Planktonic microbial communities from microbialite-bearing lakes sampled along a salinity-alkalinity gradient

Miguel Iniesto [ID],[1] **David Moreira** [ID],[1] **Karim Benzerara** [ID],[2] **Guillaume Reboul** [ID],[1] **Paola Bertolino** [ID],[1] **Rosaluz Tavera** [ID],[3] **Purificación López-García** [ID][1*]

[1]Ecologie Systématique Evolution, CNRS, Université Paris-Saclay, AgroParisTech, Orsay, France
[2]Institut de Minéralogie de Physique des Matériaux et de Cosmochimie, CNRS, Sorbonne Université, Muséum National d'Histoire Naturelle, Paris, France
[3]Departamento de Ecología y Recursos Naturales, Universidad Nacional Autónoma de México, Mexico City, Mexico

Abstract

Continental freshwater systems are particularly vulnerable to environmental variation. Climate change-induced desertification and the anthropogenic exploitation of hydric resources result in the progressive evaporation and salinization of inland water bodies in many areas of the globe. However, how this process impacts microbial communities and their activities in biogeochemical cycles is poorly known. Here, we take a space-for-time substitution approach and characterize the prokaryotic and eukaryotic microbial communities of two planktonic cell-size fractions (0.2–5 μm and 5–30 μm) from lakes of diverse trophic levels sampled along a salinity-alkalinity gradient located in the Trans-Mexican Volcanic Belt (TMVB). We applied a 16S/18S rRNA gene metabarcoding strategy to determine the microbial community composition of 54 samples from 12 different lakes, from the low-salinity lake Zirahuén to the hypersaline residual ponds of Rincón de Parangueo. Except for systems at both extremes of the salinity gradient, most lakes along the evaporation trend bear actively forming microbialites, which harbor microbial communities clearly distinct from those of plankton. Several lakes were sampled in winter and late spring and the crater lakes Alchichica and Atexcac were sampled across the water column. Physicochemical parameters related to salinity-alkalinity were the most influential drivers of microbial community structure whereas trophic status, depth, or season were less important. Our results suggest that climate change and anthropogenic-induced hydric deficit could significantly affect microbial communities, potentially altering ecosystem functioning.

Microorganisms are essential players in biogeochemical cycles. Accordingly, the effects of climate change on microbial communities may have considerable, yet unpredicted, consequences on elemental cycles at planetary scale (Cavicchioli et al. 2019). Among all ecosystems, continental freshwater

bodies are particularly vulnerable (Woodward et al. 2010; Markovic et al. 2017; Schmeller et al. 2018). This is largely due to their relatively small volumes and low buffering capacities compared with oceans and other, more stable, habitats (e.g., sediments or the subsurface). Even Lake Baikal, the largest freshwater lake by volume, is affected by climate change (Moore et al. 2009; Shimaraev and Domysheva 2012). In addition to temperature increase, several anthropogenic stressors, including pollution and hydrological exploitation, are among the major threats for local biodiversity and water quality. An important consequence is the proliferation of often harmful cyanobacterial blooms (Huisman et al. 2018). Another prominent effect is the progressive salinization of freshwater systems (Ondrasek and Rengel 2021; Cunillera-Montcusí et al. 2022). Studying the effect of progressive salinization on microbial communities is crucial to evaluate resilience, predict change and eventually apply preventive measurements for the preservation of the biodiversity and function of these ecosystems. In this context, space-for-time substitution approaches constitute

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

valuable proxies to investigate the influence of climate change on biological communities (Blois et al. 2013).

The Trans–Mexican Volcanic Belt (TMVB) is a 1000-km-long and 90–230-km-wide volcanic arc harboring numerous crater lakes (maars) that crosses the central region of Mexico from west to east around latitudes 19°–20°N (Ferrari et al. 2012). Over the years, these lakes have served as central points for the settlement of human populations that have exploited them for agricultural, fishing, or recreational activities (Faugère-Kalfon 1996). These activities combined with other anthropogenic pressures, including urban development, agriculture, and global warming, have already caused a considerable decrease in water levels (Alcocer et al. 2000), rise in turbidity, and disruption of trophic networks (Komárková and Tavera 2003; Caballero et al. 2006; Kienel et al. 2009). Water level decrease is accompanied by salinization, which in the hydrochemical context of these neutral-to-alkaline lakes often entails increased alkalinity. Accordingly, a varying evaporative history of a suite of Mexican lakes was assumed to be responsible for the measured variability of their salinities and alkalinities (Zeyen et al. 2017; Zeyen et al. 2021). Moreover, increased alkalinity appears as favorable to the precipitation of carbonates and the formation of microbialitic structures that can be very conspicuous, for instance in Lake Alchichica (Couradeau et al. 2011; Kazmierczak et al. 2011; Saghaï et al. 2015; Saghaï et al. 2016). Furthermore, the abundance and massiveness of microbialites in TMVB lakes correlate with alkalinity (Zeyen et al. 2017; Zeyen et al. 2021). The benthic microbial communities associated with microbialites in those lakes also experience a shift with increased alkalinity (Valdespino-Castillo et al. 2018; Iniesto et al. 2021b), despite the fact that they share a common microbial community core (Valdespino-Castillo et al. 2018; Iniesto et al. 2021b). However, this microbialite-associated core represents higher fractions of the community in lakes Alchichica and Atexcac, which display the highest alkalinity, suggesting that it is selected under these conditions (Iniesto et al. 2021a, 2021b). The planktonic communities of some TMVB lakes have been previously studied to a certain extent (Alcocer and Aguilar-Sierra 2019), often using classical approaches or focused on particular tropic guilds, such as specific members of phytoplankton (e.g., Sigala et al. 2017) or zooplankton, notably ciliates (Macek et al. 2009; Sánchez Medina et al. 2016). However, a joint comparative overview of prokaryotic and eukaryotic planktonic communities along a salinity gradient is still missing.

In this work, we aimed to characterize and compare the prokaryotic and eukaryotic composition of planktonic communities of two size fractions (0.2–5 and 5–30 μm) collected from 12 lakes of the TMVB along a marked salinity–alkalinity gradient. We hypothesized that this gradient is a strong determinant of plankton community composition. We analyze the environmental drivers that influence the microbial community structure including abiotic parameters but also the trophic category of lakes, often indicative of anthropization. In addition, to get insights into community dynamics and potential biotic interactions, we also compare communities and build co-occurrence networks from the Lake Alchichica water column collected in winter and late spring. Our work shows that salinity–alkalinity and trophic status are key determinants of microbial planktonic communities and suggest trends of future evolution following climate change increased evaporation levels.

Material and methods

Sample collection

Plankton samples analyzed in this work were collected using a Niskin bottle from 12 different lakes, mostly crater maars, from the Trans-Mexican volcanic belt in the regions of Puebla and Michoacán (Central Mexico) during two different campaigns: January 2012 and May 2014 (Figs. 1, S1; Supplementary Table S1). Many of these lakes are monomictic, being fully oxygenated in winter but stratified in summer (the stratification starting in May) (Armienta et al. 2008). Local parameters were measured with a multiparameter probe (Multi 350i,

Fig. 1. Mexican lakes from the Trans-Mexican volcanic belt (reddish area) sampled for this study in the regions of Puebla and Michoacán.

WTW). Alkalinity and ionic concentrations were determined from water samples by Zeyen et al. (2021). Parameters for Rincón de Parangueo were obtained from Armienta et al. (2008) and trophic parameters from Sigala et al. (2017). Sampling along the full water column was done in Lakes Alchichica (2012 and 2014) and Atextcac (2014). Five liters of water (or less, until filter saturation) were successively filtered through a 100 μm nylon mesh and a series of filters of decreasing pore diameter: 30-, 5-, and 0.2-μm pore-size Nucleopore membranes (Whatman). The 0.2- and 5-μm pore-size filters retaining cells in the 0.2–5- and 5–30-μm size fraction studied in this work were fixed in absolute ethanol and stored at $-20°$C until processing. A total of 54 samples were processed from the different lakes, depths, years, and cell fractions.

DNA purification and amplicon sequencing

DNA was purified from the biomass retained on filters soon after the return of the 2014 field trip using the Power Soil DNA Isolation Kit (MoBio) after the elimination of ethanol and rehydration of the biomass at 4°C for at least 2 h in the resuspension buffer of the kit. Prokaryotic 16S rRNA gene fragments ($\sim$ 290 bp long) covering the V4-hypervariable region were amplified using the prokaryote-specific primer set U515F (5'-GTGCCAGCMGCCGCGGTAA) and U806R (5'-GGACTACVSGGGTATCTAAT). The eukaryotic 18S rRNA gene V4-hypervariable region ($\sim$ 550 bp long fragments) was amplified using the primers EK-448F (5'- CTGAYWCAGGGAGGTAGTRA) and 18S-EUK-1134-R_UNonMet (5'-TTTAAGTTTCAGCCTTGCG) biased against Metazoa (Bower et al. 2004). Forward and reverse primers were tagged with 10-bp Molecular Identifiers (MIDs) to allow multiplexing and sorting of amplicons from different samples. The 25-μL PCR-amplification reactions contained 0.5–3 μL of eluted DNA, 1.5 mM MgCl$_2$, 0.2 mM of deoxynucleotide (dNTP) mix, 0.3 μM of each primer, and 0.5 U of the hot-start Platinum Taq DNA Polymerase (Invitrogen). PCR reactions were carried out for 35 cycles (94°C for 30 s, 55–58°C for 30–45 s, 72°C for 90 s) preceded by 2-min denaturation at 94°C, and followed by five additional minutes of polymerization at 72°C. To minimize PCR bias, five different PCR reactions were pooled for each sample. Amplicons were then purified using the QIAquick PCR purification kit (Qiagen). Amplicons were massively sequenced in 2015 using Illumina MiSeq (2 $\times$ 300 bp, paired-end) by Eurofins Genomics. Sequence statistics and accession numbers are provided in Supplementary Table S2.

Sequence analysis

We obtained 7,996,423 and 6,739,734 raw sequence-reads for 16S and 18S rDNA amplicons, respectively. These raw sequences were subsequently processed using an in-house bioinformatic pipeline. High-quality raw 16S rDNA paired-end reads were merged together according to strict criteria using FLASH (Magoc and Salzberg 2011). Cleaned merged reads with correct MIDs at each extremity were attributed to their original samples and pruned of primer + MID sequences using

"cutadapt" (Martin 2011). In the case of 18S rRNA gene sequences, we used high-quality forward reads since, due to the amplicon size, too few read pairs could be assembled reliably. VSEARCH (Rognes et al. 2016) was used in order to dereplicate high-quality merged reads and detect chimeric reads, which were excluded from further analyses. Non-chimeric high-quality merged reads were then pooled together in order to define inter-sample Operational Taxonomic Units (OTUs) using SWARM (Mahe et al. 2015). For subsequent analysis, we removed singletons (OTUs composed of one sequence). OTUs were phylogenetically classified based on sequence similarity with sequences from cultured/described organisms and environmental surveys retrieved from SILVAv138 for both prokaryotic and eukaryotic rDNA sequences (Quast et al. 2013). OTUs corresponding to chloroplasts, mitochondria, and Metazoa were removed for subsequent analyses. Sequences with low identity values were manually blasted and assigned to their best hit's taxon when they combined coverage and identity values > 80% and > 85%, respectively. After the quality-filtering process and the removal of singletons, the total of reads was grouped in 14,327 prokaryotic OTUs (1371 archaea and 12,955 bacteria) and 3189 eukaryotes (Supplementary Tables S2–S4).

Statistical and network analyses

Statistical analyses were carried out using R (R Development Core Team 2017). Diversity indexes, non-metric multidimensional scaling (NMDS) ordination analyses, and correlations were conducted using the "Vegan" R package (Oksanen et al. 2011). These tests were based on Bray–Curtis (BC) dissimilarities (Bray and Curtis 1957) between community structures in the different planktonic communities. Statistical differences between microbial communities were analyzed using a permutational multivariate ANOVA (PERMANOVA) test. A Mantel test based on the BC distance matrix and a matrix of Euclidean distances of the physico-chemical parameters observed (chemical parameters and depth) was applied to test whether microbial diversity was significantly correlated to the environmental variables. Mantel tests and canonical correspondence analyses (CCA) were calculated with the "Ade4" package (Dray and Dufour 2007). Prior to CCA analysis, we performed a forward selection of parameters with the function *ordiR2step* from the "Vegan" R package in order to avoid the effect produced by the occurrence of variables with strong correlation. Only significant factors (*p*-value from ANOVA < 0.05) were subsequently used for CCA (see Supplementary Table S5 for ANOVA analysis and variable selection performed for each CCA). To eliminate potential biases due to the nature of our data, NMDS and CCA analyses were performed using three different approaches: (1) rarefaction in order to obtain a data matrix with an equal number of sequences per sample; (2) Wisconsin standardization; and (3) frequency-based standardization. Networks of dominant OTUs in Alchichica were constructed using the

package SpiecEasi for R (Kurtz et al. 2015) with data from winter (2012) and late-spring (2014) using two approaches. First, we analyzed sparse correlations based on the model SparCC (Friedman and Alm 2012). Second, we studied modularity and degree of connectivity using the actual SPIEC-EASI (for Sparse and Compositionally Robust Inference of Microbial Ecological Networks) model, which estimates the inverse covariance matrix, creating less indirect edges.

Results and discussion

Composition of planktonic prokaryotic and eukaryotic microbial communities across space and time in TMVB lakes

To characterize and compare the composition of planktonic microbial communities in the Trans-Mexican Volcanic Belt (TMVB), we analyzed a total of 54 plankton samples, including two cell-size fractions (0.2–5 μm and 5–30 μm), from 12 TMVB lakes (Figs. 1, S1; Supplementary Tables S1, S2). With the exception of Pátzcuaro and Zirahuén, all are crater lakes (Armienta et al. 2008; Zeyen et al. 2021). All samples were collected during the same field trip in late spring (May 2014), when communities are in principle more active. We also collected samples in winter (January 2012) from four of the TMVB lakes (Alchichica, Atexcac, La Preciosa, and Quechulac) in close locations to those sampled in 2014 (Supplementary Table S1). Additionally, the water column was sampled at various depths from the surface to the bottom of Lake Alchichica (3–55-m depth) in both, winter and spring, and of Lake Atexcac (0.5–30-m depth) in spring. After sequential filtration and DNA extraction from the biomass corresponding to the aforementioned cell size fractions, we massively sequenced 16S and 18S rRNA gene amplicons to define fine-scale operational taxonomic units (OTUs) (Supplementary Table S2). The relative abundance of prokaryotic and eukaryotic sequences (used as a proxy for relative taxon abundance) and that of OTU diversity (number of OTUs per higher order taxon) is shown in Figs. 2 (prokaryotes) and 3 (eukaryotes). The detailed composition of the fraction "other archaea" "other bacteria," and "other eukaryotes" is given in Supplementary Figs. S2–S4. The diversity of eukaryotic and, especially, prokaryotic OTUs in terms of high-rank taxa appeared much more conserved across lakes and size fractions than relative abundance, which exhibited larger variation across samples from different lakes and seasons (Figs. 2, 3).

Prokaryotes

In the case of prokaryotes, bacteria dominated the planktonic communities, except for the extreme maar residual lake Rincón de Parangueo. The 0.2–5-μm fraction of this sampling site was slightly dominated by archaea, mostly haloarchaea (most of the Euryarchaeota fraction) and a much smaller proportion of Nanohaloarchaeota (Figs. 2, S2), in agreement with the hypersaline nature of the residual water body remaining after the crater lake almost fully evaporated (Alcocer and

Aguilar-Sierra 2019; Kienel et al. 2009). Archaea were present in minor proportions in the rest of the lakes, being below the detection threshold in two samples (ATX2014-3 m and LP2014, picoplanktonic fraction). Within the archaeal fraction, members of the Woesearchaeales (Nanoarchaeota) largely dominated most of the lakes (Supplementary Fig. S2). A single OTU of this group dominated Lake Alchichica samples in winter (Supplementary Table S3). Although often associated with anoxic environments (Liu et al. 2018), the presence of Woesearchaeales has been consistently observed in inland (hyper)saline bodies (Han et al. 2017; Menéndez-Serra et al. 2020; Pal et al. 2020) but also in very low salinity oligotrophic high altitude (Ortiz-Alvarez and Casamayor 2016) and boreal lakes (Juottonen et al. 2020). This attests to the wide ecological range of habitats colonized by members of these archaea and/or their hosts since, being members of the DPANN, they likely are obligatory symbionts (Castelle et al. 2018; Dombrowski et al. 2019).

The bacterial community in Rincón de Parangueo, in contrast with the rest of the samples and consistent with its much higher salinity, was dominated by Halanaerobiaeota (Firmicutes) (Supplementary Fig. S3; Supplementary Table S3). In the rest of the lakes, among bacteria, the most represented taxon was the Cyanobacteria (22.9% of the total sequenced reads), although the proportion of this group was highly variable depending on the lake, the cell fraction analyzed, and the season. For example, in Lake Alchichica, cyanobacteria were very little represented throughout the water column in winter (AL2012 samples). However, they increased their relative abundance in the picoplanktonic fraction of upper layers (3- and 10-m depth) during late spring (AL2014 samples), coincident with an oxygen peak at those depths (Figs. 2A, 4, S5), and most particularly, in the 5–30-μm size fraction, where they made up to 70% of the total prokaryotic sequences (Fig. 2C). A similar pattern was observed in Lake Atexcac, where cyanobacteria in the larger size fraction (5–30 μm) dominated the upper water column, although this trend was also observed in winter (up to 80% at 3-m depth). This suggests that large, filamentous cyanobacteria or cyanobacteria forming clusters bloomed in these lakes at least during spring. Indeed, upon inspection of the OTU taxonomic affiliation (Supplementary Table S3), sequences corresponding to the filamentous *Nodularia* spp. (Nostocaceae) were highly abundant in Alchichica at that time. In Atexcac, the *Nodularia* bloom was observed in winter, *Microcystis* being more represented in spring (Fig. 4). This corroborates and extends previous observations of recurrent *Nodularia* blooms in lakes Alchichica and Atexcac (Macek et al. 1994; Tavera and Komarek 1996; Oliva et al. 2009). Likewise, in other lakes where winter and late spring samples were analyzed, cyanobacteria were always much more abundant in the larger size fraction usually (but not always) in spring, also suggesting the bloom of filamentous species. The only exception was La Preciosa, where cyanobacteria were very abundant in the smaller cell-size fraction
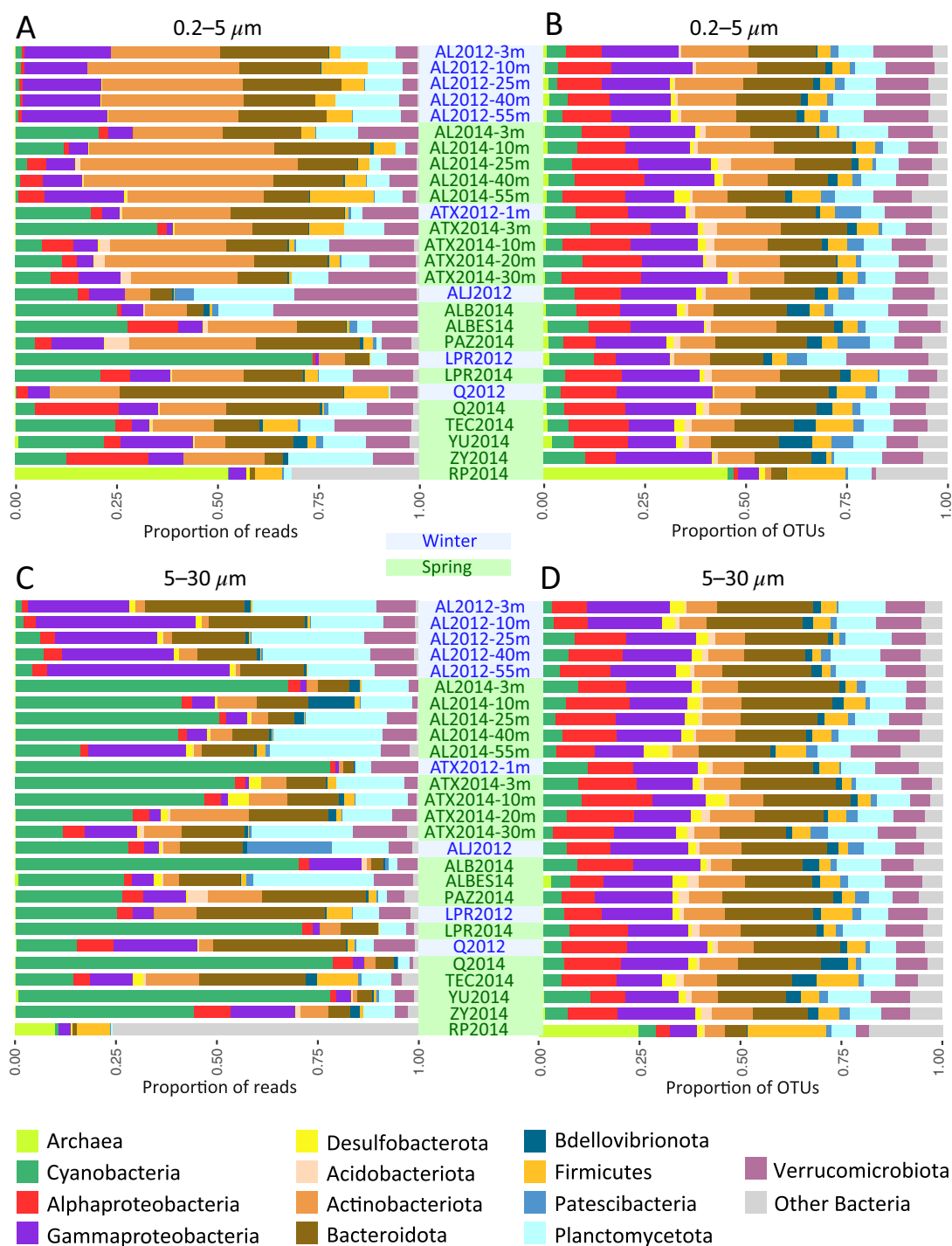
Fig. 2. Histograms showing the phylogenetic diversity and relative proportion of 16S rRNA genes amplified from planktonic samples collected from Mexican lakes. (**A**) Relative abundance of prokaryotic sequences from the 0.2–5-μm cell fraction. (**B**) Diversity shown as relative abundance of OTUs in the 0.2–5-μm fraction. (**C**) Relative abundance of prokaryotic sequences from the 5–30-μm cell fraction. (**D**) Diversity shown as relative abundance of OTUs in the 5–30-μm fraction. Detailed histograms of the categories "other archaea" and "other bacteria" are provided in, respectively, Supplementary Figs. S2 and S3. Sample descriptions are provided in Supplementary Tables S1 and S2. Lake acronyms are as in Fig. 1.

in winter (up to 70%), suggesting that, in this lake, picocyanobacteria thrived well under winter conditions (Figs. 2, 4, S5). Overall, members of the family Cyanobiaceae dominated both

planktonic fractions in many of the studied lakes. *Microcystis* blooms were also observed in Alberca de los Espinos, Pátzcuaro, Quechulac, Yuriría, and Zirahuén (Supplementary Fig. S5).
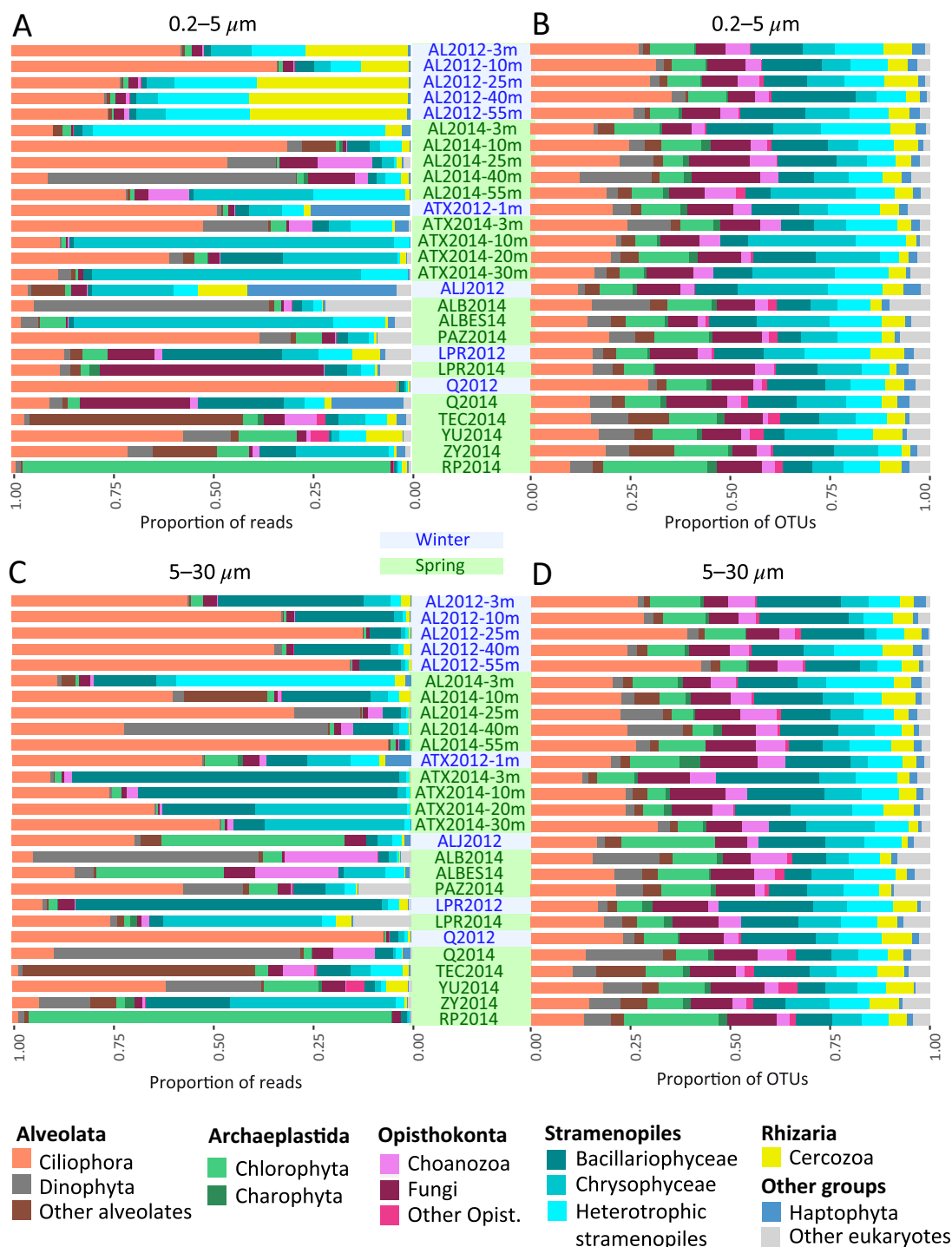
Fig. 3. Histograms showing the phylogenetic diversity and relative proportion of 18S rRNA genes amplified from planktonic samples collected from Mexican lakes. (**A**) Relative abundance of eukaryotic sequences from the 0.2–5-μm cell fraction. (**B**) Diversity shown as relative abundance of OTUs in the 0.2–5-μm fraction. (**C**) Relative abundance of eukaryotic sequences from the 5–30-μm cell fraction. (**D**) Diversity shown as relative abundance of OTUs in the 5–30-μm fraction. Detailed histograms of the category "other eukaryotes" are provided in Supplementary Fig. S4.

Bacteroidota, Planctomycetota, Actinobacteriota, Verrucomicrobiota, and in some cases, Gammaproteobacteria were also relatively abundant groups in most samples. Bacteroidota and Planctomycetota are well represented in previous surveys of freshwater lakes, including lakes such as the meromictic crater Lake Pavin or the dimictic Lake Aydat in Central France, in which they
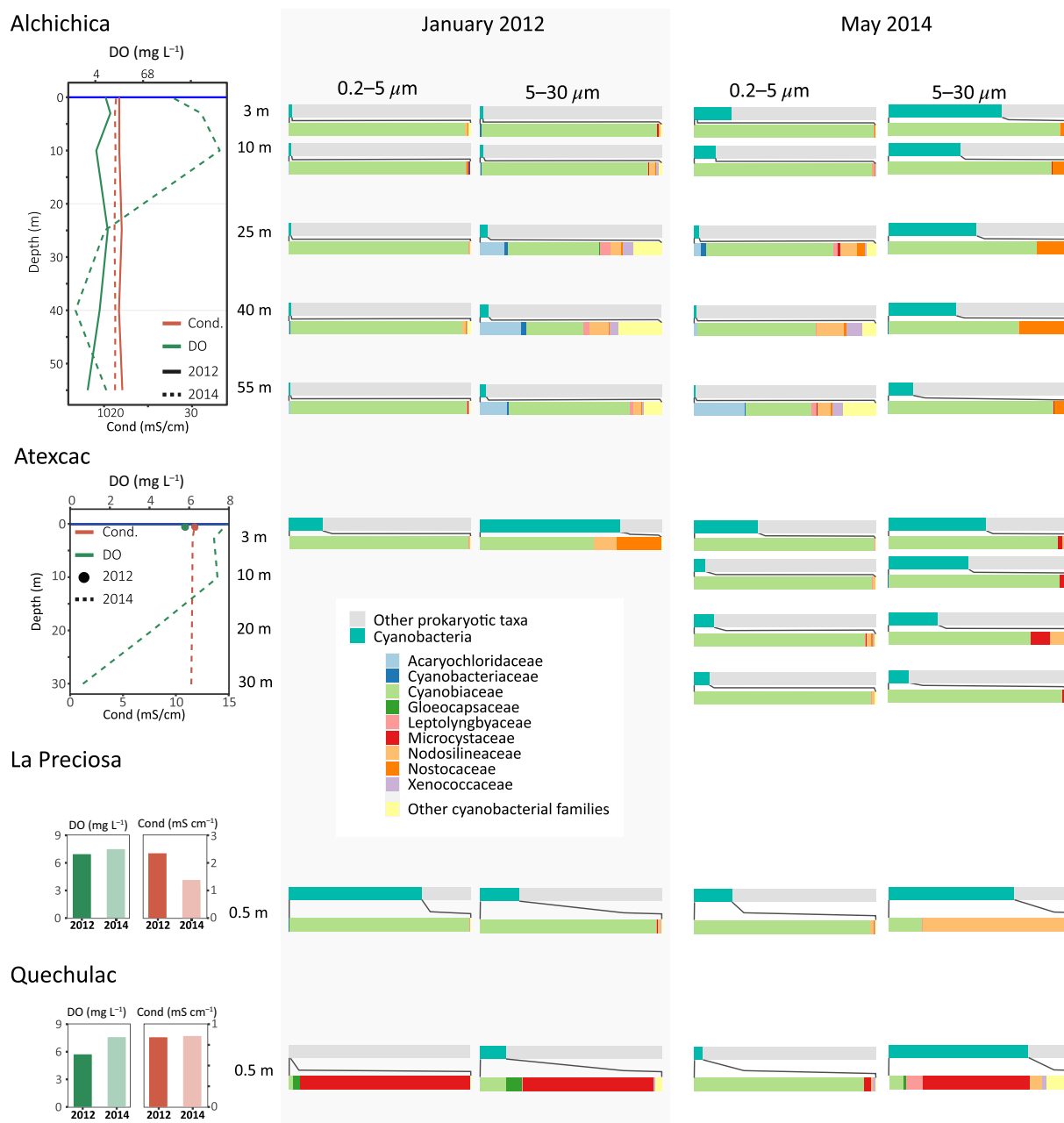
Fig. 4. Comparison of plankton cyanobacterial community structure in winter (January 2021) and late spring (May 2014) in several Trans-Mexican crater lakes. Samples were taken at different depths in the water column in lakes Alchichica and Atexcac (only late spring). Note that Lake Atexcac was already stratified in late spring 2014, while Lake Alchichica was starting the stratification period.

covered up to 95% of all sequences detected (Keshri et al. 2018) or Lake Huguangyan in the southeast of China (Hou et al. 2019). The prevalence of these taxa is also consistent with other freshwater lakes, e.g., Lake Poyang (Kong et al. 2018; Zhao et al. 2020), Lake Taihu (Zhao et al. 2017), or Lake Baikal (Kurilkina et al. 2016). Bacteroidetes were highly diverse in the TMVB lakes, with the families Balneolaceae, Flavobacteriaceae, and Saprospiraceae generally being the most represented. Members of the Balneolaceae have been

recurrently isolated from saline and alkaline environments (Xia et al. 2017). Accordingly, they are proportionally more abundant in the TMVB lakes having the highest salinity-alkalinity values (Alchichica, Atexcac, Rincón de Parangueo). Members of the Flavobacteriaceae were particularly abundant in Alchichica and Atexcac. This group of heterotrophic bacteria often blooms in lakes, usually in relation to high primary production (Eiler and Bertilsson 2007). The Planctomycetota were dominated in all lakes to the exception of Rincón del

Parangueo by members of the families Phycisphaeraceae, which are frequently associated with algae (Fukunaga et al. 2009), and Pirellulaceae. Rubinisphaeraceae were also relatively abundant in lakes Alchichica and Atexcac (Supplementary Fig. S5).

Within the classical Proteobacteria, Gammaproteobacteria were the most represented, with Alphaproteobacteria and Deltaproteobacteria (essentially Desulfobacterota) far less abundant (Fig. 2). Gammaproteobacteria showed a seasonal pattern in Lake Alchichica, where they were highly abundant in winter samples but much less so in spring (Fig. 2A,C). The diversity of families within Alpha- and Gammaproteobacteria was high (Supplementary Fig. S6). Many lineages within these two classes are deemed to be anoxygenic photosynthesizers (Imhoff et al. 2017). This is typically the case of Rhodobacteraceae, abundant in Alchichica in early winter (Supplementary Fig. S6) along with some Chromatiaceae (Supplementary Table S3) but is likely the case of diverse other proteobacterial members, suggesting their contribution to the primary production in the lake. Some of this production may be chemolithoautotrophic, since members of the Thiomicrospiraceae, typical hydrogen and sulfur-oxidizing chemolithoautotrophs (Boden et al. 2017), are relatively abundant in the large cell fraction of the winter Alchichica lake (Supplementary Fig. S6). Within the Desulfurobacterota, the Bradymonadaceae and the Geopsychrobacteraceae, occasionally the Desulfurivibrionaceae (Alchichica in the winter small cell fraction) were the most dominant taxa (Supplementary Fig. S6).

Actinobacteriota were particularly enriched in the picoplanktonic fraction. Picoplanktonic actinobacteria are frequently dominant in freshwater lakes (Garcia et al. 2014; Ghai et al. 2014; Neuenschwander et al. 2018); many of them are photoheterotrophic owing to one actinorhodopsin (Sharma et al. 2009). Many pelagic actinobacteria also bear heliorhodopsin (Flores-Uribe et al. 2019; Maresca et al. 2019) but they are not necessarily phototrophic. Heliorhodopsins seem to have a light-sensing regulatory function (Pushkarev et al. 2018; Rozenberg et al. 2021). There was a marked difference in the profile of Actinobacteria identified in Alchichica, Atexcac, and Rincón del Parangueo and the other lakes. Those lakes were characterized by a large proportion of Nitriliruptoraceae, which show a preference for saline-alkaline environments (Sorokin et al. 2009). By contrast, members of the *Candidatus* Nanopelagicaceae (Ca. Nanopelagicales), one of the most abundant groups of small-sized Actinobacteria in freshwater ecosystems (Neuenschwander et al. 2018), tended to be more abundant in the other lakes (Supplementary Fig. S7). Members of the Microbacteriaceae, frequently present in plankton and having very small genomes (Pitt et al. 2021), were also abundant in Alchichica and Atexcac (Supplementary Fig. S7). Within the Firmicutes, members of the Acholeplasmataceae were abundant in Alchichica, Atexcac, and Tecuitlapa, whereas other samples were dominated by Izemoplasmatales, Erysipelatoclostridiaceae or Paenibacillaceae

(Supplementary Fig. S7). The Verrucomicrobia also showed different diversity patterns in the different lakes and, in Alchichica, between winter and summer, with Opitutaceae (0.2–5 μm) and Rubritaleaceae (5–30 μm) being more abundant in winter and Chthoniobacteraceae in summer. Chthoniobacteraceae were also abundant in other TMVB lakes (Supplementary Fig. S7).

In the case of Lake Alchichica, where samples from the whole water column were compared in winter and summer, a pattern emerged. In general, winter samples showed a similar distribution of taxa along the water column, whereas summer samples displayed a shift of the community composition with depth (Figs. 2, S5–S7). This may reflect the period of mixing that characterizes the winter water column face to the stratified nature of the spring–summer water column in the lake (Macek et al. 1994; Alcocer 2017).

Eukaryotes

With the exception of the outlier Rincón del Parangueo, dominant eukaryotic taxa were similar in the different TMVB lakes, albeit in varying proportions. Ciliates were largely dominant in many samples, regardless of the cell size fraction (Fig. 3). They were highly diverse, with the most varied and abundant representatives belonged to the Spirotrichea and Oligohymenophorea (Supplementary Table S4). However, although ciliates often appear as dominant in many ecosystems, their relative abundance may be overestimated in difficult-to-ascertain proportions due to the large variability in ciliate rRNA gene copy number (up to several thousand copies) (Wang et al. 2017). They may prey on different organisms, such that they do not necessarily follow the picophytoplankton bloom in some of these lakes (Pestova et al. 2008). Other eukaryotic taxa of typical grazers included Cercozoa, various lineages of heterotrophic stramenopiles, and in some cases, choanoflagellates (Fig. 3; Supplementary Table S4). Colpodellids, a free-living predatory group of Apicomplexa (Simpson and Patterson 1996), were dominant in the highly eutrophic Lake Tecuitlapa (TEC).

Among phytoplanktonic eukaryotic taxa, diatoms (Bacillariophyceae), chrysophytes, and in some cases, dinoflagellates and Archaeplastida (streptophytes and mostly chlorophytes/green algae), were the most abundant taxa. Diatoms, mostly rapid-pennate and polar-centric, were extremely diverse (Supplementary Table S4). They notably occurred in the largest plankton size fractions in Lake Alchichica and, especially, in Lake Atexcac, where they could represent up to 80% of sequences (ATX2014-3m). Diatom blooms are indeed documented in this lake (Alcocer et al. 2000). By contrast, chrysophytes tended to dominate in the picoplanktonic fraction, being particularly abundant in Atexcac (Fig. 3). Among the most conspicuous dinoflagellates were the Suessiales, *Peridinium*, and *Baldinia*. Green algae were also highly diverse (Supplementary Table S4). Halophilic green algae of the genus *Dunaliella* overwhelmingly dominated the Rincón del

Parangueo sample, in agreement with the NaCl-saturation level of its residual ponds (Aranda-Gomez et al. 2013) (Fig. S1; Supplementary Table S1).

Environmental drivers of planktonic community structure

The studied TMVB lakes follow a marked salinity–alkalinity gradient, from Lake Zirahuén (salinity 0.1 psu; alkalinity 1.23) to the highly saline Rincón del Parangueo (salinity 107 psu; alkalinity 1520), a residual evaporitic system (Supplementary Table S1). Both lakes at the extremes of the salinity–alkalinity gradient lack microbialites. Microbialite-bearing lakes extended from Alberca de Michoacán (salinity 0.2 psu; alkalinity 4.3) to Lake Alchichica (salinity 8–9 psu; alkalinity 42–43) (Zeyen et al. 2021). These values are consistent with early (Alcocer and Hammer 1998; Armienta et al. 2008) and more recent (Alcocer and Bernal-Brooks 2010; Kazmierczak et al. 2011; Sigala et al. 2017) studies. Previous studies have shown that the microbialites of these lakes exhibited significantly different communities along the salinity–alkalinity gradient (Iniesto et al. 2021*b*). Although planktonic communities were significantly different from those of microbialite-associated microbial communities in NMDS analyses along axis 1 (Supplementary Fig. S8), we hypothesized that the salinity–alkalinity gradient was also a major driver of planktonic communities.

In order to compare prokaryotic and microbial eukaryotic community structure between plankton of different TMVB lakes, we used two different ordination analyses based on Bray–Curtis distances between samples: non-metric multi-dimensional scaling (NMDS) and hierarchical clustering analysis (HCA). The NMDS based on a matrix using a Wisconsin double standardization, which tends to enhance patterns among datasets, clearly separated the planktonic communities of both cell size fractions (0.2–5 μm and 5–30 μm) in lakes Alchichica and Atexcac from those of the rest of the lakes along the first axis (Fig. 5A). The effect was much more apparent for prokaryotic than for eukaryotic communities, which showed a higher dispersion (Fig. 5C). This NMDS pattern was reproduced with other types of data transformation, including simple frequencies or rarefaction prior to double standardization (Legendre and Gallagher 2001) (Supplementary Fig. S9). NMDS and HCA suggested that location (lakes) was the major determinant factor of prokaryotic community structure over cell-size fraction (Fig. 5). PERMANOVA tests confirmed that location significantly explained differences among prokaryotic communities ($p = 0.001$, $R^2 = 0.6$). Cell fraction also had a strong influence ($p = 0.001$) but, comparatively, the R^2 was much lower (0.07). In the case of eukaryotes, although the stress on the NMDS was relatively high (0.24) and samples more dispersed, statistical differences between lakes were highly significant (PERMANOVA $p = 0.001$, $R^2 = 0.44$). The similarity of prokaryotic planktonic communities observed in NMDS plots in lakes Alchichica and Atexcac was also evident in clustering analyses (Fig. 5B). As expected, eukaryotic communities did not show such a contrasted pattern (Fig. 5D).

This may relate to the fact that eukaryotes are less abundant than prokaryotes in plankton samples but likely also to the fact that they respond differently to environmental drivers. Prokaryotic communities but also eukaryotic phytoplankton are expected to be more sensitive to abiotic, physicochemical conditions whereas eukaryotic predators, rarer and more subject to stochasticity, are likely differently responsive to biotic factors (e.g., prey).

To determine the influence of the abiotic parameters, and notably the salinity–alkalinity, on planktonic community composition, we used a Mantel test in order to evaluate the correlation between two matrices (i.e., the BC distance matrix of our samples using the rarefied subsampling and the distance matrix of the environmental parameters measured for each sample). This test showed a significant correlation between the environmental parameters and both, the prokaryotic and eukaryotic community structure matrices (p values after 9999 simulations: $2e^{-04}$ and $1e^{-03}$, respectively). CCAs showed that differences in prokaryotic and eukaryotic community composition across the TMVB lakes were significantly explained by environmental factors including physicochemical variables but also trophic indicators (Fig. 6A,B; see also Supplementary Fig. S10). CCA 1 and CCA 2 collectively explained 45.1% and 24.9% of the variance for, respectively, prokaryotic and eukaryotic communities. Three major groups of samples segregated: Alchichica and Atexcac, strongly correlated with conductivity, alkalinity, major ions ($H_2SiO_4^-$, NO_3^-, Na^+, K^+, Cl^-, and SO_4^{2-}), and dissolved inorganic carbon (DIC); Tecuitlapa and Yuriría, strongly correlated with pH, dissolved oxygen; and the rest of the lakes, which showed diverse levels of anticorrelation with conductivity and alkalinity. Since the trophic status of lakes may add a layer of influence, we also included this information from a previous survey (Sigala et al. 2017) (Supplementary Fig. S10). Based mostly on chlorophyll *a* (Chl *a*) measurements, La Preciosa, Quechulac, and Zirahuén were oligotrophic, Atexcac, and Alchichica, mesotrophic, Alberca de Michoacán, eutrophic, and Tecuitlapa and Pátzcuaro, hypertrophic. CCA plots (Figs. 6A,B, S10C) showed that Tecuitlapa planktonic communities strongly correlated with Chl *a*, total phosphorous (TP), and dissolved inorganic nitrogen (DIN).

We also tried to see if particular high-rank taxa exhibited stronger correlations with specific physicochemical parameters. Although the different taxa were relatively scattered, perhaps reflecting different responses at finer taxonomic rank, some phylum- or class-level taxa clearly correlated with some variables. Among bacteria, Gammaproteobacteria and Deinococcota strongly correlated with alkalinity, sulfate, and measured cations, whereas Cyanobacteria, Myxococcota, or Bdellovibrionota correlated with dissolved oxygen and Chl *a*. Likewise, green algae strongly correlated with Chl *a*, whereas heterotrophic stramenopiles and Cercozoa strongly correlated with alkalinity, sulfate, monovalent cations, and DIC (Fig. 6C,D).
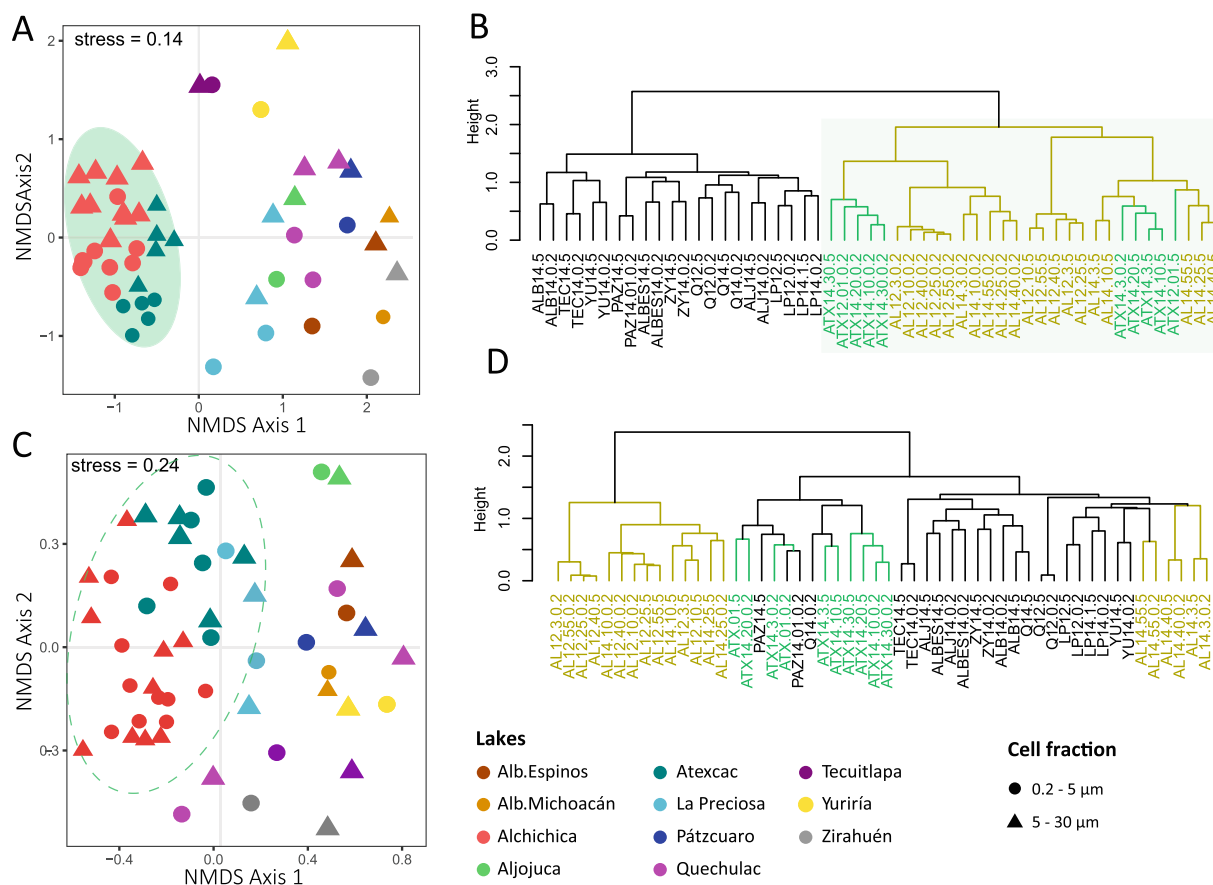
Fig. 5. Comparison of plankton samples according to their associated prokaryotic and eukaryotic community compositions. (**A**) Non-metric multi-dimensional scaling (NMDS) biplot based on Bray–Curtis distances between prokaryotic communities. (**B**) Hierarchical clustering based on 16S rRNA gene-based community composition. (**C**) NMDS biplot based on Bray–Curtis distances between eukaryotic community compositions. (**D**) Hierarchical clustering based on 18S rRNA gene-based community composition. Green-shaded areas indicate closely grouping samples from Alchichica and Atexcac. See Supplementary Fig. S9 for NMDS analyses using different data transformations (Wisconsin and normalization without rarefaction).

Collectively, our analyses establish a clear connection between planktonic community structure and the hydrogeochemical evolution of lakes along physicochemical and trophic gradients and suggest that some taxa may be more responsive than others to these environmental drivers.

Network analysis of Lake Alchichica prokaryotic plankton in contrasting seasons

In addition to abiotic parameters, biotic interactions are thought to influence community structure. Co-occurrence and/or co-exclusion of specific taxa in a given ecosystem are frequently used to hypothesize potential positive (e.g., mutualism, sequential metabolic exchange) or negative (e.g., competition) biotic interactions. Here, we aimed to unravel potential interactions between taxa in the better sampled Lake Alchichica. To do so, we focused on the most abundant prokaryotic OTUs and selected 170 OTUs being at least 1% of the microbial community in at least one sample and/or an accumulated relative abundance of 1% in all Alchichica samples. We inferred co-occurrence networks from samples collected in winter and late

spring/early summer. The late spring network was more complex, with up to 747 different edges distributed into nine different modules (more highly interconnected subgroups), while the winter network exhibited eight modules with 344 edges (Supplementary Fig. S11). However, nodes in the winter network appeared to exhibit a slightly higher degree of connections (Supplementary Fig. S12), suggesting that OTUs interact more with each other in winter. We then focused on networks of highly significant correlating OTUs (|coefficient relation (=corr)| >0.3 and $p < 0.05$). Positive correlations (co-occurrence) dominated over exclusion or other antagonistic interactions. The winter network showed 67 positive and 18 negative significant correlations between OTUs, while the spring network showed 87 positive and 15 negative significant correlations with up to 22 OTUs highly interconnected (four or more relations), slightly more than in winter (18) (Fig. 7). While the taxa the most represented (with 7 and 4 OTUs, respectively) in the spring–summer OTU network were Bacteroidota and Planctomycetota, proteobacterial OTUs were more interconnected in winter (4 Gammaproteobacteria, 3 Alphaproteobacteria,
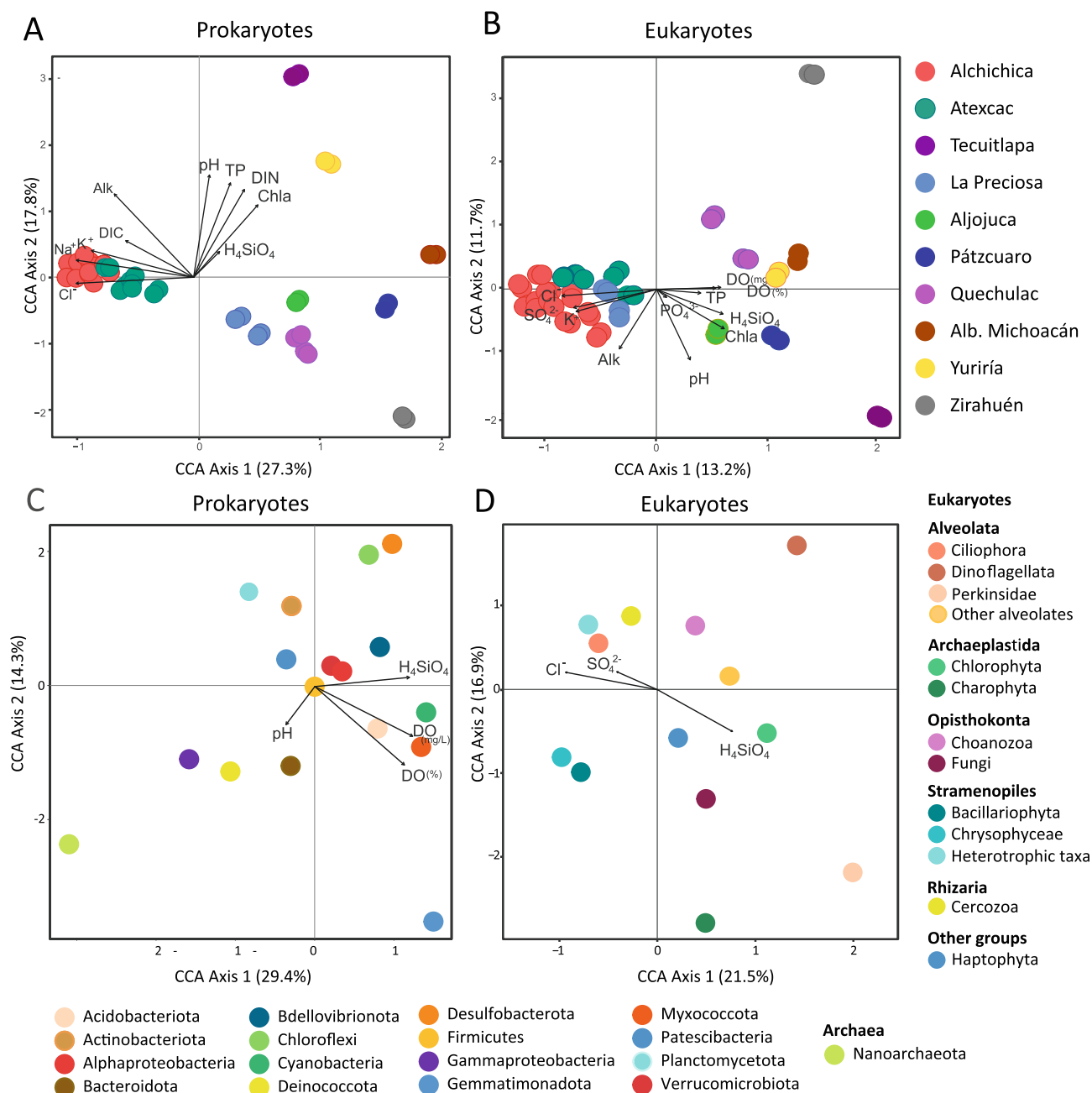
Fig. 6. Canonical-correspondence analysis (CCA) biplot of planktonic prokaryotic and microbial eukaryotic communities and taxa as a function of several environmental parameters. These include biotic and trophic-related parameters: pH, conductivity (Cond), alkalinity (measured as [HCO_3^-]), DO (% and mg/L), [Na^+], [K^+], [SiO_2], [SO_4^{2-}], [Cl^-], DIC, and nitrogen (DIN), total phosphorous (TP), and Chl *a*. Only significant factors ($p < 0.05$) were included. (**A**) CCA for prokaryotic communities. (**B**) CCA for eukaryotic communities. (**C**) CCA for prokaryotic taxa. (**D**) CCA for eukaryotic taxa. Samples from the different lakes, prokaryotic taxa, and eukaryotic groups are color-coded as indicated.

1 Desulfobacterota, and 1 Betaproteobacteria). There was no overlap between highly interconnected OTUs in the two seasons; OTUs highly interconnected in winter were substituted by a new set of highly interconnected OTUs in spring–summer. Within these 40 relevant OTUs, we identified only a few OTUs of potential autotrophic organisms according to their taxonomic affiliation (Fig. 7; Supplementary Table S6). They

included two cyanobacterial OTUs (*Nodularia*-related OTU 181361 and *Cyanobium*-related OTU 181277), one Chloroflexi (the *Roseiflexus*-related OTU 181275), two alphaproteobacteria affiliating to the Rhodobacterales (OTUs 181661, 181458) and one gammaproteobacterium affiliating to the genus *Ectothiorhodospira* (181601). In addition, OTU 181314 was 100% identical to *Thioalkalimicrobium aerophilum* (later reclassified as
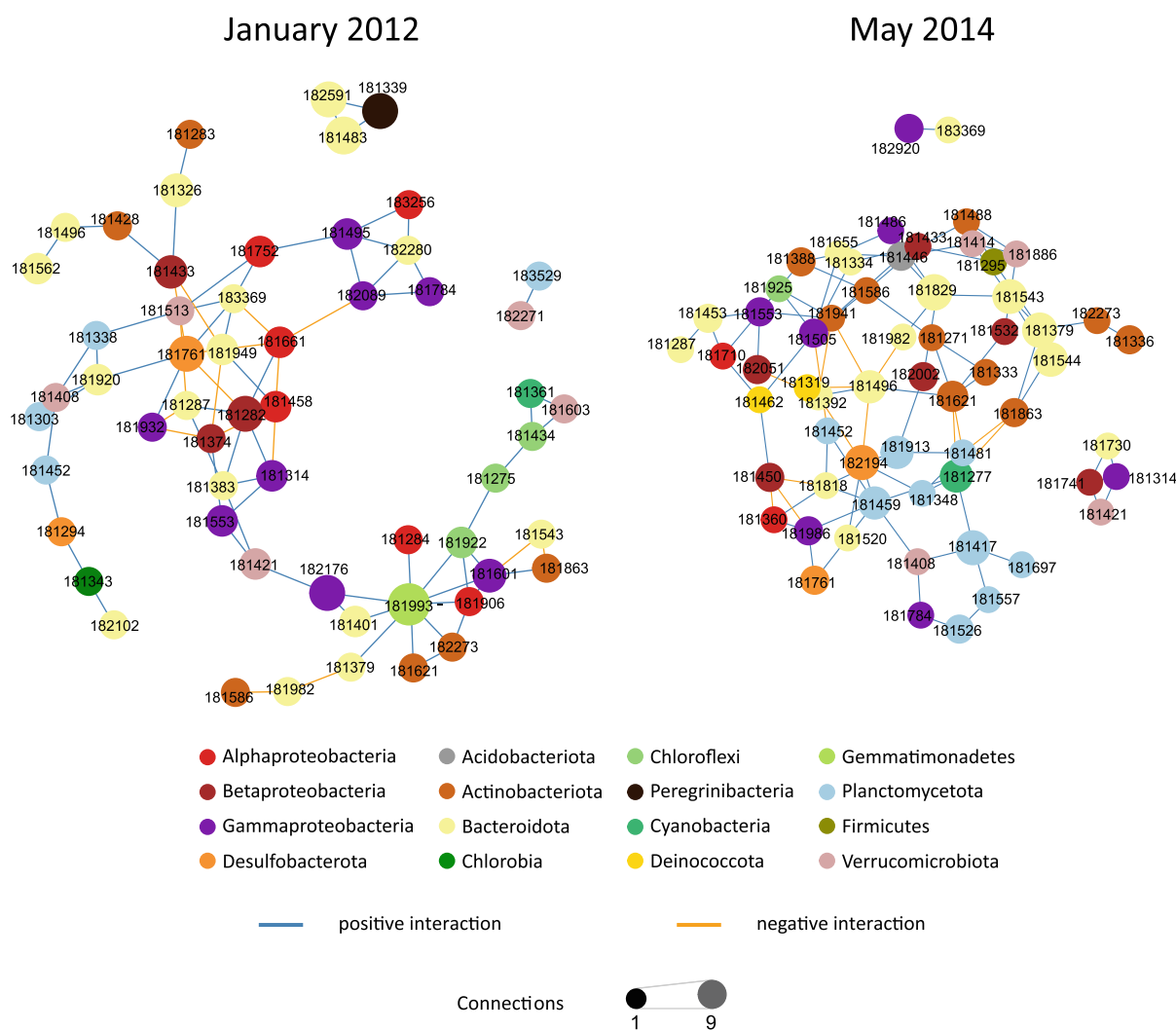
Fig. 7. SpiecEasi inverse covariance network of the most abundant OTUs in plankton from Lake Alchichica during winter and late spring. Only significant correlations (|coefficient relation (=corr)|> 0.3 and $p < 0.05$) are displayed (in blue if positive, in orange if negative). The color of the node indicates the taxonomic assignation of individual OTUs. OTU numbers are indicated (Supplementary Table S3). The distribution of the degree of connections and modularity of networks are shown in Supplementary Fig. S12.

Thiomicrospira aerophila), which is a haloalkaliphilic, litho-autotrophic sulfur-oxidizer (Loiko et al. 2002). The most inter-connected OTU (181923) was 100% identical to a member of the Gemmatimonadota BD2-11 terrestrial group (as per SILVA classification). This group as well as many others, notably photoheterotrophic, Gemmatimonadota have been identified as relevant members of temperate freshwater lakes (Mujakić et al. 2021). The rest of proteobacterial OTUs were non-photosynthetic, affiliating to the Caulobacterales, Rickettsiales, SAR11 Clade III and Sphingomonadales (Alphaproteobacteria) and Pseudomonadales, Alternomonadaceae, and Methylococcales (Gammaproteobacteria). The rest of Chloroflexi OTUs belonged to the pelagic clade (Mehrshad et al. 2018). The Chlorobi OTU belonged to the recently described Kapabacteria, which are non-phototrophic members of the group (Thiel et al. 2019).

Except for one cyanobacterial OTU identified in the spring network (Fig. 7), the rest of highly interconnected OTUs assigned to autotrophs occurred in winter. The lower presence of highly interconnected autotrophic members in late spring samples might be potentially explained by a higher diversity and variability in this season. Longer temporal series would be required to confirm whether this pattern is recurrent and whether it might reveal direct synergistic or antagonistic interactions among the identified inter-connected taxa.

Concluding remarks

Lakes are very sensitive to environmental parameter change and/or anthropogenic disturbances. In many areas of the planet, overexploitation of hydric resources and climate

change-induced desertification result in the progressive evaporation and salinization of inland water bodies. In an attempt to understand and predict how microbial communities may evolve with time under increased salinization, we applied a space-for-time substitution approach and characterized the prokaryotic and microbial eukaryotic planktonic communities in lakes of the Trans-Mexican Volcanic Belt following a salinity–alkalinity gradient. We show that significant changes in prokaryotic and microbial eukaryotic communities occur along that gradient, from the low salinity freshwater lake Zirahuén to the brines of the almost fully evaporated Rincón de Parangueo. We also show that, from the measured physicochemical parameters, and despite within-lake depth and seasonal variation, salinity, alkalinity, and related parameters are strong determinants of microbial community structure. Our study establishes a close connection between plankton microbial composition and the hydrogeochemical evolution of lakes, suggesting that they may serve as indicators of lake hydrological balance and status along evaporation processes under global climate change.

Data availability statement

Sequences have been deposited in GenBank under the BioProject number PRJNA625182, BioSample numbers SAMN29249281 to SAMN29249324 (see also Supplementary Table S2).

References

Alcocer, J. 2017. Mexican meromictic lakes: What we know so far, p. 353–375. *In* R. D. Gulati, E. S. Zadereev, and A. G. Degermendzhi [eds.], Ecology of meromictic lakes. Springer International Publishing. doi:10.1007/978-3-319-49,143-1_12

Alcocer, J., and V. Aguilar-Sierra. 2019. Biodiversity in inland waters, p. 43–75. *In* A. L. Ibáñez [ed.], Mexican aquatic environments: A general view from hydrobiology to fisheries. Springer International Publishing. doi:10.1007/s10750-010-0211-1

Alcocer, J., and F. W. Bernal-Brooks. 2010. Limnology in Mexico. Hydrobiologia **644**: 1–54. doi:10.1007/s10750-010-0211-1

Alcocer, J., and U. T. Hammer. 1998. Saline lake ecosystems of Mexico. Aquat. Ecosyst. Health Manage. **1**: 291–315. doi:10.1080/14634989808656925

Alcocer, J., E. Escobar, and A. Lugo. 2000. Water use (and abuse) and its effects on the crater-lakes of Valle de Santiago, Mexico. Lakes & Reservoirs: Sci, Pol Manage. Sustain. Use **5**: 145–149. doi:10.1046/j.1440-1770.2000.00113.x

Aranda-Gomez, J. J., and others. 2013. Active sinking at the bottom of the Rincón de Parangueo maar (Guanajuato, México) and its probable relation with subsidence faults at Salamanca and Celaya. Bol. Soc. Geol. Mex **65**: 169–188.

Armienta, M. A., and others. 2008. Water chemistry of lakes related to active and inactive Mexican volcanoes. J. Volcanol. Geotherm. Res. **178**: 249–258. doi:10.1016/j.jvolgeores.2008.06.019

Blois, J. L., J. W. Williams, M. C. Fitzpatrick, S. T. Jackson, and S. Ferrier. 2013. Space can substitute for time in predicting climate-change effects on biodiversity. Proc. Natl. Acad. Sci. USA **110**: 9374–9379. doi:10.1073/pnas.1220228110

Boden, R., and others. 2017. An evaluation of *Thiomicrospira*, *Hydrogenovibrio* and *Thioalkalimicrobium*: Reclassification of four species of *Thiomicrospira* to each *Thiomicrorhabdus* gen. nov. and *Hydrogenovibrio*, and reclassification of all four species of *Thioalkalimicrobium* to *Thiomicrospira*. Int. J. Syst. Evol. Microbiol. **67**: 1140–1151. doi:10.1099/ijsem.0.001855

Bower, S. M., R. B. Carnegie, B. Goh, S. R. M. Jones, G. J. Lowe, and M. W. S. Mak. 2004. Preferential PCR amplification of parasitic protistan small subunit rDNA from metazoan tissues. J Euk Microbiol **51**: 325–332. doi:10.1111/j.1550-7408.2004.tb00574.x

Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. **27**: 325–349. doi:10.2307/1942268

Caballero, M., and others. 2006. Present limnological conditions and recent (ca. 340 yr) palaeolimnology of a tropical Lake in the Sierra de Los Tuxtlas, Eastern Mexico. J. Paleolimnol. **35**: 83–97. doi:10.1007/s10933-005-7427-5

Castelle, C. J., C. T. Brown, K. Anantharaman, A. J. Probst, R. H. Huang, and J. F. Banfield. 2018. Biosynthetic capacity, metabolic variety and unusual biology in the CPR and DPANN radiations. Nat. Rev. Microbiol. **16**: 629–645. doi:10.1038/s41579-018-0076-2

Cavicchioli, R., and others. 2019. Scientists' warning to humanity: Microorganisms and climate change. Nat. Rev. Microbiol. **17**: 569–586. doi:10.1038/s41579-019-0222-5

Couradeau, E., and others. 2011. Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline Lake Alchichica (Mexico). PLoS One **6**: e28767. doi:10.1371/journal.pone.0028767

Cunillera-Montcusí, D., and others. 2022. Freshwater salinisation: A research agenda for a saltier world. Trends Ecol. Evol. **37**: 440–453. doi:10.1016/j.tree.2021.12.005

Dombrowski, N., J. H. Lee, T. A. Williams, P. Offre, and A. Spang. 2019. Genomic diversity, lifestyles and evolutionary origins of DPANN archaea. FEMS Microbiol. Lett **366**: fnz008. doi:10.1093/femsle/fnz008

Dray, S., and A. B. Dufour. 2007. The ade4 package: Implementing the duality diagram for ecologists. J. Statist. Softw. **22**: 1–20. doi:10.18637/jss.v022.i04

Eiler, A., and S. Bertilsson. 2007. Flavobacteria blooms in four eutrophic lakes: Linking population dynamics of freshwater bacterioplankton to resource availability. Appl. Environ. Microbiol. **73**: 3511–3518. doi:10.1128/aem.02534-06

Faugère-Kalfon, B. 1996. Entre Zacapu y río Lerma: Culturas en una zona fronteriza. Centro de Estudios Mexicanos y Centroamericanos, p. 157. doi:10.4000/books.cemca.3320

Ferrari, L., T. Orozco-Esquivel, V. Manea, and M. Manea. 2012. The dynamic history of the trans-Mexican Volcanic Belt and the Mexico subduction zone. Tectonophysics **522–523**: 122–149. doi:10.1016/j.tecto.2011.09.018

Flores-Uribe, J., and others. 2019. Heliorhodopsins are absent in diderm (gram-negative) bacteria: Some thoughts and possible implications for activity. Environ. Microbiol. Rep. **11**: 419–424. doi:10.1111/1758-2229.12730

Friedman, J., and E. J. Alm. 2012. Inferring correlation networks from genomic survey data. PLoS Comput. Biol. **8**: e1002687. doi:10.1371/journal.pcbi.1002687

Fukunaga, Y., M. Kurahashi, Y. Sakiyama, M. Ohuchi, A. Yokota, and S. Harayama. 2009. *Phycisphaera mikurensis* gen. nov., sp. nov., isolated from a marine alga, and proposal of Phycisphaeraceae fam. nov., Phycisphaerales ord. nov. and Phycisphaerae classis nov. in the phylum Planctomycetes. J. Gen. Appl. Microbiol. **55**: 267–275. doi:10.2323/jgam.55.267

Garcia, S. L., K. D. McMahon, H. P. Grossart, and F. Warnecke. 2014. Successful enrichment of the ubiquitous freshwater acI Actinobacteria. Environ. Microbiol. Rep. **6**: 21–27. doi:10.1111/1758-2229.12104

Ghai, R., C. M. Mizuno, A. Picazo, A. Camacho, and F. Rodriguez-Valera. 2014. Key roles for freshwater Actinobacteria revealed by deep metagenomic sequencing. Mol. Ecol. **23**: 6073–6090. doi:10.1111/mec.12985

Han, R., and others. 2017. Microbial community structure and diversity within hypersaline Keke salt Lake environments. Can. J. Microbiol. **63**: 895–908. doi:10.1139/cjm-2016-0773

Hou, Q., Z. Fang, Q. Zhu, and H. Dong. 2019. Microbial diversity in Huguangyan maar Lake of China revealed by high–throughput sequencing. Journal of Oceanology and Limnology **37**: 1245–1257. doi:10.1007/s00343-019-8016-1

Huisman, J., G. A. Codd, H. W. Paerl, B. W. Ibelings, J. M. H. Verspagen, and P. M. Visser. 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. **16**: 471–483. doi:10.1038/s41579-018-0040-1

Imhoff, J. F., T. Rahn, S. Künzel, and S. C. Neulinger. 2017. Photosynthesis is widely distributed among Proteobacteria as demonstrated by the phylogeny of PufLM reaction center proteins. Front. Microbiol. **8**: 2679. doi:10.3389/fmicb.2017.02679

Iniesto, M., and others. 2021*a*. Rapid formation of mature microbialites in Lake Alchichica, Mexico. Environ. Microbiol. Rep. **13**: 600–605. doi:10.1111/1758-2229.12957

Iniesto, M., and others. 2021*b*. Core microbial communities of lacustrine microbialites sampled along an alkalinity gradient. Environ. Microbiol. **23**: 51–68. doi:10.1111/1462-2920.15252

Juottonen, H., L. Fontaine, C. Wurzbacher, S. Drakare, S. Peura, and A. Eiler. 2020. Archaea in boreal Swedish lakes are diverse, dominated by Woesearchaeota and follow deterministic community assembly. Environ. Microbiol. **22**: 3158–3171. doi:10.1111/1462-2920.15058

Kazmierczak, J., S. Kempe, B. Kremer, P. López-García, D. Moreira, and R. Tavera. 2011. Hydrochemistry and microbialites of the alkaline crater lake Alchichica, Mexico. Facies **57**: 543–570. doi:10.1007/s10347-010-0255-8

Keshri, J., A. S. Pradeep Ram, P. A. Nana, and T. Sime-Ngando. 2018. Taxonomical resolution and distribution of Bacterioplankton along the vertical gradient reveals pronounced spatiotemporal patterns in contrasted temperate Freshwater Lakes. Microb. Ecol. **76**: 372–386. doi:10.1007/s00248-018-1143-y

Kienel, U., and others. 2009. First lacustrine varve chronologies from Mexico: Impact of droughts, ENSO and human activity since AD 1840 as recorded in maar sediments from Valle de Santiago. J. Paleolimnol. **42**: 587–609. doi:10.1007/s10933-009-9307-x

Komárková, J., and R. Tavera. 2003. Steady state of phytoplankton assemblage in the tropical Lake Catemaco (Mexico), p. 187–196. *In* L. Naselli-Flores, J. Padisák, and M. T. Dokulil [eds.], Phytoplankton and equilibrium concept: The ecology of steady-state assemblages, v. **502**. Springer. doi:10.1023/B:HYDR.0000004281.11917.7a

Kong, Z., W. Kou, Y. Ma, H. Yu, G. Ge, and L. Wu. 2018. Seasonal dynamics of the bacterioplankton community in a large, shallow, highly dynamic freshwater lake. Can. J. Microbiol. **64**: 786–797. doi:10.1139/cjm-2018-0126

Kurilkina, M. I., and others. 2016. Bacterial community composition in the water column of the deepest freshwater Lake Baikal as determined by next-generation sequencing. FEMS Microbiol. Ecol. **92**: fiw094. doi:10.1093/femsec/fiw094

Kurtz, Z. D., C. L. Müller, E. R. Miraldi, D. R. Littman, M. J. Blaser, and R. A. Bonneau. 2015. Sparse and compositionally robust inference of microbial ecological networks. PLoS Comput. Biol. **11**: e1004226. doi:10.1371/journal.pcbi.1004226

Legendre, P., and E. D. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia **129**: 271–280. doi:10.1007/s004420100716

Liu, X., and others. 2018. Insights into the ecology, evolution, and metabolism of the widespread Woesearchaeotal lineages. Microbiome **6**: 102. doi:10.1186/s40168-018-0488-2

Loiko, N. G., A. N. Kozlova, G. A. Osipov, and G. I. El'-Registan. 2002. Low-molecular-weight autoregulatory factors in the haloalkaliphilic bacteria *Thioalkalivibrio versutus* and *Thioalkalimicrobium aerophilum*. Microbiology **71**: 262–268. doi:10.1023/A:1015890224756

Macek, M., G. Vilaclara, and A. Lugo. 1994. Changes in protozoan assemblages structure and activity in a stratified tropical lake. Marine Microbial Food Webs **8**: 235–249.

Macek, M., J. Alcocer, A. Lugo Vazquez, M. Elena Martinez-Perez, L. Peralta Soriano, and G. Vilaclara Fatjo. 2009. Long term picoplankton dynamics in a warm-monomictic,

tropical high altitude lake. J. Limnol. **68**: 183–192. doi:10.3274/jl09-68-2-03

Magoc, T., and S. L. Salzberg. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics **27**: 2957–2963. doi:10.1093/bioinformatics/btr507

Mahe, F., T. Rognes, C. Quince, C. de Vargas, and M. Dunthorn. 2015. Swarm v2: Highly-scalable and high-resolution amplicon clustering. PeerJ **3**: e1420. doi:10.7717/peerj.1420

Maresca, J. A., and others. 2019. Light modulates the physiology of nonphototrophic Actinobacteria. J. Bacteriol. **201**: e00740-18. doi:10.1128/JB.00740-18

Markovic, D., S. F. Carrizo, O. Kärcher, A. Walz, and J. N. W. David. 2017. Vulnerability of European freshwater catchments to climate change. Glob. Chang. Biol. **23**: 3567–3580. doi:10.1111/gcb.13657

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal **17**: 10–12. doi:10.14806/ej.17.1.200

Mehrshad, M., and others. 2018. Hidden in plain sight-highly abundant and diverse planktonic freshwater Chloroflexi. Microbiome **6**: 176. doi:10.1186/s40168-018-0563-8

Menéndez-Serra, M., V. J. Ontiveros, X. Triadó-Margarit, D. Alonso, and E. O. Casamayor. 2020. Dynamics and ecological distributions of the archaea microbiome from inland saline lakes (Monegros Desert, Spain). FEMS Microbiol. Ecol. **96**: fiaa019. doi:10.1093/femsec/fiaa019

Moore, M. V., S. E. Hampton, L. R. Izmest'eva, E. A. Silow, E. V. Peshkova, and B. K. Pavlov. 2009. Climate change and the world's "Sacred Sea"—Lake Baikal, Siberia. Bioscience **59**: 405–417. doi:10.1525/bio.2009.59.5.8

Mujakić, I., and others. 2021. Common presence of phototrophic Gemmatimonadota in temperate freshwater lakes. mSystems **6**: e01241-20. doi:10.1128/mSystems.01241-20

Neuenschwander, S. M., R. Ghai, J. Pernthaler, and M. M. Salcher. 2018. Microdiversification in genome-streamlined ubiquitous freshwater Actinobacteria. ISME J. **12**: 185–198. doi:10.1038/ismej.2017.156

Oksanen, J. and others 2011. Vegan: Community Ecology Package. R package version 1.17-9. http://CRAN.R-project.org/package=vegan.

Oliva, M. G., A. Lugo, J. Alcocer, L. Peralata, and L. A. Oseguera. 2009. Planktonic bloom-forming *Nodularia* in the saline Lake Alchichica, Mexico. Nat. Resour. Environ. Issues **15**: 22 https://digitalcommons.usu.edu/nrei/vol15/iss1/22

Ondrasek, G., and Z. Rengel. 2021. Environmental salinization processes: Detection, implications & solutions. Sci. Total Environ. **754**: 142432. doi:10.1016/j.scitotenv.2020.142432

Ortiz-Alvarez, R., and E. O. Casamayor. 2016. High occurrence of Pacearchaeota and Woesearchaeota (Archaea superphylum DPANN) in the surface waters of oligotrophic high-altitude lakes. Environ. Microbiol. Rep. **8**: 210–217. doi:10.1111/1758-2229.12370

Pal, S., and others. 2020. Poorly known microbial taxa dominate the microbiome of hypersaline Sambhar Lake salterns in India. Extremophiles **24**: 875–885. doi:10.1007/s00792-020-01201-0

Pestova, D., M. Macek, and M. E. Martinez Perez. 2008. Ciliates and their picophytoplankton-feeding activity in a high-altitude warm-monomictic saline lake. Eur. J. Protistol. **44**: 13–25. doi:10.1016/j.ejop.2007.04.004

Pitt, A., J. Schmidt, U. Koll, and M. W. Hahn. 2021. *Aquiluna borgnonia* gen. nov., sp. nov., a member of a Microbacteriaceae lineage of freshwater bacteria with small genome sizes. Int. J. Syst. Evol. Microbiol **71**: 004825. doi:10.1099/ijsem.0.004825

Pushkarev, A., and others. 2018. A distinct abundant group of microbial rhodopsins discovered using functional metagenomics. Nature **558**: 595–599. doi:10.1038/s41586-018-0225-9

Quast, C., and others. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. **41**: D590–D596. doi:10.1093/nar/gks1219

R Development Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing. doi:10.4236/oalib.1107821

Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahe. 2016. VSEARCH: A versatile open source tool for metagenomics. PeerJ **4**: e2584. doi:10.7717/peerj.2584

Rozenberg, A., K. Inoue, H. Kandori, and O. Béjà. 2021. Microbial rhodopsins: The last two decades. Annu. Rev. Microbiol. **75**: 427–447. doi:10.1146/annurev-micro-031721-020452

Saghaï, A., and others. 2015. Metagenome-based diversity analyses suggest a significant contribution of non-cyanobacterial lineages to carbonate precipitation in modern microbialites. Front. Microbiol. **6**: 797. doi:10.3389/fmicb.2015.00797

Saghaï, A., and others. 2016. Comparative metagenomics unveils functions and genome features of microbialite-associated communities along a depth gradient. Environ. Microbiol. **18**: 4990–5004. doi:10.1111/1462-2920.13456

Sánchez Medina, X., M. Macek, F. Bautista-Reyes, A. Perz, P. Bonilla Lemus, and M. Chávez Arteaga. 2016. Inter-annual ciliate distribution variation within the late stratification oxycline in a monomictic lake, Lake Alchichica (Mexico). J. Limnol. **75**: 179–190. doi:10.4081/jlimnol.2016.1440

Schmeller, D. S., and others. 2018. People, pollution and pathogens - global change impacts in mountain freshwater ecosystems. Sci. Total Environ. **622–623**: 756–763. doi:10.1016/j.scitotenv.2017.12.006

Sharma, A. K., and others. 2009. Actinorhodopsin genes discovered in diverse freshwater habitats and among cultivated freshwater Actinobacteria. ISME J. **3**: 726–737. doi:10.1038/ismej.2009.13

Shimaraev, M. N., and V. M. Domysheva. 2012. Trends in hydrological and hydrochemical processes in Lake Baikal under conditions of modern climate change, v. **7**. John Wiley & Sons, Ltd, p. 43–66. doi:10.1080/20442041.2017. 1329982

Sigala, I., and others. 2017. Basic limnology of 30 continental waterbodies of the Transmexican Volcanic Belt across climatic and environmental gradients. Boletín de la Sociedad Geológica Mexicana **69**: 313–370 http://www.jstor.org/ stable/2646190

Simpson, A. G. B., and D. J. Patterson. 1996. Ultrastructure and identification of the predatory flagellate *Colpodella pugnax* Cienkowski (Apicomplexa) with a description of *Colpodella turpis* n. sp. and a review of the genus. System. Parasitol. **33**: 187–198. doi:10.1007/BF01531200

Sorokin, D. Y., S. van Pelt, T. P. Tourova, and L. I. Evtushenko. 2009. *Nitriliruptor alkaliphilus* gen. nov., sp. nov., a deep-lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of Nitriliruptoraceae fam. nov. and Nitriliruptorales ord. nov. Int. J. Syst. Evol. Microbiol. **59**: 248–253. doi:10.1099/ijs.0.002204-0

Tavera, R., and J. Komarek. 1996. Cyanoprokaryotes in the volcanic lake of Alchichica, Puebla state, Mexico. Algol. Stud. **83**: 511–538. doi:10.1127/algol_stud/83/1996/511

Thiel, V., and others. 2019. "Candidatus Thermonerobacter thiotrophicus," a non-phototrophic member of the Bacteroidetes/Chlorobi with dissimilatory sulfur metabolism in hot spring mat communities. Front. Microbiol **9**: 3159.

Valdespino-Castillo, P. M., and others. 2018. Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba. Front. Microbiol. **9**: 510. doi:10.3389/fmicb.2018.00510

Wang, C., T. Zhang, Y. Wang, L. A. Katz, F. Gao, and W. Song. 2017. Disentangling sources of variation in SSU rDNA sequences from single cell analyses of ciliates: Impact of copy number variation and experimental error. Proc Roy Soc B **284**: 20170425. doi:10.1098/rspb.2017.0425

Woodward, G., D. M. Perkins, and L. E. Brown. 2010. Climate change and freshwater ecosystems: Impacts across multiple levels of organization. Philos. Trans. R. Soc. Lond. B Biol. Sci. **365**: 2093–2106. doi:10.1098/rstb.2010.0055

Xia, J., Z. H. Xie, C. A. Dunlap, A. P. Rooney, and Z. J. Du. 2017. *Rhodohalobacter halophilus* gen. nov., sp. nov., a moderately halophilic member of the family Balneolaceae. Int. J. Syst. Evol. Microbiol. **67**: 1281–1287. doi:10.1099/ijsem.0.001806

Zeyen, N., and others. 2021. Integrative analysis of the mineralogical and chemical composition of modern microbialites from ten Mexican lakes: What do we learn about their formation? Geochim. Cosmochim. Acta **305**: 148–184. doi:10. 1016/j.gca.2021.04.030

Zeyen, N., D. Daval, P. Lopez-Garcia, D. Moreira, J. Gaillardet, and K. Benzerara. 2017. Geochemical conditions allowing the formation of modern lacustrine microbialites. Procedia Earth and Planetary Science **17**: 380–383. doi:10.1016/j. proeps.2016.12.096

Zhao, D., and others. 2017. The heterogeneity of composition and assembly processes of the microbial community between different nutrient loading Lake zones in Taihu Lake. Appl. Microbiol. Biotechnol. **101**: 5913–5923. doi:10. 1007/s00253-017-8327-0

Zhao, M., Y.-T. Ma, S.-Y. He, X. Mou, and L. Wu. 2020. Dynamics of bacterioplankton community structure in response to seasonal hydrological disturbances in Poyang Lake, the largest wetland in China. FEMS Microbiol. Ecol **96**: fiaa064. doi:10.1093/femsec/fiaa064

Conflict of Interest

The authors declare that they have no conflict of interest.