Thaumarchaeota affiliated with Soil Crenarchaeotic Group are prevalent in the alkaline soil of an alpine grassland in northwestern China

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Abstract

Purpose *Thaumarchaeota* are key players within the global nitrogen cycle. Investigations of the *Thaumarchaeota* communities are important for an integrated understanding of nitrogen nutrient cycle in soil ecosystems. Therefore, the objective of this study was to examine the presence and diversity of *Thaumarchaeota* within an alkaline soil in the Bayinbuluke alpine grassland, China.

Methods The community DNAs were directly extracted from soil samples, collected on 15 July 2014, and paired-end V5–V6 amplicons of the 16S rRNA gene were sequenced by Illumina Miseq. Sequencing reads were processed using the Quantitative Insights Into Microbial Ecology (QIIME) v. 1.8.0 pipeline. After quality control, the validated sequence reads were classified into different operational taxonomic units (OTUs) based on a 97% identity level, using the Uclust algorithm to generate stable OTUs. The longest sequence in each cluster was chosen to be the representative sequence, and sequences were annotated using the Silva rRNA database project.

Result In the analyzed grassland soil, *Thaumarchaeota* had a relative abundance of 3.65 to 51.07% of the microbial community (mean = 20.20%), representing the most dominant phylum. The *thaumarchaeal* community was dominated by the Soil Crenarchaeotic Group (SCG, 34.55 to 99.82%, mean = 95.10%), with specifically low fraction of the ammonia-oxidizing genus *Candidatus Nitrososphaera* (2.83 to 30.37%, mean = 13.10%) and remaining unclassified genus.

Conclusion Our results show *Thaumarchaeota* affiliated with SCG were prevalent in the alkaline soil of this grassland.

Keywords Alkaline soil, · Illumina amplicon sequencing, · *Thaumarchaeota* · Soil Crenarchaeotic Group · *Candidatus Nitrososphaera*

Findings

Nitrification, a core process in the global nitrogen cycle, is important for the functioning of many ecosystems (Tago et al. 2015). The *Thaumarchaeota* is involved in nitrification, and plays a key role in ammonia oxidation (Stahl and de la Torre. 2012). Therefore, investigation of the *Thaumarchaeota*

communities is important for an integrated understanding of nitrogen nutrient cycle in soil ecosystems. Furthermore, considerable attention is currently being paid to this, because of their unexpected genetic diversity in many environments. Numerous findings from ecological studies on *Thaumarchaeota* in acid soils have been well discussed in connection with physiology, in several review articles (He et al. 2012). However, our knowledge of *Thaumarchaeota* communities in alkaline soil is rather limited.

Nitrification in alkaline soils is different from that in acid and neutral soils, and the widely distributed alkaline soils of northern China are a promising location for research (Sigunga et al. 2002; Shen et al. 2008). The Bayinbuluke alpine grassland (42° 10'–43° 30' N, 82° 32'–86° 15' E) is a typical alpine grassland in the temperate arid zone of China, and lies in the middle of the southern slope of the Tianshan Mountains in the Xinjiang Uygur autonomous region, in the northwest of the country (He et al. 2014). This grassland has a typical alpine

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climate. The annual average precipitation is 265.7 mm, 78.1% of which falls in the growing season. The annual average temperature is -4.8°C , with the lowest monthly value of -27.4°C in January and a highest monthly value of 11.2°C in July (He et al. 2014).

In this study, we examined the presence and diversity of *Thaumarchaeota* in the soil from the Bayinbuluke alpine grassland. We worked at 16 sampling sites on 15 July 2014 (Fig. S1). At each site, we collected three replicated soil cores (0–20 cm) using an 8-cm-diameter soil auger, and for each core surface soil samples (0–5 cm) were taken using a slicing disc. After removing plant residues and gravels, the samples were gently and thoroughly homogenized, and immediately stored at 4°C for transportation to the laboratory.

The community DNA was directly extracted from the soil samples, using a PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. To avoid subsample bias, DNA obtained from the three subsamples per soil sample was pooled to prepare each sample's PCR template. The V5–V6 hypervariable regions of 16S rRNA gene were amplified using the universal primers (forward primer (789F): 5' TAGATACC CSSGTAGTCC 3' and reverse primer (1068R): 5' CTGACGRCRGCCATGC 3') as described previously (Su et al. 2017). The V5–V6 amplicons of 16S rRNA gene were sequenced using the paired-end method by Illumina Miseq at Personal Biotechnology Co., Ltd. (Shanghai, China). Sequencing reads (subsequently referred to as "reads") were processed using the Quantitative Insights Into Microbial Ecology (QIIME) v. 1.8.0 pipeline (He et al. 2015).

After quality control, we obtained a total of 432,820 validated sequences reads from the 16 samples, with an average of 27,051 sequence reads for each sample (the minimum from one sample was 17,886 and the maximum was 41,225). These validated sequences were classified into different operational taxonomic units (OTUs), based on an identity threshold of 97% using the Uclust algorithm (Edgar et al. 2011) to generate final OTUs (He et al. 2015). The longest sequence in each cluster was chosen to be the representative sequence, and sequences were annotated by the Silva rRNA database project (SILVA VERSION SSU11; <http://www.arb-silva.de/>). The nucleotide sequences generated by pyrosequencing were deposited in the NCBI Sequence Read Archive (SRA) database, with accession number SRP068890.

All soils were slightly alkaline, with pH values ranging from 7.99 to 8.15, and the content of total organic carbon (TOC) ranging from 14.62 to 29.50% (average 20.50%) (Table 1). *Thaumarchaeota* was the most abundant microbial phylum in all samples, and had a relative abundance of 3.65 to 51.07% of all microbial sequences among the 16 samples (average 20.20%) (Fig. 1a).

Table 1 Geographic coordinates, pH, and total organic carbon (TOC) for soil samples from 16 sampling sites in the Bayinbuluke alpine grassland, China. Samples were taken on 15 July 2014. Sites are indicated by labels B1–B16

Sites	Location	pH	TOC (%)
B1	N 42° 51' 51.01" E 84° 15' 06.73"	8.00	20.19
B2	N 42° 49' 30.84" E 84° 23' 24.22"	8.03	14.62
B3	N 42° 49' 15.69" E 84° 23' 53.50"	8.01	16.03
B4	N 42° 40' 50.86" E 84° 31' 50.60"	8.15	16.98
B5	N 42° 41' 04.51" E 84° 30' 37.74"	8.03	23.16
B6	N 42° 55' 28.14" E 83° 47' 31.01"	8.07	23.01
B7	N 42° 52' 05.95" E 83° 43' 21.12"	8.05	20.31
B8	N 42° 43' 23.27" E 83° 42' 25.04"	8.05	29.50
B9	N 42° 43' 23.29" E 83° 43' 24.02"	8.06	25.29
B10	N 42° 49' 41.80" E 84° 24' 25.30"	8.08	22.15
B11	N 43° 04' 29.87" E 84° 46' 49.09"	8.02	16.48
B12	N 43° 04' 57.69" E 85° 48' 48.48"	7.99	21.61
B13	N 43° 06' 35.11" E 85° 27' 33.77"	8.02	21.88
B14	N 43° 05' 09.51" E 85° 49' 29.60"	8.00	18.31
B15	N 43° 03' 10.28" E 85° 58' 53.20"	8.09	17.74
B16	N 43° 03' 54.52" E 85° 59' 13.04"	8.05	20.73

Our unexpected findings of the high abundance of *Thaumarchaeota* in the alkaline soils of this alpine grassland contrast with those of Ochsenreiter et al. (2003) and Lehtovirta et al. (2009), who reported that the *Thaumarchaeota* constitute only 5% of the total prokaryotic community in many soils. Two reasons may account for the phenomenon that the *Thaumarchaeota* occurred at high abundance in the Bayinbuluke alkaline grassland soil. (1) The carbon content of the soil in our study was markedly higher than that in other studies (Ochsenreiter et al. 2003; Lehtovirta et al. 2009). We found that the average TOC content of the soil was 20.50% (range 14.62 to 29.50%) (Table 1), while the study of Ochsenreiter et al. (2003) reported a TOC of only 2.6% and the TOC content is lower in another study (Lehtovirta et al. 2009). Levicnik-Höfferle et al. (2012) also reported that the *Thaumarchaea* appeared to assimilate organic matter, followed by mineralization and ammonia oxidation. (2) The use of high-throughput sequencing in our study, could explore the panorama of

Fig. 1 **a** Relative abundances of *Thaumarchaeota* at the phylum level in the soils of the Bayinbuluke alpine grassland. **b** *Thaumarchaeota* community composition based on relative abundances in the soils of the

Bayinbuluke alpine grassland. **c** Relative abundances of Soil Crenarchaeotic Group at the genus level in the soils of the Bayinbuluke alpine grassland

the diversity of soil microbes (Bik et al. 2012; Zinger et al. 2012). Our methodology contrasted with the 16S RNA surveys and real time PCR used by Ochsenreiter et al. (2003) and Lehtovirta et al. (2009); in each of these two previous studies rather small amounts of *Thaumarchaeota* were detected. Our observation could be random, because our results were obtained with soil sampled on a single occasion. Furthermore, only few alkaline soils have been studied to date. Therefore, further studies of other alkaline soils would be needed to confirm these findings.

The relative abundance analysis showed that the *Thaumarchaeota* from this alkaline alpine grassland included seven subgroups: (i) Soil Crenarchaeotic Group (SCG), (ii) Miscellaneous Crenarchaeotic Group, (iii) Marine Group I, (iv) HDBA-SITS413, (v) Marine Benthic Group B, (vi) Group C3, and (vii) Sc-EA05 (Fig. 1b). The SCG represented the major part of the thaumarchaeal group for each sample, and was the most ubiquitous sub-group: the mean relative abundance was 95.10%, with a range from 34.55 to 99.82% for the 16 samples. The other six subgroups accounted for only a low fraction of the thaumarchaeal group (4.90%).

The BLAST searches conducted with our SCG sequences revealed that *Candidatus Nitrososphaera* represented only a small part of each sample, with relative abundance ranging from

2.83 to 30.37%, with a mean of 13.10%, of the *thaumarchaeal* group; *Candidatus Nitrososphaera* was designated a major genus. The other SCG sequences corresponded to unclassified genus (Fig. 1c). *Candidatus Nitrososphaera* is an important taxa involved in the soil nitrogen cycle (Spang et al. 2012), with species being involved in nitrification by performing oxidation of ammonia (Schneider et al. 2015). In the Bayinbuluke grassland soil, most *thaumarchaeal* 16S rRNA Illumina sequence reads belonged to the ammonia-oxidizing genus *Candidatus Nitrososphaera* and other unclassified members of the SCG. Our result is in good agreement with the results from savanna soils in Kruger National Park, South Africa (Rughöft et al. 2016).

In conclusion, the present study revealed that in the alkaline soil of the Bayinbuluke alpine grassland soil in China, the *Thaumarchaeota* represented an average of 20.20% of the total soil microbial community, and was a prevalent phylum. The *Thaumarchaeota* was dominated by the SCG, with a low fraction of sequences related to the ammonia-oxidizing genus *Candidatus Nitrososphaera*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals.

Informed consent A statement regarding informed consent is not applicable for this study.

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