

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

**Anonymous Referee #2**

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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 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, and 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

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

Specific comments: title: Please add "Community composition" at the beginning. p. 16256, l. 12: operational taxonomic units p. 16256, l. 14: rather write AOA-*amoA* than just AOA because this only refers to gene abundances p. 16257, l. 13: ...in the function of their ecosystem. Which ecosystem? p. 16257, l. 22: Which temperature was the optimum temperature? please give the number here. p. 16258, l. 24: The last sentence is the conclusion of the whole work and should rather not appear in the introduction. p. 16259, l. 16-17: Why were the water samples diluted prior to storage? p. 16261, l. 20-21: Please give references for the primers A21F and A958R. p. 16265, l. 13: The differences in nitrate concentrations described here are very small. What was the detection limit of the method? p. 16265, l. 16: What is meant by ammonia rates, ammonia oxidation rates? Please specify. p. 16266, l. 13: What does "extremely similar" mean, can you give percent sequence identity here? 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. 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

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not to over-interpret the differences. p. 16269, l. 1: ...for archaeal 16S rRNA genes, please add 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? p. 16270, l. 1-2: The method section only describes DNA-based work. By which approach did the authors measure archaeal amoA transcripts? 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.

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