Influence of aphotic haloclines and euxinia on organic biomarkers and microbial communities in a thalassohaline and alkaline volcanic crater lake

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35 **Running title:** Organic biomarkers and microbial diversity in a thalassohaline lake

36

37 **Abstract**

38 Studies on microbial communities, and their associated organic biomarkers, that are found
39 thriving in the aphotic euxinic waters in modern stratified ecosystems are scarce
40 compared to those undertaken in euxinic photic zones. The Dziani Dzaha (Mayotte, Indian
41 Ocean) is a tropical, saline, alkaline crater lake that has recently been presented as a
42 modern analogue of Proterozoic Oceans due to its thalassohaline classification (having
43 water of marine origin) and specific biogeochemical characteristics. Continuous intense
44 photosynthetic production and microbial mineralization keeps most of the water column
45 permanently aphotic and anoxic preventing the development of an euxinic (sulfidic and
46 anoxic) photic zone despite a high sulfide/sulfate ratio and the presence of permanent or
47 seasonal haloclines. In this study, the molecular composition of the organic matter in Lake
48 Dziani Dzaha was investigated and compared to the microbial diversity evaluated through

16S rRNA gene amplicon sequencing, over two contrasting seasons (rainy vs. dry) that influence water column stratification. Depth profiles of organic biomarker concentrations (chlorophyll-a and lipid biomarkers) and bacterial and archaeal OTU abundances appeared to be strongly dependent on the presence of aphotic haloclines and euxinia. OTU abundances revealed the importance of specific haloalkaliphilic bacterial and archaeal assemblages in phytoplanktonic biomass recycling and the biogeochemical functioning of the lake, suggesting new haloalkaline non-phototrophic anaerobic microbial precursors for some of the lipid biomarkers. Uncultured Firmicutes from the family Syntrophomonadaceae (Clostridiales), and Bacteroidetes from the ML635J-40 aquatic group, emerged as abundant chemotrophic bacterial members in the anoxic or euxinic waters, and were probably responsible for the production of short-chain *n*-alkenes, wax esters, diplopterol and tetrahymanol. Halocline-dependent euxinia also had a strong impact on the archaeal community which was dominated by Woesearchaeota in the sulfide-free waters. In the euxinic waters, methanogenic Euryarchaeota from the Methanomicrobia, Thermoplasmata and WSA2 classes dominated and were likely at the origin of common hydrocarbon biomarkers of methanogens (phytane, pentamethyl-eicosenes, and partially hydrogenated squalene).

Key words: Haline and aphotic euxinia, lipid biomarkers, bacterial and archaeal diversity, Syntrophomonadaceae, Bacteroidetes ML635J-40 aquatic group, WSA2 methanogenic Euryarchaeota.

1. INTRODUCTION

The reliability of lipid biomarkers that are assigned to specific biological origins in the environment is dependent on previous characterization of similar lipids from isolated and cultured (micro)organisms. The use of lipid biomarkers to reconstruct the paleo-biosphere and paleo-environments through the analysis of fossil sedimentary records is essentially based on the principle of actualism (uniformitarianism), that assumes that biosynthetic pathways have not evolved drastically over time within the different domains of the tree of life (Brocks & Pearson, 2005). However, the number of (micro)organisms isolated from the environment and available in pure cultures is still limited, and over the past few decades, the use of lipid biomarkers has largely benefited from phylogenetic trees and culture-independent techniques applied to natural communities (Brocks & Banfield, 2009). Great advances have been made with comparative and environmental genomics, which have demonstrated that certain classes of lipids can be produced by a much greater diversity of organisms than previously envisaged (Pearson, Flood Page, Jorgenson, Fischer, & Higgins, 2007; Brocks & Banfield, 2009; Pearson, 2014; Banta, Wei, & Welander, 2015). As an alternative, the comparison of lipid biomarker abundance with the composition of the microbial community, as determined by genetic methods, can potentially help to elucidate the biological origin of certain lipids (Villanueva et al., 2014). Investigating the possible origin(s) of lipid biomarkers can be particularly challenging in specific environments such as saline and/or stratified water bodies including soda lakes, meromictic lakes, and closed basins, where a significant part of the microbial community is generally composed of, as-yet-uncharacterized, or non-isolated organisms (Overmann, Beatty, & Hall, 1996; Sorokin et al., 2014; Hamilton et al., 2016). These ecosystems are characterized by seasonal or permanent physico-chemical gradients which divide the

95 water column into at least two or more physically- and chemically- contrasting
96 compartments hosting diverse microbial communities that are adapted to specific
97 ecological niches. Typically, phototrophic organisms such as (pico)cyanobacteria deliver
98 fixed carbon to vertically organized chemolitho- and chemoorgano-trophic bacterial and
99 archaeal communities that then recycle a major part of the sinking organic matter (Sorokin
100 et al., 2014; Sorokin, Banciu, & Muyzer, 2015). In stratified sulfur-rich environments where
101 light reaches anoxic waters, the presence of an euxinic (sulfidic and anoxic) photic zone
102 further allows dense populations of purple and green sulfur anoxygenic phototrophic
103 bacteria to develop and impact the carbon and sulfur cycles (Overmann et al., 1996;
104 Hamilton et al., 2014). These conditions resemble, at least regionally, those that have
105 prevailed over several periods of the Earth's history, such as the Mesoproterozoic and
106 Phanerozoic biotic crises, when parts of the ocean were oxygen-depleted and partly
107 euxinic (Grice et al., 2005; Lyons, Anbar, Severmann, Scott, & Gill, 2009). For this reason
108 contemporaneous stratified and euxinic ecosystems are often considered to be good
109 models for the past ocean (Meyer & Kump, 2008). The structure and lipid biomarkers of
110 the microbial communities thriving in such euxinic ecosystems have been regularly
111 investigated, and lipid biomarkers specific to photic zone euxinia and associated
112 chemoclines have been characterized and used for paleo-environmental reconstructions
113 (Grice et al., 2005; French, Rocher, Zumberger, & Summons, 2015). Surprisingly, much
114 less is known about the microbial communities thriving in aphotic euxinic waters (i.e.,
115 below the euxinic photic zone or in euxinic ecosystems deprived of an euxinic photic zone)
116 or their potential specific organic biomarkers, although such ecological niches often
117 represent a major part of euxinic ecosystems (Klepac-Ceraj et al., 2012; Hamilton et al.,
118 2016).

Lake Dziani Dzaha (Mayotte, Indian Ocean) is a tropical volcanic crater lake formed ca. 6-7 kyr ago. It has recently been presented as a new contemporaneous analogue of Proterozoic oceans based on its thalassohaline classification (having water of marine origin), microbial functioning, biogeochemical characteristics, and the presence of one deep permanent and one shallow seasonal halocline (Leboulanger et al., 2017; Bernard et al., 2019; Cadeau et al., 2020; Sarazin et al., 2020). The apparent absence of hydrological connections with the surrounding Indian Ocean makes it a very peculiar saline lake, functioning as an endorheic basin, where the water composition has been modified through time by biological and climatic/environmental processes to attain its current characteristics (Sarazin et al., 2020). These include strong alkalinity, elevated pH, low sulfate content, and continuous massive rates of organic matter production and mineralization which limit light penetration and maintain a permanently anoxic and aphotic water column below ca. 1 m depth (Leboulanger et al., 2017; Hugoni et al., 2018; Bernard et al., 2019; Sarazin et al., 2020). Depending on the season (rainy vs. dry), a strong halocline develops at ca. 2 m depth, i.e., below the limited photic zone, and sulfidic conditions intensify in the anoxic waters below. However, the combination of the sun-shielding impact of the huge cyanobacterial biomass and the strong halocline precludes the photic zone overlapping with euxinic waters and thus prevents the development of an euxinic photic zone.

Previous studies on the thriving microbial diversity in Lake Dziani Dzaha have shown that the photosynthetic community is strongly dominated by the cyanobacteria *Arthrospira fusiformis* and the picoeukaryote green algae *Picocystis salinarum* which are responsible for the permanent green colour of the surface waters (Leboulanger et al., 2017; Bernard et al., 2019). A metabarcoding approach has demonstrated that the bacterial and archaeal

diversity is shaped by the contrasting physico-chemical conditions observed in the different compartments of the water column, showing both typical and atypical features when compared to other saline alkaline ecosystems (Hugoni et al. 2018). In addition to *A. fusiformis* and *P. salinarum*, the archaeal phylum Woesearchaeota dominates the microbial assemblage in the shallow oxygenated surface waters. This phylum remains dominant in the anoxic waters deprived of sulfides, whereas methanogenic Euryarchaeota and the chemotrophic bacterial phyla Firmicutes and Bacteroidetes take over the microbial diversity in anoxic and sulfidic (euxinic) waters. In line with the low sulfate content of the lake waters (<3mM in non-sulfidic waters and undetected in euxinic waters) and the absence of an euxinic photic zone, sulfate-reducing bacteria and anoxygenic phototrophic sulfur bacteria do not develop significantly in this ecosystem (Leboulanger et al., 2017; Hugoni et al., 2018).

In this study, the composition of the organic matter (OM) in Lake Dziani Dzaha was investigated and depth concentration profiles of selected biomarkers present in the suspended OM were compared to a more in-depth analysis of the bacterial and archaeal diversity (down to the class or order levels) for the two contrasting seasons that influence water column stratification and euxinia (rainy vs. dry). The microbial organic signature and community structure of the different compartments of the water column, suggest an uncommon microbial origin for certain lipid biomarkers and highlight the importance of specific bacterial and archaeal assemblages that develop below seasonal or permanent aphotic and euxinic haloclines, in the biogeochemical functioning of this saline and alkaline lake.

2. MATERIALS AND METHODS

2.1 Site description, water column geochemistry and sampling

Dziani Dzaha is a crater lake located on the French 'Petite Terre' island of the Mayotte Comoros archipelago, in the western Indian Ocean (Figs. 1A, B). Situated close to the ocean (ca. 0.2 km; Fig. 1C), it is likely that this inland lake was formed during the most recent phreatomagmatic eruption (between 7.5 and 4 ky BP; Zinke, Reijmer, & Thomassin, 2003). Lake Dziani Dzaha has been defined as a thalassohaline ecosystem because its dissolved mineral composition originates predominantly from seawater (Leboulanger et al., 2017; Sarazin et al., 2020), in contrast to athalassohaline ecosystems where the dissolved mineral composition originates from the evaporation of continental water. The unique seawater chemistry of the lake has evolved through time, in a closed system, to attain its current (poly)extremophilic characteristics, with alkalinity 100 times that of seawater, elevated pH (9.1-9.4), saline to hypersaline conditions (34-71 g L⁻¹), low sulfate concentrations (0-3 mM) and high dissolved organic carbon (DOC) concentrations (4-8 mmol L⁻¹) (Sarazin et al., 2020). The water temperature ranges between 27 and 33°C all year round. The seasonality, the huge cyanobacterial production (with *Arthrospira fusiformis* representing ca. 98% of the biomass) and recycling by the microbial communities are the main drivers of the present-day dynamics of the water chemistry of Lake Dziani Dzaha (Leboulanger et al., 2017; Bernard et al., 2019). Due to its small catchment area (ca. 0.5 km²) relative to its size (ca. 0.24 km²; Figs. 1C, D) and the absence of connected rivers, the lake is only marginally influenced by watershed inputs. During the rainy season, from November to March, the lake receives intense rainfall, which rapidly induces strong water column stratification characterized by a seasonal halocline at ca. 2 m depth and euxinic waters below, where sulfide concentrations reach up to 6.5 mM. During the dry season, from April to October, rainfall is scarce, and strong

191 evaporation from the lake induces a 0.7-1 m decrease in the water level and, ultimately
192 (3-4 months after the end of precipitations), the disappearance of the seasonal halocline.
193 For simplicity, both periods are referred to as 'with' and 'without' seasonal stratification,
194 respectively. However, whatever the period, lake waters become rapidly anoxic below the
195 surface due to limited wind mixing, owing to the steep crater slopes, and the intense
196 mineralization of the huge phytoplanktonic productivity which prevents light penetration
197 and photosynthetic O₂ production below a depth of 1 m (Leboulanger et al., 2017). The
198 maximum water depth of Lake Dziani Dzaha ca. 18 m, is found in a pit to the east of the
199 lake (Fig. 1E), where an additional (permanent) halocline is observed (Fig. 2).

200 Sampling was performed at the pit site (Fig. 1E) during two field surveys, one conducted
201 during October 2014 (around the end of the dry season, i.e., without seasonal
202 stratification) and the other, in April 2015 (around the end of the wet period, i.e., with
203 seasonal stratification). Prior to water sampling, pH, dissolved oxygen, temperature, and
204 conductivity depth profiles were recorded using a YSI 6600XLM probe. These profiles
205 enabled the sampling strategy to be adapted for organic biomarkers and microbial
206 diversity analyses depending on the depth of the seasonal and permanent haloclines.

207 Water was then sampled using a horizontal Niskin bottle to provide an appropriate vertical
208 resolution. H₂S/HS⁻ concentrations (i.e., soluble sulfide concentrations (ΣS(-II))), were
209 determined in the field by colorimetry using a AQUALYTIC SpectroDirect
210 spectrophotometer and Merck reagent kits. For lipid analyses, 500 to 1000 mL of lake
211 water was filtered onto pre-combusted GF/F filters, and for total chlorophyll-a (Chl-a)
212 analysis 5 mL water samples were conditioned similarly. For microbial diversity
213 determination, 20 mL water samples were first filtered through 3 µm polycarbonate filters
214 before being filtered again onto 0.2 µm polycarbonate filters. All filters were stored at -

20°C until analysis. The total phytoplankton abundance was determined by flow cytometry and cell counting as described in Bernard et al. (2019).

2.2. Chlorophyll-a and lipid biomarker analyses

Chl-a concentration was determined, on the sampling day, by spectrophotometry as described by Ritchie (2006). The samples were placed into polystyrene tubes with 3 mL 96% ethanol and ultrasonified for 30 min in an ice bath. The closed tubes were then stored overnight at 4°C to complete pigment extraction in the dark. The extracts were filtered and transferred into spectrophotometric optical glass cuvettes, and absorption was measured over the visible light range (400-800 nm) using an Aqualytic AL800 spectrophotometer.

Lipids were extracted by ultrasonication with methanol-dichloromethane (MeOH-DCM 2:1, v/v, ×2), methanol-DCM (1:1, v/v, ×2), and DCM (×1). The combined extracts were concentrated by rotary evaporation and, following the removal of elemental sulfur using activated copper curls, the total lipid extract (TLE) was evaporated to dryness under a N₂ flux. The TLE was then chromatographed over a column of silica gel (60 Merck silica gel) and fractions of increasing polarity were eluted using n-hexane (hydrocarbons), 5% ethyl acetate in hexane (wax esters), and 20 % ethyl acetate in hexane (alcohols). Each fraction was concentrated, spiked with an internal standard, and alcohols were silylated using a mixture of pyridine/BSTFA (1:1, v/v, 45 min at 50°C) prior to gas chromatography (GC) and GC-mass spectrometry (GC-MS) analyses.

GC analyses were performed on a HP-6890 Series gas chromatograph equipped with a cool on-column injector and a flame ionization detector. GC-MS analyses were performed on a HP 6890 Series Plus gas chromatograph equipped with a cool on-column injector and coupled to an Agilent 5975C (VL MSD) mass spectrometer. Compound separation

was achieved with a fused silica capillary column (30 m × 0.25 mm) coated with HP-5MS (0.25 µm film thickness) using helium as the carrier gas (constant flow, 1 mL min⁻¹). The injector temperature was programmed as follows: 60°C (held 0.5 min), 60 to 300°C (200°C min⁻¹), 300°C (held 1 min). The oven temperature program was set as: 60°C (held 0.5 min), 60 to 130°C (20°C min⁻¹), 130 to 300°C (4°C min⁻¹), and 300°C (held 55 min). Electron impact mass spectra were recorded at 70 eV in full scan mode in the m/z range 50-700. The different lipid biomarkers were identified based on their characteristic mass spectral fragmentations, mass spectra libraries from the manufacturer, and comparison with data in the literature (e.g., ten Haven, Rohmer, Rullkötter, & Bisseret, 1989).

2.3. DNA extraction, sequencing, and bioinformatics analyses

Methods employed here are detailed in Hugoni et al. (2018). Briefly, for each sampling depth, DNA extractions were conducted on both 3 µm and 0.2 µm filters using the Power Water DNA isolation kit according to the manufacturer's instructions (MoBio laboratories). Bacterial and archaeal 16S rRNA genes were amplified in triplicate using 357F/926R and 519F/915R primers, respectively (Hugoni et al., 2018), and amplicons were sequenced on an Illumina HiSeq 2500 (300bp PE, GATC Biotech, Konstanz, Germany).

Bioinformatic analyses were conducted using FROGS (Escudié et al., 2018) and consisted of merging the 3µm and 0.2µm fractions, removing low quality sequences, dereplication, clustering using SWARM, removing chimeras using VSEARCH, filtering sequences representing less than 0.005%, and affiliated against the Silva database (v.128). To compare samples with each other, we applied a normalization threshold of 124,779 sequences per sample for Bacteria and 37,529 for Archaea.

OTU richness and diversity was estimated using Chao1 and Shannon indexes,

respectively. Canonical correspondence analysis (CCA) was performed to assess the relationships between the bacterial and archaeal taxonomic groups and environmental parameters. Sequences were considered at the «Phylum» level and pooled for each sampling date. CCA was performed using 5 environmental factors (temperature, salinity, oxygen, $\text{H}_2\text{S}/\text{HS}^-$ concentration and pH) with the taxonomic phyla abundance using the VEGAN package (<http://cran.r-project.org/web/packages/vegan/index.html>) in R.

3. RESULTS

3.1. Geochemistry of the water column at the time of sampling

The physico-chemical characteristics of the water column in Lake Dziani Dzaha at the end of the dry and rainy seasons (i.e., without and with seasonal stratification; Fig. 2) were consistent with previous observations made at similar times of the year (Leboulanger et al., 2017). The lake water temperature remained stable (around 30°C) from the bottom of the oxic-anoxic transition zone, down to the water-sediment interface, but within the first two meters below the lake surface a thermal gradient was present (Fig. 2A). The pH of the water was constant with depth (average pH ~9.2) and only showed a systematic decrease below the seasonal and the permanent haloclines (ca. -0.4 and -0.2 pH, respectively). Water salinity was more dependent on the season (Fig. 2A) and in the absence of seasonal stratification, the salinity was 64 psu from the surface down to 14 m depth increasing to 71 psu below the deep halocline. In the presence of the seasonal halocline, the upper part of the water column (i.e., above the halocline) was less salty (43-45 psu) due to rainfall dilution (Fig. 2A). Below this halocline, the salinity shifted back to ca. 65 psu down to the deep halocline at ca. 14 m depth, then below this, increased again reaching the same salinity as observed during the dry season (Fig. 2B). Whatever the

season, the oxygen concentration rapidly decreased from the oversaturated surface waters to the completely anoxic waters. The oxygen penetration depth fluctuated daily, but oxygen was never present below ca. 1.5 m depth (Fig. 2B). The oxygen penetration depth, therefore, did not coincide with the seasonal halocline set at a depth of between 1.8 and 2.2 m during the rainy period. The photic zone was also limited permanently as 1% Photosynthetic Active Radiation (PAR) was reached at a depth of less than 0.5 m. During the rainy season, euxinic conditions developed below the seasonal halocline, with $\text{H}_2\text{S}/\text{HS}^-$ concentrations reaching >2 mM down to the sediment interface (Fig. 2B) and CH_4 concentrations approaching saturation (ca. 1.7 mM; Sarazin et al., 2020). During the dry season, sulfides were present in much lower concentrations ($\text{H}_2\text{S}/\text{HS}^- < 0.16$ mM) in the anoxic waters, except below the deep permanent halocline where $\text{H}_2\text{S}/\text{HS}^-$ concentrations were higher (>2.8 mM) than those measured between 2 and 14 m depth in the presence of the seasonal halocline. Both seasonal and permanent haloclines were light-deprived and thus considered aphotic.

3.2. Chlorophyll-a and lipid biomarkers

Due to the permanent hypereutrophic status of the lake, Chl-a concentrations in the surface oxic waters (0-1m) ranged between 600 and 750 $\mu\text{g L}^{-1}$ irrespective of season (Fig. 3). During the dry season, in the absence of the seasonal halocline, Chl-a concentrations remained roughly stable to a depth of 7 m and then dropped significantly deeper down, especially across the deep halocline (Fig. 3). Conversely, in the presence of the seasonal halocline (the wet season), Chl-a concentrations decreased by ca. 80% within the first 6 m, and then remained stable down to the water-sediment interface. The concentration profile of free phytol (Fig. 3), the hydrolyzed form of the isoprenoid chain of

Chl-*a*, closely followed that of Chl-*a* during both sampling periods but higher values were reached (ca. 250 $\mu\text{g L}^{-1}$) in the surface waters during the rainy season than during the dry season (ca. 55 $\mu\text{g L}^{-1}$).

The GC-MS analysis of the different lipid fractions revealed the presence of lipid biomarkers which, based on their non-isoprenoidal structures and depth concentration profiles, could be attributed to the anaerobic bacterial populations thriving in the lake (see discussion). Mono-unsaturated *n*-alkenes with 19 or 21 carbon atoms were systematically detected in the anoxic and/or euxinic layers of the water column. Their concentration remained low (maxima of 0.24 $\mu\text{g L}^{-1}$ for C_{19:1} and 2.5 $\mu\text{g L}^{-1}$ for C_{21:1}) but, whatever the sampling period, both compounds were absent from the oxygenated water layer with concentrations generally rising in the euxinic waters (Fig. 4A). A range of long chain wax esters (WE) was also observed throughout the water column. Trace amounts of saturated C₃₀ and C₃₂ WE were detected in the surface oxygenated waters but the concentration and structural diversity of WE significantly increased in the sulfidic waters below (Figs. 4B; S1). Here, WE were composed of linear or methyl-branched C₁₄-C₂₀ acyl chains esterified to linear or methyl-branched C₁₄-C₁₈ alcohol chains and contained 0 to 3 double bonds (Fig. S1). The concentration of C-odd WE reached ca. 13 $\mu\text{g L}^{-1}$ in euxinic waters where the systematic appearance of C-odd WE with pentadecyl and heptadecyl (sometimes branched) moieties was striking (Figs. 4B; S1). During the rainy season, C-odd WE concentrations were at a maximum below the seasonal halocline, dropping sharply with depth, before increasing again below the deep halocline. Most of the alkyl chains constitutive of the C-odd WE were retrieved in their free form in the alcohol fractions (i.e., C-odd possibly branched *n*-alkanols). Like C-odd WE, these alcohols systematically

appeared below the (seasonal or deep) halocline, but their concentrations did not decrease between the two haloclines during the stratified period (Fig. 4B).

In addition to free phytol and C-odd *n*-alkanols, the pentacyclic triterpenoids diplopterol and tetrahymanol were among the main alcohols observed in the water column of Lake Dziani Dzaha. Remarkably, these triterpenols were mainly observed in the sulfidic part of the water column (Fig. 4C) regardless of seasonal stratification. This was especially true for tetrahymanol whose concentration systematically rose below the seasonal and/or permanent halocline (Fig. 4C). Diplopterol was also present in most compartments of the water column but, unlike tetrahymanol, its concentration only increased below the deep halocline in both sampling periods, suggesting a distinct biological precursor for these two triterpenols. As for most of the bacterial biomarkers, the maximum concentrations of diplopterol and tetrahymanol were observed at the bottom of the pit during the stratified period, reaching ca. 20 and 11 $\mu\text{g L}^{-1}$, respectively.

Few GC-amenable archaeal lipid biomarkers were observed in the lipid fractions investigated, and their occurrence seemed to depend on the sampling period and/or the physico-chemical characteristics of the water. Archaeol, and to a lesser extent phytane, were detected during both sampling periods (Fig. 5). In the absence of a seasonal halocline, phytane was only detected in the euxinic waters below the deep halocline whereas archaeol was present in low concentrations at all depths but increased in the deep euxinic waters (Fig. 5B). Phytane and archaeol concentrations were much higher during the rainy season and their occurrence was correlated with the presence of $\text{H}_2\text{S}/\text{HS}^-$. At this time, their concentration profiles were similar, showing an increase below the seasonal halocline, and an even stronger increase below the deep halocline, where they reached concentrations of ca. 1 and 12 $\mu\text{g L}^{-1}$, respectively (Fig. 5).

A range of pentamethyl-eicosenes (PMI) and partially hydrogenated squalenes (hydrosqualenes) with 3 to 4 and 1 to 4 double bonds respectively, were also detected in the euxinic waters during the stratified period. Hydrosqualenes were present in much higher concentrations than PMI, the sum of all isomers reaching up to 8.2 $\mu\text{g L}^{-1}$ and 0.9 $\mu\text{g L}^{-1}$, respectively. The depth concentration profiles of both classes of compounds mirrored those of phytane and archaeol, showing an increase below the seasonal and/or permanent halocline (Fig. 5).

3.3. Bacterial and archaeal diversity

In order to get an in-depth analysis of the microbial diversity in Lake Dziani Dzaha, sequencing data acquired previously from whole microbial community at a high taxonomic level (Hugoni et al., 2018) were reinvestigated separately (Bacteria and Archaea) and down to the class or order taxonomic level (Figs. 6; 7). Whatever the sampling period (end of the dry or the rainy season), cyanobacterial OTUs strongly dominated from the surface down to the first halocline, accounting for 87% of the bacterial sequences above the permanent deep halocline during the dry season, and for 71% of the bacterial sequences above the seasonal halocline during the rainy season (Fig. 6A). Apart from cyanobacteria, the most abundant bacterial phyla were Bacteroidetes and Firmicutes, and to a lesser extent Alphaproteobacteria and Actinobacteria. Non-affiliated bacterial sequences represented at most 5% of the total sequences regardless of season or depth (Fig. 6A). Interestingly, the bacterial community structure did not seem to be modified across the oxic-anoxic transition zone whereas a strong shift was systematically observed across the transition to euxinic waters both with or without the seasonal halocline. Bacteroidetes and Firmicutes became strongly dominant in the euxinic waters where together they

382 represented 65% of the bacterial sequences below the deep halocline during the dry
383 season and 73% of bacterial sequences below the seasonal halocline during the rainy
384 season (Fig. 6A). A further insight into Firmicutes diversity showed the overwhelming
385 dominance of an unknown genus of anaerobic Clostridiales from the family
386 Syntrophomonadaceae at all depths during both sampling periods (Figs. 6B; S2). A small
387 decrease in the proportion of this genus was however observed in euxinic waters.
388 Bacteroidetes appeared to be more diversified than Firmicutes and were significantly
389 influenced by the presence of haloclines (Fig. 6C). In the absence of $\text{H}_2\text{S}/\text{HS}^-$,
390 Bacteroidales belonging to the ML635J-40 aquatic group, Flavobacteriales,
391 Sphingobacteriales and two non-cultivated orders (III and ML602M-17) were the dominant
392 Bacteroidetes whereas, in the euxinic waters, the ML635J-40 aquatic group systematically
393 took over the whole Bacteroidetes abundance during both seasons, representing on
394 average 90% of the Bacteroidetes sequences.

395 Whatever the season, the archaeal community was essentially composed of
396 Woesearchaeota and Euryarchaeota, which together represented between 91 and 97%
397 of the total archaeal sequences (Fig. 7A). Woesearchaeota dominated the archaeal
398 assemblages above the deep halocline during the dry season (64 to 74% of archaeal
399 sequences) and above the seasonal halocline during the rainy season (66 to 69% of
400 archaeal sequences), whereas Euryarchaeota became systematically dominant in the
401 euxinic waters below (70-80% of archaeal sequences). Further analysis of Euryarchaeota
402 diversity showed the predominance of the methanogenic class WSA2 in all depths
403 irrespective of the sampling season, representing between 40 and 95% of the total
404 sequences of Euryarchaeota (Fig. 7B). Methanobacteria, Methanomicrobia and
405 Thermoplasmata were the other lineages of Euryarchaeota represented in the non-sulfidic

compartments of the water column contributing <10 to 50% of the total euryarchaeal sequences depending on the presence of the seasonal halocline. The number of sequences related to Methanobacteria significantly decreased in the euxinic waters whereas Methanomicrobia and Thermoplasmata were the predominant classes of Euryarchaeota along with WSA2.

Using a Canonical Correspondence Analysis, the influence of the main physico-chemical parameters (pH, salinity, temperature, dissolved oxygen, and $\text{H}_2\text{S}/\text{HS}^-$) on the distribution of bacterial and archaeal classes along the water column was further investigated. The first two axes represented more than 95% of the total variance (ca. 89 and 7% respectively; Fig. 8). Samples grouped into two well-separated groups highlighted the strong impact of the presence of sulfides and a significant influence of salinity on the composition of the bacterial and archaeal communities. Cyanobacteria, Alphaproteobacteria, Actinobacteria and Woesearchaeota appeared to be mostly related to pH, temperature, and dissolved oxygen, while Firmicutes, Bacteroidetes and Euryarchaeota were mostly related to $\text{H}_2\text{S}/\text{HS}^-$ and salinity (Fig. 8).

4. DISCUSSION

4.1 Chemocline-dependent microbial community composition

The microorganisms that thrive in saline-alkaline lakes are considered as a special category of extremophiles. They are haloalkaline microorganisms and their biodiversity depends on the specific physico-chemical characteristics of the ecosystem encountered. Salinity, oxygen, and pH have been established as important factors for shaping the microbial communities in soda lakes, while striking differences in diversity have also been

reported between the oxic mixolimnion and the euxinic hypolimnion/monimolimnion of alkaline and non-alkaline stratified ecosystems (Humayoun, Bano, & Hollibaugh, 2003; Klepac-Ceraj et al., 2012; Lanzén et al., 2013; Hamilton et al., 2016). This is also the case for Lake Dziani Dzaha where microbial diversity, evaluated through changes in OTU abundances, appears significantly constrained by the presence of aphotic and euxinic haloclines⁻ (Figs. 6; 7; 8). However, unlike most stratified ecosystems including soda lakes, the peculiarity of Lake Dziani Dzaha is i) the absence of euxinic photic zone, where light and sulfides meet and support the development of anoxygenic phototrophic sulfur bacteria and, ii) the apparent absence of sulfate-reducing bacteria due to the low sulfate content of the lake waters.

The massive phytoplanktonic biomass of Lake Dziani Dzaha is mainly composed of the filamentous cyanobacterium *Arthrospira fusiformis* (representing 97 to 99% of the total biomass) and, to a much lesser extent, the unicellular eukaryote *Picocystis salinarum* and non-heterocytous filamentous cyanobacteria from the orders Oscillatoriales and Synechococcales (Leboulanger et al., 2017; Bernard et al. 2019). Despite the permanent hypereutrophic status of the lake, a much lower number of 16S rRNA gene sequences affiliated with cyanobacteria were observed below the (seasonal) halocline (Fig. 6A) regardless of the sampling period. This was accompanied by lower phytoplanktonic cell density (Fig. S3) suggesting an efficient anaerobic biodegradation of the settling phytoplanktonic biomass. Whilst the anaerobic digestion of OM under saline and alkaline conditions has been shown to be very effective, it is a very complex and poorly understood process (Sorokin et al., 2015; Nolla-Ardèvol, Strous, & Tegetmeyer, 2015), and little is known about the biodegradation processes that occur in the presence of high concentrations of sulfides in the absence of sulfate and light. This case is of particular

interest since $\text{H}_2\text{S}/\text{HS}^-$ is present at low concentration (or absent) in the biogas that is produced during the anaerobic digestion of OM under alkaline conditions (Van Leerdam et al., 2008; Nolla-Ardèvol et al., 2015).

Apart from cyanobacteria, the marked differences in bacterial and archaeal diversity observed on either side of the seasonal or permanent halocline, indicates the occurrence of distinct haloalkaliphilic microbial populations adapted to specific ecological niches that require specific conditions to develop (Fig. 8). Dominant in Lake Dziani Dzaha, Firmicutes, Bacteroidetes and to a lesser extent Alphaproteobacteria are bacterial phyla commonly observed in alkaline environments (Jones et al., 1998; Wani et al., 2006; Sorokin et al., 2014; Rojas et al., 2018; Vavourakis et al., 2018). Their role in OM degradation under saline and alkaline conditions has been recognized (Sorokin et al., 2015; Nolla-Ardèvol et al., 2015 and references therein). However, a number of peculiar features characterize the bacterial community of Lake Dziani Dzaha. First, the overwhelming dominance among Firmicutes of a single (yet unknown) genus of anaerobes belonging to the family *Syntrophomonadaceae* among Clostridiales (Figs. 6B; S2A). Prevailing at all depths, this unknown haloalkaliphile genus of Firmicutes did not appear significantly hampered by the presence of high concentrations of $\text{H}_2\text{S}/\text{HS}^-$, and was sometimes accompanied by other members of the same family from the genera *Dethiobacter* and '*Candidatus contubernalis*', especially in the euxinic waters (Fig. S2A). Clostridiales are primary or secondary hydrolytic anaerobes regularly observed in alkaline and soda lakes (Sorokin et al., 2014; 2015), belonging to the family *Syntrophomonadaceae*. They are known to use carboxylic acids as a source of energy, and have been increasingly found in haline and non-haline alkaline sediments. For example, several phylotypes of Clostridiales belonging to, or phylogenetically close to, the *Syntrophomonadaceae* were predominantly retrieved

478 from stratified sediments from Russian hypersaline soda lakes (Sorokin et al., 2014;
479 Vavourakis et al., 2018) and have been reported as a major constituent of the bacterial
480 community inhabiting the alkaline, low-salt, shoreline anaerobic sediments of Mono Lake
481 (Rojas et al., 2018). *Synthrophomonadaceae* from the genus *Dethiobacter* have also been
482 recovered from a mixture of soda lake sediments (Sorokin, Tourova, Mussmann, &
483 Muyzer, 2008) and this genus has been shown to contribute to carbon cycling in the hyper-
484 euxinic waters of the stratified Mahoney Lake (British Columbia; Hamilton et al., 2016).
485 Interestingly, different cultured and uncultured members of the family
486 *Synthrophomonadaceae* including the genera *Dethiobacter* and '*Candidatus*
487 *contubernalis*' have been reported to oxidize acetate anaerobically through syntrophic
488 acetate oxidation (SAO) with hydrogenotrophic sulfate-reducing bacteria (SRB) or
489 methanogenic Archaea in several Russian hypersaline soda lakes (Timmers et al., 2018).
490 In Lake Dziani Dzaha, a syntrophic association between acetate oxidizing
491 *Synthrophomonadaceae* and hydrogenotrophic methanogenic archaea appears to be a
492 likely scenario based on 1) the strong similarities in *Synthrophomonadaceae*
493 assemblages observed here and in soda lakes where SAO occurs and, 2) the co-
494 occurrence of potential adequate archaeal partners (Figs. 6; 7 and discussion below). An
495 association between *Synthrophomonadaceae* and hydrogenotrophic SRB in Lake Dziani
496 Dzaha appears less likely, due to the near absence of sulfates and SRB in the anoxic
497 waters where (poly)sulfides prevail. This is particularly interesting when considering a
498 possible analogy between Lake Dziani Dzaha and the Early Proterozoic Oceans where
499 low oxygen concentrations in the atmosphere and surface waters are thought to have
500 prevented sulfate production by oxidative weathering, thus strongly limiting microbial
501 sulfate reduction to the benefit of other sulfur cycling processes (Philippot et al., 2007).

Another remarkable characteristic of the bacterial community of Lake Dziani Dzaha is the dominant proportion, among Bacteroidetes, of the uncultured ML635J-40 aquatic group from the family Bacteroidales. From a general point of view, this family is part of the Cytophaga-Flavobacterium-Bacteroides group (FCB group now called Sphingobacteria) which is more commonly observed in soda lakes and alkaline environments where it participates in the degradation of macromolecules such as carbohydrates and proteins (Humayoun et al., 2003; Wani et al., 2006). Interestingly enough, Bacteroidetes closely related to the ML635J-40 aquatic group have been shown to dominate the bacterial community in alkaline anaerobic reactors that have been inoculated with soda lake sediments and fed with the cyanobacterium *Arthrospira* (Nolla-Ardèvol et al., 2015). Based on metagenomic and metatranscriptomic analyses, the ML635J-40 aquatic group was further demonstrated to play a major role in the breakdown of the phytoplanktonic substrate (Nolla-Ardèvol et al., 2015). This same order of Bacteroidales has also been reported in different soda lakes (Humayoun et al., 2003; Wani et al., 2006; Lanzén et al., 2013) and in ultrabasic serpentinite spring (Brazelton et al., 2017), where they appear to be among the most important bacterial taxa. The ecological role of the ML635J-40 aquatic group is not yet fully understood but, in addition to being involved in the hydrolysis and degradation of complex OM, this group might supply hydrogen gas to methanogens and catalyze the oxidation of formate to CO₂ and of glycine to NH₃ (Nolla-Ardèvol et al., 2015; Brazelton et al., 2017). Such primordial biogeochemical implications of the ML635J-40 aquatic group can be envisaged beneath the haloclines of Lake Dziani Dzaha where the highest numbers of OTUs related to this Bacteroidales group were observed (Fig. 6C), along with a sharp decrease in cyanobacterial biomass (Figs. 3; 6A) and CH₄ and NH₃ production (Sarazin et al., 2020).

The archaeal diversity in Lake Dziani Dzaha is mainly composed of Woesearchaeota and Euryarchaeota and, like bacterial diversity, also presents some striking features. The strong dominance of Woesearchaeota in the non-euxinic waters (Fig. 6A) is in accordance with the widespread distribution of this phylum in anoxic and saline aquatic environments and sediments (Casamayor, Triadó-Margarit, & Castañeda, 2013). In Lake Dziani Dzaha, however, Woesearchaeota appear to be poorly adapted to high concentrations of $\text{H}_2\text{S}/\text{HS}^-$, confirmed by their drastic decrease in euxinic waters. Woesearchaeota belong to the DPANN superphylum of Archaea which groups together ecologically and has genetically diverse archaeal lineages (Castelle et al., 2015). Most DPANN Archaea have a small genome size with limited metabolic capacities, and Woesearchaeota are predicted to have saccharolytic and fermentative and/or symbiotic lifestyles (Castelle et al., 2015) that are consistent with the conditions prevailing in the water column of Lake Dziani Dzaha. Putative bacterial hosts of Woesearchaeota and ecological interaction networks in Lake Dziani Dzaha still remain to be investigated, but it is noteworthy that the abundance and distribution of this uncultured archaeal phyla perfectly mirrors those of cyanobacteria (Figs. 6A; 7A).

A second remarkable feature of the archaeal community of Lake Dziani Dzaha was the high abundance of methanogenic Euryarchaeota found at all depths, and their systematic prevalence in euxinic waters (Fig. 7). This possibly reflects limited competition for growth substrates (acetate, pyruvate, lactate, H_2 , etc.) due to the near absence of SRB in the lake. The diversity of methanogenic Euryarchaeota seems partly influenced by the absence of sulfate and the presence of strong concentrations of $\text{H}_2\text{S}/\text{HS}^-$ below the chemoclines (Fig. 7B). Shifts in diversity were only observed in the proportions of Methanobacteria and Thermoplasmata, which respectively decreased and increased in

the euxinic waters, where members of Methanomicrobia and WSA2 constituted the major part (>85%) of the methanogenic community (Fig. 7B). The hydrogenotrophic genus *Methanocalculus* was the second most abundant genus of Methanomicrobia in Lake Dziani Dzaha (following an unknown genus) at all depths (Fig. S2B). *Methanocalculus* is a common and occasionally dominant producer of CH₄ in haline and/or alkaline environments (Ollivier et al., 1998; Lanzén et al., 2013; Nolla-Ardèvol et al., 2015; Rojas et al., 2018). This archaeon is able to use CO₂ or formate for the production of CH₄ and has been recently reported as a possible hydrogenotrophic partner in SAO (Timmers et al., 2018 and references therein). Although the number of sequences related to *Methanocalculus* did not increase systematically in the euxinic waters of Lake Dziani Dzaha (Fig. S2B), where CH₄ concentration approaches saturation (Sarazin et al., 2020), the presence of this genus along the water column suggests a major biogeochemical role, especially in CH₄ production and, possibly, in acetate oxidation through syntrophic associations with *Syntrophomonadaceae* (Figs. 6; 7; Timmers et al., 2018). *Methanocalculus* has been shown to be an active methanogen during the anaerobic incubation in alkaline conditions of bioreactors containing soda lake sediments and *Arthrospira* biomass (Nolla-Ardèvol et al., 2015). A remarkable distinction with Lake Dziani Dzaha, however, is that the CH₄-rich biogas produced under such conditions was almost sulfide-free, whereas strong concentrations in H₂S/HS⁻ and CH₄ co-existed below the halocline and the deep chemocline in Lake Dziani Dzaha (Sarazin et al., 2020) indicating that strong concentrations of sulfides may not hamper methanogenesis under haloalkaline conditions.

Another striking characteristic of the Euryarchaeota community structure in Lake Dziani Dzaha was the systematic dominance of the WSA2 class in all depths whatever the

structure of the water column. Based on metagenomic data from different WSA2 populations, the methanogenic potential of this enigmatic new class of Euryarchaeota is thought to be restricted to methylated thiol reduction (Nobu, Narihiro, Kuroda, Mei, & Liu, 2016). The absence of pathways for CO₂-reducing and acetoclastic methanogenesis in the investigated genomes and their heterotrophic, auxotrophic, and ammonia-dependent nature, suggests that WSA2 can only inhabit anaerobic eutrophic environments with other active methanogens, but that they have an essential ecological role for syntrophy and for linking the carbon and sulfur cycles (Nobu et al., 2016). Such an unusual lifestyle perfectly fits the biogeochemical characteristics of the euxinic waters of Lake Dziani Dzaha, explaining the overwhelming presence of WSA2 (Fig. 7B). Methanogenesis by WSA2 through methylated thiol reduction may be especially efficient when CO₂-reducing methanogenesis (notably performed by *Methanocalculus*) becomes unfavorable at low H₂ concentrations.

4.2 Inferences on the microbial origin of lipid biomarkers

As a great part of the bacterial and archaeal populations thriving in Lake Dziani Dzaha are uncharacterized and isolated populations are absent, it is difficult to assign the biological origin of some of the lipid biomarkers detected in the particulate OM. Possible precursors can be inferred by comparing the quantitative distribution of these lipid compounds with the composition of the microbial community as a function of water-depth and physico-chemical characteristics of the water column. This approach remains indirect and requires caution due to possible biases (e.g., differential degradation rates of lipids and DNA, choice of primers, genes present but not expressed, multiple origins of lipid

597 biomarkers, varying lipid content of cells, etc.), but can help to clarify the biological origin
598 of some of the compounds.

599 Linear unsaturated hydrocarbons (*n*-alkenes) are commonly observed in lacustrine
600 settings (De Mesmay, Grossi, Williamson, Kajula, & Derenne, 2007). Short-chain
601 homologues (<*n*-C₂₂) are typically used as biomarkers of microalgae and of some
602 prokaryotes, typically cyanobacteria (Volkman et al., 1998 and references therein). The
603 depth profiles of the C₁₉ and C₂₁ mono-unsaturated *n*-alkenes in the water column of Lake
604 Dziani Dzaha however, point towards a prokaryotic origin distinct from cyanobacteria
605 (Figs. 4A; 6A). Non-isoprenoid unsaturated hydrocarbons are produced within the
606 bacterial domain, but there are few reports in the literature possibly because such lipids
607 are regularly overlooked due to their low cell concentration and/or to the use of inadequate
608 analytical procedures (Nichols, Nichols, & McMeekin, 1995; Cario, Grossi, Schaeffer, &
609 Oger, 2015). C₂₂ to C₃₁ alkenes with an eventual iso- or anteiso- methyl branch have been
610 reported in members of the family Micrococcaceae (Finnerty, 1989) whereas C₂₉ to C₃₃ *n*-
611 alkenes with one to three unsaturation have been characterized in the phototrophic green
612 non-sulfur bacterium *Chloroflexus aurantiacus* in Hot Spring microbial mats (Shiea et al.,
613 1990). Also, *n*-C₂₃ monoenes are important biomarkers in the anaerobic microbial mats
614 developing in Black Sea methane seeps (Thiel, Peckmann, Schmale, Reitner, &
615 Michaelis, 2001) and a *n*-C_{31:9} polyene has been reported in unidentified psychrophilic
616 bacterial strains isolated from Antarctic Sea ice (Nichols et al., 1995). Remarkably, the
617 occurrence of *n*-alkenes in Bacteria often appears to be associated with extreme
618 environmental conditions, which may indicate an adaptive role of these lipids as has been
619 proposed for extremophilic Archaea (Cario et al., 2015; Tourte, Schaeffer, Grossi, & Oger,
620 2020). This is supported in Lake Dziani Dzaha where the systematic increase in the

concentration of the C₁₉ and C₂₁ *n*-alkenes in the aphotic and anoxic waters suggests it originates from non-phototrophic haloalkaline anaerobic bacteria, that are adapted to euxinic conditions. The comparison of *n*-alkenes depth profiles with the number of bacterial sequences, further suggests a new biological precursor for these lipids within (yet unknown) Firmicutes or the Bacteroidales uncultured ML635J-40 aquatic group (Figs. 4A; 6).

Long chain wax esters (WE) can be produced by bacteria, plants, zooplankton, and some other eukaryotes (Bianchi, 1995; Albers, Kattner, & Hagen, 1996; Alvarez, 2016). In Lake Dziani Dzaha, the sharp increase in concentration and structural diversity of C-odd WE observed in the euxinic waters over both seasons (Fig. 4B) supports a significant WE production by haloalkaline non-phototrophic anaerobic bacteria specifically adapted to the presence of sulfides. WE have been reported to be produced by different genera of Gram negative (hydrocarbon-degrading) Gammaproteobacteria, Gram positive Actinobacteria and filamentous anoxygenic phototrophic bacteria (van der Meer et al., 2010; Alvarez, 2016 and references therein). Among these families, Actinobacteria were detected at all depths during both seasons, but their minor contribution to the bacterial community and their proportional decrease below the aphotic and euxinic chemoclines did not support this bacterial class as a potential source of C-odd WE in the lake. The comparison of WE concentration profiles with the number of bacterial sequences, would rather suggest a new biological source of these lipids within the Firmicutes or the uncultured ML635J-40 aquatic group from the family Bacteroidales, potentially linked to the S cycle. Interestingly, a recent survey of 8 282 bacterial genomes showed that the key enzyme involved in WE synthesis in bacteria, WS/DGAT (Wax Ester Synthase/Acyl Coenzyme A:Diacylglycerol Acyltransferase), is present in 673 species, mostly belonging to the phylum Actinobacteria

645 and the phylum Proteobacteria, but also in a few members from the FCB group (Wang et
646 al., 2019). The investigated Firmicutes did not have WS/DGAT enzymes (Wang et al.,
647 2019). This would make the uncultured ML635J-40 aquatic group from the family
648 Bacteroidales a candidate for the origin of WE in the aphotic and anoxic waters of Lake
649 Dziani Dzaha. The production of WE by non-phototrophic strict anaerobic bacteria is
650 unprecedented, which would further extend the potential biological precursors and
651 environmental significance of such lipids. The decrease in concentration in C-odd WE
652 observed in between the two haloclines during the rainy season (Fig. 4B) was not mirrored
653 in the structure of the bacterial community (Fig. 6). This may either reflect the existence
654 of two distinct populations of WE-producing bacteria each associated with a specific
655 halocline, or a WE production/consumption dependent on the varying conditions
656 encountered with depth. Indeed, bacterial WE generally constitute storage compounds
657 that can have diverse functions in cells, mostly related to a metabolic adaptation to
658 environmental stress or fluctuating environmental growth conditions (Grossi et al., 2010;
659 Alvarez, 2016). The alkyl building blocks of the C-odd WE were also retrieved in their free
660 form (i.e., potentially branched C-odd alkanols) in the euxinic waters but, unlike WE, their
661 concentration remained roughly stable downwards in the presence of the seasonal
662 halocline. Short-chained *n*-alkanols can be produced by both eukaryotes and bacteria
663 (Meyers, 2003), but their depth profiles and carbon chain length distribution in Lake Dziani
664 Dzaha clearly support a bacterial precursor. Whether or not C-odd alkanols and WE share
665 a common source is difficult to predict since bacterial WE can be produced either
666 autotrophically, or heterotrophically from available alkyl substrates (Grossi et al., 2010).
667 Hopanoids are specific membrane constituents of some bacteria, though such
668 compounds have also been reported in plants (Volkman, 2005). Based on the study of

669 pure bacterial strains, the biosynthesis of hopanoids has long been considered restricted
670 to oxygen-respiring microorganisms and a limited number of facultative anaerobes
671 (Rohmer, Bouvier-Nave, & Ourisson, 1984; Neunlist, Bissere, & Rohmer, 1988; Ourisson
672 & Rohmer, 1992), but the capacity of some strict anaerobic strains to produce simple (e.g.,
673 diploptene, diplopterol) and elongated (hopanepolyols) bacteriohopanoids was later
674 demonstrated. So far, the anaerobes known to synthesize hopanoids are sulfate-reducing
675 Deltaproteobacteria from the genus *Desulfovibrio* (Blumenberg et al., 2006), iron-oxidizing
676 deltaproteobacteria from the genus *Geobacter* (Härtner, Straub, & Kannenberg, 2005)
677 and Planctomycetes performing the anaerobic oxidation of ammonium (Sinninghe Damsté
678 et al., 2004). However, environmental gene surveys have shown that in natural systems,
679 most bacteria producing hopanoids come from phyla, orders, or classes that have not
680 been identified or characterized metabolically (Pearson, 2014). In Lake Dziani Dzaha, the
681 constant occurrence of diplopterol from the surface waters down to the pit deep halocline,
682 whatever the sampling period, does not distinguish between aerobic or (facultative)
683 anaerobic production of this compound in the upper part of the water column. But the
684 marked increase in the concentration of this hopanol below the deep halocline (Fig. 4C),
685 supports the existence of a new anaerobic bacterial source of hopanoids able to thrive
686 under strong euxinic and haloalkaline conditions, potentially belonging to Bacteroidetes.
687 Since the first identification of tetrahymanol in ciliates from the genus *Tetrahymena*, and
688 its subsequent report from different marine and freshwater ciliates (Harvey & McManus,
689 1989), the occurrence in the sedimentary record of this pentacyclic triterpenol or its
690 molecular fossil gammacerane, has generally been assigned as originating from
691 bacterivorous Protozoa, feeding at microbially active oxic-anoxic interfaces and defined
692 as an indication of a stratified water column, typical for settings with photic zone euxinia

693 (ten Haven et al., 1989; Sinninghe Damsté et al., 1995). However, tetrahymanol has also
694 been observed in anoxygenic phototrophic Alphaproteobacteria (Kleemann et al., 1990),
695 and the presence of a gene encoding for a tetrahymanol synthase (Ths) was recently
696 identified in 104 bacterial genomes belonging to Alphaproteobacteria, aerobic
697 methanotrophic Gammaproteobacteria and sulfate-reducing Deltaproteobacteria,
698 confirming a more widespread potential to produce tetrahymanol in the bacterial domain
699 than previously thought (Banta et al., 2015). The presence of tetrahymanol in Lake Dziani
700 Dzaha is in line with its regular report in alkaline, anoxic, evaporative, and stratified
701 environments (e.g., ten Haven et al., 1989; Thiel et al., 1997; Reinhardt et al., 2019).
702 Flagellated unicellular eukaryotes (ciliates) belonging to the phyla Excavata and
703 Ciliophora are present in Lake Dziani Dzaha (Hugoni et al., 2018) and potentially account
704 for the presence of tetrahymanol in some parts of the water column, especially in the non-
705 sulfidic waters. However, the number of sequences assigned to ciliates collapses and
706 tends toward zero below the halocline during the stratified period (Hugoni et al., 2018)
707 when tetrahymanol concentrations show a three-fold increase (Fig. 4C), making ciliates
708 an unlikely source of this lipid biomarker in the euxinic compartment of the lake. There,
709 tetrahymanol most likely originates from non-phototrophic anaerobic bacteria, capable of
710 developing at high salinity, high alkalinity and potentially involved in the S cycle. The
711 strong co-variation of Firmicutes and Bacteroidetes OTUs with tetrahymanol
712 concentration suggests that this triterpenol originated from one of these two bacterial
713 lineages (Figs. 4 and 6). Genes encoding for Ths have not been detected within genomes
714 of Firmicutes but are present in one Bacteroidetes genome (Banta et al., 2015), supporting
715 yet unknown anaerobic haloalkaline members of this phylum as a potential source of
716 tetrahymanol in Lake Dziani Dzaha.

717

718 The diversity of GC-amenable archaeal lipid biomarkers detectable in the particulate OM
719 appeared limited relative to bacterial diversity in Lake Dziani Dzaha, but fit with the
720 apparent simplicity of the archaeal biodiversity of the lake. Archaeol is a common archaeal
721 biomarker which does not exhibit strong species specificity, and is widely present in
722 methanogens and halophiles (Russell, 1989; Koga, Nishihara, Morii, & Agakawa-
723 Matsushita, 1993; Tourte et al., 2020). The comparison of archaeol concentration profile
724 with archaeal OTUs indeed suggested a major production of this lipid by Euryarchaeota
725 in Lake Dziani Dzaha (Figs 5A; 7B). Although the lipidome of Woesearchaeota has not
726 yet been investigated due to the lack of available cultures, our data does not support
727 archaeol as a major lipid constituent in this newly described archaeal phylum. Phytane,
728 saturated and unsaturated PMI and partially hydrogenated squalenes are more specific
729 of methanogens (Tornabene et al., 1979; Risatti, Rowland, Yon, & Maxwell, 1984;
730 Schouten, van der Maarel, Huber, & Sinninghe Damsté, 1997). The detection of these
731 compounds only in the euxinic waters of Lake Dziani Dzaha agree with the distribution of
732 methanogenic Euryarchaeota OTUs and suggests the production of these isoprenoid
733 hydrocarbons by members of Methanomicrobia, Thermoplasmata and WSA2. PMI and
734 partially hydrogenated squalenes, occurred only when the seasonal halocline was
735 present, further indicating the development of a specific methanogenic assemblage in the
736 euxinic waters during the rainy season characterized by enhanced CH₄ production
737 (Sarazin et al., 2020).

738

739 5. CONCLUSIONS

740 The stratified saline and alkaline ecosystem Dziani Dzaha, exhibits a unique microbial
741 community mostly composed of uncultured bacterial and archaeal lineages. Apart from
742 the massive development of the cyanobacteria *Arthrospira* in the restricted oxygenated
743 water layer, Firmicutes and Bacteroidetes dominate the bacterial community while
744 Woesearchaeota and methanogenic Euryarchaeota represent most of archaeal lineages.
745 The diversity of the microbial community in this hypereutrophic and polyextremophilic lake
746 appears to be shaped by the physico-chemical composition of the water column, and more
747 specifically, by the presence of aphotic haloclines and euxinic waters. In the latter case,
748 the microbial diversity is much lower than in non-sulfidic waters and is strongly dominated
749 by an unknown genus of Syntrophomonadaceae (affiliated with Firmicutes_Costridiales)
750 and the ML635J-40 aquatic group of Bacteroidetes, associated with three groups of
751 methanogenic Euryarchaeota from the Methanomicrobia, Thermoplasmata and WSA2
752 classes. Such dominance suggests essential roles of these microbial groups in anaerobic
753 carbon and sulfur cycling likely to be promoted by specific (yet still uninvestigated)
754 ecological interactions. This is in accordance with the suspected strong recycling of the
755 cyanobacterial biomass across aphotic sulfide gradients whatever the seasonally driven
756 structure of the water column (presence/absence of the seasonal halocline). Our results
757 however do not support anoxygenic photosynthesis as a significant biogeochemical
758 process in Dziani Dzaha, contrary to many past and present sulfidic environments.

759 The sharp changes in bacterial and archaeal diversity induced by the presence of aphotic
760 and euxinic haloclines in Lake Dziani Dzaha were generally mirrored by changes in
761 specific lipid biomarker concentrations, questioning the biological origin of some of these
762 compounds , and supporting an unprecedented microbial origin for some of them. C₁₉ and
763 C₂₁ *n*-alkenes, WE and pentacyclic triterpenes including diplopterol and tetrahymanol on

one hand, and phytane, saturated and unsaturated PMI and partially hydrogenated squalenes on the other hand appeared to be produced by haloalkaline non-phototrophic anaerobic bacteria and archaea, respectively. These anaerobes appear to be adapted to and/or are dependent on strong euxinic conditions. The precise biological precursors of these lipid biomarkers in Lake Dziani Dzaha remain to be characterized by enrichment cultures or strain isolation. It is, however, likely that the aforementioned bacterial compounds are produced by yet unknown members of Syntrophomonadaceae within Firmicutes and/or the Bacteroidales uncultured ML635J-40 aquatic group, and the archaeal biomarkers by yet unknown methanogenic Euryarchaeota notably belonging to the recently proposed WSA2 class. The possibility for tetrahymanol to be produced by non-phototrophic anaerobic bacteria in Lake Dziani Dzaha does not hamper its possible use (or of its molecular fossil gammacerane) as a marker of stratification in the sedimentary record. A better understanding of the specific environmental conditions linked to its production in stratified ecosystems would however improve our understanding of its occurrence and biogeochemical significance throughout Earth's history.

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Data Availability Statement

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

REFERENCES

- Albers, C. S., Kattner, G., & Hagen, W. (1996). The compositions of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: evidence of energetic adaptations. *Marine Chemistry* 55, 347-358.
- Alvarez, H. M. (2016). Triacylglycerol and wax ester-accumulating machinery in prokaryotes. *Biochimie*, 120, 28-39.
- Banta, A. B., Wei, J. H., & Welander, P. V. (2015). A distinct pathway for tetrahymanol synthesis in bacteria. *Proceedings of the National Academy of Sciences*, 112, 13478-13483.
- Bernard, C., Escalas, A., Villeriot, N., Agogu, H., Hugoni, M., Duval, C., ... Troussellier, M. (2019). Very low phytoplankton diversity in a tropical saline-alkaline lake, with co-dominance of *Arthrospira fusiformis* (Cyanobacteria) and *Picocystis salinarum* (Chlorophyta). *Microbial Ecology*, 78, 603-617.
- Bianchi, G. (1995). Plant waxes. In: Hamilton R. J. (Ed.) Waxes: Chemistry, Molecular Biology and Functions. The Oily Press, Dundee, chapter 5.

810 Blumenberg, M., Krüger, M., Nauhaus, K., Talbot, H. M., Oppermann, B. I., Seifert, R., ...
811 Michaelis, W. (2006). Biosynthesis of hopanoids by sulfate-reducing bacteria (genus
812 *Desulfovibrio*). *Environmental Microbiology*, 8, 1220-1227.

813 Brazelton, W. J., Thornton, C. N., Hyer, A., I. Twing, K. I., Longino, A. A., Lang, S. Q., ...
814 Schrenk, M. O. (2017). Metagenomic identification of active methanogens and
815 methanotrophs in serpentinite springs of the Voltri Massif, Italy. *PeerJ*, 5, e2945.

816 Brocks, J. J., & Pearson, A. (2005). Building the biomarker tree of life. *Reviews in*
817 *Mineralogy and Geochemistry*, 59, 233-258.

818 Brocks, J. J., & Banfield, J. (2009). Unravelling ancient microbial history with community
819 proteogenomics and lipid geochemistry. *Nature Reviews Microbiology*, 7, 601-609.

820 Cadeau, P., Jézéquel, D., Leboulanger, C., Fouilland, E., Le Floc'h, E., Chaduteau, C., ...
821 Ader, M. (2020). Carbon isotope evidence for large methane emissions to the
822 Proterozoic atmosphere. *Scientific Reports*, 10, 18186.

823 Cario, A., Grossi, V., Schaeffer, P., & Oger, P. M. (2015). Membrane homeoviscous
824 adaptation in the piezo-hyperthermophilic archaeon *Thermococcus barophilus*.
825 *Frontiers in Microbiology*, 6, 1152.

826 Casamayor, E. O., Triadó-Margarit, X., & Castañeda, C. (2013). Microbial biodiversity in
827 saline shallow lakes of the Monegros desert, Spain. *FEMS Microbiology Ecology*, 85,
828 503-518.

829 Castelle, C. J., Wrighton, K. C., Thomas, B. C., ,Hug, L. A., Brown, C. T., Wilkins, M. J.,
830 ... Banfield, J. F. (2015). Genomic expansion of domain Archaea highlights roles for
831 organisms from new phyla in anaerobic carbon cycling. *Current Biology*, 25, 1-12.

832 de Mesmay, R., Grossi, V., Williamson, D., Kajula, & Derenne, S. (2007). Novel mono-,
833 di- and tri-unsaturated very long chain (C₃₇-C₄₃) *n*-alkenes in alkenone-free lacustrine
834 sediments (Lake Masoko, Tanzania). *Organic Geochemistry*, 38, 323-333.

835 Escudié F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., ... Pasca G.
836 (2018). FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics*, 34, 1287-
837 1294.

838 Finnerty, W. R. (1989). Microbial lipid metabolism. In: Ratledge, C. & Wilkinson, S. G.
839 (Eds.), *Microbial Lipids*, Vol. 2. Academic Press, London, pp. 525-566.

840 French, K. L., Rocher, D., Zumberger, J. E., & Summons, R. E. (2015). Assessing the
841 distribution of sedimentary C₄₀ carotenoids through time. *Geobiology*, 13, 139-151.

842 Grice, K., Cao, C., Love, G. D., Böttcher, M. E., Twitchett, R. J., Grosjean, E., ... Jin, Y.
843 (2005). Photic zone euxinia during the Permian-Triassic superanoxic event. *Science*,
844 307, 706-709.

845 Grossi, V., Yakimov, M. M., Al Ali, B., Tapilatu, Y., Cuny, P., Goutx, M., ..., Tamburini, C.
846 (2010). Hydrostatic pressure affects membrane and storage lipid compositions of the
847 piezotolerant hydrocarbon-degrading *Marinobacter hydrocarbonoclasticus* strain #5.
848 *Environmental Microbiology*, 12, 2020-2033.

849 Hamilton, T. L., Bovee, R. J., Thiel, V., Sattin, S. R., Mohr, W., Schaperdoth, I., ...
850 Macalady, J. L. (2014). Coupled reductive and oxidative sulfur cycling in the
851 phototrophic plate of a meromictic lake. *Geobiology*, 12, 451-468.

852 Hamilton, T. L., Bovee, R. J., Sattin, S. R., Mohr, W., Gilhooly III, W. P., Lyons, T. W.,
853 Pearson, A., & Macalady, J. L. (2016). Carbon and sulfur cycling below the chemocline
854 in a meromictic lake and the identification of a novel taxonomic lineage in the FCB
855 Superphylum *Candidatus Aegiribacteria*. *Frontiers in Microbiology*, 7, 598.

856 Härtner, T., Straub, K. L., & Kannenberg, E. (2005). Occurrence of hopanoid lipids in
857 anaerobic *Geobacter* species. *FEMS Microbiology Letters*, 243, 59–64.

858 Harvey H. R., & McManus, G. B. (1991). Marine ciliates as a widespread source of
859 tetrahymanol and hopan-3- β -ol in sediments. *Geochimica et Cosmochimica Acta*, 55,
860 3387-3390.

861 Hugoni, M., Escalas, A., Bernard, C., Nicolas, S., Jezequel, D., Vazzoler, F., ... Agogu,
862 H. (2018). Spatiotemporal variations in microbial diversity across the three domains of
863 life in a tropical thalassohaline lake (Dziani Dzaha, Mayotte Island). *Molecular Ecology*,
864 27, 4775-4786.

865 Humayoun, S. B., Bano, N., & Hollibaugh, J. T. (2003). Depth distribution of microbial
866 diversity in Mono Lake, a meromictic soda lake in California. *Applied and*
867 *Environmental Microbiology*, 69, 1030-1042.

868 Jones, B. E., Grant, W. D., Duckworth, A. W., Owenson, G. G. (1998). Microbial diversity
869 of soda lakes. *Extremophiles*, 2, 191-200.

870 Kleemann, G., Poralla, K., Englert, G., Kjsen, H., Liaaen-Jensen, S., Neunlist, S., &
871 Rohmer, M. (1990). Tetrahymanol from the phototrophic bacterium
872 *Rhodopseudomonas palustris*: first report of a gammacerane triterpene from a
873 prokaryote. *Journal of General Microbiology*, 136, 2551-2553.

874 Klepac-Ceraj, V., Hayes, C. A., Gilhooly, W. P., Lyons, T. W., Kolter, R., & Pearson, A.
875 (2012). Microbial diversity under extreme euxinia: Mahoney Lake, Canada.
876 *Geobiology*, 10, 223-235.

877 Koga, Y., Nishihara, M., Morii, H., & Agakawa-Matsushita, M. (1993). Ether polar lipids of
878 methanogenic bacteria: structures, comparative aspects, and biosyntheses.
879 *Microbiological Reviews*, 57, 164-182.

880 Lanzén, A., Simachew, A., Gessesse, A., Chmolowska, D., Jonassen, I., Øvreas, L.
881 (2013). Surprising prokaryotic and eukaryotic diversity, community structure and
882 biogeography of Ethiopian soda lakes. *PLoS One*, 8, e72577.

883 Leboulanger, C., Agogué, H., Bernard, C., Bouvy, M., Carré, C., Cellamare, M., ...
884 Lavergne, C. (2017). Microbial diversity and cyanobacterial production in Dziani Dzaha
885 crater lake, a unique tropical thalassohaline environment. *PloS one*, 12, e0168879.

886 Lyons, T. W., Anbar, A. D., Severmann, S., Scott, C., & Gill, B. C. (2009) Tracking euxinia
887 in the ancient ocean: a multiproxy perspective and Proterozoic case study. *Annual*
888 *Review of Earth and Planetary Sciences*, 37,507-534.

889 Magoč, T., & Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to
890 improve genome assemblies. *Bioinformatics*, 27, 2957-2963.

891 Meyers, P. A. (2003). Applications of organic geochemistry to paleolimnological
892 reconstructions: a summary of examples from the Laurentian Great Lakes. *Organic*
893 *geochemistry*, 34, 261-289.

894 Meyer, K. M., & Kump, L. R. (2008). Oceanic euxinia in Earth history: causes and
895 consequences. *Annual Review of Earth and Planetary Sciences*, 36, 251-88.

896 Neunlist, S., Bissert, P., & Rohmer, M. (1988). The hopanoids of the purple non-sulfur
897 bacteria *Rhodopseudomonas palustris* and *Rhodopseudomonas acidophila* and the
898 absolute configuration of bacteriohopanetetrol. *European Journal of Biochemistry*,
899 171, 243–252.

900 Nichols, D. S., Nichols, P. D., & McMeekin, T. A. (1995). A new n-C_{31:9} polyene
901 hydrocarbon from Antarctic bacteria. *FEMS Microbiology Letters*, 125, 281-286.

902 Nobu, M. K., Narihiro, T., Kuroda, K., Mei, R., & Liu, W.-T. (2016). Chasing the elusive
903 Euryarchaeota class WSA2: genomes reveal a uniquely fastidious methyl-reducing
904 methanogen. *The ISME Journal*, 10, 2478-2487.

905 Nolla-Ardèvol, V., Strous, M., & Tegetmeyer, H. E. (2015). Anaerobic digestion of the
906 microalga *Spirulina* at extreme alkaline conditions: biogas production, metagenome,
907 and metatranscriptome. *Frontiers in Microbiology*, 6, 597.

908 Ollivier, B., Fardeau, M.-L., Cayol, J.-L., Magot, M., Patel, B. K., Prensier, G., & Garcia,
909 J.-L. (1998). *Methanocalculus halotolerant*, gen. nov., sp. nov., isolated from an oil-
910 producing well. *International Journal of Systematic Bacteriology*, 48, 821-828.

911 Ourisson, G., & Rohmer, M. (1992). Hopanoids. 2. Biohopanoids: a novel class of bacterial
912 lipids. *Accounts of Chemical Research*, 25, 403-408.

913 Overmann, J., Beatty, J. T. & Hall, K. J. (1996). Purple sulfur bacteria control the growth
914 of aerobic heterotrophic bacterioplankton in a meromictic salt lake. *Applied and*
915 *Environmental Microbiology*, 62, 3251-3258.

916 Pearson, A. (2014). Lipidomics for Geochemistry. In: Holland, H. D. & Turekian, K. K. C
917 (Eds.), *Treatise on Geochemistry* (2nd ed.). Elsevier, Oxford, vol. 12, pp. 291-336.

918 Pearson, A., Flood Page, S. R., Jorgenson, T. L., Fischer, W. W., & Higgins, M. B. (2007).
919 Novel hopanoid cyclases from the environment. *Environmental Microbiology*, 9, 2175-
920 2180.

921 Philippot, P., van Zuilen, M., Lepot, K., Thomazo, C., Farquhar, J., & van Kranendonk, M.
922 J. (2007). Early Archaean microorganisms preferred elemental sulfur, not sulfate.
923 *Science*, 317, 1534-1537.

924 Reinhardt, M., Goetz, W., Duda, J.-P., Heim, C., Reitner, J., Thiel, V. (2019). Organic
925 signatures in Pleistocene cherts from Lake Magadi (Kenya), implications for early
926 Earth hydrothermal deposits. *Biogeosciences*, 16, 2443-2465.

927 Risatti, J. B., Rowland, S. J., Yon, D. A., & Maxwell, J. R. (1984). Stereochemical studies
928 of acyclic isoprenoids--XII. Lipids of methanogenic bacteria and possible contributions
929 to sediments. *Organic Geochemistry*, 6, 93-104.

930 Ritchie, R. J. (2006). Consistent sets of spectrophotometric chlorophyll equations for
931 acetone, methanol and ethanol solvents. *Photosynthesis Research*, 89, 27-41.

932 Rohmer, M., Bouvier-Nave, P., & Ourisson, G. (1984). Distribution of hopanoid triterpenes
933 in prokaryotes. *Microbiology*, 130, 1137-1150.

934 Rojas, P., Rodríguez, N., de la Fuente, V., Sánchez-Mata, D., Amils, R., Sanz J. L., 2018).
935 Microbial diversity associated to the anaerobic sediments of a soda lake (Mono Lake,
936 CA). *Canadian Journal of Microbiology*, 64, 385-392.

937 Russel, N. J. (1989). Adaptive modifications in membranes of halotolerant and halophilic
938 microorganisms. *Journal of Bioenergetics and Biomembranes*, 21, 93-113.

939 Sarazin, G., Jézéquel, D., Leboulanger, C., Fouilland, E., Le Floc'h, E., Bouvy, M., ...
940 Ader, M. (2020). Geochemistry of an endorheic thalassohaline ecosystem: the Dziani
941 Dzaha crater lake (Mayotte Archipelago, Indian Ocean). *Compte Rendus*
942 *Geosciences*, 352, 559-577.

943 Schouten, S., van der Maarel, M. J. E. C., Huber, R., & Sinninghe Damsté, J. S. (1997).
944 2,6,10,15,19-Pentamethylicosenes in *Methanolobus bombayensis*, a marine
945 methanogenic archaeon, and in *Methanosarcina mazei*. *Organic Geochemistry*, 26,
946 409-414.

947 Sinninghe Damsté, J. S., Rijpstra, I. C., Schouten, S., Fuerst, J.A., Jetten, M. S. M., &
948 Strous, M. (2004). The occurrence of hopanoids in planctomycetes: implications for
949 the sedimentary biomarker record. *Organic Geochemistry*, 35, 561-566.

950 Sinninghe Damsté, J. S., Kenig, F., Koopmans, M. P., Köster, J., Schouten, S., Hayes, J.
951 M., & de Leeuw, J. W. (1995). Evidence for gammacerane as an indicator of water
952 column stratification. *Geochimica et Cosmochimica Acta*, 59, 1895-1900.

953 Sorokin, D. Y., Tourova, T. P., Mussmann, M., Muyzer, G. (2008). *Dethiobacter*
954 *alkaliphilus* gen. nov. sp. nov., and *Desulfurivibrio alkaliphilus* gen. nov. sp. nov. - two
955 novel representatives of reductive sulfur cycle from soda lakes. *Extremophiles*, 12,
956 431-439.

957 Sorokin, D. Y., Berben, T., Melton, E. D., Overmars, L., Vavourakis, C. D., & Muyzer, G.
958 (2014). Microbial diversity and biogeochemical cycling in soda lakes. *Extremophiles*,
959 18, 791-809.

960 Sorokin, D., Banciu, H. L., & Muyzer, G. (2015). Functional microbiology of soda lakes.
961 *Current Opinion in Microbiology*, 25, 88-96.

962 Shiea, J., Brassell, S.C., & Ward, D.M. (1990). Mid-chain branched mono- and dimethyl
963 alkanes in hot spring cyanobacterial mats: A direct biogenic source for branched
964 alkanes in ancient sediments? *Organic Geochemistry* 15, 223-231.

965 ten Haven, H. L., Rohmer, M., Rullkötter, J., & Bissleret, P. (1989). Tetrahymanol, the most
966 likely precursor of gammacerane, occurs ubiquitously in marine sediments.
967 *Geochimica et Cosmochimica Acta*, 53, 3073-3079.

968 Thiel, V., Jenisch, A., Landmann, G., Reimer, A., & Michaelis, W. (1997). Unusual
969 distributions of long-chain alkenones and tetrahymanol from the highly alkaline Lake
970 Van, Turkey. *Geochimica et Cosmochimica Acta*, 61, 2053-2064.

971 Thiel, V., Peckmann, J., Schmale, O., Reitner, J., & Michaelis, W. (2001). A new straight-
972 chain hydrocarbon biomarker associated with anaerobic methane cycling. *Organic*
973 *Geochemistry*, 32, 1019-1023.

974 Timmers, P. H. A., Vavourakis, C. D., Kleerebezem, R., Sinninghe Damsté, J. S. S.,
975 Muyzer, G., Stams, A. J. M., Sorokin, D.Y., & Plugge, C. M. (2018). Metabolism and
976 occurrence of methanogenic and sulfate-reducing syntrophic acetate oxidizing
977 communities in haloalkaline environments. *Frontiers in Microbiology*, 9, 3039.

978 Tornabene, T. G., Langworthy T. A., Holzer G., & Oro J. (1979). Squalenes, phytenes and
979 other isoprenoids as major neutral lipids of methanogenic and thermoacidophilic
980 "archaebacteria". *Journal of Molecular Evolution*, 13, 73-83.

981 Tourte, M., Schaeffer, P., Grossi, V., & Oger, P.M. (2020). Functionalized Membrane
982 Domains: An Ancestral Feature of Archaea? *Frontiers in Microbiology*, 11, 526.

983 van der Meer, M. T., Klatt, C. G., Wood, J., Bryant, D. A., Bateson, M. M., Lammerts, L.,
984 & Ward, D. M. (2010). Cultivation and genomic, nutritional, and lipid biomarker
985 characterization of *Roseiflexus* strains closely related to predominant in situ
986 populations inhabiting Yellowstone hot spring microbial mats. *Journal of bacteriology*,
987 192, 3033-3042.

988 van Leerdam, R. C., Bonilla-Salinas, M., de Bok, F. A. M., Bruning, H., Lens, P. N. L.,
989 Stams, A. J. M., & Janssen, A. J. H. (2008). Anaerobic methanethiol degradation and
990 methanogenic community analysis in an alkaline (pH 10) biological process for
991 liquefied petroleum gas desulfurization. *Biotechnology and Bioengineering*, 101, 691-
992 701.

993 Vavourakis, C. D., Andrei, A.-S., Mehrshad, M., Ghai, R., & Sorokin, D. Y. (2018). A
994 metagenomics roadmap to the uncultured genome diversity in hypersaline soda lake
995 sediments. *Microbiome*, 6, 168.

996 Villanueva, L., Besseling, M., Rodrigo-Gámiz, M., Rampen, S. W., Verschuren, D., &
997 Sinninghe Damsté, J. S. (2014). Potential biological sources of long chain alkyl diols
998 in a lacustrine system. *Organic Geochemistry*, 68, 27-30.

999 Volkman, J. K. (2005). Sterols and other triterpenoids: source specificity and evolution of
1000 biosynthetic pathways. *Organic Geochemistry*, 36, 139-159.

1001 Volkman, J. K., et al 1998. Microalgal biomarkers: A review of recent research
1002 developments. *Organic Geochemistry* 29, 1163-1179.

1003 Wang, L., Yang, J., Huang, Y., Liu, Q., Xu, Y., Piao, X., & Wise, M. J. (2019). Systematic
1004 analysis of metabolic pathway distributions of bacterial energy reserves. *Genes*,
1005 *Genomes*, *Genetics*, 9, 2489-2496.

1006 Wani, A. A., Surakasi, V. P., Siddarth, J., Raghavan, R. G., Patole, M. S., Ranade, D., &
1007 Shouche, Y. S. (2006). Molecular analyses of microbial diversity associated with the
1008 Lonar soda lake in India: an impact crater in a basalt area. *Research in Microbiology*,
1009 157, 928-937.

1010 Zinke, J., Reijmer, J.J.G., & Thomassin, B. A. (2003). Systems tracts sedimentology in
1011 the lagoon of Mayotte associated with the Holocene transgression. *Sedimentary*
1012 *Geology*, 160, 57-79.

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FIGURE CAPTIONS

FIGURE 1 Lake Dziani Dzaha location (**A & B**), size (**C & D**), and bathymetry (**E**).

FIGURE 2 Water column physico-chemical characteristics of Lake Dziani Dzaha (pit) at the time of sampling at the end of the dry (November 2014) and rainy (April 2015) seasons. **A)** pH, temperature and salinity; **B)** diurnal dissolved O₂ and H₂S/HS⁻. Note that following the end of the rainy season, the seasonal halocline may last for 2 to 3 additional months before disappearing.

FIGURE 3 Depth concentration profiles of chlorophyll-a and free phytol in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

FIGURE 4 Depth concentration profiles of **A)** C_{19:1} and C_{21:1} *n*-alkenes, **B)** summed C-odd *n*-alkanols and C-odd wax esters and **C)** diplopterol and tetrahymanol in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

FIGURE 5 Depth concentration profiles of phytane and summed PMI **A)** and archaeol and summed hydrosqualenes **B)** in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

FIGURE 6 Relative abundance (% of sequences) of bacterial phyla **A)** and among Firmicutes **B)** and Bacteroidetes **C)** orders in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

FIGURE 7 Relative abundance (% of sequences) of archaeal phyla **A)** and among euryarchaeal classes **B)** in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

FIGURE 8 Canonical Correspondence Analysis (CCA) between physico-chemical characteristics of Lake Dziani Dzaha (pH, salinity, temperature, O₂ and H₂S/HS⁻) and the most abundant (>1% of pooled sequences) bacterial and archaeal phylum. NS = without seasonal halocline, S = with seasonal halocline.

Supplementary figures

FIGURE S1 Partial GC-MS chromatograms (TIC) of the wax esters (WE) present in Lake Dziani Dzaha particulate organic matter collected at 0.2m (oxic), 6m (anoxic and non-sulfidic) and 17.4m (anoxic and sulfidic) at the end of the dry season (absence of seasonal halocline).

FIGURE S2 Relative abundance (% of sequences) of genera present among the family of Syntrophomonadaceae **A)** and the class of Methanomicrobia **B)** in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

FIGURE S3 Depth concentration profiles of phytoplankton abundance in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

Fig. 1

Fig. 2

Fig. 3 revised

Without seasonal stratification
(dry season)

With seasonal stratification
(rainy season)

A

B

C

Fig. 4

Fig. 5

With seasonal stratification (dry season)

Without seasonal stratification (rainy season)

A

B

among Firmicutes (Order level)

C

among Bacteroidetes (Order level)

Fig. 6

With seasonal stratification
(dry season)

Without seasonal stratification
(rainy season)

A

B

among Euryarchaeota (Class level)

Fig. 7

Fig. 8

A **B** **C** **Fig. S1**

With seasonal stratification
(dry season)

Without seasonal stratification
(rainy season)

A

among Syntrophomonadaceae (Genus level)

B

among Methanomicrobia (Genus level)

Fig. S2

Fig. S3 new