

***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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Terrestrial geothermal environments are very important settings for research on biogeochemical cycle of elements. Ammonia oxidation is the first and rate-limiting step of nitrification in nature environments. The manuscript by Chen et al describes a study on composition of ammonia-oxidizing archaea and their contribution to nitrification in a high-temperature hot spring. Their results showed that AOA were widely involved in nitrification whereas bacterial *amoA* was not detected in studied hot spring, indicating dominance of archaea in driving the nitrogen cycle in terrestrial geothermal environments. The results are very important for our understanding on N biogeochemical

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cycle in hot springs.

However, I have some concerns as listed below: 1. P16L5: “A weak but significant correlation was found between the abundances of the archaeal amoA and gross nitrification rates, which were consistent with the results reported by Isobe et al. (2012)”. P16L20: “By conducting correlation analysis between the gross nitrification rates and abundances of amoA in the two samples”. It is not a scientific way to described statistical correlation on only two samples. 2. As the manuscript showed that the cell-specific nitrification rates were estimated to be in the range of 0.41 to 0.79 fmol N cell⁻¹ h⁻¹, which is consistent with earlier estimates in estuary environments. These results are two magnitude higher than those for AOA in reported US hot springs (0.008-0.01 fmolN cell⁻¹ h⁻¹; Dodsworth et al., 2011). In P14L7, the author said “The ammonia or ammonium concentration and temperature are controlling factors of the distribution of AOA”, and P14L17 “The ammonia concentration and potential activity of AOA and AOB showed an obvious positive correlation”. The pH and Temperature showed no significant difference between the GXS hot spring (Temp:77 degree C, pH7.7, NH₄⁺ concentration:102.61 μg L⁻¹, amoA copies: 2.75-9.80×10⁵ gene copies g⁻¹ of dry weight) and the GBS hot spring (Temp:81 degree C, pH7.2, NH₄⁺ concentration: 663 μg L⁻¹, amoA copies: 3.5-3.9×10⁸ gene copies g⁻¹ of dry weight). However, the GBS hot spring possesses high amoA gene copies and NH₄⁺ concentration. Such ammonia oxidation difference between the authors’ and Dodsworth et al. (2011) is of interest. The author should include this point into the discussion on controlling factors of cell-specific nitrification rates. 3. P3L21: “A thermophilic autotrophic AOA Ca. N. yellowstonii”: the bracket should be removed. In addition, I have some technique queries as follows: 1. Page 6 line 4-7, how many bottles for each experiment treatment? 2. Page 9, section 2.7, the qPCR conditions should be at least briefly given here. In addition, the qPCR efficiency should also be presented. 3. Page 10, line 12, did the authors forget archaeal probe here? There is Arch915 probe targeting total archaea in table 1. 4. Page 23, line 24-26, based on Fig.3, I cannot get the information on cell relative abundance of Crenarchaea. The cell shown in Fig. 3 are all Crenarchaea (I assume

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the green ones are). Are the two dyes for archaea and Crenarchaea probes same or different? If different, two pictures should be taken at the same place for total archaea and Crenarchaea, which will reveal whether the observed cells are Crenarchaea or other group of archaea. If same, how did the authors distinguish crenarchaea cells from others?

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