

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

Sala David <sup>1</sup>, Grossi Vincent <sup>1,\*</sup>, Agogu  H l ne <sup>2</sup>, Leboulanger Christophe <sup>3</sup>, J z quel Didier <sup>4,5</sup>, Sarazin G rard <sup>4</sup>, Antheaume Ingrid <sup>1</sup>, Bernard Cecile <sup>6</sup>, Ader Magali <sup>4</sup>, Hugoni Myl ne <sup>7,8,9</sup>

<sup>1</sup> Univ Lyon, Universit  Claude Bernard Lyon 1CNRS ENS LUJM, LGL-TPE Villeurbanne , France

<sup>2</sup> LIENSs, UMR 7266 La Rochelle Universit  - CNRS La Rochelle, France

<sup>3</sup> MARBEC, Univ Montpellier, IRD CNRS Ifremer S te , France

<sup>4</sup> Universit  de Paris, Institut de Physique du Globe de Paris Paris, France

<sup>5</sup> INRAE & Universit  Savoie Mont Blanc, UMR CARRTEL Thonon-les-Bains, France

<sup>6</sup> UMR 7245 Mol cules de Communication et Adaptations des Microorganismes (MCAM) MNHN-CNRS Mus um National d'Histoire Naturelle Paris, France

<sup>7</sup> Univ Lyon, Universit  Claude Bernard Lyon 1CNRS INRAE VetAgro SupUMR Ecologie Microbienne Villeurbanne , France

<sup>8</sup> Univ Lyon, INSA Lyon CNRS UMR 5240 Microbiologie Adaptation et Pathog nie Villeurbanne , France

<sup>9</sup> Institut Universitaire de France Paris , France

\* Corresponding author : Vincent Grossi, email address : [vincent.grossi@univ-lyon1.fr](mailto:vincent.grossi@univ-lyon1.fr)

### Abstract :

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

**Keywords** : bacterial and archaeal diversity, bacteroidetes ML635J-40 aquatic group, haline and aphotic euxinia, lipid biomarkers, syntrophomonadaceae, 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

water column into at least two or more physically- and chemically- contrasting compartments hosting diverse microbial communities that are adapted to specific ecological niches. Typically, phototrophic organisms such as (pico)cyanobacteria deliver fixed carbon to vertically organized chemolitho- and chemoorgano-trophic bacterial and archaeal communities that then recycle a major part of the sinking organic matter (Sorokin et al., 2014; Sorokin, Banciu, & Muyzer, 2015). In stratified sulfur-rich environments where light reaches anoxic waters, the presence of an euxinic (sulfidic and anoxic) photic zone further allows dense populations of purple and green sulfur anoxygenic phototrophic bacteria to develop and impact the carbon and sulfur cycles (Overmann et al., 1996; Hamilton et al., 2014). These conditions resemble, at least regionally, those that have prevailed over several periods of the Earth's history, such as the Mesoproterozoic and Phanerozoic biotic crises, when parts of the ocean were oxygen-depleted and partly euxinic (Grice et al., 2005; Lyons, Anbar, Severmann, Scott, & Gill, 2009). For this reason contemporaneous stratified and euxinic ecosystems are often considered to be good models for the past ocean (Meyer & Kump, 2008). The structure and lipid biomarkers of the microbial communities thriving in such euxinic ecosystems have been regularly investigated, and lipid biomarkers specific to photic zone euxinia and associated chemoclines have been characterized and used for paleo-environmental reconstructions (Grice et al., 2005; French, Rocher, Zumberger, & Summons, 2015). Surprisingly, much less is known about the microbial communities thriving in aphotic euxinic waters (i.e., below the euxinic photic zone or in euxinic ecosystems deprived of an euxinic photic zone) or their potential specific organic biomarkers, although such ecological niches often represent a major part of euxinic ecosystems (Klepac-Ceraj et al., 2012; Hamilton et al., 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<sup>-1</sup>), low sulfate concentrations (0-3 mM) and high dissolved organic carbon (DOC) concentrations (4-8 mmol L<sup>-1</sup>) (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 km<sup>2</sup>) relative to its size (ca. 0.24 km<sup>2</sup>; 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

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

Sampling was performed at the pit site (Fig. 1E) during two field surveys, one conducted during October 2014 (around the end of the dry season, i.e., without seasonal stratification) and the other, in April 2015 (around the end of the wet period, i.e., with seasonal stratification). Prior to water sampling, pH, dissolved oxygen, temperature, and conductivity depth profiles were recorded using a YSI 6600XLM probe. These profiles enabled the sampling strategy to be adapted for organic biomarkers and microbial diversity analyses depending on the depth of the seasonal and permanent haloclines. Water was then sampled using a horizontal Niskin bottle to provide an appropriate vertical resolution. H<sub>2</sub>S/HS<sup>-</sup> concentrations (i.e., soluble sulfide concentrations (ΣS(-II))), were determined in the field by colorimetry using a AQUALYTIC SpectroDirect spectrophotometer and Merck reagent kits. For lipid analyses, 500 to 1000 mL of lake water was filtered onto pre-combusted GF/F filters, and for total chlorophyll-a (Chl-a) analysis 5 mL water samples were conditioned similarly. For microbial diversity determination, 20 mL water samples were first filtered through 3 µm polycarbonate filters 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, x2), methanol-DCM (1:1, v/v, x2), and DCM (x1). 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 N<sub>2</sub> 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<sup>-1</sup>). The injector temperature was programmed as follows: 60°C (held 0.5 min), 60 to 300°C (200°C min<sup>-1</sup>), 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<sup>-1</sup>), 130 to 300°C (4°C min<sup>-1</sup>), 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  $\text{CH}_4$  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<sub>19:1</sub> and 2.5  $\mu\text{g L}^{-1}$  for C<sub>21:1</sub>) 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 C<sub>30</sub> and C<sub>32</sub> 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 C<sub>14</sub>-C<sub>20</sub> acyl chains esterified to linear or methyl-branched C<sub>14</sub>-C<sub>18</sub> 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

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

Whatever the season, the archaeal community was essentially composed of Woesearchaeota and Euryarchaeota, which together represented between 91 and 97% of the total archaeal sequences (Fig. 7A). Woesearchaeota dominated the archaeal assemblages above the deep halocline during the dry season (64 to 74% of archaeal sequences) and above the seasonal halocline during the rainy season (66 to 69% of archaeal sequences), whereas Euryarchaeota became systematically dominant in the euxinic waters below (70-80% of archaeal sequences). Further analysis of Euryarchaeota diversity showed the predominance of the methanogenic class WSA2 in all depths irrespective of the sampling season, representing between 40 and 95% of the total sequences of Euryarchaeota (Fig. 7B). Methanobacteria, Methanomicrobia and 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 H<sub>2</sub>S/HS<sup>-</sup>) 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 H<sub>2</sub>S/HS<sup>-</sup> 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<sup>-</sup> (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

from stratified sediments from Russian hypersaline soda lakes (Sorokin et al., 2014; Vavourakis et al., 2018) and have been reported as a major constituent of the bacterial community inhabiting the alkaline, low-salt, shoreline anaerobic sediments of Mono Lake (Rojas et al., 2018). *Synthrophomonadaceae* from the genus *Dethiobacter* have also been recovered from a mixture of soda lake sediments (Sorokin, Tourova, Mussmann, & Muyzer, 2008) and this genus has been shown to contribute to carbon cycling in the hyper-euxinic waters of the stratified Mahoney Lake (British Columbia; Hamilton et al., 2016). Interestingly, different cultured and uncultured members of the family *Synthrophomonadaceae* including the genera *Dethiobacter* and '*Candidatus contubernalis*' have been reported to oxidize acetate anaerobically through syntrophic acetate oxidation (SAO) with hydrogenotrophic sulfate-reducing bacteria (SRB) or methanogenic Archaea in several Russian hypersaline soda lakes (Timmers et al., 2018). In Lake Dziani Dzaha, a syntrophic association between acetate oxidizing *Synthrophomonadaceae* and hydrogenotrophic methanogenic archaea appears to be a likely scenario based on 1) the strong similarities in *Synthrophomonadaceae* assemblages observed here and in soda lakes where SAO occurs and, 2) the co-occurrence of potential adequate archaeal partners (Figs. 6; 7 and discussion below). An association between *Synthrophomonadaceae* and hydrogenotrophic SRB in Lake Dziani Dzaha appears less likely, due to the near absence of sulfates and SRB in the anoxic waters where (poly)sulfides prevail. This is particularly interesting when considering a possible analogy between Lake Dziani Dzaha and the Early Proterozoic Oceans where low oxygen concentrations in the atmosphere and surface waters are thought to have prevented sulfate production by oxidative weathering, thus strongly limiting microbial 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 CO<sub>2</sub> and of glycine to NH<sub>3</sub> (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 CH<sub>4</sub> and NH<sub>3</sub> 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,  $\text{H}_2$ , 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 CH<sub>4</sub> 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 CO<sub>2</sub> or formate for the production of CH<sub>4</sub> 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 CH<sub>4</sub> concentration approaches saturation (Sarazin et al., 2020), the presence of this genus along the water column suggests a major biogeochemical role, especially in CH<sub>4</sub> production and, possibly, in acetate oxidation through syntrophic associations with *Synthrophomonadaceae* (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 CH<sub>4</sub>-rich biogas produced under such conditions was almost sulfide-free, whereas strong concentrations in H<sub>2</sub>S/HS<sup>-</sup> and CH<sub>4</sub> 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 CO<sub>2</sub>-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 CO<sub>2</sub>-reducing methanogenesis (notably performed by *Methanocalculus*) becomes unfavorable at low H<sub>2</sub> 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



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

Linear unsaturated hydrocarbons (*n*-alkenes) are commonly observed in lacustrine settings (De Mesmay, Grossi, Williamson, Kajula, & Derenne, 2007). Short-chain homologues (<*n*-C<sub>22</sub>) are typically used as biomarkers of microalgae and of some prokaryotes, typically cyanobacteria (Volkman et al., 1998 and references therein). The depth profiles of the C<sub>19</sub> and C<sub>21</sub> mono-unsaturated *n*-alkenes in the water column of Lake Dziani Dzaha however, point towards a prokaryotic origin distinct from cyanobacteria (Figs. 4A; 6A). Non-isoprenoid unsaturated hydrocarbons are produced within the bacterial domain, but there are few reports in the literature possibly because such lipids are regularly overlooked due to their low cell concentration and/or to the use of inadequate analytical procedures (Nichols, Nichols, & McMeekin, 1995; Cario, Grossi, Schaeffer, & Oger, 2015). C<sub>22</sub> to C<sub>31</sub> alkenes with an eventual iso- or anteiso- methyl branch have been reported in members of the family Micrococcaceae (Finnerty, 1989) whereas C<sub>29</sub> to C<sub>33</sub> *n*-alkenes with one to three unsaturation have been characterized in the phototrophic green non-sulfur bacterium *Chloroflexus aurantiacus* in Hot Spring microbial mats (Shiea et al., 1990). Also, *n*-C<sub>23</sub> monoenes are important biomarkers in the anaerobic microbial mats developing in Black Sea methane seeps (Thiel, Peckmann, Schmale, Reitner, & Michaelis, 2001) and a *n*-C<sub>31:9</sub> polyene has been reported in unidentified psychrophilic bacterial strains isolated from Antarctic Sea ice (Nichols et al., 1995). Remarkably, the occurrence of *n*-alkenes in Bacteria often appears to be associated with extreme environmental conditions, which may indicate an adaptive role of these lipids as has been proposed for extremophilic Archaea (Cario et al., 2015; Tourte, Schaeffer, Grossi, & Oger, 2020). This is supported in Lake Dziani Dzaha where the systematic increase in the

concentration of the C<sub>19</sub> and C<sub>21</sub> *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

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

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

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

The diversity of GC-amenable archaeal lipid biomarkers detectable in the particulate OM appeared limited relative to bacterial diversity in Lake Dziani Dzaha, but fit with the apparent simplicity of the archaeal biodiversity of the lake. Archaeol is a common archaeal biomarker which does not exhibit strong species specificity, and is widely present in methanogens and halophiles (Russell, 1989; Koga, Nishihara, Morii, & Agakawa-Matsushita, 1993; Tourte et al., 2020). The comparison of archaeol concentration profile with archaeal OTUs indeed suggested a major production of this lipid by Euryarchaeota in Lake Dziani Dzaha (Figs 5A; 7B). Although the lipidome of Woesearchaeota has not yet been investigated due to the lack of available cultures, our data does not support archaeol as a major lipid constituent in this newly described archaeal phylum. Phytane, saturated and unsaturated PMI and partially hydrogenated squalenes are more specific of methanogens (Tornabene et al., 1979; Risatti, Rowland, Yon, & Maxwell, 1984; Schouten, van der Maarel, Huber, & Sinninghe Damsté, 1997). The detection of these compounds only in the euxinic waters of Lake Dziani Dzaha agree with the distribution of methanogenic Euryarchaeota OTUs and suggests the production of these isoprenoid hydrocarbons by members of Methanomicrobia, Thermoplasmata and WSA2. PMI and partially hydrogenated squalenes, occurred only when the seasonal halocline was present, further indicating the development of a specific methanogenic assemblage in the euxinic waters during the rainy season characterized by enhanced CH<sub>4</sub> production (Sarazin et al., 2020).

## 5. CONCLUSIONS

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

The sharp changes in bacterial and archaeal diversity induced by the presence of aphotic and euxinic haloclines in Lake Dziani Dzaha were generally mirrored by changes in specific lipid biomarker concentrations, questioning the biological origin of some of these compounds, and supporting an unprecedented microbial origin for some of them. C<sub>19</sub> and

C<sub>21</sub> *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.

## **Acknowledgments**

Field work permission was given by the 'Conservatoire du Littoral et des Rivages Lacustres' (Antenne Océan Indien). We are grateful to Alexandra and Laurent at "Les Couleurs" (Mayotte) for transforming their Guest House into a 'field laboratory' and for helpful and friendly assistance. We thank two anonymous reviewers for their comments and Tracy Bentley for careful English editing which enabled us to improve the manuscript. The Molecular Core Facilities at LIENSs laboratory is acknowledged. This work was



supported by Total Corporate Foundation, Total E&P (unconventional R&D program) and the French National Research Agency (project DZIANI, ANR-13-BS06-0001).

### **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 e, 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.

- Blumenberg, M., Krüger, M., Nauhaus, K., Talbot, H. M., Oppermann, B. I., Seifert, R., ... Michaelis, W. (2006). Biosynthesis of hopanoids by sulfate-reducing bacteria (genus *Desulfovibrio*). *Environmental Microbiology*, 8, 1220-1227.
- Brazelton, W. J., Thornton, C. N., Hyer, A., I. Twing, K. I., Longino, A. A., Lang, S. Q., ... Schrenk, M. O. (2017). Metagenomic identification of active methanogens and methanotrophs in serpentinite springs of the Voltri Massif, Italy. *PeerJ*, 5, e2945.
- Brocks, J. J., & Pearson, A. (2005). Building the biomarker tree of life. *Reviews in Mineralogy and Geochemistry*, 59, 233-258.
- Brocks, J. J., & Banfield, J. (2009). Unravelling ancient microbial history with community proteogenomics and lipid geochemistry. *Nature Reviews Microbiology*, 7, 601-609.
- Cadeau, P., Jézéquel, D., Leboulanger, C., Fouilland, E., Le Floc'h, E., Chaduteau, C., ... Ader, M. (2020). Carbon isotope evidence for large methane emissions to the Proterozoic atmosphere. *Scientific Reports*, 10, 18186.
- Cario, A., Grossi, V., Schaeffer, P., & Oger, P. M. (2015). Membrane homeoviscous adaptation in the piezo-hyperthermophilic archaeon *Thermococcus barophilus*. *Frontiers in Microbiology*, 6, 1152.
- Casamayor, E. O., Triadó-Margarit, X., & Castañeda, C. (2013). Microbial biodiversity in saline shallow lakes of the Monegros desert, Spain. *FEMS Microbiology Ecology*, 85, 503-518.
- Castelle, C. J., Wrighton, K. C., Thomas, B. C., ,Hug, L. A., Brown, C. T., Wilkins, M. J., ... Banfield, J. F. (2015). Genomic expansion of domain Archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. *Current Biology*, 25, 1-12.

- de Mesmay, R., Grossi, V., Williamson, D., Kajula, & Derenne, S. (2007). Novel mono-, di- and tri-unsaturated very long chain (C<sub>37</sub>-C<sub>43</sub>) *n*-alkenes in alkenone-free lacustrine sediments (Lake Masoko, Tanzania). *Organic Geochemistry*, *38*, 323-333.
- Escudié F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., ... Pasca G. (2018). FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics*, *34*, 1287-1294.
- Finnerty, W. R. (1989). Microbial lipid metabolism. In: Ratledge, C. & Wilkinson, S. G. (Eds.), *Microbial Lipids*, Vol. 2. Academic Press, London, pp. 525-566.
- French, K. L., Rocher, D., Zumberger, J. E., & Summons, R. E. (2015). Assessing the distribution of sedimentary C<sub>40</sub> carotenoids through time. *Geobiology*, *13*, 139-151.
- Grice, K., Cao, C., Love, G. D., Böttcher, M. E., Twitchett, R. J., Grosjean, E., ... Jin, Y. (2005). Photic zone euxinia during the Permian-Triassic superanoxic event. *Science*, *307*, 706-709.
- Grossi, V., Yakimov, M. M., Al Ali, B., Tapilatu, Y., Cuny, P., Goutx, M., ..., Tamburini, C. (2010). Hydrostatic pressure affects membrane and storage lipid compositions of the piezotolerant hydrocarbon-degrading *Marinobacter hydrocarbonoclasticus* strain #5. *Environmental Microbiology*, *12*, 2020-2033.
- Hamilton, T. L., Bovee, R. J., Thiel, V., Sattin, S. R., Mohr, W., Schaperdoth, I., ... Macalady, J. L. (2014). Coupled reductive and oxidative sulfur cycling in the phototrophic plate of a meromictic lake. *Geobiology*, *12*, 451-468.
- Hamilton, T. L., Bovee, R. J., Sattin, S. R., Mohr, W., Gilhooly III, W. P., Lyons, T. W., Pearson, A., & Macalady, J. L. (2016). Carbon and sulfur cycling below the chemocline in a meromictic lake and the identification of a novel taxonomic lineage in the FCB Superphylum *Candidatus Aegiribacteria*. *Frontiers in Microbiology*, *7*, 598.

- Härtner, T., Straub, K. L., & Kannenberg, E. (2005). Occurrence of hopanoid lipids in anaerobic *Geobacter* species. *FEMS Microbiology Letters*, *243*, 59–64.
- Harvey H. R., & McManus, G. B. (1991). Marine ciliates as a widespread source of tetrahymanol and hopan-3- $\beta$ -ol in sediments. *Geochimica et Cosmochimica Acta*, *55*, 3387-3390.
- Hugoni, M., Escalas, A., Bernard, C., Nicolas, S., Jezequel, D., Vazzoler, F., ... Agogu e, H. (2018). Spatiotemporal variations in microbial diversity across the three domains of life in a tropical thalassohaline lake (Dziani Dzaha, Mayotte Island). *Molecular Ecology*, *27*, 4775-4786.
- Humayoun, S. B., Bano, N., & Hollibaugh, J. T. (2003). Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. *Applied and Environmental Microbiology*, *69*, 1030-1042.
- Jones, B. E., Grant, W. D., Duckworth, A. W., Owenson, G. G. (1998). Microbial diversity of soda lakes. *Extremophiles*, *2*, 191-200.
- Kleemann, G., Poralla, K., Englert, G., Kj osen, H., Liaaen-Jensen, S., Neunlist, S., & Rohmer, M. (1990). Tetrahymanol from the phototrophic bacterium *Rhodospseudomonas palustris*: first report of a gammacerane triterpene from a prokaryote. *Journal of General Microbiology*, *136*, 2551-2553.
- Klepac-Ceraj, V., Hayes, C. A., Gilhooly, W. P., Lyons, T. W., Kolter, R., & Pearson, A. (2012). Microbial diversity under extreme euxinia: Mahoney Lake, Canada. *Geobiology*, *10*, 223-235.
- Koga, Y., Nishihara, M., Morii, H., & Agakawa-Matsushita, M. (1993). Ether polar lipids of methanogenic bacteria: structures, comparative aspects, and biosyntheses. *Microbiological Reviews*, *57*, 164-182.

- Lanzén, A., Simachew, A., Gessesse, A., Chmolowska, D., Jonassen, I., Øvreas, L. (2013). Surprising prokaryotic and eukaryotic diversity, community structure and biogeography of Ethiopian soda lakes. *PLoS One*, 8, e72577.
- Leboulanger, C., Agogué, H., Bernard, C., Bouvy, M., Carré, C., Cellamare, M., ... Lavergne, C. (2017). Microbial diversity and cyanobacterial production in Dziani Dzaha crater lake, a unique tropical thalassohaline environment. *PloS one*, 12, e0168879.
- Lyons, T. W., Anbar, A. D., Severmann, S., Scott, C., & Gill, B. C. (2009) Tracking euxinia in the ancient ocean: a multiproxy perspective and Proterozoic case study. *Annual Review of Earth and Planetary Sciences*, 37,507-534.
- Magoč, T., & Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27, 2957-2963.
- Meyers, P. A. (2003). Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. *Organic geochemistry*, 34, 261-289.
- Meyer, K. M., & Kump, L. R. (2008). Oceanic euxinia in Earth history: causes and consequences. *Annual Review of Earth and Planetary Sciences*, 36, 251-88.
- Neunlist, S., Bisseret, P., & Rohmer, M. (1988). The hopanoids of the purple non-sulfur bacteria *Rhodopseudomonas palustris* and *Rhodopseudomonas acidophila* and the absolute configuration of bacteriohopanetetrol. *European Journal of Biochemistry*, 171, 243–252.
- Nichols, D. S., Nichols, P. D., & McMeekin, T. A. (1995). A new n-C<sub>31:9</sub> polyene hydrocarbon from Antarctic bacteria. *FEMS Microbiology Letters*, 125, 281-286.

- Nobu, M. K., Narihiro, T., Kuroda, K., Mei, R., & Liu, W.-T. (2016). Chasing the elusive Euryarchaeota class WSA2: genomes reveal a uniquely fastidious methyl-reducing methanogen. *The ISME Journal*, 10, 2478-2487.
- Nolla-Ardèvol, V., Strous, M., & Tegetmeyer, H. E. (2015). Anaerobic digestion of the microalga *Spirulina* at extreme alkaline conditions: biogas production, metagenome, and metatranscriptome. *Frontiers in Microbiology*, 6, 597.
- Ollivier, B., Fardeau, M.-L., Cayol, J.-L., Magot, M., Patel, B. K., Prensier, G., & Garcia, J.-L. (1998). *Methanocalculus halotolerant*, gen. nov., sp. nov., isolated from an oil-producing well. *International Journal of Systematic Bacteriology*, 48, 821-828.
- Ourisson, G., & Rohmer, M. (1992). Hopanoids. 2. Biohopanoids: a novel class of bacterial lipids. *Accounts of Chemical Research*, 25, 403-408.
- Overmann, J., Beatty, J. T. & Hall, K. J. (1996). Purple sulfur bacteria control the growth of aerobic heterotrophic bacterioplankton in a meromictic salt lake. *Applied and Environmental Microbiology*, 62, 3251-3258.
- Pearson, A. (2014). Lipidomics for Geochemistry. In: Holland, H. D. & Turekian, K. K. C (Eds.), *Treatise on Geochemistry* (2<sup>nd</sup> ed.). Elsevier, Oxford, vol. 12, pp. 291-336.
- Pearson, A., Flood Page, S. R., Jorgenson, T. L., Fischer, W. W., & Higgins, M. B. (2007). Novel hopanoid cyclases from the environment. *Environmental Microbiology*, 9, 2175-2180.
- Philippot, P., van Zuilen, M., Lepot, K., Thomazo, C., Farquhar, J., & van Kranendonk, M. J. (2007). Early Archaean microorganisms preferred elemental sulfur, not sulfate. *Science*, 317, 1534-1537.

- Reinhardt, M., Goetz, W., Duda, J.-P., Heim, C., Reitner, J., Thiel, V. (2019). Organic signatures in Pleistocene cherts from Lake Magadi (Kenya), implications for early Earth hydrothermal deposits. *Biogeosciences*, 16, 2443-2465.
- Risatti, J. B., Rowland, S. J., Yon, D. A., & Maxwell, J. R. (1984). Stereochemical studies of acyclic isoprenoids--XII. Lipids of methanogenic bacteria and possible contributions to sediments. *Organic Geochemistry*, 6, 93-104.
- Ritchie, R. J. (2006). Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynthesis Research*, 89, 27-41.
- Rohmer, M., Bouvier-Nave, P., & Ourisson, G. (1984). Distribution of hopanoid triterpenes in prokaryotes. *Microbiology*, 130, 1137-1150.
- Rojas, P., Rodríguez, N., de la Fuente, V., Sánchez-Mata, D., Amils, R., Sanz J. L., 2018). Microbial diversity associated to the anaerobic sediments of a soda lake (Mono Lake, CA). *Canadian Journal of Microbiology*, 64, 385-392.
- Russel, N. J. (1989). Adaptive modifications in membranes of halotolerant and halophilic microorganisms. *Journal of Bioenergetics and Biomembranes*, 21, 93-113.
- Sarazin, G., Jézéquel, D., Leboulanger, C., Fouilland, E., Le Floc'h, E., Bouvy, M., ... Ader, M. (2020). Geochemistry of an endorheic thalassohaline ecosystem: the Dziani Dzaha crater lake (Mayotte Archipelago, Indian Ocean). *Compte Rendus Geosciences*, 352, 559-577.
- Schouten, S., van der Maarel, M. J. E. C., Huber, R., & Sinninghe Damsté, J. S. (1997). 2,6,10,15,19-Pentamethylcosenes in *Methanlobus bombayensis*, a marine methanogenic archaeon, and in *Methanosarcina mazei*. *Organic Geochemistry*, 26, 409-414.

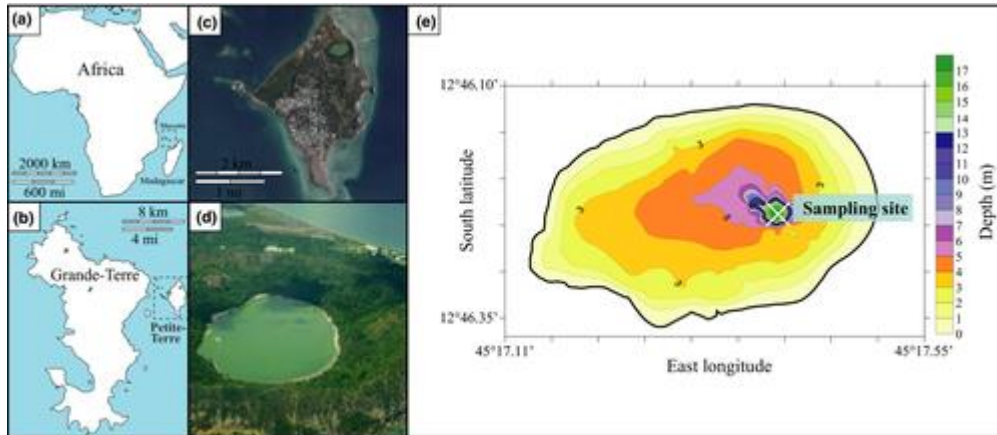
- Sinninghe Damsté, J. S., Rijpstra, I. C., Schouten, S., Fuerst, J.A., Jetten, M. S. M., & Strous, M. (2004). The occurrence of hopanoids in planctomycetes: implications for the sedimentary biomarker record. *Organic Geochemistry*, 35, 561-566.
- Sinninghe Damsté, J. S., Kenig, F., Koopmans, M. P., Köster, J., Schouten, S., Hayes, J. M., & de Leeuw, J. W. (1995). Evidence for gammacerane as an indicator of water column stratification. *Geochimica et Cosmochimica Acta*, 59, 1895-1900.
- Sorokin, D. Y., Tourova, T. P., Mussmann, M., Muyzer, G. (2008). *Dethiobacter alkaliphilus* gen. nov. sp. nov., and *Desulfurivibrio alkaliphilus* gen. nov. sp. nov. - two novel representatives of reductive sulfur cycle from soda lakes. *Extremophiles*, 12, 431-439.
- Sorokin, D. Y., Berben, T., Melton, E. D., Overmars, L., Vavourakis, C. D., & Muyzer, G. (2014). Microbial diversity and biogeochemical cycling in soda lakes. *Extremophiles*, 18, 791-809.
- Sorokin, D., Banciu, H. L., & Muyzer, G. (2015). Functional microbiology of soda lakes. *Current Opinion in Microbiology*, 25, 88-96.
- Shiea, J., Brassell, S.C., & Ward, D.M. (1990). Mid-chain branched mono- and dimethyl alkanes in hot spring cyanobacterial mats: A direct biogenic source for branched alkanes in ancient sediments? *Organic Geochemistry* 15, 223-231.
- ten Haven, H. L., Rohmer, M., Rullkötter, J., & Bisserset, P. (1989). Tetrahymanol, the most likely precursor of gammacerane, occurs ubiquitously in marine sediments. *Geochimica et Cosmochimica Acta*, 53, 3073-3079.
- Thiel, V., Jenisch, A., Landmann, G., Reimer, A., & Michaelis, W. (1997). Unusual distributions of long-chain alkenones and tetrahymanol from the highly alkaline Lake Van, Turkey. *Geochimica et Cosmochimica Acta*, 61, 2053-2064.



- Thiel, V., Peckmann, J., Schmale, O., Reitner, J., & Michaelis, W. (2001). A new straight-chain hydrocarbon biomarker associated with anaerobic methane cycling. *Organic Geochemistry*, 32, 1019-1023.
- Timmers, P. H. A., Vavourakis, C. D., Kleerebezem, R., Sinninghe Damsté, J. S. S., Muyzer, G., Stams, A. J. M., Sorokin, D.Y., & Plugge, C. M. (2018). Metabolism and occurrence of methanogenic and sulfate-reducing syntrophic acetate oxidizing communities in haloalkaline environments. *Frontiers in Microbiology*, 9, 3039.
- Tornabene, T. G., Langworthy T. A., Holzer G., & Oro J. (1979). Squalenes, phytanes and other isoprenoids as major neutral lipids of methanogenic and thermoacidophilic "archaeobacteria". *Journal of Molecular Evolution*, 13, 73-83.
- Tourte, M., Schaeffer, P., Grossi, V., & Oger, P.M. (2020). Functionalized Membrane Domains: An Ancestral Feature of Archaea? *Frontiers in Microbiology*, 11, 526.
- van der Meer, M. T., Klatt, C. G., Wood, J., Bryant, D. A., Bateson, M. M., Lammerts, L., & Ward, D. M. (2010). Cultivation and genomic, nutritional, and lipid biomarker characterization of *Roseiflexus* strains closely related to predominant in situ populations inhabiting Yellowstone hot spring microbial mats. *Journal of bacteriology*, 192, 3033-3042.
- van Leerdam, R. C., Bonilla-Salinas, M., de Bok, F. A. M., Bruning, H., Lens, P. N. L., Stams, A. J. M., & Janssen, A. J. H. (2008). Anaerobic methanethiol degradation and methanogenic community analysis in an alkaline (pH 10) biological process for liquefied petroleum gas desulfurization. *Biotechnology and Bioengineering*, 101, 691-701.

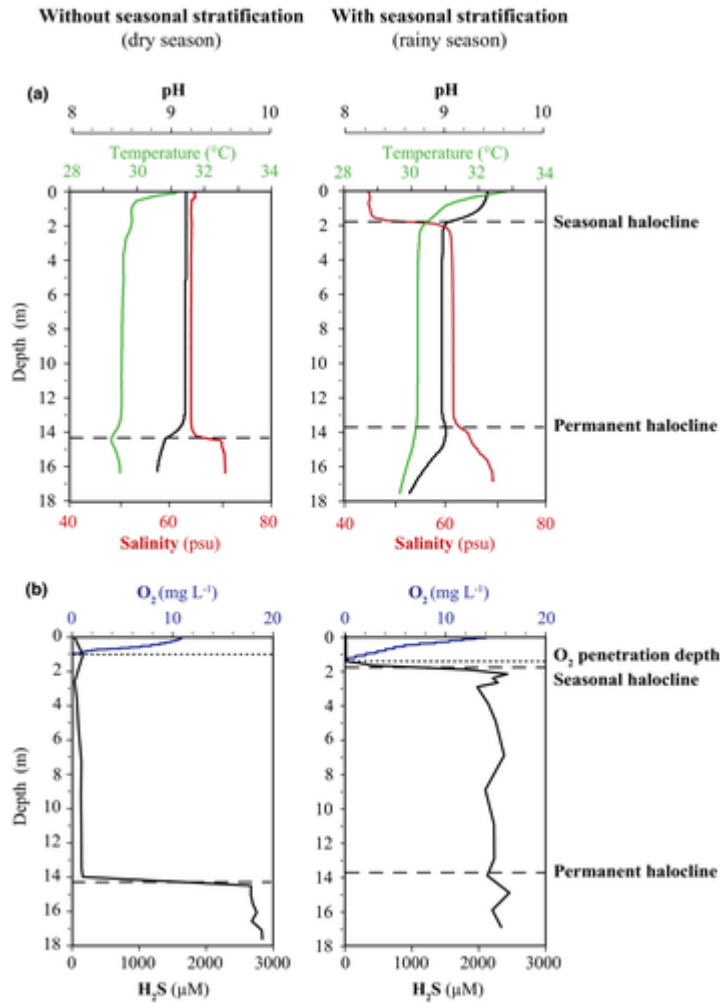
- Vavourakis, C. D., Andrei, A.-S., Mehrshad, M., Ghai, R., & Sorokin, D. Y. (2018). A metagenomics roadmap to the uncultured genome diversity in hypersaline soda lake sediments. *Microbiome*, 6, 168.
- Villanueva, L., Besseling, M., Rodrigo-Gámiz, M., Rampen, S. W., Verschuren, D., & Sinninghe Damsté, J. S. (2014). Potential biological sources of long chain alkyl diols in a lacustrine system. *Organic Geochemistry*, 68, 27-30.
- Volkman, J. K. (2005). Sterols and other triterpenoids: source specificity and evolution of biosynthetic pathways. *Organic Geochemistry*, 36, 139-159.
- Volkman, J. K., et al 1998. Microalgal biomarkers: A review of recent research developments. *Organic Geochemistry* 29, 1163-1179.
- Wang, L., Yang, J., Huang, Y., Liu, Q., Xu, Y., Piao, X., & Wise, M. J. (2019). Systematic analysis of metabolic pathway distributions of bacterial energy reserves. *Genes, Genomes, Genetics*, 9, 2489-2496.
- Wani, A. A., Surakasi, V. P., Siddarth, J., Raghavan, R. G., Patole, M. S., Ranade, D., & Shouche, Y. S. (2006). Molecular analyses of microbial diversity associated with the Lonar soda lake in India: an impact crater in a basalt area. *Research in Microbiology*, 157, 928-937.
- Zinke, J., Reijmer, J.J.G., & Thomassin, B. A. (2003). Systems tracts sedimentology in the lagoon of Mayotte associated with the Holocene transgression. *Sedimentary Geology*, 160, 57-79.

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

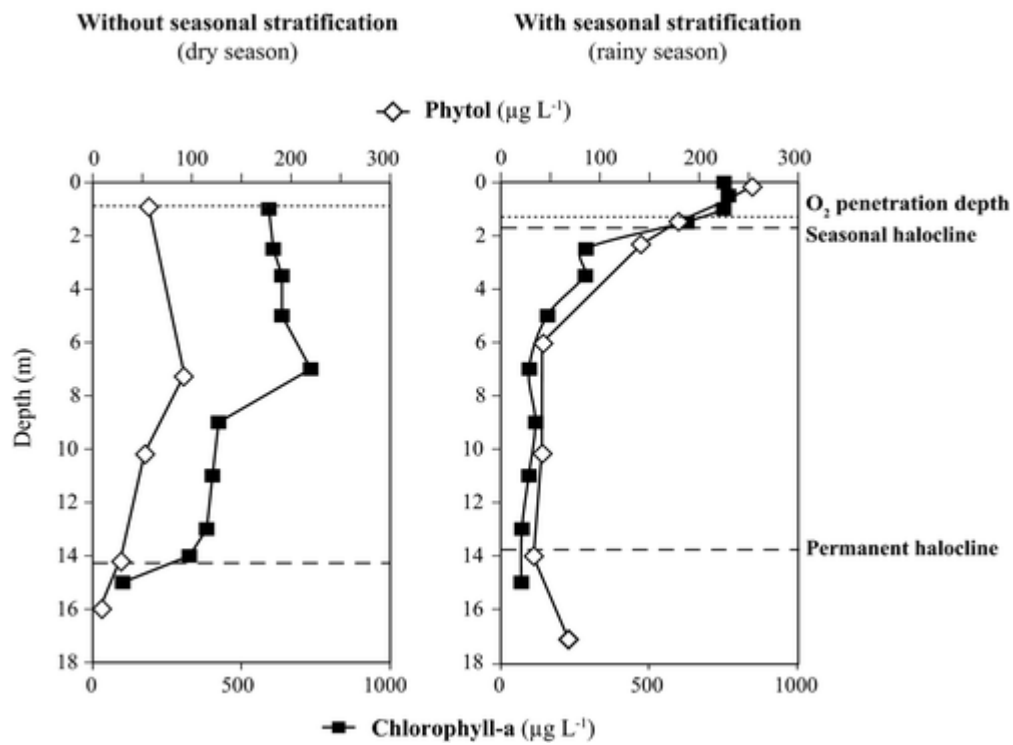


**FIGURE 2** Water column physico-chemical characteristics of Lake Dziani Dzaha (pit) at the time of sampling at the end of the dry (November 2014) and rainy (April 2015) seasons.

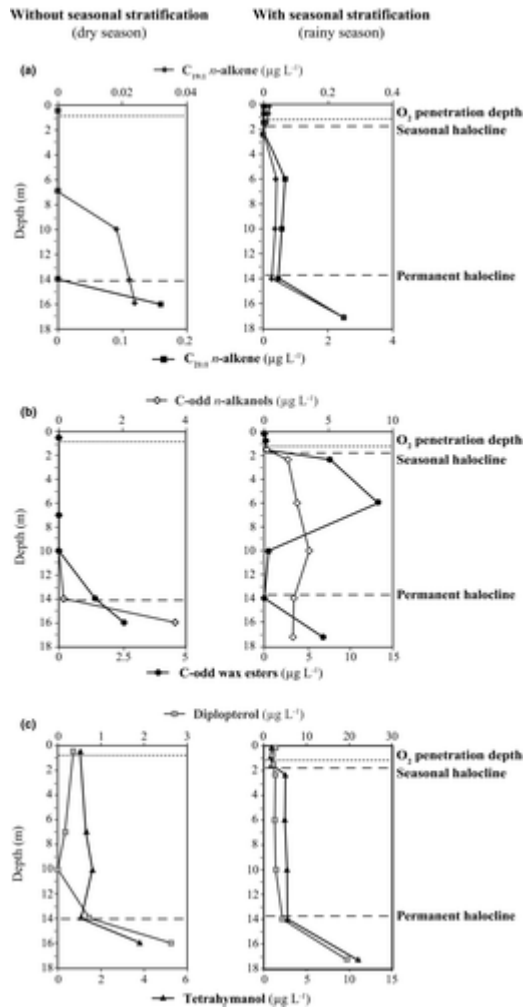
**A)** pH, temperature and salinity; **B)** diurnal dissolved  $O_2$  and  $H_2S/HS^-$ . Note that following the end of the rainy season, the seasonal halocline may last for 2 to 3 additional months before disappearing.



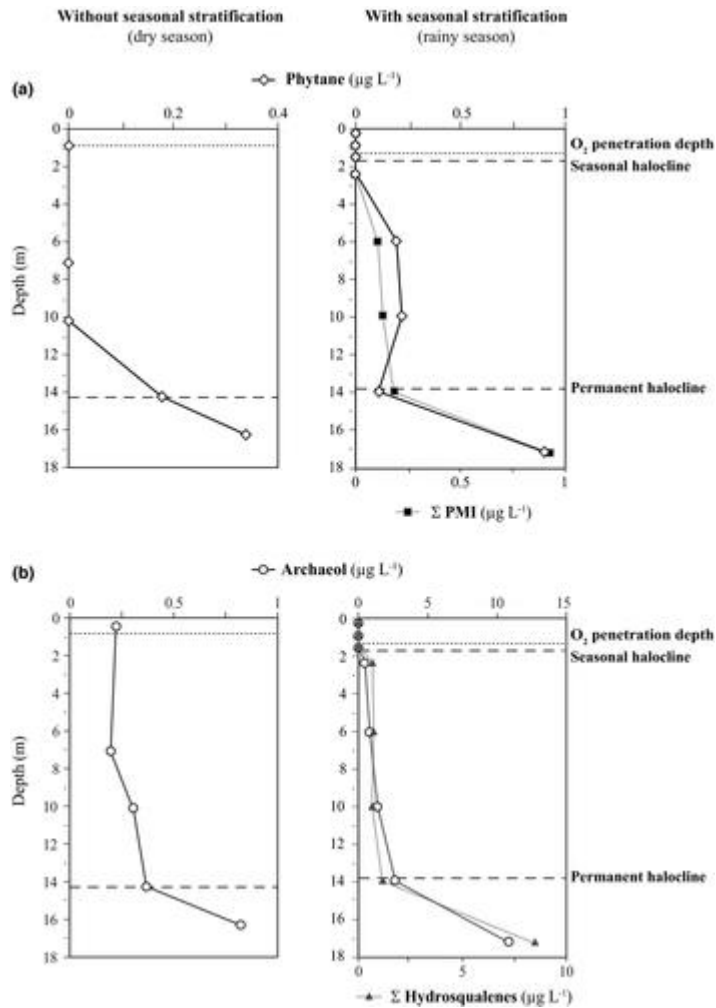
**FIGURE 3** Depth concentration profiles of chlorophyll-a and free phytol in Lake Dziani Dzaha (pit) as a function of seasonal stratification.



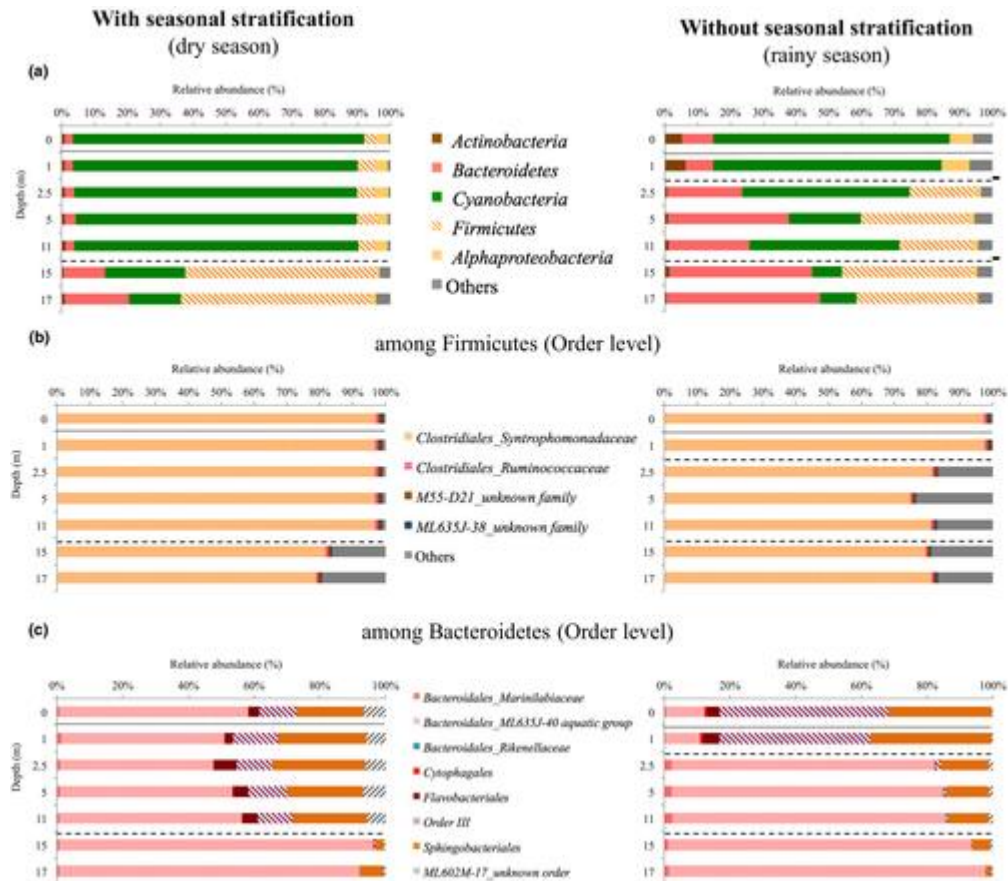
**FIGURE 4** Depth concentration profiles of **A)**  $C_{19:1}$  and  $C_{21:1}$  *n*-alkenes, **B)** summed C-odd *n*-alkanols and C-odd wax esters and **C)** diplopterol and tetrahymanol in Lake Dziani Dzaha (pit) as a function of seasonal stratification.



**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.

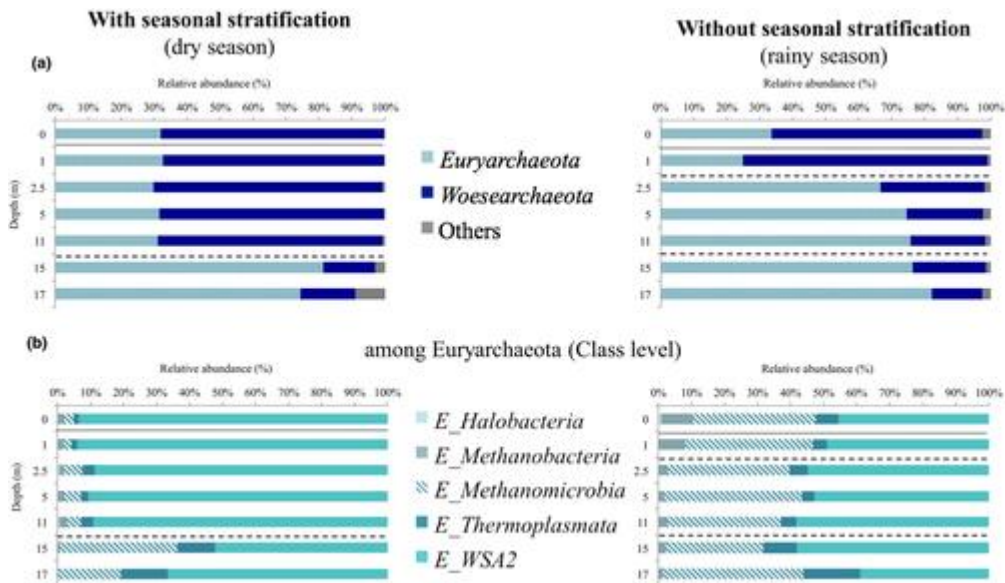


**FIGURE 6** Relative abundance (% of sequences) of bacterial phyla **A)** and among Firmicutes **B)** and Bacteroidetes **C)** orders in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

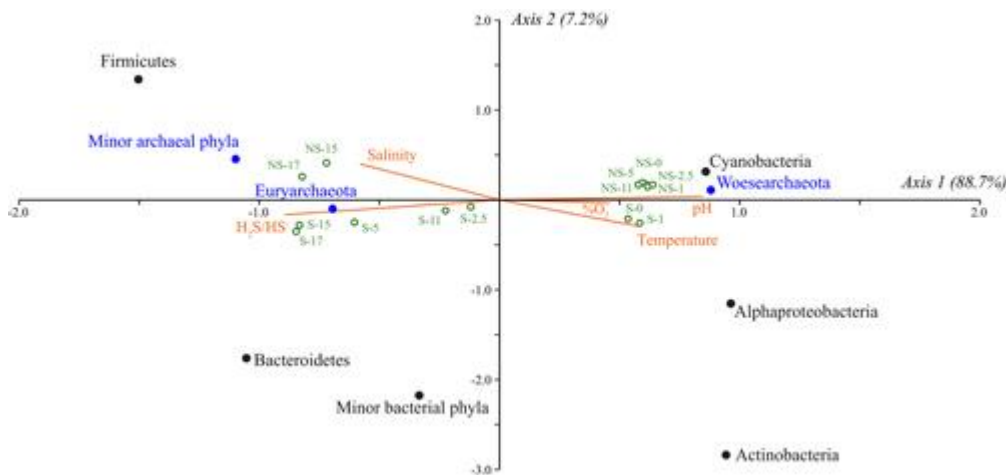




**FIGURE 7** Relative abundance (% of sequences) of archaeal phyla **A**) and among euryarchaeal classes **B**) in Lake Dziani Dzaha (pit) as a function of seasonal stratification.

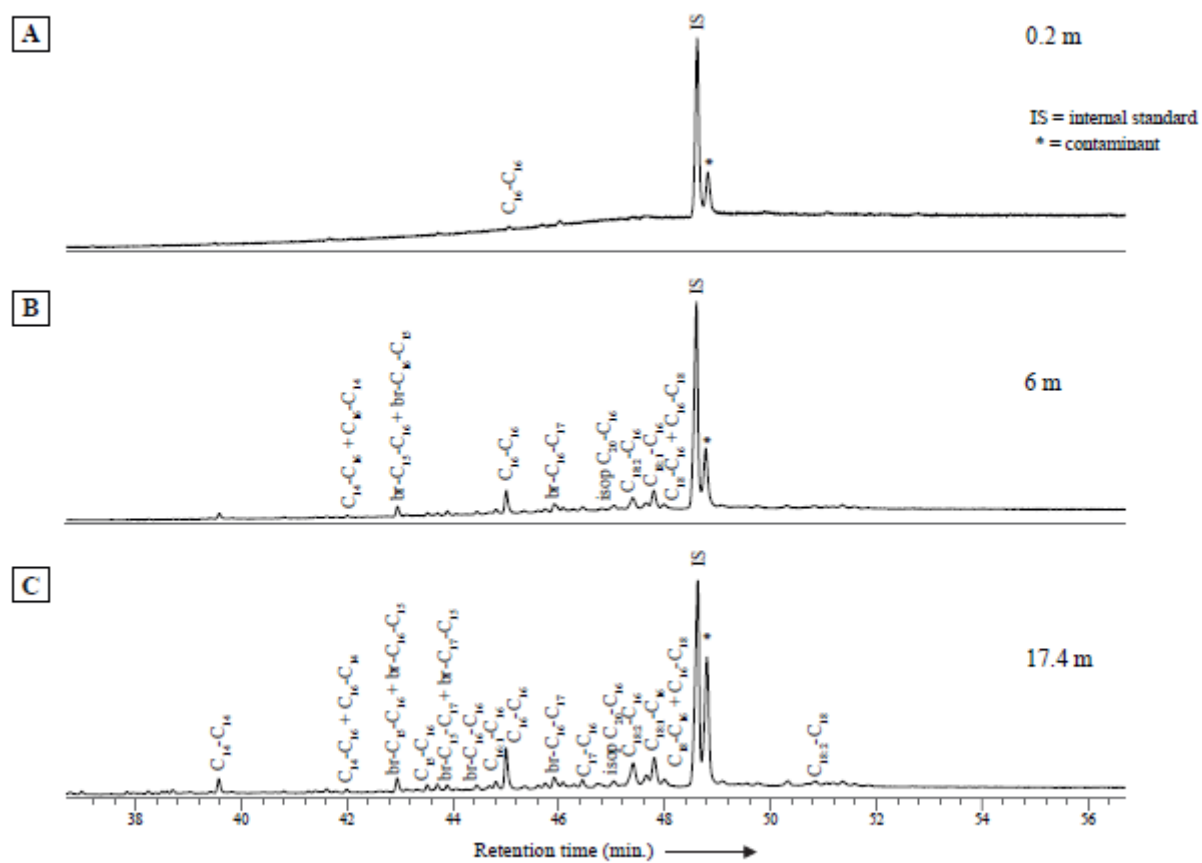


**FIGURE 8** Canonical Correspondence Analysis (CCA) between physico-chemical characteristics of Lake Dziani Dzaha (pH, salinity, temperature, O<sub>2</sub> and H<sub>2</sub>S/HS<sup>-</sup>) and the most abundant (>1% of pooled sequences) bacterial and archaeal phylum. NS = without seasonal halocline, 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.

