Effects of salinity and nutrients on sedimentary bacterial communities in oligosaline Lake Bosten, northwestern China

Jiangyu Dai^{1,2}, Xiangming Tang¹, Guang Gao^{1,*}, Dan Chen^{1,2}, Keqiang Shao¹,
Xianlei Cai^{1,2}, Lei Zhang^{1,2}

¹State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology,
Chinese Academy of Sciences, Nanjing 210008, PR China

²Graduate School of Chinese Academy of Sciences, Beijing 100049, PR China

ABSTRACT: Lake Bosten, a large oligosaline lake in arid northwestern China, has evolved from a freshwater lake and is influenced by eutrophication processes. Since sediments record past limnic changes, the analysis of sedimentary bacteria in Lake Bosten may help elucidate bacterial responses to both salinization and eutrophication. We characterized the intrasystem distribution of sedimentary bacterial communities between freshwater and oligosaline regions of Lake Bosten using denaturing gradient gel electrophoresis and 16S rRNA gene clone libraries. Both principal component analysis and redundancy analysis revealed that salinity was the dominant and pronounced intrasystem factor that shaped the sedimentary bacterial community composition in Lake Bosten. In total, 349 clones and 185 distinct operational taxonomic units were acquired from 4 clone libraries. *Proteobacteria* (41.5%) was the most abundant phylum, followed by *Bacteroidetes* (13.5%), *Chloroflexi* (8.9%), *Firmicutes* (8.6%) and others. The higher bacterial diversities and proportions of *Beta*- and *Deltaproteobacteria* in the oligosaline regions than in the freshwater region reflected the responses of sedimentary bacteria to increased salinity and nutrient levels. The scarceness of typical freshwater bacteria, the relatively high percentage (12.3% of total bacteria) of putative sulfate-reducing bacteria and a high percentage of unclassified bacteria in the sediments all highlight the uniqueness of sedimentary bacterial communities in the early stages of salinization and eutrophication.

KEY WORDS: Lake Bosten · Sediment · Salinity · Nutrient · Bacterial community composition

— Resale or republication not permitted without written consent of the publisher —

INTRODUCTION

In inland areas, limnic ecosystems are frequently used as models for studying the response of aquatic bacterial communities to dominant environmental factors, such as salinity (Wu et al. 2006, Jiang et al. 2007, Wang et al. 2011, Tang et al. 2012), nitrogen and phosphorus levels (Tang et al. 2010) and organic matter (Fujii et al. 2012). Most of the lakes that have previously been studied are either freshwater or saline, and only a few studies have focused on oligo-

saline lakes (Wu et al. 2006, Liu et al. 2010, Tang et al. 2012). In arid regions of Central Asia, many freshwater lakes have evolved to be oligosaline in the past 30 yr due to climatic change and uncontrolled resource exploitation (Bai et al. 2011). However, the impact of these environmental changes on bacterial communities may be accurately recorded in limnic sediments (Chen et al. 1999). Sedimentary bacteria, as a result of their high cell abundance ($>10^8$ cells g^{-1}) (Schmidt et al. 1998, Danovaro et al. 2002), play a crucial role in ecological and biogeochemical pro-

*Corresponding author. Email: guanggao@niglas.ac.cn

cesses (Nealson 1997). The characteristics of sedimentary bacteria, including their biomass and community structure, are determined by the variability in physicochemical and biotic parameters, and can thus be regarded as representative indicators of environmental change (Zhang et al. 2008). Although salinity is regarded as one of the most important global determinants of bacterial communities (Lozupone & Knight 2007), it is not well known whether increases in salinity shape sedimentary bacterial communities in oligosaline lakes.

Lake Bosten, the largest oligosaline lake in the arid region of China, is representative of an oligosaline lake that evolved from a freshwater lake (Bai et al. 2011). The freshwater inflows supplied by melting ice, precipitation and groundwater in the mountainous regions of Tianshan (Xiao et al. 2010) helped shape the freshwater region of this lake and shift the salinity gradient from freshwater to oligosaline in this lake (Tang et al. 2012). Meanwhile, a trophic gradient from oligotrophic to mesotrophic also formed due to anthropogenic agricultural and tourism activities (Xie et al. 2011). Such a complex ecosystem is an ideal model to study the responses of bacterial communities to gradients in salinity and nutrients (Tang et al. 2012). However, little is known about the responses of sedimentary bacterial community composition (BCC) to increased salinity and nutrient levels in oligosaline lakes or which bacterial phylotypes are particularly sensitive to such changes.

To address the aforementioned issues, in the present study, we (1) investigated whether salinity and/or nutrient levels were the main environmental parameters shaping the sedimentary BCC, by analyzing the spatial distribution of sedimentary BCC in response to salinity and nutrient gradients in Lake Bosten, and (2) identified the dominant bacterial phylotypes that are sensitive to alterations in salinity and nutrient levels in sediments. Our results provide a framework for understanding the sedimentary BCC in oligosaline environments in the arid region of China and shed light on responses of sedimentary BCC to the processes of salinization and eutrophication.

MATERIALS AND METHODS

Sampling sites

Lake Bosten (86°40'–87°26'E, 41°56'–42°14'N) spans 55 km from east to west and 25 km from north to south, covering an area of ~1100 km² (Mischke & Wünnemann 2006). Over the course of a year, the

average water depth is 7 m, with a maximum depth of 16 m (Sai et al. 2011). Water stratification in the lake depends mainly on the inflow of meltwater from the Kaidu River and the outflow through the Kongqi River which ends in the Tarim Basin (Mischke & Wünnemann 2006). Sixteen sites across the lake were chosen for collecting sediment samples on 23 and 24 August 2010 (Fig. 1). Of these sampling sites, Sites L and M were located near the Kaidu River, which is an isolated freshwater region. All the other sites were located in oligosaline regions, which are influenced by human activities in agriculture or tourism. For example, Sites D and E were near the tourist area of the Jinsha Shore along the eastern lakeshore. Sites G and H were influenced by saline and eutrophic irrigation drainage from the northern lakeshore.

Sample collection and physicochemical analysis

The temperature, pH, dissolved oxygen (DO), salinity and total dissolved solids (TDS) of the lowest layer of water overlying the sediments were measured *in situ* using a YSI 6600 Multi-Parameter Water Quality Sonde. Surface sediment (0 to 5 cm) samples from all sites were collected using a 1/16 m² Petersen grab sampler and stored in a refrigerated container with ice bags until analysis. All analyses were conducted within 24 h of sampling. Aliquots of sediment samples were stored at –80°C for bacterial cell counting and DNA extraction. Interstitial water from the sediment samples was collected by centrifuging samples at 1000 × *g* for 5 min and then used to determine the concentrations of chloride (Cl[–]) and sulfate (SO₄^{2–}) with an ICS 2000 Ion Chromatography sys-

Fig. 1. Location of sampling sites in Lake Bosten, north-western China

tem (Thermo Scientific). The total organic carbon (TOC) in the sediment samples was determined using the standard $K_2Cr_2O_7$ oxidation-reduction titration method, the total nitrogen (TN) was determined by the standard Kjeldahl method, and the total phosphorus (TP) was colorimetrically determined after $HClO_4$ - H_2SO_4 digestion, using the standard ascorbic-acid method (Lu 1999).

Bacterial cell counting

The bacterial cell-counting protocol was modified from Martins's method (Martins et al. 2009). Aliquots of the stored sediment samples were first freeze-dried. Next, the freeze-dried sediment samples (1 g) were each fixed in 5 ml 4 % formaldehyde for 2 h, centrifuged at $10\,000 \times g$ for 5 min, washed with 5 ml PBS buffer (10 mM NaH_2PO_4 [pH 7.2], 120 mM NaCl and 10 mM sodium pyrophosphate) and centrifuged again as above. The reprocessed samples were stored in a mixture of 5 ml PBS buffer and 5 ml 100 % ethanol at $-20^\circ C$. To separate the bacteria from the sediment particles, aliquots of the reprocessed samples (5 ml) were sonicated for 90 s at 30 W with a 5 s pulse mode and then allowed to rest for 5 min to precipitate the sediment particles. One microliter of the sonicated sample was then gradually diluted to 1:2500 with sterile water in sequential 10-fold dilutions. Aliquots of 5 ml of the diluted samples were stained for 10 min in a glass filtering cylinder (Millipore) with 4',6-diamidino-2-phenylindole (final concentration of 5 mg l^{-1}) (Porter & Feig 1980, Gao et al. 2007). After 10 min, the stained sample was filtered through a $0.2\text{ }\mu\text{m}$ pore-size polycarbonate filter (Millipore) and washed briefly with sterile water. The filtered membrane was mounted on a slide and observed with a Zeiss epifluorescent microscope (Carl Zeiss). For each filter, 20 fields were photographed randomly over the entire filter and counted manually. The final bacterial cell counts were calculated per gram of dry sediment.

DNA extraction and denaturing gradient gel electrophoresis (DGGE) analysis

DNA was extracted from 0.25 g freeze-dried sediment using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories), as described by the manufacturer, and diluted to a final volume of 50 μl .

PCR amplification for DGGE was carried out using the 16S rRNA gene specific primer 341F, with a 40 bp GC-clamp attached to its 5' end, and the reverse

primer 518R (Muyzer et al. 1993). PCR was conducted with $1\times$ PCR buffer, 0.6 mM dNTPs, 1.5 mM $MgCl_2$, 0.1 μM of each primer, 2.5 U *rTaq* polymerase (TaKaRa) and sterilized water in a final volume of 50 μl . The PCR conditions were as follows: denaturation at $95^\circ C$ for 5 min; 35 cycles of 45 s at $94^\circ C$, 45 s at $55^\circ C$ and 1 min at $72^\circ C$; a final extension step of 5 min at $72^\circ C$. Triplicate PCR products for each sample were mixed and loaded onto an 8 % polyacrylamide gel (acrylamide/bis-acrylamide: 37.5:1) in $1\times$ TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA, pH 8.0) using a denaturing gradient ranging from 35 % to 55 % (100 % denaturant contained 7 M urea and 40 % deionized formamide). Electrophoresis was performed at 100 V and $60^\circ C$ for 18 h with a DGGE-2001 system (C.B.S. Scientific). Next, the gel was stained with a 1:10 000 dilution of SYBR Green I solution (AMRESCO) for 30 min and then rinsed with Milli-Q water. The gel image was photographed with an Omega 10 Gel image analysis system (UltraLum).

16S rRNA gene clone library construction

Four bacterial 16S rRNA clone libraries were constructed for sediments taken from Sites D, G, L and N (Fig. 1). These sites were chosen based on the Shannon-Wiener index (H') values of the DGGE-binary matrix data, which showed that the H' values were relatively higher at these selected sites in comparison to other sites in the same clustering group. 16S rRNA gene sequences were amplified from extracted environmental DNA samples using the eubacterial forward primer 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and the universal reverse primer 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') (Newton et al. 2006). The PCR reaction mixture was the same as that in PCR for DGGE (see above). PCR was performed under the following conditions: denaturation at $95^\circ C$ for 5 min; 30 cycles of $94^\circ C$ for 45 s, $52^\circ C$ for 90 s and $72^\circ C$ for 1 min; an additional 10 min of final extension at $72^\circ C$.

Triplicate PCR products for each sample were mixed and loaded onto a 1 % agarose gel. The target band was excised for each sample, and the gel fragments were purified with an AxyPrep™ Gel Extraction Kit (Axygen Biosciences) following the manufacturer's instructions. Purified PCR products were each cloned into pMD® 19T vectors using the pMD® 19T Vector Kit (TaKaRa), according to the manufacturer's instructions. The plasmid containing the ligated PCR product was then transformed into *Escherichia coli* Top 10 supercompetent cells and

incubated at 37°C by shaking at 150 rpm for 1 h in an incubator. The incubated cells were then spread onto prepared solid Luria-Bertani (LB) medium plates and grown at 37°C for 16 h. Randomly selected white colonies were incubated at 37°C for 8 h in liquid LB media to increase the yield of the plasmid DNA, respectively (Sambrook & Russell 2001). The incubated liquid media samples were each then reamplified with the PCR program described above using the primers RV-M (5'-GAG CGG ATA ACA ATT TCA CAC AGG-3') and M13-47 (5'-CGC CAG GGT TTT CCC AGT CAC GAC-3') to verify the insert sizes and discard false positive clones.

16S rRNA gene sequence analysis

Sequencing was conducted using an automated 3730 DNA capillary sequencer (Applied Biosystems). All partial 16S rRNA sequences were edited manually using the software BioEdit Sequence Alignment Editor (v.7.0.5.3) (Hall 1999) and examined for chimeric artifacts using the software Mallard (v.1.02) (Ashelford et al. 2006). The chimeric sequences were excluded from further analysis, and the remaining sequences were then blasted with the most similar 16S rRNA sequences from GenBank. The Ribosomal Database Project II (RDP II; <http://rdp.cme.msu.edu/>) classifier was used to assign taxonomic identities to our 16S rRNA sequences. When the sequence similarity was $\geq 95\%$, which is the value corresponding to the separation of genera (Ludwig et al. 1998), the sequences were categorized as belonging to a member of the same genus as the most similar clone in the NCBI database. Sequences that had similarities to the closest clones or strains that were $<95\%$ were labeled as 'unclassified'.

To examine the phylotypes of the available sequences in more detail, all of the available sequences in each clone library were first aligned using the software program Clustal X 1.83 (Thompson et al. 2002). Each alignment was then analyzed using the nucleic acid sequence distance matrix program (DNAdist, v.3.66) (Felsenstein 1993), and the output of this program was subsequently analyzed with DOTUR (Schloss & Handelsman 2005) to compute the OTUs, Chao 1 richness estimator (Chao1), Shannon index (H) and coverage index (C). Coverage was calculated with the equation $C = [1 - (n_1/N)] \times 100$, in which n_1 is the number of single-occurrence phylotypes within a library, and N is the total number of clones in the library. Estimators were calculated using a genetic distance level of 0.03.

Phylogenetic trees for sedimentary *Alpha*-, *Beta*-, *Gamma*- and *Deltaproteobacteria* were constructed using the Molecular Evolutionary Genetics Analysis (MEGA) software package (v.5.0) (Tamura et al. 2011). Node support was assessed by generating and summarizing 1000 bootstrap replicates, and evolutionary distances were computed using a Jukes-Cantor model.

Statistical analyses

A principle components analysis (PCA) was conducted to identify differences in physicochemical parameters, while a redundancy analysis (RDA) (Ter Braak 1986) was used to examine the influence of environmental parameters on the sedimentary bacterial community structure. All the environmental parameters and the DGGE peak area data were $\log_{10}(x + 1)$ -transformed, with the exception of temperature and pH. PCA of physicochemical parameters was conducted with SPSS 16.0 for Windows (IBM). RDA was conducted with the software CANOCO 4.5 (Ter Braak & Šmilauer 2002). Environmental parameters were identified by forward selection using the Monte Carlo test, with 499 permutations under the reduced model. Clustering analysis of the DGGE-binary matrix data was performed using the GelCompar II software (Applied Maths), based on Dice distances. In addition, the 4 constructed clone libraries were compared statistically using the software J-LIBSHUFF (Schloss et al. 2004) to distinguish differences among them.

Nucleotide sequence accession numbers

All bacterial 16S rRNA gene sequences generated for the present study were deposited in GenBank under the accession numbers JN104732 to JN105080.

RESULTS

Physicochemical parameters and bacterial cell abundances

Spatial distribution of the investigated physicochemical parameters in the sediments and overlying water are shown in Fig. S1 in the Supplement at www.int-res.com/articles/suppl/a069p123_supp.pdf. There were significant environmental gradients from the freshwater region to the oligosaline re-

gions, as revealed by the lower values of most physicochemical parameters at the freshwater sites in comparison with the oligosaline sites. We also found significant positive correlations among a number of the environmental parameters. For instance, sulfate was strongly correlated with chloride ($r = 0.817$, $n = 16$, $p < 0.001$), as was TDS with salinity ($r = 0.999$, $n = 16$, $p < 0.001$). From the PCA results, the first PCA component explained 58.6% of the total variance of the physicochemical parameters, while 71.4% of the total variance was explained by including the second PCA component. Salinity and TDS contributed more to the first component, while TP contributed more to the second component. The third component, which accounted for only 10.3% of the total variance, reflected the parameters of chloride and sulfate.

Bacterial abundances at different sites ranged from 1.53×10^{10} to 3.77×10^{10} cells per gram of dry sediment, with the abundances at the freshwater sites (Sites L and M) consistently lower than at the oligosaline sites (Fig. 2). The only significant correlation between bacterial abundances and physicochemical parameters was that between the overlying water DO concentration and bacterial abundance ($r = 0.535$, $p = 0.033$, $n = 16$).

Clustering analysis of DGGE profiles and RDA analysis

The DGGE profiles of bacterial community structure in the sediments of Lake Bosten are shown in

Fig. 2. Spatial distribution of sedimentary bacterial cell abundances, dissolved oxygen concentrations and salinity at the 16 sites investigated within Lake Bosten. The values for freshwater Sites L and M are shaded

Fig. 3. According to the marked bands in the gel image, the H' of bacterial communities varied from 2.76 at Site M to 3.55 at Site N. A clustering analysis of DGGE profiles based on Dice distances indicated significant heterogeneity in the sedimentary bacterial community structure. We recovered 4 major groups of sites using a similarity cutoff of 60%. Groups 1, 2 and 3 belonged to the oligosaline regions, while Group 4 (Sites L and M) was localized to the freshwater region of the lake. Additionally, there was a significant Pearson correlation between the H' and salinity (Fig. 4).

Our RDA examined the significance of salinity, TP, DO, sulfate and chloride in relation to the sedimentary bacterial community structure in Lake Bosten (Fig. 5). These 5 significantly correlated variables accounted for 56.0% of the sedimentary bacterial community variance in Lake Bosten. Salinity was the most important factor and accounted for 15.0% of the total variance in bacterial community structure ($p = 0.006$, $F = 2.40$). Additionally, TP ($p = 0.018$, $F = 2.24$), DO ($p = 0.024$, $F = 2.19$), sulfate ($p = 0.028$, $F = 2.14$) and chloride ($p = 0.038$, $F = 1.92$) accounted for 12.0, 11.0, 10.0 and 8.0% of the sedimentary bacterial community variance, respectively.

Diversity estimators of bacterial communities

OTU identifications, Chao1, H and coverage values for the clone libraries are summarized in Table S1 in the Supplement at www.int-res.com/articles/suppl/a069p123_suppl.pdf. A total of 185 OTUs were acquired from the 349 nonchimeric sequences with a similarity of $\geq 97\%$. In the present study, the estimated coverage of each clone library was moderately high, ranging from 67.0 to 72.0%. The highest diversity (Chao1 and H) was at Site D where the sedimentary TP content was the highest among all the investigated sites, whereas the lowest was measured at Site L in the freshwater region. J-LIBSHUFF was used to test the significant differences among the 4 clone libraries. The clone library at the freshwater Site L was significantly different from the other 3 clone libraries at oligosaline sites (see Table S2 in the Supplement).

Fig. 3. UPGMA dendrogram of DGGE banding patterns based on the Dice similarity coefficient. The cutoff of the similarity level is indicated by the dashed line

Taxonomic identification of 16S rRNA gene sequences

The relative proportions of the taxonomic affiliations of 16S rRNA sequences at the phylum level within each library are shown in Fig. 6A. *Proteobacteria* was the dominant phylum at all 4 sites, with the relative proportions of 49.5, 35.4 and 55.1% in the samples from oligosaline Sites D, G and N, respectively, yet with the lowest relative proportion (25.6%) at the freshwater Site L, in contrast to the other 3 oligosaline sites.

Fig. 4. Statistically significant linear regression between salinity and the Shannon-Wiener index (H') of the DGGE-binary matrix data. The regression line is solid, and the dotted lines indicate the 95% confidence intervals

A total of 145 *Proteobacteria*-affiliated sequences were acquired from the 4 clone libraries, which yielded 63 unique OTUs (including *Alphaproteobacteria*, 8 OTUs; *Betaproteobacteria*, 13 OTUs; *Gammaproteobacteria*, 17 OTUs; and *Deltaproteobacteria*, 25 OTUs) at the identity level of $\geq 97\%$. The relative proportions of the 4 proteobacterial classes from each clone library are shown in Fig. 6B. *Betaproteobacteria* and *Deltaproteobacteria* were the dominant proteobacterial classes in the 4 clone libraries, with the relative proportions of *Betaproteobacteria* ranging from 24.4% at Site D to 42.9% at Site N and the *deltaproteobacteria* proportions ranging from 26.7% at Site D to 46.4% at Site G. In total, 36.6% of the proteobacterial sequences were affiliated with *Delta*-*proteobacteria*, and *Betaproteobacteria* accounted for 32.4% of the total proteobacterial sequences.

According to the phylogenetic analysis of *Proteobacteria*-affiliated sequences in Lake Bosten, some specific genera-affiliated sequences were identified, as indicated in Fig. S2 in the Supplement. *Alphaproteobacteria* harbored 7 specific genera clusters:

Fig. 5. Redundancy analysis (RDA) of sedimentary bacterial community structure in relation to the significantly correlated physicochemical parameters in sediments at Sites A to P of Lake Bosten. DO: dissolved oxygen; TP: total phosphorus; Sulfate: SO_4^{2-} ; Chloride: Cl^-

Fig. 6. Relative proportions of (A) the identified bacterial phyla and (B) proteobacterial classes recovered from the 4 16S rDNA gene clone libraries constructed from Sites D, G, L and N of Lake Bosten

Methylocella, *Blastochloris*, *Bacterium* Ellin335, *Rhodobacter*, *Magnetospirillum*, *Roseomonas* and *Deftuviococcus*, of which the genus *Methylocella* is facultatively methanotrophic (Dedysh et al. 2005). Only 1 OTU was affiliated with *Rhodospirillales*, which was unclassified at the genus level (see Fig. S2A).

Five specific genera clusters of *Betaproteobacteria* were present in the sediments of Lake Bosten: *Georgfuchsia*, *Thiobacillus*, *Burkholderia*, *Leptothrix* and *Rhodoferrax* (see Fig. S2B). Only the *Rhodoferrax* cluster belonged to the typical freshwater bacterial genera (Zwart et al. 2002), accounting for 14.3% and 4.8% of the *Betaproteobacteria* at the freshwater Site L and the oligosaline Site N, respectively. As a sulfur-oxidizing bacterium (Robertson & Kuenen 2006), the genus *Thiobacillus* was dominant at Sites G and N, with relative proportions accounting for 50.0 and 76.2% of the total *Betaproteobacteria*, respectively. Additionally, there were large proportions of unclassified *Betaproteobacteria*, with proportions relative to total *Betaproteobacteria* ranging from 14.3% at Site N to 81.8% at Site D (Fig. 7A).

For *Gammaproteobacteria*, 9 clusters of specific genera were identified: *Thiohalocapsa*, *Marichromatium*, *Thioalkalicoccus*, *Methylocaldum*, *Methylobacter*, *Steroidobacter*, *Halomona*, *Psychrobacter* and *Legionella*. The dominant genus *Psychrobacter* (39.4% of *Gammaproteobacteria*) was only found at Site D. Moreover, only 1 gammaproteobacterial OTU was retrieved from the freshwater Site L (see Fig. S2C).

Six genera clusters were grouped into *Deltaproteobacteria*: *Desulfobacter*, *Desulfobulbus*, *Geobacter*, *Desulfobacca*, *Myxobacter* and *Nannocystis* (see Fig. S2D). Of these, the genus *Desulfobacter* was dominant and was found in each library (Fig. 7B). Four of these genera (*Desulfobacter*, *Desulfobulbus*, *Geobacter* and *Desulfobacca*) belonged to the putative sulfate-reducing bacteria (SRB). The sequences affiliated with these putative SRB accounted for 12.3% of the total bacteria surveyed and were the dominant members of *Deltaproteobacteria*, with percentages of 91.7, 76.9, 77.8 and 88.2% for Sites D, G, L and N, respectively.

In contrast with the 3 oligosaline sites, *Bacteroidetes* were most abundant at Site L (23.3% of the total bacteria at Site L) (Fig. 6A). There were 5 genera clusters identified at the freshwater Site L: *Flavobacter*, *Sphingobacter*, *Cytophaga*, *Niastella* and *Algoriphagus*, while only *Sphingobacter* and *Cytophaga* were identified at the oligosaline sites (see Fig. S3A in the Supplement). In addition, 67.4% of the *Bacteroidetes* sequences belonged to the unclassified *Sphingobacteriales*.

Chloroflexi were found in each library, with the relative proportions ranging from 2.2% at Site L to 16.5% at Site G (Fig. 6A). *Caldilinea* was the only identified genus, and most of the *Chloroflexi* sequences were unidentified at the genus level, with the relative proportion of >80.0% in each library (see Fig. S3B). Similar to the *Bacteroidetes* group, members of *Firmicutes* were also most abundant, with a

Fig. 7. Relative proportions of the identified genera and the unclassified groups of (A) *Betaproteobacteria* and (B) *Deltaproteobacteria* in the 4 16S rDNA gene clone libraries constructed from Sites D, G, L and N of Lake Bosten

proportion of 22.2% of the total bacteria at Site L, when compared to the other 3 oligosaline sites (Fig. 6A). Five genera belonging to this group were identified in our study: *Bacillus*, *Brochothrix*, *Carnobacter*, *Clostridium* and *Eubacter*, of which *Clostridium* was the most abundant genus (see Fig. S3C). In addition, sequences affiliated with the phyla *Chlorobi*, *Nitrospirae*, *Planctomycetes* and *Candidate division* were also recovered in all 4 libraries (Fig. 6A).

DISCUSSION

Effects of salinity and nutrients on sedimentary bacterial community structures

During the past 20 yr, the hydrochemical condition of Lake Bosten has become the sodium sulfate type, and the salinity content (or TDS) has been mainly attributed to the sulfate and chloride sodium salts present in the lake (Li & Yuan 2002). In the present study, sulfate and chloride were important parameters that influenced the heterogeneity of sedimentary bacterial communities in the lake (Fig. 5), and might be the direct factors influencing certain groups of sedimentary bacteria. This was also supported by the relatively high proportion of putative SRB in the sediments of Lake Bosten. However, salinity is an integrated variable that reflects the global level of aquatic dissolved solids concentrations (Lozupone &

Knight 2007, Wang et al. 2011). Measurements of the water salinity in Lake Bosten have revealed that most regions of the lake are oligosaline (Xie et al. 2011). Although salinity levels in Lake Bosten are much lower than those in hypersaline lakes (Wu et al. 2006, Xie et al. 2011), salinity has become an obvious index of the gradual alteration of this ecosystem. PCA of the environmental parameters in our study demonstrated that salinity is the dominant factor shaping the microbial habitats of Lake Bosten.

The freshwater inflow of the Kaidu River shapes the freshwater region in the western district of Lake Bosten, which can be seen in the physicochemical properties of Sites L and M (see Fig. S1). Intriguingly, the sedimentary bacterial community structures at Sites L and M grouped together, separated from the other oligosaline sites (Fig. 3). In addition, the BCC at the freshwater Site L differed significantly from the other 3 oligosaline sites (see Table S2). Both the bacterial cell abundances (Fig. 2) and the DGGE based Shannon-Wiener index (Fig. 4) indicated that bacterial abundances and diversities increase along the salinity gradient from freshwater to oligosaline. In the present study, salinity was the most significant parameter influencing the bacterial community structures (Fig. 5). These results, combined with a significant positive correlation between salinity and the DGGE based Shannon-Wiener index (Fig. 4), demonstrate the effect of salinity on the sedimentary bacterial communities, which is consistent with pre-

vious work conducted on bacterioplankton in Lake Bosten (Tang et al. 2012).

According to the sedimentary TN, TP and TOC concentrations in our investigation and based on previous reports on water of this lake (Xie et al. 2011), it is clear that Lake Bosten is facing a new ecological crisis—namely, eutrophication. Agricultural wastewater, rich in nutrients, is contaminating the lake water. These nutrients are deposited in the surface sediment and contribute to the increase in nutrient levels throughout the entire lake. In the present study, TN, TP and TOC concentrations at freshwater sites were all lower than at oligosaline sites (see Fig. S1). Moreover, TP was the most significant factor shaping the sedimentary bacterial communities in Lake Bosten in the present study (Fig. 5). This is in addition to its known role in affecting the bacterioplankton distribution in the lake (Tang et al. 2012), which indicated the response of sedimentary bacterial communities to the nutrients gradient in Lake Bosten.

Moreover, DO was strongly correlated with bacterial community structures and cell abundances in our study (Figs. 2 & 5). Oxygen is an important factor for regulating the metabolic activity of bacteria and is known to be an indispensable electron acceptor in many biochemical processes driven by bacteria (Nealson 1997). In Lake Bosten, the stream flow and allochthonous inputs of soil particles from the Kaidu River disturb the water of the freshwater region and decrease the oxygen concentration in the bottom water, which was an important reason for the lower bacterial abundances at the freshwater sites than at other oligosaline sites.

Effects of salinity and nutrients on bacterial community composition

Proteobacteria was the most abundant phylum, with *Betaproteobacteria* and *Deltaproteobacteria* predominating in all 4 libraries (Fig. 6). *Betaproteobacteria* are globally distributed in freshwater lakes, where they numerically dominate the cell biomass and bacterial types (Zwart et al. 2002, Tang et al. 2010). The presence and dominance of *Betaproteobacteria* may be related to their ability to quickly respond to changes in available nutrients, such as dissolved organic carbon (Newton et al. 2006). In Lake Bosten, the higher proportions of *Betaproteobacteria* at the 3 oligosaline sites compared to the freshwater Site L might be related to the more abundant sedimentary nutrients, including TOC, TN and

TP, in oligosaline regions (see Fig. S1 in the Supplement). Moreover, *Thiobacillus*, mostly found in mine wastewater-contaminated habitats (Fortin et al. 1996, Robertson & Kuenen 2006), was a dominant genus that was recovered at the oligosaline sites. This provides additional evidence for the sedimentary bacterial response to the enrichment of pollutants, including nutrients.

In the present study, most of the identified betaproteobacterial clusters were distinct from typical freshwater clusters previously characterized (Zwart et al. 2002), an observation that may be attributed to the oligosaline environment in Lake Bosten. However, the low abundance of *Alphaproteobacteria*, which are known to be competitive at low nutrient levels (Pinhassi & Berman 2003), may be attributed to the high concentrations of nutrients in the sediments within Lake Bosten.

Gammaproteobacteria are generally more abundant in saltwater environments, such as oceans (Glöckner et al. 1999, Rusch et al. 2007, Biers et al. 2009) or saline lakes (Wu et al. 2006), than in freshwater environments (Wu et al. 2007, Mueller-Spitz et al. 2009). Indeed, the majority of the gammaproteobacterial clusters we identified have previously been sampled from saline environments. For instance, members of the genera *Psychrobacter*, *Steroidobacter* and *Halomonas*, which are often found in saltwater environments (Bowman et al. 1997), were recovered in oligosaline regions of Lake Bosten in our study. Moreover, the higher taxon richness of *Gammaproteobacteria* at the oligosaline sites than at the freshwater Site L supports the bacterial response to the salinity gradient in Lake Bosten.

Most of the *Deltaproteobacteria* we identified in Lake Bosten are affiliated with the putative SRB, which have been found to exist almost exclusively in various anoxic environments, such as estuarine and coastal marine sediments (Purdy et al. 2002), Antarctic marine sediments (Bowman & McCuaig 2003), saline lake sediments (Koizumi et al. 2004) and freshwater wetland sediments (Ikenaga et al. 2010). Other studies have suggested that SRB are highly active and that they participate in anaerobic decomposition and the transformation of deposited organic matter in surface sediments (Soltwedel & Vopel 2001, Bowman & McCuaig 2003). The relatively high percentage of SRB might be due to the high concentrations of sedimentary sulfate and TOC, which can provide an ideal habitat for these functional bacteria.

Bacteroidetes were more abundant at the freshwater Site L than at any of the other 3 oligosaline sites. Site L is located near the mouth of the Kaidu

River, and the freshwater inflow mixed with large amounts of particles flows into the western region of Lake Bosten, while the other 3 sites are in the eastern district with relatively low turbidity (water turbidity data not shown). *Bacteroidetes* is composed of a large proportion of particle-associated bacteria, which are especially common in freshwater lakes (Simon et al. 2002, Lemarchand et al. 2006, Tang et al. 2010). Because of the low hydrodynamic intensity in Lake Bosten, particles and associated *Bacteroidetes* were likely deposited on the surface sediment. *Bacteroidetes* are thought to play a critical role in freshwater environments by processing complex molecules into simpler compounds. Similarly, *Firmicutes* displayed a relatively high proportion at Site L, with *Clostridium* being the dominant genus. *Clostridium* is saprotrophic and is usually found in soils (Elshahed et al. 2008). Such a relatively high proportion of saprotrophic bacteria at Site L may be attributed to the soil particles carried by the inflow of the Kaidu River. Moreover, the lower relative proportion of *Firmicutes* at each of the 3 oligosaline sites is consistent with the findings of a previous study in the oligosaline Lake Namco, which is located on the Tibetan Plateau (Liu et al. 2010).

Numerous 16S rRNA sequences in the present study could not be identified at the genus level. For example, most of the *Betaproteobacteria*-, *Deltaproteobacteria*-, *Bacteroidetes*- and *Chloroflexi*-affiliated sequences were not classified to the known genera with a similarity level of $\geq 95\%$. These unclassified bacterial phylotypes may reflect the unique bacterial communities in this oligosaline lake in the early stage of salinization and eutrophication and may even include unknown species unique to these transitioning environments.

Although there are several other bacterial phyla that may have participated in various biochemical processes in the sediments of Lake Bosten, the heterogeneous distribution of these dominant phyla between the freshwater and oligosaline regions revealed the response of the sedimentary BCC to increases in salinity and nutrient levels, which demonstrates that salinity and nutrients are the most important factors controlling sedimentary BCC in oligosaline lakes in the arid region of China.

CONCLUSIONS

The present work is the first report characterizing the sedimentary BCC in the oligosaline Lake Bosten. Our results demonstrate that salinity shapes the sed-

imentary BCC in this oligosaline lake. *Proteobacteria* was the dominant phylum in Lake Bosten, with the classes *Betaproteobacteria* and *Deltaproteobacteria* being the most abundant. The responses of the sedimentary BCC to gradients in salinity and nutrient levels were reflected in both the higher bacterial diversities and higher percentages of *Proteobacteria* in oligosaline regions than in the freshwater region. The low percentage of typical freshwater bacteria, the presence of a relatively high proportion of SRB, and relatively high proportions of unclassified bacteria indicate the unique nature of sedimentary BCC in this oligosaline lake in the early stages of salinization and eutrophication. Our study has provided fundamental information about the sedimentary BCC of the oligosaline Lake Bosten, located in the arid region of China. This lake can now serve as a model for future studies examining environmental changes from freshwater/oligotrophic to oligosaline/mesotrophic conditions. However, additional studies of limnic bacterial diversity and their function in arid-region lakes are still needed to better understand the influences of climatic drought and human activities on aquatic ecosystems.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (grant 41171388), the Special Environmental Research Funds for Public Welfare of the State Environmental Protection Administration (grant 201309041) and the National Water Pollution Control and Management of Science and Technology Major Projects (grant 2009ZX07106-004). We thank the 3 anonymous reviewers and the editor for their thoughtful and constructive suggestions.

LITERATURE CITED

- Ashelford KE, Chuzhanova NA, Fry JC, Jones AJ, Weightman AJ (2006) New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. *Appl Environ Microbiol* 72:5734–5741
- Bai J, Chen X, Li J, Yang L, Fang H (2011) Changes in the area of inland lakes in arid regions of central Asia during the past 30 years. *Environ Monit Assess* 178:247–256
- Biers EJ, Sun S, Howard EC (2009) Prokaryotic genomes and diversity in surface ocean waters: interrogating the global ocean sampling metagenome. *Appl Environ Microbiol* 75:2221–2229
- Bowman JP, McCuaig RD (2003) Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. *Appl Environ Microbiol* 69:2463–2483
- Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA (1997) Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* 63:3068–3078
- Chen FH, Shi Q, Wang JM (1999) Environmental changes documented by sedimentation of Lake Yiema in arid

- China since the Late Glaciation. *J Paleolimnol* 22: 159–169
- Danovaro R, Manini E, Dell'Anno A (2002) Higher abundance of bacteria than of viruses in deep Mediterranean sediments. *Appl Environ Microbiol* 68:1468–1472
- Dedysh SN, Knief C, Dunfield PF (2005) *Methylocella* species are facultatively methanotrophic. *J Bacteriol* 187: 4665–4670
- Elshahed MS, Youssef NH, Spain AM, Sheik C and others (2008) Novelty and uniqueness patterns of rare members of the soil biosphere. *Appl Environ Microbiol* 74: 5422–5428
- Felsenstein J (1993) PHYLIP: phylogenetic inference package, version 3.5c. University of Washington, Seattle, WA
- Fortin D, Davis B, Beveridge T (1996) Role of *Thiobacillus* and sulfate-reducing bacteria in iron biocycling in oxic and acidic mine tailings. *FEMS Microbiol Ecol* 21:11–24
- Fujii M, Kojima H, Iwata T, Urabe J, Fukui M (2012) Dissolved organic carbon as major environmental factor affecting bacterioplankton communities in mountain lakes of eastern Japan. *Microb Ecol* 63:496–508
- Gao G, Qin B, Sommaruga R, Psenner R (2007) The bacterioplankton of Lake Taihu, China: abundance, biomass, and production. *Hydrobiologia* 581:177–188
- Glöckner FO, Fuchs BM, Amann R (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl Environ Microbiol* 65:3721–3726
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Ikenaga M, Guevara R, Dean AL, Pisani C, Boyer JN (2010) Changes in community structure of sediment bacteria along the Florida coastal Everglades marsh-mangrove-seagrass salinity gradient. *Microb Ecol* 59:284–295
- Jiang H, Dong H, Yu B, Liu X, Li Y, Ji S, Zhang CL (2007) Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. *Environ Microbiol* 9:2603–2621
- Koizumi Y, Kojima H, Oguri K, Kitazato H, Fukui M (2004) Vertical and temporal shifts in microbial communities in the water column and sediment of saline meromictic Lake Kaiike (Japan), as determined by a 16S rRNA-based analysis, and related to physicochemical gradients. *Environ Microbiol* 6:622–637
- Lemarchand C, Jardillier L, Carrias JF, Richardot M, Debroas D, Sime-Ngando T, Amblard C (2006) Community composition and activity of prokaryotes associated to detrital particles in two contrasting lake ecosystems. *FEMS Microbiol Ecol* 57:442–451
- Li W, Yuan L (2002) On the water-salt change of Bosten Lake in Xinjiang. *J Lake Sci* 14:223–227 (in Chinese)
- Liu X, Yao T, Kang S, Jiao N, Zeng Y, Liu Y (2010) Bacterial community of the largest oligosaline lake, Namco on the Tibetan Plateau. *Geomicrobiol J* 27:669–682
- Lozupone CA, Knight R (2007) Global patterns in bacterial diversity. *Proc Natl Acad Sci USA* 104:11436–11440
- Lu R (1999) Analytical methods of soil agrochemistry. China Agricultural Science and Technology Press, Beijing
- Ludwig W, Strunk O, Klugbauer S, Klugbauer N and others (1998) Bacterial phylogeny based on comparative sequence analysis (review). *Electrophoresis* 19:554–568
- Martins JL, Silveira TS, Silva KT, Lins U (2009) Salinity dependence of the distribution of multicellular magnetotactic prokaryotes in a hypersaline lagoon. *Int Microbiol* 12:193–201
- Mischke S, Wünnemann B (2006) The Holocene salinity history of Bosten Lake (Xinjiang, China) inferred from ostracod species assemblages and shell chemistry: possible palaeoclimatic implications. *Quat Int* 154–155:100–112
- Mueller-Spitz SR, Goetz GW, McLellan SL (2009) Temporal and spatial variability in nearshore bacterioplankton communities of Lake Michigan. *FEMS Microbiol Ecol* 67: 511–522
- Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* 59:695–700
- Nealson KH (1997) Sediment bacteria: Who's there, what are they doing, and what's new? *Annu Rev Earth Planet Sci* 25:403–434
- Newton RJ, Kent AD, Triplett EW, McMahon KD (2006) Microbial community dynamics in a humic lake: differential persistence of common freshwater phylotypes. *Environ Microbiol* 8:956–970
- Pinhassi J, Berman T (2003) Differential growth response of colony-forming alpha- and gamma-proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. *Appl Environ Microbiol* 69:199–211
- Porter K, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 25: 943–948
- Purdy KJ, Embley TM, Nedwell DB (2002) The distribution and activity of sulphate reducing bacteria in estuarine and coastal marine sediments. *Ant Van Leeuwenhoek* 81:181–187
- Robertson LA, Kuenen JG (2006) The genus *Thiobacillus*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) *Prokaryotes*. Springer, New York, NY, p 812–827
- Rusch DB, Halpern AL, Sutton G, Heidelberg KB and others (2007) The Sorcerer II global ocean sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS Biol* 5:e77
- Sai B, Huang J, Xie G, Feng L, Hu S, Tang X (2011) Response of planktonic bacterial abundance to eutrophication and salinization in Lake Bosten, Xinjiang. *J Lake Sci* 23:934–941 (in Chinese)
- Sambrook J, Russell DW (2001) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Press, New York, NY
- Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* 71:1501–1506
- Schloss PD, Larget BR, Handelsman J (2004) Integration of microbial ecology and statistics: a test to compare gene libraries. *Appl Environ Microbiol* 70:5485–5492
- Schmidt JL, Deming JW, Jumars PA, Keil RG (1998) Constancy of bacterial abundance in surficial marine sediments. *Limnol Oceanogr* 43:976–982
- Simon M, Grossart HP, Schweitzer B, Ploug H (2002) Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat Microb Ecol* 28:175–211
- Soiltwedel T, Vopel K (2001) Bacterial abundance and biomass in response to organism-generated habitat heterogeneity in deep-sea sediments. *Mar Ecol Prog Ser* 219: 291–298

- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731–2739
- Tang X, Gao G, Chao J, Wang X, Zhu G, Qin B (2010) Dynamics of organic-aggregate-associated bacterial communities and related environmental factors in Lake Taihu, a large eutrophic shallow lake in China. *Limnol Oceanogr* 55:469–480
- Tang X, Xie G, Shao K, Sai B, Chen Y, Gao G (2012) Influence of salinity on the bacterial community composition in Lake Bosten, a large oligosaline lake in arid northwestern China. *Appl Environ Microbiol* 78: 4748–4751
- Ter Braak CJF (1986) Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67:1167–1179
- Ter Braak CJF, Šmilauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca, NY
- Thompson JD, Gibson T, Higgins DG (2002) Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc Bioinformatics* 2:1–22
- Wang J, Yang D, Zhang Y, Shen J, van der Gast C, Hahn MW, Wu Q (2011) Do patterns of bacterial diversity along salinity gradients differ from those observed for macro-organisms? *PLoS ONE* 6:e27597
- Wu QL, Zwart G, Schauer M, Kamst-van Agterveld MP, Hahn MW (2006) Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. *Appl Environ Microbiol* 72:5478–5485
- Wu X, Xi W, Ye W, Yang H (2007) Bacterial community composition of a shallow hypertrophic freshwater lake in China, revealed by 16S rRNA gene sequences. *FEMS Microbiol Ecol* 61:85–96
- Xiao M, Wu F, Liao H, Li W, Lee X, Huang R (2010) Characteristics and distribution of low molecular weight organic acids in the sediment porewaters in Bosten Lake, China. *J Environ Sci (China)* 22:328–337
- Xie G, Zhang J, Tang X, Cai Y, Gao G (2011) Spatio-temporal heterogeneity of water quality (2010–2011) and succession patterns in Lake Bosten during the past 50 years. *J Lake Sci* 23:837–846 (in Chinese)
- Zhang R, Thiagarajan V, Qian PY (2008) Evaluation of terminal-restriction fragment length polymorphism analysis in contrasting marine environments. *FEMS Microbiol Ecol* 65:169–178
- Zwart G, Crump BC, Kamst-van Agterveld MP, Hagen F, Han SK (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquat Microb Ecol* 28:141–155

*Editorial responsibility: Rutger de Wit,
Montpellier, France*

*Submitted: September 28, 2012; Accepted: March 13, 2013
Proofs received from author(s): May 7, 2013*