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Community response of microbial primary producers to salinity is primarily driven by nutrients in lakes

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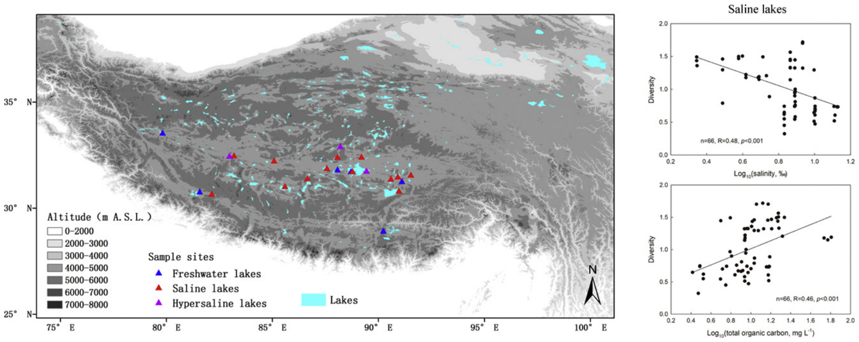
HIGHLIGHTS

- Microbial primary producers (MPP) diversity is constrained by salinity in lakes.
- Higher nutrients mitigated the salinity constraints on MPP abundance and diversity.
- Nutrient mitigating effect was more pronounced in saline than in freshwater lakes.
- MPP community compositions substantially varied with salinity.

GRAPHICAL ABSTRACT



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ABSTRACT

Higher microbial diversity was frequently observed in saline than fresh waters, but the underlying mechanisms remains unknown, particularly in microbial primary producers (MPP). MPP abundance and activity are notably constrained by high salinity, but facilitated by high nutrients. It remains to be ascertained whether and how nutrients regulate the salinity constraints on MPP abundance and community structure. Here we investigated the impact of nutrients on salinity constraints on MPP abundance and diversity in undisturbed lakes with a wide salinity range on the Tibetan Plateau. MPP community was explored using quantitative PCR, terminal restriction fragment length polymorphism and sequencing of cloning libraries targeting form IC *cbbL* gene. The MPP community structure was sorted by salinity into freshwater (salinity<1‰), saline (1‰ < salinity<29‰) and hypersaline (salinity>29‰) lakes. Furthermore, while MPP abundance, diversity and richness were significantly constrained with increasing salinity, these constraints were mitigated by enhancing total organic carbon (TOC) and total nitrogen (TN) contents in freshwater and saline lakes. In contrast, the MPP diversity increased significantly with the salinity in hypersaline lakes, due to the mitigation of enhancing TOC and TN contents and salt-tolerant MPP taxa. The mitigating effect of nutrients was more pronounced in saline than in freshwater and hypersaline lakes. The MPP compositions varied along salinity, with *Betaproteobacteria* dominating both the freshwater and saline lakes and *Gammaproteobacteria* dominating the hypersaline lakes. We concluded that high nutrients could mitigate the salinity constraining effects on MPP abundance, community richness and diversity. Our findings offer a novel insight into the salinity effects on primary producers and highlight the interactive effects of salinity and nutrients on MPP in lakes. These findings can be used as a baseline to illuminate the effects of increased anthropogenic activities altering nutrient dynamics on the global hydrological cycle and the subsequent responses thereof by MPP communities.

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1. Introduction

Microbial primary producers (MPP) are responsible for approximately 50% (~50 Pg C yr^{-1}) of the annual global net primary production in aquatic ecosystems; although, they only account for only 0.2% of global primary producer biomass (Field et al., 1998; Falkowski et al., 2004; Baumert and Petzoldt, 2008; Basu and Mackey, 2018). Carbon fixation performed by MPP is thus a key process in aquatic ecosystems, including inland lakes (Duarte et al., 2008; Engel et al., 2019).

MPP abundance and their carbon-fixing capacity are primarily driven by bottom-up abiotic resources across the freshwater, marine and terrestrial systems (Elser et al., 2007; Yuan et al., 2012; Larson and Belovsky, 2013; Seekell et al., 2015; Gusha et al., 2019). Degradation of organic matter releases synchronously multiple nutrients back to aquatic ecosystems, e.g. C, N and P (Finzi et al., 2011; Bratkic et al., 2012; Chen et al., 2018). Organic matter thus represents the nutrient status and fertility in ecosystems (Zarin et al., 1998; Quayle and Convey, 2006; Chen and Wang, 2019). In addition to nutrient limitation, salinity is a strong driver of microbial community and primary producers in aquatic ecosystems (Wu et al., 2006; Lozupone and Knight, 2007; Al-Said et al., 2017; Wang et al., 2018). Salinity usually constrains microbial abundance, diversity and primary production in aquatic environments (Liu et al., 2013; Zhong et al., 2016; Lindsay et al., 2019). Contrasting findings were frequently observed, indicating that microbial community diversity was higher in more saline habitats (Lozupone and Knight, 2007; Zhong et al., 2016). Therefore, the salinity effect on microbial communities is more complicated than previously assumed, and other environmental factors, such as nutrients (Larson and Belovsky, 2013; Van Horn et al., 2014; Ferreira et al., 2019) and temperature (Bergstrom et al., 2013; Yvon-Durocher et al., 2015; Stefanidou et al., 2018), also regulate the microbial response to salinity. There is a need to understand the interactive effects of salinity and nutrients, as inland lakes are currently or in the near future impacted by enhanced nutrient inputs. For example, the inputs of terrestrial dissolved organic carbon, nitrogen deposition and phosphorous into aquatic ecosystems have been significantly increased by climate change and human activities (Anderson, 1991; Shaver et al., 2000; Tranvik and Jansson, 2002; Hole and Engardt, 2008; Conley et al., 2009).

Herein we used a RubisCO large subunit gene (IC *cbbL*) as a proxy to investigate MPP community abundance and diversity along wide salinity and nutrient gradients in lakes on the Tibetan Plateau. Form I is the most pervasive type of RubisCO which can be further divided into four clades of IA, IB, IC and ID (Tabita et al., 2008). The gene *cbbL* is highly conserved and has been widely used as phylogenetic biomarker to investigate the diversity of MPP community in a variety of ecosystems (John et al., 2007; Kong et al., 2012; Martinez-Garcia et al., 2012).

Tibetan Plateau is the largest and highest (>4000 m above sea level, a.s.l.) plateau on Earth and hosts thousands of pristine lakes with a total area of 4.5×10^4 km^2 accounting for 49.5% of the lake area in China (Wan et al., 2014). These lakes contain stratified biogeochemical gradients, such as salinity, nutrients and pH (Yang et al., 2016; Liu et al., 2018), and therefore provide an ideal study ground to test hypotheses on the relevance of environmental stresses for biodiversity. Microbial survival under high salinity requires more energy for a variety of processes which would be supplied by a robust photosynthetic apparatus (Oren, 1999; Sudhir and Murthy, 2004; Ashraf and Harris, 2013). Oligotrophic conditions would limit the organisms' ability to build proteins/enzymes for maintaining high rates of photosynthesis (Oren, 1999; Harpole et al., 2011; Ashraf and Harris, 2013). Nutrients are thus particularly important for MPP survival and growth in lake waters with high salinity. We thus hypothesized that (i) salinity is a major environmental driver of MPP abundance, community diversity and structure, and (ii) the salinity constraints on MPP community are dependent on nutrients.

2. Materials and methods

2.1. Study sites and field sampling

Twenty-two lakes were investigated across the Tibetan Plateau at altitudes ranging from 4280 to 4858 m above sea level (Table S1). Surface waters were sampled using a 2.5-liter sampler in three replicates in July and August 2015. One-liter of water was concentrated onto Millipore membrane filters (0.22 μm pore size) at 0.6 atm filtering pressure. The filters were frozen at −20 °C and delivered to the laboratory in cooler with ice bags and stored at −80 °C until DNA extraction.

2.2. Environmental factor analysis

Water salinity, pH and dissolved oxygen were measured by the Professional Plus (Pro Plus) Multiparameter Instrument (YSI, USA) in situ. Lake water total organic carbon and total nitrogen concentrations were measured after filtering (0.45 μm pore size) by total organic carbon analyzer and total nitrogen measuring unit (TOC-V$_{CPH}$ and TNM-1, Shimadau, Japan), respectively. Water temperature was the mean during daytime of July and August 2015, which was extracted from previous study (Wan et al., 2017).

2.3. DNA extraction

The frozen filters were cut into pieces and total environmental DNA was extracted using a FastDNA® SPIN Kit for soil (MP Biomedical, USA) as described previously (Kong et al., 2012). The purity and concentration of the DNA were examined by Nanodrop® ND-2000 UV–Vis Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and 1.2% agarose gels.

2.4. Real-time quantitative PCR

In our previous studies, we found that form IC MPP substantially outnumbered other types (forms IAB and ID) in Tibetan Plateau environments (Guo et al., 2015; Zhao et al., 2018). In the current study form IAB was not successfully quantified for the low abundance, while ID was substantially lower abundance than IC by appropriately 100 folds (Fig. S1). We thus concentrated on form IC MPP abundance and community structure in the study. Form IC *cbbL* gene abundance was quantified by real-time quantitative PCR (qPCR) with primer set IC-F/IC-R (Alfreider et al., 2009), using a Light Cycler 480 II instrument (Roche, Switzerland). qPCR was conducted as our previous study with a temperature program of 35 cycles of 30 s at 95 °C, 30 s at 64 °C, 40 s at 72 °C (Zhao et al., 2018). A standard curve was generated using plasmid containing target gene fragments (Guo et al., 2015).

2.5. Terminal restriction fragment length polymorphism analysis

MPP community structure was determined by terminal restriction fragment length polymorphism (T-RFLP). PCR reactions were performed in triplicate for each sample on an Applied Biosystems (ABI, USA) instrument using the same primer set as qPCR, with the exception that the forward primer was labeled with 6-carboxy-fluorescein at the 5′ end (5′-FAM). The labeled PCR products were gel-purified using an AxyPrep DNA purification kit (Axygen, CA, USA). The purified products were digested with restriction endonuclease *Msp*I (Takara, Japan) at 37 °C for 3 h (Guo et al., 2015; Zhao et al., 2018), and then the reactions were stopped by incubation at 65 °C for 20 min.

The digested products were purified using Sigma-Aldrich Spin Post Reaction Clean-Up columns (Sigma, USA), and a portion was mixed with deionized formamide and the internal standard GeneScan-1000 LIZ (Applied Biosystems, USA). The mixtures were denatured for 3 min at 95 °C, and the DNA fragments were size separated using a 3130xl Genetic Analyzer (Applied Biosystems, USA). T-RFLP profiles were determined using Gene Scan analysis software (Applied Biosystems, USA).

2.6. Clone library construction, sequencing and phylogenetic analysis

Clone libraries of form IC *cbbL* gene were constructed for three lakes (lake Bong Co, salinity 0.14‰; Zhari Namco, salinity 10.02‰; and Pongyin Co, salinity 85.28‰) to analyze the MPP community compositions. The PCR program and primer set of the form IC *cbbL* gene were same as the qPCR. The purified PCR products were ligated into pGEM-T Easy vector (Promega, Madison, USA), and then transformed into *Escherichia coli* DH5α competent cells. A total number of 94 clones were sequenced using an ABI model 3730xl DNA analyzer (Applied Biosystems, USA).

Sequence alignments were performed using CLUSTALW in MEGA6.0 (Tamura et al., 2013). Sequences with >97% nucleotide similarity were grouped into the same operational taxonomic unit (OTU) using the Mothur program v.1.33.3 (Schloss et al., 2009). Phylogenetic tree was constructed in MEGA6.0 using the neighbor-joining method with the maximum composite likelihood model. Sequences generated in this study have been deposited in the National Center for Biotechnology Information GenBank (NCBI) under accession numbers MK058319-MK058349.

2.7. Statistical analysis

Figures and curve fittings were generated by Sigmaplot 12.5 (Systat Software, Inc., CA, USA). Correlations between MPP gene abundance, Shannon diversity, richness, evenness and environmental factors were determined using Pearson correlation in Sigmaplot 12.5 (Systat Software, Inc., CA, USA) and partial correlations were assessed by SPSS 23 (SPSS Inc., Armonk, NY, USA). Prior to the Pearson correlation and partial correlation analysis, all the data of environmental factors and gene abundance were $\log_{10}$ transformed. MPP community structure based on T-RFLP peaks were determined by canonical correspondence analysis (CCA) using CANOCO 5 (Microcomputer Power, Ithaca, NY). The classification of lakes based on environmental factors was determined by principal component analysis using CANOCO 5. Variation partition analysis (VPA) was performed to determine the individual contribution of each environmental factor to the MPP community variation using CANOCO 5 (Guo et al., 2015). MPP diversity (Shannon-wiener index), richness (the number of terminal restriction fragments) and evenness (Pielou-evenness) indexes were calculated from T-RFLP profiles using the vegan package in R (Zhao et al., 2017).

3. Results

3.1. Environmental factors in lake waters

Salinity levels of lake surface waters ranged from 0.07 to 85.28‰, with a pH range of 8.78–10.00 (Table S1). Total organic carbon (TOC) and total nitrogen (TN) contents were 2.48–66.69 mg L^{-1} and 0.62–7.61 mg L^{-1}, respectively. Principal component analysis based on all the environmental factors distinguished these lakes into three categories: freshwater (salinity<1‰), saline (1‰ < salinity<29‰) and hypersaline (salinity>29‰) lakes (Fig. S2). Among all the environmental factors, salinity played a key role in categorizing the lakes. Salinity exhibited significantly positive and negative correlations with pH ($r = 0.74$, $p < 0.001$) and dissolved oxygen (DO) ($r = -0.61$, $p < 0.001$, Table S2), respectively. TOC and TN positively correlated with salinity, pH and water temperature (Table S2).

3.2. Microbial primary producer abundance along salinity and nutrient gradients

MPP abundance was quantified using qPCR of the form IC *cbbL* gene, which has been shown to dominate MPP communities on the Tibetan Plateau (Guo et al., 2015; Zhao et al., 2018). Form IC *cbbL* gene abundance ranged from 10^5 to 10^9 copies L^{-1} water, and the peak was observed in lake Guogen Co (salinity 4.15‰) with 1.8×10^9 copies L^{-1} water. Form IC *cbbL* gene abundance varied along the salinity gradient, however, the relationship between salinity and MPP community abundance was distinct between the three lake categories of freshwater (salinity<1‰), saline lakes (1‰ < salinity<29‰), and hypersaline lakes (salinity>29‰). In freshwater lakes, *cbbL* gene abundance increased linearly with salinity (Fig. 1A), while in saline lakes, *cbbL* gene abundance covaried negatively with salinity levels (Fig. 1B). In contrast, form IC *cbbL* gene abundance and salinity did not exhibit a significant relationship in hypersaline lake waters (Fig. 1C).

To further investigate whether additional environmental factors could contribute to the distinct patterns observed in MPP abundance between the three salinity categories, Pearson correlations between the gene abundance and environmental factors were calculated. The results demonstrated that form IC *cbbL* gene abundance was positively correlated with water salinity, pH, temperature, TOC and TN contents in the freshwater lakes (all $p < 0.05$, Table 1). In the saline lakes, *cbbL* gene abundance negatively correlated with salinity and pH, but positively correlated with water temperature (all $p < 0.01$, Table 1). In hypersaline lakes, the *cbbL* gene abundance positively correlated with pH, TOC and TN, and negatively correlated with DO (all $p < 0.05$, Table 1). To further confirm the positive correlations of form IC *cbbL* gene abundance with TOC and TN contents in the lake waters, log-transformed gene abundance was plotted as a function of nutrient content. In agreement with the Pearson correlations, gene abundance exhibited significantly positive correlations with TOC and TN contents in both freshwater and hypersaline lakes (Fig. S3). In saline lakes, the gene abundance exhibited a marginally positive correlation with TN content ($p = 0.08$). Partial correlations for *cbbL* gene abundance with environment factors while controlling the effect of salinity were tested. The results showed that pH significantly influenced form IC *cbbL* gene abundance in freshwater and hypersaline lakes. Temperature exhibited a marginally ($p = 0.07$) and significantly ($p < 0.05$) positive correlation with form IC *cbbL* gene abundance in freshwater and saline lakes, respectively (Table S3).

3.3. Microbial primary producer community diversity along salinity and nutrient gradients

All detected T-RFs were included to calculate the MPP diversity (Shannon diversity), richness (the number of T-RFs) and evenness (Pielou-evenness). The three indexes exhibited distinct patterns, which were dependent on the salinity lake classifications (Fig. 2). The diversity significantly decreased along the increasing salinity in freshwater and saline lakes (Fig. 2A–B), but significantly increased in hypersaline lakes (Fig. 2C). In contrast, the MPP richness was relatively stable in freshwater and hypersaline lakes (Fig. 2D and F), but significantly decreased with increasing salinity in saline lakes (Fig. 2E). MPP evenness followed a similar pattern as the diversity (Fig. 2G–I).

To further illustrate the effects of other environmental factors (except salinity) on the three community indexes, Pearson correlations of diversity, richness and evenness with physicochemical factors were calculated. Community richness positively correlated with water temperature and TOC content in freshwater lakes (Table S4), while diversity and evenness exhibited significantly positive correlations with water temperature and TOC content (all $p < 0.05$), and the richness marginally correlated with the temperature ($p = 0.07$) in saline lakes. Last, community richness correlated positively with TOC and TN contents in hypersaline lakes (all $p < 0.05$).

To further demonstrate the nutrient effects on the MPP community, the three community indexes were curve-fitted as a function of TOC and TN contents. TOC significantly increased richness, but failed to enhance the diversity or evenness in either the freshwater or hypersaline lakes (Fig. 3). Contrarily, the nutrient effects on MPP community were

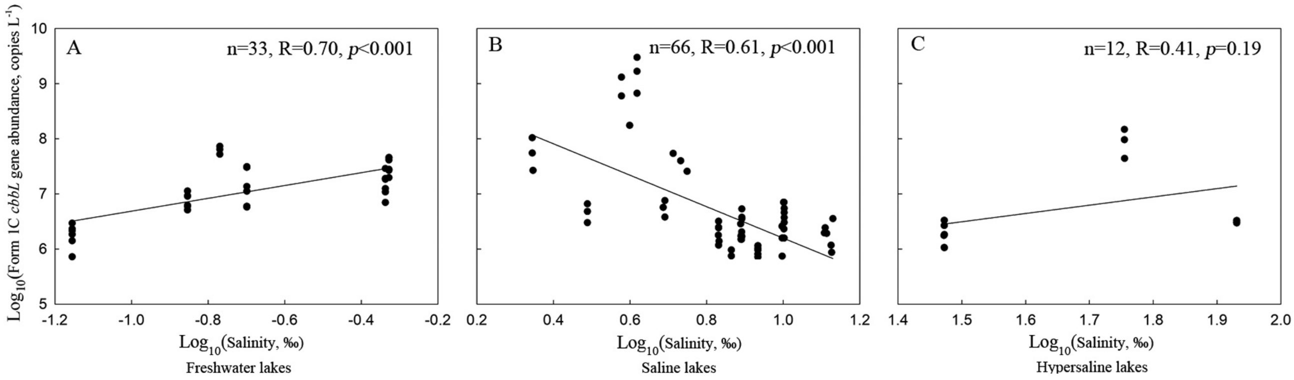

Fig. 1. Abundance of form IC microbial primary producers (indicated by *cbbL* gene) in lake waters along the salinity gradient. All the data of salinity and form IC *cbbL* gene abundance were log$_{10}$ transformed.

substantially pronounced in saline lakes. TOC significantly increased the diversity, evenness (all $p < 0.01$, Fig. 3B and H) and richness in saline lakes (Fig. 3E). The effects of TN on the three indexes were similar to TOC in freshwater and hypersaline lakes (Fig. S4). Compared to TOC, TN slightly increased the three indexes in saline lakes. To further elucidate the effects of salinity versus other environmental factors on the MPP community indexes, partial correlation analysis was performed. While controlling the effect of salinity, the effects of environmental factors on MPP diversity, richness and evenness were tested. The results showed that pH positively correlated with the MPP diversity and evenness in freshwater and saline lakes ($p < 0.05$, Table S5), and TOC positively correlated with the diversity and richness in freshwater and saline lakes ($p < 0.05$).

3.4. Microbial primary producer community structure in lake waters

The MPP community structure was screened by T-RFLP profiles. Canonical correspondence analysis (CCA) revealed that the MPP community structure was distinguished by salinity, and categorized into freshwater, saline and hypersaline lakes (Fig. 4). The MPP community variation was driven primarily by salinity and nutrients (TN and TOC). The impact of both salinity and nutrients was more pronounced at the interface between saline and hypersaline lakes. Variation partitioning analysis (VPA) disentangled the individual contribution of each environmental factor to the MPP community variation. The results showed that salinity individually explained the community variation by 8% across all lakes, and other environmental factors also contributed to explain the variation by 1.5–2.8%, with the exception of TOC (Table 2). In freshwater lakes, the MPP microbial community was primarily driven by salinity (22.6%), while pH, water temperature, TOC and TN individually explained by 7.4%, 7.5%, 5.1% and 3.0%, respectively. Given that salinity, pH and dissolved oxygen (DO) co-varied (Table S2), salinity exclusively drove the MPP community structure in saline and hypersaline lakes.

Table 1
Pearson correlations of form IC *cbbL* gene abundance with environmental factors.

Environmental factors	Form IC *cbbL* gene abundance (copies L^{-1})		
	Freshwater lakes[a]	Saline lakes[b]	Hypersaline lakes[c]
Salinity (‰)	0.696***	−0.609***	0.408
pH	0.777***	−0.315**	0.956***
Dissolved oxygen (mg L^{-1})	−0.203	−0.019	−0.910***
Water temperature (°C)	0.728***	0.374**	−0.503
Total organic carbon (mg L^{-1})	0.609***	0.112	0.791**
Total nitrogen (mg L^{-1})	0.434*	0.218	0.594*

All the data of environmental factors and form IC *cbbL* gene abundance were log$_{10}$ transformed.
[a] $n = 33$.
[b] $n = 66$.
[c] $n = 12$.
* $p \le 0.05$.
** $p \le 0.01$.
*** $p \le 0.001$.

3.5. Phylogenetic analysis of form IC cbbL gene in lake waters

Clone libraries of form IC *cbbL* gene were constructed for freshwater (Bong Co, 0.14‰ salinity), saline (Zhari Namco, 10.02‰) and hypersaline (Pongyin Co, 85.28‰) lakes, respectively. A total of 30 OTUs were identified for *cbbL* gene. All sequences were phylogenetically affiliated with four clusters (Fig. 5). The four clusters belonged to three phyla, which were *Alphaproteobacteria* (cluster III), *Betaproteobacteria* (cluster I and II) and *Gammaproteobacteria* (cluster IV). *Betaproteobacteria* dominated the form IC MPP community in freshwater and saline lakes and *Gammaproteobacteria* dominated the hypersaline lake, while *Alphaproteobacteria* accounted for a low relative abundance in these waters. Particularly, OTU1 (GenBank accession number MK058319) belonging to *Nitrosomonadales* dominated the form IC community in saline lake waters (69% relative frequency) and shared the highest nucleic acid similarity with deglaciated soil clones at Muztag glacier. OTU2 (GenBank accession number MK058320) belonging to *Burkholderiales* shared high similarity with glacier melt water clones and dominated the freshwater samples (51% relative abundance). OTU3 (GenBank accession number MK058321) belonging to *Thioalkalivibrio versutus* dominated the hypersaline water MPP with 85% relative frequency, and shared high similarity with clones of hypersaline soda lake sediments (Kovaleva et al., 2011).

4. Discussion

4.1. Abundance of microbial primary producers in Tibetan Plateau lake waters

Form IC MPP were abundant in Tibetan Plateau lake waters (Fig. 1). Compared with the Antarctic lakes (Kong et al., 2012), mesotrophic lakes (Alfreider et al., 2018) and soils (Guo et al., 2015; Lynn et al., 2017), the Tibetan Plateau lake waters exhibited similar or even higher MPP abundance. Previous studies demonstrated that the MPP were active and played an important role in carbon fixation in high salinity environments (Vavourakis et al., 2016), and they contribute to over 46% of primary production in oceans (Field et al., 1998; Falkowski et al., 2004). Therefore, the abundant MPP suggested a high CO_2-fixing potential in the Tibetan Plateau lakes.

Our study showed that salinity and nutrients both played key roles in driving MPP abundance in the lake waters (Figs. 1, S3 and Table 1). Salinity correlated with a significant loss in MPP abundance in saline lakes (Fig. 1), in agreement with previous findings in aquatic

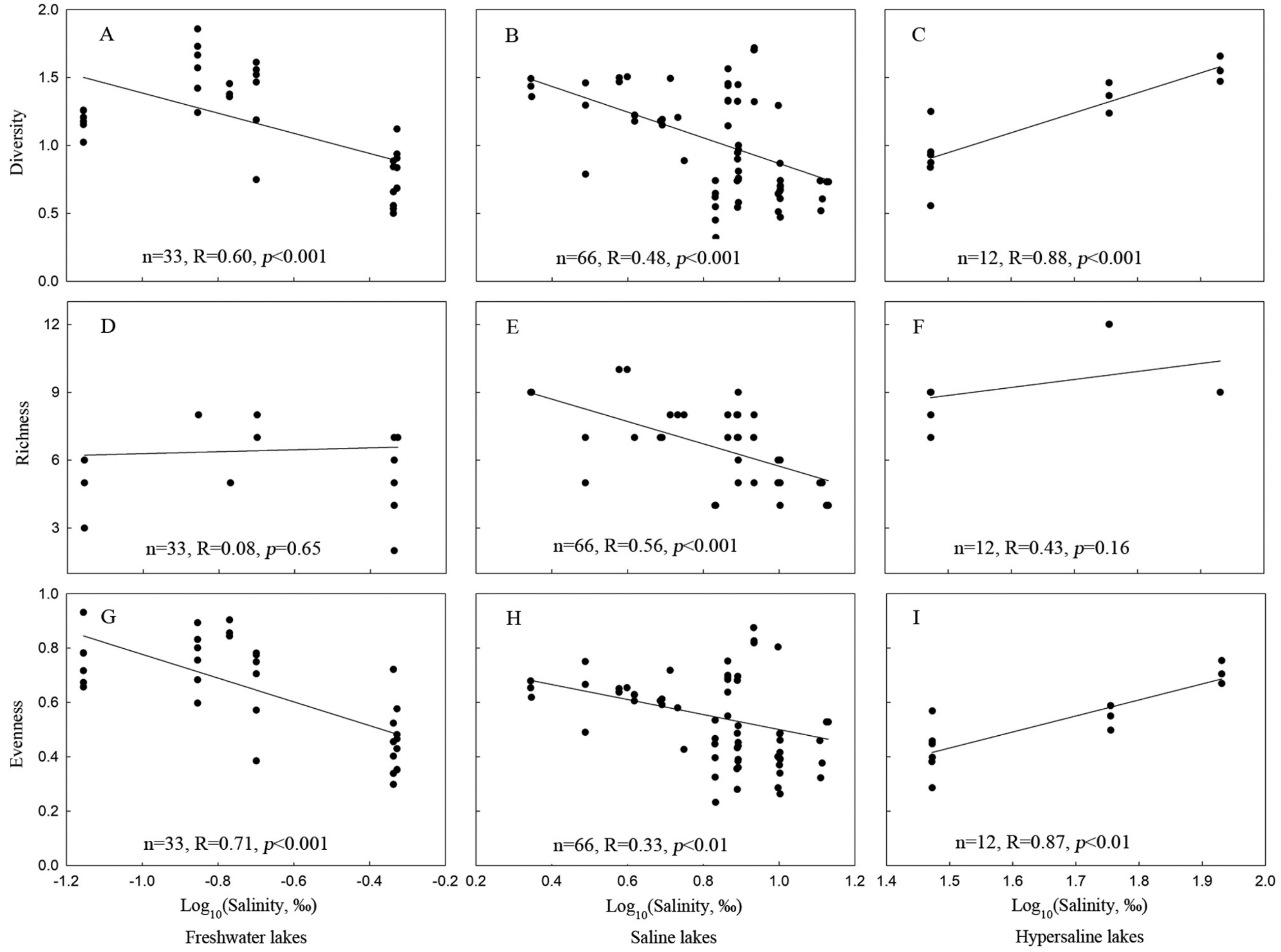

Fig. 2. Form IC microbial primary producer diversity (A, B, C), richness (D, E, F) and evenness (G, H, I) in lake waters along the salinity gradient. All the data of salinity were $\log_{10}$ transformed.

ecosystems (Liu et al., 2013, 2019; Zhong et al., 2016). However, positive correlation of MPP abundance with nutrient content (TOC and TN) in freshwater and hypersaline lakes as well as with water temperature in freshwater and saline lakes (Tables 1, S3) suggest that higher nutrient availability and more favorable temperatures can mitigate the salinity constraint. This is consistent with that increased nutrients, particularly nitrogen and phosphorus, stimulate MPP abundance and rates of primary production in aquatic ecosystems (Elser et al., 2009; Bergstrom et al., 2013; Sanders et al., 2015; Teufel et al., 2017). Nutrients usually increased microbial abundance by facilitating cell growth and division (Huang et al., 2012; Martínez-Macías et al., 2018). For example, organic matter addition facilitated microbial growth and biomass in high pH and salinity soils (Kamble et al., 2014). Form IC MPP are typically facultative autotrophs (Fig. 5) (Alfreider et al., 2009; Yuan et al., 2012; Guo et al., 2015; Zhao et al., 2018) and could utilize an array of different carbon sources, such as *Rubrivivax gelatinousus* (Nagashima et al., 2012). Alternative carbon and energy sources would be advantageous under high salinity, which is particularly stressful for autotrophic energy generating pathways (Sudhir and Murthy, 2004; Ashraf and Harris, 2013).

In contrast with the saline lakes, salinity did not constrain MPP abundance in either the freshwater or hypersaline lakes, which could be due to the following reasons. In freshwaters the nutrients and favorable temperature mitigated the salinity constraint or low salinity did not exert enough constraint effects. In hypersaline waters the MPP were adapted to salt, thus their abundance was not constrained by hypersalinity, but facilitated by higher nutrient availability (TN and TOC). This hypothesis is supported by the observation that the MPP in the hypersaline waters were phylogenetically affiliated to those retrieved from hypersaline soda lakes (Sorokin et al., 2001; Kovaleva et al., 2011), belonging to *Gammaproteobacteria* (Fig. 5). *Gammaproteobacteria* were halophilic and widely observed in hypersaline habitats (Logares et al., 2013; Liu et al., 2018; Rath et al., 2018).

4.2. Diversity of microbial primary producer community in Tibetan Plateau lake waters

Our results demonstrated that MPP community diversity, richness and evenness decreased with enhancing salinity in the lake waters (Fig. 2). In contrast, nutrients and warmer temperatures mitigated the salinity constraints (Fig. 3 and Tables S4–S5), and the mitigating effects were more pronounced in saline lakes than in freshwater and hypersaline lakes. These results are consistent with previous observations that increased nutrient supply enhanced phytoplankton richness in a saline lake (Larson and Belovsky, 2013), and *cbbL* diversity in soils (Selesi et al., 2005; Yuan et al., 2015). The results imply that MPP diversity is constrained by high salinity only if they experience nutrients limitation or other stresses. The nutrient mitigation could be due to that organic matter decomposition provided rich nitrogen and phosphorus and labile substrates for mixotrophs (Xiao et al., 2014; Yuan et al., 2015; Wu et al., 2017), which subsequently increased the MPP diversity. Our results are contrary to most previous findings that increasing salinity was more commonly observed to either have no effect or decrease overall bacterial or eukaryotic diversity (Benlloch et al., 2002; Foti et al., 2008; Hollister et al., 2010; Larson and Belovsky, 2013; Rath et al., 2018). In addition, our findings explain the previous phenomena that

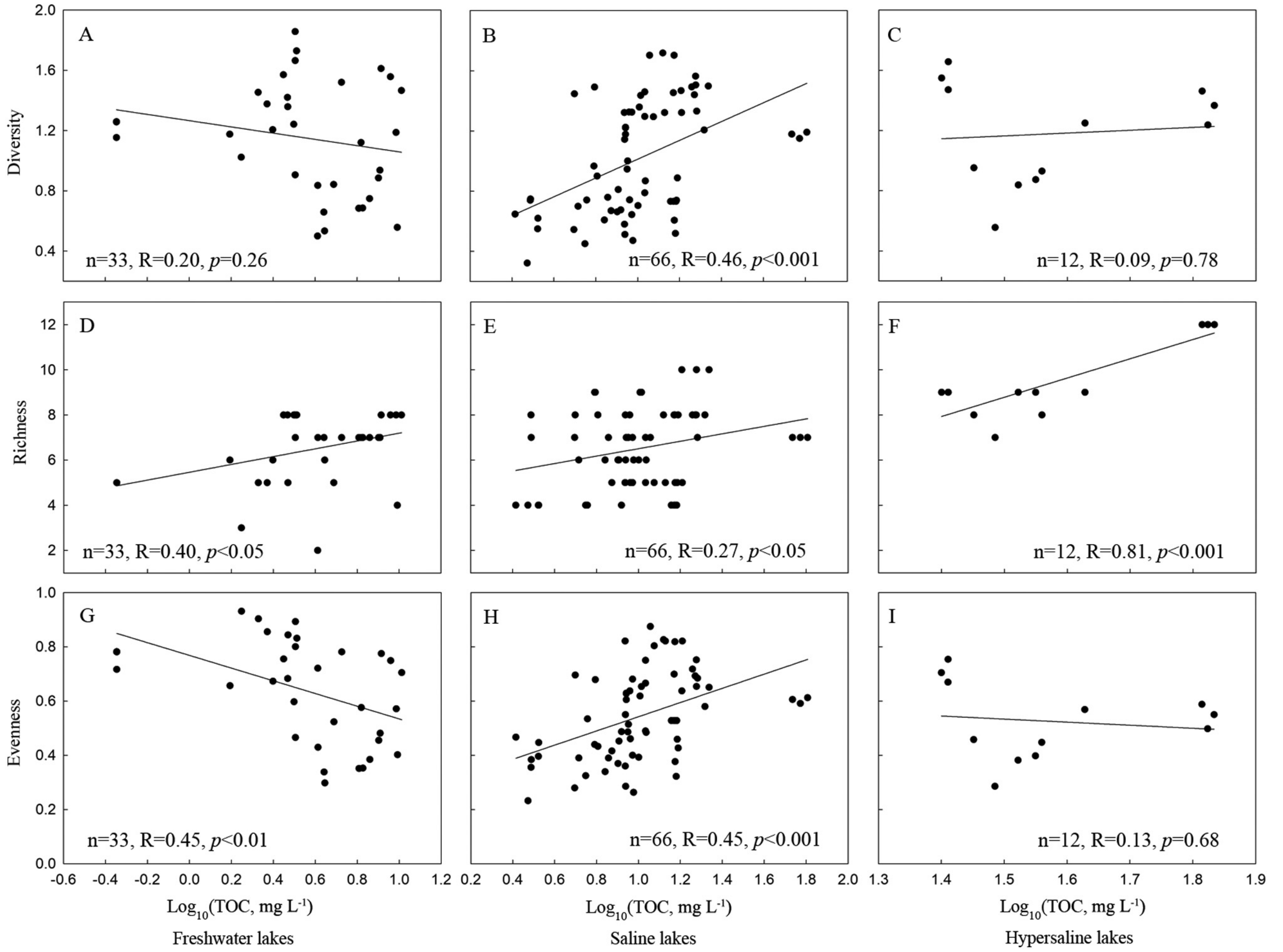

Fig. 3. Form IC microbial primary producer diversity (A, B, C), richness (D, E, F) and evenness (G, H, I) in lake waters along the total organic carbon (TOC) gradient. All the data of TOC contents were $\log_{10}$ transformed.

the richness of cyanobacteria and bacterioplankton are not negatively impacted by increasing salinity in Tibetan Plateau lakes (Wu et al., 2006, 2009).

Interestingly, the MPP diversity in hypersaline lakes was similar to or even higher than freshwater and saline lakes on the Tibetan Plateau. A similar finding was observed in prokaryote diversity in Tibetan Plateau lake waters (Zhong et al., 2016). This could be attributed to that the hypersaline lakes were rich in nutrients and their MPP taxa were more halotolerant (such as *Thioalkalivibrio versutus*) (Fig. 5) (Sorokin et al., 2001). Thus the higher nutrients coupling with MPP compositions with high salt-tolerance collectively contributed to the high diversity and richness in the hypersaline lakes.

4.3. Microbial primary producer community structure and composition in Tibetan Plateau lake waters

Our results demonstrated that salinity played a dominant role in driving the MPP community structure, while nutrients and water temperature also played important roles (Figs. 4, S2 and Table 2). The interactive effects of nutrient availability and abiotic stresses were previously observed in microbial community diversity (Wu et al., 2006; Bergstrom et al., 2013; Gusha et al., 2019). Microbial community structure was strongly influenced by the ability of each taxa to tolerate salinity constraint (Larson and Belovsky, 2013), with the freshwater taxa could rarely survive in saline waters (Logares et al., 2009, 2013). Microbial community structure could be driven by favoring the growth of copiotrophic (fast-growing but low C use efficiency) taxa and reducing the abundance of oligotrophic (slow-growing, high C use efficiency) taxa (Van Horn et al., 2014; Leff et al., 2015). It was observed that high organic carbon content and nutrient availability facilitated the growth of mixotrophs (Yuan et al., 2012) in soils. Additionally, nutrients could regulate the temperature dependence of metabolic rates, abundance and productivity in phytoplankton (Maranon et al., 2018; Gusha et al., 2019). Higher nutrients may also assist the MPP in maintaining stable or even higher carbon fixation rates (Young et al., 2015; Maranon et al., 2018). Thus, nutrients could directly and indirectly drive MPP community. In addition to higher nutrients, favorable temperatures frequently drove microbial community structure in aquatic ecosystems (Fuhrman et al., 2006; Gilbert et al., 2009; Pagaling et al., 2009). Temperature was reported to control biological activity through its favorable effect on metabolic rates (Cross et al., 2015; Maranon et al., 2018). The temperature facilitating effect was obvious in our study because of the low temperatures in Tibetan Plateau lakes, which ranged from 6 to 24 °C during Summer (Wan et al., 2017).

The MPP community variation was consistent with the compositional shifts along the salinity gradient. *Betaproteobacteria* dominated freshwater and saline lakes, while *Gammaproteobacteria* dominated hypersaline lakes (Fig. 5). These microbes were phylogenetically close to clones from cold and saline habitats. Salinity was the predominant factor regulating the microbial compositions on a global scale (Lozupone and Knight, 2007). It was assumed that salinity increase may lead to the replacement of suboptimal salinity-adapted taxa by the better adapted (Wu et al., 2006; Jiang et al., 2007) and hypersalinity exerts strong phylogentic selection on MPP.

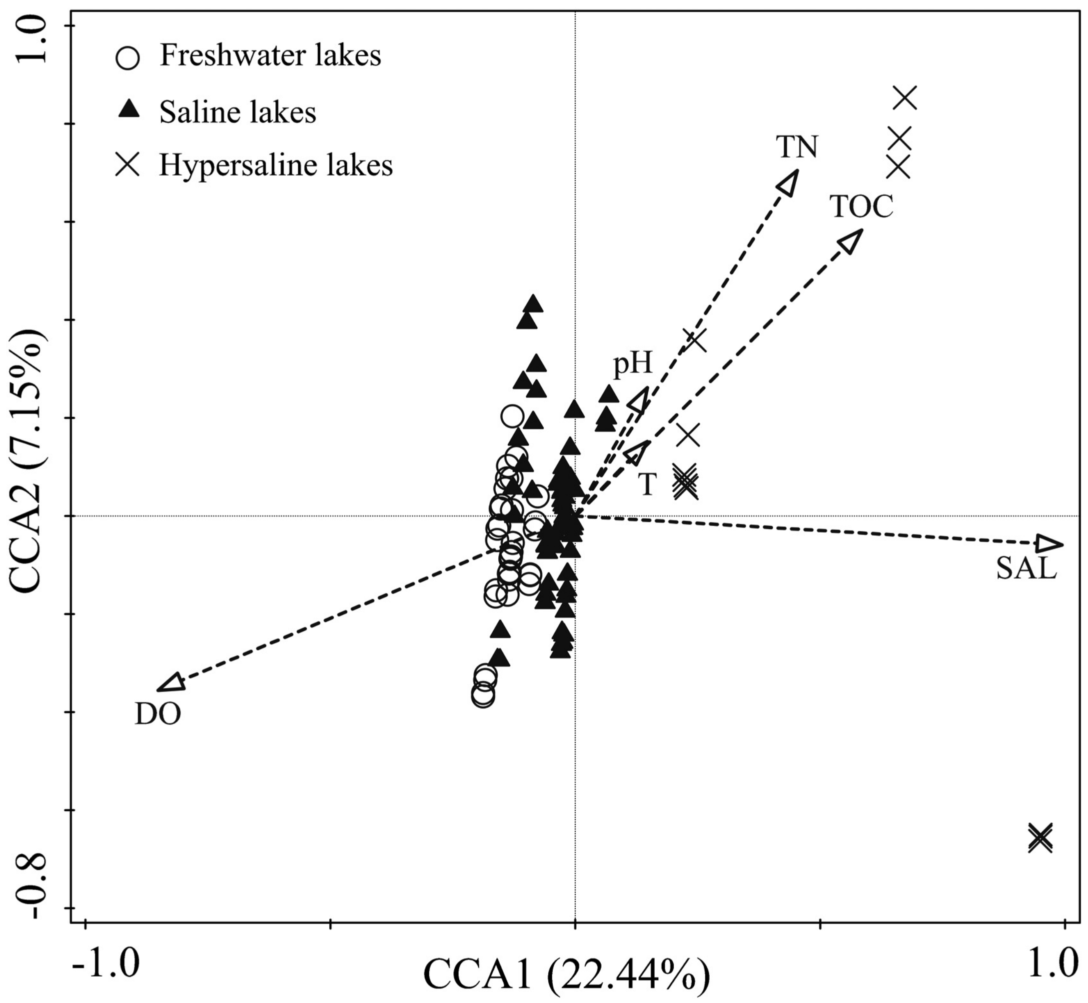

Fig. 4. Canonical correspondence analysis (CCA) of form IC microbial primary producer community structure based on T-RFLP profiles. SAL, salinity (‰); T, water temperature (°C); DO, dissolved oxygen (mg L^{-1}); TOC, total organic carbon (mg L^{-1}); TN, total nitrogen (mg L^{-1}).

5. Conclusions

In summary, we provided a large-scale investigation of the MPP community abundance, diversity and their response to salinity and nutrients in surface lake waters located in the Tibetan Plateau. Our findings demonstrated that while the MPP community abundance and diversity were constrained by salinity, nutrients availability contributed to the responses of MPP to salinity and mitigated the salinity constraints. The nutrients mitigating effects were more pronounced in saline waters than in fresh and hypersaline waters. Our study provided a new insight into the interactive effects of salinity and nutrients on primary producers in aquatic ecosystems in general, and in particular to understudied, low-latitude polar lakes. Given the enormous CO_2-fixing amount by MPP in aquatic ecosystems, there is an urgent need to study MPP roles in carbon cycling and their response to various environmental factors. This baseline will be beneficial in predicting MPP community and carbon cycling responses in aquatic ecosystems to climate change.

Table 2
Individual and combined contributions of environmental factors to form IC microbial primary producer community structure in the Tibetan Plateau lakes.

Environmental factors	Individual or combined contribution			
	All lakes[a]	Freshwater lakes[b]	Saline lakes[c]	Hypersaline lakes[d]
Salinity (‰)	8.0**	22.6**	7.2**	17.4**
pH	2.6**	7.4**	5.0**	–
Dissolved oxygen (mg L^{-1})	2.0**	–	4.8**	–
Water temperature (°C)	2.8**	7.5**	–	–
Total organic carbon (mg L^{-1})	–	5.1**	–	–
Total nitrogen (mg L^{-1})	1.5**	3.0*	–	–
Combined effect of all factors (%)	32.8**	59.7**	15.6**	48.8**

Variation partitioning analysis (VPA) tested the correlation between T-RFLP data of form IC *cbbL* gene and environmental factors.
Significant difference was determined by Monte Carlo test with 1000 permutations.
[a] $n = 111$.
[b] n = 33.
[c] n = 66.
[d] $n = 12$.
* $p \leq 0.05$.
** $p \leq 0.01$, and missing values indicate non-significant correlations ($p > 0.05$).

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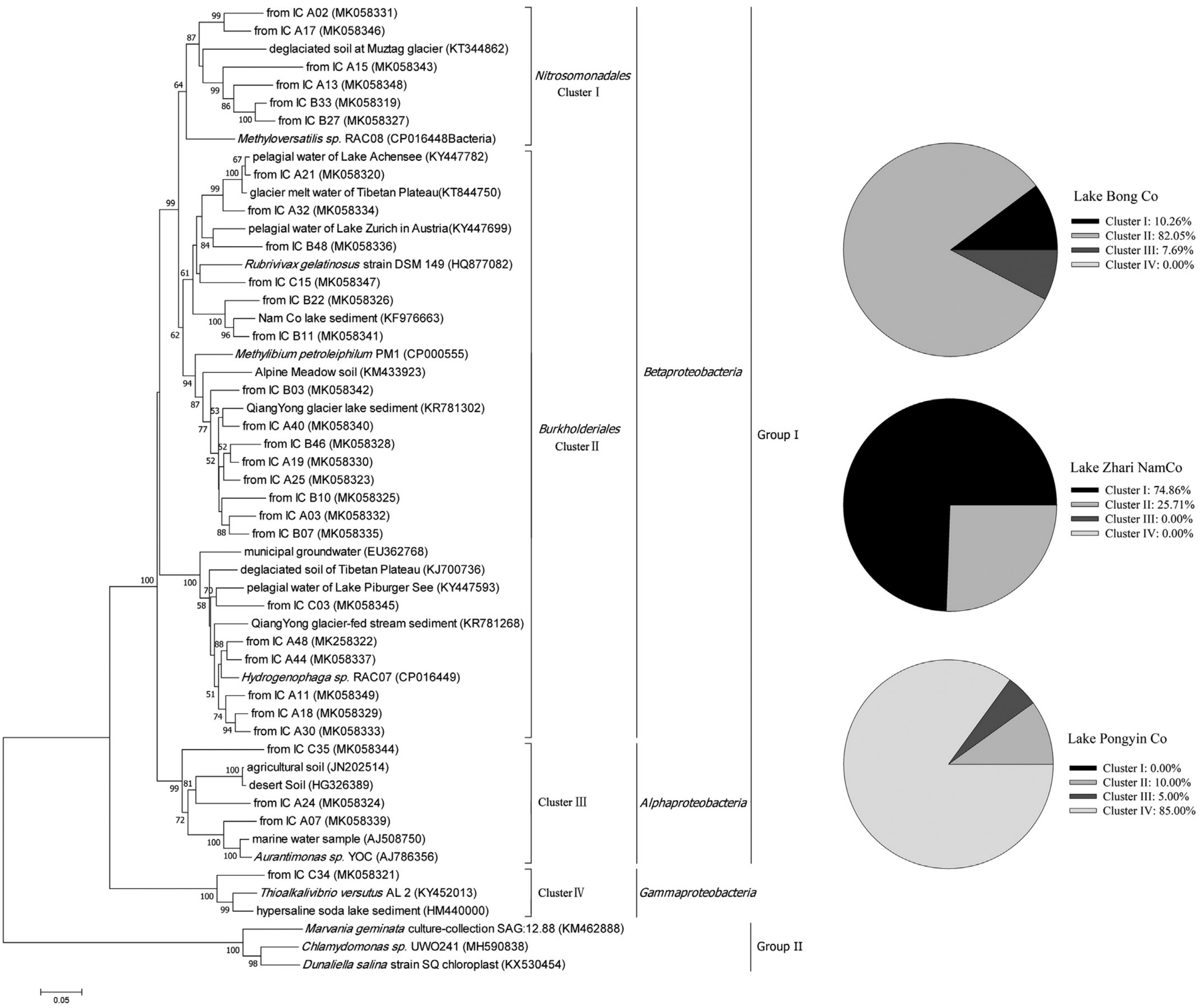

Fig. 5. Neighbor-joining phylogenetic tree of form IC *cbbL* gene sequences retrieved from environmental DNA in lake waters. Bar, 0.05 substitutions per nucleotide position. The name of water sample was named as the start of clone name (A, lake Bong Co, salinity 0.14‰; B, lake Zhari Namco, salinity 10.05‰; C, lake Pongyin Co, salinity 85.28‰) followed by the clone number.

Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134001.

References

Alfreider, A., Vogt, C., Geiger-Kaiser, M., Psenner, R., 2009. Distribution and diversity of autotrophic bacteria in groundwater systems based on the analysis of RubisCO genotypes. Syst. Appl. Microbiol. 32, 140–150. https://doi.org/10.1016/j.syapm.2008.11.005.

Alfreider, A., Grimus, V., Luger, M., Ekblad, A., Salcher, M.M., Summerer, M., 2018. Autotrophic carbon fixation strategies used by nitrifying prokaryotes in freshwater lakes. FEMS Microbiol. Ecol. 94, 1–12. https://doi.org/10.1093/femsec/fiy163.

Al-Said, T., Al-Ghunaim, A., Rao, D.V.S., Al-Yamani, F., Al-Rifaie, K., Al-Baz, A., 2017. Salinity-driven decadal changes in phytoplankton community in the NW Arabian Gulf of Kuwait. Environ. Monit. Assess. 189, 17. https://doi.org/10.1007/s10661-017-5969-4.

Anderson, J.M., 1991. The effects of climate change on decomposition processes in grassland and coniferous forests. Ecol. Appl. 1, 326–347. https://doi.org/10.2307/1941761.

Ashraf, M., Harris, P.J.C., 2013. Photosynthesis under stressful environments: an overview. Photosynthetica 51, 163–190. https://doi.org/10.1007/s11099-013-0021-6.

Basu, S., Mackey, K., 2018. Phytoplankton as key mediators of the biological carbon pump: their responses to a changing climate. Sustainability 10, 869. https://doi.org/10.3390/su10030869.

Baumert, H.Z., Petzoldt, T., 2008. The role of temperature, cellular quota and nutrient concentrations for photosynthesis, growth and light–dark acclimation in phytoplankton. Limnologica 38, 313–326. https://doi.org/10.1016/j.limno.2008.06.002.

Benlloch, S., Lopez-Lopez, A., Casamayor, E.O., Ovreas, L., Goddard, V., Daae, F.L., Smerdon, G., Massana, R., Joint, I., Thingstad, F., Pedros-Alio, C., Rodriguez-Valera, F., 2002. Prokaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. Environ. Microbiol. 4, 349–360. https://doi.org/10.1046/j.1462-2920.2002.00306.x.

Bergstrom, A.-K., Faithfull, C., Karlsson, D., Karlsson, J., 2013. Nitrogen deposition and warming - effects on phytoplankton nutrient limitation in subarctic lakes. Glob. Chang. Biol. 19, 2557–2568. https://doi.org/10.1111/gcb.12234.

Bratkic, A., Sturm, M., Faganeli, J., Ogrinc, N., 2012. Semi-annual carbon and nitrogen isotope variations in the water column of Lake Bled, NW Slovenia. Biogeosciences 9, 1–11. https://doi.org/10.5194/bg-9-1-2012.

Chen, J.G., Yang, H.Q., Zeng, Y., Guo, J.Y., Song, Y.L., Ding, W., 2018. Combined use of radiocarbon and stable carbon isotope to constrain the sources and cycling of particulate organic carbon in a large freshwater lake, China. Sci. Total Environ. 625, 27–38. https://doi.org/10.1016/j.scitotenv.2017.12.275.

Chen, S.J., Wang, D., 2019. Responses of decomposition rate and nutrient release of floating-leaved and submerged aquatic macrophytes to vertical locations in an urban lake (Nanhu Lake, China). Chem. Ecol. 35, 431–444. https://doi.org/10.1080/02757540.2019.1584615.

Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., 2009. Controlling Eutrophication: Nitrogen and Phosphorus. American Association for the Advancement of Science.

Cross, W.F., Hood, J.M., Benstead, J.P., Huryn, A.D., Nelson, D., 2015. Interactions between temperature and nutrients across levels of ecological organization. Glob. Chang. Biol. 21, 1025–1040. https://doi.org/10.1111/gcb.12809.

Duarte, C.M., Prairie, Y.T., Montes, C., Cole, J.J., Striegl, R., Melack, J., Downing, J.A., 2008. CO2 emissions from saline lakes: a global estimate of a surprisingly large flux. J. Geophys. Res. Biogeosci. 113. https://doi.org/10.1029/2007jg000637.

Elser, Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x.

Elser, Kyle, M., Steger, L., Nydick, K.R., Baron, J.S., 2009. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology 90, 3062–3073. https://doi.org/10.1890/08-1742.1.

Engel, F., Drakare, S., Weyhenmeyer, G.A., 2019. Environmental conditions for phytoplankton influenced carbon dynamics in boreal lakes. Aquat. Sci. 81, 12. https://doi.org/10.1007/s00027-019-0631-6.

Falkowski, P.G., Katz, M.E., Knoll, A.H., Quigg, A., Raven, J.A., Schofield, O., Taylor, F.J.R., 2004. The evolution of modern eukaryotic phytoplankton. Science 305, 354–360. https://doi.org/10.1126/science.1095964.

Ferreira, A., Garrido-Amador, P., Brito, A.C., 2019. Disentangling environmental drivers of phytoplankton biomass off Western Iberia. Front. Mar. Sci. 6, 17. https://doi.org/10.3389/fmars.2019.00044.

Field, C.B., Behrenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281, 237–240. https://doi.org/10.1126/science.281.5374.237.

Finzi, A.C., Austin, A.T., Cleland, E.E., Frey, S.D., Houlton, B.Z., Wallenstein, M.D., 2011. Responses and feedbacks of coupled biogeochemical cycles to climate change: examples from terrestrial ecosystems. Front. Ecol. Environ. 9, 61–67. https://doi.org/10.1890/100001.

Foti, M.J., Sorokin, D.Y., Zacharova, E.E., Pimenov, N.V., Kuenen, J.G., Muyzer, G., 2008. Bacterial diversity and activity along a salinity gradient in soda lakes of the Kulunda Steppe (Altai, Russia). Extremophiles 12, 133–145. https://doi.org/10.1007/s00792-007-0117-7.

Fuhrman, J.A., Hewson, I., Schwalbach, M.S., Steele, J.A., Brown, M.V., Naeem, S., 2006. Annually reoccurring bacterial communities are predictable from ocean conditions. Proc. Natl. Acad. Sci. U. S. A. 103, 13104–13109. https://doi.org/10.1073/pnas.0602399103.

Gilbert, J.A., Field, D., Swift, P., Newbold, L., Oliver, A., Smyth, T., Somerfield, P.J., Huse, S., Joint, I., 2009. The seasonal structure of microbial communities in the Western English Channel. Environ. Microbiol. 11, 3132–3139. https://doi.org/10.1111/j.1462-2920.2009.02017.x.

Guo, G., Kong, W., Liu, J., Zhao, J., Du, H., Zhang, X., Xia, P., 2015. Diversity and distribution of autotrophic microbial community along environmental gradients in grassland soils on the Tibetan Plateau. Appl. Microbiol. Biotechnol. 99, 8765–8776. https://doi.org/10.1007/s00253-015-6723-x.

Gusha, M.N.C., Dalu, T., Wasserman, R.J., McQuaid, C.D., 2019. Zooplankton grazing pressure is insufficient for primary producer control under elevated warming and nutrient levels. Sci. Total Environ. 651, 410–418. https://doi.org/10.1016/j.scitotenv.2018.09.132.

Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E.S., Elser, J.J., Gruner, D.S., Hillebrand, H., Shurin, J.B., Smith, J.E., 2011. Nutrient co-limitation of primary producer communities. Ecol. Lett. 14, 852–862. https://doi.org/10.1111/j.1461-0248.2011.01651.x.

Hole, L., Engardt, M., 2008. Climate change impact on atmospheric nitrogen deposition in northwestern Europe: a model study. AMBIO J. Hum. Environ. 37, 9–17. https://doi.org/10.1579/0044-7447(2008)37[9,CCIOAN]2.0.CO;2.

Hollister, E.B., Engledow, A.S., Hammett, A.J., Provin, T.L., Wilkinson, H.H., Gentry, T.J., 2010. Shifts in microbial community structure along an ecological gradient of hypersaline soils and sediments. ISME J. 4, 829–838. https://doi.org/10.1038/ismej.2010.3.

Huang, X., Huang, Z., Wen, W., Yan, J., 2012. Effects of nitrogen supplementation of the culture medium on the growth, total lipid content and fatty acid profiles of three microalgae (Tetraselmis subcordiformis, Nannochloropsis oculata and Pavlova viridis). J. Appl. Phycol. 25, 129–137. https://doi.org/10.1007/s10811-012-9846-9.

Jiang, H., Dong, H., Yu, B., Liu, X., Li, Y., Ji, S., Zhang, C.L., 2007. Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. Environ. Microbiol. 9, 2603–2621. https://doi.org/10.1111/j.1462-2920.2007.01377.x.

John, D.E., Wang, Z.A., Liu, X., Byrne, R.H., Corredor, J.E., Lopez, J.M., Cabrera, A., Bronk, D.A., Tabita, F.R., Paul, J.H., 2007. Phytoplankton carbon fixation gene (RuBisCO) transcripts and air-sea CO2 flux in the Mississippi River plume. ISME J. 1, 517–531. https://doi.org/10.1038/ismej.2007.70.

Kamble, P.N., Gaikwad, V.B., Kuchekar, S.R., Baath, E., 2014. Microbial growth, biomass, community structure and nutrient limitation in high pH and salinity soils from Pravaranagar (India). Eur. J. Soil Biol. 65, 87–95. https://doi.org/10.1016/j.ejsobi.2014.10.005.

Kong, W., Ream, D.C., Priscu, J.C., Morgan-Kiss, R.M., 2012. Diversity and expression of RubisCO genes in a perennially ice-covered Antarctic lake during the polar night transition. Appl. Environ. Microbiol. 78, 4358–4366. https://doi.org/10.1128/aem.00029-12.

Kovaleva, O.L., Tourova, T.P., Muyzer, G., Kolganova, T.V., Sorokin, D.Y., 2011. Diversity of RuBisCO and ATP citrate lyase genes in soda lake sediments. FEMS Microbiol. Ecol. 75, 37–47. https://doi.org/10.1111/j.1574-6941.2010.00996.x.

Larson, C.A., Belovsky, G.E., 2013. Salinity and nutrients influence species richness and evenness of phytoplankton communities in microcosm experiments from Great Salt Lake, Utah, USA. J. Plankton Res. 35, 1154–1166. https://doi.org/10.1093/plankt/fbt053.

Leff, J.W., Jones, S.E., Prober, S.M., Barberan, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schuetz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proc. Natl. Acad. Sci. U. S. A. 112, 10967–10972. https://doi.org/10.1073/pnas.1508382112.

Lindsay, M.R., Johnston, R.E., Baxter, B.K., Boyd, E.S., 2019. Effects of salinity on microbialite-associated production in Great Salt Lake, Utah. Ecology 100, 14. https://doi.org/10.1002/ecy.2611.

Liu, K.S., Liu, Y.Q., Han, B.P., Xu, B.Q., Zhu, L.P., Ju, J.T., Jiao, N.Z., Xiong, J.B., 2019. Bacterial community changes in a glacial-fed Tibetan lake are correlated with glacial melting. Sci. Total Environ. 651, 2059–2067. https://doi.org/10.1016/j.scitotenv.2018.10.104.

Liu, W., Jiang, H., Yang, J., Wu, G., 2018. Gammaproteobacterial diversity and carbon utilization in response to salinity in the lakes on the Qinghai–Tibetan Plateau. Geomicrobiol J. 35, 392–403. https://doi.org/10.1080/01490451.2017.1378951.

Liu, Y., Yao, T., Jiao, N., Zhu, L., Hu, A., Liu, X., Gao, J., Chen, Z.-Q., 2013. Salinity impact on bacterial community composition in five high-altitude lakes from the Tibetan Plateau, Western China. Geomicrobiol J. 30, 462–469. https://doi.org/10.1080/01490451.2012.710709.

Logares, R., Brate, J., Bertilsson, S., Clasen, J.L., Shalchian-Tabrizi, K., Rengefors, K., 2009. Infrequent marine-freshwater transitions in the microbial world. Trends Microbiol. 17, 414–422. https://doi.org/10.1016/j.tim.2009.05.010.

Logares, R., Lindstrom, E.S., Langenheder, S., Logue, J.B., Paterson, H., Laybourn-Parry, J., Rengefors, K., Tranvik, L., Bertilsson, S., 2013. Biogeography of bacterial communities exposed to progressive long-term environmental change. ISME J. 7, 937–948. https://doi.org/10.1038/ismej.2012.168.

Lozupone, C.A., Knight, R., 2007. Global patterns in bacterial diversity. Proc. Natl. Acad. Sci. U. S. A. 104, 11436–11440. https://doi.org/10.1073/pnas.0611525104.

Lynn, T.M., Ge, T., Yuan, H., Wei, X., Wu, X., Xiao, K., Kumaresan, D., Yu, S.S., Wu, J., Whiteley, A.S., 2017. Soil carbon-fixation rates and associated bacterial diversity and abundance in three natural ecosystems. Microb. Ecol. 73, 645–657. https://doi.org/10.1007/s00248-016-0890-x.

Maranon, E., Lorenzo, M.P., Cermeno, P., Mourino-Carballido, B., 2018. Nutrient limitation suppresses the temperature dependence of phytoplankton metabolic rates. ISME J. 12, 1836–1845. https://doi.org/10.1038/s41396-018-0105-1.

Martinez-Garcia, M., Swan, B.K., Poulton, N.J., Gomez, M.L., Masland, D., Sieracki, M.E., Stepanauskas, R., 2012. High-throughput single-cell sequencing identifies photoheterotrophs and chemoautotrophs in freshwater bacterioplankton. ISME J. 6, 113–123. https://doi.org/10.1038/ismej.2011.84.

Martínez-Macías, R., Meza-Escalante, E., Serrano-Palacios, D., Gortáres-Moroyoqui, P., Ruíz-Ruíz, P.E., Ulloa-Mercado, G., 2018. Effect of fed-batch and semicontinuous regimen on Nannochloropsis oculata grown in different culture media to high-value products. J. Chem. Technol. Biotechnol. 93, 585–590. https://doi.org/10.1002/jctb.5405.

Nagashima, S., Kamimura, A., Shimizu, T., Nakamura-Isaki, S., Aono, E., Sakamoto, K., Ichikawa, N., Nakazawa, H., Sekine, M., Yamazaki, S., Fujita, N., Shimada, K., Hanada, S., Nagashima, K.V.P., 2012. Complete genome sequence of phototrophic betaproteobacterium Rubrivivax gelatinosus IL144. J. Bacteriol. 194, 3541–3542. https://doi.org/10.1128/jb.00511-12.

Oren, A., 1999. Bioenergetic aspects of halophilism. Microbiol. Mol. Biol. Rev. 63, 334–348.

Pagaling, E., Wang, H., Venables, M., Wallace, A., Grant, W.D., Cowan, D.A., Jones, B.E., Ma, Y., Ventosa, A., Heaphy, S., 2009. Microbial biogeography of six salt lakes in Inner Mongolia, China, and a salt lake in Argentina. Appl. Environ. Microbiol. 75, 5750–5760. https://doi.org/10.1128/aem.00040-09.

Quayle, W.C., Convey, P., 2006. Concentration, molecular weight distribution and neutral sugar composition of DOC in maritime antarctic lakes of differing trophic status. Aquat. Geochem. 12, 161–178. https://doi.org/10.1007/s10498-005-3882-x.

Rath, K.M., Fierer, N., Murphy, D.V., Rousk, J., 2018. Linking bacterial community composition to soil salinity along environmental gradients. ISME J. https://doi.org/10.1038/s41396-018-0313-8.

Sanders, R.W., Cooke, S.L., Fischer, J.M., Fey, S.B., Heinze, A.W., Jeffrey, W.H., Macaluso, A.L., Moeller, R.E., Morris, D.P., Neale, P.J., Olson, M.H., Pakulski, J.D., Porter, J.A., Schoener, D.M., Williamson, C.E., 2015. Shifts in microbial food web structure and productivity after additions of naturally occurring dissolved organic matter: results from large-scale lacustrine mesocosms. Limnol. Oceanogr. 60, 2130–2144. https://doi.org/10.1002/lno.10159.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/aem.01541-09.

Seekell, D.A., Lapierre, J.F., Ask, J., Bergstrom, A.K., Deininger, A., Rodriguez, P., Karlsson, J., 2015. The influence of dissolved organic carbon on primary production in northern lakes. Limnol. Oceanogr. 60, 1276–1285. https://doi.org/10.1002/lno.10096.

Selesi, D., Schmid, M., Hartmann, A., 2005. Diversity of green-like and red-like ribulose-1,5-bisphosphate carboxylase/oxygenase large-subunit genes (cbbL) in differently managed agricultural soils. Appl. Environ. Microbiol. 71, 175–184. https://doi.org/10.1128/aem.71.1.175-184.2005.

Shaver, G.R., Canadell, J., Chapin, F.S., Gurevitch, J., Harte, J., Henry, G., Ineson, P., Jonasson, S., Melillo, J., Pitelka, L., Rustad, L., 2000. Global warming and terrestrial ecosystems: a conceptual framework for analysis. Bioscience 50, 871–882. https://doi.org/10.1641/0006-3568(2000)050[0871,gwatea]2.0.co;2.

Sorokin, D.Y., Lysenko, A.M., Mityushina, L.L., Tourova, T.P., Jones, B.E., Rainey, F.A., Robertson, L.A., Kuenen, G.J., 2001. Thioalkalimicrobium aerophilum gen. nov., sp. nov. and Thioalkalimicrobium sibericum sp. nov., and Thioalkalivibrio versutus gen. nov., sp. nov., Thioalkalivibrio nitratis sp.nov., novel and Thioalkalivibrio denitrificancs sp. nov., novel obligately alkali. Int. J. Syst. Evol. Microbiol. 51, 565–580.

Stefanidou, N., Genitsaris, S., Lopez-Bautista, J., Sommer, U., Moustaka-Gouni, M., 2018. Effects of heat shock and salinity changes on coastal Mediterranean phytoplankton in a mesocosm experiment. Mar. Biol. 165 (14). https://doi.org/10.1007/s00227-018-3415-y.

Sudhir, P., Murthy, S.D.S., 2004. Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42, 481–486. https://doi.org/10.1007/s11099-005-0001-6.

Tabita, F.R., Satagopan, S., Hanson, T.E., Kreel, N.E., Scott, S.S., 2008. Distinct form I, II, III, and IV Rubisco proteins from the three kingdoms of life provide clues about Rubisco evolution and structure/function relationships. J. Exp. Bot. 59, 1515–1524. https://doi.org/10.1093/jxb/erm361.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. https://doi.org/10.1093/molbev/mst197.

Teufel, A.G., Li, W., Kiss, A.J., Morgan-Kiss, R.M., 2017. Impact of nitrogen and phosphorus on phytoplankton production and bacterial community structure in two stratified Antarctic lakes: a bioassay approach. Polar Biol. 40, 1007–1022. https://doi.org/10.1007/s00300-016-2025-8.

Tranvik, L.J., Jansson, M., 2002. Climate change - terrestrial export of organic carbon. Nature 415, 861–862. https://doi.org/10.1038/415861b.

Van Horn, D.J., Okie, J.G., Buelow, H.N., Gooseff, M.N., Barrett, J.E., Takacs-Vesbach, C.D., 2014. Soil microbial responses to increased moisture and organic resources along a salinity gradient in a polar desert. Appl. Environ. Microbiol. 80, 3034–3043. https://doi.org/10.1128/aem.03414-13.

Vavourakis, C.D., Ghai, R., Rodriguez-Valera, F., Sorokin, D.Y., Tringe, S.G., Hugenholtz, P., Muyzer, G., 2016. Metagenomic insights into the uncultured diversity and physiology of microbes in four hypersaline soda lake brines. Front. Microbiol. 7, 1–18. https://doi.org/10.3389/fmicb.2016.00211.

Wan, W., Xiao, P., Feng, X., Li, H., Ma, R., Duan, H., Zhao, L., 2014. Monitoring lake changes of Qinghai-Tibetan Plateau over the past 30 years using satellite remote sensing data. Chin. Sci. Bull. 59, 1021–1035. https://doi.org/10.1007/s11434-014-0128-6.

Wan, W., Li, H., Xie, H.J., Hong, Y., Long, D., Zhao, L.M., Han, Z.Y., Cui, Y.K., Liu, B.J., Wang, C.G., Yang, W.T., 2017. A comprehensive data set of lake surface water temperature over the Tibetan Plateau derived from MODIS LST products 2001-2015. Scientific Data 4, 1–10. https://doi.org/10.1038/sdata.2017.95.

Wang, N., Xiong, J., Wang, X.C., Zhang, Y., Liu, H., Zhou, B., Pan, P., Liu, Y., Ding, F., 2018. Relationship between phytoplankton community and environmental factors in landscape water with high salinity in a coastal city of China. Environ. Sci. Pollut. Res. 25, 28460–28470. https://doi.org/10.1007/s11356-018-2886-1.

Wu, Q.L., Zwart, G., Schauer, M., Kamst-van Agterveld, M.P., Hahn, M.W., 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. https://doi.org/10.1128/aem.00767-06.

Wu, Q.L., Chatzinotas, A., Wang, J., Boenigk, J., 2009. Genetic diversity of eukaryotic plankton assemblages in Eastern Tibetan Lakes differing by their salinity and altitude. Microb. Ecol. 58, 569–581. https://doi.org/10.1007/s00248-009-9526-8.

Wu, X., Ge, T., Yan, W., Zhou, J., Wei, X., Chen, L., Chen, X., Nannipieri, P., Wu, J., 2017. Irrigation management and phosphorus addition alter the abundance of carbon dioxide-fixing autotrophs in phosphorus-limited paddy soil. FEMS Microbiol. Ecol. 93, 1–10. https://doi.org/10.1093/femsec/fix154.

Xiao, K.-Q., Bao, P., Bao, Q.-L., Jia, Y., Huang, F.-Y., Su, J.-Q., Zhu, Y.-G., 2014. Quantitative analyses of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) large-subunit genes (cbbL) in typical paddy soils. FEMS Microbiol. Ecol. 87, 89–101. https://doi.org/10.1111/1574-6941.12193.

Yang, J., Ma, L.a., Jiang, H., Wu, G., Dong, H., 2016. Salinity shapes microbial diversity and community structure in surface sediments of the Qinghai-Tibetan Lakes. Sci. Rep. 6, 1–6. https://doi.org/10.1038/srep25078.

Young, J.N., Goldman, J.A.L., Kranz, S.A., Tortell, P.D., Morel, F.M.M., 2015. Slow carboxylation of Rubisco constrains the rate of carbon fixation during Antarctic phytoplankton blooms. New Phytol. 205, 172–181. https://doi.org/10.1111/nph.13021.

Yuan, H., Ge, T., Wu, X., Liu, S., Tong, C., Qin, H., Wu, M., Wei, W., Wu, J., 2012. Long-term field fertilization alters the diversity of autotrophic bacteria based on the ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO) large-subunit genes in paddy soil. Appl. Microbiol. Biotechnol. 95, 1061–1071. https://doi.org/10.1007/s00253-011-3760-y.

Yuan, H., Ge, T., Chen, X., Liu, S., Zhu, Z., Wu, X., Wei, W., Whiteley, A.S., Wu, J., 2015. Abundance and diversity of CO2-assimilating bacteria and algae within red agricultural soils are modulated by changing management practice. Microb. Ecol. 70, 971–980. https://doi.org/10.1007/s00248-015-0621-8.

Yvon-Durocher, G., Allen, A.P., Cellamare, M., Dossena, M., Gaston, K.J., Leitao, M., Montoya, J.M., Reuman, D.C., Woodward, G., Trimmer, M., 2015. Five years of experimental warming increases the biodiversity and productivity of phytoplankton. PLoS Biol. 13. https://doi.org/10.1371/journal.pbio.1002324.

Zarin, D.J., Johnson, A.H., Thomas, S.M., 1998. Soil organic carbon and nutrient status in old-growth montane coniferous forest watersheds, Isla Chiloe, Chile. Plant Soil 201, 251–258. https://doi.org/10.1023/a:1004375920790.

Zhao, K., Kong, W., Khan, A., Liu, J., Guo, G., Muhanmmad, S., Zhang, X., Dong, X., 2017. Elevational diversity and distribution of ammonia-oxidizing archaea community in meadow soils on the Tibetan Plateau. Appl. Microbiol. Biotechnol. 101, 7065–7074. https://doi.org/10.1007/s00253-017-8435-x.

Zhao, K., Kong, W., Wang, F., Long, X.-E., Guo, C., Yue, L., Yao, H., Dong, X., 2018. Desert and steppe soils exhibit lower autotrophic microbial abundance but higher atmospheric CO2 fixation capacity than meadow soils. Soil Biol. Biochem. 127, 230–238. https://doi.org/10.1016/j.soilbio.2018.09.034.

Zhong, Z.-P., Liu, Y., Miao, L.-L., Wang, F., Chu, L.-M., Wang, J.-L., Liu, Z.-P., 2016. Prokaryotic community structure driven by salinity and ionic concentrations in Plateau Lakes of the Tibetan Plateau. Appl. Environ. Microbiol. 82, 1846–1858. https://doi.org/10.1128/aem.03332-15.