Biogeosciences Discuss., 12, 16255–16283, 2015
www.biogeosciences-discuss.net/12/16255/2015/
doi:10.5194/bgd-12-16255-2015
© Author(s) 2015. CC Attribution 3.0 License.

Biogeosciences Discussions
Open Access

This discussion paper is/has been under review for the journal Biogeosciences (BG).
Please refer to the corresponding final paper in BG if available.

Composition of ammonia-oxidizing archaea and their contribution to nitrification in a high-temperature hot spring

S. Chen, X.-T. Peng, H.-C. Xu, and K.-W. Ta

Sanya Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences, Sanya 572000, China

Received: 1 September 2015 – Accepted: 10 September 2015 – Published: 2 October 2015

Correspondence to: X. Peng (xtpeng@sidsse.ac.cn)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

The oxidation of ammonia by microbes and associated organisms has been shown to occur in diverse natural environments. However, the contribution of ammonia-oxidizing archaea to nitrification in high-temperature environments remains unclear. Here, we studied in situ ammonia oxidation rates and the abundance of ammonia-oxidizing archaea (AOA) in surface and bottom sediments at 77 °C in the Gongxiaoshe hot spring, Tengchong, Yunnan, China. The in situ ammonia oxidation rates measured by the ^{15}N–NO_3^- pool dilution technique in the surface sinter and bottom sediments were 4.8 and 5.3 nmol N g^{-1} h^{-1}, respectively. Relative abundances of Crenarchaea in both samples were determined by fluorescence in situ hybridization (FISH). Phylogenetic analysis of 16S rRNA genes showed high sequence similarity to thermophilic "*Candidatus Nitrosocaldus yellowstonii*", which represented the most abundant operation taxonomic units (OTU) in both sediments. Furthermore, bacterial amoA was not detected in this study. Quantitative PCR (qPCR) indicated that AOA and 16S rRNA genes were present in the range of 2.75 to 9.80×10^5 and 0.128 to 1.96×10^8 gene copies g^{-1} sediment. The cell-specific nitrification rates were estimated to be in the range of 0.41 to 0.79 fmol N archaeal $cell^{-1}$ h^{-1}, which is consistent with earlier estimates in estuary environments. This study demonstrated that AOA were widely involved in nitrification in this hot spring. It further indicated the importance of archaea rather than bacteria in driving the nitrogen cycle in terrestrial geothermal environments.

1 Introduction

Geothermal systems harbor phylogenetically and functionally distinct microbial communities under their wide range of physical and chemical conditions. Previous studies have demonstrated that thermophilic, chemotrophic and phototrophic microorganisms are ubiquitous in global geothermal environments, as they play an important role in the biogeochemical cycle of elements (Pierson et al., 1999; Huber et al., 2000; Shock

BGD
12, 16255–16283, 2015

et al., 2010). Recently, many studies have elucidated nitrogen metabolism and cycling in geothermal ecosystems (Dodsworth et al., 2011b; Nishizawa et al., 2013; Gerbl et al., 2014). In such systems, there has been evidence of microbial communities oxidizing ammonia, the first and rate-limiting step of nitrification (Reigstad et al., 2008; Dodsworth et al., 2011a). The ammonia monooxygenase subunit A (amoA) gene can be used to quantify and characterize ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in natural environments. When metagenomic and laboratory isolation methods were performed on samples, the results indicated that mesophilic crenarchaea significantly oxidized ammonia. However, the function of mesophilic crenarchaea in its ecosystem was previously unknown. Moreover, it has been found that AOA exceed AOB in abundance of by several orders of magnitude in many terrestrial and marine systems (Wuchter et al., 2006; Leininger et al., 2006; Mincer et al., 2007). Previous studies suggested that AOA play a significant role in the function of their ecosystem.

Following the discovery that the archaeal amoA gene is ubiquitous in moderate environments, more attention has been paid to the AOA in higher-temperature environments. Since the occurrence of a putative archaeal amoA gene in geothermal environments was first reported by Weidler et al. (2007) and Spear et al. (2007), thaumarchaeota possessing ammonia monooxygenase (AMO) have been obtained from terrestrial hot springs in the USA, China and Russia; these species harbor AOA amoA gene markers (Pearson et al., 2008; Zhang et al., 2008). A thermophilic autotrophic AOA *Ca. N. yellowstonii*) was enriched from a hot spring at an optimum temperature; thereafter, ammonia oxidation was carried out in the temperature range of 65 to 72°, representing a separate ammonia-oxidizing lineage (De la Torre et al., 2008). Reigstad et al. (2008) measured the nitrification rates in an acidic hot spring in situ, suggesting that considerable oxidation of ammonia occurs in many terrestrial hot springs. Dodsworth et al. (2011b) measured the oxidation rate of ammonia in two US Great Basin hot springs, indicating that this process was driven by ammonia-oxidizing archaea. Data from these studies serve as strong evidence supporting the notion that

BGD
12, 16255–16283, 2015

archaeal ammonia oxidizers play an important role in the biogeochemical nitrogen cycle of terrestrial, geothermal environments. However, further investigations are needed to ascertain the abundance and diversity of ammonia-oxidizing archaea in these extremely high temperature biotopes, as well as the rate of nitrification catalyzed by these species in the aforementioned biotopes.

In the Ruidian geothermal area, Gongxiaoshe is a large, circumneutral site that is dominated by carbonate-depositing springs. A variety of studies have focused on the microbial communities thriving in the Gongxiaoshe hot spring, as the water of this hot spring is enriched with ammonia (Zhang et al., 2008; Hou et al., 2013; Edwards et al., 2013), indicating that ammonia oxidation and possibly other N transformations may be widespread at this site. However, very few studies have explicitly characterized the abundance and community composition of AOA or determined the extent to which the thermophilic ammonia-oxidizing microorganisms of this hot spring are involved in nitrification.

In this study, our aim was to conduct quantitative analyses of the ammonia-oxidizing microorganism populations and their contribution to nitrification in the Gongxiaoshe hot spring. To determine the nitrification activity in hot springs under in situ high-temperature conditions, the ^{15}N pool dilution technique was employed to trace the nitrate produced from ammonia oxidation in the sediments (surface and bottom) of the hot spring. The rate measurements were correlated with the censuses of microbial populations and the amoA functional gene in the surface and bottom samples. Fluorescence in situ hybridization (FISH) and quantitative PCR (qPCR) specific for ammonia oxidizing microorganisms were performed in this process. Our results imply that nitrification is driven by archaea rather than by bacteria in the geothermal environment.

BGD
12, 16255–16283, 2015

2 Materials and methods

2.1 Site description and chemical measurements

Gongxiaoshe is an octagonal pool with a concrete wall and iron railing. Around the pool margins, the area is filled with sinter characterized as a $CaCO_3$ precipitate. The surfaces of the sinter are either partially or completely covered with dark-green microbial mats (Fig. 1). At the bottom of the hot spring, there is loose cream-colored sediment containing carbonates. The sinter samples from the pool margins and sediments from the bottom of the spring, designated SS (surface sinter) and BS (bottom sediment), respectively, were collected using sterile equipment in April 2013. During transportation, all of the samples were packed with dry ice. After transporting the samples to the laboratory, they were stored in a freezer at −80 °C for further analysis.

Temperature and pH were measured in situ in the hot water spring. Temperature was determined with an iButton thermometer (DS1922T, Dallas Semiconductor, USA). The pH was measured using a pH meter (SevenGo™ pH meter SG2, Mettler Toledo, USA). Water samples for cation and anion analysis were filtered through a syringe filter with a 0.22 µm filtration membrane; these samples were diluted 10 times with deionized water and stored in 100 mL polypropylene bottles in the field because an analysis was carried out after 2 days. The cation concentrations were determined using an IRIS Advantage ICP-AES, whereas the anion (F^-, SO_4^{2-}, Cl^-) concentrations were determined using an ion chromatography system (DIONEX ICS-1500, Thermo Scientific, USA). The HCO_3^- concentration was measured using the Gran titration method (Appelo and Postma, 1996). The NH_4^+-N and NO_3^--N concentrations were determined using a nutrient analyzer (Micromac 1000, Partech, UK).

2.2 ^{15}N stable isotope tracing of nitrification activity

Gross N nitrification rates were determined by the ^{15}N pool dilution technique. All of the nitrification measurements were conducted in 500 mL polycarbonate culture flasks

BGD
12, 16255–16283, 2015

(Nalgene) with a silicone plug that contained 400 mL of mud (~ 1/3 sediment by volume). Two subsamples were collected from the bottom and surface sediments with 350 µL of $K^{15}NO_3$ (485 µmol L^{-1}, at 10 % ^{15}N). For each sample, two experiments were conducted to measure the in situ nitrification activity: A1 (SS slurry + $^{15}NO_3^-$) and A2 (BS slurry + $^{15}NO_3^-$). Two experiments involving ionic ammonium were conducted to determine the potential nitrification activity: B1 (SS slurry + $^{15}NO_3^-$ + $^{14}NH_4^+$) and B2 (BS slurry + $^{15}NO_3^-$ + $^{14}NH_4^+$). The reactors were incubated near the in situ conditions of the hot spring water at 77 °C for 30 and 120 min. At certain time intervals (e.g., 30, 120 min), 80 mL aliquots were collected from A1, A2, B1, and B2 with sterile serological pipettes and transferred to acid-cleaned 250 mL polypropylene bottles. Prior to filtration, 40 mL of KCl (3 M) was added to each sample bottle, and the samples were shaken at 120 rpm for 1 h and then centrifuged at 1600 × *g* for 10 min (Reigstad et al., 2008). The supernatant was filtered through a syringe filter containing a 0.22 µm filtration membrane; the supernatant was subsequently stored in acid-cleaned 60 mL polypropylene bottles at 4 °C, and analysis was performed after 2 days.

In the laboratory, the concentrations of NH_4^+ and NO_3^- in the filtrate were determined by a nutrient analyzer (Micromac-1000, UK). The NO_3^- ($^{15}NO_3^-$ and $^{14}NO_3^-$) ions of the filtrates were converted to N_2O by denitrifying bacteria (*Pseudomonas aureofaciens*) lacking N_2O reductase activity, and N_2O was quantified by coupled gas chromatography–isotope ratio mass spectrometry (GC-IRMS) (Dodsworth et al., 2011a). The gross nitrification rates were calculated using the equations of Barraclough (1991), as were the concentrations and N isotope ratios of NO_3^- in the samples incubated for 30 and 120 min, respectively.

2.3 DNA extraction and purification

DNA was extracted by the SDS-based extraction method described by Zhou et al. (1996), with some modifications. Briefly, approximately 5 g samples were frozen with liquid nitrogen and milled three times. Then the powdered samples were mixed

BGD
12, 16255–16283, 2015

with 13.5 mL of DNA extraction buffer and 100 µL of proteinase K (10 mg ml^{-1}) in tubes; these tubes were horizontally shaken at 225 rpm for 30 min at 37 °C. After shaking, 1.5 mL of 20 % SDS was added, and the samples were incubated in a water bath; the temperature of the water bath was maintained at 65 °C for 2 h. During this period, the tubes were subjected to gentle end-over-end inversions every 15 to 20 min. The supernatant fluids were collected after subjecting the tubes to centrifugation at 6000 × *g* for 10 min at room temperature; the collected supernatant tubes were subsequently transferred into 50 mL centrifuge tubes. The supernatant fluids were mixed with an equal volume of chloroform : isoamyl alcohol solution (24 : 1, vol/vol). The aqueous phase was recovered by centrifugation and precipitated with a 0.6 volume of isopropanol at room temperature; this process was carried out for at least 1 h. Crude nucleic acids were obtained by centrifugation at 16 000 × *g* for 20 min at room temperature; these crude nucleic acids were washed with cold 70 % ethanol and resuspended in sterile deionized water; the final volume of this solution was 100 µL. The crude nucleic acids were purified with a Cycle-Pure Kit (Omega, USA). These crude nucleic acids were then resuspended in the elution buffer, and the final volume of the solution mixture was 50 µL; this solution was stored at −80 °C.

2.4 PCR and clone library construction

16S rDNA was amplified with purified genomic DNA as templates and universal bacterial primers. The primer pairs A21f (5'-TTC CGG TTG ATC CCT G CCG GA-3') and A958R (5'-CCC GGC GTT GAA TC AAT T-3') were chosen for archaea, and Eubac27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and Eubac1492r (5'-GGT TAC CTT GTT ACG ACT T-3') were chosen for bacteria. In a total volume of 50 µL, the reactions were performed using 1.25 U of Taq DNA polymerase (Takara, Japan). The amplification conditions were as follows: an initial denaturation was carried out at 94 °C for 4 min, and then, the same denaturation was continued at 94 °C for 1 min. Thereafter, annealing was carried out at 55 °C for 45 s, while extension was conducted at 72 °C for 60 s;

BGD
12, 16255–16283, 2015

the process was repeated for 30 cycles, followed by a final extension step at 72 °C for 10 min. The PCR products were excised after being separated by gel electrophoresis; a gel-extraction kit (Omega, USA) was used to purify the products in accordance with the manufacturer's instructions. The purified PCR products were cloned into pMD20-T vectors (Takara, Japan) and transformed into competent *Escherichia coli* DH5α cells. To select the positive clones, colony PCR was used to determine the presence of correctly sized inserts containing vector-specific primers M13f (5'-GTA AAA CGA CGG CCA G-3') and M13r (5'-CAG GAA ACA GCT ATG AC-3').

2.5 Sequencing and phylogenetic analysis

All of the clones were sequenced by the dideoxynucleotide chain-termination method. In this procedure, an ABI 3730 capillary electrophoresis sequencer (Applied Biosystems Inc., USA) was coupled with the ***T*** vector universal primers M13f and M13r. The whole sequence of each clone was spliced using DNAMAN software (version 6.0), and the vector sequences were deleted; the presence of chimeras was checked using the Greengenes chimera check tool (Bellerophon server) (Huber et al., 2004). The program DOTUR was used to determine the operation taxonomic units (OTUs) for each sequence; 97 % similarity was considered as the cut-off for the chimeric sequences. To find closely related sequences in the GenBank and EMBL databases for phylogenetic analysis, none of the chimeric sequences were submitted to the advanced BLAST search program. Phylogenetic trees were constructed using the neighbor-joining method and the software MEGA (version 5.05). A bootstrap analysis was used to provide confidence estimates of the tree topologies.

2.6 Amplification of amoA (ammonia monooxygenase subunit A)-related sequences

Archaeal amoA gene fragments were amplified using the primer pair Arch-amoAF (5'-STA ATG GTC TGG CTT AGA CG-3') and Arch-amoAR (5'-GCG GCC ATC CAT CTG

BGD
12, 16255–16283, 2015

TAT GT-3') (Francis et al., 2005). Bacterial amoA genes were also tested using the bacterial primer sets amoA 1F (5'-GGG GTT TCT ACT GGT GGT-3') and amoA 2R (5'CCC CTC KGS AAA GTT CCT TC-3') (Rotthauwe et al., 1997). PCR cycling was performed by the method of Francis et al. (2005). In this method, PCR products from SS and BS were recovered from the gel slices using a gel-extraction kit (Omega, USA) in accordance with the manufacturer's instructions. The purified PCR products from each type of sample were cloned into the pMD20-T vectors (Takara, Japan) and transformed into competent *Escherichia coli* DH5α cells. Cloning and sequencing were performed according to the above-mentioned process. Forty to fifty randomly selected colonies per sample were analyzed for the presence of insert archaeal amoA gene sequences.

2.7 Quantification of 16S rDNA and amoA genes

The numbers of 16S rDNA and archaeal amoA genes were determined by an ABI 7500 real-time PCR system (Applied Biosystems Inc., USA); the SYBR Green I method was used for this analysis. The 20 µL reaction mixture contained 1 µL of template DNA (10 ng), a 0.15 µM concentration of each primer, and 10 µL of Power SYBR Green PCR master mix (Applied Biosystems Inc., USA); this reaction mixture was analyzed with ROX and SYBR Green I. Melting curve analysis was performed after amplification, and the cycle threshold was set automatically using system 7500 **Software v2.0 Patch 6**. Primers targeting different genes were selected according to previous studies (Muyzer et al., 1993; Øvreas et al., 1998; Treusch et al., 2005) and are listed in Table 1.

2.8 Sample processing for FISH

To visualize AOA in situ, FISH was performed according to the procedure described by Orphan et al. (2002, 2009). Small aliquots of sediment were fixed overnight at 4 °C using 2 % formaldehyde in 1 × PBS ([145 mM NaCl, 1.4 mM NaH_2PO_4, 8 mM Na_2HPO_4 (pH = 7.4)]; these aliquots of sediments were washed twice with 1 × PBS and stored at −20° in an ethanol : PBS (1 : 1, vol/vol) medium. The total supernatant was filtered

BGD
12, 16255–16283, 2015

through a polycarbonate filter (Millipore) under low vacuum (< 5 psi; 1 psi = 6.89 kPa). Filters were cut into suitably sized pieces and transferred onto untreated, round, 1 in. glass slides. The transfer of filters onto glass slides was performed according to the procedure described by Murray et al. (1998). In this process, 5 µL of a 1 × PBS solution was spotted onto a glass slide that was scored with a diamond pen prior to mapping, and half of the freshly prepared filter was used to invert the sample onto the slide; this inverted sample was then air-dried. Prior to FISH, the samples on the glass slides were treated with an EtOH dehydration series (50, 75, and 100 % EtOH), dried, and stored at −20 °C. Hybridization and wash buffers were prepared according to the procedure described by Pernthaler et al., 2001. Here, 20 µL of hybridization buffer containing 35 or 20 % formamide was added to the samples on the glass slides. FITC or CY3-labeled oligonucleotide Cren679 probes described by Stahl and Amann (1991) and Labrenz et al. (2010), respectively, were added to the hybridization buffer so that the final solution had a concentration of 5 ng µL^{-1}.

The hybridization mixtures on the slides were incubated for 1.5 h at 46 °C in a pre-moistened chamber. After hybridization, the slides were transferred into a preheated wash buffer and incubated for an additional 15 min at 48 °C. The samples were rinsed in distilled water and air-dried in the dark. The microscopic images of the hybridized samples were recorded on a Leica imager (Leica, DMI 4000B, Germany).

2.9 Nucleotide sequence accession numbers

All of the small-subunit rRNA gene sequences and the amoA sequences were deposited in the GenBank/EMBL nucleotide sequence database under the following accession numbers: KP784719 to KP784760 for partial 16S rRNA gene sequences and KP994442 to KP994448 for the amoA sequences.

BGD
12, 16255–16283, 2015

3 Results

3.1 Water chemistry

The hot water of the spring had a temperature of 77.06 °C and pH of 7.7; it contained Ca (20.25 mg L^{-1}), K (41.97 mg L^{-1}), Mg (3.986 mg L^{-1}), Na (313.3 mg L^{-1}), SiO_2 (130.3 mg L^{-1}), HCO_3^- (963 mg L^{-1}), NH_4^+–N (102.61 µg L^{-1}), NO_3^-–N (7.68 µg L^{-1}), F^- (9.158 mg L^{-1}), Cl^- (418.9 mg L^{-1}) and SO_4^{2-} (24.96 mg L^{-1}). The hot spring was previously categorized as a Na–HCO_3 spring (Zhang et al., 2008) because of the high concentration of alkaline metal ions (K, Na, and Ca).

3.2 Ammonia oxidation rates

In the surface and bottom sediments (without NH_4^+ stimulation), the ammonia oxidation rates calculated from the ^{15}N–NO_3^- pool dilution data were 4.8 ± 0.2 and 5.3 ± 0.5 nmol N g^{-1} h^{-1}, respectively. In the samples with no added NH_4^+, the nitrate concentration increased from 2.84 ± 2 to 3.25 ± 2 µM in the surface sediments and from 2.33 ± 3 to 2.62 ± 3 µM in the bottom sediments. These results agreed with the incubation experiments amended with NH_4^+. The nitrate concentration increased continuously with the addition of NH_4^+, and the ammonia rates recorded in the surface sediments and bottom sediments (with NH_4^+) were 5.7 ± 0.6 and 7.1 ± 0.8 nmol N g^{-1} h^{-1}, respectively.

3.3 Archaeal community composition and phylogenetic analysis

Phylogenetic analysis showed the distribution of the clone sequences into three monophyletic groups: Thaumarchaeota, Crenarchaeota, and Euryarchaeota (Fig. 4). In this study, the most abundant archaeal phylum was Thaumarchaeota. Among them, two phylotypes, SS-A19 and BS-A1, were the most dominant archaeal lineage, representing 89 and 86 % of the cloned archaeal sequences in surface and bottom sediments, respectively. These sequences were closely related to the thermophilic, au-

BGD
12, 16255–16283, 2015

totrophic, ammonia-oxidizing archaeal "*Ca. N. yellowstonii*" (de la Tarre et al., 2008). The seven archaeal OTUs found here belonged to Crenarchaeota, which contains sequences recovered from hydrothermal vents and hot spring environments. In addition, two phylotypes (BS-A47 and BS-A8) that were branched with uncultured sequences belonged to *Desulfurococcales*, which was also recovered from sediments of the hot spring. Euryarchaeota also occurred in both the sediments, but with relatively low abundances. Phylotype BS-A80 is associated with *Geoglobus ahangari*, which belongs to *Archaeoglobales* and is capable of oxidizing organic acids (Kashefi et al., 2002). SS-A12, which represents four clones recovered from the surface sediments, showed 93 % similarity to an uncultured archaeal clone that was recovered from the Spring River. SS-A47 belonged to the *Thermoplasmatales* that were 96 % similar to their nearest neighbor sequence, which were collected from the Spring River. The other euryarchaeotal sequences were extremely similar to their uncultured counterparts, which were mostly recovered from high-temperature geothermal environments.

3.4 Community analysis of AOA

In this study, a total of 113 archaeal amoA gene fragments were obtained from the two samples. They were all branched within the three distinct clusters of archaeal amoA sequences: cluster A, with subclusters A1 to A2; cluster B; and cluster C (Fig. 5). Cluster A contained four phylotypes (57 % of total OTUs) representing 68 sequences (60 % of the total sequences). This cluster had two major clades whose bootstrap confidence value was 99 %. Clade A1 contained phylotypes SS-AOA-4 and BS-AOA-22, which branched with large numbers of sequences recovered from the sediments and water samples in the marine or fresh environments. The other clade, A2, has two phylotypes representing 44 sequences. OTU BS-AOA-62 contained 18 sequences, which was closely related to sequences from soil. The clone SS-AOA-76 clustered within clade A2 and showed up to 99 % sequence identity to an uncultured archaeon clone GHL2_S_AOA_19 (JX488447) obtained from lake sediment.

BGD
12, 16255–16283, 2015

Cluster B had 1 phylotype (SS-AOA-65) with 11 sequences (12 % of the total sequences). The closely related sequences in this cluster included characteristic crenarchaeotal group sequences that were obtained from alpine soil. Another clone, MX_3_OCT_18 (DQ501052), from estuary sediment was 96 % similar.

Cluster C contained two phylotypes (BS-AOA-15 and SS-AOA-50) with 34 sequences (30 % of the total sequences). They were closely related to the geothermal water sequences, with 95–99 % similarity. Furthermore, cluster C mainly represented previously described ThAOA/HWCG III (Prosser and Nicol, 2008). Notably, the recently reported amoA gene sequence of "*Ca. N. yellowstonii*" (EU239961) (De la Torre et al., 2008) showed 85 % sequence identity to clones BS-AOA-15 and SS-AOA-50.

3.5 Quantitative PCR

The qPCR results (Fig. 2b) indicated that the abundance of the archaeal 16S rRNA gene in the two samples was similar, ranging from 1.28 to 1.96×10^7 gene copies g^{-1} of dry weight of sediments. However, the abundance of the bacterial 16S rRNA gene varied greatly, ranging from 6.86×10^6 to 4.25×10^8 gene copies g^{-1} of dry weight of sediments (Fig. S2 in the Supplement). The copy numbers of archaeal amoA genes in the bottom sediments (9.80×10^5 gene copies g^{-1} of dry weight) are 3 times higher than those of the surface sediments (2.75×10^5 gene copies g^{-1} of dry weight). The copy numbers of the archaeal 16S rRNA genes in the bottom sediments were significantly higher than those of the bacterial 16S rRNA genes, with a ratio of 28.57. However, in surface sediments, the ratio of bacterial 16S rRNA genes to archaeal 16S rRNA genes is 3.32.

3.6 FISH

FISH was used to analyze the relative abundance of Crenarchaea in two samples. As expected, most metabolically active Crenarchaea cells and aggregated cells were detected by FISH probes (Cren679) (Fig. 3). Based on the fluorescence signals, a high

BGD
12, 16255–16283, 2015

abundance of crenarchaea in the hot spring sediments harbored amoA genes, providing strong evidence supporting the important role of Crenarchaea in the oxidation of ammonia.

4 Discussion

4.1 Environmental factors affecting the occurrence of ammonia-oxidizing microorganisms

The ammonia or ammonium concentration and temperature are controlling factors of the distribution of AOA. The thermophilic AOA amoA genes that were retrieved from hot springs mediate ammonia oxidation at temperatures of 45 to 97 °C (de la Torre et al., 2008; Hatzenpichler et al., 2008; Jiang et al., 2010). So far, only two enrichments of thermophilic ammonia-oxidizing archaeota (i.e., *Ca. N. yellowstonii* and *C. Nitrososphaera gargensis*) have been shown to perform nitrification at high temperatures. The Gongxiaoshe hot spring, with a high temperature of 77 °C, does not favor AOB, which is in accord with the observation that it is difficult for AOB to exist in a high-temperature geothermal environment (40–55 °C) (Lebedeva et al., 2005; Hatzenpichler et al., 2008).

The ammonia concentration and potential activity of AOA and AOB showed an obvious positive correlation. Martens-Habbena et al. (2009) found that the affinity of "*Candidatus Nitrosopumilus maritimus*" strain SCM1 for ammonia/ammonium was among the highest affinities reported for microbial substrates, as it exceeds that of AOB more than 200-fold. Their study also proved the existence of oligotrophic ammonia oxidizers among thaumarchaeota. Moreover, they provided evidence for the ability of thaumarchaeota to compete for ammonia in oligotrophic hot spring environments (Hatzenpichler et al., 2008). Because AMO in AOA has a much higher affinity for the substrate compared to a similar process in AOB, it has been suggested that archaeal amoA genes were detected only in the Gongxiaoshe hot spring.

BGD
12, 16255–16283, 2015

4.2 Composition and abundance of AOA

The rarefaction curves (Fig. S3) for archaeal and amoA genes in the surface and bottom sediment samples reached a plateau, and their coverage values were relatively high (89–99 %). This result indicated that a large part of the archaeal/amoA diversity at this spring was probably included in the archaeal/amoA clone libraries. The majority of archaeal sequences were closely related to "*Ca. N. yellowstonii*", a known AOA, which may be responsible for the oxidation of ammonia in this spring.

In this study, phylogenetic analyses of archaea amoA genes showed that *Ca. N. yellowstonii* dominated in both of the samples. This result also agreed with previous hot spring observations reported by Dodsworth et al. (2011b) and Hou et al. (2013). According to the sequences retrieved from NCBI, cluster A2 and cluster B were closely related to the cluster soil, whereas cluster A1 was related to the cluster marine and cluster sediments published in previous studies (Francis et al., 2005; Park et al., 2006). Based on the analysis of the real-time PCR and FISH methods, our data indicate that the abundance of AOA is relatively high in both samples. The archaeal amoA gene copy numbers ranged from 2.75 to 9.8×10^5 per gram dry weight of sediments in this study. This is comparable to the abundance in other hot water springs (10^4–10^5 copies g^{-1}; Dodsworth et al., 2011b) but is lower than the abundance of the archaeal amoA gene in non-thermal environments, such as paddy rhizosphere soil (10^6–10^7 copies g^{-1}; Chen et al., 2008) and marine sediments (10^7–10^8 copies g^{-1}; Park et al., 2008). This result indicates that AOB is absent or is a minority in this hot spring ecosystem. A predominance of archaeal amoA genes vs. bacterial amoA genes indicated that ammonia oxidation may be due to the activity of archaea in the Gongxiaoshe hot spring.

4.3 The role of AOA in the nitrification of terrestrial geothermal environments

In the experiments carried out in this study, the gross nitrification rates of the hot spring varied slightly between 4.8 and 7.1 nmol N g^{-1} h^{-1}. The ammonia oxidation rates in bottom sediments (5.3 ± 0.5 nmol N g^{-1} h^{-1}) were slightly higher than those observed in

BGD
12, 16255–16283, 2015

surface sediments (4.8 ± 0.2 nmol N g^{-1} h^{-1}). This result agrees with the distribution of archaeal amoA transcripts, which were found to be in higher abundance in the bottom sediment than in the surface sediment. 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). In contrast to the surface sediments' unamended NH_4^+ (4.8 ± 0.2 nmol N g^{-1} h^{-1}), the ammonia oxidation rate appeared to be stimulated after amendment with NH_4^+ (1 M) (5.7 ± 0.6 nmol N g^{-1} h^{-1}). There are indications that the ammonia concentration is an important factor affecting the rates of nitrification (Hatzenpichler et al., 2008). Similarly, the rates of ammonia oxidation in the bottom sediments were increased by the addition of NH_4^+. Moreover, the rates reported here were comparable with those observed in the two US Great Basin hot springs (5.5–8.6 nmol N g^{-1} h^{-1}; Dodsworth et al., 2011b) and in two acidic (pH = 3, T = 85 °C) Iceland hot springs (2.8–7.0 nmol NO_3^- g^{-1} h^{-1}; Reigstad et al., 2008). However, the rates reported in this study were lower than those observed in some wetland sediments and agricultural soils (85–180 nmol N g^{-1} h^{-1}; White and Reddy, 2003; Booth et al., 2005). Furthermore, most studies specifically mention the ammonia oxidation rates, rather than complete nitrification (ammonia oxidation plus nitrite oxidation). Those studies neither determined the occurrence of NO_2^- oxidation nor accounted for the possibility of nitrate loss by denitrification (Dodsworth et al., 2011a).

By conducting correlation analysis between the gross nitrification 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 and 0.79 fmol N $cell^{-1}$ h^{-1} in the surface and bottom sediments of the hot spring, respectively. These results are much higher than those for AOA in US hot springs (0.008–0.01 fmol N $cell^{-1}$ h^{-1}; Dodsworth et al., 2011b). When the nitrification rates in the North Sea and Colne estuary were measured in situ, it was found that they had maximum values of 0.3 fmol N $cell^{-1}$ h^{-1} (Wuchter et al., 2006) and 3.1 fmol N $cell^{-1}$ h^{-1}

BGD
12, 16255–16283, 2015

(Li et al., 2014), respectively. Therefore, it can be deduced that the estimated activity of AOA in the Gongxiaoshe hot spring is similar to the activity of AOA in an estuary environment. The changes in the amoA gene abundance were specifically associated with the high nitrification rates in the two sediment samples, thereby implying that AOA plays an important role in the nitrogen cycle of Gongxiaoshe hot water spring.

5 Conclusions

Combination of ^{15}N–NO_3^- pool dilution and molecular analyses demonstrates that the oxidation of ammonia by AOA occurs actively in the high-temperature Gongxiaoshe geothermal system. The observation of the presence of considerable in situ nitrification rates in the hot spring is likely due to two dominant groups in the hot springs that include phylotypes that are closely related to the autotrophic AOA "*Ca. N. yellowstonii*". The detection of archaeal amoA genes and the absence of AOB indicate that archaeal ammonia oxidizers, rather than AOB, may significantly contribute to the nitrification in the Gongxiaoshe geothermal systems. Our results shed light on the importance of AOA in driving the oxidation of ammonia in high-temperature environments, which may be ubiquitous in other terrestrial hot springs in the world.

The Supplement related to this article is available online at doi:10.5194/bgd-12-16255-2015-supplement.

References

Appelo, C. A. J. and Postma, D.: Geochemistry, Groundwater, and Pollution, Balkema, Rotterdam, 1996.

Beman, J. M. and Francis, C. A.: Diversity of ammonia oxidizing archaea and bacteria in the sediments of a hypernutrified subtropical estuary: Bahìa del Toìbari, Mexico, Appl. Environ. Microb., 72, 7767–7777, 2006.

BGD
12, 16255–16283, 2015

Beman, J. M., Popp, B. N., and Francis, C. A.: Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California, ISME J., 2, 429–441, 2008.

Chen, X. P., Zhu, Y. G., Xia, Y., Shen, J. P., and He, J. Z.: Ammonia oxidizing archaea: important players in paddy rhizosphere soil?, Environ. Microbiol., 10, 1978–1987, 2008.

Barraclough, D.: The use of mean pool abundances to interpret ^{15}N tracer experiments, Plant Soil, 131, 89–96, 1991.

Bernander, R. and Poplawski, A.: Cell cycle characteristics of thermophilic archaea, J. Bacteriol., 179, 4963–4969, 1997.

Booth, M. S., Stark, J. M., and Rastetter, E.: Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data, Ecol. Monogr., 75, 139–157, 2005.

De la Torre, J., Walker, C., Ingalls, A., Koenneke, M., and Stahl, D.: Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol, Environ. Microbiol., 10, 810–818, 2008.

Dodsworth, J. A., Hungate, B., Torre, J., Jiang, H., and Hedlund, B. P.: Measuring nitrification, denitrification, and related biomarkers in continental geothermal ecosystems, Method. Enzymol., 486, 171–203, 2011a.

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, 2011b.

Edwards, T. A., Calical, N. A., Huang, D. A., Manoharan, N., Hou, W. G., Huang, L. Q., Panosyan, H., Dong, H. L., and Hedlund, B. P.: Cultivation and characterization of thermophilic *Nitrospira* species from geothermal springs in the US Great Basin, China, and Armenia, FEMS Microbiol. Ecol., 85, 283–292, 2013.

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., and Oakley, B. B.: Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean, P. Natl. Acad. Sci. USA, 102, 14683–14688, 2005.

Francis, C. A., Beman, J., and Kuypers, M.: New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation, Appl. Environ. Microb., 1, 19–27, 2007.

Gerbl, F. W., Weidler, G. W., Wanek, W., Erhardt, A., and Stan-Lotter, H.: Thaumarchaeal ammonium oxidation and evidence for a nitrogen cycle in a subsurface radioactive thermal spring in the Austrian Central Alps, Front. Microbiol., 5, 1–17, 2014.

BGD
12, 16255–16283, 2015

Gontang, E. A., Fenical, W., and Jensen, P. R.: Phylogenetic diversity of gram-positive bacteria cultured from marine sediments, Appl. Environ. Microb., 73, 3272–3282, 2007.
Hanada, S., Takaichi, S., Matsuura, K., and Nakamura, K.: *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes, Int. J. Syst. Evol. Micr., 52, 187–193, 2002.
Haouari, O., Fardeau, M. L., Cayol, J. L., Fauque, G., Casiot, C., Elbaz-Poulichet, F., Hamdi, M., Joseph, M., and Ollivier, B.: *Thermodesulfovibrio hydrogeniphilus* sp. nov., a new thermophilic sulphate-reducing bacterium isolated from a Tunisian hot spring, Syst. Appl. Microbiol., 31, 38–42, 2008.
Hatzenpichler, R., Lebedeva, E., Spieck, E., Stoecker, K., Richter, A., Daims, H., and Wagner, M.: A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring, P. Natl. Acad. Sci. USA, 105, 2134–2139, 2008.
Hou, W. G., Wang, S., Dong, H. L., Jiang, H. C., Briggs, B. R., Peacock, J. P., Huang, Q. Y., Huang, L. Q., Wu, G., Zhi, X. Y., Li, W. J., Dodsworth, J. A., Hedlund, B. P., Zhang, C. L., Hartnett, H. E., Dijkstra, P., and Hungate, B. A: A comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan province China using 16S rRNA gene pyrosequencing, PLoS ONE, 8, e53350, doi:10.1371/journal.pone.0053350, 2013.
Huber, R., Huber, H., and Stetter, K. O.: Towards the ecology of hyperthermophiles: biotopes, new isolation strategies and novel metabolic properties, FEMS Microbiol. Rev., 24, 615–623, 2000.
Huber, T., Faulkner, G., and Hugenholtz, P.: Bellerophon: a program to detect chimeric sequences in multiple sequence alignments, Bioinformatics, 20, 2317–2319, 2004.
Isobe, K., Koba, K., Suwa, Y., Ikutani, J., Fang, Y. T., Yoh, M., Mo, J. M., Otsuka, S., and Senoo, K.: High abundance of ammonia-oxidizing archaea in acidified subtropical forest soils in southern China after long-term N deposition, FEMS Microbiol. Ecol., 80, 193–203, 2012.
Jiang, H. C., Huang, Q. Y., Dong, H. L., Wang, P., Wang, F. P., Li, W. J., and Zhang, C. L.: RNA-based investigation of ammonia oxidizing archaea in hot springs of Yunnan Province, China, Appl. Environ. Microb., 76, 4538–4541, 2010.
Kanokratana, P., Chanapan, S., Pootanakit, K., and Eurwilaichitr, L.: Diversity and abundance of bacteria and archaea in the Bor Khlueng hot spring in Thailand, J. Basic Microb., 44, 430–444, 2004.
Kasahara, Y. and Hattori, T.: Analysis of bacterial populations in a grassland soil according to rates of development on solid media, FEMS Microbiol. Ecol., 86, 95–102, 1991.

BGD
12, 16255–16283, 2015

Kashefi, K., Tor, J. M., Holmes, D. E., Gaw Van Praagh, C. V., Reysenbach, A. L., and Lovley, D. R.: *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor, Int. J. Syst. Evol. Micr., 52, 719–728, 2002.

Labrenz, M., Sintes, E., Toetzke, F., Zumsteg, A., Herndl, G., Seidler, M., and Jurgens, K.: Relevance of a crenarchaeotal subcluster related to *Candidatus Nitrosopumilus maritimus* to ammonia oxidation in the suboxic zone of the central Baltic Sea, ISME J., 4, 1496–1508, 2010.

Lebedeva, E. V., Alawi, M., Fiencke, C., Namsaraev, B., Bock, E., and Spieck, E.: Moderately thermophilic nitrifying bacteria from a hot spring of the Baikal rift zone, FEMS Microbiol. Ecol., 54, 297–306, 2005.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G., Prosser, J., Schuster, S., and Schleper, C.: Archaea predominate among ammonia-oxidizing prokaryotes in soils, Nature Lett., 442, 806–809, 2006.

Li, J. L., Nedwell, D. B., Beddow, J., Dumbrell, A. J., McKew, B. A., Thorpe, E. L., and Whiteby, C.: amoA gene abundances and nitrification potential rates suggest that benthic ammonia-oxidizing bacteria (AOB) not archaea (AOA) dominate N cycling in the Colne estuary, UK, Appl. Environ. Microb., 81, 159–165, 2015.

Mincer, T. J., Church, M. J., Taylor, L. T., Preston, C., Karl, D. M., and Delong, E. F.: Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre, Environ. Microbiol., 9, 1162–1175, 2007.

Murray, A. E., Preston, C. M., Massana, R., Taylor, L. T., Blakis, A., Wu, K., and DeLong, E. F.: Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica, Appl. Environ. Microb., 64, 2585–2595, 1998.

Muyzer, G., de Waal, E. C., and Uitterlinden, A. G.: Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl. Environ. Microb., 59, 695–700, 1993.

Nishizawa, M., Koba, K., Makabe, A., Yoshida, N., Kaneko, M., Hirao, S., Ishibashi, J. I., Yamanaka, T., Shibuya, T., Kikuchi, T., Hirai, M., Miyazaki, J., Nunoura, T., and Takai, K.: Nitrification-driven forms of nitrogen metabolism in microbial mat communities thriving along an ammonium-enriched subsurface geothermal stream, Geochim. Cosmochim. Ac., 113, 152–173, 2013.

BGD
12, 16255–16283, 2015

Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D., and DeLong, E. F.: Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments, P. Natl. Acad. Sci. USA, 99, 7663–7668, 2002.

Orphan, V. J., Turk, K. A., Green, A. M., and House, C. H.: Patterns of ^{15}N assimilation and growth of methanotrophic ANME-2 archaea and sulfate-reducing bacteria within structured syntrophic consortia revealed by FISH-SIMS, Environ. Microbiol., 11, 1777–1791, 2009.

Øvreas, L., Jensen, S., Daae, F. L., and Torsvik, V.: Microbial community changes in a perturbed agricultural soil investigated by molecular and physiological approaches, Appl. Environ. Microb., 64, 2739–2742, 1998.

Park, H. D., Wells, G. F., Bae, H., Griddle, C. S., and Francis, C. A.: Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors, Appl. Environ. Microb., 72, 5643–5647, 2006.

Park, S. J., Park, B. J., and Rhee, S. K.: Comparative analysis of archaeal 16S rRNA and amoA genes to estimate the abundance and diversity of ammonia-oxidizing archaea in marine sediments, Extremophiles, 12, 605–615, 2008.

Pearson, A., Huang, Z., Ingalls, A. E., Romanek, C. S., Wiegel, J., Freeman, K. H., Smittenberg, R. H., and Zhang, C. L.: Nonmarine crenarchaeol in Nevada hot springs, Appl. Environ. Microb., 70, 5229–5237, 2004.

Pearson, A., Pi, Y., Zhao, W., Li, W., Li, Y.-L., Inskeep, W., Perevalova, A., Romanek, C., Li, S. G., and Zhang, C. L.: Factors controlling the distribution of archaeal tetraethers in terrestrial hot springs, Appl. Environ. Microb., 74, 3523–3532, 2008.

Peng, X., Xu, H., Jones, B., Chen, S., and Zhou, H.: Silicified virus-like nanoparticles in an extreme thermal environment: implications for the preservation of viruses in the geological record, Geobiology, 11, 511–526, 2013.

Pernthaler, J., Glockner, F. O., Schonhuber, W., and Amann, R.: Fluorescence in situ hybridization with rRNA-targeted oligonucleotide probes, in: Methods in Microbiology: Marine Microbiology, edited by: Paul, J. H., Academic Press, San Diego, San Francisco, New York, Boston London, Sydney, Tokyo, 207–226, 2001.

Pierson, B. K., Parenteau, M. N., and Griffin, B. M.: Phototrophs in high-iron-concentration microbial mats: physiological ecology of phototrophs in an iron-depositing hot spring, Appl. Environ. Microb., 65, 5474–5483, 1999.

Pommerening-Röser, A., Juretschko, S., Schmid, M. C., Koops. H. P., and Wagner, M.: Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA

BGD
12, 16255–16283, 2015

and amoA sequence analysis: implications for molecular diversity surveys, Appl. Environ. Microb., 66, 5368–5382, 2000.
Prosser, J. I. and Nicol, G. W.: Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment, Environ. Microbiol., 10, 2931–2941, 2008.
Reigstad, L., Richter, A., Daims, H., Urich, T., Schwark, L., and Schleper, C.: Nitrification in terrestrial hot springs of Iceland and Kamchatka, FEMS Microbiol. Ecol., 64, 167–174, 2008.
Rotthauwe, J. H., Witzel, K. P., and Liesack, W.: The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations, Appl. Environ. Microb., 63, 4704–4712, 1997.
Santoro, A. E., Francis, C., de Sieyes, N., and Boehm, A.: Shifts in the relative abundance of ammonia-oxidizing bacteria and archaea across physicochemical gradients in a subterranean estuary, Environ. Microbiol., 10, 1068–1079, 2008.
Shock, E. L., Holland, M., Meyer-Dombard, D., Amend, J. P., Osburn, G. R., and Fischer, T. P.: Quantifying inorganic sources of geochemical energy in hydrothermal ecosystems, Yellowstone National Park, USA, Geochim. Cosmochim. Ac., 74, 4005–4043, 2010.
Spear, J. R., Barton, H. A., Robertson, C. E., Francis, C. A., and Pace, N. R.: Microbial community biofabrics in a geothermal mine adit, Appl. Environ. Microb., 73, 6172–6180, 2007.
Stahl, D. A. and Amann, R.: Development and application of nucleic acid probes, in: Nucleic Acid Techniques in Bacterial Systematics, edited by: Stackebrandt, E. and Goodfellow, M., John Wiley & Sons, Ltd., Chichester, England, UK, 205–248, 1991.
Treusch, A., Leininger, S., Kletzin, A., Schuster, S., Klenk, H., and Schleper, C.: Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling, Environ. Microbiol., 7, 1985–1995, 2005.
White, J. R. and Reddy, K. R.: Potential nitrification and denitrification rates in a phosphorous-impacted subtropical peatland, J. Environ. Qual., 32, 2436–2443, 2003.
Wuchter, C., Abbas, B., Coolen, M. J. L., Herfort, L., van Bleijswijk, J., Timmers, P., Strous, M., Teira, V., Herndl, G. J., Middelburg, J. J., Schouten, S., and Damste, J. S. S: Archaeal nitrification in the ocean, P. Natl. Acad. Sci. USA, 33, 12317–12322, 2006.
Zhang, C. L., Ye, Q., Huang, Z., Li, W., Chen, J., Song, Z., Zhao, W., Bagwell, C., Inskeep, W. P., Ross, C., Gao, L., Wiegel, J., Romanek, C. S., Shock, E. L., and Hedlund, B. P.: Global occurrence of archaeal amoA genes in terrestrial hot springs, Appl. Environ. Microb., 74, 6417–6426, 2008.

BGD
12, 16255–16283, 2015

Zhang, G., Liu, C. Q., Liu, H., Jin, Z., Han, G., and Li, L.: Geochemistry of the Rehai and Ruidian geothermal waters, Yunnan province, China, Geothermics, 37, 73–83, 2008.
Zhou, J., Bruns, M., and Tiedje, J.: DNA recovery from soils of diverse composition, Appl. Environ. Microb., 62, 316–322, 1996.

BGD
12, 16255–16283, 2015

Table 1. FISH probes and quantitative PCR primer sets used in this study.

Application	Probe/ primer set	Specificity	Sequence (5'-3')	FA(%)/ AT(°)*	Reference
FISH	Arch915	archaea	GTGCTCCCCCGCCAATTCCT	20	Stahl (1991)
	Cren679	crenarchaeota	TTTTACCCCTTCCTTCCG	35	Labrenz et al. (2010)
qPCR	518F	bacteria	CCAGCAGCCGCGGTAAT	57	Muyzer et al. (1993)
	786R		GATTAGATACCCTGGTAG		
	344F	archaea	ACGGGGCGCAGCAGGCGCGA	60	Øvreas et al. (1998)
	518R		ATTACCGCGGCTGCTGG		
	amo196F	archaeal	GGWGTKCCRGGRACWGCMAC	60	Treusch et al. (2005)
	amo277R	amoA	CRATGAAGTCRTAHGGRTADCC		

* FA: formamide.
AT: annealing temperature.

BGD
12, 16255–16283, 2015

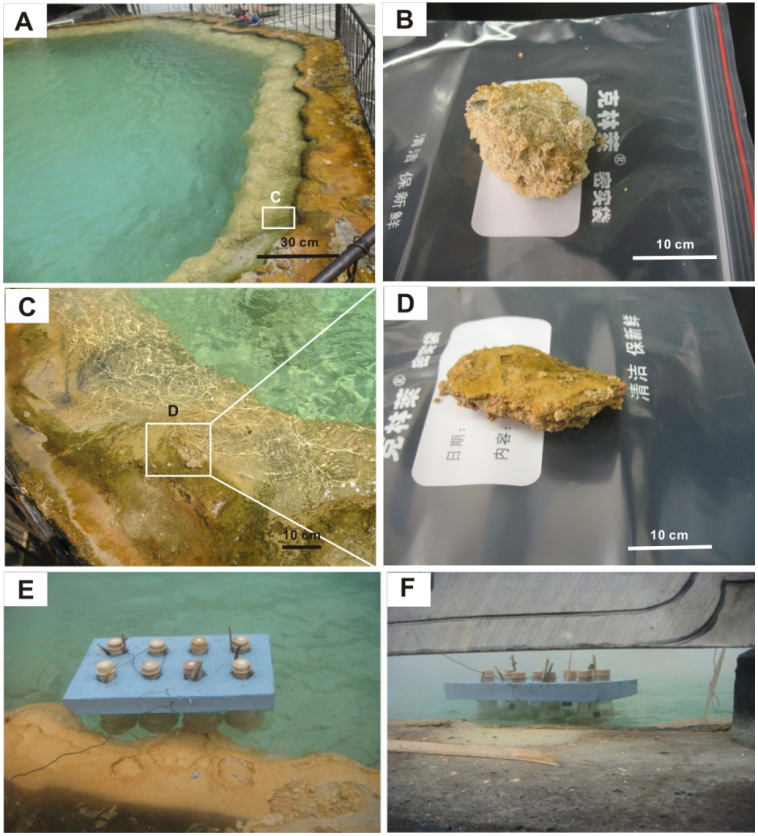

Figure 1. The Gongxiaoshe hot spring, located in the Ruidian geothermal area. **(a)** A full view of the spring; **(b)** bottom sediments of the hot spring, designated BS; **(c)** an enlarged view of the white box from Fig. 1a, surface sinter of the hot spring; **(d)** surface sinter of the hot spring designated SS; and (**e, f**) in situ nitrification activity and potential nitrification activity experiments in the field.

BGD
12, 16255–16283, 2015

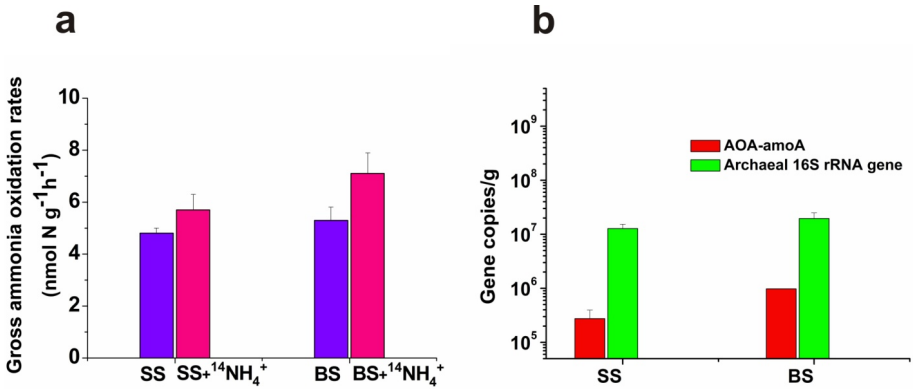

Figure 2. (a) Gross ammonia oxidation rates calculated from $^{15}N–NO_3^-$ pool dilution experiments on amended (add $^{14}NH_4^+$) or unamended SS and BS sediment slurries. The amendment with "$^{15}NO_3$" represents in situ nitrification activity, while $^{15}NO_3$ plus $^{14}NH_4$ is considered as potential nitrification activity. Bars represent the mean and standard error of the mean ($n = 3$) for 30 and 120 min of incubation. **(b)** Abundance of archaeal 16S rRNA genes and archaeal amoA genes for SS and BS samples collected from Gongxiaoshe hot spring. Data are expressed as gene copies per gram of sediment (dry weight). Error bars represent the standard deviation of the mean ($n = 3$).

BGD
12, 16255–16283, 2015

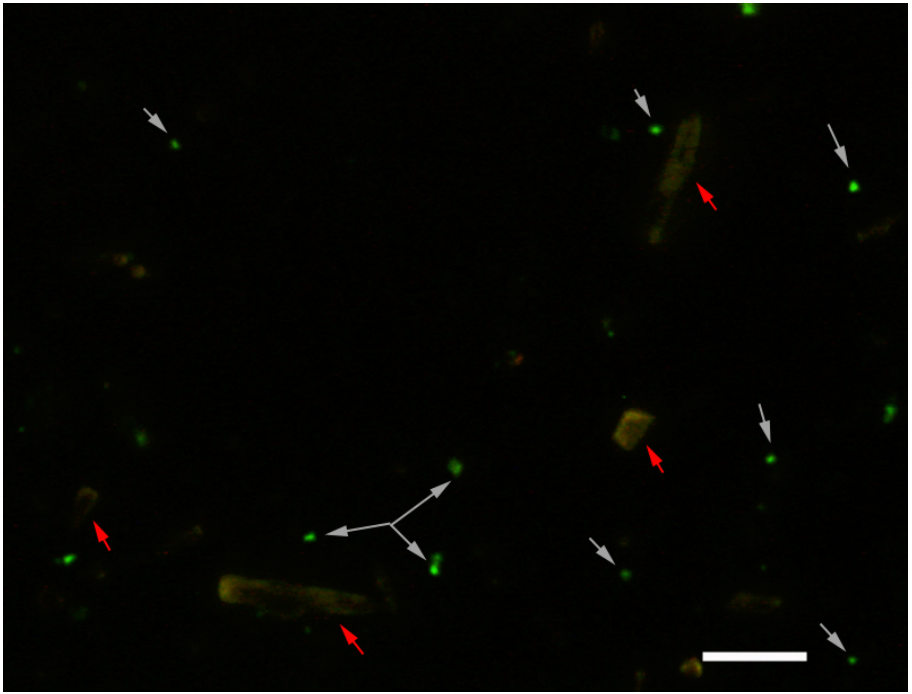

Figure 3. Epifluorescence photomicrograph of Crenarchaeota cells and cell aggregates. (White and red arrows show the cells and carbonate crystals, respectively. Scale bar corresponds to 20 µm.)

BGD
12, 16255–16283, 2015

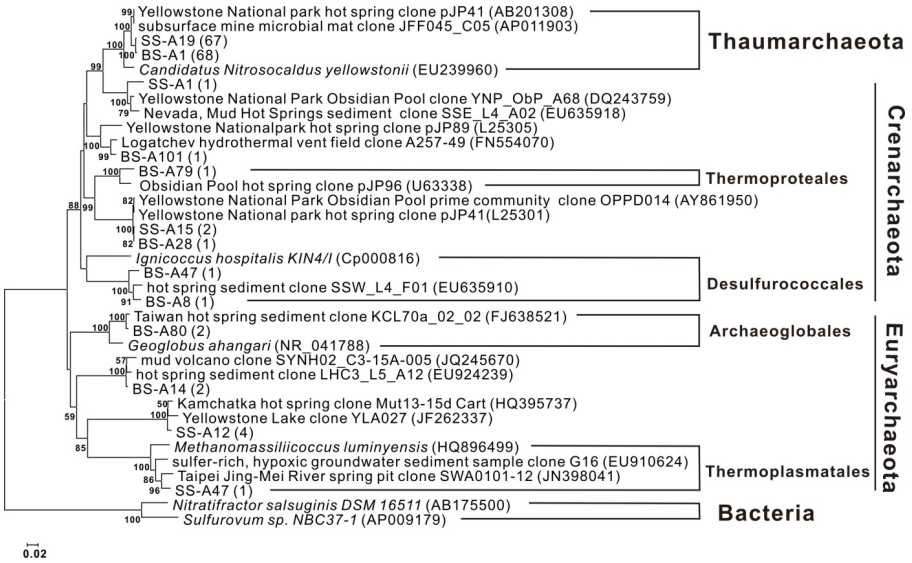

Figure 4. Archaeal phylogenetic tree based on 16S rRNA gene sequences, including various 16S rRNA gene clones obtained from the Gongxiaoshe hot spring sediments (SS and BS). The tree is constructed using the neighbor-joining method, and bootstrap confidence values over 50 % (1000 replicates) are shown. The scale bar represents the expected number of changes per nucleotide position.

BGD
12, 16255–16283, 2015

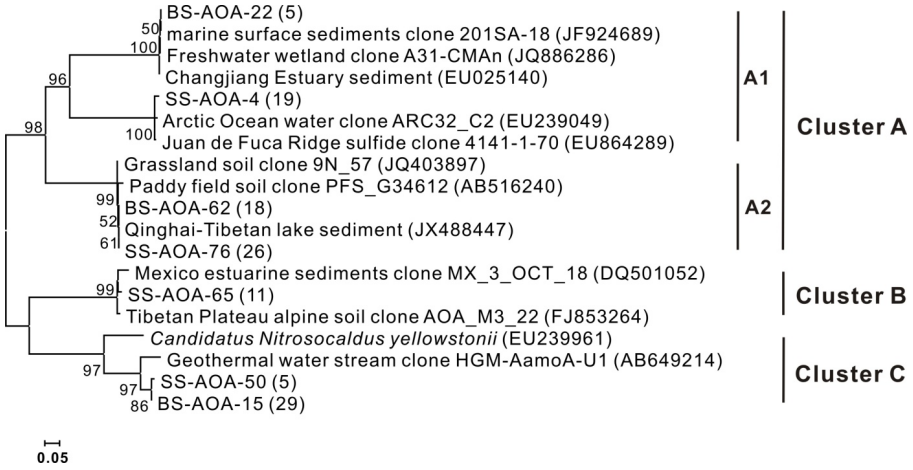

Figure 5. The phylogenetic tree of archaeal amoA genes is cloned from the Gongxiaoshe hot spring sediments (SS and BS). The tree is constructed using the neighbor-joining method, and bootstrap confidence values over 50 % (1000 replicates) are shown. The scale bar represents the expected number of changes per nucleotide position.

BGD
12, 16255–16283, 2015