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Spatiotemporal variations in microbial diversity across the three domains of life in a tropical thalassohaline lake (Dziani Dzaha, Mayotte Island)

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Abstract :

Thalassohaline ecosystems are hypersaline environments originating from seawater in which sodium chloride is the most abundant salt and the pH is alkaline. Studies focusing on microbial diversity in thalassohaline lakes are still scarce compared with those on athalassohaline lakes such as soda lakes that have no marine origin. In this work, we investigated multiple facets of bacterial, archaeal and eukaryotic diversity in the thalassohaline Lake Dziani Dzaha using a metabarcoding approach. We showed that bacterial and archaeal diversity were mainly affected by contrasting physicochemical conditions retrieved at different depths. While photosynthetic microorganisms were dominant in surface layers, chemotrophic phyla (Firmicutes or Bacteroidetes) and archaeal methanogens dominated deeper layers. In contrast, eukaryotic diversity was constant regardless of depth and was affected by seasonality. A detailed focus on eukaryotic communities showed that this constant diversity profile was the consequence of the high predominance of Picocystis salinarum, while nondominant eukaryotic groups displayed seasonal diversity turnover. Altogether, our results provided an extensive description of the diversity of the three domains of life in an unexplored extreme environment and showed clear differences in the responses of prokaryotic and eukaryotic communities to environmental conditions.

Keywords : Extreme environment, Thalassohaline lake, Metabarcoding, Eukaryotes, Archaea, Bacteria

Introduction

Hypersaline environments are widely distributed on Earth, representing a variety of aquatic ecosystems that include lakes, deep-sea basins, lagoons and solar salterns, in which salt concentrations are higher than that of seawater (McGenity & Oren, 2012; Ventosa, 2006; Ventosa et al., 2008). These ecosystems are often also characterized by other extreme environmental conditions, such as low oxygen concentrations, high or low temperatures and, sometimes, high alkalinity (Ventosa, 2006). Hypersaline ecosystems are classified into two different types according to the origin of their water. In thalassohaline ecosystems, the water has a marine origin, and its chemical composition reflects that of seawater, with sodium chloride as the major salt and circumneutral to alkaline pH values. In athalassohaline ecosystems such as soda lakes, the origin of the water is not marine, and its chemical composition results from the dissolution of mineral salt deposits or from concentration due to evaporation of dissolved elements originating from rock weathering. The water composition of athalassohaline ecosystems can thus be dominated by potassium, magnesium, sodium and carbonate ions depending on the surrounding geology, topography and climatic conditions (Youssef et al., 2012).

Many investigations have been conducted on athalassohaline systems, and soda lakes in particular, especially in tropical areas where elevated temperature and high incident solar radiation place soda lakes among the most productive aquatic ecosystems on Earth (Grant, 2006; Grant & Jones, 2016; Jones et al., 1998; Sorokin et al., 2014). These prodigious rates of photosynthetic primary production are notably associated with Cyanobacteria from the genera *Arthrospira*, *Cyanospira*, *Geitlerinema*, *Leptolyngbya* or *Anabaenopsis* (Grant & Jones, 2016; Krienitz & Schagerl, 2016); unicellular green algae such as *Picocystis salinarum* or *Dunaliella viridis* (Krienitz & Schagerl, 2016); and/or anoxygenic phototrophic bacteria (Ballot et al., 2004; Lewis, Wurtsbaugh, & Paerl, 2011). The rates of primary productivity in such systems can reach up to 10 gC cm^{-2} day^{-1}, delivering fixed Carbon to haloalkaliphilic communities, such as aerobic and anaerobic chemoorganotrophic

bacteria and archaea (Grant et al., 1990; Leboulanger et al., 2017; Melack & Kilham, 1974). The dominant prokaryotes retrieved from soda lakes have been affiliated with Alphaproteobacteria, Gammaproteobacteria, Firmicutes and Bacteroidetes (Aguirre-Garrido et al., 2016; Dimitriu et al., 2008; Sorokin et al., 2014), coexisting with abundant Euryarchaeota (Mesbah et al., 2007; Ochsenreiter et al., 2002; Vavourakis et al., 2016).

In contrast, continental thalassohaline lakes that are permanently landlocked with hardly any exchange with the sea have been the focus of a limited number of investigations (Makhdoumi-Kakhki et al., 2012; Naghoni et al., 2017). Lake Dziani Dzaha (Mayotte) has been recently classified as a thalassohaline lake and presents several atypical features that make it an excellent model to examine the genetic structure and dynamics of microbial communities across environmental niches (Leboulanger et al., 2017, Gérard et al., 2018).

First, the Lake Dziani Dzaha water column is mainly inhabited by microorganisms, as aquatic metazoans are rare or absent, making it essentially a microbial ecosystem. Furthermore, this crater lake is characterized by a permanent anoxic zone below ~1.5 m depth and has a deep chemocline at approximately 14 m, with a narrow depression reaching 18 m deep, below which H_2S/HS^-, NH_4^+/NH_3 and CH_4 concentrations reach values as high as 5, 4 and 1 mM, respectively. Moreover, the alternation in Mayotte of a rainy (November-April) and a dry period (June-October) modifies the stratification pattern of the water column. During the rainy period, the upper layer is diluted, creating a seasonal chemocline at a depth of approximately 2 m, below which a saline, anoxic and sulfidic zone can be observed. During the dry period, this chemocline disappears, and the density of the surface layer increases through water evaporation, leading to an unstratified state of the water column. During this unstratified period, the sulfidic zone is restricted to below the deep chemocline (*i.e.*, below 14 m), but the water column remains anoxic below a depth of 1.5 m despite mixing.

The aim of this study was to determine the influence of the physicochemical conditions in the Lake Dziani Dzaha water column on microbial diversity and community assembly. Previous studies reported that salinity is one of the key environmental factors shaping the composition and structure of aquatic microbial communities (Canfora et al., 2014; Lozupone & Knight, 2007). Here, we hypothesized that the distinct environmental niches provided by the spatial and temporal shifts in the physicochemical characteristics of Lake Dziani Dzaha shape its microbial diversity. To verify this hypothesis, we used a multiseasonal sampling strategy conducted across two consecutive years (2014 and 2015) at different depths of the water column (from 0 to 17 m), for which we characterized both prokaryotic (including Archaea and Bacteria) and eukaryotic diversity using a metabarcoding approach. This study provided the first description of the spatiotemporal dynamics of the complete microbial assemblage in a tropical thalassohaline lake.

Materials and Methods

Study site, sampling, and environmental parameters

Lake Dziani Dzaha is a volcanic crater lake situated on the Petite Terre Island of Mayotte situated in the Comoros archipelago (Western Indian Ocean). Its surface is 0.24 km^2 with a mean depth of ~3.5 m except for the eastern part of the lake, which is characterized by a narrow pit reaching a maximum depth of 18 m. The elevation of the lake surface is close to the average sea level. This lake

is characterized by notable salinity (ranging from 34 to 71 psu), slight alkalinity (0.23 mol/L) and a permanent green color due to high primary production (Leboulanger et al., 2017). Four sampling campaigns were conducted to cover both annual and seasonal variations in environmental conditions. Two contrasting periods, the stratified period (April, end of the rainy season) and the unstratified period (October-November, end of the dry season), were sampled over two consecutive years (2014 and 2015). Water samples were collected along a depth profile (0.5 m, 1 m, 2.5 m, 5 m, 11 m, 15 m and 17 m depth) located above the deepest point of the lake, using a horizontal 1.2-L Niskin bottle. Water samples were kept in the dark in a coolbox, and processed within 2 hours following sampling for both chemical and microbiological analyses. Vertical profiles for pH, dissolved O_2, temperature and conductivity were determined using either a MPP350 probe connected to a Multi 350i data logger (WTW GmbH) or a YSI 6600 probe. The salinity was calculated based on conductivity. Soluble sulfide levels (ΣS(-II), hereafter referred to H_2S/HS^-) were determined by colorimetry in the field lab using an Aqualytic SpectroDirect spectrophotometer and Merck reagent kits.

DNA extraction and Illumina sequencing

Because of clogging effect, a prefiltration of water subsample (20mL) through 3µm pore-size polycarbonate filters (Millipore), that was conserved, was necessary before collection on 0.2µm pore-size polycarbonates filters (Millipore, pressure <10kPa) and storage at -20°C until nucleic acid extraction. DNA extractions were conducted separately on both 3-µm and 0.2-µm filters, using the Power Water DNA Isolation Kit (MoBio Laboratories) according to the manufacturer's recommendation. The DNA quality was checked by 1% (w/v) agarose gel electrophoresis and quantified using NanoDrop. The V3-V5 region of the 16S rRNA genes was amplified in triplicate for Bacteria and Archaea using the universal primers 357F (Schuurman et al., 2004) and 926R (Walters et al., 2016) and 519F and 915R (Hugoni et al., 2015), respectively. For *Eukaryota*, the V4 region of the 18S rRNA genes was amplified in triplicate using the universal primer 515F (Caporaso et al., 2011) and the eukaryotic primer 951R (Lepère et al., 2016).

PCR mix consisted in 0.5 U of Taq DNA polymerase (Invitrogen), 1X PCR Buffer, 1.5 mM of $MgCl_2$, 0.8 µM of each primer, 0.2 mM of each dNTP, 8 µg of BSA (New England Biolabs) and 20 ng of genomic DNA, in a final volume of 30 µl. All amplifications were carried out on a Biorad C1000 thermal cycler (Biorad) using a PCR program for Archaea composed of 10 min at 94°C, 35 cycles of 1 min at 94 °C, 1 min at 58°C, and 1 min 30 sec at 72 °C, followed by 10 min at 72°C. For Bacteria, it consisted in 3 min at 95°C, 35 cycles of 45 sec at 95 °C, 45 sec at 50°C, and 1 min 30 sec at 72 °C, followed by 5 min at 72°C. For Eukaryota, it consisted in 10 min at 94°C, 35 cycles of 1 min at 94 °C, 1 min at 55°C, and 1 min 30 sec at 72 °C, followed by 10 min at 72°C. Each primer set contained the two Illumina overhanging adapter sequences (TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG and GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G) allowing the construction of amplicon libraries by a two-step PCR.

Additionally, DNA extraction was carried out without any biological matrix and was considered a negative control to evaluate ambient and kit products contamination. High-throughput sequencing was achieved after pooling PCR triplicates using HiSeq Rapid Run 300 bp PE technology on an Illumina HiSeq 2500 system (GATC Biotech, Konstanz, Germany).

Sequence processing

Bacterial and archaeal 16S rRNA and eukaryotic 18S rRNA paired-end reads from both 3-µm and 0.2-µm filters were pooled and further merged with a maximum of 10% mismatches in the overlap region using FLASH (Magoč & Salzberg, 2011). Denoising procedures consisted in discarding reads containing ambiguous bases (N) or reads outside the range of expected length (*i.e.*, 450 to 580 bp for bacterial 16S rRNA genes, 370 to 580 bp for archaeal 16S rRNA genes and 250 to 420 bp for eukaryotic 18S rRNA genes). After dereplication, sequences were clustered using SWARM (Mahé et al., 2014) with a local clustering threshold. We removed chimeric sequences using VSEARCH (Rognes et al., 2016) but also sequences representing less than 0.005% of the total number of sequences (Bokulich et al., 2013) along with singletons. The eukaryotic dataset was manually curated for metazoan sequences (data not shown). Taxonomic affiliation was performed with both RDP Classifier (Wang et al., 2007) and BLASTN+ (Camacho et al., 2009) against the 128 SILVA database (Pruesse et al., 2007). This procedure was automated in the FROGS pipeline (Escudié et al., 2017). Contaminant Operational Taxonomic Units (OTUs) identified from the control samples were removed, and samples were randomly resampled to lowest number of retrieved sequences per sample, i.e. 124,779, 37,529 and 36,840 sequences for bacteria, archaea and eukaryota, respectively, to allow comparisons between samples.

Statistical analyses

For each domain of life (Bacteria, Archaea and Eukaryota), we tested the relative importance of different sources of temporal and spatial variability (year, season and depth) on the composition of microbial communities using nonparametric permutation-based multivariate analysis of variance (PERMANOVA, function *adonis* in R package *vegan*; Anderson, 2001) on abundance-based Bray-Curtis dissimilarity matrices. We also created an ordination of the samples using an NMDS approach on Bray-Curtis dissimilarity matrices (function *metaMDS* in R package *vegan*). We used an additive diversity-partitioning framework (Belmaker et al., 2008) to decompose the total observed diversity and express it as the sum of the diversity observable at various scales. In a first step, we decomposed the total diversity (*γ-diversity*) into the sum of interseason differences, interdepth differences and diversity in local communities with: *γ-diversity* = *β-InterSeason* + *β-InterDepth* + *α-LocalCommunities*. The approach is presented in greater detail elsewhere (Escalas et al., 2013, 2017). This decomposition was performed on diversity estimated using Rao's quadratic entropy on presence-absence and abundance data. This method provided diversity estimates expressed in equivalent number of species (Jost, 2007) and tended to be influenced by highly abundant species when abundances were taken into account. In a second step, we decomposed the total diversity (*γ-diversity*) along the depth profile and for each season separately into the diversity in local communities and differences across communities: *γ-diversity* = *β-diversity* + *α-diversity*.

Results

Physico-chemical characteristics of the water column

This study was conducted in the pit zone of the lake, characterized by the maximum depth registered (18 m). Except for the surface layer ranging from 0 to 2 m, the water column was completely anoxic all year long (Figure 1). This zone was characterized by a deep chemocline located at 14 m, which isolated the deepest water layer from the rest of the water column. During the stratified period, a second seasonal chemocline was observed at a depth of approximately 2 m that separated the intermediate water layer from the surface layer. The physicochemical structure of the water column thus alternated seasonally between two layers during the unstratified period versus three layers during the stratified period.

The establishment of the seasonal chemocline at a depth of approximately 2 m was due to the increase in rainfall at the beginning of the rainy season that diluted the upper layer of the water column, reducing its salinity (34 and 45 psu in Apr. 2014 and Apr. 2015, respectively), resulting in haline stratification (Figure 1). Below this halocline, the salinity increased up to approximately 65 psu in the intermediate layer, the temperature stabilized to approximately 30.2°C, and H_2S/HS^- concentrations reached 5900 and 2500 µM in 2014 and 2015, respectively.

In its unstratified state (corresponding to the dry season), the water column was homogeneous with respect to H_2S/HS^- with low concentrations (mean 187 µM). The water had a mean salinity of 64.2 psu from the surface to the deep chemocline (14 m), while temperature varied in the first 2 m (average value of 31.6°C, from 31 to 30.2°C in 2014 and from 32.7 to 30.3°C in 2015), and O_2 disappeared within the first 2 m. Hence, despite mixing, the water column remained anoxic below a depth of 1.5 m.

The deepest layer of the lake (below the deep chemocline) was characterized by slight drops in temperature and pH (up to 0.5°C and 0.35 pH units, respectively) and by an increase in salinity (up to 8 psu).

Microbial community structure in Lake Dziani Dzaha

This work investigated changes in the microbial community diversity of the three domains of life from the pelagic ecosystem in Lake Dziani Dzaha across time (year and season) and space (water column depth). Rarefaction curves indicated that most of the diversity was captured in the present work (Supplementary Figure 1). The relative importance of these two dimensions on microbial dynamics differed among the considered domains (Bacteria, Archaea and Eukaryota).

Depth, and thus vertical physicochemical gradients, was the main factor influencing the composition of prokaryotic communities (this factor is here considered independently), notably for Bacteria, for which it explained 47% of variation in community composition (Table 1). For Archaea, the influences of season and year on community composition were closer to the effect of depth (15, 12 and 21% of variation, respectively, factors considered independently, Table 1). In contrast, the composition of eukaryotic microbial communities was mostly influenced by the season and to a lesser extent by year and depth (35, 12 and 6% of variation, respectively, Table 1). Differences in the relative importance of time and space effects on microbial community composition are presented in Figure 2.

When considering all samples together, the richness in the normalized datasets consisted in 242 bacterial, 109 archaeal and 87 eukaryotic OTUs. When pooling the three domains together, the total microbial richness ranged from 221 to 359 OTUs per sample, with an average of 301 ± 37 OTUs. We used a diversity-partitioning approach to explore the multiple facets of microbial diversity across multiple scales. We estimated the total microbial diversity (*i.e.*, *γ-diversity*) associated with each domain of life using Rao's quadratic entropy (Table 2). When using presence-absence data, we found that bacterial communities exhibited the highest *γ-diversity* in the system (Supplementary Figure 2), with 230.6 OTUs, followed by Archaea with 97.9 OTUs and Eukaryota with 41.9 OTUs. When using relative abundance data, archaeal communities were the most diverse ($\gamma_{\text{-diversity}}$ = 8.9), followed by bacterial ($\gamma_{\text{-diversity}}$ = 3.7) and eukaryotic ($\gamma_{\text{-diversity}}$ = 1.4) communities.

An additive decomposition of *γ-diversity* was used to determine whether the total observed diversity derived from the average diversity within communities (*i.e.*, $\alpha_{LocalCommunities}$) or from the differences between communities across seasons (*i.e.*, $\beta_{InterSeason}$) and depths (*i.e.*, $\beta_{InterDepth}$) (Figs. 3.A and B). Overall, local communities represented most of the total diversity ($\alpha_{LocalCommunities}$ > 76.5% on average). When using presence-absence data, *β-diversity* ($\beta_{InterSeason} + \beta_{InterDepth}$) represented a higher proportion of *γ-diversity* in eukaryotic communities (36.6%), while it contributed a smaller proportion in archaeal (20.2%) and bacterial (14.6%) communities. When using abundance data, we observed higher contributions of *β-diversity* to *γ-diversity* for Archaea (34.9%) and Bacteria (31.6%), while the value for Eukaryota dropped to 3%. Further, differences along the depth profile ($\beta_{InterDepth}$) contributed more to the total diversity than interseason differences ($\beta_{InterSeason}$), with 20.4 and 3.1% of $\gamma_{Diversity}$, respectively.

The present work showed that the diversity distribution along the depth profile differed greatly between the three domains of life (Figs. 3.C and D). The bacterial diversity increased with depth, from very low values near the surface (0 to 2.5 m) to a sharp increase at the bottom of the water column (15 to 17 m). Thus, the distribution of abundance between OTUs in these communities was more balanced as the depth increased. The decomposition of diversity revealed a very low degree of differentiation between communities at the top of the water column, while deeper communities tended to be more variable across season. In contrast, archaeal communities exhibited maximum diversity in the first meter and a sharp drop in diversity below this depth. This reverse

pattern corresponded to the increasing dominance of a smaller number of archaeal taxa with increasing depth. For archaeal communities, we observed higher *β-diversity* in the upper layers, but there was always a certain degree of differentiation from a given depth across time (year and season). Eukaryotic communities showed a very low and constant diversity along the depth profile (due to the hyperdominance of *Picocystis* OTUs). The proportion of eukaryotic *β-diversity* observed was strikingly lower than that for the other domains, and furthermore, this low proportion was observed at all depths.

Sampling seasons also have a differential impact on the estimated diversity of the three domains of life (Figure 3D). Bacterial communities appeared to be more diverse during the stratified period, with *γ-diversity* estimates more than twofold higher in this season. This was notably due to more dissimilar communities, with *β-diversity* representing 36.7% of *γ-diversity* during the stratified period compared to only 15.5% during the unstratified period. The diversity of archaeal and eukaryotic communities was more stable across seasons, and these communities exhibited smaller changes in their composition, notably for eukaryotic organisms where *β-diversity* represented less than 3% of *γ-diversity*.

Microbial community composition in Lake Dziani Dzaha

The taxonomic composition of the microbial assemblages from the three domains of life was investigated according to seven different depths for both stratified and unstratified periods in 2014 and 2015.

During the stratified period, the bacterial assemblage was dominated by Cyanobacteria affiliated with *Arthrospira fusiformis* from a depth of 0.5 m to 2.5 m, representing on average 48.1% of the total eukaryotic and prokaryotic sequences in 2014 and 2015 (Figure 4). In this surface layer, Eukaryota affiliated with *Picocystis salinarum* (15.5% of total sequences) and *Archaea* affiliated with *Woesearchaeota* (13.7% of total sequences from 0. 5 m to 1 m) were also present. The number of *P. salinarum* sequences was constant through the whole water column, while *A. fusiformis* and *Woesearchaeota* sequence numbers decreased drastically with depth (dropping to 5.2% and 2.9% of total sequences, respectively, in the deeper layers, *i.e.*, 15 and 17 m). Other microorganisms such as *Bacteroidia* within Bacteroidetes, *Clostridia* within Firmicutes or *Methanomicrobia* and *WSA2* within Euryarchaeota increased with depth, ranging from 0.5 to 18.9%, 0.3 to 34.5%, 1.7 to 4.9% and 1 to 7.2%, respectively, based on the average sequences for the three domains of life in 2014 and 2015.

In contrast, during the unstratified period, *A. fusiformis* clearly dominated the total assemblage from the surface to a depth of 11 m, with abundances representing 48% of the total sequences on average, followed by *P. salinarum* (13.9% of total sequences) and Woesearchaeota (representing 12.6% of total sequences). Other phyla were less represented in this 0 to 11 m zone, such as Euryarchaeota *WSA2*_WCHA1-57 and an Euryarchaeota unknown class, *Bacteroidia, Clostridia, Alphaproteobacteria* and eukaryotic *Jakobida*. In deeper waters (close to 15 and 17 m), a shift was observed, characterized by a decrease in *A. fusiformis* associated with an increase in *Clostridia* and *Bacteroidia* and in Euryarchaeota affiliated with *WSA2* and *Methanomicrobia* (Figure 4).

A detailed analysis of nondominant Eukaryota (*i.e.*, non-*P. salinarum* sequences) revealed the presence of contrasting phyla during the two seasons (Figure 5). During the stratified period, for both years, the nondominant eukaryotic assemblage was dominated by ciliates (*Ciliophora*) mainly assigned to the *Intramacronucleata* subphylum, from the surface to a depth of 2.5 m. Additionally, this class represented one-third of the eukaryotic sequences at a depth of 17 m in 2015. Several groups, such as fungal ascomycetes, increased in relative abundance with depth in 2014, shifting from 0.22% of sequences in the surface layer to 1.44% in the bottom layer. Other groups, such as Blastocystae within Stramenopiles, followed the opposite trend and decreased in relative abundance with depth (from 1.00 to 0% of sequences from the surface a depth of to 17 m in 2014). During the unstratified period, different groups were observed, with an unknown group of Jakobida (i.e. flagellate) being the dominant class, representing between 16.48 and 0.18% of eukaryotic sequences from the surface to a depth of 17 m in 2014 and between 7.07 and 3.45% in 2015. Interestingly, an unknown group in the Ancyromonadida class codominated the nondominant eukaryotic assemblage with Jakobida in 2015, accounting for 10.62 to 16.23% of eukaryotic sequences.

Discussion

The present work constitutes the first characterization of prokaryotic and eukaryotic microbial assemblages in a tropical thalassohaline lake using high-throughput Illumina sequencing.

Our study provided new insights into the biodiversity of microbial communities at three different scales by including the diversity (i) of local communities (*α-diversity)*, (ii) between communities (*β-diversity*) and (iii) of the whole system (*γ-diversity*). We showed that the contribution of *α-LocalCommunities* to *γ-diversity* was important (approximately 76.5%), outweighing that of *β-InterSeason* and *β-InterDepth*, thus supporting the idea of consistently high diversity across local microbial communities (OTU richness = 301 ± 37 OTUs on average). However, *β-diversity* represented a variable part of *γ-diversity* depending on the domain of life and the type of matrix considered (*i.e.*, presence-absence *versus* abundance matrix).

While the results were relatively similar for Archaea and Bacteria, there was a striking difference in the contribution of *β-InterDepth* to *γ-diversity* in eukaryotic communities with presence-absence (32.2%, Table 2) *versus* abundance data (2.1%). This finding reflected the fact that, although the list of detected OTUs might change between depths, the abundance distribution across these OTUs at each depth was skewed toward a single OTU (*P. salinarum*), such that the eukaryote communities could almost be considered as composed of a single OTU, which was not the case for prokaryotes (*cf*. low *γ-diversity* estimates for Eukaryota in Figs. 3B, C and D). To gain insights into the eukaryotic diversity in a community almost entirely consisting in *P. salinarum*, we decided to focus on the nondominant eukaryotic members and observed a seasonal turnover with clear shifts in eukaryotic classes. Approximately 200 protists have been described in hypersaline ecosystems, mainly based on cultivation methods (Hauer & Rogerson, 2005), and investigations into their diversity, ecology and community complexity through the use of molecular techniques are recent (Edgcomb et al., 2009; Stock et al., 2012). Nonetheless, microbial Eukaryota are an essential component of microbial food webs and thus play a central role in local ecosystem functioning and global biogeochemical cycles (Edgcomb & Bernhard, 2013). Previous work conducted on hypersaline

environments reported protistan species turnover due to saline boundaries (Casamayor et al., 2002; Filker et al., 2017). The present work clearly showed the dominance of the autotrophic green algae *P. salinarum* but also the seasonal turnover of nondominant heterotrophic eukaryotes such as ciliates (*i.e.*, *Ciliophora*) and flagellates (*e.g., Jakobida*, *Stramenopiles*). Previous studies have shown that eukaryotes could be active in the anoxic zone of the non-saline lake Pavin (Lepère et al., 2016) where ciliates, fungi and *Haptophyceae* were the dominant phylotypes; but also in hypersaline environments, with phylotypes related to fungi, ciliates, stramenopiles, flagellates and jakobids being detected through 18S rRNA transcript analysis in the deep hypersaline anoxic Thetis and Atalante basins (Alexander et al., 2008; Stoeck et al., 2010).

Jakobids have been studied primarily because of their evolutionary importance and their uncertain phylogenetic placement among eukaryotes (Archibald et al., 2017). This group has been detected in different oxygen-poor, sulfide-rich and hypersaline ecosystems (Stock et al., 2009; Weber et al., 2014), exhibiting H_2S/HS^- concentrations (0 to 100 µM) close to those retrieved in Lake Dziani Dzaha during the unstratified period (67-328 µM H_2S/HS^- above the deep chemocline). When H_2S/HS^- concentrations increased during the stratified period (up to 5900 µM), jakobids were almost absent from the eukaryotic assemblage, suggesting that high H_2S/HS^- concentrations are toxic to those microorganisms.

Ciliates are widely distributed in aqueous ecosystems on Earth and mostly occurred above the chemoclines during the stratified period in Lake Dziani Dzaha (their detection at 17 m below the deep chemocline in 2014 is considered to be an artifact due to sediment resuspension during sampling). Those microorganisms are considered the top predators in microbial food webs where metazoans are absent (Archibald et al., 2017). In a given habitat, ciliate assemblages could be specific to certain biotic and abiotic characteristics of the environment, driven primarily by H_2S/HS^- toxicity or resource availability (Bick & World Health Organization, 1972; Lynn, 2008). In the present case, we hypothesized that ciliate and flagellate shifts occurring seasonally could be related to prey shifts in the water column and/or differences in salinity tolerance.

In prokaryotic communities, changes in the identity of detected species were relatively small when compared to the total diversity (*i.e., β-diversity* represented 20.2% and 14.6% of *γ-diversity* with presence-absence data in Archaea and Bacteria, respectively). However, the distribution of abundances among the OTUs varied greatly according to depth and season (*e.g.*, the contribution of *β-diversity* was 34.9% and 31.6% for Archaea and Bacteria, respectively). Overall, this pattern suggested that while eukaryotic communities were relatively more variable in their composition, prokaryotic communities were more dynamic in terms of changes in OTU abundance across seasons and depths. Those results were confirmed by the comparison of community composition along the depth profile during the two seasons (Figure 4). Moreover, the diversity decomposition according to season revealed higher *γ-diversity* during the stratified period, especially for Bacteria, which is in accordance with the presence of environmental clines generating different ecological niches and ultimately allowing greater community differentiation. Overall, the structure and composition of microbial assemblages were highly influenced by the sampling year, the season and depth considered, and the physicochemical structure of the water column. During the stratified period, prokaryotic and eukaryotic community composition differed between the surface (0.5 to 1 m) and mid-water column (2.5 to 11 m), suggesting an impact of the seasonal chemocline at 2 m. Moreover, a shift in bacterial community structure was also reported at the deep chemocline situated at a

depth of 14 m. During the unstratified period, the community composition was homogeneous along the water column (because of water convection), shifting only below the deep chemocline.

As observed in soda lakes, photosynthetic organisms dominated the microbial assemblage (Grant & Jones, 2016; Krienitz & Schagerl, 2016), particularly in the upper layer. In Lake Dziani Dzaha, the photosynthetic communities were dominated by Cyanobacteria from the *Arthrospira* genus (also named *Spirulina*) and Eukaryota from the *Picocystis* genus. *Arthrospira* consisted in alkaliphilic filamentous Cyanobacteria, which form dense masses seasonally or permanently (Ballot et al., 2004; Sili et al., 2012). Interestingly, a recent characterization using a polyphasic approach of strains isolated from Lake Dziani Dzaha reported that the unusual straight morphotype of *Arthrospira* strains was affiliated with *Arthrospira fusiformis* and *Picocystis* strains with *Picocystis salinarum* (Cellamare et al., 2018). *P. salinarum* and *A. fusiformis* are highly productive primary producers that are often reported as numerically abundant in microbial communities of saline habitats, such as soda lakes of the East African Rift Valley (Krienitz et al., 2012) or Mono Lake, USA (Hollibaugh et al., 2001). A recent study conducted on bioreactors inoculated with alkaline sediments fed by *Arthrospira* biomass showed that the produced organic matter was hydrolyzed mainly by the Bacteroidetes ML635J-40 aquatic group, while methane was biologically produced (via the hydrogenotrophic pathway) by an archaeal community dominated by *Methanocalculus* (Nolla-Ardèvol et al., 2015). This microbial consortium is highly relevant in the case of Lake Dziani Dzaha, where *Methanomicrobia* is strongly dominated by the genus *Methanocalculus*. In addition, the enrichment of *Methanocalculus* with depth is concomitant to the enrichment of Bacteroidetes affiliated with the *Bacteroidia*_ML635J-40 aquatic group. This group has been formerly identified in soda lakes and alkaline environments, such as Mono Lake in United States (Humayoun et al., 2003), Magadi Lake in Africa (Baumgarte, 2003) and the Lonar crater lake in India (Wani et al., 2006). Our results showed that multidomain metabarcoding is an interesting approach to unravel previously known relationships between domains of life.

In addition, sulfur cycle-related organisms were retrieved in the water column of Lake Dziani Dzaha. Firmicutes represented an important fraction of the relative abundance (*i.e.*, number of sequences) in the lake, whereas Deltaproteobacteria were far less represented and could be considered almost absent. Previous studies demonstrated potential competition occurring between methanogenic and sulfidogenic microorganisms (Visser et al., 1996). Under natural conditions, and in the presence of nonlimited levels of sulfate, methanogens are generally poor competitors when compared with sulfate-reducers, especially in marine sediments, while methanogens tend to be the dominant scavengers of hydrogen and acetate in low-sulfate environments (Raskin et al., 1996). In Lake Dziani Dzaha, sulfate concentrations were relatively low in the surface layers (2.5 mM), that is, above the 2-m-depth seasonal chemocline during the stratified period and above the 14-m-depth deep chemocline during unstratified periods. Additionally, sulfate was not detected below these two chemoclines, and H_2S/HS^- was present instead. The low to null sulfate concentrations could explain the low occurrence of sulfate-reducing microorganisms, such as *Deltaproteobacteria*. The fact that higher concentrations of H_2S sometimes occurred below both chemoclines than the sulfate concentration in surface waters might be due to the production of H_2S by fermentative pathways performed by heterotrophic microorganisms using R-SH-enriched organic matter or alternative processes involved in the sulfur cycle, such as the reduction of elemental sulfur.

Conclusions

Thalassohaline lakes are extreme environments where highly adapted microbial communities flourish. Understanding their microbial ecology is key to providing insights into ecosystem functioning, resilience and stability. The present work is the first to assess, with an in-depth metabarcoding approach of the three domains of life, how total microbial diversity is impacted by seasonal and interannual variations across a vertical gradient of the water column in a thalassohaline lake. By decomposing biodiversity at a fine scale in Lake Dziani Dzaha, we highlighted that the richness and diversity of Bacteria, Archaea and Eukaryota are differentially impacted by the physicochemical structure of the water column. Eukaryotic communities were relatively more variable in their composition than prokaryotic communities, which were more dynamic in terms of changes in OTU abundance across seasons and depths. The eukaryotic turnover observed in this lake and its comparison to the prokaryotic patterns could be of great importance for understanding this ecosystem's trophic network. Finally, we identified some microbial classes, or even taxa, that could be related to biogeochemical processes occurring in Lake Dziani Dzaha; these findings will need to be confirmed experimentally in the future.

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Data accessibility

The sequence data generated in this study were deposited on the European Nucleotide Archive (ENA) browser (http://www.ebi.ac.uk/ena/data/) under accession number: PRJEB24947.

Author Contributions

CB, DJ, CL, MB, MA, HA designed research
MH, CB, DJ, GS, CL, MB, PG, FV, MA, HA performed research
MH, AE, CB, SN, DJ, GS, MT, HA analyzed data
MH, AE, MA, HA, MT wrote the paper

Figures and Tables Legends

Figure 1. Vertical profiles of environmental parameters recorded during 4 sampling campaigns

Salinity (psu), temperature (°C), pH, O_2 (%) and H_2S/HS^- (µM) profiles were recorded during the stratified and the unstratified periods in 2014 and 2015.

Figure 2. Differences in microbial community structure

NMDS ordination performed on Bray-Curtis dissimilarity matrices. The legend is presented in the bottom right corner of the panel. Circle size indicates the depth. Blue circles represent the stratified periods, and orange circles represent the unstratified periods. Black-surrounded circles represent 2014, while unsurrounded circles represent 2015.

Figure 3. Additive decomposition of microbial diversity

Decomposition of total diversity (*γ-Diversity*) estimated using Rao's quadratic entropy into local community diversity (*α-LocalCommunities*) and differences between communities (*β-InterSeason* and *β-InterDepth*). This was done with presence-absence data (A) and using OTU relative abundances (B). Decomposition of total diversity (*γ-Diversity*) estimated using Rao's quadratic entropy on abundance data into local community diversity (α) and differences between communities (β), along the depth profile (C) and between seasons (D).

Figure 4. Relative abundance (% of sequences) of bacterial, archaeal and eukaryotic phyla

Data were collected in (A) 2014 and (B) 2015 during both stratified and unstratified periods. In the legend at the right of the panel, Archaea are indicated as A_, Bacteria as B_, and Eukaryota as E_.

Figure 5. Relative abundance (% of sequences) of eukaryotic phyla, except *Picocystis*

Data were collected in (A) 2014 and (B) 2015 during both stratified and unstratified periods. The detection of ciliates at a depth of 17 m below the deep chemocline is considered an artifact due to sediment resuspension during sampling.

Supplementary Figure 1. Rarefaction curves

Rarefaction curves for each sample were calculated for bacterial, archaeal and eukaryotic datasets pooled.

Supplementary Figure 2. Additive decomposition of microbial diversity

Decomposition of total diversity (*γ-Diversity*) estimated using Rao's quadratic entropy on presence-absence data into local community diversity (α) and differences between communities (β) along the depth profile (A) and between seasons (B).

Table 1. Effects of environmental factors on microbial community composition

Differences between groups were tested by using PERMANOVA analysis of Bray-Curtis dissimilarity matrices.

Table 2. Additive decomposition of microbial diversity

γ-Diversity was estimated using Rao's quadratic entropy, and decomposition was performed according to Escalas *et al.* (2017). The number in parenthesis corresponds to the percentage of the *γ-Diversity* represented by each component.

	Bacteria			Archaea			Eukaryota		
Factor	**F.value**	**R^2**	**p.value**	**F.value**	**R^2**	**p.value**	**F.value**	**R^2**	**p.value**
Year	4.99	0.07	0.020	6.62	0.12	0.004	8.77	0.12	0.001
Season	6.65	0.09	0.009	7.80	0.15	0.001	25.19	0.35	0.001
Depth	35.67	0.47	0.001	11.00	0.21	0.001	4.51	0.06	0.006
Year:Season	1.89	0.03	0.150	3.31	0.06	0.020	4.87	0.07	0.004
Year:Depth	3.96	0.05	0.038	1.96	0.04	0.114	1.71	0.02	0.151
Season:Depth	2.14	0.03	0.130	1.63	0.03	0.164	3.64	0.05	0.018
Year:Season:Depth	0.31	0.00	0.773	0.68	0.01	0.605	3.10	0.04	0.030
Residuals	NA	0.26	NA	NA	0.38	NA	NA	0.28	NA

	γ-diversity	***α-LocalComm.***	***β-InterSeason***	***β-InterDepth***
Presence-absence				
Bacteria	230.6	196.9 *(85.4)*	3.4 *(1.5)*	30.3 *(13.1)*
Archaea	97.9	78.1 *(79.8)*	1.3 *(1.3)*	18.5 *(18.9)*
Eukaryota	41.9	26.6 *(63.4)*	1.8 *(4.4)*	13.5 *(32.2)*
Abundance				
Bacteria	3.7	2.5 *(68.4)*	0.1 *(3.0)*	1.1 *(28.6)*
Archaea	8.9	5.8 *(65.0)*	0.7 *(7.6)*	2.4 *(27.3)*
Eukaryota	1.4	1.4 *(97.0)*	0.01 *(0.9)*	0.03 *(2.1)*