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Research Article

RESEARCH ARTICLE

Salinity drives archaeal distribution patterns in high altitude lake sediments on the Tibetan Plateau

Yongqin Liu[1,2,*], John C. Priscu[3], Jinbo Xiong[4], Ralf Conrad[5], Trista Vick-Majors[3], Haiyan Chu[6] and Juzhi Hou[1]

[1]Key Laboratory of Tibetan Environment Changes and Land Surface Processes, Institute of Tibetan Plateau Research, Beijing 100101, China, [2]CAS Center for Excellence in Tibetan Plateau Earth Sciences, Chinese Academy of Sciences, Beijing 100101, China, [3]Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717, USA, [4]Faculty of Marine Sciences, Ningbo University, Ningbo 315211, China, [5]Max Planck Institute for Terrestrial Microbiology, Marburg 35043, Germany and [6]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

*Corresponding author: Institute of Tibetan Plateau, Chinese Academy of Science, No. 16, Lincuo Rd., Beijing 100101, China. Tel: 86-10-84097122; E-mail: yqliu@itpcas.ac.cn

One sentence summary: We represent a comprehensive investigation of archaeal diversity in the pristine lake sediments across the Tibetan Plateau, and found salinity is the key factor controlling archaeal community diversity and composition.

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ABSTRACT

Archaeal communities and the factors regulating their diversity in high altitude lakes are poorly understood. Here, we provide the first high-throughput sequencing study of Archaea from Tibetan Plateau lake sediments. We analyzed twenty lake sediments from the world's highest and largest plateau and found diverse archaeal assemblages that clustered into groups dominated by methanogenic Euryarchaeota, Crenarchaeota and Halobacteria/mixed euryarchaeal phylotypes. Statistical analysis inferred that salinity was the major driver of community composition, and that archaeal diversity increased with salinity. Sediments with the highest salinities were mostly dominated by Halobacteria. Crenarchaeota dominated at intermediate salinities, and methanogens were present in all lake sediments, albeit most abundant at low salinities. The distribution patterns of the three functional types of methanogens (hydrogenotrophic, acetotrophic and methylotrophic) were also related to changes in salinity. Our results show that salinity is a key factor controlling archaeal community diversity and composition in lake sediments on a spatial scale that spans nearly 2000 km on the Tibetan Plateau.

Keywords: archaea; salinity; lake sediment; Tibetan Plateau

INTRODUCTION

Archaea are widely distributed on Earth, and occupy a variety of niches, including extreme environments such as salt brines, hot springs, acidic environments and anoxic environments, as well as being abundant in soils, lakes and oceans (DeLong and Pace 2001; Chaban, Ng and Jarrell 2006). This group of organisms play critical roles in global climate change, as the methanogenic archaea produce about 85% of the greenhouse gas methane on our planet (Cavicchioli 2011). The diversity and ecology of Archaea

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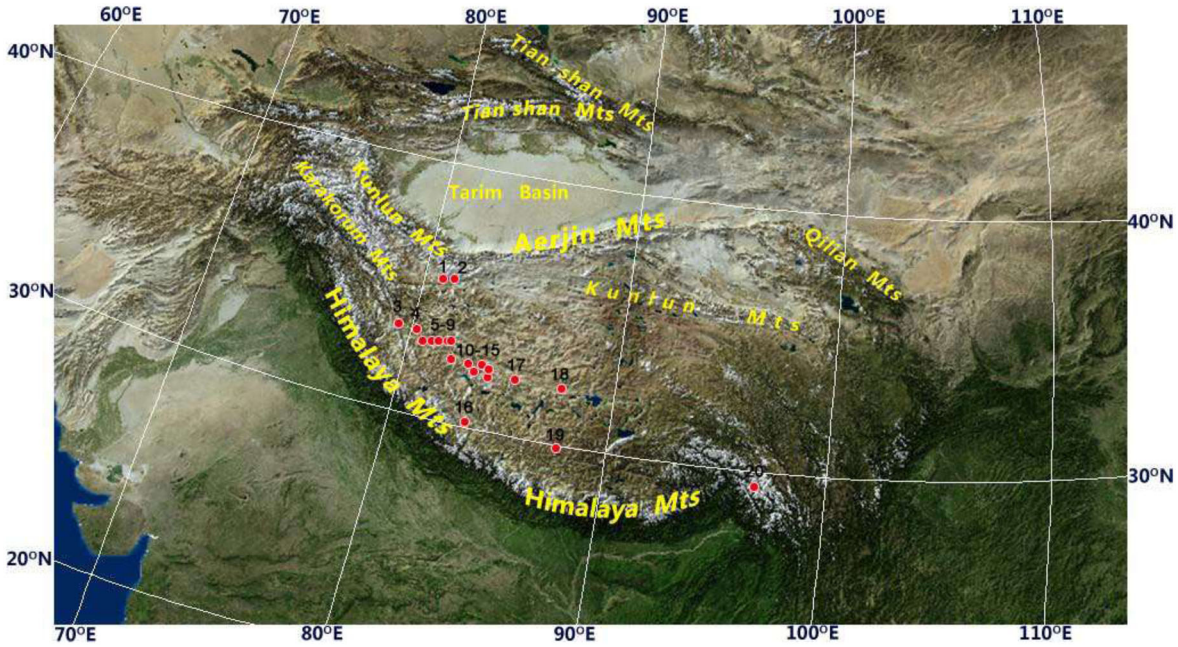

Figure 1. Locations of the 20 investigated lakes on the Tibetan Plateau. (1) Sumxi (SMX), (2) Longmuco (LC), (3) Bangongco (BGC), (4) Aiyongco (AYC), (5) Lubuco1 (LBC1), (6) Lubuco2 (LBC2), (7) Kunzhongco1 (KZC1), (8) Kunzhongco2 (KZC2), (9) Rebangco (RBC), (10) Awongco (AWC), (11) Zhacang Chaka1 (ZCCK1), (12) Zhacang Chaka2 (ZCCK2), (13) Zhacang Chaka3 (ZCCK3), (14) Bieruozeco (BRZC), (15) Darebuco (DRBC), (16) Gongzhuco (GZC), (17) Dongco (DC), (18) Dazeco (DZC), (19) Langco (LC) and (20) Ranwuco (RWC).

have received increasing attention due to their importance to biogeochemical cycles (Robertson *et al.* 2005; Cavicchioli 2011). Globally, salinity has been shown to be important in controlling archaeal distribution patterns (Auguet, Barberan and Casamayor 2010), while controls on local scales vary across environments. In soils, the relative abundance of Archaea is related to the ratio of carbon to nitrogen (Bates *et al.* 2011), while in marine systems, archaeal diversity seems to be strongly influenced by oceanic circulation, depth and temperature (Galand *et al.* 2009; Biller *et al.* 2012). Lake archaeal communities have been studied across a range of altitudes and latitudes (Schwarz, Eckert and Conrad 2007; Auguet and Casamayor 2008; Jiang *et al.* 2008; Pouliot *et al.* 2009; Hu *et al.* 2010; Swan *et al.* 2010; Auguet *et al.* 2012), where their diversity is often higher than that in sea water and soils (Auguet, Barberan and Casamayor 2010) and is affected by chemical gradients (Jiang *et al.* 2008; Pouliot *et al.* 2009; Hu *et al.* 2010; Swan *et al.* 2010).

Auguet and Casamayor (2008), in a study of 10 oligotrophic high mountain cold-water lakes in the Central Pyrenees (Spain), showed that they were important habitats for freshwater Crenarchaeota. By contrast, the ecology of Archaea in the sediments of high mountain lakes has received less attention (but see Jiang *et al.* 2008; Jiang *et al.* 2009a,b) despite the greater archaeal phylogenetic and metabolic diversity that has been proposed to be characteristic of sediment habitats (Torsvik, Ovreas and Thingstad 2002; Tranvik *et al.* 2009).

Here, we investigated archaeal communities along a salinity gradient from the sediments of 20 high altitude (>3850 m above sea level; a.s.l.) lakes spanning the Tibetan Plateau. The Tibetan Plateau is the highest plateau on Earth, with an average height of 4000 m a.s.l. and encompasses a total lake area of more than 50 900 km^2. Most lakes of the Plateau are removed from human habitation, and their pristine nature and range of geochemical conditions provide excellent conditions for studying the natural diversity and ecology of Archaea. To date, studies of the Tibetan Plateau archaeal communities have been limited to four individual hypersaline lake sediments from the northeastern Plateau, which, based on phospholipid fatty acid analysis and/or 16S rRNA clone libraries, provided limited resolution of phylogenetic diversity (PD) (Dong *et al.* 2006; Jiang *et al.* 2008). Consequently, little is known about the archaeal community composition in these pristine high altitude lake sediments. We used a high-resolution bar-coded pyrosequencing technique to describe the composition of archaeal assemblages in high altitude lake sediments across the Tibetan Plateau in the context of their different environmental conditions.

METHODS

Sampling sites and collection

We investigated surface sediments from 20 lakes located along an east-west transect on the Tibetan Plateau (Fig. 1): Sumxi (SMX), Longmuco (LC), Bangongco (BGC), Aiyongco (AYC), Lubuco1 (LBC1), Lubuco2 (LBC2), Kunzhongco1 (KZC1), Kunzhongco2 (KZC2), Rebangco (RBC), Awongco (AWC), Zhacang Chaka1 (ZCCK1), Zhacang Chaka2 (ZCCK2), Zhacang Chaka3 (ZCCK3), Bieruozeco (BRZC), Darebuco (DRBC), Gongzhuco (GZC), Dongco (DC), Dazeco (DZC), Langco (LC) and Ranwuco (RWC). The elevation range of the lakes (3850–4795 m a.s.l.) encompassed transitions from subalpine conifer to alpine steppe to alpine desert ecosystems. Annual average precipitation in the area ranges from 62 to 836 mm, and air temperature from −8.5°C to +8°C (Wang and Dou 1998). Lakes located in the eastern and central part of the transect were surrounded by meadows, while those in the western part were mostly surrounded by rocky landscapes. The lakes ranged from fresh, to saline, to hypersaline conditions with a salinity gradient from 0.3 to 340 g L^{-1} and ranged in surface area from 8 to 245 km^2 (Table S1, Supporting

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Information). Lakes LBC1 and 2, KZC1 and 2 and ZCCK1, 2 and 3 are connected during high water level.

A surface sediment sample (0–5 cm) was collected from each lake between 6 June and 5 July 2010 at or near the site of maximum water depth using a sediment grab sampler. The sediments were refrigerated upon collection and shipped to the laboratory where they were stored at 4°C for sediment geochemical characterization, and at −80°C for genomic DNA extraction.

Measurement of physico-chemical parameters

The pH and salinity values were measured in slurries of 1:1 weight ratio of sediment to distilled water. Concentrations of sodium (Na), magnesium (Mg), iron (Fe) and SiO_2 were measured using inductively coupled plasma mass spectrometry. Total carbon (TC) and total nitrogen (TN) were measured by CN Analyzer (Vario Max CN, Elementar, Germany). Phosphorus (TP) was measured following sample hydrolyzation followed by measurement of phosphate by the molybdate method (Solorzano and Sharp 1980). Lake water samples for sulfate measurements were collected from all lakes; unfortunately eight of the sample bottles were broken in transit. The remaining 12 samples were used to quantify sulfate using an ion chromatograph system (Thermofisher 900). GPS coordinates were recorded at each sampling point (range from N 29°13′ to N 34°36′, E 79°47′ to E 96°47′). Geographic pairwise distances were calculated from these positions using the following website algorithm: http://www.nhc.noaa.gov/gccalc.shtml.

DNA extraction and purification

Community DNA was extracted from sediments using the FastDNA® Spin kit (Bio 101, Carlsbad, CA, USA) according to the manufacturer's protocol. The raw DNA was purified using a 1% (w/v) agarose gel. DNA bands were excised from the gel, extracted using an Agarose Gel DNA purification kit (TaKaRa), and quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Purified DNA was stored at −20°C until use.

Archaeal 16S rRNA gene amplification and 454 sequencing

Aliquots (50 ng) of purified DNA from each sample were used as template for amplification of the hypervariable V3 regions of archaeal 16S rRNA genes (Yu *et al.* 2008). The archaeal specific primer Arch334F (ACGGGGYGCAGCAGGCGCGA) contained the Roche 454 'A' pyrosequencing adapter, and a unique 7-bp barcode sequence, while primer Arch915R (GTGCTCCCCCGCCAATTCCT) contained the Roche 454 'B' sequencing adapter at the 5′-end of each primer. Each sample was amplified in triplicate in 50 μl reaction mixture under the following conditions: 94°C for 5 min, followed by 10 cycles of touchdown PCR (denaturation at 94°C for 30 s, annealing for 30 s with an 1°C/cycle temperature decrease from 65°C and extension at 72°C for 45 s), followed by 25 cycles of regular PCR (94°C for 30 s, 55°C for 30 s and 72°C for 45 s, with a final extension at 72°C for 10 min). PCR products were pooled and purified by Agarose Gel DNA purification kit (TaKaRa) as described above.

PCR products from each sample were combined in equimolar ratio in a single tube and run on a Roche FLX 454 pyrosequencing machine (Roche Diagnostics Corporation, Branford, CT, USA), producing reads from the forward direction Arch334F.

Processing of pyrosequencing data

Sequence data were processed using the Quantitative Insights Into Microbial Ecology (QIIME, v1.5.0) pipeline (http://qiime.sourceforge.net/; Caporaso *et al.* 2010). Specifically, archaeal sequences with the same barcode were assigned into a discrete sample, and then the barcode and primer sequences were removed. The reads were truncated at any site of more than three sequential bases receiving a Phred quality score (Q) < 25 and any read containing ambiguous base calls, <200 bp was discarded. Subsequently, chimeras were removed using USARCH (Edgar *et al.* 2011). These procedures removed 298 607 low quality sequences, accounting for 27.4% of the raw sequences. Archaeal phylotypes were identified using uclust (Edgar 2010) and assigned to operational taxonomic units (OTUs, 97% similarity). Representative sequences from each phylotype were aligned using PyNAST (DeSantis *et al.* 2006a), and the most abundant sequence in the OTU was selected as representative sequence. Taxonomic identity of each phylotype was determined using the Greengenes database (DeSantis *et al.* 2006b) via the RDP classifier (Wang *et al.* 2007). After taxonomies were assigned, unclassified OTUs (0.043%) and those affiliated with Bacteria (0.07%) were removed from the dataset prior to subsequent analysis. The remaining OTUs could be classified to at least the archaeal phylum level. To correct for unequal sampling effort, we used a randomly selected subset of 3300 sequences per sample, resulting in the removal of some singletons. The rarefying procedure was repeated 20×. Archaeal diversity and community distances were calculated using the mean of the 20× subsampling. The differences in overall community composition between each pair of samples were determined using the UniFrac distance (Lozupone and Knight 2005), which provides a more robust index of community distance than taxon-based methods. The sequences generated in this study have been deposited in the NCBI Sequence Read Archive under the accession number SRP068531.

Diversity estimations and statistical analyses

The alpha diversities were estimated as richness (number of phylotypes), Shannon index (H′) and PD. Phylogenetic diversities (PD) were estimated using Faith's index, which calculates the sum of the phylogenetic distances of all the branches that are members of the corresponding minimum spanning path (Faith 1992). The relationships of the diversity indices with geochemical features were tested using correlation analysis with SPSS 17.0 for Windows (SPSS Inc., an IBM Company, US). Correlations between the UniFrac distances and sediment characteristics or geographical distances were calculated by Mantel test in PASSaGE (Rosenberg and Anderson 2011). Nonmetric multidimensional scaling (NMDS) was used to evaluate the overall differences between microbial communities in R v.2.8.1 with the vegan package. For cluster analysis, a distance matrix was computed from the abundance of OTUs present in each community using Bray–Curtis similarity, and a dendrogram was inferred with an unweighted pair-group average algorithm (UPGMA) as implemented in the program Past (Hammer, Harper and Ryan 2001).

RESULTS

Physical and geochemical characteristics

The geographical distances between individual lakes ranged from 4 to 1670 km. The physico-chemical conditions in the

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Table 1. Diversity estimates of archaeal communities based on 3300 sequences per sample.

Map id.	Lake	Detected no. of OTUs (richness of phylotype)	Phylogenetic diversity (PD)	Shannon index (H′)
1	SMXC	323	28.685	4.688
2	LMC	238	17.461	4.016
3	BGC	130	12.791	2.590
4	AYC	430	24.876	5.441
5	KZC2	330	22.551	5.699
6	LBC1	286	21.121	5.358
7	KZC1	265	17.866	4.712
8	LBC2	317	22.323	5.625
9	RBC	477	36.149	6.592
10	AWC	452	32.06	5.634
11	ZCCK1	642	30.115	6.978
12	ZCCK2	606	33.614	6.966
13	ZCCK3	635	37.129	6.643
14	BRZC	436	34.239	6.209
15	DRBC	168	15.678	2.509
16	GZC	560	32.556	6.895
17	DC	343	26.316	5.245
18	DZC	435	37.741	6.323
19	LC	414	31.023	6.324
20	RWC	489	34.63	6.363

Note: only significant correlations ($P < 0.05$) were listed. - means no significant correlations.

20 sediment samples covered a broad range (Table S1, Supporting Information), particularly with regard to salinity (0.3 g L^{-1} in SMXC to 41.9 g L^{-1} in ZCCK2) and carbon to nitrogen ratio (12 in LC to 190 in LMC). Lake water salinity ranged from 0.3 g L^{-1} in RWC to 308 g L^{-1} in ZCCK3. The pH values ranged from neutral (pH 6.9 in LBC1) to alkaline (pH 10.4 in DZC). TC content was between 0.3% and 13.2%, TN between 0.02% and 0.93% and total phosphorus between 0.2% and 0.8%. The contents of Na, Mg, Fe and SiO_2 were in a range of 4–40, 10–85, 8–45 and 0.1-15 g kg^{-1}, respectively. The salinities of sediment and water were significantly correlated (Pearson correlation, $r = 0.84$, $P < 0.001$) and the concentration of Na was significantly correlated with the salinity of the lake water ($r = 0.511$, $P = 0.021$) and the sediment ($r = 0.802$, $P < 0.001$). Sulfate concentrations measured in the water of 12 out of the 20 lakes ranged between 0.01 g L^{-1} and 39.6 g L^{-1}. Concentrations were highest in the saline lakes DRBC, DZC, AYC, AWC, BRZC and DC (2.7, 7.4, 8.5, 10.0, 16.5 and 39.6 g L^{-1}, respectively).

Diversity of the archaeal communities

After removing low quality sequences, a total of 133 987 reads were obtained from the 20 sediment samples, with an average of 6699 sequences per sample. Average read length was 429 bp after trimming the primers. Only 0.07% of the sequences were affiliated to Bacteria, the rest were Archaea, indicating the high specificity of our chosen primers.

Among the 20 lake sediments, 14% to 69% of the OTUs were unique to each sample (Table S2, Supporting Information). The percentages of overlapping OTUs ranged from 0.3% to 36.5% (average = 6.6%). In particular, those lakes that were connected during times of high water level (LBC1 and 2, KZC1 and 2 and ZCCK1-3) showed a relatively large overlap (36.2%, 27.0% and 12.0%, respectively). The archaeal communities were highly diverse, with numbers of OTUs (richness of phylotypes) ranging from 130 to 642 per 3300 sequences (Table 1). Richness was significantly correlated with the salinities of both lake water ($r = 0.515$, $P = 0.02$) and sediment ($r = 0.676$, $P = 0.001$), while Shannon indices (H′) correlated only with sediment salinity ($r = 0.464$, $P = 0.039$). PD were significantly correlated with sediment pH values ($r = 0.482$, $P = 0.032$) and marginally correlated with sediment salinity ($r = 0.411$, $P = 0.072$). Among the mineral components of the sediments (Table S1, Supporting Information), only Na was significantly correlated with richness ($r = 0.679$ $P = 0.001$), H′($r = 0.494$ $P = 0.027$), and PD ($r = 0.608$ $P = 0.004$). No significant relationship was observed between richness, H′ or PD and concentrations of TC and nitrogen, and C/N ratio.

Composition and distribution of archaeal taxa

The archaeal communities in the sediment samples were predominately composed of the methanogenic classes Methanomicrobia and Methanobacteria, the halophillic class Halobacteria, the class Thermoplasmata of the phylum Euryarchaeota, and the phylum Crenarchaeota (Fig. 2). The methanogenic classes, the Halobacteria, and the Crenarchaeota accounted for 36%, 29% and 27% of all archaeal reads, respectively. The relative abundances of these archaeal taxa were correlated with different sediment characteristics. Specifically, the percentage of the Halobacteria significantly ($r = 0.735$, $P < 0.001$) correlated with sediment salinity. The percentage of methanogenic classes was positively ($r = 0.622$, $P = 0.003$) correlated with total N content and negatively ($r = -0.507$, $P = 0.023$) with sediment pH. The relative contribution of the Crenarchaeota was correlated with the concentration of SiO_2 ($r = 0.468$, $P = 0.038$).

Cluster analysis of the archaeal community compositions separated the 20 samples into three main clusters (Fig. 2). Two of these clusters were dominated by Euryarchaeota, and one by Crenarcheota. The sediments in the first cluster (KZC1,2, LBC 1,2 and BGC) were dominated by methanogens (Euryarchaeota); those in the second cluster (DC, SMXC, DZC, LMC, LC and RWC) were dominated by the Soil Crenarchaeotic Group (SCG); those in the third cluster contained mainly a mixture of different Euryarchaea and Crenarchaeota. The Euryarchaeota in this

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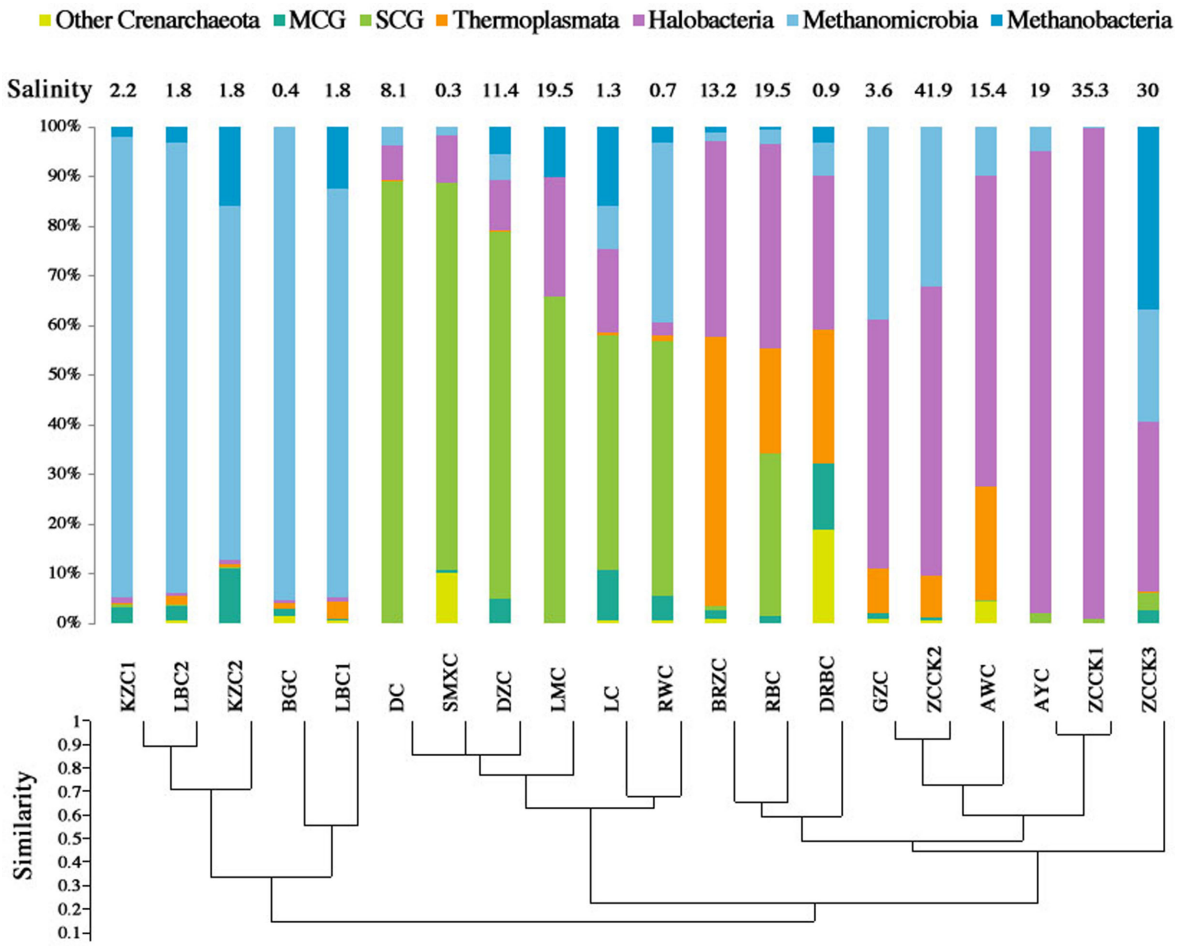

Figure 2. Relative proportions of the archaeal classes within each lake sediment. SCG and MCG represent Soil Crenarchaeotic Group and Miscellaneous Crenarchaeotic Group, respectively. Cluster analysis of archaeal community composition was done using Bray–Curtis similarity; the dendrogram was inferred from an unweighted pair-group average algorithm (UPGMA).

sediment cluster were dominated by Halobacteria, but also contained Thermoplasmata and methanogens.

Methanogens

Methanogen sequences were widely distributed across all sediment samples. Eighty five percent of the methanogens belonged to the class Methanomicrobia and the rest to the class Methanobacteria. The methanogens grouped into 16 genera and seven taxonomically unclassified groups (Table S3, Supporting Information). The genera *Methanosaeta, Methanoregula, Methanobacterium* and *Methanosarcina* were relatively abundant, accounting for 32%, 19%, 11% and 10% of the total methanogenic sequences, while the 12 other genera each accounted for less than 5% of the methanogenic sequences in each sample. The acetoclastic genus *Methanosaeta* was the most ubiquitous, occurring in 18 of the 20 sediments, followed by *Methanolobus* (17 lakes), which can use methanol to produce methane (Liu and Whitman 2008). *Methanolobus* was the primary methanogenic genus in AWC and DC, accounting for 85% and 88% of all the methanogenic sequences, respectively. The hydrogenotrophic genera *Methanobacterium, Methanoregula* and *Methanolinea*, which are commonly encountered in freshwater lakes (Borrel *et al.* 2011), were observed in 16, 14 and 12 of the lakes, respectively. The genus *Methanocella*, which is prevalent in rice soil but rare in lake sediments (Conrad, Erkel and Liesack 2006), was found in 10 lakes.

Methanogenic genera can be classified into three functional types according to substrate utilization, i.e. (i) hydrogenotrophic methanogens, which reduce CO_2 to CH_4 using H_2 as reducing agent; (ii) methylotrophic methanogens which use methylated compounds such as methanol, trimethylamine, dimethylsulfide, to produce CH_4; and (iii) acetotrophic methanogens which dismutate acetate to CH_4 and CO_2 (Liu and Whitman 2008). We calculated the relative contributions of these three functional types to the lake sediment archaeal communities (Fig. 3). We grouped all the sequences of the genus *Methanosarcina* into acetotrophic methanogens, although *Methanosarcina* species can also utilize H_2 and CO_2 to produce CH_4.

There was no significant correlation between the relative abundances of the three functional types with salinity, TC, TN or pH, but general distribution patterns were related to salinity. Hydrogenotrophic and acetotrophic methanogens occurred in sediments with relatively low salinities (0.4 to 3.5 g L^{-1}), where they dominated the total archaeal community (Fig. 3). However, they also occurred in two hypersaline lake sediments (ZCCK1, ZCCK2) with salinities of 30 and 42 g L^{-1} (Fig. 3). As the percentage of the hydrogenotrophic methanogenic genera typically decreased with salinity, the opposite trend occurred for the acetotrophic genera (Fig. S1, Supporting Information).

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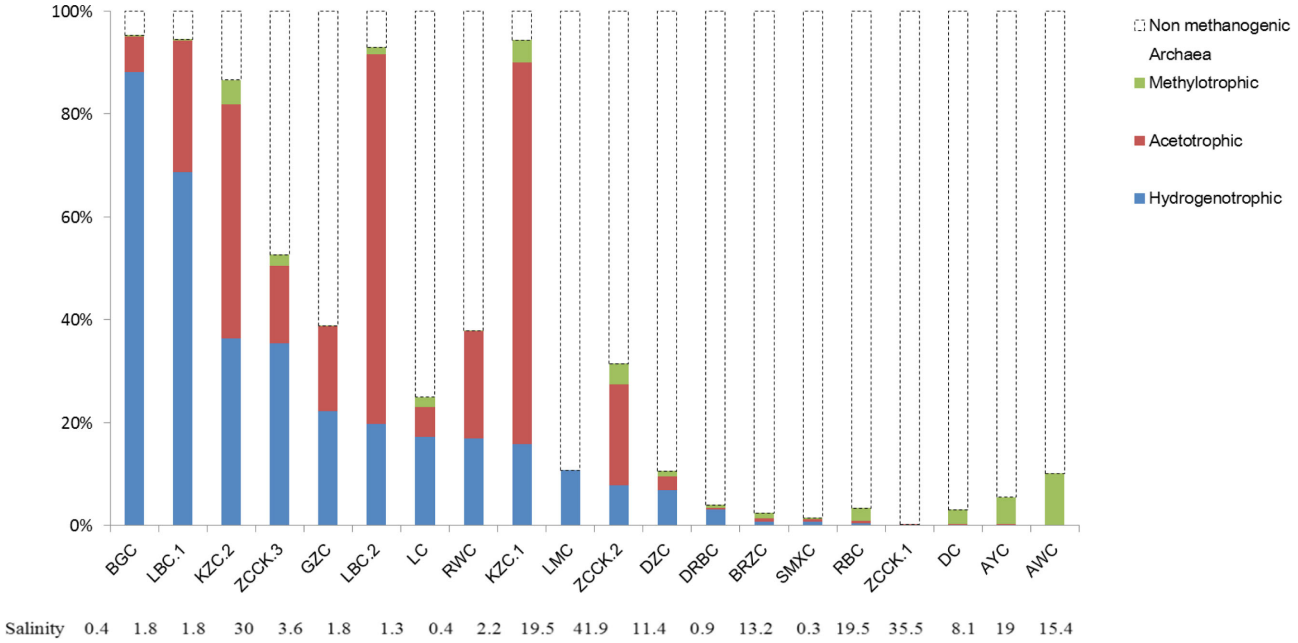

Figure 3. Relative proportions of hydrogenotrophic, acetotrophic and methylotrophic methanogens relative to total archaea in each lake. Columns were arranged in decreasing order of the proportions of hydrogenotrophic methanogens. Sediment salinities are indicated on the *x*-axis. The hydrogenotrophic methanogens include the genera *Methanocella*, *Methanocalculus*, *Methanolinea*, *Methanoregula*, *Methanoculleus*, *Methanomicrobium*, *Methanospirillum*, *Methanobacterium*, *Methanobrevibacter* and *Methanosphaera*, and the taxonomically unclassified groups Rice cluster II, LR-13, GOM Arc1 and WCHA2-08. The acetotrophic methanogens include the genera *Methanosaeta* and *Methanosarcina*. The methylotrophic methanogens include the genera *Methanohalophilus*, *Methanolobus*, *Methanomethylovorans* and *Methanosalsum*.

Methylotrophic methanogens most often occurred in sediments with salinities between 8.1 and 19.5 g L^{-1}, and comprised a relatively small percentage of the total archaeal community (Fig. 3). Although we have no data for sediment sulfate concentrations, the relative abundances of methylotrophs were significantly correlated with lake water sulfate concentrations ($r = 0.58$, $P = 0.046$).

Halophiles

Many different halophiles exist in the Tibetan lake sediments. The numbers of halobacterial OTUs in a given sediment sample ranged from 11 to 628 with an average of 197 (Table S4, Supporting Information), and exhibited a significant correlation with sediment salinity ($r = 0.809$, $P < 0.001$). The sequences primarily belonged to the family Halobacteriaceae (76% of the halobacterial sequences), and the remainder belonged to Deep Sea Hydrothermal Vent Gp 6 (DSHV) and Miscellaneous groups. The sequences in the Halobacteriaceae were divided into 26 genera and five other groups (Table S4, Supporting Information). The genus *Halorubrum* was widely distributed across 11 saline and four freshwater lakes, but was primarily found in hypersaline lakes (salinity 11–41.9 g L^{-1}; 79% of sequences in the genus). The most abundant OTU was 98.6% similar to *Halorubrumaidingense*, an extremely halophilic archaeon from a solar saltern (Cui *et al.* 2006). Sequences related to the genus *Natronorubrum* were the second most abundant, but were not widely dispersed; 87% of the sequences were restricted to only two lake sediments (AWC and AYC, salinity both 19.5 g kg^{-1}). The most abundant *Natronorubrum* sequence was similar to that of a strain of *Natronorubrumbangense* (NR˙028252, 97.4%), which was isolated from a Tibetan lake sediment (Xu, Zhou and Tian 1999). Sequences of the DSHV group were the only cosmopolitan halophilic group, with representatives in all 20 sediments accounting for 0.2% to 19% (average 5%) of all sequences in the seven freshwater lakes and 0.2% to 22% (average 7%) of all sequences in the 13 saline lakes (Tables S1 and 4, Supporting Information).

Crenarchaeota

The Crenarchaeota existed in all sediments and dominated the archaeal sequences in six of the lakes (SMXC, RWC, LC, DC, DZC and LMC) with moderate salinities (0.3 to 19.5 g L^{-1}). The crenarchaeotal sequences were affiliated with the SCG, the Miscellaneous Crenarchaeotic Group (MCG), Group C3, and the Marine Benthic GroupB (MBGB) (Fig. 2; Table S5, Supporting Information). Among these different groups, SCG accounted for 81% of the Crenarchaeotic sequences and was abundant (average 94 ± 6%) in six lakes whose archaeal community was dominated by Crenarchaeota (>56%). The most abundant sequences belonging to SCG accounted for 45% of total Crenarchaeota and were closely related (96.2% similarity) to *Nitrososphaeragargensis*(GU797786), an ammonia-oxidizing archaeon isolated from a hot spring(Spang *et al.* 2010). Crenarchaeota belonging to MCG accounted for 11% of the crenarchaotal sequences. They made up the majority of the Crenarchaeota (average 74 ± 24%) in lakes with methanogens as most numerous archaea (>59%). Groups C3 and MBGB primarily occurred in saline lakes with a high percentage of *Halobacteria* (Table S5, Supporting Information).

Thermoplasmata

The Thermoplasmata only accounted for 7.6% of all archaeal sequences, and occurred in 18 of 20 lakes. However, the Thermoplasmata were abundant in three hypersaline lake sediments (salinity >13 g L^{-1}; BRZC, AWC, RBC), where they accounted for 54%, 23% and 27% of the total, respectively, and in one freshwater sediment (DRBC, 21% of total). In the other 14 lakes, the Thermoplasmata accounted for less than 10% of total

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Table 2. Mantel test for the archaeal community compositions.

Factors	Archaeal community composition	
	r	*p*
Altitude	0.055	0.339
Area	−0.105	0.716
C/N	0.106	0.171
Mg	0.052	0.241
Na	0.048	0.255
P	−0.161	0.861
pH	−0.062	0.609
Geographic distance	0.021	0.782
Lake water salinity	−0.082	0.618
Sediment salinity	**0.530***	**0.035***
TC	−0.182	0.509
TN	−0.115	0.725

*indicates significance.

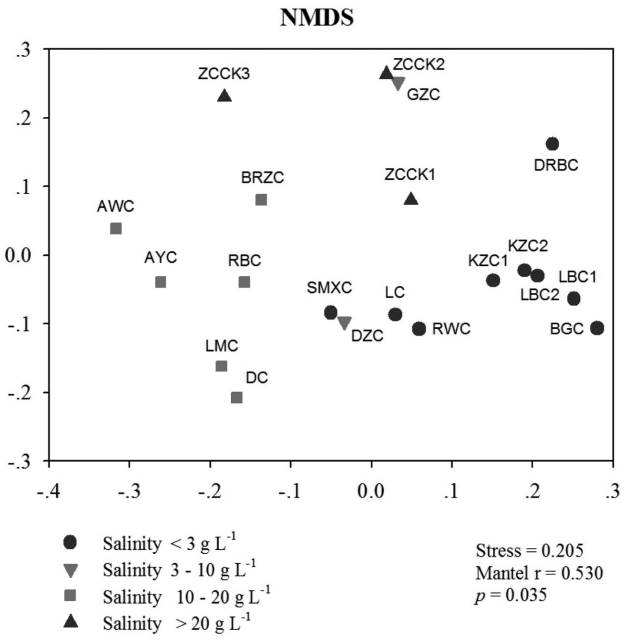

Figure 4. NMDS analysis of the archaeal communities based on Bray–Curtis distances of detected OTU abundances, with symbols coded by sediment salinity category as in Fig. 1. Mantel test further revealed the significant correlation between community composition and salinity.

sequences. Ninety one percent of the Thermoplasmata were affiliated with four taxonomically unclassified groups (34% Marine Group, 28% AMOS1A-4113-D04, 17% KTK and 12% CCA47), which are often associated with marine or high salinity environments (Sorensen *et al.* 2005; Ferrer *et al.* 2011). Sequences belonging to the terrestrial Group accounted for 6% of total Thermoplasmata sequences.

Linking archaeal communities to sediment properties

Mantel tests of OTUs and environmental indices showed that the archaeal community structures in the different lake sediments were not correlated with the surface area, the altitude or the water salinity of the different lakes, or with the geographic distance between the lakes (Table 2). However, archaeal community composition was correlated ($r = 0.53$, $P = 0.035$) to the salinities of the lake sediments (Table 2). This result was further supported by NMDS demonstrating that the archaeal communities clustered according to sediment salinity (Fig. 4).

DISCUSSION

Salinity has been reported as one of the most important factors influencing the archaeal composition within a lake sediment core (Jiang *et al.* 2007; Swan *et al.* 2010), and locally between lakes (Jiang *et al.* 2009a; Hu *et al.* 2010), as well as across different aquatic environments including lakes, ocean, rivers and hot springs (Biller *et al.* 2012). Moreover, salinity was found as one of the principal forces driving archaeal community composition on a global scale (Auguet, Barberan and Casamayor 2010). We found salinity to be similarly important in our regional examination of lake sediments across the Tibetan Plateau.

Previous studies on lowland aquatic systems and soils have shown that salinity decreases archaeal richness and diversity (Bowman *et al.* 2000; Hollister *et al.* 2010) and that Shannon H′ values tend to decrease as salinity increases (Hollister *et al.* 2010). We found that, in our Tibetan lake sediments, salinity did not impose an overall limit on archaeal communities' diversity. Rather, phylotype richness, H′ and PD were positively correlated with sediment salinity. The same trend was also observed for the bacterial richness in the water of Tibetan lakes (Wang *et al.* 2011). Similar patterns in upland archaeal and bacterial richness, in combination with the opposite trend in lowland lakes, indicate that the selection factors acting on microbial populations in the isolated high altitude lakes of the Tibetan plateau may be different from those in the lowland.

Archaeal communities in the Tibetan Plateau lakes sediments clustered into groups dominated by methanogenic Euryarchaeota, Crenarchaeota and Halobacteria/mixed euryarchaealphylotypes (Fig. 2). Community compositions (Fig. 4) and dominant groups (Fig. 2) clustered according to the salinity categories. Further evidence for this pattern is provided by the analysis of similarity (Global $F = 0.509$, $P = 0.002$). Sediments with the highest salinities (>15 g L^{-1}) were usually dominated by Halobacteria .The Crenarchaeota dominated at intermediate salinities (0.3 to 19.5 g L^{-1}), in contrast to previous studies, which found that Crenarcheota were more common in freshwater than in saline sediments (Ochsenreiter *et al.* 2003; Jiang *et al.* 2007).

Diverse methanogenic lineages were present in all of the lake sediment samples and accounted for more than 50% of the total sequences in five of the lakes. Methanogens generally dominated at low salinity (0.4 to 2.2 g L^{-1}), although some lineages were dominant at salinities up to 30 g L^{-1} (Fig. 2). Additionally, Thermoplasmata-related Archaea, which dominated in three of the lakes in our study, were recently identified as a seventh order of methanogens (Paul *et al.* 2012). The MCG, one of the dominant Crenarchaeota group in our study lakes, were recently found to metabolize methane (Evans *et al.* 2015). The methanogenic archaea play key roles in biogeochemical carbon cycling in soils and oceans (Cavicchioli 2006; Conrad 2007) and the dominance of methanogenic archaea in our study suggests important roles in sediments of Tibetan lakes.

The distribution patterns of the three functional types of methanogens (putatively hydrogenotrophic, acetotrophic and methylotrophic) indicated that CH_4 production pathways varied among the lakes and were influenced by salinity. Usually, hydrogenotrophic and acetotrophic methanogenesis

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represent the terminal processes in the anaerobic degradation of organic carbon in freshwater sediments (Conrad and Claus 2005). The relative abundances of hydrogenotrophic and acetotrophic methanogens have been found to be affected by environmental factors such as pH (Phelps and Zeikus 1984), temperature (Conrad *et al*. 1989) and substrate concentration (Chan *et al*. 2005), but these factors did not influence either functional group in the Tibetan sediments. Instead, the distributions of hydrogenotrophic and acetotrophic methanogens were related to salinity. The hydrogenotrophic methanogens decreased in abundance as salinity increased, while the trend was opposite for acetotrophic methanogens. Methylotrophs dominated the methanogenic groups in the hypersaline lake sediments where acetotrophic methanogens became less abundant (AWC, AYC and DC; Fig. 3), although they also existed in the non-saline sediments that were dominated by hydrogenotrophic and acetotrophic methanogens.

The relative abundance of methylotrophic methanogens among total methanogens was significantly correlated with the sulfate concentration of the overlying lake water, suggesting that these methanogens might be important contributors to methane production in sediments with high sulfate content. Sulfate reducers can outcompete methanogens for common substrates, such as H_2 or acetate, a phenomenon common in marine sediments (Ward and Winfrey 1985). However, sulfate reducers do not usually compete for methylated compounds, allowing methylotrophic methanogenesis to be carried out in spite of high sulfate concentrations (Oremland and Polcin 1982; Oremland 1988).

Anaerobic methanotrophic archaea (ANME) are often found in association with methanogenic archaea, but no sequences from our study clustered with ANME. Instead, members of the MCG tended to co-vary with methanogenic Archaea in the lake sediments. MCG have been implicated in methane cycling in marine sediments (Biddle *et al*. 2006). Kubo *et al*. (2012) found that MCG distribution did not co-vary with energy availability from methane and sulfate, but Archaea in MCG-rich sediments were found to utilize degradation products of fossil organic matter (Kubo *et al*. 2012). Our data indicated a potential relationship between MCG and methanogens in high altitude lake and, in combination with the abundance of methanogenic Archaea, indicate that the presence of an active methane cycle is likely in these lakes.

In conclusion, our study represents a comprehensive investigation of archaeal diversity in the sediments of pristine lakes across the Tibetan Plateau. Archaeal community composition was influenced most strongly by salinity, rather than pH and geographic distance, which are important for bacterial communities (Xiong *et al*. 2012), suggesting that the distribution patterns of different microbial groups are shaped by different factors in lake sediments. The results document high diversity of methanogens, Crenarchaeota, Halobacteria and Thermoplasmata. Methanogens occurred in sediments of all lakes, but the distribution patterns of the three functional groups of methanogens (hydrogenotrophic, acetotrophic and methylotrophic) were controlled by salinity. The cosmopolitan distribution of functionally diverse methanogens in these lakes indicates that the archaeal communities may be a significant source of CH_4. Considered with the substantial atmospheric methane input already calculated for Tibetan Plateau wetlands (450 Gg yr^{-1}; Ding, Cai and Wang 2004), our data reveals that microbial activity and methane efflux from the region may have significant impacts on global climate, with salinity controlling the functional groups responsible.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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