Biogeosciences Discuss., 12, C9374–C9378, 2016
www.biogeosciences-discuss.net/12/C9374/2016/

Biogeosciences Discussions

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

S. Chen et al.

chans@sidsse.ac.cn

Received and published: 24 January 2016

H. Jiang, Received and published: 9 December 2015.

We greatly appreciate Dr. Jiang'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

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 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.

Reply:

We agree with the reviewer's valuable comments and have rephrased the sentences as follows.

"High abundance of ammonia-oxidizing archaea corresponds to high ammonia oxidation rate, which is consistent with the results reported by Isobe et al. (2012)".

"To understand the relationship between the gross nitrification rates and abundances of amoA in the two samples, we specifically estimated the contribution of archaeal cells to nitrification"

(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 cor-

relation". 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, amoA copies: 2.75-9.8*105 gene copies g-1 of dry weight) and the GBS hot spring (Temp:81 degree C, pH7.2, NH4+ concentration: 663 μg/L, 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.

Reply:

We are very grateful for the reviewer's constructive comments. More discussions have been made to focus on controlling factors of cells specific nitrification rates in revised manuscript as follows.

To understand the relationship between the ammonia oxidation rates and abundances of amoA in the two samples, we specifically estimated the contribution of archaeal cells to nitrification. By assuming two amoA copies per cell (Bernander and Poplawski, 1997) and by comparing the ammonia oxidation rates with the qPCR results of AOA amoA per gram, the cell-specific nitrification rates were estimated to be 0.41 fmol N cell-1h-1 and 0.79 fmol N cell-1h-1 in the surface and bottom sediments of the hot spring, respectively. These results are much higher than those for AOA in the GBS hot spring [0.008-0.01 fmol N cell-1h-1(Dodsworth et al., 2011b)]. It is interesting that although the GBS hot spring possesses higher amoA gene copies (3.5-3.9 $\times$ 108 gene copies g-1 of dry weight) and higher NH4+ concentration (663 μg L-1), it exhibits a lower cell-specific nitrification rate than Gongxiaoshe hot spring. This may imply that both the abundance of AOA and the NH4+ concentration are not important factors that control the cell-specific nitrification rates in high-temperature hot spring environments. The difference in cell-specific nitrification rates between the Gongxiaoshe hot spring and the GBS hot spring may reflect the difference of AOA population structure in those two hot springs (Gubry-Rangin et al., 2011; Pester et al., 2012). In line with

this AOA heterogeneity, cell-specific nitrification rates do not reflect the overall AOA abundance or NH4+ concentration in these AOA-dominated hot springs. Alves et al. (2013) reported a similar case that soil dominated by AOA (clade A) exhibited the lowest nitrification rates, in spite of harboring the largest AOA populations.

(3). P3L21: "A thermophilic autotrophic AOA Ca. N. yellowstonii)": the bracket should be removed.

Reply:

We have removed it in the revised manuscript.

(4). Page 6 line 4-7, how many bottles for each experiment treatment?

Reply:

We set up reactors in duplicate for four experiments, eight bottles for each experiment. We have added this information in the revised manuscript.

(5). Page 9, section 2.7, the qPCR conditions should be at least briefly given here. In addition, the qPCR efficiency should also be presented.

Reply:

Thanks for the reviewer's valuable suggestion. We have added following information on the qPCR conditions in the revised manuscripts.

The PCR conditions were 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.

The efficiencies of the qPCR runs were 87.8-105.6% (R2=0.992-0.999) for 16S rDNA and 102% (R2=0.998) for AOA.

(6). Page 10, line 12, did the authors forget archaeal probe here? There is Arch915 probe targeting total archaea in table1.

Reply:

We did not use archaeal probe to perform FISH experiment. We apologize for this confusion. We have deleted relevant information in table 1.

(7). Page 13, line 24-26, based on Fig.3, I cannot get the information on cell relative abundance of Crenarchaea. The cells shown in Fig. 3 are all Crenarchaea (I assume 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?

Reply:

We apologize for this confusion. The abundance of archaeal amoA genes in this study is determined by qPCR, instead of FISH. We only use Cren679 probe to detect Crenarchaea cells in the samples, so the green cells observed in Fig. 3 are all Crenarchaea. We have added a sentence to make this point more clearly in the revised paper.

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