RESEARCH ARTICLE

A comparison of pelagic, littoral, and riverine bacterial assemblages in Lake Bangongco, Tibetan Plateau

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Abstract

Lakes of the Tibetan Plateau lack direct anthropogenic influences, providing pristine high-altitude (> 4000 m) sites to study microbial community structure. We collected samples from the pelagic, littoral, and riverine zones of Lake Bangongco, located on the western side of the Plateau, to characterize bacterial community composition and geochemistry in three distinct, but hydrologically connected aquatic environments during summer. Bacterial community composition differed significantly among zones, with communities changing from riverine zones dominated by *Bacteroidetes* to littoral and pelagic zones dominated by *Gammaproteobacteria*. Community composition was strongly related to the geochemical environment, particularly concentrations of major ions and total nitrogen. The dominance of *Gammaproteobacteria* in the pelagic zone contrasts with typical freshwater bacterial communities as well as other lakes on the Tibetan Plateau.

Introduction

The Tibetan Plateau has experienced rapid increases in temperature over the past three decades, resulting in increased glacier melt across the region (Cui & Graf, 2009). At 2×10^6 km^2, it is the largest and highest (average ~4500 m above mean sea level) plateau on our planet and contains many glacial meltwater-fed lakes generally characterized by low nutrients, temperature and productivity, and high exposure to UV radiation (Wang & Dou, 1998). Increasing glacier melt in this alpine environment will lead to changes in hydrology and lake structure in the coming decades, which may impact microbial community structure and biogeochemical function. Lakes on the eastern and southern parts of the Plateau have microbial communities typically dominated by *Bacteroidetes* and *Cyanobacteria* (Dong *et al.*, 2006; Liu *et al.*, 2009, 2010; Xing *et al.*, 2009), making them unique from

typical, low-altitude lakes, which are usually dominated by *Betaproteobacteria* (Newton *et al.*, 2011). Lake Bangongco, which we discuss here, is located in the lesser studied western part of the Plateau.

Most studies of freshwater bacterial diversity have focused on inter- and intralake comparisons (i.e. depth profiles or lake transects) (e.g. Lindstrom & Bergstrom, 2004; Allgaier *et al.*, 2007; Van der Gucht *et al.*, 2007) and how biotic and abiotic factors affect bacterial communities. Biotic (competition with, and predation by, other planktonic organisms) and abiotic factors (e.g. pH, salinity, and temperature) have been addressed in a number of studies, and their role in structuring bacterial communities is relatively well understood (Yannarell & Triplett, 2005; Sawstrom *et al.*, 2007; Lymer *et al.*, 2008). Fewer investigations have focused on the linkages between riverine, littoral, and pelagic environments. It has been shown that lake bacterial communities can be seeded by

riverine inputs and that the relationship between lake and river bacterial communities is influenced strongly by hydrology (Lindstrom & Bergstrom, 2004; Crump *et al.*, 2007, 2012). Ultimately, a comprehensive understanding of the holistic effects of hydrology, biotic, and abiotic factors is required to determine the mechanisms structuring bacterial communities in lakes.

We hypothesized that hydrologic connectivity has a major influence on bacterial abundance and diversity in the habitats associated with Lake Bangongco and that geochemical differences between these habitats further define bacterial community structure and abundance within the pelagic, littoral, and riverine zones. Most limnological studies in the Tibetan Plateau have been conducted in the eastern portion of the Plateau, which has much higher plant cover and precipitation than in the west (Zheng, 1996). Our work, which focuses on the western portion of the plateau, provides a much needed benchmark for monitoring microbial community structure and function in this rapidly changing environment. The current study is the first comprehensive microbial and geochemical investigation of a large alpine freshwater lake on the western Tibetan Plateau.

Materials and methods

Study site

The western Tibetan Plateau is a cold desert located in the rain shadow of the Kunlun and Karakorum ranges. Precipitation [annual mean = 62 mm (Wang & Dou, 1998)] occurs primarily from June to September associated with convective storms or as rare monsoon rains (Fontes *et al.*, 1996). Mean annual air temperatures in the region range from −4 to −2 °C (Wang & Dou, 1998).

Lake Bangongco (33°26′-33°58′N, 78°25′-79°56′E, 4241 m above mean sea level) lies in a long fault valley

and has a surface area of 604 km^2 (Fig. 1). Its maximal depth (41.3 m) occurs near the eastern shore. The Chiao Ho, Nam Chu, and Makha rivers (Fig. 1) are the primary sources of water to the lake. The source of the Chiao Ho River is melt water from the Mawang Kangri Glacier in the north. The Nam Chu River flows into the eastern part of the basin, while the River Makha flows into the southern end of Lake Bangongco from the Kang Ti Su Shan Glacier (Fontes *et al.*, 1996). The lake outflow is in the west, toward India.

Wang & Dou (1998) showed a marked gradient in total dissolved solids (TDS) from east to west across Lake Bangongco. The eastern part of the lake where the rivers enter is relatively fresh (TDS = 0.7 g L^{-1}) and increases to 2.8 g L^{-1} in the middle of the lake and 19.6 g L^{-1} in the far western portion of the lake, which lies in India. Lake Bangongco is typically ice-covered from late October through mid-April.

Sampling

All sampling was conducted from 1 to 6 July 2010. Profiles of temperature and specific (25 °C) conductivity in the pelagic zone of the lake (sites P0-P38; Fig. 1) were made using a Hydrolab DS5 water quality sonde (Hach, Loveland, CO). Density was estimated from these data using the UNESCO Equations of state for seawater (Millero & Poisson, 1981). Five samples (P0, P10, P20, P30, and P38, where numbers indicated depths) at this pelagic site were collected for chemical and biologic analyses using a discrete water sample bottle. Surface water samples were also collected from logistically accessible regions of the shallow (< 50 cm) littoral zone (sites L1 to L8) and the Nam Chu inlet river (sites R1, R2, R3) (Fig. 1) using clean Nalgene HDPE bottles.

Duplicate 2 mL aliquots from each sample were fixed immediately with 1.5% glutaraldehyde for bacterial
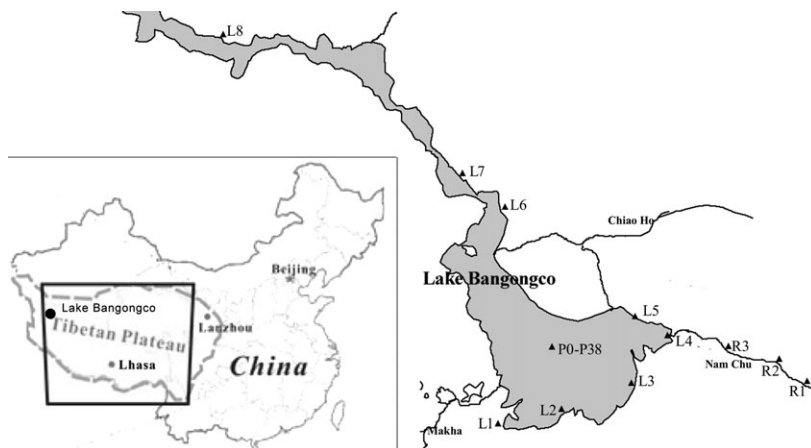

Fig. 1. Location of Lake Bangongco and sampling sites. Sites P0, P10, P20, P30, and P38 refer to pelagic depths 0, 10, 20, 30, and 38 m, respectively. Sites L1 to L8 represent littoral zones, and sites R1, R2, and R3 are riverine zones.

enumeration. One liter of sample water was filtered immediately after collection through a 0.22-μm polycarbonate filter (Millipore, Bedford, MA) for genomic analysis. Samples (500 mL) for chlorophyll *a* (chl *a*) determination were filtered onto glass-fiber filters (Whatman GF/F) and frozen in liquid nitrogen. Upon returning to our laboratory, these samples were stored at −80 °C until analysis (within 30 days). Duplicate 100 mL aliquots for the measurement of total dissolved organic carbon (DOC), total nitrogen (TN), and major ions were collected using 1% HCl leached, deionized water rinsed (3 ×), and combusted (450 °C for > 3 h) 20-mL glass bottles from each sample and frozen at −20 °C until analysis.

Bacterial abundance

Bacterial abundance was analyzed using a flow cytometer (Beckman Coulter, Epics Altra II) equipped with an external quantitative sample injector (Harvard Apparatus PHD 2000) using SYBR Green I (Molecular Probes) nucleic acid stain (Jiao *et al.*, 2005). All measurements were completed within 1 month after sampling to minimize accrued cell loss (Sato *et al.*, 2006). Duplicate samples were measured with a relative standard deviation lower than 10%. Flow cytometry data were collected in list mode and analyzed with CytoWin 4.1 software (http://www.sb-roscoff.fr/phyto).

Physicochemical parameters

Following filtration (Whatman GF/F), major ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-}), total nitrogen (TN), and dissolved organic carbon (DOC) were determined according to standard methods (Greenberg *et al.*, 1992). DOC and TN were analyzed using a TOC-Vcph (Shimadzu Corp., Japan). Each sample for DOC and TN was measured three times with a relative standard deviation lower than 10%. Ions were analyzed with a Thermofisher ion chromatograph system 900. Chl *a* was measured using a spectrofluorometer (Shimadzu Corp.,) following an overnight freeze-thaw extraction in 90% acetone (Tett *et al.*, 1975). The spectrofluorometer was calibrated using standard chl *a* solutions. pH was measured in the field on freshly collected samples using a calibrated probe.

Molecular methods

Community DNA was extracted using the UltraClean Soil DNA kit (MoBio, San Diego, CA) according to the manufacturer's instructions. DNA size and integrity were checked in a 0.8% agarose gel stained with SYBR Green I, and the concentration and purity of DNA were measured using a Bio-Rad SmartSpec Plus spectrophotometer (Bio-Rad Laboratories, Hercules). The bacterial 16S rRNA genes were amplified with the primer sets 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-ACG GGC GGT GTG TRC-3′). The reaction mixture (30 μL) consisted of 1 U of LA Taq (TaKaRa Co., Dalian, China), 0.2 mM each dNTP, 3 μL of 10 × Buffer, 0.15 mM of each primer, and 1 μL (~10 ng DNA) of template. The PCR incubation included an initial denaturation step at 94 °C for 5 min followed by 26 cycles of 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1.5 min, and then a final extension at 72 °C for 8 min.

The PCR products were purified using an agarose gel DNA purification kit (TaKaRa Co.,), ligated into a pGEM-T vector (TaKaRa Co.,), and then transformed into *E. coli* DH5α. The presence of inserts was checked using ampicillin resistance selection and colony PCR. About 100 clones from each library were randomly selected for sequencing.

Phylogenetic analysis

All sequences obtained were examined for chimeric artifacts using the Pintail tool (http://www.bioinformatics-toolkit.org). Clones identified as potential chimeras were discarded. Each sequence library was analyzed with DOTUR (Schloss & Handelsman, 2005) and grouped into operational taxonomic units (OTUs) with 97% and 98% similarity cutoff. We used 97% similarity cutoff primarily to compare our results with other publications that used this value; 98% similarity cutoff was used for all other statistics in the manuscript to describe the bacterial community composition. Sequences were assigned to the genus level with 80% confidence using the 'Classifier' program of RDP (Cole *et al.*, 2005). The closest sequences were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) using BLASTn. Neighbor-joining tree illustrating 16S rRNA gene sequences of the four most abundant genera (genus *Flavobacterium* in *Bacteroidetes* and genera *Psychrobacter*, *Acinetobacter,* and *Pseudomonas* in *Gammaproteobacteria*) amplified from Lake Bangongco and riverine and sequences (> 1200 nt) of cultivated bacteria retrieved from GenBank using 531 bp were performed within ARB (Ludwig *et al.*, 2004). The 16S rRNA gene sequences were aligned against the SILVA reference database (Pruesse *et al.*, 2007). The tree was rooted using *Thermotoga maritima* as the outgroup.

Statistical analysis

Richness was estimated using the nonparametric model of Chao: $S = S_{obs} + (a^2/2b)$, where S is the Chao 1 richness estimator, S_{obs} is the observed number of OTUs, a is the number of OTUs observed only once, and b is the

number of OTUs observed only twice (Chao, 1984). Coverage of clone libraries was calculated using the equation: $Coverage = [1- (N/Individuals)] \times 100\%$, where N is the number of clones that occurred only once (Kemp & Aller, 2004). Shannon diversity (H) was calculated with PAST v1.92 statistical package, and differences among the pelagic, littoral, and riverine zones were determined with the nonparametric Kruskal–Wallis test included with the PAST v1.92 statistical package (Hammer *et al.*, 2001).

Bacterial community patterns were analyzed using non-metric multidimensional scaling (nMDS) based on Bray–Curtis dissimilarity. Analyses of similarity (ANOSIM) were performed to determine the differences in bacterial communities from different groups of samples. The OTU tables were standardized using Hellinger transformation. To evaluate correlations between bacterial community patterns in nMDS ordination and environmental parameters, Spearman correlation coefficients were calculated for each axis nMDS score and the environmental parameter values. All analyses were carried out with R v2.14 with the packages vegan (Oksanen *et al.*, 2009) and gplot (Warnes *et al.*, 2009).

Nucleotide sequence accession numbers

Representative sequences of the partial 16S rRNA gene sequences, based on 98% cutoff, have been deposited in GenBank under accession numbers: JN866187–JN866514.

Results

Limnological data

The pelagic zone of the lake was weakly stratified both thermally and chemically (Fig. 2) during the sampling period (mid-July). A layer of relatively fresh warm water was present in the upper 2 m, which suggests the advection of low ionic strength river water across the surface of the lake. A second layer was apparent between 2 and 14 m, and a metalimnion existed between 14 and 22 m, which overlaid colder hypolimnetic water. The overall density structure of the water column was stable. The pH ranged from 8.5 to 9.4 with no clear depth trend. DOC, TN, and chl *a* ranged from 2.1 to 4.1 mg L^{-1}, 0.3 to 0.6 mg L^{-1}, and 0.4 to 0.9 μg L^{-1}, respectively, and all peaked at the 30 m sample depth (Fig. 3a). Bacterial abundance was 0.8×10^5 at the surface and increased with depth reaching 9.4×10^5 cell mL^{-1} at 38 m (Fig. 3a). Na^+ and Cl^- were the dominant ions at all depths and increased in concentration, along with SO_4^{2-} and Mg^{2+}, with depth (Fig. 3b).

Environmental variables in the littoral zone varied dramatically among sites (Fig. 3a and b). DOC and TN at
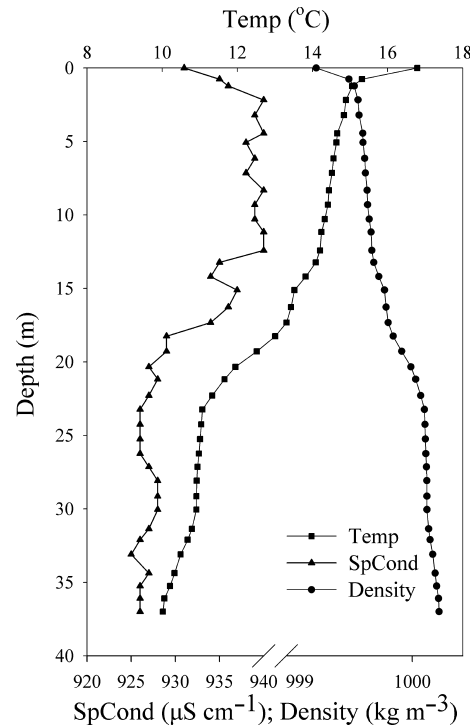

Fig. 2. Temperature, specific (25 °C) conductivity, and density profile of the Lake Bangongco water column measured at pelagic site P.

L1 were more than 10 times higher than measured in the other sites. Ion concentrations varied more than 10-fold between the highest (L1 and L8) and lowest (L3) concentrations. Across the littoral zone, chl *a* concentration varied between 7.7 μg L^{-1} (L4) and 0.3 μg L^{-1} (L8), bacterial abundances were between 1.4×10^5 (L1) and 16.3×10^5 cells mL^{-1} (L2), and pH values ranged from 8.2 to 9.2. Bacterial abundance, DOC, TN, and all ion concentrations were considerably higher at site L1 presumably because this site is separated from the main lake during the dry season when water levels are low. River water was characterized by relatively low bacterial abundance, chl *a*, DOC, and ion concentrations (except Ca^{2+}), similar to the pelagic surface water (Fig. 3a and b).

The DOC, Na^+, K^+, Mg^{2+}, Ca^{2+}, and Cl^- values were significantly different among the pelagic, littoral, and riverine zones (Kruskal–Wallis test; $P = 0.003, 0.015, 0.024, 0.024, 0.029$, and 0.001, respectively), whereas TN and chl *a* were not significantly different among the three zones (Kruskal–Wallis test; $P = 0.11$ and 0.41, respectively).

Richness and diversity

A total of 1590 16S rRNA gene clones were sequenced from the 16 sample sites. After quality checking and removal of chimeric sequences, 1537 remained and were
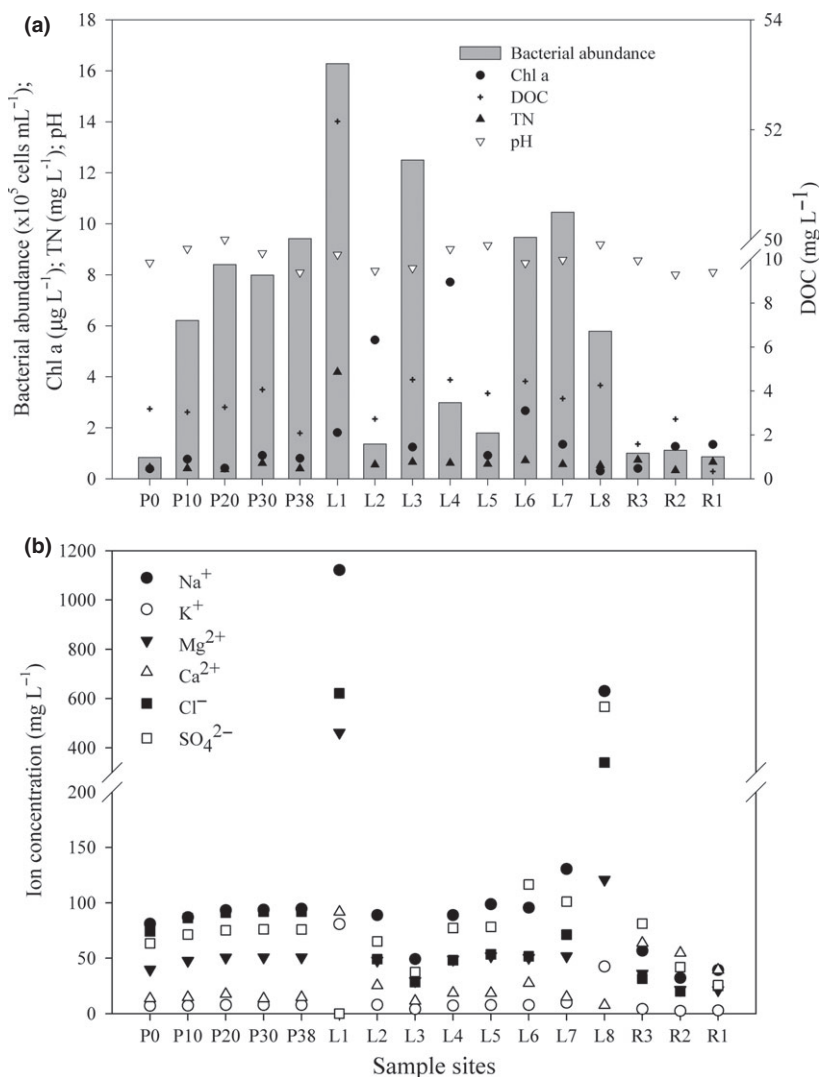
Fig. 3. Bacterial abundance (10^5 cells mL^{-1}), chl a (µg L^{-1}), DOC (mg L^{-1}), TN (mg L^{-1}), and pH (a); major ion concentrations (mg L^{-1}) at pelagic and littoral sites within Lake Bangongco and in the associated riverine zone (b).

grouped into 328 OTUs with 98% similarity cutoff. (Supporting information, Table S1). For ease of comparison with other studies, we used the 97% cutoff (282 OTUs) to calculate richness and diversity, which were similar to calculations using 98% (data not shown). Coverage for the 16 libraries varied from 62% to 89% (Table 1).

Taxonomic richness (Chao1) estimated for the libraries from all habitats varied from 35 to 659 (Table 1). No significant differences in Chao1 diversity were observed among pelagic, littoral, and riverine zones (Kruskal–Wallis; $P = 0.598$). Chao1 in the pelagic zone averaged 187 ± 264 ($\pm$ SD; range 54–659), and values were highest at 10 m and lowest at the surface. Chao1 was relatively consistent across the littoral zone, ranging from 39 to 75 with an average ($\pm$ SD) of 60 ± 14. Chao1 diversity in the riverine water (average ($\pm$ SD) = 95 ± 59; range 35–152) was intermediate between the littoral and pelagic zones. Pearson correlation analysis revealed that

Chao1 richness in the different habitats within the lakes was not significantly related to other environmental parameters ($P > 0.05$).

The community Shannon diversity index (H value) ranged from 1.3 to 3.6 across all samples collected (Table 1) with average ($\pm$ SD) values of 3.0 ± 0.2, 2.5 ± 0.7, and 2.7 ± 0.8 in the pelagic, littoral, and riverine samples, respectively. H values compared over three zones were not statistically different ($P = 0.385$, Kruskal–Wallis test).

Comparison of bacterial communities between zones

Riverine communities overall were dominated by *Bacteroidetes*, whereas the littoral and pelagic communities were both dominated by *Gammaproteobacteria* (Fig. 4). The riverine samples showed a succession from upstream (R1)

to downstream (R3) of communities dominated by *Bacteroidetes* and *Betaproteobacteria* to *Bacteroidetes* and *Gammaproteobacteria*. Ninety-one percent of clones in the riverine water grouped with the genus *Flavobacterium*, within the phylum *Bacteroidetes*. Thirty-five percent of sequences in this genus were affiliated with *F. aquatile* (Fig. S1).

Eighty-nine percent of the *Gammaproteobacteria* clones from the three zones in total were related to the genera

Table 1. Diversity indices of 16S rRNA gene clone libraries from the pelagic, littoral, and riverine zones of Lake Bangongco

Habitat	Site	Taxa	Individuals	Coverage (%)	*Chao 1*	Shannon H
Pelagic	P 0	29	94	85	54	3.0
	P 10	46	92	62	659	3.4
	P 20	35	96	79	68	3.1
	P 30	31	83	78	54	2.8
	P 38	35	95	76	101	2.8
Littoral	L1	34	93	80	64	3.0
	L2	36	100	82	54	3.1
	L3	19	97	89	39	1.7
	L4	18	100	87	60	1.3
	L5	28	100	86	44	2.4
	L6	34	97	78	71	2.7
	L7	42	98	77	75	3.4
	L8	27	100	83	75	2.5
Riverine	R1	50	95	63	152	3.6
	R2	25	99	89	35	1.9
	R3	35	98	74	98	2.6

Psychrobacter, *Acinetobacter*, and *Pseudomonas*. These genera occurred in the three habitats with differing frequency. *Psychrobacter* accounted for 40% and 22% of all sequences in littoral and riverine habitats, respectively, but were comparatively rare in the pelagic zone, accounting for only 0.6% of all sequences. Conversely, the genus *Acinetobacter* existed primarily in pelagic habitats, whereas *Pseudomonas* was consistently found across all habitats, but in relatively low abundance (Fig. 5 and Table S2). Fifty-eight percent of sequences in the genus *Acinetobacter* were assigned to two OTUs (B3_0_005 and B3_0_016) and were most closely related to *A. beijerinckii* and *A. lwoffii* (Fig. S1). Eighty-one percent of sequences in the genus *Psychrobacter* were similar to *P. namhaensis* (Fig. S1).

ANOSIM of the 16 sampling sites showed that the bacterial communities from pelagic, littoral, and riverine habitats were significantly different ($r_{\text{pelagic-river}} = 0.536$, $P = 0.017$; $r_{\text{pelagic-littoral}} = 0.582$, $P = 0.001$; and $r_{\text{river-littoral}} = 0.518$, $P = 0.036$). Over half of the clones from the pelagic, littoral, and riverine habitats (63%, 61%, and 59%, respectively) were found only in their respective region of collection. Overlap between pelagic and riverine habitats was particularly weak, with only 3 shared OTUs, while 26 OTUs overlapped between pelagic and littoral zones and 19 between littoral and riverine zones (Fig. 6).

Three-dimensional nMDS of the bacterial communities at each of the sample sites clustered the samples into three groups: pelagic, littoral, and riverine (Fig. 7). Only
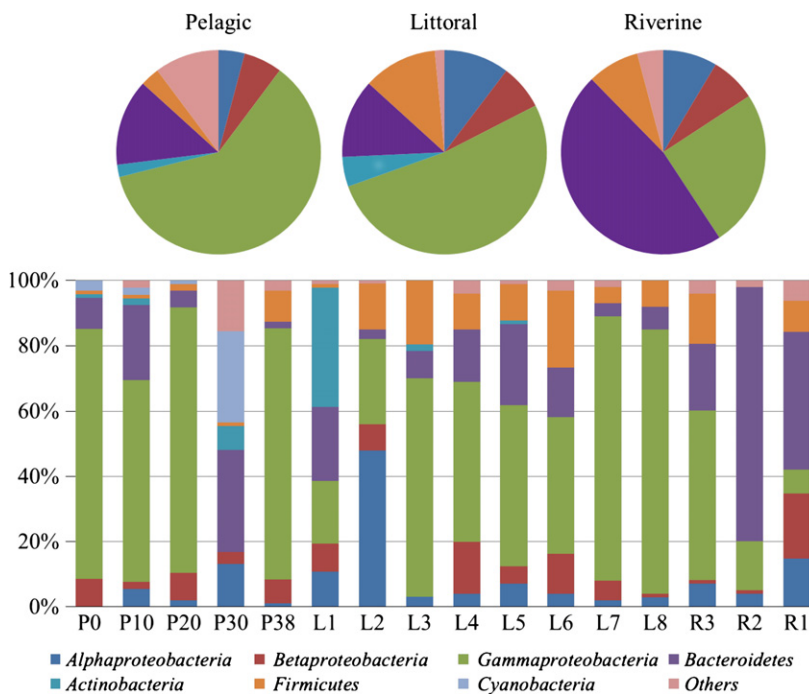

Fig. 4. Bacterial diversity (percent of total) in pelagic and littoral zones within Lake Bangongco and the associated riverine zone. The pie charts denote the combined diversity within each zone.

Fig. 5. Relative percent of the genera *Psychrobacter*, *Acinetobacter*, and *Pseudomonas* of total Gammaproteobacterial clones in pelagic and littoral zones within Lake Bangongco and the associated riverine zone.
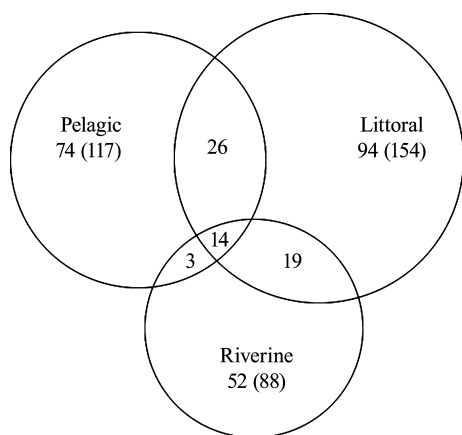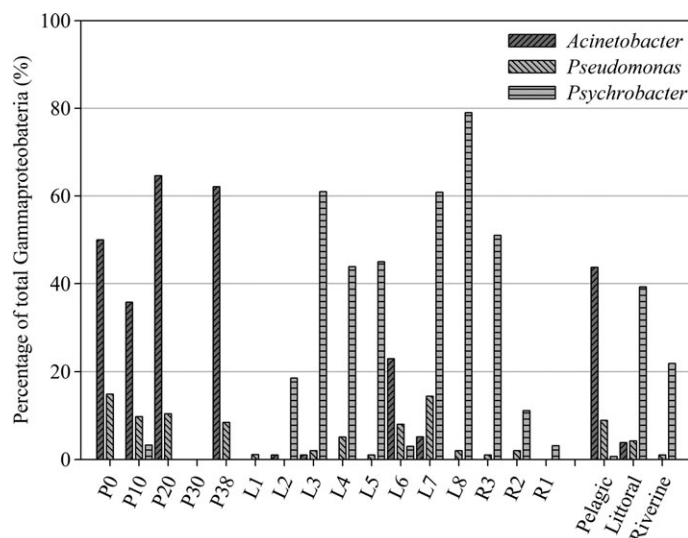

Fig. 6. Venn diagram showing the number of shared OTUs among pelagic, littoral, and riverine zones. Numbers in parentheses indicate the total number of OTUs in each zone. Shared OTU numbers among different zones were calculated in R.

the L1 sample site did not cluster with the other littoral samples. Axis 1 was significantly ($P < 0.05$) negatively correlated with Mg^{2+} ($r = -0.518$), Cl^- ($r = -0.805$) concentrations and bacterial abundance($r = -0.508$), whereas Axis 2 was significantly ($P < 0.001$) negatively correlated with TN ($r = -0.792$). Axis 3 was significantly ($P < 0.05$) negatively correlated with the ions Na^+ ($r = -0.547$) and K^+ ($r = -0.609$) as well as Mg^{2+} ($r = -0.547$) and Cl^- ($r = -0.559$).

Discussion

Hydraulic connectivity has been shown to increase the similarity of bacterial species in certain lakes and their associated watersheds (Lindstrom & Bergstrom, 2004;

Lindstrom *et al.*, 2006; Crump *et al.*, 2012). Riverine dispersal mechanisms are especially important in lakes with short (< 10 year) water residence times (Lindstrom *et al.*, 2006; Crump *et al.*, 2012), whereas lakes with residence times > 10 years have been shown to have lower similarity between communities (Lindstrom & Bergstrom, 2004). The hydraulic retention time of Lake Bangongco has yet to be determined accurately, but is estimated at 15–20 years based on the hydrodynamic similarities to another Tibetan lake (Tian *et al.*, 2008). This residence time makes it likely that postdispersal events, such as species-sorting (Leibold *et al.*, 2004), impact community structure to a greater degree than riverine input of new organisms. This contention is supported in Lake Bangongco by results from three-dimensional nMDS analysis (Fig. 7) showing that bacteria clustered into three distinct groups according to habitat and the fact that few OTUs were shared between the three hydraulically connected habitats (Figs 4 and 6). Collectively, these results indicate that dispersal from riverine input had little direct influence on the community structure of the littoral and the pelagic habitats in the lake, and postdispersal mechanisms likely structure bacterial communities in Lake Bangongco.

The availability of nutrients can impact bacterial community structure, and while soluble reactive phosphorus is typically at or below detection (< 1.0 µgP L^{-1}) in lakes of the Tibetan Plateau (Y. Liu, unpublished data), TN was significantly correlated with Axis 2 in the nMDS. Axis 2 mainly separated P30 from the other pelagic sites and L1 from all other sites. Indeed, TN concentrations were substantially higher at L1 than at any of the other sites, while P30 represented a peak in TN in the water column, relative to the other depths sampled. Site P30 community structure was characterized by increased
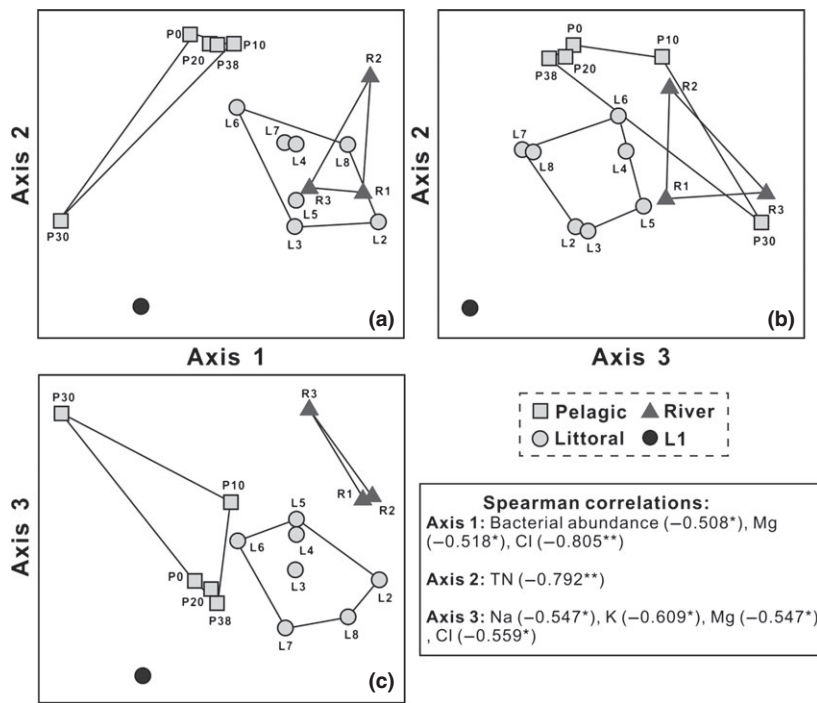
Fig. 7. Three-dimensional nMDS plot for the bacterial communities at different sampling sites (a, axes 1 and 2; b, axes 1 and 3; c, axes 2 and 3). The Spearman correlation coefficients of the axes with various environmental variables are provided in the insert. A single asterisk indicates significance at the 0.05 level; two asterisks denote significance at the 0.01 level.

incidence of cyanobacterial sequences (28% of total) and the near absence of *Gammaproteobacteria*. In addition to elevated TN, P30 was also higher in DOC and chl *a*, indicating a potential link between diversity, primary production, and nutrients at this depth in the pelagic zone. This pattern is similar to one found in Lake Puma Yumco on the southern Tibetan Plateau, where a change in bacterial community composition coincided with peaks in DOC and chl *a* and the abundance of different primary producers (Liu *et al.* 2009).

Significantly different ion concentrations in the pelagic, littoral, and riverine habitats combined with the correlation of major ions with nMDS axes 1 and 3 (Fig. 7) suggest that ion concentrations are an important factor controlling bacterial community composition in this system. Ionic strength (salinity) has been found to be a key factor controlling bacterial community composition and diversity in other systems, including some in the Tibetan Plateau (Bouvier & del Giorgio, 2002; Lozupone & Knight, 2007; Logares *et al.*, 2009, 2012; Wang *et al.*, 2011). Diversity in Lake Bangongco (Chao1) was higher on average than that reported from lakes on the southern and eastern Tibetan Plateau (Xing *et al.*, 2009), which lie in a warmer, wetter region with higher vegetation cover, but diversity in Lake Bangongco was not correlated with ion concentration or any other environmental parameter.

The bacterial community in the littoral and pelagic waters of Lake Bangongco differed from that reported for a majority of other lakes. *Betaproteobacteria*, *Actinobacteria*,

Bacteroidetes, and *Cyanobacteria*, which often dominate lakes around the world, including other lakes on the Tibetan Plateau (Zwart *et al.*, 2002; Liu *et al.*, 2009, 2010; Xing *et al.*, 2009; Newton *et al.*, 2011), accounted for only 2–19% of the clone libraries from Lake Bangongco. Instead, *Gammaproteobacteria*, which are typically abundant in the ocean and saline lakes (Methe *et al.*, 1998; Biers *et al.*, 2009; Newton *et al.*, 2011), dominated the littoral and pelagic zones of Lake Bangongco (55%, Figs 4 and 5) where TDS only reaches ~2.4 g L^{-1}. We note that our results are derived from clone library data, which can underestimate diversity and miss rare ($\prec 0.1\%$) taxa (Kemp & Aller, 2004; Sogin *et al.*, 2006). Owing to this potential bias, we limited our diversity comparison to previous studies that also utilized clone libraries to determine bacterial diversity and abundance (Urbach *et al.*, 2001; Zwart *et al.*, 2002; Wu *et al.*, 2006; Liu *et al.*, 2009; Xing *et al.*, 2009; Newton *et al.*, 2011).

Gammaproteobacteria have been suggested to be 'tourists' brought in to lakes from the surrounding environment (Newton *et al.*, 2011). However, their high relative abundance and diversity in the Lake Bangongco habitats imply that they are not simply transient but are actively growing and dividing there (Fig. 5, Table S2). The three zones were dominated by different genera, with *Acinetobacter* accounting for most of the *Gammaproteobacteria* in the pelagic zone, while *Psychrobacter* accounted for nearly all of the sequences from the riverine zone, and *Psychrobacter* mixed with *Pseudomonas* and *Acinetobacter* in the littoral zone.

The soils around Lake Bangongco were not examined as a part of our study, but cold-adapted *Pseudomonas* spp. have been found to be prevalent in alpine soils (Meyer *et al.*, 2004), and *Psychrobacter* spp. are found in permafrost soils of the Arctic, Siberia, and the Tibetan Plateau (Bakermans *et al.*, 2003; Bai *et al.*, 2006; Kim *et al.*, 2010), suggesting a terrestrial signal in the riverine and littoral zones. The shift away from potentially terrestrial sequences in the pelagic zone indicates that while terrestrial inputs of *Gammaproteobacteria* may provide the initial seed to Lake Bangongco, postdispersal mechanisms determine which lineages persist and dominate in each zone.

Our study provides the first comprehensive data set on a large alpine lake on the western Tibetan Plateau. Warming climate on the Tibetan Plateau (Kang *et al.*, 2010) and subsequent loss of glacier mass (Yao *et al.*, 2012) are enhancing water flow to glacier-fed lakes in the region. As glacial-melt inputs increase and lake levels rise, we expect the physical and chemical characteristics of Lake Bangongco, and other lakes of the Tibetan Plateau, to change significantly. Our data indicate that resulting changes in ion and nitrogen concentrations could lead to shifts in bacterial community structure. Because the Tibetan Plateau serves as the main water storage reservoir for Asia, providing fresh water to more than one billion people, this study will serve as a benchmark for future work on bacterial community composition in lakes of the region as climate continues to warm.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Neighbor-joining tree illustrating 16S rRNA gene sequences of the four most abundant genera (genus *Flavobacterium* in *Bacteroidetes* and genera *Psychrobacter, Acinetobacter,* and *Pseudomonas* in *Gammaproteobacteria*) amplified from Lake Bangongco and riverine, and sequences(> 1200 nt) of cultivated bacteria retrieved from GenBank.

Table S1. Type sequences for OTUs (98% similarity) deposited in GenBank representing 1537 clones distributed across different sites within Lake Bangonco (P0-38 and L1-8) and the riverine zone of the Nam Chu River (R1,2, and 3).

Table S2. Distribution of OTUs in the four most abundant genera: genus *Flavobacterium* in *Bacteroidetes* and genera *Psychrobacter*, *Acinetobacter,* and *Pseudomonas* in *Gammaproteobacteria*.