogenetic trees. The new phylogenetic trees have also been added in revised manuscript (Figure 4 and Figure S2).

(4). Page 16262, line 25. All the "amoA" should be italics.

Reply: We have changed it in revised manuscript.

(5). Page 16263, line 11-20. How specific these qPCR reactions are?

Reply: The PCR conditions are as follows: 10 min at 50°C, 2 min at 95°C; 40 cycles consisting of 15 s at 95°C and 1 min at 60°C; 15 s at 95°C, 1 min at 60°C, and 15 s at 95°C to make the melting curve. We have added it in revised manuscript.

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(6). Page 16263, line 11-20. What are the efficiencies and R2 values of the qPCR assays?

Reply: The efficiencies of the qPCR runs are 87.8-105.6% (R2=0.992-0.999) for 16S rDNA and 102% (R2=0.998) for AOA. We have added it in the revised manuscript.

(7). Page 16267, line 1-4. What is the meaning of the phylotype close to soil AOA? Does it mean that the soil might be one source of the hot spring AOA? Should do some discussion here.

Reply: It is interesting that some phylotypes from Gongxiaoshe hot spring are closely related to soil AOA. One possibility is that some of the amoA genes obtained in this study may derive from soil AOA, particularly those sequences in cluster Nitrosotalea and cluster Nitrososphaera, which have been widely found in sediments and soils. Those AOA from soil might have evolved multiple times and have adapted to high-temperature environments. We have added some discussion in the revised manuscript.

(8). Page 16267 line 16. Xie et al.(2014, EM) has shown similar results by lipid marker. Should be mentioned here.

Reply: Thanks for the reviewer's suggestion. We have added it in the revised manuscript.

(9). Page 16268 line 5-25. This paragraph just generally described the AOA research in geothermal hot springs. No discussion about your own data. Should be specific about how the environmental factors controlling the AOA in Gongxiaoshe hot spring.

Reply: We are very grateful for the reviewer's comments. A specific paragraph has been added in the revised manuscript to discuss the environmental factors (e.g. temperature and ammonia concentration) controlling the AOA in Gongxiaoshe hot spring as follows.

Temperature is likely a very important factor controlling microbial community structure in Gongxiaoshe hot spring. This interpretation is supported by the results of qPCR (Fig.

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2b and Fig. S2). The sediment samples from the bottom of pool (T=77 °C) are dominated by Archaea, whereas the sediment samples from the margin of pool (T=55 °C) are dominated by Bacteria. In addition, no AOB were detected in both bottom and margin samples, indicating that it might be difficult for AOB to inhabit in high-temperature hot spring environments (Lebedeva et al., 2005; Hatzenpichler et al., 2008). Additionally, the abundance of AOA amoA gene in bottom sediments is slightly higher than that in margin sediments, reflecting that although AOA can adapt to a wide range of temperature, higher temperature could be more favorable to the growth of AOA (de la Torre, et al., 2008; Hatzenpichler et al., 2008; Jiang et al., 2010). Ammonia concentration may be another factor that influences the potential activity of AOA and AOB in hot springs. Because AMO in AOA has a much higher affinity for the substrate compared to a similar process in AOB, the ability of AOA to compete for ammonia in oligotrophic hot spring environments is also quite higher than that of AOB (Hatzenpichler et al., 2008). In Gongxiaoshe hot spring, the ammonia concentration is 102.61 μg L-1 that is lower compared to other hot springs with high ammonia concentrations. This relatively low ammonia concentration may also be one of the reasons for the absence of AOB in Gongxiaoshe hot spring.

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