



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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1 **Spatio-temporal variations of microbial diversity across the**
2 **three domains of life in a tropical soda lake**
3 **(Dziani Dzaha, Mayotte Island)**
4

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24 Bacteria

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

26 **Originality-Significance Statement**

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

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48

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.

68

69 **Introduction**

70 Soda lakes are extreme environments widely distributed on Earth, mainly in arid
71 and semi-arid regions, such as the Rift Valley in Africa, United-States, Canada, Iran, China
72 or Mexico and in dry steppe regions like in Russia (Sorokin *et al.*, 2014; Grant and Jones,
73 2016). The name “soda” is derived from the chemical composition of their waters, which
74 are considered alkaline because of the high concentration of dissolved inorganic carbon and
75 the presence of sodium as the major cation, so that upon nearly complete evaporation
76 Na_2CO_3 (called soda) could precipitate. These characteristics are often associated with
77 elevated pH values (9.5 to 12) and high salinity waters (>50 psu). As a result of such drastic
78 environmental conditions, soda lakes are inhabited by microorganisms adapted to high
79 alkalinity and salinity, unlimited supplies of dissolved inorganic carbon and sometimes low
80 oxygen availability. These adaptations relied on molecular mechanisms (Ochsenreiter *et al.*,
81 2002; Mesbah *et al.*, 2009), as for example modifications of their membrane structure, the
82 use of transporter proteins or the synthesis of osmo-protectants (Sorokin *et al.*, 2014; Grant
83 and Jones, 2016). Previous studies clearly showed that salinity is one of the key
84 environmental factor shaping the composition and structure of aquatic microbial
85 communities on a global scale (Lozupone and Knight, 2007; Canfora *et al.*, 2014). As a
86 consequence, the microbial diversity in soda lakes is limited to salt-tolerant specialists that
87 are key players of biogeochemical cycles occurring in those ecosystems (*e.g.* carbon,
88 nitrogen and sulfur cycles).

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

92 notably associated with Cyanobacteria and/or anoxygenic phototrophic Bacteria (*Ballot et*
93 *al.*, 2004; *Lewis et al.*, 2011). The primary productivity can reach up to $10 \text{ gC.cm}^{-2}.\text{day}^{-1}$,
94 delivering fixed carbon to haloalkaliphilic communities, as aerobic and anaerobic
95 chemoorganotrophic Bacteria and Archaea (*Melack and Kilham*, 1974; *Grant et al.*, 1990).
96 Most of the studies investigating microbial communities in soda lakes revealed dense
97 populations of primary producers affiliated with unicellular and filamentous Cyanobacteria,
98 such as the genera *Arthrospira*, *Cyanospira*, *Geitlerinema*, *Leptolyngbya* or *Anabaenopsis*
99 (*Grant and Jones*, 2016; *Krienitz and Schagerl*, 2016). At higher salinity (>50 psu),
100 unicellular green algae like *Picocystis salinarum* or *Dunaliella viridis* were also retrieved
101 (*Krienitz and Schagerl*, 2016). Studies focusing on prokaryotes reported the dominance of
102 Alphaproteobacteria, Gammaproteobacteria, Firmicutes and Bacteroidetes (*Dimitriu et al.*,
103 2008; *Sorokin et al.*, 2014; *Aguirre-Garrido et al.*, 2016), associated with abundant
104 Euryarchaeota in hypersaline soda lakes (*Ochsenreiter et al.*, 2002; *Mesbah et al.*, 2007;
105 *Vavourakis et al.*, 2016). Although salt-adapted organisms derive from all the three
106 domains of life, most studies investigated the prokaryotic fraction of the microbial
107 assemblage (*Ghai et al.*, 2011; *Ventosa et al.*, 2015; *Kambura et al.*, 2016), while very few
108 considered the eukaryotic diversity associated with those ecosystems (*Krienitz et al.*, 2012).
109 Over the past decade, high-throughput sequencing technologies have been successful in
110 describing relative low-diversity ecosystems such as extreme environments, by giving
111 snapshots of the overall community structure and gaining new insights into uncultivated
112 microorganisms genome (*Ghai et al.*, 2011; *Narasingarao et al.*, 2012; *Vavourakis et al.*,
113 2016).

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

116 origin makes it a peculiar soda lake, which water composition has been modified by
117 biological and geothermal activities up to current conditions: extremely high alkalinity
118 (0.23 mol/L), elevated salinity (ranging from 34 to 71 psu) and pH (9.1 to 9.4). This lake
119 presents extremely and constantly high rates of organic matter production and
120 mineralisation (Leboulanger *et al.*, 2017). The alternation of a rainy (Nov.-Apr.) and a dry
121 season (June-Oct.) induces a saline and seasonal stratification of the water column, caused
122 by the dilution of the upper layer by rainfalls, which creates a chemocline at around 2.25
123 meter depth. This stratification usually persists until August, when a combination of
124 temperature decrease and salinity increase by evaporation lead to an increase of the density
125 of the surface layer. The water column is permanently anoxic below about 1 to 2m depth,
126 irrespectively of the water column stratification, with rare episodes when anoxia reaches
127 the surface of the water column. A second chemocline was systematically identified in the
128 pit at a depth around 14m, below which $\text{H}_2\text{S}/\text{HS}^-$, $\text{NH}_4^+/\text{NH}_3$ and CH_4 concentrations were
129 systematically high and reached values of up to 5, 4 and 1 mM, respectively. In this
130 ecosystem, the aquatic food web is very simple and characterized by permanent
131 cyanobacterial blooms (mainly *Arthrospira*), high prokaryotic biomass depending on the
132 season, and an absence of metazoans apart from endemic flies (Leboulanger *et al.*, 2017).
133 Thus, this singular ecosystem can be considered as an excellent microbial model to
134 examine the genetic structure and dynamics of microbial communities. Through a multi-
135 seasonal survey conducted across two consecutive years (2014; 2015) at different depths of
136 the water column (from 0 to 17m), we aimed at testing whether the microbial diversity
137 inhabiting the lake Dziani Dzaha is stable in time and space. By providing an exhaustive
138 and simultaneous characterization of both prokaryotic (including Archaea and Bacteria) and
139 eukaryotic microorganisms using Illumina metabarcoding sequencing, the present work is

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

142 **Results**

143 *Physico-chemical characteristics of the water column*

144 This study was conducted in the pit zone of the lake, characterized by the maximum depth
145 registered (18m). The surface layer of the water column was oxic down to 2m while the
146 conditions were completely anoxic all year long below that limit (Figure 1). The water
147 column was characterized by a permanent chemocline at 14m, that isolated the deepest
148 water layer, and by a seasonal one that separated the intermediate water layer from the
149 surface layer. The physico-chemical structure of the water column thus alternated
150 seasonally with two layers of water in the non-stratified season versus three during the
151 stratified season.

152 The apparition of a seasonal chemocline and a stratified state, occurring at
153 approximately 2m was due to changes in rainfall regime at the beginning of the rainy
154 season (Leboulanger *et al.*, 2017). The rainfall effect resulted in a dilution of the upper
155 layer of the water column, reducing its salinity (34 to 45 psu, Apr. 2014 and Apr. 2015
156 respectively) and resulting in a haline stratification at *ca.* 2m depth (Figure 1). Below this
157 halocline, the salinity rises up to approximately 65 psu in the intermediate layer, the
158 temperature stabilizes around 30.2 °C and the H₂S/HS⁻ concentrations reach up to 5900 and
159 2500 μM, in 2014 and 2015, respectively.

160 A non-stratified state of the water column was also recorded, with homogeneous
161 concentrations of H₂S (mean 187 μM), temperature (mean 31.6 °C), salinity (mean 64.2
162 psu) and pH from surface to the deeper chemocline (14m).

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

166

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

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

172 Depth was the main factor influencing the composition of prokaryotic communities,
173 notably for Bacteria for which it explained 47% of variation in community composition
174 (Table 1). For Archaea, the influences of season and year on community composition were
175 closer to the effect of depth (15, 12 and 21% of variation, respectively, Table 1). On the
176 contrary, the composition of eukaryotic microbial communities was mostly influenced by
177 the sampling season and to a lesser extent by year and depth (35, 12 and 6% of variation,
178 respectively, Table 1). The differences in the relative importance of time and space effects
179 on microbial communities composition are presented in Figure 2.

180 When considering all samples together, the richness in normalized datasets
181 consisted in 242 bacterial, 109 archaeal and 87 eukaryotic OTUs. When pooling the three
182 domains together, total microbial richness ranged from 221 to 359 OTUs per sample, with
183 an average of 301 ± 37 OTUs. Then we used a diversity partitioning approach to explore the
184 multiple facets of microbial diversity across multiple scales. We estimated the total
185 microbial diversity (*i.e.* γ -diversity) associated with each domain of life using Rao'
186 quadratic entropy (Table 2). When using presence-absence data, we found that bacterial

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

196 An additive decomposition of γ -diversity was used to determine whether the total
197 observed diversity arose from the average diversity within communities (*i.e.*
198 $\alpha_{LocalCommunities}$) or the differences between communities across seasons (*i.e.* $\beta_{InterSeason}$) and
199 across depth (*i.e.* $\beta_{InterDepth}$) (Figure 3.A and B). Overall, local communities represented
200 most of the total diversity ($\alpha_{LocalCommunities} > 76.5\%$ on average). When using presence-
201 absence data, we observed that β -diversity ($\beta_{InterSeason} + \beta_{InterDepth}$) represented a higher
202 proportion of γ -diversity in eukaryotic communities (36.6%), while it contributed in a
203 smaller proportion in archaeal (20.2%) and bacterial (14.6%) communities. When using
204 abundance data, we observed higher contributions of β -diversity to γ -diversity for Archaea
205 (34.9%) and Bacteria (31.6%) while the value for Eukaryota dropped to 3%. Further, we
206 found that differences along the depth profile ($\beta_{InterDepth}$) contributed more to the total
207 diversity than inter-season differences ($\beta_{InterSeason}$), with 20.4 and 3.1% of γ Diversity,
208 respectively.

209 The present work showed that the diversity distribution along the depth profile
210 differed greatly between the three domains of life (Figure 3.C and D). The bacterial

211 diversity increased with depth, from very low values near the surface (0 to 2.5m) to a sharp
212 increase at the bottom of the water column (15 to 17m). Thus the distribution of abundance
213 between OTUs in these communities was more balanced as the depth increased. The
214 decomposition of diversity reveals that there was a very low degree of differentiation
215 between communities at the top of the water column while deeper communities tended to
216 be more variable. On the contrary, archaeal communities exhibited maximum diversity in
217 the first meter and a sharp drop of diversity below this depth. This reverse pattern
218 corresponded to the increasing dominance of a smaller number of archaeal taxa with
219 increasing depth. For archaeal communities, we observed a higher β -diversity in the upper
220 layers but there was always a certain degree of differentiation from a given depth across
221 time (year and season). Eukaryotic communities showed a very low and constant diversity
222 along the depth profile (*i.e.* due to the hyper dominance of *Picocystis* OTUs). The
223 proportion of eukaryotic β -diversity observed was strikingly lower than for other domains
224 and this was the case at every depth.

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

233

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

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

238 During the stratified period the bacterial assemblage was dominated by
239 Cyanobacteria affiliated with *Arthrospira fusiformis* from 0.5m to 2.5m-depth, representing
240 on average 48.1% of the total eukaryotic and prokaryotic sequences in 2014 and 2015
241 (Figure 4). In this surface layer, Eukaryota affiliated with *Picocystis salinarum* (about
242 15.5% of total sequences) and Archaea affiliated with *Woesearchaeota* (from 0.5m to 1m,
243 about 13.7% of total sequences) were also present. The number of *Picocystis* sequences
244 was constant through the whole water column, while *Arthrospira* and *Woesearchaeota*
245 sequence numbers decreased drastically with depth (falling on average to 5.2% and 2.9% of
246 total sequences, respectively, in the deeper layer, i.e. 15 and 17m). Other microorganisms
247 like Bacteroidetes affiliated with *Bacteroidia*, Firmicutes affiliated with *Clostridia*,
248 Archaea affiliated with *WSA2*, or Euryarchaeota affiliated with *Methanomicrobia*, became
249 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%
250 of total eukaryotic and prokaryotic sequences on average from 0.5 m to 17m, in 2014 and
251 2015.

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

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

261

262 **Discussion**

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

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

282 of a single OTU (*cf.* low γ -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.* β -diversity represented 20.2% and
285 14.6% of γ -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 β -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 γ -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 H₂S, O₂ or pH, and ultimately allowed higher
295 communities differentiation. Some differences in γ -diversity were also observed according
296 to depth, with decreasing bacterial and increasing archaeal γ -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

306 14m-depth. Interestingly, during the non-stratified period the community composition also
307 followed the physico-chemical structure of the water column. It was homogeneous along
308 most of the water column, shifting only at the 14m depth permanent chemocline. Overall,
309 prokaryotic communities composition were mainly influenced by depth while eukaryotic
310 ones were mostly influenced by seasons.

311 As observed in other soda lakes, photosynthetic organisms dominated the microbial
312 assemblage (Grant and Jones, 2016; Krienitz and Schagerl, 2016), particularly in upper
313 layer. In lake Dziani Dzaha, the photosynthetic communities were dominated by
314 Cyanobacteria from the *Arthrospira* genus (also named *Spirulina*) and Eukaryota from the
315 *Picocystis* genus. *Arthrospira* consisted in alkaliphilic filamentous Cyanobacteria, which
316 formed dense masses seasonally or permanently (Ballot *et al.*, 2004; Sili *et al.*, 2012).
317 Interestingly, a recent characterization using a polyphasic approach of strains isolated from
318 lake Dziani Dzaha, reported that *Arthrospira* strains were affiliated to *Arthrospira*
319 *fusiformis* and *Picocystis* strains to *Picocystis salinarum* (Cellamare *et al.*, Submitted). In
320 lake Dziani Dzaha, *Arthrospira fusiformis* shows an unusual straight morphotype
321 suggesting that it might be adapted to the peculiar environmental conditions identified in
322 this lake. This is also consistent with the retrieval of 15.2% of total sequences on average
323 corresponding to *Picocystis salinarum*, although the lake was considered as dominated in
324 biomass by Cyanobacteria (Leboulanger *et al.*, 2017). *P. salinarum* and *A. fusiformis* are
325 highly productive primary producers often reported as numerically abundant in microbial
326 communities of saline habitats, such as soda lakes of the East African Rift Valley (Krienitz
327 *et al.*, 2012) or Mono Lake, USA (Hollibaugh *et al.*, 2001). A recent study conducted on
328 bioreactors inoculated with alkaline sediments fed by *Arthrospira* biomass showed that the
329 produced organic matter was hydrolyzed mainly by Bacteroidetes ML635J-40 aquatic

330 group while methane was biologically produced (hydrogenotrophic pathway) by an
331 archaeal community dominated by *Methanocalculus* (Nolla-Ardèvol *et al.*, 2015). This
332 microbial consortium is highly relevant in the case of lake Dziani Dzaha, where the
333 *Methanomicrobia* is strongly dominated by the genus *Methanocalculus*. In addition, the
334 enrichment in *Methanocalculus* with depth is concomitant to the enrichment in
335 Bacteroidetes affiliated with *Bacteroidia*_ML635J-40 aquatic group. This group has been
336 formerly identified in soda lakes and alkaline environments such as the Mono lake
337 (Humayoun *et al.*, 2003), Magadi lake (Baumgarte, 2003) and the Lonar crater lake (Wani
338 *et al.*, 2006). Previous work conducted on soda lake sediments reported the presence of the
339 genus *Methanocalculus* responsible for hydrogenotrophic methanogenesis able to use both
340 CO₂ and formate to produce CH₄ in highly saline conditions (Zhilina *et al.*, 2013; Sorokin,
341 Abbas, Merkel, *et al.*, 2015). Syntrophic acetate-dependent methanogenesis has been
342 reported in a co-culture of a novel high-salt tolerant and obligatory syntrophic member of
343 *Clostridiales* and *Methanocalculus natrophilus* (Sorokin, Abbas, Geleijnse, *et al.*, 2015).
344 Our results clearly showed that multi-domain metabarcoding approaches could reveal
345 biological processes observed in natural ecosystems that also occurred in experimental
346 conditions.

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

354 succeeded in the isolation and characterization from soda lake sediments (Mongolia) of a
355 *Dethiobacter alkaliphilus* strain which is anaerobic, sulfidogenic and chemolithotrophic
356 (Melton *et al.*, 2017); this strain is phylogenetically close to *C. contubernalis* (also isolated
357 from a soda lake in Russia (Zhilina *et al.*, 2005)). Among the later candidate division, *C.*
358 *contubernalis alkalaceticum* was isolated and demonstrated as one partner of the
359 sulfidogenic association with a hydrogenotrophic, alkaliphilic sulfate-reducing
360 *Desulfonatum* and/or *Desulfonatovibrio* (Zhilina *et al.*, 2005). In this syntrophic
361 association, *C. contubernalis alkalaceticum* is able to oxidize acetate (an important terminal
362 product formed and accumulated in the course of fermentation of organic matter by the
363 anaerobic microbial community), ethanol, serine or fructose. Previous studies demonstrated
364 the competition potentially occurring between methanogenic and sulfidogenic
365 microorganisms (Visser *et al.*, 1996). In natural conditions, and in the presence of non-
366 limited levels of sulfate, methanogens are generally poor competitors compared with
367 sulfato-reducers, especially in marine sediments, while methanogens tend to be the
368 dominant scavengers of hydrogen and acetate in low-sulfate environments (Raskin *et al.*,
369 1996). In lake Dziani Dzaha, sulfate concentrations were relatively low in the surface layers
370 (3.5 mM), that is above the 2m deep seasonal chemocline during the stratified period and
371 above the 14m depth permanent chemocline during non-stratified periods. Additionally,
372 sulfate was not detected below these two chemoclines and H₂S/HS⁻ was present instead.
373 The low to null sulfate concentrations could explain the low occurrence of sulfato-reducers
374 microorganisms, such as *Deltaproteobacteria*. The sometimes high concentration of H₂S
375 compared to sulfate in the anoxic part of the water column during stratified seasons might
376 be due to fermentative pathways performed by heterotrophic microorganisms using R-SH-

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

379 Other less documented phyla were detected in lake Dziani Dzaha and could
380 represent a gene reservoir for undeciphered functional diversity. Indeed, Woesearchaeota
381 DHVEG-6 were initially described in anoxic environments such as saline and sediments
382 habitats (Dojka *et al.*, 1998; Großkopf *et al.*, 1998) and were recently demonstrated as
383 inhabiting surface waters of oligotrophic alpine lakes, suggesting a wide range of
384 environmental adaptations (Ortiz-Alvarez and Casamayor, 2016). Moreover, a recent study
385 showed that Woesearchaeota probably encompasses two phyla, splitted into *Pacearchaeota*
386 and Woesearchaeota (Castelle *et al.*, 2015). Genome analysis of the Woesearchaeota AR20
387 revealed the absence of most of the core biosynthetic pathways, suggesting a parasitic or
388 symbiotic lifestyle for this microorganism (Castelle *et al.*, 2015). Thus it could be
389 interesting to build ecological interaction networks to investigate the potential consortia of
390 OTUs affiliated with Woesearchaeota along with their putative bacterial hosts. Other partial
391 genomes affiliated with *Pacearchaeota* (AR13) and *Woesearchaeota* (AR3 and 4) are
392 predicted to have saccharolytic and fermentative lifestyle, consistent with environmental
393 conditions retrieved in the water column of lake Dziani Dzaha. Another less abundant
394 group, named WSA2 and belonging to Euryarchaeota, was also identified in the water
395 column, and was recently attributed with a peculiar methanogenic metabolism by
396 metagenomes analyses (Nobu *et al.*, 2016). Indeed, these authors reconstructed eight draft-
397 genomes and revealed the presence of classical Mcr protein (methylated thiol coenzyme M
398 methyltransferase) with an absence of conventional CO₂ reduction to CH₄ and acetyl-CoA
399 synthase pathway, indicating that neither CO₂ nor acetate can serve as substrates. Thus
400 WSA2 may only be capable of catabolism through the demethylation of methylated thiols

401 for CH₄ generation. Those Archaea possess neither carbon fixation modules nor type I
402 coenzyme M indicating that they might require organic carbon sources to grow in a
403 heterotrophic way; however, this carbon way is very rare for methanogens, suggesting that
404 WSA2 might cooperate with other active methanogens. Taken together, these features are
405 congruent with a lifestyle adapted to organic-rich anaerobic environments, such as
406 wastewater treatment sludge (Wilkins *et al.*, 2015) or lake Dziani Dzaha water column,
407 where fermentation and methanogenesis might produce the required carbon sources, amino
408 acids, ammonia and putatively free coenzyme M.

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

420

421 **Conclusions**

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

424 functioning, resilience and stability. The present work is the first to assess, with an in-depth
425 3-domains-of-life metabarcoding approach, how total microbial diversity is impacted by
426 season and inter-annual variations, across a vertical gradient of the water column. By
427 decomposing biodiversity at fine scale in lake Dziani Dzaha, we highlighted that richness
428 and diversity of Bacteria, Archaea and Eukaryota are differently impacted by the physico-
429 chemical structure of the water column. We showed that eukaryotic communities were
430 relatively more variable in their composition compared with prokaryotic communities that
431 were more dynamic in terms of changes in OTUs abundance across season and depth.
432 Finally, we identified some microbial classes, or even taxa, that could be related to
433 biogeochemical processes occurring in lake Dziani Dzaha and that would need to be
434 confirmed experimentally in the future.

435

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447 **Experimental Procedures**

448 *Study site, sampling, and environmental parameters*

449 Lake Dziani Dzaha is an alkaline and saline lake situated on the Mayotte Island (Western
450 Indian Ocean). Its surface is 0.24 km² with the mean depth about 2.9 meters except the
451 eastern part of the lake characterized by a pit reaching a maximum depth of 18 m. The
452 elevation of the lake surface is close to the average sea level. This lake occupies a volcanic
453 crater and is characterized by an important salinity (ranging from 34 to 71 psu), a very high
454 alkalinity (0.23 mol/L), and a permanent green color. Four surveys were conducted in order
455 to cover both annual and seasonal variations in environmental conditions. Two contrasted
456 seasons were sampled: the stratified period (April, end of rainy season) and the non-
457 stratified period (October-November, end of the dry season), over two consecutive years,
458 (2014 and 2015). Water samples were collected along a depth profile located at the deepest
459 point of the lake (0.5m, 1m, 2.5m, 5m, 11m, 15m and 17m depth), using a horizontal 1.2L
460 Niskin bottle. Water samples were processed on return to the field laboratory within 2
461 hours. Vertical profiles for pH, dissolved O₂, temperature and conductivity were taken
462 using either a MPP350 probe connected to a Multi 350i data logger (WTW GmbH) or a
463 YSI 6600 probe. The salinity was calculated using conductivity measured in the vertical
464 profiles.

465

466 *DNA extraction and Illumina sequencing*

467 Because of clogging effect, a prefiltration of water subsample (20mL) through 3µm pore-
468 size polycarbonate filters (Millipore) was necessary before collection on 0.2µm pore-size
469 polycarbonates filters (Millipore, pressure <10kPa) and storage at -20°C until nucleic acid

470 extraction. DNA extractions were conducted separately on both 3 μ m and 0.2 μ m filters,
471 using the Power Water DNA isolation kit (MoBio Laboratories) as described by the
472 manufacturer for maximum yield. The DNA quality was checked by 1% (w/v) agarose gel
473 electrophoresis and quantified using NanoDrop. Amplification of the V3-V5 region of the
474 16S rRNA genes was performed in triplicate for *Bacteria* and *Archaea*, using the universal
475 primers 357F (Schuurman *et al.*, 2004) and 926R (Walters *et al.*, 2016) and 519F and 915R
476 (Hugoni *et al.*, 2015), respectively. Amplification of the V4 region of the 18S rRNA genes
477 was performed in triplicate using the universal primer 515F (Caporaso *et al.*, 2011) and the
478 eukaryotic primer 951R (TTG-GYR-AAT-GCT-TTC-GC). Additionally, a DNA extraction
479 was carried out without any biological matrix and considered as a negative control to
480 evaluate ambient contaminations. High-throughput sequencing was achieved after pooling
481 the PCR-triplicates and a multiplexing step, using a HiSeq Rapid Run 300bp PE technology
482 on an Illumina HiSeq 2500 system (GATC Biotech, Konstanz, Germany).

483

484 ***Sequence processing***

485 Bacterial and archaeal 16S rRNA and eukaryotic 18S rRNA paired-end reads from both
486 3 μ m and 0.2 μ m filters were pooled and further merged with a maximum of 10%
487 mismatches in the overlap region using FLASH (Magoč and Salzberg, 2011). Denoising
488 procedures consisted in discarding reads containing ambiguous bases (N) or outside the
489 range of expected length (*i.e.* 450 to 580 bp for bacterial 16S rRNA, 370 to 580 bp for
490 archaeal 16S rRNA, and 250 to 420bp eukaryotic 18S rRNA). After dereplication,
491 sequences were clustered using SWARM (Mahé *et al.*, 2014) that uses a local clustering
492 threshold. We removed chimeric sequences using VSEARCH (Rognes *et al.*, 2016) but
493 also sequences representing less than 0.005% of the total number of sequences (Bokulich *et*

494 *al.*, 2013) along with singletons. The eukaryotic dataset was cured for metazoans sequences
495 (data not shown). Taxonomic affiliation was performed with both RDP Classifier (*Wang et*
496 *al.*, 2007) and Blastn+ (*Camacho et al.*, 2009) against the 128 SILVA database (*Pruesse et*
497 *al.*, 2007). This procedure was automated in the FROGS pipeline (*Escudié et al.*, 2017).
498 Contaminant OTUs identified from the control samples were removed and samples were
499 randomly resampled to 41,044, 39,960 and 10,868 sequences, for *Bacteria*, *Archaea* and
500 *Eukaryota*, respectively, in order to allow comparisons.

501

502 ***Statistical analyses***

503 For each domain of life (*Bacteria*, *Archaea* and *Eukaryota*), we tested the effect of
504 environmental factors (year, season and depth) on the composition of microbial
505 communities using non-parametric permutation-based multivariate analysis of variance
506 (PERMANOVA, function *adonis* in R package *vegan*, (Anderson, 2001)) on abundance-
507 based (Bray-Curtis) dissimilarity matrices. We also realized an ordination of the samples
508 using NMDS approach on Bray-Curtis dissimilarity matrices (function *metaMDS* in R
509 package *vegan*).

510 We used an additive diversity-partitioning framework (*Belmaker et al.*, 2008) to
511 decompose the total observed diversity and express it as the sum of the diversity observable
512 at various scales. In a first step, we decomposed the total diversity (γ -diversity) into the sum
513 of inter-season differences, inter-depth differences and diversity in local communities with:
514 γ -diversity = β -InterSeason + β -InterDepth + α -LocalCommunities. The approach is
515 presented in greater details elsewhere (*Escalas et al.*, 2013, 2017). This decomposition was
516 performed on diversity estimated using Rao's quadratic entropy on presence-absence and
517 abundance data. This method provides diversity estimates expressed in equivalent number

518 of species (Jost, 2007) and tend to be influenced by highly abundant species when
519 abundances are taken into account. In a second step, we decomposed the total diversity (γ -
520 *diversity*) along the depth profile and for each season separately into the diversity in local
521 communities and differences across communities: γ -*diversity* = β -*diversity* + α -*diversity*.
522 This decomposition was performed on abundance data.

523

524 ***Data deposition***

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

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709 **Figures and Tables Legends**

710

711 **Figure 1. Vertical profiles of environmental parameters recorded during the 4** 712 **sampling campaigns**

713 Salinity (psu), temperature (°C), pH, O₂ (%) and H₂S (μM) profiles were recorded during
714 the stratified periods in 2014 and 2015, and during the non-stratified periods in 2014 and
715 2015.

716

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

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

722

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

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

730

731 **Figure 4. Relative abundance (% of sequences) of bacterial, archaeal and eukaryotic** 732 **phyla**

733 Data were collected in (A) 2014 and (B) 2015 during both stratified and non-stratified
734 periods. In the legend on the right of the panel, Archaea were mentioned as A_, Bacteria as
735 B_, and Eukaryota as E_.

736

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

738 Decomposition of total diversity (γ -Diversity) estimated using Rao' quadratic entropy on
739 presence-absence data into local communities diversity (α) and differences between
740 communities (β), along the depth profile (C) and between seasons (D).

741

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

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

745

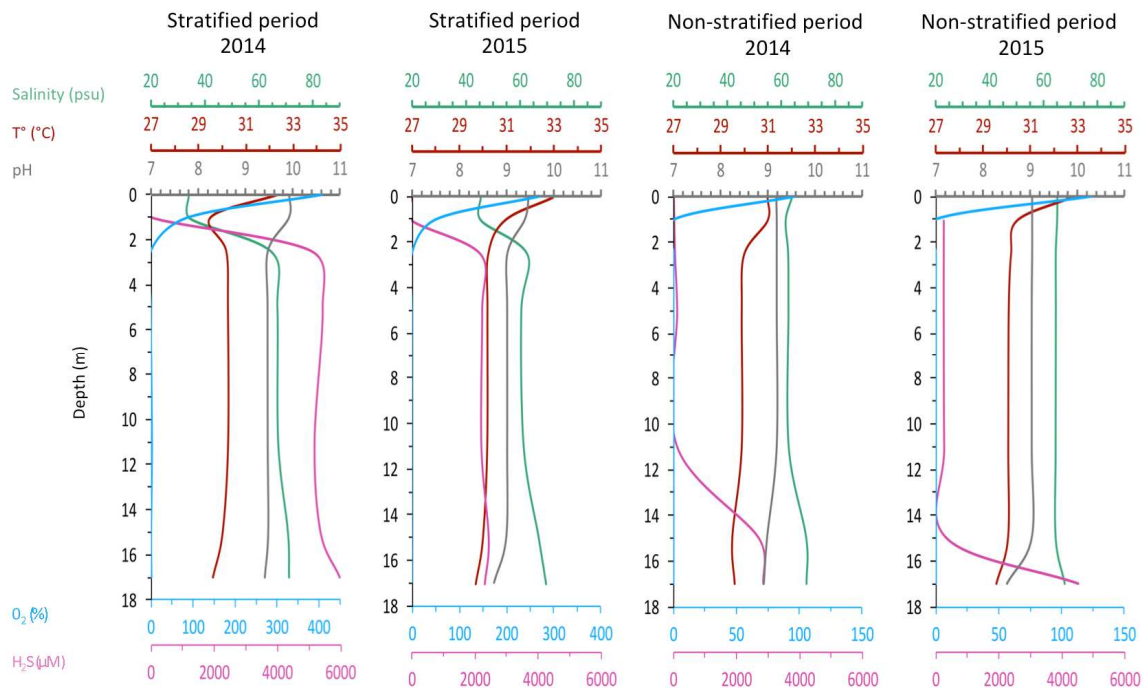
746 **Table 2. Additive decomposition of microbial diversity**

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

750

751

752



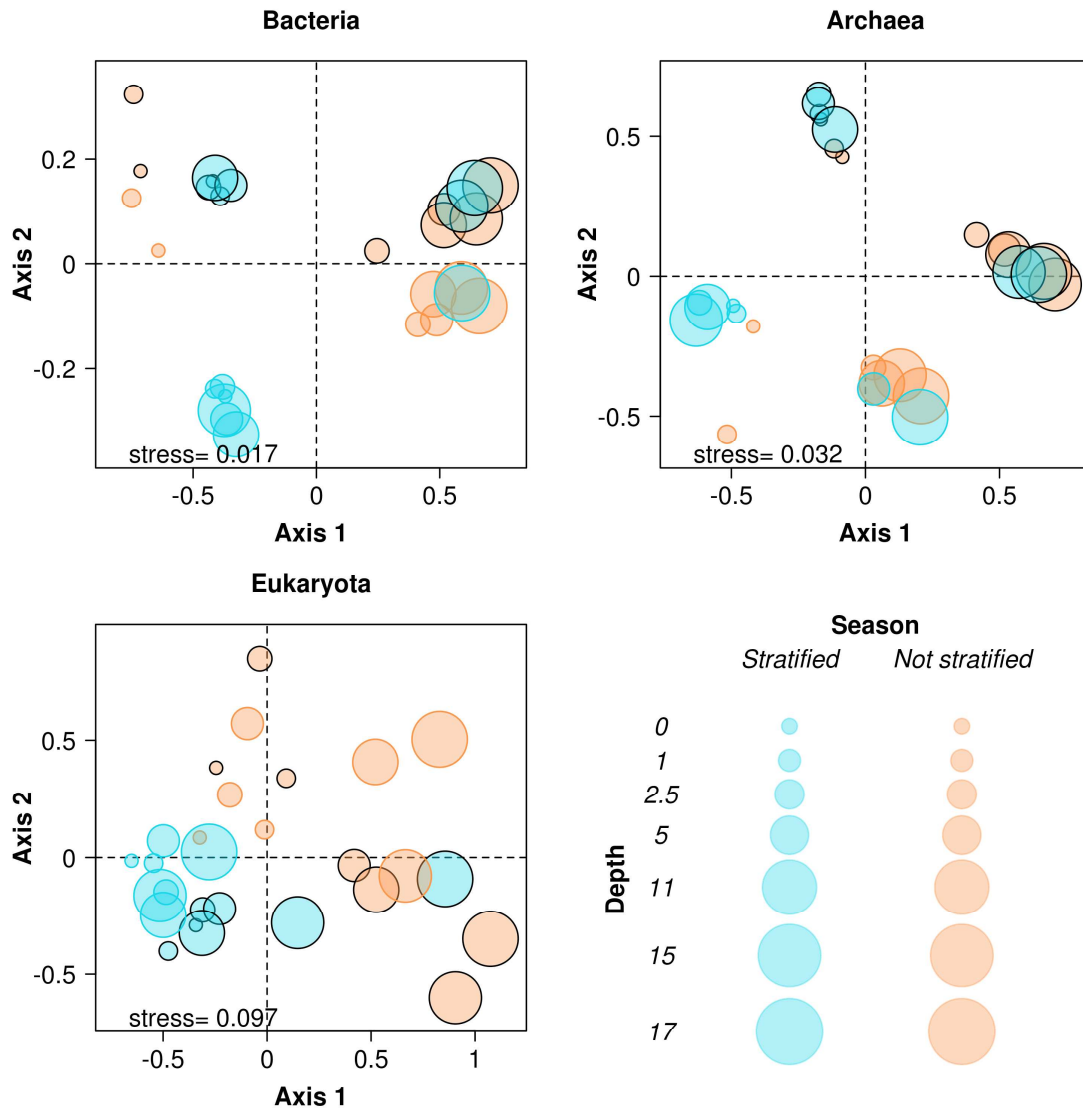
753

754

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

757 Salinity (psu), temperature (°C), pH, O₂ (%) and H₂S (µM) profiles were recorded during
 758 the stratified periods in 2014 and 2015, and during the non-stratified periods in 2014 and
 759 2015.

760



761

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

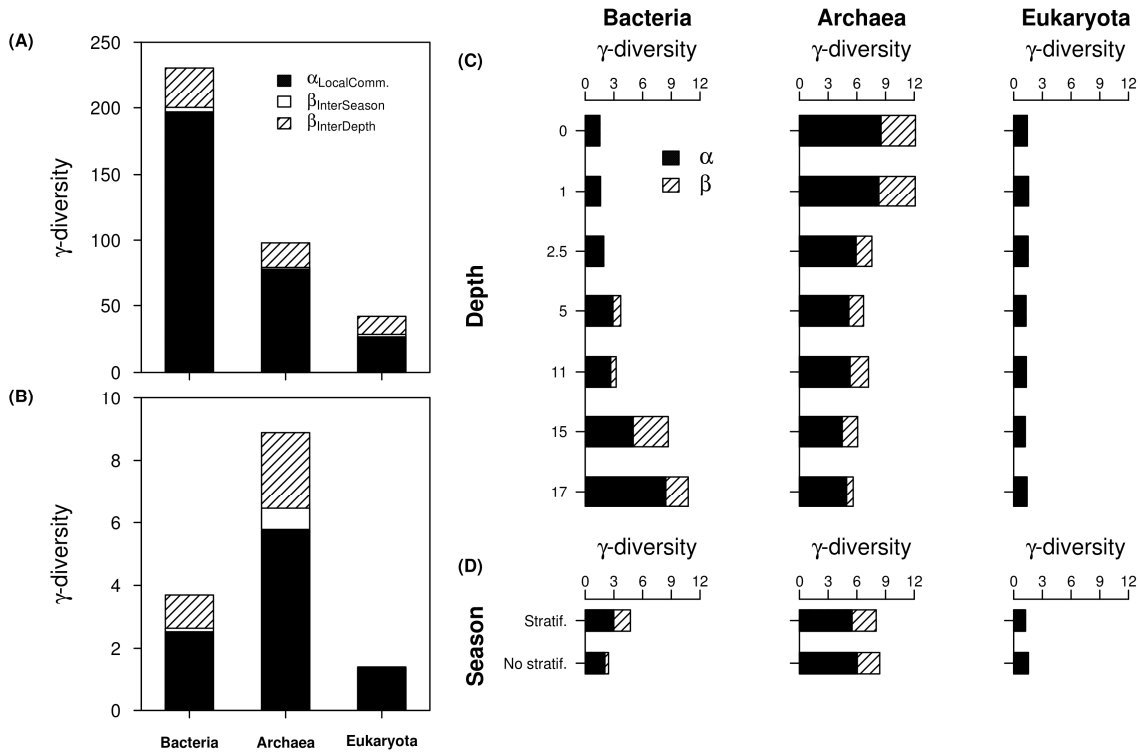
763 NMDS ordination performed on Bray-Curtis dissimilarity matrices. The legend is presented

764 in the bottom right corner of the panel. The size of circles indicated the depth. Blue circles

765 represented the stratified periods and orange ones the non-stratified periods. Black-

766 surrounded circles reported the 2014-year while non-surrounded circles represented 2015.

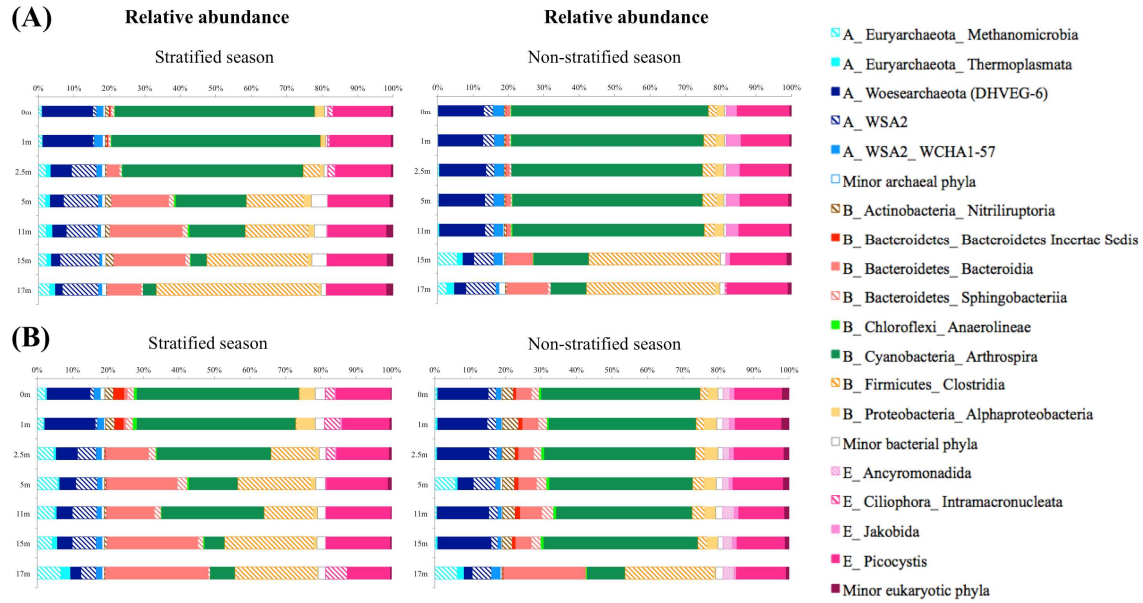
767



768

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 771 local communities diversity (α -LocalCommunities) and differences between communities
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 775 differences between communities (β), along the depth profile (C) and between seasons (D).

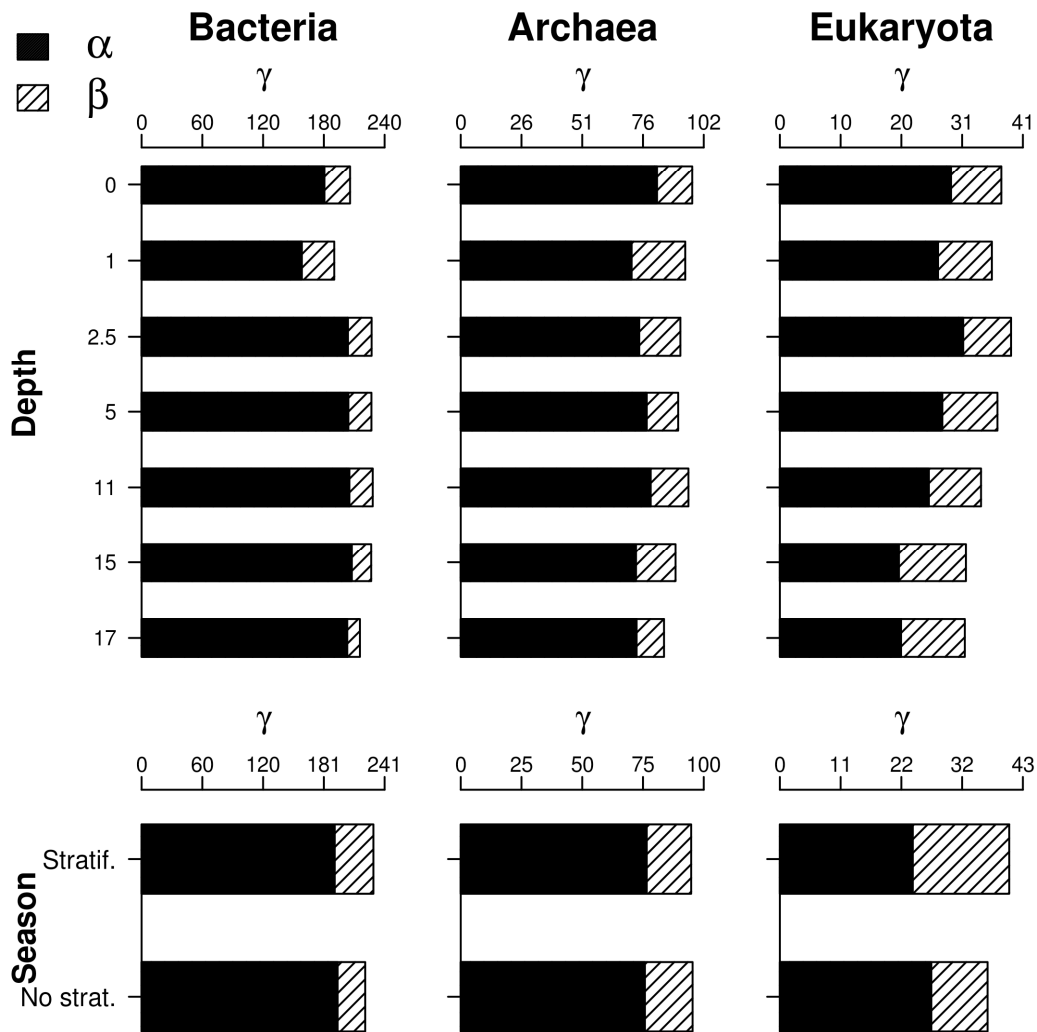


776

777 **Figure 4. Relative abundance (% of sequences) of bacterial, archaeal and eukaryotic**
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779 Data were collected in (A) 2014 and (B) 2015 during both stratified and non-stratified
 780 periods. In the legend on the right of the panel, Archaea were mentioned as A_, Bacteria as
 781 B_, and Eukaryota as E_.

782



783

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

785 Decomposition of total diversity (γ -Diversity) estimated using Rao' quadratic entropy on
 786 presence-absence data into local communities diversity (α) and differences between
 787 communities (β), along the depth profile (C) and between seasons (D).

788

Table 1

Factor	Bacteria			Archaea			Eukaryota		
	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

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Table 2

	γ -diversity	α -LocalComm.	β -InterSeason	β -InterDepth
<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)