Methane related changes in prokaryotes along geochemical profiles in sediments of Lake Kinneret (Israel)

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Abstract. Microbial methane oxidation is the primary control on the emission of the greenhouse gas methane into the atmosphere. In terrestrial environments, aerobic methanotrophic bacteria are largely responsible for this process. In marine sediments a coupling of anaerobic oxidation of methane (AOM) with sulfate reduction, often carried out by a consortium of anaerobic methanotrophic archaea (ANME) and sulfate reducing bacteria, consumes almost all methane produced within marine sediments. Motivated by recent evidence for AOM with iron(III) in Lake Kinneret sediments, the goal of the present study was to link the geochemical gradients in the lake porewater to the microbial community structure. Screening of archaeal 16S rRNA gene sequences revealed a shift from hydrogenotrophic to acetoclastic methanogens with depth. The observed changes in microbial community structure suggest possible direct and indirect mechanisms for the

AOM coupled to iron reduction in deep sediments. Members of the *Nitrospirales* order increased with depth, suggesting their involvement in iron reduction together with *Geobacter* genus and "reverse methanogenesis". An indirect mechanism through sulfate and ANMEs seems less probable due to the absence of ANME sequences. This is despite the abundant sequences related to sulfate reducing bacteria (*Deltaproteobacteria*) together with the occurrence of *dsr*AB in the deep sediment that could indicate the production of sulfate (disproportionation) from S^0 for sulfate-driven AOM. The presence of the functional gene *pmo*A in the deep anoxic sediment together with sequences related to *Methylococcales* suggests the existence of a second unexpected indirect pathway - aerobic methane oxidation pathway in an anaerobic environment.

1. Introduction

Chemical profiles in the porewater of aquatic sediments reflect the sequence of microbially mediated redox reactions that are driven by the availability of both, electron donors and of suitable electron acceptors. The latter are depleted in the order of decreasing chemical potential, beginning with oxygen and proceeding through nitrate, manganese and iron oxides, and then sulfate. Below the main zone of sulfate reduction, the fermentation of organic carbon leads to the formation of methane (CH_4) by the process of methanogenesis (Froelich et al., 1979).

The produced methane is isotopically depleted in ^{13}C, with values of ~−50 to −110‰ (Schoell, 1988), and the residual dissolved inorganic carbon (DIC) pool is enriched by an isotopic fractionation factor (ε) of 50 to 70‰ (e.g., (Borowski et al., 2000; Whiticar, 1999)). When the produced methane diffuses into a zone with a suitable electron acceptor, it can be consumed by microbial oxidation (methanotrophy), the main process by which the important greenhouse methane is prevented from escaping into the atmosphere. While in the terrestrial environment, aerobic methanotrophy is the dominant process (Chistoserdova et al., 2005), in anaerobic marine sediments archaea are found to consume the majority of upward diffusing methane coupled to sulfate reduction (Knittel and Boetius, 2009; Thauer, 2010; Valentine, 2002).

Evidence from lipids and from fluorescence *in situ* hybridization (FISH) showed that a consortium of archaea and sulfate reducing bacteria are involved in this anaerobic methane oxidation (AOM) (Boetius et al., 2000; Hinrichs et al., 1999; Orphan et al., 2001). To date, three groups of anaerobic methanotrophic archaea (ANME), named ANME-1, ANME-2, and ANME-3, are known to perform sulfate driven AOM (Niemann et al., 2006; Orphan et al., 2002). Recently Milucka et al. (2012) demonstrated AOM mediated solely by archaea, where the archaea was shown to oxidize the methane and reduce the sulfate to elemental sulfur. Disproportionating bacteria, also involved in this mechanism, oxidize and reduce this elemental sulfur to sulfate and sulfide, respectively. The carbon isotopic fractionation factor (ε) for this methanotrophic process was shown to be in the range of 4-30‰ (Kinnaman et al., 2007; Whiticar, 1999). Enrichment cultures of ANME from different environments showed a carbon isotopic fractionation of 12-39‰ (Holler et al., 2009).

Other electron acceptors were recently shown to drive AOM. Nitrite driven AOM by oxygenic bacteria was observed in two different freshwater ecosystems in Netherlands (Ettwig et al., 2009; Raghoebarsing et al., 2006) and also in peatlands (Zhu et al., 2012). Beal et al. (Beal et al., 2009) showed the potential of manganese and iron-driven AOM in marine sediments, and Egger et al., (2014) showed it in brackish costal sediments. In our recent study (Sivan et al., 2011), we provided *in situ* geochemical evidence for AOM coupled to microbial iron reduction below the main

methanogenesis zone in Lake Kinneret (LK) sediments, where dissolved sulfate and nitrate are absent. However, the mechanism that is responsible for this process was not investigated. The goal of the present study is to explore the possible microbial communities that may be involved in the iron driven AOM in LK sediments. This is accomplished by combining chemical and isotope analyses of porewater samples along a depth profile from LK sediments with molecular biological techniques. By using specific functional genes related to dissimilatory sulfate reductase (*dsr*) for sulfate reducers (Klein et al., 2001), methyl-coenzyme M reductase (*mcr*) for methanogens and anaerobic methanotrophs (Hallam et al., 2003) and particulate methane monooxygenase (*pmmo*) for aerobic methanotrophs (McDonald et al., 2008) we pinpoint the most likely candidate for this relatively unknown process.

1.1. Study site

Located in northern Israel, Lake Kinneret (LK, Fig.1) is a warm monomictic subtropical lake. Typical concentrations of major electron acceptors in the water column during the mixed period are 35–50 μM nitrate and 600 μM sulfate (Adler et al., 2011; Nishri et al., 2000; Serruya et al., 1974). In the spring, the newly formed epilimnion is characterized by increasing temperatures and enhanced phytoplankton development, while in the hypolimnion heterotrophic microorganisms gradually deplete oxygen and then nitrate. Organic matter degradation by bacterial iron and manganese reduction takes place below the thermocline in the summer, and in the end of the stratification period, sulfate reduction starts in the bottom water. In the upper part of the sediment, sulfate reduction is the dominant microbial process year round, and below depth of 5 cm this process is mostly replaced by methanogenesis (Adler et al., 2011; Eckert and Conrad, 2007). Total iron content ($Fe_{(tot)}$) in the sediment increases with depth till 18 cm and then remains uniform around 550 μmol/g Dry Weight (Eckert, 2000). Mn(II) concentration is about 13 μmol/g Dry Weight in the sediment (Serruya, 1971). The total carbon (C_{tot}) in the solid phase shows a decrease from 14 μmol/g Dry Weight in the top part of the sediment to 8 μmol/g Dry Weight in the deep part of the sediment (~30 cm) (Eckert, 2000).

2. Material and Methods

2.1. Sampling

Several sediment cores were collected from the center of the lake (Station A, Fig. 1) at a water depth of ~42 m (maximum lake water column depth) at different times using Perspex tubes, measuring 55 cm long by 5 cm in diameter, with a gravity corer. The cores were stored in the dark at 4°C until they were sliced (on the same day or the day after). Core sampling for the microbial

community study took place in December 2009. Over a dozen of porewater chemical and isotope profiles were conducted seasonally (every 3-4 months) from 2007 to 2013. The slight seasonal changes allowed using typical geochemical profiles (Adler et al., 2011) in order to sample for the microbiology communities in the different electron acceptors zones and to correlate between the microbial sampling and the geochemical profiles.

2.2. Geochemical analyses

Cores were divided into 2-cm slices from top to bottom under a constant flow of N_2 using a slicing device. About 1.5 ml of each sediment slice was transferred into N_2-flushed crimp bottles containing 5 ml of 1.5 N NaOH for the headspace measurements of CH_4 and $\delta^{13}C_{CH4}$ (after Sivan et al., 2011). CH_4 concentrations were measured on a SHIMADZU gas chromatograph (GC 8IF) equipped with a FID detector at a precision of 2 $\mu mol \cdot L^{-1}$.

Porewater was extracted immediately from each slice by centrifugation at 27,000 g at 4ºC in a N_2 atmosphere, and the supernatant was filtered through 0.45-µm filters. A 1-ml subsample was fixed with Ferrozine and analyzed for dissolved Fe(II) (Stookey, 1970). Four millimeters of subsample were poured into an acidified vial (1 ml of 0.5N nitric acid) to measure dissolved Mn(II). The sample was analyzed via an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Elan DRC II, Perkin Elmer) at a precision of ±10%. For sulfide profiles, 1 ml of subsample was added to zinc acetate and hydrogen sulfide concentrations were determined by the methylene blue method with MDL of the method is 1 µM (Cline, 1969). For sulfate measurements, 5 ml of the subsamples were analyzed with a Dionex DX500 high-pressure liquid chromatograph with a precision of ±3%. Iron isotope analysis was done by acidifying the subsamples with 10% HCl for one week (to dissolve any precipitated iron) and then purifying them by anion exchange chromatography (Borrok et al., 2007). $\delta^{56}Fe$ was measured on a Neptune multi-collector ICP-MS in high resolution mode according to standard methods and standardized against isotopic reference material (IRMM-014) with a precision of ±0.1‰ (John and Adkins, 2010). Total lipids were extracted using the Bligh–Dyer procedure (Bligh and Dyer, 1959) with solvent mixture of 2:1:0.8 (methanol:dichloromethane:buffer). $\delta^{13}C$ of the total lipid extraction (TLE) was measured on an Elemental Analyzer Isotopic Ratio Mass Spectrometer (EA-IRMS) with a precision of 0.1%.

2.3. DNA extraction and quantitative PCR (qPCR) amplification from sediment samples

Part of the sediment slices were kept frozen at -20ºC for the microbial work. Samples from three different depths (0–3 cm representing the sulfate reduction zone, 6–9 cm representing the methanogenesis zone, and 29–32 cm representing the deep AOM zone) were defrosted. Those depth zones were chosen based on the geochemical profiles sampled at different times, showing quasi

steady state and stabilization of the sediments (Adler et al., 2011; Sivan et al., 2011). Therefore, microbial community sampling of the three depth zones at a single time point should represent the different microbial habitats as they pertain to the respective geochemical zones. Total genomic DNA was extracted from the sediment samples using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Solana Beach, CA). Genomic DNA was eluted using 60 µl of elution buffer and stored at −20°C. Concentrations of DNA were determined via UV-Vis spectrophotometry (ND-1000 NanoDrop Technologies, Wilmington, DE) (sulfate reduction zone 22ng/µl, methanogenesis zone 35.8ng/µl and AOM zone 14ng/µl).

Quantification of functional genes were performed using the *mcrA* primers (Luton et al., 2002), F 'GGTGGTGTMGGATTCACACARTAYGCWACAG' and R 'TTCATTGCRTAGTTWGGRTAGTT', *dsrA* primers, DSR1F 'ACSCACTGGAAGCACG' (Wagner et al., 1998) and RH3-dsr-R 'GGTGGAGCCGTGCATGTT' (Ben-Dov et al., 2007) and *pmoA* primers, a189F 'GGNGACTGGGACTTCTGG' and mb661R 'CCGGMGCAACGTCYTTACC' (Yan et al., 2006) with a ABI prism 7000 Sequence Detection System (Applied Biosystems). The qPCR reaction consisted of 10 µL of Absolute Blue SYBR Green ROX, 150 nM each of forward and reverse primers and 5 µL of each DNA template. Thermal cycling conditions were as follows: 15 minutes at 95^{o}C for enzyme activation, followed by 40 rounds of 15 seconds at 95^{o}C for denaturation and 1 minute at 60^{o}C for annealing/extension. To verify that the used primer pair produced only a single specific product, a dissociation protocol was added after thermo cycling, to determine dissociation of the PCR products from 60^{o}C to 95^{o}C. Standards for the calibration curves for quantification were made using pGEM-T Easy plasmid cloned with 1.9 kb *dsrAB* and amplicons of the *mcrA* and *pmoA* encoding for functional genes at a known concentration with six serial dilution points (in steps of tenfold). All runs included a no-template control. Plasmid standards and environmental samples were simultaneously assayed in triplicates. The ABI prism 7000 Sequence Detection System and SDS Software were used for data analysis. qC values were exported into a Microsoft Excel Work sheet for further statistical analysis.

2.4. Sequence analysis

454 tag-encoded FLX amplicon pyrosequencing was performed by the Research and Testing Laboratory (Lubbock, Texas, USA) as previously described (Dowd et al., 2008). The bacterial and archaeal 16S rRNA gene primers that were used are 28F 'GAGTTTGATCNTGGCTCAG' and 519R 'GTNTTACNGCGGCKGCTG' and Arch349F 'GYGCASCAGKCGMGAAW' and Arch806R 'GGACTACVSGGGTATCTAAT' respectively.

Data analysis was made using two different methodologies (MOTHUR and SILVA ngs). The initial trimming of the sequences was made by MOTHUR v1.33 (Schloss et al., 2009) and generated around 300 bp length of sequences. The trimmed sequences were taken to further analysis by MOTHUR (Schloss et al., 2011) using SILVA.nr_v119 database. MOTHUR 454 pipeline filtration and denoising remove from the analysis sequences that were <150 bp, when they contained homopolymers longer than 8 bp, ambiguous bases, more than one mismatch to barcode sequences or more than two mismatches to the forward primer sequence. We further removed sequences that did not align in the same nucleotide position on the reference database.

Most of the bacterial sequences were in the same region, however the archaeal sequences were spaced between two different regions. We used one position window that was dominant in the top sample and implied it on the other archaeal analysis in order to better compare the dataset between other samples of the same position window. Identical sequences were grouped and then were aligned against SILVA.nr_v119. Chimeras were removed using MOTHUR CHIMERA UCHIME (Edgar et al., 2011). The lengths of the remaining sequences were around 200 bp. A further screening step (pre-cluster) was applied to reduce sequencing noise by clustering reads differing by only one base every 100 bases (Huse et al., 2010). In addition, the rest of the sequences were classified in order to remove eukaryote, mitochondria and chloroplast classified sequences. The remaining sequences were used to generate a distance matrix and clustering into operational taxonomic units (OTUs) defined at 97% cutoff using the average neighbor algorithm. The OTUs were classified using SILVA.nr_v119 database with a confidence threshold of 80%. Alpha diversity was calculated by MOTHUR using the remaining sequences at 97% similarity. The beta diversity was calculated using comparable data, the number of sequences per sample was made equal through subsampling (Table 1and 2).

The second program which we used for analysis was SILVA ngs pipeline (Quast et al., 2013). The trimmed sequences from mother were Aligner against the SILVA SSU rRNA seed. Sequences shorter than 50 aligned nucleotides and with more than 2% of ambiguities or 2% of homopolymers, respectively, were removed. The sequences that were not aligned as being putative contaminations/artifacts were removed. Then sequences were clustered to OTUs with 98% similarity and classified by local nucleotide BLAST search against SILVA database v119. To filter out low identity and artificial BLAST hits, hits for which the function '(%sequence identity +%alignment coverage)/2' did not exceed the value of 93% were discarded. Sequences with weak low score were classified as 'No Relatives' and will be disregarded from now on. Standard deviation between the percentages of the two classification showed that at the phylum level there are small differences between the two pipelines (supplementary Table 1). In the order classification, the standard deviations increased but the sequences still showed close similarity. Estimates of

phylotype richness, diversity coverage and similarity were calculated according to the abundance-based coverage estimate (ACE), Chao's estimator (Chao, 1984; Chao and Ma, 1993), the Shannon diversity index, Good's coverage (Good, 1953) were calculated in MOTHUR (Table 1and 2). The distribution and abundance matrix of the OTUs was normalized to the sample with the smallest number of reads by randomly resampling of the MOTHUR data set for statistical comparisons. Raw sequencing data was deposited in the MG-RAST (metagenomics.anl.gov) archive.

2.5. Microbial community structure related to the environment conditions

To estimate community similarity among samples, we used PC-ORD 6 software. Subsample OTU data from MOTHUR was used to create a distance matrix based on the Sørensen (Bray–Curtis) dissimilarities of the OTU composition of the samples. The data was normalized to percentages before the analysis. Community relationships were visualized using principal coordinate analysis (PCoA) based on this distance matrix. Different environmental variables were added as well (CH_4, $\delta^{13}C_{CH4}$, Fe(II), δ^{56}Fe(II), Mn(II), H_2S, SO_4^{2-},dissolved organic carbon (DOC), $\delta^{13}C_{TLE}$, NH_4^+ and PO_4^{3-}) in order to identify potential explanatory variables. The environmental vector were applied and projected to the PCoA ordination. In addition, Venn diagrams for graphical descriptions of unshared and shared OTUs between the three samples were constructed using MOTHUR.

3. Results

3.1. Porewater profiles

This study focused on microbial community shifts along the porewater profiles of electron acceptor gradients related to the methane cycle. To characterize shifts in main electron acceptors with depth, over a dozen porewater profiles were performed seasonally from station A (Fig. 1). The shown profiles (Fig. 2) are representatives of the slight seasonal trends. Sulfate and sulfide profiles show depletion from ~100 μM at the top part of the sediment, to below detection limits within the upper 15 cm (Fig. 2A). This typical concave curvature profile of sulfate profile in the porewater indicates intensive sulfate reduction in the upper few centimeters of the sediment throughout the year. A typical LK methane concentration profile (Fig. 2B) shows increase from 250 μM at the water-sediment interface to a maximum of about 1.25 mM in the depth range of 7 to 15 cm, and then a decrease below 15 cm depth. The profile of $\delta^{13}C_{CH4}$ (Fig. 2B) shows a decrease from −60‰ at a depth of 1 cm to about −65‰ at a depth of 7 cm and then an increase in the deeper sediments to

a maximum value of −53.5‰ at a depth of 25 cm. Also, the profile of δ13CTLE (Fig. 2C) shows a decrease in this deepest part of the sediment.

Manganese and iron oxides are the most probable electron acceptors in the deep part of the sediment where methane is decreased. Dissolved Mn(II) concentration (Fig. 2D) increased from 5 μM at the top of the sediment to a plateau of about 23 μM from depths of 23 to 36 cm. The dissolved Fe(II) concentration profile also showed an increase with depth (Fig. 2D), however following a different pattern. In the upper 15 cm, dissolved Fe(II) concentrations were below the detection limit, and gradually increased below 15 cm to about 90 μM at a depth of 36 cm. The δ^{56}Fe profile (Fig. 2C) showed a decrease with depth from 0.5‰ in the upper part to −1.7% to −2.3% in the deep part of the sediment.

3.2. Sediment microbial communities

To study the sediment depth-dependent microbial community shifts, 16S rRNA gene sequences of the prokaryotic community from three different depth zones (0–3, 6–9 and 29–32 cm, see arrows in Fig. 2a) were extracted and analyzed. As mentioned above, these three zones represent the main sulfate reduction zone, the methanogenesis zone, and the deep iron driven AOM zone, respectively. The diverse bacterial and archaeal communities at these depth zones varied in composition and richness throughout the sediment. A total of 13227 and 4881 bacterial and archaeal sequences representing 3852 and 705 OTUs (cut-off value of 98%), respectively, were identified by SILVA database project. The normalized Shannon and Chao index were used as proxies for diversity and richness, respectively (Table 1 and 2). The upper layer showed lower diversity (Shannon index, 3.91 and 1.8 for bacteria and archaea) and richness (Chao index, 925.7 and 83.4 for bacteria and archaea) than the other depths. Moreover, the bacterial coverage of the upper layer was the highest (91%), while that of the deeper layers was about 80%. The archaeal coverage was almost the same (~88%) in all the sampled layers. Most of our sequences were affiliated with uncultured microorganisms mainly from various sediment environments.

Although the bacterial OTUs were distributed over 43 phyla, we present here only the phyla that were over 1% sequences of the 454 library. The most abundant phyla were: *Proteobacteria*, *Chloroflexi, Nitrospirae, Bacteroidetes, Firmicutes* and *Chlorobi* observed in at least one of the libraries (Fig. 3).

The communities of microorganisms in the top layer (0-3cm) of the sediment had less sequence overlap with those of the deeper layers (70 and 33 shared bacterial sequences with the middle and bottom layers respectively) and of the deeper layers (Supplementary Fig 1and 2). The most dominant (~48%) phylum in this bacterial community was *Proteobacteria*; class *Gammaproteobacteria* (91%) and the genera *Acinetobacter* (28%) and *Pseudomonas* (14%) (Fig.

3). The second most abundant (38%) phylum was *Firmicutes*; class *Clostridia* (21%) and *Bacilli* (17%), family *Clostridiaceae* (15%) and genus *Bacillus* (14%). Further sequences were related to other phyla and order (Fig. 3 and supplementary Table 1).

In the archaeal community the sequences number were much lower, however the coverage calculation shows about 90% coverage. The dominant phylum in the upper layer was *Euryarchaeota* (98%), and the rest of the 2% was *Thaumarchaeota*. *Methanomicrobia* (92%) was the dominant class of *Euryarchaeota* divided between genera of *Methanoregula* (58%), *Methanosaeta* (22%) and *Methanolinea* (11%). The other abundant classes of *Euryarchaeota* were *Halobacteria* (2%) and *Thermoplasmata* (3%). Marine Benthic Group B (MBG-B 0.7%) and Miscellaneous Crenarchaeotic Group (MCG 1%) (Fig. 4 and supplementary Table 1).

The middle layer (6-9cm) was the richest and most diverse in its bacterial and archaeal populations comparing to the other layers, but with the lowest community coverage (bacterial 77% and archaeal 86%). The dominant phyla in this depth were divided to *Proteobacteria* (40%), *Chloroflexi* (14%), *Bacteroidetes* (7%), *Chlorobi* (5%) and *Nitrospirae* (4%). The dominant classes in *Proteobacteria* were *Deltaproteobacteria* (68%), *Betaproteobacteria* (14%) and *Gammaproteobacteria* (14%). Sva0485 (8%), *Syntrophobacterales* (7%), *Desulfarculales* (3%) and *Desulfuromonadales* (2%) were the dominant affiliated order in *Deltaproteobacteria*. *Methylococcaceae* family of *Gammaproteobacteria* had 1% affiliated sequences. The dominant family in *Chloroflexi* was *Anaerolineaceae* (6%). *Ignavibacteriales* (4%) order was the dominant in *Chlorobi*. *Nitrospirae* increased from the upper layer and was mainly represented by the *Nitrospiraceae* (4%) family. Further sequences were related to other phyla and order (Fig. 3, supplementary Table 1).

The archaeal community was the richest and most diverse in the middle zone. The dominant phyla were *Euryarchaeota* (96%) while *Thaumarchaeota* has the rest of the 4%. *Euryarchaeota* was divided between 3 dominant classes: *Methanomicrobia* (78%), *Thermoplasmata* (11%) and *Halobacteria* (6%). *Methanomicrobia* was divided between *Methanoregula* (19%), *Methanosaeta*(50%) and *Methanolinea* (10%) genus's. *Thermoplasmata* was divided between Marine Benthic Group D (5%) and Terrestrial Miscellaneous Gp(TMEG) (3%). *Halobacteria* dominant family was Deep Sea Hydrothermal Vent Gp 6(DHVEG-6) (6%). Marine Benthic Group B (MBGB 1.5%) and Miscellaneous Crenarchaeotic Group (MCG 1.5%) (Fig. 4 and supplementary table 1).

The bottom layer (29-32 cm) bacterial community dominant phyla included: *Proteobacteria* (39%), *Chloroflexi* (19%) and *Nitrospirae* (8%). *Proteobacteria* were divided to three main classes: *Deltaproteobacteria* (20%), *Gammaproteobacteria* (10%) and *Betaproteobacteria* (6%). Sva0485 (11%) and *Syntrophobacterales* (4%) were the dominant affiliated order in *Deltaproteobacteria*.

311 The dominant family in *Chloroflexi* was *Anaerolineaceae* (7%) and *Ignavibacteriales* (4%) order is
312 the dominant *Chlorobi*. *Nitrospirae* increased from the upper layer and was mainly represented by
313 *Nitrospiraceae* (8%) family. Further sequences were related to other phyla and order (Fig. 3,
314 supplementary Table 1)

315 At this depth, the dominant archaeal phyla were *Euryarchaeota* (98%) while *Thaumarchaeota*
316 has the rest of the 2%. *Euryarchaeota* was divided between 3 dominant classes: *Methanomicrobia*
317 (86%), *Thermoplasmata* (6%) and *Halobacteria* (5%). *Methanomicrobia* was divided between
318 *Methanoregula* (16%), *Methanosaeta* (58%) and *Methanolinea* (11%) genus's. *Thermoplasmata*
319 was divided between Marine Benthic Group D (MBG-D)(5%) and Terrestrial Miscellaneous
320 Gp(TMEG) (3%) family. *Halobacteria* dominant family was Deep Sea Hydrothermal Vent Gp
321 6(DHVEG-6) (1%). Marine Benthic Group B (MBG-B 1%) and Miscellaneous Crenarchaeotic
322 Group (MCG 0.6%) (Fig. 4 and supplementary table 1). The detailed description of the major
323 communities in the sediment is described at the discussion and the appendix. No ANME sequences
324 were detected even though specific primers (ANME2C-AR468F, ANME3-1249, ANME1-395F,
325 ANME1-1417, ANME3-140F, ANME3-1249, ANME2a-426 and ANME2a-1242R, see Miyashita
326 et al., 2009) were used.

327 **3.3. Depth comparison of microbial communities**

328 An ordination plot from the 454 pyrosequencing by MOTHUR subsample OTUs dataset was
329 derived from Principal coordinate's analysis (PCoA) of the bacterial and archaeal. The PCoA
330 display the similarities and differences between the bacterial and archaeal communities varied with
331 depth (Fig. 5a and 5b respectively). The PCoA of bacteria and archaea show very similar separation
332 between the communities of different layers related to the different environment conditions of each
333 layer. The two deeper layers are on the same distant on the X axis showing relatively more
334 similarity than the upper layer. The vectors of the sulfate and sulfide are correlative with
335 communities of the top layer, while methane, $\delta^{13}C_{TLE}$ and $\delta^{56}Fe(II)$ were more associated to the
336 communities of the middle layer. The communities in the bottom layer were more correlated to
337 Fe(II), Mn(II) and NH_4^+. The Venn diagram shows also that more OTUs from the deep layers were
338 shared than between the upper layer and the deeper layers (Supplementary Fig 1 and 2).

339 **3.4. Functional gene profile**

340 In order to better understand the abundance of microbial functionality in the different depth we
341 used qPCR for different functional genes. The functional genes *mcrA* and *dsrA* had very similar
342 pattern - low concentrations in the upper layer ($9*10^5 \pm 6.4*10^4$ and $1*10^6 \pm 1.7*10^5$ respectively) and
343 highest concentrations ($6.9*10^6 \pm 6.7*10^5$ and $6.9*10^6 \pm 9.9*10^4$) in the middle layer. *pmoA* gene

showed also the same pattern, however with lower concentration ($2.3*10^5 \pm 9.7*10^3$ to $1.6*10^6 \pm 1.7*10^5$) (Fig. 6).

4. Discussion

In this study of LK sediments we investigated changes in the microbial diversity associated with porewater geochemistry and the transition of the dominant electron acceptors with depth (0-40 cm). The geochemical porewater profiles in LK (Fig. 2) suggest that the sediment can be broadly divided into three different regions of microbial processes: the upper 5 cm which is dominated by sulfate reduction, the methanogenesis zone between 5 to 17 cm, and the deep sediments, dominated by anaerobic oxidation of methane coupled to iron reduction. This division of the sediment and the deep iron-driven AOM processes were confirmed by *in situ* profiles of methane, $\delta^{13}C_{CH4}$, sulfate, and ferrous iron and subsequent use of a numerical mass conservation model (based on the geochemical profiles of DIC and $\delta^{13}C_{DIC}$ (Adler et al., 2011) and a set of geochemical incubation experiments conducted in our previous work (Sivan et al., 2011).

The mechanisms that enable the process of AOM via iron reduction can be characterized by the existence of specific prokaryotic populations at each depth and their resemblance to similar environments with a distinct characterization. There are only few studies that analyzed microbial communities relative to geochemical zones in fresh water sediments (Deutzmann and Schink, 2011; Koizumi et al., 2004; Ye et al., 2009). Therefore, this study can deepen the information on microbial community shifts under different electron acceptors conditions, especially those related to the methane cycle. It is also the first study in LK that divides the sediment to different layers and attribute significantly to the diversity of the microbial ecology in the different zones.

Pipeline of SILVA ngs was used for the alpha-diversity to get more classified sequences and better coverage of the classification and population. However, for the beta-diversity the data from the MOTHUR pipeline applied to get more statistical results without the bias of different sample size. The changes between the pipeline results for the alpha diversity were not large in the phyla level (standard deviation of ~0.5%) but increased with higher taxonomy levels.

4.1. Sediment geochemical conditions

In the upper layer of sediment (0-3 cm), sulfate concentrations were the highest and decreased with depth as particulate organic matter from the water column accumulates, making organic carbon degradation available (Hadas and Pinkas, 1992). Adler et al. (2011) calculated that the bulk of bacterial sulfate reduction occurs in the top 1 cm of sediment, a finding that was supported by the

microbial work of Hadas and Pinkas, (1992). Using sulfide and particulate organic carbon measurements, Eckert and Conrad. (2007) showed that sulfate reduction accounts for most of the mineralization of organic matter in the upper 15 cm of sediment. Methane concentrations in the upper layer were relatively low and probably reached from upward diffusion as methanogens are outcompeted by sulfate reducers there (Lovley and Klug, 1983). The depletion in methane concentrations and the decline $\delta^{13}C_{TLE}$ values and the heavier isotopic values of $\delta^{13}C_{CH4}$ in the upper layer (Fig 2) compared to the methanogenic zone may be explained by AOM via sulfate reduction, although no ANME sequences were found using specific primers and Fluorescent *in situ* hybridization methods or by our phylogenetic alignment of 16S rRNA gene libraries at any depth in the sediment.

Below the sulfate reduction zone, in the middle layer of sediment (6-9 cm), methane reached its maximum concentrations (Fig. 2B). The low values of $\delta^{13}C_{CH4}$ in the methanogenesis zone are typical due to the large carbon isotope fractionation that occurs during methane production, thereby leaving the produced methane isotopically light and the DIC isotopically heavy. Below the maximum methane production zone (around 20 cm), methane concentrations began to decrease while $\delta^{13}C_{CH4}$ values increased (Fig. 2B), an outcome that may be due to the AOM process, which leaves residual methane isotopically heavier. Depleted $\delta^{13}C_{TLE}$ (Fig. 2C) in the deep part of the sediment also supports the presence of AOM with the production of light biomass from methane oxidation. In addition, although all the suitable electron acceptors at this depth were depleted, both Fe(II) and Mn(II) increased (Fig. 2D). Also, $\delta^{56}Fe$ values (Fig. 2C) of dissolved iron in the deep sediment were isotopically negative, similar to sediments with active dissimilatory bacterial iron reduction (Severmann et al., 2006) and are an indication of active iron reduction in the deep sediments and not just diffusion. The increase in Fe(II) concentrations below a depth of 15 cm was probably supported by the absence of sulfide. The potential of highly reactive Fe(III) oxides to drive AOM in LK was also supported by our set of mesocosm incubation studies (Sivan et al., 2011). Since manganese oxide concentrations were very low (~0.04%) throughout the LK sediment column (Serruya et al., 1974), iron oxide seemed to play a bigger role in this AOM process.

4.2 Concept for methane cycle mechanisms

A few possible mechanisms for the novel process of iron driven AOM in the deep part of the sediment can be suggested based on the geochemical profiles and the microbial communities. A possible direct process could be through new, currently unknown bacteria/archaea that reduce iron and utilize methane. Indirect processes could include: 1) Reduction of Fe(III) oxides by oxidation of sulfide (in pyrite or FeS minerals) to sulfur intermediates, and then disproportionation to sulfide and sulfate (such as in (Holmkvist et al., 2011)), and/or sulfate driven AOM but without ANME. 2)

Reduction of Fe(III) by utilizing H_2 (Lovley, 1991), could consume most of produced H_2 in the deep sediment. Thus creating a low concentration of H_2 which drives methanogenesis backwards ("reverse methanogenesis") (Hallam et al., 2004; Hoehler et al., 1994) by the methanogens in the deep sediment. 3) An oxygenic methane oxidation pathway in an anaerobic environment as described by Ettwig et al. (2010), when methane is oxidized by oxygen that is released from iron oxides.

Changes in the microbial population shown in the present study may support the deep iron driven AOM mechanism (presented by the sample from 29-32cm). The bacterial diversity in this depth was lower than in the methanogenesis zone (6-9 cm) but higher than in the upper layer (0-3 cm) of the sediment. The changes in bacterial diversity with depth could be related to the availability of different electron acceptors and of organic matter (Nam et al., 2008). However, there is an overlap between bacterial and archaeal sequences in the middle and the bottom layers (PCoA and Venn diagram), indicating that the environmental conditions are affecting the processes. Alpha diversity indexes (Table 1and 2) were compared to previous studies conducted only in the upper layer of the sediment of LK using different methods (Schwarz et al., 2007a; Wobus et al., 2003), and showed a similarity in the upper parts of the sediment. However, the deeper layers in this study showed higher diversity and richness than the previous studies (Nusslein et al., 2001; Schwarz et al., 2007a, 2007b) and was close to other studies using 454 sequencing (Hollister et al., 2010; Röske et al., 2012).

The direct mechanism of iron reduction coupled with methane oxidation could be performed by a single microorganism, as methanogens have the ability to reduce iron (Van Bodegom et al., 2004; Bond et al., 2002) however not with methane as the electron donor. This direct mechanism could be performed by unique methanotrophy like MBG-D (*Thermoplasmata* class) or by MCG/MBG-B (MCG may represent as new archaeal phylum (Lloyd et al., 2013) or as a sister lineage with *Thaumarchaeota* (Meng et al., 2014). However MBG-B is still classified as *Thaumarchaeota* (Marlow et al., 2014)), to which some of our sequences were similar. About 5% of the archaeal sequences in the middle and deep layers were affiliated to MBG-D, however MCG and MBG-B showed much lower representation and thus are not discussed. Members of the MBG-D have been shown to exist in a variety of freshwater and marine environments (Beal et al., 2009; Borrel et al., 2012), and it is the most widely encountered, uncultured lineage in freshwater lake sediments. Even though their metabolism is unknown, hypotheses about their functionalities are based on the environments in which they were found. Methanogenesis was suggested, as they were found in deep lake sediments with high methane concentrations (Borrel et al., 2012), and they were also hypothesized to be involved in AOM, as they were found in AOM zones (Schubert et al., 2011) and in marine seep sediment (Beal et al., 2009). In other environments, in which methane concentrations

were low, the utilization of waste products, intermediates, or dead cells by MBG-D was also suggested (Smith et al., 1975). Recently, a single cell genomics study showed that members of MBG-D were capable of exogenous protein degradation in cold anoxic environments (Lloyd et al., 2013).

Another option is a consortium of iron reducing microbes together with methanotrophs or with methanogens capable of "reverse methanogenesis". The well-known iron reducers are *Geobacter* genus, however only about 1% of the sequences in the middle and bottom layers or the cores were affiliated to the *Geobacter* genus members. In the deep layer the most abundant class was Sva0485. Sequences of the SVa485 order were similar to those found in different aquatic environments, but the metabolic functions of members of this order are not clear. *Pelobacter carbinolious* (Lovley et al., 1995), a member of SVa485 is capable of Fe(III) and sulfur reduction. *Desulfuromonadales,* which was found in our sequences in the deep parts of the sediment, was shown as S^0 respiring (Pjevac et al., 2014) and may also reduce Fe(III) and Mn(IV) in marine surface sediments (Lovley, 2006). Schwarz et al. (2007a) showed that in the upper part of LK sediment *Deltaproteobacteria* was one of the dominant classes, and that most of them were affiliated with acetate oxidizing sulfate reducing bacteria, that outcompete the acetoclastic methanogens. Even though *Deltaproteobacteria* are best known for their sulfate reduction metabolism, they can shift their metabolism in response to depleted sulfate concentrations (Plugge et al., 2011).

In addition, our results suggest that *Desulfuromonadales* of *Deltaproteobacteria* class could be also involved in indirect mechanisms of disproportionation of sulfur together with other *Deltaproteobacteria* sulfate reducer. The *dsrAB* functional gene shows that sulfate reducers are present at the same level of the core where *mcrA* functional genes (Fig. 6) of methanogens or methanotrophs are found in the deep part of the sediment. Additionally, the presence of the *Deltaproteobacteria* at the deepest sediment depth and the observed accumulation of acetate with depth (data not shown) could indicate their exploitation of a different metabolic path in the deep sediment than in the upper part of the sediment.

Other options for functioning iron reducers include members of the *Nitrospirae* phylum that increased with depth. Indeed, sequences from our samples were classified to *Nitrospirales* order in *Nitrospirea* and were most abundant in the bottom layer of the sediment (7%). Part of our sequences were similar to those found in a previous study (Schwarz et al., 2007b). Using SIP-RNA, Schwarz et al., (2007b) showed not only that *Nitrospirae* were present, but also that they were functionally bioactive. Although *Nitrospirae* is a known nitrate oxidizer (Ehrich et al., 1995), the conditions of this environment suggest that it utilized another metabolic pathway. *Nitrospirae* also include the iron reducing candidates such as *Magnetobacterium bavaricum* (Spring et al., 1993) and sulfur reducers (Sonne-Hansen and Ahring, 1999). Part of our sequences were aligned to uncultured

clone (98%) from freshwater sediment which enhanced degradation of phenanthrene and pyrene by amorphous ferric hydroxide (Yan et al., 2012).

The "reverse methanogenesis" pathway could be carried out by the dominant deep layer methanogens *Methanosaeta*. *Methanosaeta* are acetoclastic methanogens, which are able to grow only on acetate (Jetten et al., 1990). *Methanosaeta* was also shown to be the dominant active methanogens in the upper layer in previous studies of LK (Schwarz et al., 2007a, 2007b) and in other meso to eutrophic freshwater lakes (Glissman et al., 2004; Koizumi et al., 2003). Yamada et al. (2014) showed that *Methanosaetacan* also have the ability to reduce ferrihydrite with H_2 as the electron source. The *Methanosaeta concilii* was the most similar cultured acetoclastic methanogen (96%) to our sequences which was observed also in the previous study. *Methanosaeta* could perform a different metabolic process or may have been inhibited. The other methanogens that could perform the "revers methanogenesis" are from the *Methanomicrobiales* order. The *Methanolinea* genus of *Methanomicrobiales* a hydrogenotrophic methanogen was observed in constant percentages for all depths. Members of the *Methanoregula* genus, a genus that is also hydrogenotrophic methanogen, decreased with depth in the core. qPCR analysis of *mcrA* functional gene of methanogens/anaerobic methanotroph shows that in the middle layer methanogens were more abundant than in the upper and bottom layers (Fig 6). ANME were not found in the sediment using specific primers and Fluorescent *in situ* hybridization methods. An additional reason for not finding any ANME sequences in our samples is likely because 454 sequences related to methanogens were found in high percentages and they are probably dominant in the qPCR analysis of *mcrA* gene observed in the deep layer.

The additional indirect mechanism of anaerobic methane oxidation via an oxygenic pathway was shown clearly by the *pmoA* functional gene. The *pmoA* concentration in the deep part of the sediment was higher than in the upper part, indicating an oxygenic pathway (Fig. 6). However, pMMO is a homologue enzyme of ammonia monooxygenase and might be sequenced together with pMMO (Tavormina et al., 2011). The source of pMMO could be *Methylococcales* (~1%), of *Gammaproteobacteria*. which were observed in the middle and bottom sediment layers. *Methylococcales* are aerobic methanotrophs, which some members were present within the sediments and overlying water column from dysoxic, methane-rich vent and seep systems (Tavormina et al., 2008). The most abundant order in the deep layer was HOC36 (5%), which is an uncultured *Gammaproteobacteria*. However, when compared to NCBI data base, it was found to be closely related to uncultured LK clones (99%) and to cultured *Methylocaldum* sp. (94%) (Bodrossy et al., 1997), which is a Thermophilic methanotroph isolated from landfill cover soil. However, bias of *pmoA* with ammonia monooxygenase could be caused by *Thaumarchaeota*, Betaproteobacteria or Nitrospirae, which were found in all depth.

515 *Thaumarchaeota* comprise not only all known archaeal ammonia oxidizers, but also several
516 clusters of environmental sequences representing microorganisms with unknown energy
517 metabolisms (Pester et al., 2011). Members of *Thaumarchaeota* phylum could have monooxgenases
518 like enzymes that are able to capture methane due to the enzymes' phylogenetic proximities to
519 methane monooxgenases, but that activity requires the necessary downstream metabolic pathway. If
520 they could have that ability, they would have been good candidates for group of methanotrophic
521 archaea. This indirect pathway can be similar to that found in the NC10 phylum (Zhu et al., 2012),
522 which produces oxygen via the reduction of nitrite and the oxidation of methane, but with iron
523 oxides. Also many bacterial ammonia oxidizer sequences were found in our environment.
524 Ammonium profiles show increase with depth mainly due to decomposition of the organic matter,
525 and theoretically the large amount of ammonium could be oxidized by ferric iron minerals and
526 produce nitrite (Clement et al., 2005). Maybe ammonia monooxygenase can function for
527 ammonium up take for iron reduction and for methane up take for oxidation, but that is only a
528 speculation. However, no nitrate or nitrite was detected in the deep sediment, moreover no NC10
529 phylum (Ettwig et al., 2010) was observed in our sequences.
530 To summarize, this study attempted to correlate between the performed geochemical and
531 microbial profiles in lake sediments. The geochemical data suggest three main depth related zones
532 of electron acceptor activities in the sediment: sulfate reduction, methanogenesis and a novel, deep
533 iron-driven AOM. The prokaryotic analysis provided clues regarding the microorganisms that may
534 be involved in this novel process and the metabolic paths that occur throughout the microbial
535 assemblage. For AOM via iron reduction to occur, a number of potential pathways and their
536 combinations have been suggested. Orders that become enriched (Sva0485, *Methanosarcinales* and
537 *Nitrospirales*) with depth can be assumed to participate in the AOM process either directly or
538 indirectly. A possible direct process could be through new, currently unknown bacteria/archaea that
539 reduce iron and utilize methane, which may be carried out by a MBG-D as a methanotroph in a
540 consortium with an iron reducer like *Geobacter*. Possible indirect processes could be Fe(III)
541 reduction by sulfide, oxidation of the sulfide to elemental sulfur and other sulfur intermediates and
542 then disproportionation to sulfide and sulfate and sulfate driven AOM. However, this process is less
543 likely because ANMEs were not found. It could also be via reduction of Fe(III) by utilizing H_2,
544 creating a low concentration of H_2 and driving "reverse methanogenesis". Fe(III) reduction
545 processes could be carried out by *Nitrospirae* and/or *Deltaproteobacteria*. Members of both groups
546 can reduce iron while in a consortium with methanogenic *Methanosarcinales*/ *Methanomicrobiales*,
547 or can reduce the iron with sulfur minerals, creating sulfate (see above) that *Deltaproteobacteria*
548 can utilize while in a consortium with a MBG-D as a methanotrophic archaea (Schubert et al.,
549 2011). An oxidation of methane coupled to iron reduction pathway as described by Ettwig et al.,

(2010) could occur by *Thaumarchaeota* or *Nitrospirae* with a monooxygenase enzyme that can utilize methane while using iron oxides to generate the oxygen needed to oxidize the methane. Further research involving even larger sample of the microbial community and characterization of more diverse functional genes will provide better indication for the composition of the microbial communities at the different depths. Also, microbial and geochemical experiments have the potential to give more clues on the mechanism of this novel iron driven AOM.

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Tables

Table 1. Bacterial sequences used for classification in SILVA ngs and for subsample in MOTHUR for the alpha diversity.

Bacteria	# seq SILVA ngs	OTUs SILVA ngs	# seq MOTHUR	OTUs MOTUR	coverage	invsimpson	chao	ace	shannon
0-3 cm	3631	599	2588	388	0.92	10/10.8/11.7	805/925.7/1092.8	1246.5/1384.0/1546.7	3.83/3.91/3.98
6-9 cm	4641	1735	3365	1337	0.77	286.6/315.9/351.8	2437.9/2673.0/2958.4	3876.8/4135.2/4420.0	6.48/6.53/6.57
29-32 cn	5038	1516	3615	1214	0.81	127.8/142.4/160.6	2104.4/2318.7/2583.1	3047.7/3259.5/3495.4	6.13/6.19/6.24

Table 2. Archaeal sequences used for classification in SILVA ngs and for subsample in MOTHUR for the alpha diversity.

Archaea	# seq SILVA ngs	OTUs SILVA ngs	# seq MOTHUR	OTUs MOTHUR	coverage	invsimpson	chao	ace	shannon
0-3 cm	288	48	268	32	0.90	2.5/2.9/3.5	53.9/83.4/164.5	232.8/344.6/520.4	1.6/1.8/2.0
6-9 cm	1382	184	1408	200	0.86	8.4/10.2/13.2	39.0/131.4/220.4	144.7/195.2/277.4	2.87/3.05/3.22
29-32 cm	3110	383	2829	245	0.88	7.9/9.2/10.9	73.5/104.8/181.7	133.2/190.9/289.6	2.58/2.74/2.91

Figure Captions:

Figure 1: Schematic location of Lake Kinneret. Numbers correspond to altitude measured in meters from the level of Lake Kinneret. Cores were taken from station A (taken from Hambright et al., 2004).

Figure 2: Geochemical porewater profiles in LK sediment. **A.** profile of SO_4^{-2} (black circles) and sulfide (gray diamonds) in the porewater **B**. Headspace measurements of methane (gray circles) and $\delta^{13}C_{CH4}$ (black squares) in the sediments **C.** $\delta^{13}C$ of total lipids extraction (TLE) (black diamonds) from the sediment, and $\delta^{56}Fe$ (gray circles) of the dissolved iron in the porewater. **D**. Electron acceptor profiles of dissolved Fe(II) (gray triangles) and Mn (II) (black squares) in the porewater,**.** Black arrows indicate the sampled sections for 16S rRNA gene analysis. The presented methane profile was taken two weeks prior to the sampling for the microbial communities on December 2009. Sampling for Fe(II), Mn(II) and SO_4^{-2} profiles was carried out between 2007 and 2011 and for sulfide from 2007 to 2013. The $\delta^{13}C_{CH4}$ and $\delta^{56}Fe$ profiles were performed four months prior to the microbial sampling on August 2009.

Figure 3: Classification of bacterial sequences using SILVA ngs pipeline. Phyla and classes distribution of sequences of the 454 sequencing at the different depths above 1% in the communities.

Figure 4: Classification of archaeal sequences using SILVA ngs pipeline. Phyla and orders distributions of sequences of the 454 sequencing at the different depths above 1% in the communities.

Figure 5: Principal coordinate analysis (PCoA) of the **A**. bacterial and **B.** archaeal community matrix (based on operational taxonomic units) and vector fitting of the environmental geochemical

conditions in the different depth. The bacterial community from the upper layer is labeled with red triangle, the middle layer is labeled with green triangle and bottom layer is labeled with blue triangle**.**

Figure 6: profiles of functional genes from the 3 different depths. Green bar represent *pmoA* functional gene of aerobic methanotroph. Red bar represent *dsrAB* functional gene of sulfate reducers and blue bar represent *mcrA* functional gene from methanogens and anaerobic methanotrophs.

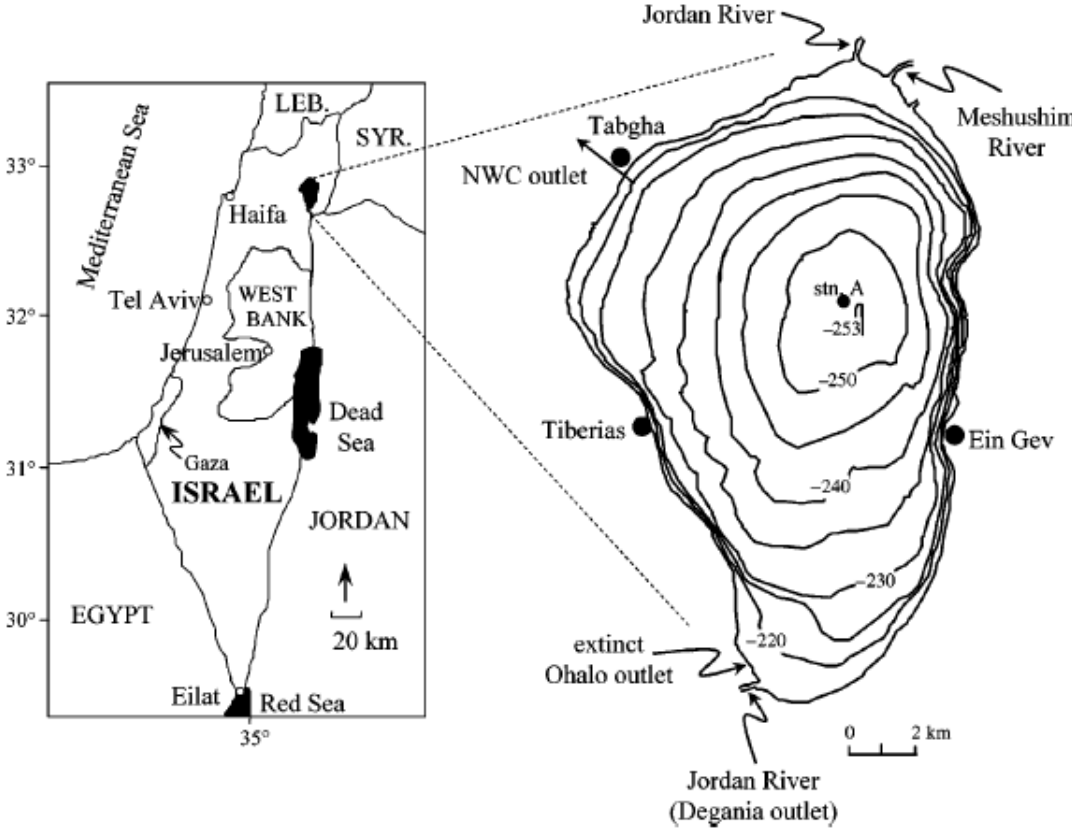


Figure 1.

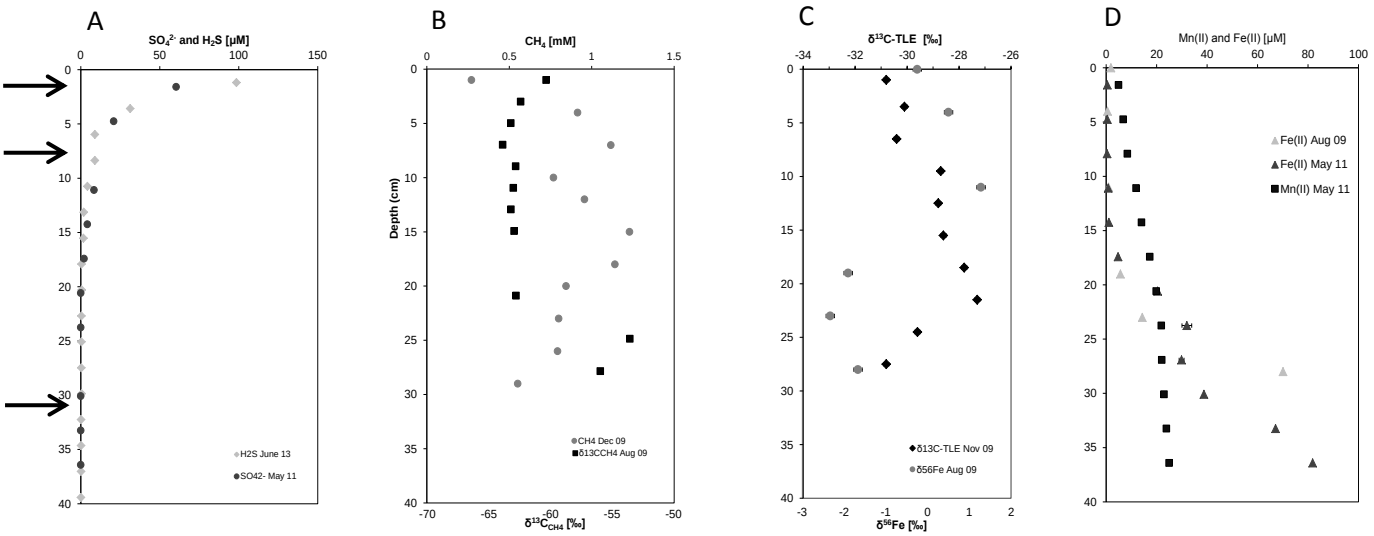


Figure 2.

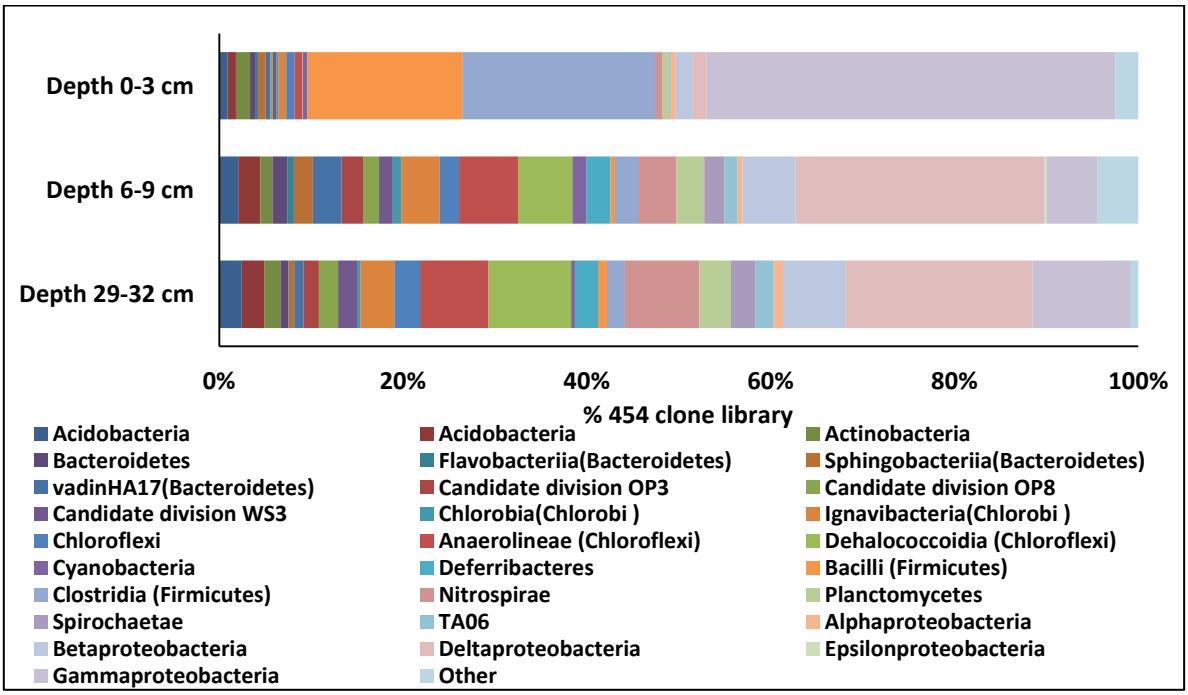


Figure 3.

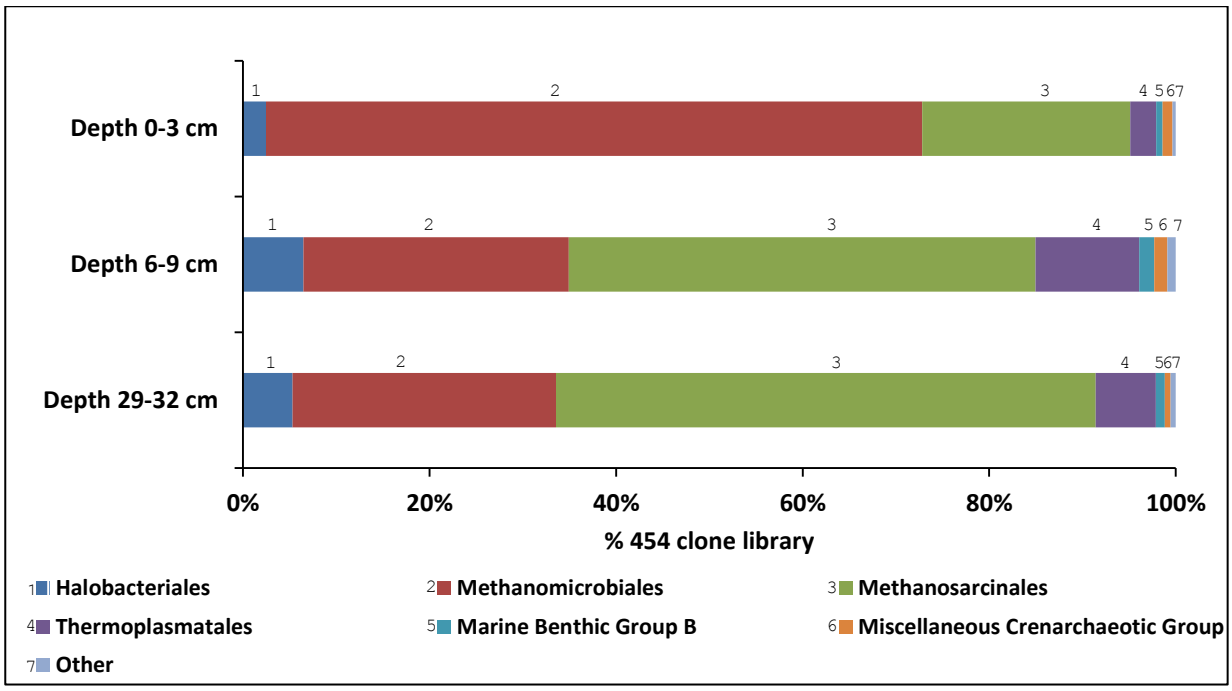
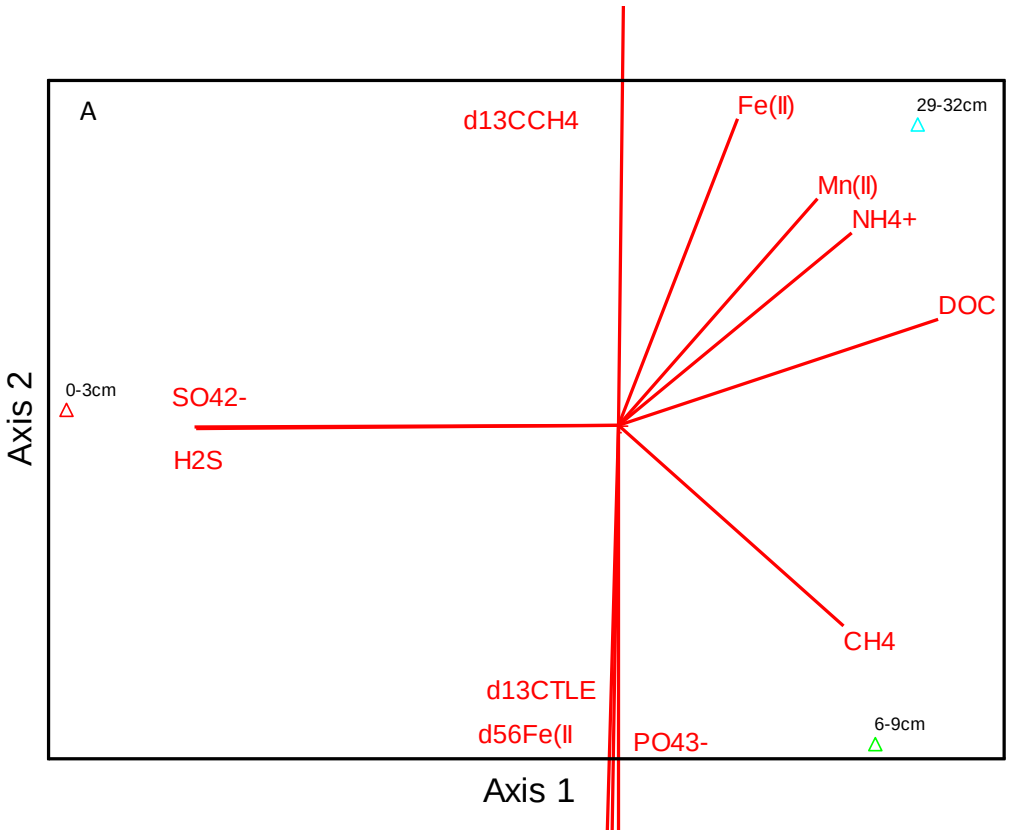
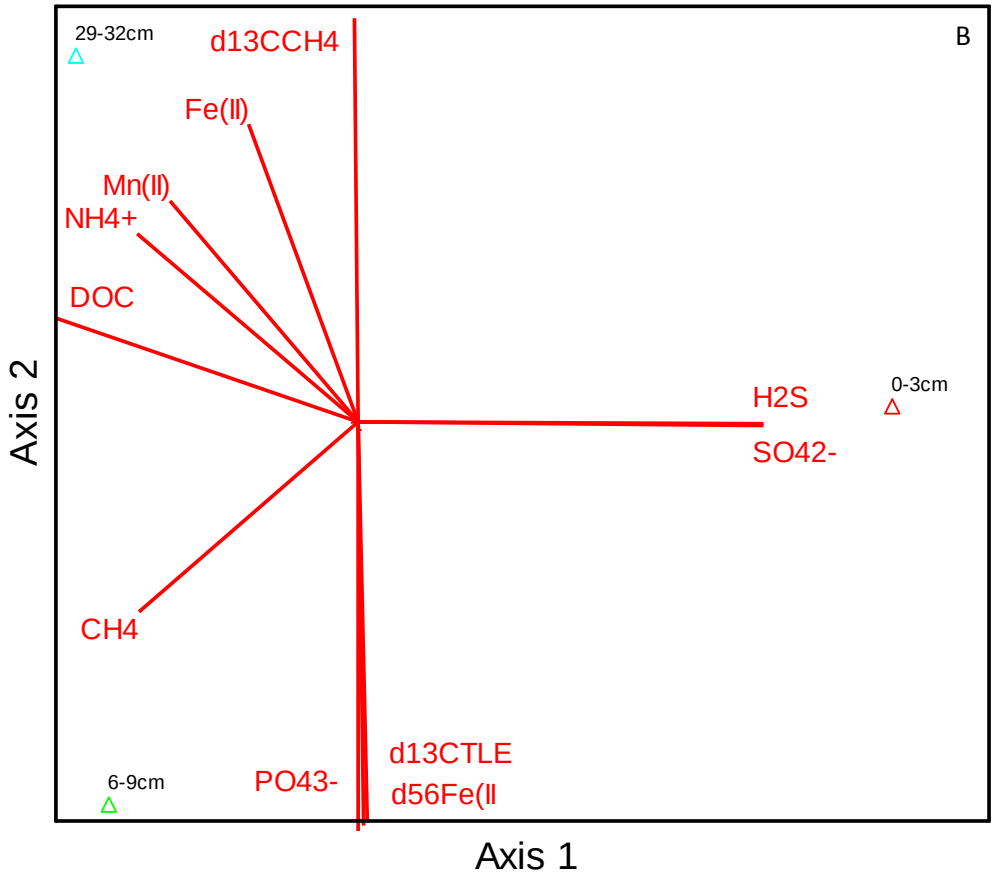

Depth 0-3 cm
Depth 6-9 cm
Depth 29-32 cm
0%
20%
40%
60%
80%
100%
% 454 clone library
1 Halobacteriales
2 Methanomicrobiales
3 Methanosarcinales
4 Thermoplasmatales
5 Marine Benthic Group B
6 Miscellaneous Crenarchaeotic Group
7 Other

Figure 4.


A
d13CCH4
Fe(II)
29-32cm
Mn(II)
NH4+
DOC
0-3cm
SO42-
H2S
CH4
d13CTLE
d56Fe(II
PO43-
6-9cm
Axis 2
Axis 1


29-32cm
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H2S
0-3cm
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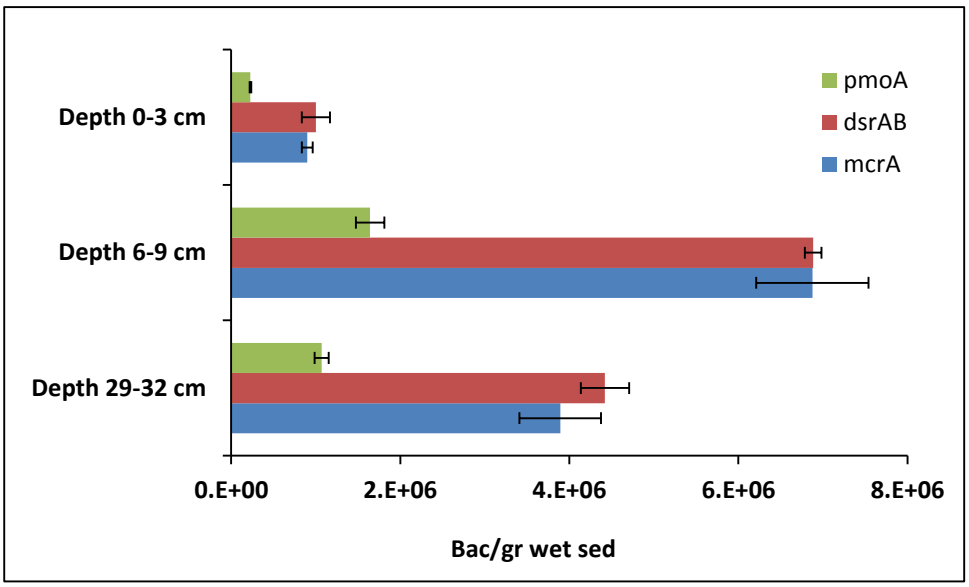
Axis 2
CH4
d13CTLE
d56Fe(II
6-9cm
PO43-
Axis 1

Figure 5.


pmoA
dsrAB
mcrA
Depth 0-3 cm
Depth 6-9 cm
Depth 29-32 cm
0.E+00
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4.E+06
6.E+06
8.E+06
Bac/gr wet sed

871

872 **Figure 6**

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Supplementary

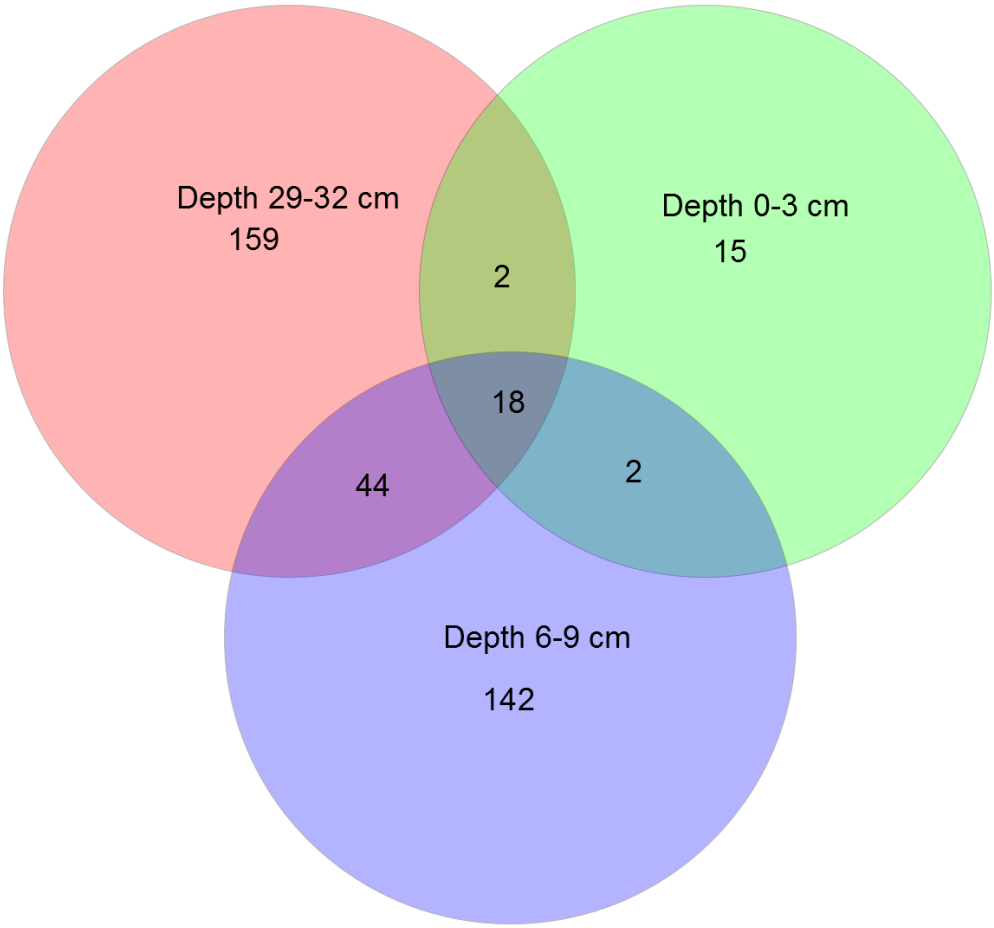
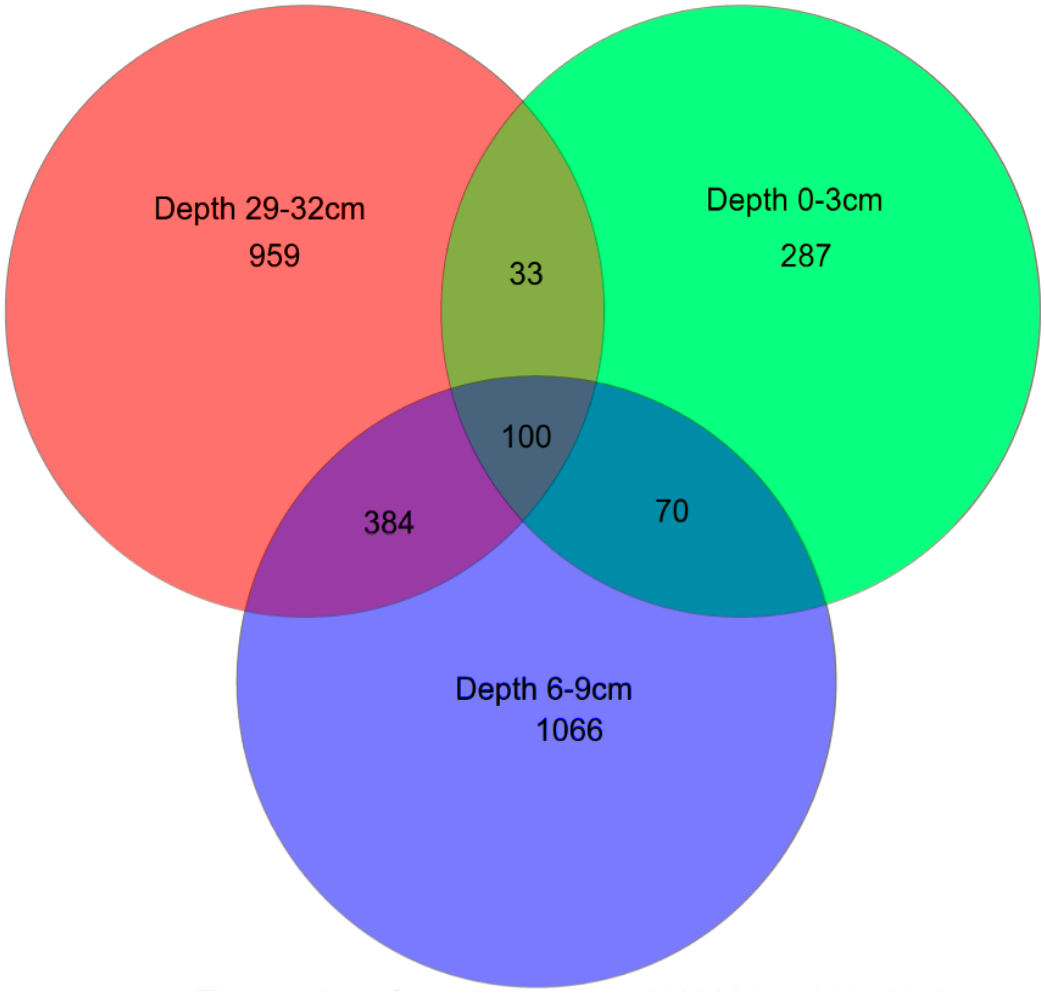


Figure 1

Supplementary Fig. 1. Venn diagram showing archaeal sequences overlap between the different depths. The green circle represents the top sample (0-3 cm), the blue circle represents the middle sample (6-9 cm) and the red circle represents the bottom sample (29-32 cm).



884

885 **Figure 2**

886 **Supplementary Fig. 2.** Venn diagram showing bacterial sequences overlap between the different
887 depths. The green circle represents the top sample (0-3 cm), the blue circle represents the middle
888 sample (6-9 cm) and the red circle represents the bottom sample (29-32 cm).

889

Supplementary Table 1. The classification percentage and number of sequences of archaea A. phylum, B. class and C. order by SILVA ngs.

Archaea	0-3 cm		6-9 cm		29-32 cm	
Phylum	# seq	%	# seq	%	# seq	%
Euryarchaeota	283	98.3	1430	96.4	3048	98.0
Thaumarchaeota	5	1.7	53	3.6	62	2.0

Archaea	0-3 cm		6-9 cm		29-32 cm	
Class	# seq	%	# seq	%	# seq	%
AK59					1	0.0
AK8			1	0.1	4	0.1
Group C3			2	0.1	2	0.1
Halobacteria	7	2.4	96	6.5	165	5.3
Marine Benthic Group B	2	0.7	24	1.6	30	1.0
Marine Group I					2	0.1
Methanobacteria	1	0.3				
Methanomicrobia	266	92.4	1164	78.5	2679	86.1
Miscellaneous Crenarchaeotic Group	3	1.0	21	1.4	19	0.6
pSL12			2	0.1	3	0.1
South African Gold Mine Gp 1(SAGMCG-1)			3	0.2	1	0.0
Thermoplasmata	9	3.1	170	11.5	204	6.6

Archaea	0-3 cm		6-9 cm		29-32 cm	
Order	# seq	%	# seq	%	# seq	%
20a-9			5	0.3	3	0.1
ANME-1					1	0.0
Cenarchaeales					2	0.1
Halobacteriales	7	2.4	96	6.5	165	5.3
Methanobacteriales	1	0.3				
Methanomicrobiales	202	70.1	422	28.5	878	28.2
Methanosarcinales	64	22.2	742	50.0	1800	57.9
Thermoplasmatales	8	2.8	165	11.1	201	6.5
WCHA1-57	1	0.3				
Uncultured	5	1.7	53	3.6	60	1.9

Supplementary Table 2. The classification percentage and number of sequences of bacterial A. phylum, B. class and C. order by SILVA ngs.

Bacteria	0-3 cm		6-9 cm		29-32 cm	
Phylum	# seq	%	# seq	%	# seq	%
Acidobacteria	33	0.9	112	2.4	123	2.4
Actinobacteria	56	1.5	61	1.3	90	1.8
Armatimonadetes	21	0.6	12	0.3	14	0.3
Bacteroidetes	80	2.2	349	7.5	126	2.5
BD1-5	2	0.1	2	0.0	2	0.0
Caldiserica					2	0.0
Candidate division BRC1	2	0.1	14	0.3	8	0.2
Candidate division JS1	1	0.0	2	0.0		
Candidate division KB1					3	0.1
Candidate division OD1			1	0.0	18	0.4
Candidate division OP11	2	0.1	17	0.4	1	0.0
Candidate division OP3	1	0.0	108	2.3	84	1.7
Candidate division OP8	9	0.2	81	1.7	106	2.1
Candidate division SR1			4	0.1		
Candidate division TM7	6	0.2	5	0.1		
Candidate division WS3	15	0.4	70	1.5	103	2.0
Chlorobi	38	1.0	235	5.1	206	4.1
Chloroflexi	65	1.8	672	14.5	969	19.2
CKC4	1	0.0				
Cyanobacteria	18	0.5	69	1.5	21	0.4
Deferribacteres	1	0.0	122	2.6	126	2.5
Deinococcus-Thermus	1	0.0	8	0.2	9	0.2
Elusimicrobia			7	0.2	13	0.3
Fibrobacteres			20	0.4	9	0.2
Firmicutes	1376	37.9	140	3.0	152	3.0
Gemmatimonadetes	2	0.1	15	0.3	15	0.3
Hyd24-12	4	0.1	8	0.2		
GOUTA4					4	0.1
JL-ETNP-Z39			1	0.0	3	0.1
Lentisphaerae	12	0.3	38	0.8	10	0.2
Nitrospirae	31	0.9	195	4.2	409	8.1
NPL-UPA2	1	0.0	33	0.7	5	0.1
OC31			1	0.0		
Planctomycetes	22	0.6	140	3.0	172	3.4
Proteobacteria	1762	48.5	1837	39.6	1954	38.8
SHA-109	6	0.2	6	0.1	1	0.0
Spirochaetae	1	0.0	102	2.2	134	2.7
Synergistetes					1	0.0
TA06	5	0.1	66	1.4	102	2.0
Tenericutes	13	0.4	1	0.0	1	0.0
Thermotogae			8	0.2	1	0.0
TM6	19	0.5	27	0.6	21	0.4
Verrucomicrobia	3	0.1	39	0.8	20	0.4
WCHB1-60	22	0.6	13	0.3		

Bacteria	0-3 cm		6-9 cm		29-32 cm	
CLASS	# seq	%	# seq	%	# seq	%
Acidimicrobiia	5	0.1	4	0.1	2	0.0
Acidobacteria	26	0.7	80	1.7	83	1.6
Actinobacteria	7	0.2	5	0.1	5	0.1
Alphaproteobacteria	23	0.6	25	0.5	50	1.0
Anaerolineae	31	0.9	296	6.4	375	7.4
Ardenticatenia			1	0.0		
ARKDMS-49			2	0.0		
ARKICE-90			11	0.2		
Bacilli	611	16.8	23	0.5	49	1.0
Bacteroidia			5	0.1	1	0.0
BD2-2	2	0.1	16	0.3	8	0.2
Betaproteobacteria	65	1.8	268	5.8	344	6.8
BS5	3	0.1	7	0.2		
BSV13			9	0.2	1	0.0
Caldilineae	1	0.0	8	0.2	3	0.1
Caldisericia					2	0.0
Chlorobia	6	0.2	42	0.9	18	0.4
Chloroplast	3	0.1	42	0.9	4	0.1
Clostridia	761	21.0	116	2.5	99	2.0
Coriobacteriia	3	0.1	4	0.1	7	0.1
Cyanobacteria	13	0.4	22	0.5	7	0.1
Cytophagia	1	0.0	4	0.1	8	0.2
Deferribacteres	1	0.0	122	2.6	126	2.5
Dehalococcoidia	2	0.1	276	5.9	453	9.0
Deinococci	1	0.0	8	0.2	9	0.2
Deltaproteobacteria	57	1.6	1257	27.1	1022	20.3
DEV055	6	0.2	9	0.2		
Elusimicrobia			7	0.2	13	0.3
Epsilonproteobacteria			12	0.3	1	0.0
Erysipelotrichia					3	0.1
FFCH16263	1	0.0			2	0.0
Fibrobacteria			20	0.4	9	0.2
Flavobacteriia	9	0.2	34	0.7	3	0.1
Gammaproteobacteria	1614	44.5	257	5.5	536	10.6
Gemmatimonadetes	2	0.1	15	0.3	15	0.3
Holophagae	5	0.1	24	0.5	29	0.6
Ignavibacteria	32	0.9	193	4.2	188	3.7
JG30-KF-CM66			12	0.3	6	0.1
KD4-96	9	0.2	5	0.1	4	0.1
Ktedonobacteria	1	0.0				
LD1-PB3			1	0.0	1	0.0
Lentisphaeria	1	0.0	3	0.1		
MB-A2-108	6	0.2	4	0.1	14	0.3
MBMPE71			1	0.0	1	0.0
Melainabacteria	2	0.1	1	0.0	4	0.1
ML635J-21			4	0.1	6	0.1
Mollicutes	13	0.4	1	0.0	1	0.0
MSB-5B2					4	0.1
MSBL3			6	0.1		
Negativicutes	4	0.1	1	0.0	1	0.0
Nitrospira	31	0.9	195	4.2	409	8.1
OM190	1	0.0	4	0.1	2	0.0
Oligosphaeria			7	0.2	5	0.1
OPB35 soil group	3	0.1	25	0.5	11	0.2
OPB41	20	0.6	21	0.5	35	0.7
Opitutae			12	0.3	7	0.1
PBS-III-20	2	0.1	4	0.1		
Phycisphaerae	11	0.3	101	2.2	130	2.6
Pla3 lineage			10	0.2	7	0.1
Pla4 lineage			9	0.2	8	0.2
Planctomycetacia	10	0.3	15	0.3	19	0.4
Proteobacteria Incertae Sedis			1	0.0	1	0.0
S085					1	0.0
SB-1			7	0.2	10	0.2
SB-5	19	0.5	33	0.7	12	0.2
S-BQ2-57 soil group					1	0.0
SHA-26			7	0.2	14	0.3
Spartobacteria			1	0.0	1	0.0
Sphingobacteriia	30	0.8	95	2.0	30	0.6
Spirochaetes	1	0.0	102	2.2	134	2.7
Subgroup 22	2	0.1	8	0.2	11	0.2
Synergistia					1	0.0
TA18	3	0.1	4	0.1		
Thermoleophilia	14	0.4	23	0.5	25	0.5
Thermotogae			8	0.2	1	0.0
TK10					2	0.0
vadinHA17	18	0.5	145	3.1	51	1.0
vadinHA49					5	0.1
VC2.1 Bac22			1	0.0		
Verrucomicrobiae			1	0.0		
WCHB1-41			1	0.0	2	0.0
WCHB1-32	1	0.0			1	0.0
uncultured	138	3.8	538	11.6	585	11.6

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Bacteria	0-3 cm		6-9 cm		29-32 cm	
Order	# seq	%	# seq	%	# seq	%
10bav-F6					2	0.0
43F-1404R			2	0.0	3	0.1
Acidimicrobiales	5	0.1	4	0.1	2	0.0
Alteromonadales			9	0.2	14	0.3
Amsterdam-1B-07			1	0.0	4	0.1
Anaerolineales	31	0.9	296	6.4	375	7.4
B1-7BS			1	0.0		
B276-D12			1	0.0		
Bacillales	602	16.6	23	0.5	47	0.9
Bacteroidales			5	0.1	1	0.0
BD2-11 terrestrial group			2	0.0	5	0.1
Bdellovibrionales			34	0.7	13	0.3
BP-U1C-1g10			1	0.0		
Burkholderiales	56	1.5	86	1.9	208	4.1
C86					2	0.0
Caldilineales	1	0.0	8	0.2	3	0.1
Caldisericales					2	0.0
Campylobacterales			12	0.3	1	0.0
Caulobacterales	12	0.3	1	0.0	1	0.0
CCM11a			9	0.2	6	0.1
Chlorobiales	6	0.2	42	0.9	18	0.4
Chromatiales	3	0.1	11	0.2	21	0.4
Chthoniobacterales			1	0.0	1	0.0
Clostridiales	760	20.9	107	2.3	99	2.0
Coriobacteriales	3	0.1	4	0.1	7	0.1
Corynebacteriales	1	0.0	1	0.0	3	0.1
Cytophagales	1	0.0	3	0.1		
D8A-2	1	0.0				
Deferribacterales	1	0.0	122	2.6	126	2.5
Dehalococcoidales			6	0.1	2	0.0
Deltaproteobacteria Incertae Sedis	2	0.1	55	1.2	2	0.0
Desulfarculales	12	0.3	132	2.8	62	1.2
Desulfobacterales	13	0.4	118	2.5	88	1.7
Desulfuromonadales			80	1.7	41	0.8
Erysipelotrichales					3	0.1
Fibrobacterales			18	0.4	6	0.1
Flavobacteriales	9	0.2	34	0.7	3	0.1
Frankiales	1	0.0	2	0.0	3	0.1
FS117-23B-02			2	0.0	4	0.1
FW22	1	0.0	2	0.0	2	0.0
Gaiellales	11	0.3	19	0.4	20	0.4
Gammaproteobacteria Incertae Sedis			4	0.1	4	0.1
Gastranaerophilales			1	0.0	4	0.1
Gemmatimonadales	2	0.1	13	0.3	10	0.2
GIF3			2	0.0	7	0.1
GIF9			117	2.5	251	5.0

GR-WP33-30	1	0.0	14	0.3	34	0.7
Haloplasmatales	13	0.4				
HOC36	2	0.1	72	1.6	267	5.3
Holophagae Incertae Sedis			5	0.1	12	0.2
Holophagales			2	0.0	2	0.0
Hydrogenophilales			48	1.0	44	0.9
Ignavibacteriales	32	0.9	193	4.2	188	3.7
KCLunmb-38-53	1	0.0	9	0.2		
KD3-62	1	0.0	8	0.2	9	0.2
KI89A clade			4	0.1	5	0.1
Lactobacillales	2	0.1			2	0.0
Legionellales	5	0.1	17	0.4	15	0.3
Lineage IIb					4	0.1
Lineage IIc			5	0.1	4	0.1
Lineage IV			1	0.0	1	0.0
MD2894-B20			1	0.0	3	0.1
Methylococcales	9	0.2	70	1.5	69	1.4
Methylophilales			27	0.6	10	0.2
Micrococcales	1	0.0	2	0.0	1	0.0
mle1-8	6	0.2	5	0.1	8	0.2
MSB-3A7 sediment group			3	0.1	3	0.1
MSBL8			1	0.0		
MSBL5	1	0.0	139	3.0	169	3.4
MSBL9	1	0.0	22	0.5	59	1.2
MVP-21	1	0.0				
MVP-88					1	0.0
Myxococcales	1	0.0	82	1.8	45	0.9
NB1-n			1	0.0	1	0.0
Nitrosomonadales	2	0.1	36	0.8	22	0.4
Nitrospirales	31	0.9	195	4.2	409	8.1
ODP1230B30.09					1	0.0
Oceanospirillales			1	0.0		
Oligosphaerales			7	0.2	5	0.1
Opitutales			12	0.3	7	0.1
Order II (Bacteroidetes)			1	0.0	5	0.1
Order Incertae Sedis			2	0.0		
PBS-18			3	0.1	1	0.0
PeM15	1	0.0				
Phycisphaerales	2	0.1	10	0.2	10	0.2
Pla1 lineage			18	0.4	17	0.3
Planctomycetales	10	0.3	15	0.3	19	0.4
Propionibacteriales	3	0.1				
possible order 07			2	0.0	3	0.1
Pseudomonadales	1523	41.9	1	0.0		
Rhizobiales	6	0.2	16	0.3	45	0.9
Rhodobacterales	1	0.0	1	0.0		
Rhodocyclales	4	0.1	43	0.9	29	0.6
Rhodospirillales			3	0.1	1	0.0
Rickettsiales	3	0.1	4	0.1		

S15A-MN16	1	0.0	18	0.4	14	0.3
S-70			3	0.1		
SAR324 clade(Marine group B)			2	0.0		
SC-I-84	2	0.1	27	0.6	31	0.6
Sh765B-AG-111					2	0.0
Sh765B-TzT-29			1	0.0	3	0.1
SHA-43			2	0.0	6	0.1
Selenomonadales	4	0.1	1	0.0	1	0.0
Solirubrobacterales	3	0.1	4	0.1	5	0.1
Sphingobacteriales	30	0.8	95	2.0	30	0.6
Sphingomonadales	1	0.0			3	0.1
Spirochaetales	1	0.0	98	2.1	133	2.6
Streptomycetales					1	0.0
Subgroup 17	1	0.0	3	0.1		
Subgroup 18	3	0.1	27	0.6	21	0.4
Subgroup 23	2	0.1	6	0.1	7	0.1
Subgroup 4	2	0.1				
Subgroup 6	20	0.6	31	0.7	25	0.5
Subgroup 7	2	0.1	3	0.1		
Subgroup 13			5	0.1	5	0.1
Subgroup 19			1	0.0	13	0.3
Subgroup 2			1	0.0	6	0.1
Subgroup 21			9	0.2		
Subgroup 25			1	0.0	6	0.1
Subgroup 3			1	0.0	1	0.0
Subgroup 11					1	0.0
Subgroup 9					1	0.0
SubsectionII (Cyanobacteria)					1	0.0
SubsectionI (Cyanobacteria)	6	0.2	8	0.2	3	0.1
SubsectionIV (Cyanobacteria)	7	0.2	14	0.3	3	0.1
Sva0485	20	0.6	390	8.4	546	10.8
Synergistales					1	0.0
Syntrophobacterales	8	0.2	347	7.5	183	3.6
SZB30	4	0.1	9	0.2	7	0.1
Thermoanaerobacterales			9	0.2		
Thermotogales			8	0.2	1	0.0
Thiotrichales	3	0.1	5	0.1	3	0.1
TPD-58	1	0.0	6	0.1	8	0.2
TRA3-20	1	0.0				
vadinBA26			8	0.2	16	0.3
Verrucomicrobiales			1	0.0		
Vampirovibrionales	2	0.1				
VAN12	7	0.2				
Victivallales	1	0.0	3	0.1		
WD2101 soil group			1	0.0		
Xanthomonadales	65	1.8	50	1.1	129	2.6
uncultured	237	6.5	951	20.5	816	16.2

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Appendix

The major bacterial populations in the sediment:

Proteobacteria

Proteobacteria was the most dominant bacterial phylum in all the depths. Their percentage was slowly decreases with depth however the composition within its classes were changing with depth. *Proteobacteria* is also among the most abundant phyla in LK (Schwarz et al., 2007a) and in other freshwater sediments (Tamaki et al., 2005; Wobus et al., 2003). *Proteobacteria* is a very diverse phylum, divided in to number of classes which most of them have been observed in LK sediments.

Betaproteobacteria

Betaproteobacteria percentages were slowly increasing with depth. They are comprised of chemoheterotrophs and chemoautotrophs which derive nutrients from decomposition of organic material**.** *Burkholderiales* was the dominant order in *Betaproteobacteria* and increased with depth (1.5% to 4%). Schwarz et al. (2007a) showed that *Burkholderiales* is active in the upper sediment of LK. Members of this group are able of anaerobic oxidation of acetate with (per)chlorate as electron acceptor (Yoshida et al., 2005) or oxidize hydrogen in deep subserface(Orcutt et al., 2011). *Hydrogenophilales* and *Nitrosomonadales* orders were observed in lower abundant but in the middle and deep layer of the sediment. *Hydrogenophilales* can utilize hydrogen (Ontiveros-Valencia et al., 2013) and *Hydrogenophilaceae* family are obligatory chemolithoautotrophic, aerobic or facultative anaerobic sulfur oxidizers and reduction of nitrate (Kelly and Wood, 2000) which are often found also in wastewater treatment systems(Luo et al., 2011). *Nitrosomonadales* are freshwater bacteria which presumed to have a role in ammonia oxidation and/or denitrification processes (Edlund et al., 2008). McBeth et al. (2013) also showed that *Nitrosomonadales* are commonly found as freshwater Fe oxidizers. It should be noted that the upper sediment may contain denitrifying bacteria that arrived from the water column where nitrification conditions exists throughout the year.

Gammaproteobacteria

Our sequences which were affiliated to the *Gammaproteobacteria* have different phylogenetic classification distribution in each layer. The upper layer was dominated by *Pseudomonadales* (42%) which were divided to 2 main genera: *Acinetobacter* (28%) and *Pseudomonas* (14%). *Acinetobacter* was shown to have the ability to degrade organic matter. Our sequences which similar to *Acinetobacter genus were closest to Acinetobacter lwoffii (*99%*)* found in sewage and growndwater (Nazina et al., 2000). our sequences classified as *Pseudomonas were* similar to *Pseudomonas stutzeri* (99%) which has the ability to oxidize a large variety of organic compounds, and some

members also involved in nitrification and denitrification, degradation of aromatic compounds, and nitrogen fixation (Chen et al., 2011). In the middle and bottom layers *Methylococcales* order was observed (~1%). *Methylococcales* are aerobic methanotrophs which some members were present within the sediments and overlying water column from dysoxic, methane-rich vent and seep systems (Tavormina et al., 2008). The most abundant order in the deep layer was HOC36 (5%) which is an uncultured *Gammaproteobacteria*. however when compared to NCBI data base it was closly related to uncultured LK clones (99%) and to cultured *Methylocaldum* sp. (94%) (Bodrossy et al., 1997) which was a Thermophilic methanotroph isolated from landfill cover soil.

Chlorobi

The Chlorobi, green sulfur bacteria, are a group of anoxygenic photosynthetic bacteria observed frequently in anoxic microbial mats and meromictic lakes. *Chlorobi* in found in deeper layers of the sediment since they support higher H_2S concentrations and require less light intensity. They photosynthesize using H_2S as electron donor, although they can also grow photoheterotrophically, and some of them form symbiotic consortia with heterotrophic partners that are widely distributed in chemoclines of meromictic lakes throughout the world (Briée et al., 2007). *Chlorobi* metabolic features include fixing CO_2, fixing N_2 and oxidize sulfide or other reduced sulfur compounds (Liu et al., 2012). Most our sequences were classified to *Ignavibacteria* order which capable of organoheterotrophy under both oxic and anoxic conditions.

Firmicutes

Firmicutes are mostly abundant in the upper part of the sediment. Our sequences were classified to 2 main orders: *Bacillales* and *Clostridia*. *Bacillales* have been observed in diverse environments and with diverse metabolic functions. Our sequences were closely related to a few species: *Bacillus thioparans* (99%) which grew chemolithoautotrophically by oxidation of thiosulfate to sulfate and was found in deep sea sediment (Pérez-Ibarra et al., 2007). Bacillus subterraneus (99%) utilize amorphous iron(III), Mn(II)(IV), nitrate, nitrite and fumarate as electron acceptors (Kanso et al., 2002). *Clostridia* are anaerobic bacteria which play an important role in the biogeochemical cycling of carbon, sulfur and iron. Clostridia are key microbial drivers in iron reduction in estuarine sediments and acid mine drainage environments(Shah et al., 2014). Part of our sequences were closely related to *Clostridium tunisiense* (96%) which uses elemental sulfur but not sulfate, thiosulfate, nor sulfite as terminal electron acceptors(Thabet et al., 2004).

Bacteroidetes

Sequences of *Bacteroidetes* were mostly abundant in the middle layer of the LK sediment core. *Bacteroidetes* are known as hydrolytic fermentative bacteria, they are major utilizers of high-

molecular-mass dissolved organic matter in marine ecosystems (Cottrell and Kirchman, 2000). Therefore, fresh organic matter (high in high molecular mass dissolved organic matter) that descends from the water column could be utilized by the *Bacteroidetes* present in the upper part of the sediment.

The major archaeal populations in the sediment:

The distribution of the population was described above. However the environmental conditions and metabolic functionality of our sequences were not shown. The uncultured clones similar to our sequences might help indicate on microbial process of the dominant sequences in our samples.

Euryarchaeota

Methanomicrobia

The majority of our sequences were classified within *Euryarchaeota*, in *Methanomicrobia* class. Our 16S rRNA sequences were divided between *Methanomicrobiales and Methanosarcinales* order. *Methanosaeta* genus of *Methanosarcinales* increases with depth. *Methanosaeta* are acetoclastic methanogens which only able to grow on acetate even at low concentrations(Jetten et al., 1990). *Methanosaeta* was also shown in previous studies of LK (Schwarz et al., 2007a, 2007b) and in other meso to eutrophic freshwater lakes (Glissman et al., 2004; Koizumi et al., 2003). Yamada et al. (2014) showed that *Methanosaetacan* also have the ability to reduce ferrihydrite with H_2 as the electron source. The closest relative cultured acetoclastic methanogen to our sequences was *Methanosaeta concilii* (96%) similar results to the previous studies. *Methanolinea* genus of *Methanomicrobiales* which is hydrogenotrophic methanogen was observed in constant percentages for all depths. *Methanoregula* genus which is also hydrogenotrophic methanogen is decreasing with depth. *Methanoregula, Methanosaeta and Methanolinea* represent 60% of sequences of methanogens retrieved from freshwater lakes (Borrel et al., 2011).

Thermoplasmata

The dominant family in *Thermoplasmata* is The Marine Benthic Group D and DHVEG-1 (MBG-D). Members of the MBG-D have been shown to exist in a variety of freshwater and marine environments (Beal et al., 2009; Borrel et al., 2012), and it is the most widely encountered, uncultured lineage in freshwater lake sediments. Even though their metabolism is unknown, hypotheses about their functionalities are based on the environments in which they were found. Methanogenesis was suggested, as they were found in deep lake sediments with high methane concentrations (Borrel et al., 2012), and they were also hypothesized to be involved in AOM, as they were found in AOM zones (Schubert et al., 2011) and in marine seep sediment (Beal et al., 2009). However, in other environments, in which methane concentrations were low, the utilization

of waste products, intermediates, or dead cells by MBG-D was also suggested (Smith et al., 1975). Recently, a single cell genomics study showed that members of MBG-D were capable of exogenous protein degradation in cold anoxic environments (Lloyd et al., 2013).

In addition Terrestrial Miscellaneous Gp(TMEG) family was observed only in the middle and bottom layers of the sediment. This lineage was includes clones from the terrestrial subsurface and from soils, marine sediments and freshwater lakes (Teske and Sørensen, 2008). Sequences closely related to this group were found in sediment influenced by sulfer-rich, hypoxic groundwater and aquatic sediment samples.

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