



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

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## Spatio-temporal variations of microbial diversity across the three domains of life in a tropical soda lake (Dziani Dzaha, Mayotte Island)

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**Spatio-temporal variations of microbial diversity across the  
three domains of life in a tropical soda lake  
(Dziani Dzaha, Mayotte Island)**

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**Running title:** Microbial characterization of a soda lake

## Originality-Significance Statement

Many collaborative projects have recently emerged to characterize microbial life on Earth. Indeed, microorganisms are key players of biogeochemical cycles in diverse ecosystems and understanding how diversity is related to ecosystem functioning is a challenge in microbial ecology. Advances in sequencing technologies and analytic techniques now provide efficient tools to address crucial microbial ecology concepts. In this study we explore the multiple facets of microbial diversity associated with an extreme ecosystem (high salinity, pH, alkalinity and high rates of organic matter production and mineralization): lake Dziani Dzaha (Mayotte). The present work is the first to assess, with an in-depth 3-domains-of-life metabarcoding approach (Eukaryota, Archaea and Bacteria), how total microbial diversity is impacted by season and inter-annual variations, across a vertical gradient corresponding to different physico-chemical state of the water column. By decomposing biodiversity at fine scale in lake Dziani Dzaha, we highlighted that richness and diversity of Bacteria, Archaea and Eukaryota are differently impacted by environmental variations. We suggested that eukaryotic communities were relatively more variable in their composition in contrast to prokaryotic communities that were more dynamic in terms of changes in OTUs abundance across season and depth. Finally, we identified some microbial classes, or even taxa, that could be related to biogeochemical processes occurring in lake Dziani Dzaha that needed to be confirmed experimentally.

## 49    **Abstract**

50    Extreme environments, like soda lakes, represent excellent models to study how drastic  
51    environmental conditions shape microbial diversity. In these ecosystems, microorganisms  
52    significantly contribute to biogeochemical cycles, but their community structure and spatio-  
53    temporal dynamics remain largely unexplored. In this study, we monitored the 3-domains-  
54    of-life composing the microbial communities of Lake Dziani Dzaha (Mayotte), a tropical  
55    saline alkaline lake. Using a diversity partitioning approach, we revealed that most of the  
56    microbial diversity was hosted in local microbial communities, with a comparatively lower  
57    contribution of between-communities differences to the overall diversity. Nonetheless, the  
58    diversity of the whole system was clearly impacted by the presence of chemoclines,  
59    inducing a stratification of the water column into different ecological niches, allowing  
60    significant communities differentiation across spatio-temporal scales. A detailed analysis of  
61    Bacteria, Archaea and Eukaryota showed that depth, season, and sampling year impacted  
62    differentially the establishment of each domain of life. Indeed, photosynthetic  
63    microorganisms were abundant in surface layers while deeper layers were dominated by  
64    chemotrophic microorganisms from Firmicutes and Bacteroidetes phyla, and by putative  
65    methanogenic lineages such as Woesearchaeota. Our findings highlighted the importance of  
66    considering multi-domain microbial processes and interactions in time and space to better  
67    understand their impact on ecosystem functioning.

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## Introduction

Soda lakes are extreme environments widely distributed on Earth, mainly in arid and semi-arid regions, such as the Rift Valley in Africa, United-States, Canada, Iran, China or Mexico and in dry steppe regions like in Russia (Sorokin *et al.*, 2014; Grant and Jones, 2016). The name “soda” is derived from the chemical composition of their waters, which are considered alkaline because of the high concentration of dissolved inorganic carbon and the presence of sodium as the major cation, so that upon nearly complete evaporation  $\text{Na}_2\text{CO}_3$  (called soda) could precipitate. These characteristics are often associated with elevated pH values (9.5 to 12) and high salinity waters (>50 psu). As a result of such drastic environmental conditions, soda lakes are inhabited by microorganisms adapted to high alkalinity and salinity, unlimited supplies of dissolved inorganic carbon and sometimes low oxygen availability. These adaptations relied on molecular mechanisms (Ochsenreiter *et al.*, 2002; Mesbah *et al.*, 2009), as for example modifications of their membrane structure, the use of transporter proteins or the synthesis of osmo-protectants (Sorokin *et al.*, 2014; Grant and Jones, 2016). Previous studies clearly showed that salinity is one of the key environmental factor shaping the composition and structure of aquatic microbial communities on a global scale (Lozupone and Knight, 2007; Canfora *et al.*, 2014). As a consequence, the microbial diversity in soda lakes is limited to salt-tolerant specialists that are key players of biogeochemical cycles occurring in those ecosystems (*e.g.* carbon, nitrogen and sulfur cycles).

In spite of apparent extreme conditions, soda lakes are considered among the most productive aquatic ecosystems, especially in tropical areas with elevated temperature and high incident solar radiations. Prodigious rates of photosynthetic primary production were

notably associated with Cyanobacteria and/or anoxygenic phototrophic Bacteria (Ballot *et al.*, 2004; Lewis *et al.*, 2011). The primary productivity can reach up to 10 gC.cm<sup>-2</sup>.day<sup>-1</sup>, delivering fixed carbon to haloalkaliphilic communities, as aerobic and anaerobic chemoorganotrophic Bacteria and Archaea (Melack and Kilham, 1974; Grant *et al.*, 1990). Most of the studies investigating microbial communities in soda lakes revealed dense populations of primary producers affiliated with unicellular and filamentous Cyanobacteria, such as the genera *Arthrospira*, *Cyanospira*, *Geitlerinema*, *Leptolyngbya* or *Anabaenopsis* (Grant and Jones, 2016; Krienitz and Schagerl, 2016). At higher salinity (>50 psu), unicellular green algae like *Picocystis salinarum* or *Dunaliella viridis* were also retrieved (Krienitz and Schagerl, 2016). Studies focusing on prokaryotes reported the dominance of Alphaproteobacteria, Gammaproteobacteria, Firmicutes and Bacteroidetes (Dimitriu *et al.*, 2008; Sorokin *et al.*, 2014; Aguirre-Garrido *et al.*, 2016), associated with abundant Euryarchaeota in hypersaline soda lakes (Ochsenreiter *et al.*, 2002; Mesbah *et al.*, 2007; Vavourakis *et al.*, 2016). Although salt-adapted organisms derive from all the three domains of life, most studies investigated the prokaryotic fraction of the microbial assemblage (Ghai *et al.*, 2011; Ventosa *et al.*, 2015; Kambura *et al.*, 2016), while very few considered the eukaryotic diversity associated with those ecosystems (Krienitz *et al.*, 2012). Over the past decade, high-throughput sequencing technologies have been successful in describing relative low-diversity ecosystems such as extreme environments, by giving snapshots of the overall community structure and gaining new insights into uncultivated microorganisms genome (Ghai *et al.*, 2011; Narasingarao *et al.*, 2012; Vavourakis *et al.*, 2016).

Lake Dziani Dzaha, located in Mayotte Island, is a small crater lake (*ca.* 0.24 km<sup>2</sup>) with shallow water (mean depth: 3 m, except for a pit at 18 m depth). Its probable seawater

origin makes it a peculiar soda lake, which water composition has been modified by biological and geothermal activities up to current conditions: extremely high alkalinity (0.23 mol/L), elevated salinity (ranging from 34 to 71 psu) and pH (9.1 to 9.4). This lake presents extremely and constantly high rates of organic matter production and mineralisation (Leboulanger *et al.*, 2017). The alternation of a rainy (Nov.-Apr.) and a dry season (June-Oct.) induces a saline and seasonal stratification of the water column, caused by the dilution of the upper layer by rainfalls, which creates a chemocline at around 2.25 meter depth. This stratification usually persists until August, when a combination of temperature decrease and salinity increase by evaporation lead to an increase of the density of the surface layer. The water column is permanently anoxic below about 1 to 2m depth, irrespectively of the water column stratification, with rare episodes when anoxia reaches the surface of the water column. A second chemocline was systematically identified in the pit at a depth around 14m, below which  $\text{H}_2\text{S}/\text{HS}^-$ ,  $\text{NH}_4^+/\text{NH}_3$  and  $\text{CH}_4$  concentrations were systematically high and reached values of up to 5, 4 and 1 mM, respectively. In this ecosystem, the aquatic food web is very simple and characterized by permanent cyanobacterial blooms (mainly *Arthrospira*), high prokaryotic biomass depending on the season, and an absence of metazoans apart from endemic flies (Leboulanger *et al.*, 2017). Thus, this singular ecosystem can be considered as an excellent microbial model to examine the genetic structure and dynamics of microbial communities. Through a multi-seasonal survey conducted across two consecutive years (2014; 2015) at different depths of the water column (from 0 to 17m), we aimed at testing whether the microbial diversity inhabiting the lake Dziani Dzaha is stable in time and space. By providing an exhaustive and simultaneous characterization of both prokaryotic (including Archaea and Bacteria) and eukaryotic microorganisms using Illumina metabarcoding sequencing, the present work is



the first to describe the spatio-temporal dynamics of the complete microbial assemblage of an extremely original soda lake.

## 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 (18m). The surface layer of the water column was oxic down to 2m while the conditions were completely anoxic all year long below that limit (Figure 1). The water column was characterized by a permanent chemocline at 14m, that isolated the deepest water layer, and by a seasonal one that separated the intermediate water layer from the surface layer. The physico-chemical structure of the water column thus alternated seasonally with two layers of water in the non-stratified season versus three during the stratified season.

The apparition of a seasonal chemocline and a stratified state, occurring at approximately 2m was due to changes in rainfall regime at the beginning of the rainy season (Leboulanger *et al.*, 2017). The rainfall effect resulted in a dilution of the upper layer of the water column, reducing its salinity (34 to 45 psu, Apr. 2014 and Apr. 2015 respectively) and resulting in a haline stratification at *ca.* 2m depth (Figure 1). Below this halocline, the salinity rises up to approximately 65 psu in the intermediate layer, the temperature stabilizes around 30.2 °C and the H<sub>2</sub>S/HS<sup>-</sup> concentrations reach up to 5900 and 2500 µM, in 2014 and 2015, respectively.

A non-stratified state of the water column was also recorded, with homogeneous concentrations of H<sub>2</sub>S (mean 187 µM), temperature (mean 31.6 °C), salinity (mean 64.2 psu) and pH from surface to the deeper chemocline (14m).

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

### ***Microbial community structure in lake Dziani Dzaha***

This work investigated the spatial and temporal changes in the microbial communities diversity of the three life domains from the pelagic ecosystem of the lake Dziani Dzaha. The relative importance of these two dimensions of the microbial dynamics differed among the considered life domain (Bacteria, Archaea, Eukaryota).

Depth was the main factor influencing the composition of prokaryotic communities, 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, Table 1). On the contrary, the composition of eukaryotic microbial communities was mostly influenced by the sampling season and to a lesser extent by year and depth (35, 12 and 6% of variation, respectively, Table 1). The differences in the relative importance of time and space effects on microbial communities composition are presented in Figure 2.

When considering all samples together, the richness in normalized datasets consisted in 242 bacterial, 109 archaeal and 87 eukaryotic OTUs. When pooling the three domains together, total microbial richness ranged from 221 to 359 OTUs per sample, with an average of  $301 \pm 37$  OTUs. Then 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.*  $\gamma$ -diversity) associated with each domain of life using Rao' quadratic entropy (Table 2). When using presence-absence data, we found that bacterial

communities exhibited the highest  $\gamma$ -diversity in the system, with 230.6 OTUs, followed by Archaea with 97.9 OTUs and Eukaryota with 41.9 OTUs. When using relative abundance data, we observed that archaeal communities were the most diverse ( $\gamma$ -diversity = 8.9), followed by bacterial ( $\gamma$ -diversity = 3.7) and eukaryotic ( $\gamma$ -diversity = 1.4) communities. This change in the diversity estimates between the two methods is due to the fact that the distribution of abundance between OTUs was more even in archaeal communities compared with bacterial and eukaryotic ones, which were dominated by a single OTU representing most of the dataset (*i.e.* *Arthrospira fusiformis* and *Picocystis salinarum*, respectively).

An additive decomposition of  $\gamma$ -diversity was used to determine whether the total observed diversity arose from the average diversity within communities (*i.e.*  $\alpha_{LocalCommunities}$ ) or the differences between communities across seasons (*i.e.*  $\beta_{InterSeason}$ ) and across depth (*i.e.*  $\beta_{InterDepth}$ ) (Figure 3.A and B). Overall, local communities represented most of the total diversity ( $\alpha_{LocalCommunities} > 76.5\%$  on average). When using presence-absence data, we observed that  $\beta$ -diversity ( $\beta_{InterSeason} + \beta_{InterDepth}$ ) represented a higher proportion of  $\gamma$ -diversity in eukaryotic communities (36.6%), while it contributed in a smaller proportion in archaeal (20.2%) and bacterial (14.6%) communities. When using abundance data, we observed higher contributions of  $\beta$ -diversity to  $\gamma$ -diversity for Archaea (34.9%) and Bacteria (31.6%) while the value for Eukaryota dropped to 3%. Further, we found that differences along the depth profile ( $\beta_{InterDepth}$ ) contributed more to the total diversity than inter-season 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 (Figure 3.C and D). The bacterial

diversity increased with depth, from very low values near the surface (0 to 2.5m) to a sharp increase at the bottom of the water column (15 to 17m). Thus the distribution of abundance between OTUs in these communities was more balanced as the depth increased. The decomposition of diversity reveals that there was a very low degree of differentiation between communities at the top of the water column while deeper communities tended to be more variable. On the contrary, archaeal communities exhibited maximum diversity in the first meter and a sharp drop of 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 a higher  $\beta$ -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 (*i.e.* due to the hyper dominance of *Picocystis* OTUs). The proportion of eukaryotic  $\beta$ -diversity observed was strikingly lower than for other domains and this was the case at every depth.

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

#### ***Microbial community composition in lake Dziani Dzaha***

The taxonomic composition of the 3-domains-of-life microbial assemblages was investigated according to seven different depths in 2014 and 2015, for both stratified and non-stratified state.

During the stratified period the bacterial assemblage was dominated by Cyanobacteria affiliated with *Arthrospira fusiformis* from 0.5m to 2.5m-depth, 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* (about 15.5% of total sequences) and Archaea affiliated with *Woesearchaeota* (from 0.5m to 1m, about 13.7% of total sequences) were also present. The number of *Picocystis* sequences was constant through the whole water column, while *Arthrospira* and *Woesearchaeota* sequence numbers decreased drastically with depth (falling on average to 5.2% and 2.9% of total sequences, respectively, in the deeper layer, i.e. 15 and 17m). Other microorganisms like Bacteroidetes affiliated with *Bacteroidia*, Firmicutes affiliated with *Clostridia*, Archaea affiliated with *WSA2*, or Euryarchaeota affiliated with *Methanomicrobia*, became abundant with depth, ranging from 0.5 to 18.9%, 0.3 to 34.5% and 1 to 7.2 and 1.7 to 4.9% of total eukaryotic and prokaryotic sequences on average from 0.5 m to 17m, in 2014 and 2015.

In contrast, during the non-stratified period, *Arthrospira* clearly dominated the total assemblage from the surface to 11m depth, with abundances representing 48% of the total sequences on average, followed by *Picocystis* (13.9% of total sequences) and *Woesearchaeota* (representing 12.6% of total sequences). Other phyla were less represented in this 0 to 11m zone, such as Archaea\_ *WSA2* from WCHA1-57 and unknown class, *Bacteroidia*, *Clostridia*, *Alphaproteobacteria* and eukaryotic *Jakobida*. In deeper waters (close to 15 and 17m) a shift was observed with a decrease in *Arthrospira* associated with

an increase of *Clostridia* and *Bacteroidia*, but also in Archaea affiliated with WSA2 and *Methanomicrobia* (Figure 4).

## Discussion

The present work constitutes the first characterization of the whole microbial assemblage associated with the tropical lake Dziani Dzaha, by including both prokaryotic (Archaea and Bacteria) and eukaryotic compartments. The use of high-throughput Illumina sequencing, through two consecutive years and during stratified and non-stratified periods, gave the opportunity to fully describe microbial taxonomic groups inhabiting a simple functional microbial network where most grazers were absent.

Our study provided new insights into the biodiversity of microbial communities at three different scales including diversity (i) of local communities ( $\alpha$ -diversity), (ii) between communities ( $\beta$ -diversity) and (iii) of the whole system ( $\gamma$ -diversity). We showed that  $\alpha$ -*LocalCommunities* contribution to  $\gamma$ -diversity was important (about 76.5%) outweighing  $\beta$ -*InterSeason* and  $\beta$ -*InterDepth*, thus supporting the idea of consistently high diversity across local microbial communities (OTU richness =  $301 \pm 37$  OTUs on average). However,  $\beta$ -diversity represented a variable part of  $\gamma$ -diversity depending on the domain of life and the type of matrix considered (i.e. presence-absence vs abundance matrix). A striking example was the difference in the contribution of  $\beta$ -*InterDepth* to  $\gamma$ -diversity observed in eukaryotic communities with presence-absence (32.2%, Table 2) versus abundance data (2.1%). This reflected the fact that, although the list of detected OTUs might change between depths, the abundance distribution across these OTUs at each depth was so skewed toward a single OTU (*Picocystis salinarum*), that the communities could almost be considered as composed

282 of a single OTU (*cf.* low  $\gamma$ -diversity estimates for Eukaryota in Figure 3. B, C and D). On  
283 the other hand, for prokaryotic communities, change in the identity of detected species was  
284 relatively small compared to the total diversity (*i.e.*  $\beta$ -diversity represented 20.2% and  
285 14.6% of  $\gamma$ -diversity with presence-absence data in Archaea and Bacteria, respectively).  
286 However, the distribution of abundance among the OTUs varied greatly according to depth  
287 and season (*i.e.* contribution of  $\beta$ -diversity was 34.9% and 31.6% for Archaea and Bacteria,  
288 respectively). Overall, these trends suggested that while eukaryotic communities were  
289 relatively more variable in their composition, prokaryotic communities were more dynamic  
290 in terms of changes in OTUs abundance across season and depth. Moreover, the diversity  
291 decomposition according to the season revealed that  $\gamma$ -diversity was higher during the  
292 stratified period, especially for Bacteria, which is in accordance with the presence of  
293 environmental clines in the water column. In fact, these clines generated different  
294 ecological niches through changes in H<sub>2</sub>S, O<sub>2</sub> or pH, and ultimately allowed higher  
295 communities differentiation. Some differences in  $\gamma$ -diversity were also observed according  
296 to depth, with decreasing bacterial and increasing archaeal  $\gamma$ -diversity. Those results were  
297 confirmed through the analysis of community composition along the depth profile during  
298 the stratified period compared to the non-stratified one. Indeed, the structure and  
299 composition of microbial assemblages were highly influenced by the sampling year, the  
300 season and the depth considered, and thus ultimately by the physico-chemical structure of  
301 the water column. During the stratified period, the community composition differed  
302 between surface water samples (0.5m and 1m depth) and water samples from 2.5 to 11m,  
303 suggesting that the oxic zone associated with the seasonal chemocline at 2m impacted the  
304 community structure for both prokaryotes and microbial eukaryotes. Moreover, a shift in  
305 bacterial community structure was also reported at the permanent chemocline situated at



14m-depth. Interestingly, during the non-stratified period the community composition also followed the physico-chemical structure of the water column. It was homogeneous along most of the water column, shifting only at the 14m depth permanent chemocline. Overall, prokaryotic communities composition were mainly influenced by depth while eukaryotic ones were mostly influenced by seasons.

As observed in other soda lakes, photosynthetic organisms dominated the microbial assemblage (Grant and Jones, 2016; Krienitz and Schagerl, 2016), particularly in 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 formed 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 *Arthrospira* strains were affiliated to *Arthrospira fusiformis* and *Picocystis* strains to *Picocystis salinarum* (Cellamare *et al.*, Submitted). In lake Dziani Dzaha, *Arthrospira fusiformis* shows an unusual straight morphotype suggesting that it might be adapted to the peculiar environmental conditions identified in this lake. This is also consistent with the retrieval of 15.2% of total sequences on average corresponding to *Picocystis salinarum*, although the lake was considered as dominated in biomass by Cyanobacteria (Leboulanger *et al.*, 2017). *P. salinarum* and *A. fusiformis* are highly productive primary producers 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 Bacteroidetes ML635J-40 aquatic



group while methane was biologically produced (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 the *Methanomicrobia* is strongly dominated by the genus *Methanocalculus*. In addition, the enrichment in *Methanocalculus* with depth is concomitant to the enrichment in Bacteroidetes affiliated with *Bacteroidia*\_ML635J-40 aquatic group. This group has been formerly identified in soda lakes and alkaline environments such as the Mono lake (Humayoun *et al.*, 2003), Magadi lake (Baumgarte, 2003) and the Lonar crater lake (Wani *et al.*, 2006). Previous work conducted on soda lake sediments reported the presence of the genus *Methanocalculus* responsible for hydrogenotrophic methanogenesis able to use both CO<sub>2</sub> and formate to produce CH<sub>4</sub> in highly saline conditions (Zhilina *et al.*, 2013; Sorokin, Abbas, Merkel, *et al.*, 2015). Syntrophic acetate-dependent methanogenesis has been reported in a co-culture of a novel high-salt tolerant and obligatory syntrophic member of *Clostridiales* and *Methanocalculus natrophilus* (Sorokin, Abbas, Geleijnse, *et al.*, 2015). Our results clearly showed that multi-domain metabarcoding approaches could reveal biological processes observed in natural ecosystems that also occurred in experimental conditions.

In addition to microorganisms responsible for organic matter recycling, sulfur cycle related organisms were also retrieved in the water column of lake Dziani Dzaha. Firmicutes represent an important fraction of the relative abundance (*i.e.* number of sequences) in lake Dziani Dzaha, whereas Deltaproteobacteria were far less represented and can be considered as almost absent. Among Firmicutes and particularly Clostridiales, the main family represented in the lake Dziani Dzaha was *Syntrophomonadaceae*, which is affiliated with the unknown genera *Dethiobacter*, *Alkaliphilus* or *Candidatus contubernalis*. Recent work

succeeded in the isolation and characterization from soda lake sediments (Mongolia) of a *Dethiobacter alkaliphilus* strain which is anaerobic, sulfidogenic and chemolithotrophic (Melton *et al.*, 2017); this strain is phylogenetically close to *C. contubernalis* (also isolated from a soda lake in Russia (Zhilina *et al.*, 2005)). Among the later candidate division, *C. contubernalis alkalaceticum* was isolated and demonstrated as one partner of the sulfidogenic association with a hydrogenotrophic, alkaliphilic sulfate-reducing *Desulfonatronum* and/or *Desulfonatovibrio* (Zhilina *et al.*, 2005). In this syntrophic association, *C. contubernalis alkalaceticum* is able to oxidize acetate (an important terminal product formed and accumulated in the course of fermentation of organic matter by the anaerobic microbial community), ethanol, serine or fructose. Previous studies demonstrated the competition potentially occurring between methanogenic and sulfidogenic microorganisms (Visser *et al.*, 1996). In natural conditions, and in the presence of non-limited levels of sulfate, methanogens are generally poor competitors compared with sulfato-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 (3.5 mM), that is above the 2m deep seasonal chemocline during the stratified period and above the 14m depth permanent chemocline during non-stratified 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 sulfato-reducers microorganisms, such as *Deltaproteobacteria*. The sometimes high concentration of  $H_2S$  compared to sulfate in the anoxic part of the water column during stratified seasons might be due to fermentative pathways performed by heterotrophic microorganisms using R-SH-

enriched organic matter or alternative processes involved in sulfur cycle in this lake such as reduction of elemental sulfur.

Other less documented phyla were detected in lake Dziani Dzaha and could represent a gene reservoir for undeciphered functional diversity. Indeed, Woesearchaeota DHVEG-6 were initially described in anoxic environments such as saline and sediments habitats (Dojka *et al.*, 1998; Großkopf *et al.*, 1998) and were recently demonstrated as inhabiting surface waters of oligotrophic alpine lakes, suggesting a wide range of environmental adaptations (Ortiz-Alvarez and Casamayor, 2016). Moreover, a recent study showed that Woesearchaeota probably encompasses two phyla, splitted into *Pacearchaeota* and Woesearchaeota (Castelle *et al.*, 2015). Genome analysis of the Woesearchaeota AR20 revealed the absence of most of the core biosynthetic pathways, suggesting a parasitic or symbiotic lifestyle for this microorganism (Castelle *et al.*, 2015). Thus it could be interesting to build ecological interaction networks to investigate the potential consortia of OTUs affiliated with Woesearchaeota along with their putative bacterial hosts. Other partial genomes affiliated with *Pacearchaeota* (AR13) and *Woesearchaeota* (AR3 and 4) are predicted to have saccharolytic and fermentative lifestyle, consistent with environmental conditions retrieved in the water column of lake Dziani Dzaha. Another less abundant group, named WSA2 and belonging to Euryarchaeota, was also identified in the water column, and was recently attributed with a peculiar methanogenic metabolism by metagenomes analyses (Nobu *et al.*, 2016). Indeed, these authors reconstructed eight draft-genomes and revealed the presence of classical Mcr protein (methylated thiol coenzyme M methyltransferase) with an absence of conventional CO<sub>2</sub> reduction to CH<sub>4</sub> and acetyl-CoA synthase pathway, indicating that neither CO<sub>2</sub> nor acetate can serve as substrates. Thus WSA2 may only be capable of catabolism through the demethylation of methylated thiols

for CH<sub>4</sub> generation. Those Archaea possess neither carbon fixation modules nor type I coenzyme M indicating that they might require organic carbon sources to grow in a heterotrophic way; however, this carbon way is very rare for methanogens, suggesting that WSA2 might cooperate with other active methanogens. Taken together, these features are congruent with a lifestyle adapted to organic-rich anaerobic environments, such as wastewater treatment sludge (Wilkins *et al.*, 2015) or lake Dziani Dzaha water column, where fermentation and methanogenesis might produce the required carbon sources, amino acids, ammonia and putatively free coenzyme M.

Interestingly, in this ecosystem, the microbial eukaryotic compartment is also characterized by the presence of some taxa affiliated with bacterivorous flagellates like *Jakoba jakobida*, classically retrieved in marine and hypersaline ecosystems (Rodríguez-Ezpeleta *et al.*, 2007). Some other taxa were affiliated with ciliates, such as *Spirotrichea*. Taken together, our results suggest that in this extreme ecosystem the trophic network can be clearly defined as microbial, but is not exclusively composed of prokaryotes. In this microbial trophic network, Eukaryota might play important roles by controlling prokaryotic and *Picocystis salinarum* communities through grazing pressure. Moreover, we could certainly explain the ecological success of *Arthrospira fusiformis* by the absence of metazoan predators, like shrimps or flamingos, characteristic of other hypersaline ecosystems (Krienitz and Schagerl, 2016).

## Conclusions

Soda lakes are extreme environments where highly adapted microbial communities flourish. Understanding their microbial ecology is key in providing insights into ecosystem

functioning, resilience and stability. The present work is the first to assess, with an in-depth 3-domains-of-life metabarcoding approach, how total microbial diversity is impacted by season and inter-annual variations, across a vertical gradient of the water column. By decomposing biodiversity at fine scale in lake Dziani Dzaha, we highlighted that richness and diversity of Bacteria, Archaea and Eukaryota are differently impacted by the physico-chemical structure of the water column. We showed that eukaryotic communities were relatively more variable in their composition compared with prokaryotic communities that were more dynamic in terms of changes in OTUs abundance across season and depth. Finally, we identified some microbial classes, or even taxa, that could be related to biogeochemical processes occurring in lake Dziani Dzaha and that would need to be confirmed experimentally in the future.

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## Experimental Procedures

### *Study site, sampling, and environmental parameters*

Lake Dziani Dzaha is an alkaline and saline lake situated on the Mayotte Island (Western Indian Ocean). Its surface is 0.24 km<sup>2</sup> with the mean depth about 2.9 meters except the eastern part of the lake characterized by a pit reaching a maximum depth of 18 m. The elevation of the lake surface is close to the average sea level. This lake occupies a volcanic crater and is characterized by an important salinity (ranging from 34 to 71 psu), a very high alkalinity (0.23 mol/L), and a permanent green color. Four surveys were conducted in order to cover both annual and seasonal variations in environmental conditions. Two contrasted seasons were sampled: the stratified period (April, end of rainy season) and the non-stratified period (October-November, end of the dry season), over two consecutive years, (2014 and 2015). Water samples were collected along a depth profile located at the deepest point of the lake (0.5m, 1m, 2.5m, 5m, 11m, 15m and 17m depth), using a horizontal 1.2L Niskin bottle. Water samples were processed on return to the field laboratory within 2 hours. Vertical profiles for pH, dissolved O<sub>2</sub>, temperature and conductivity were taken using either a MPP350 probe connected to a Multi 350i data logger (WTW GmbH) or a YSI 6600 probe. The salinity was calculated using conductivity measured in the vertical profiles.

### *DNA extraction and Illumina sequencing*

Because of clogging effect, a prefiltration of water subsample (20mL) through 3µm pore-size polycarbonate filters (Millipore) 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 3µm and 0.2µm filters, using the Power Water DNA isolation kit (MoBio Laboratories) as described by the manufacturer for maximum yield. The DNA quality was checked by 1% (w/v) agarose gel electrophoresis and quantified using NanoDrop. Amplification of the V3-V5 region of the 16S rRNA genes was performed 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. Amplification of the V4 region of the 18S rRNA genes was performed in triplicate using the universal primer 515F (Caporaso *et al.*, 2011) and the eukaryotic primer 951R (TTG-GYR-AAT-GCT-TTC-GC). Additionally, a DNA extraction was carried out without any biological matrix and considered as a negative control to evaluate ambient contaminations. High-throughput sequencing was achieved after pooling the PCR-triplicates and a multiplexing step, using a HiSeq Rapid Run 300bp 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 3µm and 0.2µm filters were pooled and further merged with a maximum of 10% mismatches in the overlap region using FLASH (Magoč and Salzberg, 2011). Denoising procedures consisted in discarding reads containing ambiguous bases (N) or outside the range of expected length (*i.e.* 450 to 580 bp for bacterial 16S rRNA, 370 to 580 bp for archaeal 16S rRNA, and 250 to 420bp eukaryotic 18S rRNA). After dereplication, sequences were clustered using SWARM (Mahé *et al.*, 2014) that uses 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 cured for metazoans 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 OTUs identified from the control samples were removed and samples were randomly resampled to 41,044, 39,960 and 10,868 sequences, for *Bacteria*, *Archaea* and *Eukaryota*, respectively, in order to allow comparisons.

### **Statistical analyses**

For each domain of life (*Bacteria*, *Archaea* and *Eukaryota*), we tested the effect of environmental factors (year, season and depth) on the composition of microbial communities using non-parametric permutation-based multivariate analysis of variance (PERMANOVA, function *adonis* in R package *vegan*, (Anderson, 2001)) on abundance-based (Bray-Curtis) dissimilarity matrices. We also realized an ordination of the samples using 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 ( $\gamma$ -diversity) into the sum of inter-season differences, inter-depth differences and diversity in local communities with:  $\gamma\text{-diversity} = \beta\text{-InterSeason} + \beta\text{-InterDepth} + \alpha\text{-LocalCommunities}$ . The approach is presented in greater details 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 provides diversity estimates expressed in equivalent number



of species (Jost, 2007) and tend to be influenced by highly abundant species when abundances are taken into account. In a second step, we decomposed the total diversity ( $\gamma$ -diversity) along the depth profile and for each season separately into the diversity in local communities and differences across communities:  $\gamma$ -diversity =  $\beta$ -diversity +  $\alpha$ -diversity. This decomposition was performed on abundance data.

**Data deposition**

The sequence data generated in this study were deposited here: <http://www.ebi.ac.uk/ena/data/view/PRJEB24947>.

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## Figures and Tables Legends

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

Salinity (psu), temperature (°C), pH, O<sub>2</sub> (%) and H<sub>2</sub>S (μM) profiles were recorded during the stratified periods in 2014 and 2015, and during the non-stratified periods in 2014 and 2015.

### Figure 2. Differences in the structure of microbial communities

NMDS ordination performed on Bray-Curtis dissimilarity matrices. The legend is presented in the bottom right corner of the panel. The size of circles indicated the depth. Blue circles represented the stratified periods and orange ones the non-stratified periods. Black-surrounded circles reported the 2014-year while non-surrounded circles represented 2015.

### Figure 3. Additive decomposition of microbial diversity

Decomposition of total diversity ( $\gamma$ -Diversity) estimated using Rao' quadratic entropy into local communities diversity ( $\alpha$ -LocalCommunities) and differences between communities ( $\beta$ -InterSeason and  $\beta$ -InterDepth). This was done with presence-absence data (A) and using OTU relative abundances (B). Decomposition of total diversity ( $\gamma$ -Diversity) estimated using Rao' quadratic entropy on abundance data into local communities diversity ( $\alpha$ ) and differences between communities ( $\beta$ ), 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 non-stratified periods. In the legend on the right of the panel, Archaea were mentioned as A\_, Bacteria as B\_, and Eukaryota as E\_.

### **Supplementary Figure 1. Additive decomposition of microbial diversity**

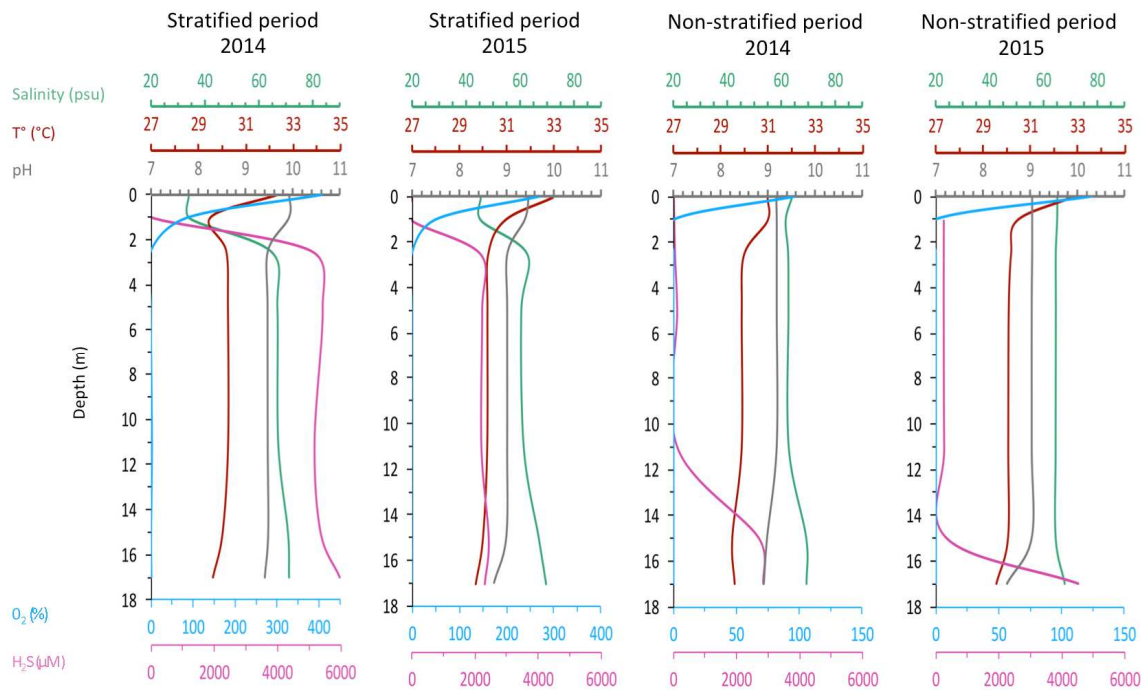
Decomposition of total diversity ( $\gamma$ -Diversity) estimated using Rao' quadratic entropy on presence-absence data into local communities diversity ( $\alpha$ ) and differences between communities ( $\beta$ ), along the depth profile (C) and between seasons (D).

### **Table 1. Effect of environmental factors on microbial communities composition**

The differences between groups were tested using PERMANOVA analysis on Bray-Curtis dissimilarity matrices.

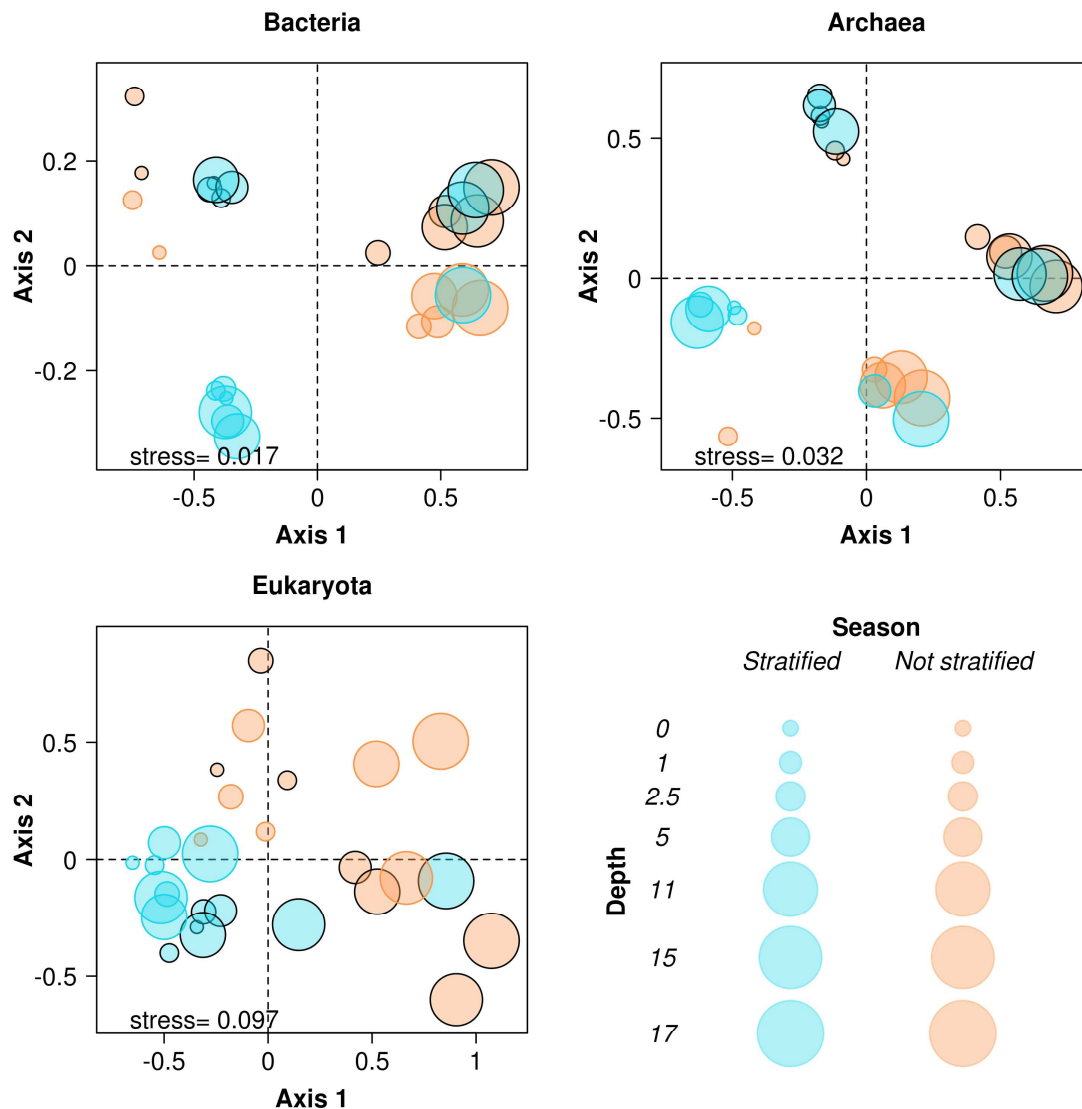
### **Table 2. Additive decomposition of microbial diversity**

$\gamma$  -Diversity was estimated using Rao' quadratic entropy and the decomposition was done according to Escalas *et al.* (2017). Number in parenthesis corresponds to the percentage of the  $\gamma$ -Diversity represented by each component.



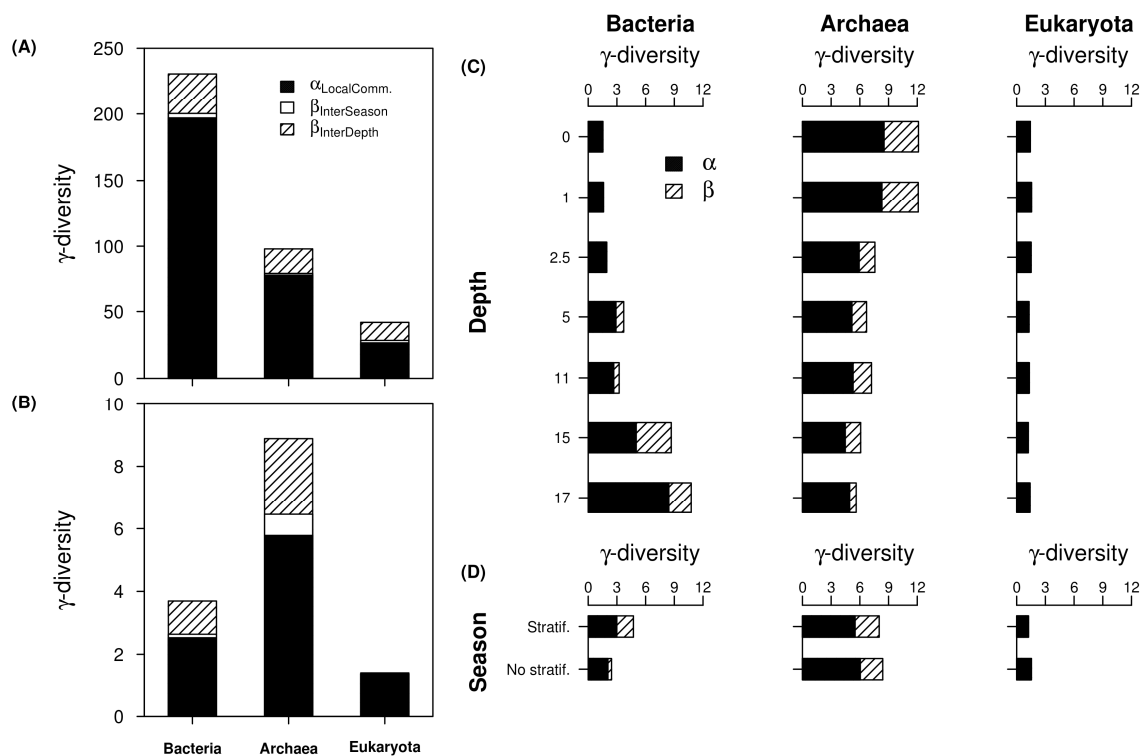
**Figure 1. Vertical profiles of environmental parameters recorded during the 4 sampling campaigns**

Salinity (psu), temperature (°C), pH, O<sub>2</sub> (%) and H<sub>2</sub>S (μM) profiles were recorded during the stratified periods in 2014 and 2015, and during the non-stratified periods in 2014 and 2015.



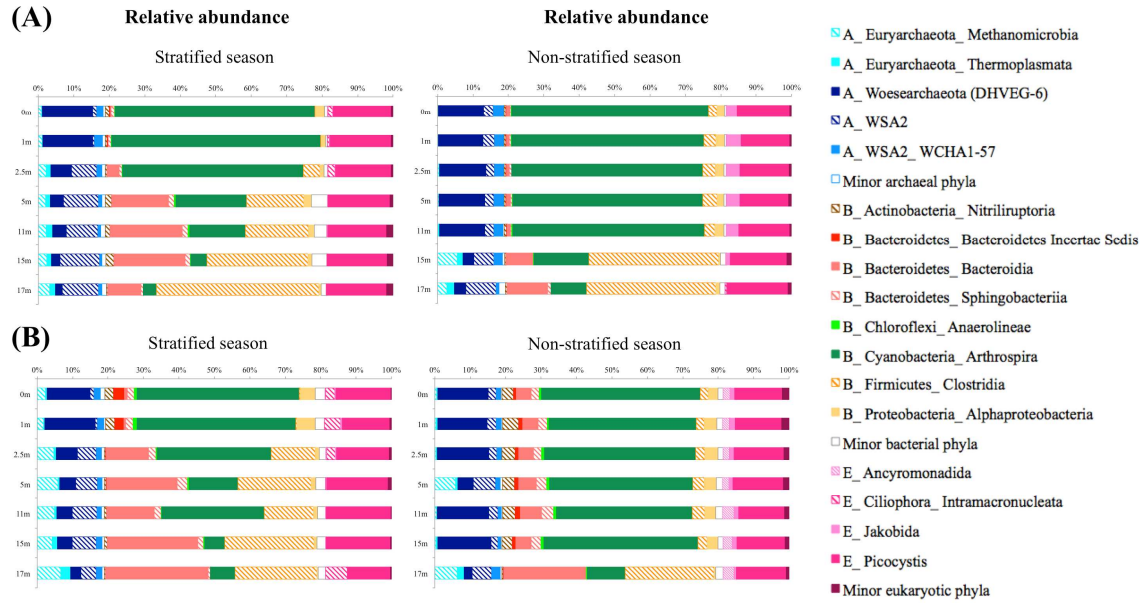
**Figure 2. Differences in the structure of microbial communities**

NMDS ordination performed on Bray-Curtis dissimilarity matrices. The legend is presented in the bottom right corner of the panel. The size of circles indicated the depth. Blue circles represented the stratified periods and orange ones the non-stratified periods. Black-surrounded circles reported the 2014-year while non-surrounded circles represented 2015.



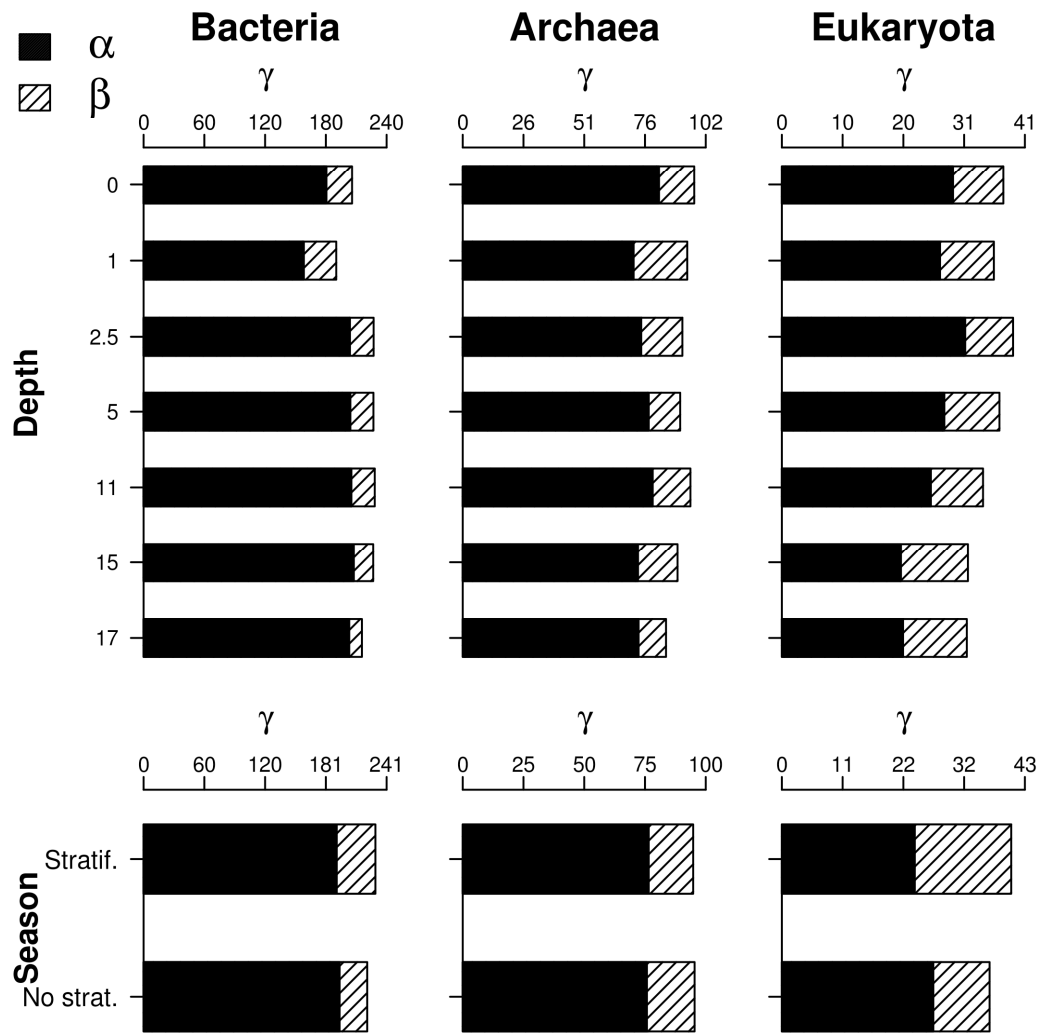
**Figure 3. Additive decomposition of microbial diversity**

Decomposition of total diversity ( $\gamma$ -Diversity) estimated using Rao' quadratic entropy into local communities diversity ( $\alpha$ -LocalCommunities) and differences between communities ( $\beta$ -InterSeason and  $\beta$ -InterDepth). This was done with presence-absence data (A) and using OTU relative abundances (B). Decomposition of total diversity ( $\gamma$ -Diversity) estimated using Rao' quadratic entropy on abundance data into local communities diversity ( $\alpha$ ) and differences between communities ( $\beta$ ), 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 non-stratified periods. In the legend on the right of the panel, Archaea were mentioned as A\_, Bacteria as B\_, and Eukaryota as E\_.



**Supplementary Figure 1. Additive decomposition of microbial diversity**

Decomposition of total diversity ( $\gamma$ -Diversity) estimated using Rao' quadratic entropy on presence-absence data into local communities diversity ( $\alpha$ ) and differences between communities ( $\beta$ ), along the depth profile (C) and between seasons (D).

Table 1

Factor	Bacteria			Archaea			Eukaryota		
	F.value	R <sup>2</sup>	p.value	F.value	R <sup>2</sup>	p.value	F.value	R <sup>2</sup>	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



Table 2

	<i>γ-diversity</i>	<i>α-LocalComm.</i>	<i>β-InterSeason</i>	<i>β-InterDepth</i>
<i>Presence-absence</i>				
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)
<i>Abundance</i>				
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)