Recording of climate and diagenesis through sedimentary DNA and fossil pigments at Laguna Potrok Aike, Argentina

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Abstract

Aquatic sediments record past climatic conditions while providing a wide range of ecological niches for microorganisms. In theory, benthic microbial community composition should depend on environmental features and geochemical conditions of surrounding sediments, as well as ontogeny of the subsurface environment as sediment degraded. In principle, DNA in sediments should be composed of ancient and extant microbial elements persisting at different degrees of preservation, although to date few studies have quantified the relative influence of each factor in regulating final composition of total sedimentary DNA assemblage. Here geomicrobiological and phylogenetic analyses of a Patagonian maar lake were used to indicate that the different sedimentary microbial assemblages derive from specific lacustrine regimes during defined climatic periods. Two climatic intervals (Mid-

Holocene, 5 ka BP; Last Glacial Maximum, 25 ka BP) whose sediments harboured active microbial populations were sampled for a comparative environmental study based on fossil pigments and 16S rRNA gene sequences. The genetic assemblage recovered from the Holocene record revealed a microbial community displaying metabolic complementarities that allowed prolonged degradation of organic matter to methane. The series of *Archaea* identified throughout the Holocene record indicated an age-related stratification of these populations brought on by environmental selection during early diagenesis. These characteristics were associated with sediments resulting from endorheic lake conditions and stable pelagic regime, high evaporative stress and concomitant high algal productivity. In contrast, sulphate-reducing bacteria and lithotrophic *Archaea* were predominant in sediments dated from the Last Glacial Maximum, in which pelagic clays alternated with fine volcanic material characteristic of a lake level highstand and freshwater conditions, but reduced water column productivity. Comparison of sedimentary DNA composition with that of fossil pigments suggested that post-depositional diagenesis resulted in a rapid change in the initial nucleic acid composition and overprint of phototrophic communities by heterotrophic assemblages with preserved pigment compositions. Long DNA sequences (1400-900 bp) appeared to derive from intact bacterial cells, whereas short fragments (290-150 bp) reflected extracellular DNA accumulation in ancient sediments. We conclude that sedimentary DNA obtained from lacustrine deposits provides essential genetic information to complement paleoenvironmental indicators and trace post-depositional diagenetic processes over tens of millennia. However, it remains difficult to estimate the time lag between original deposition of lacustrine sediments and establishment of the final composition of the sedimentary DNA assemblage.

1 Introduction

Lacustrine sediments represent excellent archives of past environmental conditions (Meyers and Lallier-Vergès, 1999), while providing a wide range of ecological niches for sedimentary microbes resulting in complex composition of sedimentary DNA. Initial climatic conditions influence the flux and geochemical make up of organic and inorganic material deposited at the lake bottom (Meyers and Ishiwatari, 1993; Meyers and Teranes, 2001), while microbial activity in the water column (Chen et al., 2008) and after deposition (Freudenthal et al., 2001; Lehmann et al., 2002) further refine the nature of sediments and associated microbial biota. Finally, evolution of sediment environments during early diagenesis is expected to select for

the final composition of entombed microbial consortia (Nelson et al., 2007; Zhao et al., 2008).

DNA from ancient sediments has already been successfully employed to study the succession of species as a result of environmental changes in lacustrine settings (Coolen and Gibson, 2009). For example, wet and warm climates result in high bacterial abundance and diversity in the sediment, whereas cold and dry climates favour lower abundance and diversity of microbes (Dong et al., 2010; Vuillemin et al., 2013a). Similarly, changes in terrestrial plant cover along climate-related environmental gradients influence sedimentary microbes via variations in erosion and export of mineral soil and organic matter (OM) to lakes (Clark and Hirsch, 2008). Shifts in lake salinity, as well as modifications of the water column regime, further induce large changes in bacterial populations (Coolen et al., 2006; Coolen et al., 2008), while differences in the age and composition (lability) of sedimentary OM can also create distinct bacterial niches (Nelson et al., 2007). Despite the fact that the composition of sedimentary microorganisms shows a strong correspondence to geological and geochemical conditions at the time of deposition in marine environments (Inagaki et al., 2003), little is known about the relative influence of extant environmental conditions and post-depositional sedimentary processes as controls of microbial assemblage composition in deep lacustrine sedimentary settings (Vuillemin et al., 2013b). Moreover, persistent activity of microbes in sediments following burial can further modify geochemical conditions via diagenesis (Inagaki et al., 2006) and alter extant bacterial populations to lead to selective preservation of prior sedimentary assemblages (Miskin et al., 1998; Boere et al., 2011a, 2011b). Therefore, the composition of microbial communities in deep sedimentary environments arises from a combination of climatic conditions at the time of deposition, sediment provenance, diagenetic modifications and metabolic activity and distribution of microbial populations (Ariztegui et al., 2015; Kallmeyer et al., 2015).

This paper tests the hypothesis that the sedimentary DNA assemblage potentially records climatic in-lake processes, sedimentary environments and post-depositional alterations associated with subsurface microbial communities. We compare phylogenetic signatures with pigment data reflecting planktonic production by algae and phototrophic bacteria in an unproductive glacial environment (ca. 25,000 years ago) to those characteristic of the productive Holocene (ca. 5,000 years ago). Moreover, the detection of in situ microbial activity within sediments from the Holocene and Last Glacial Maximum (LGM) provides a

way to assess the persistence of sedimentary DNA over time and discriminate nucleic acid sequences of the initial microbial assemblages at the time of deposition (Anderson-Carpenter et al., 2011; Jørgensen et al., 2012) from those arising from diagenetic processes following entombment (Freudenthal et al., 2001).

In this contribution, we take advantage of previous paleoclimatic reconstructions (Gebhardt et al., 2012; Kliem et al., 2013) and blend these results with new pigment data. We also complement geomicrobiological investigations (Vuillemin et al., 2013b and 2014a) with selected phylogenetic data using 16S rRNA gene libraries to focus on discrete horizons in LGM and Holocene. This approach allows us to compare variations in sedimentary DNA over the last 25,000 years in response to both past environmental conditions and geochemical evolution of the sediments. Finally, we established six archaeal clone libraries at regular intervals throughout the microbially-active sediments of the Holocene period to evaluate the recording of population changes with depth and during diagenesis.

2 Material and methods

2.1 Study site

Laguna Potrok Aike is a maar lake located in southern Patagonia, Argentina (Fig. 1A) within the Pali Aike volcanic field (Coronato et al., 2013). Due to the persistent influence of Westerly winds in the area (Mayr et al., 2007), the lake is polymictic and, at present, the water column does not exhibit thermal stratification in any season. The basin has a maximum depth of 100 m (Fig. 1B), while mean annual temperatures range from 4 to 10 °C. The water column is fully oxic (220 µM) down to 80 m depth, where oxygen concentrations rapidly decrease to 60 µM in the last 20 m. Conditions are thus oxic but become microoxic at the water-sediment interface (Zolitschka et al., 2006), likely due to the steep morphology of the maar and currents in the profundal zone (Kastner et al., 2010). Oxygen penetration within surface sediment is likely restricted to the first mm (Vuillemin et al., 2013a). This hydrologically-closed basin contains a sedimentary record of the climatic regime in southernmost South America in which changes in the Westerly winds and ice cap distribution in the Andes regulate variations in regional environmental conditions and in-lake conditions (Fig. 2) such as mixing and hydrological balance (Mayr et al., 2007 and 2013; Ohlendorf et

al., 2013). During wetter periods, elevated nutrient influx enhances lake primary productivity in the lake (Recasens et al., 2012), as well as colonization of the sediments by microbes (Vuillemin et al., 2013a).

In the framework of the ICDP-PASADO project, a 100-m-long by 7-cm-wide hydraulic piston core (Ohlendorf et al., 2011) was collected and sampled for a detailed geomicrobiological study of the lacustrine subsurface biosphere (Vuillemin et al., 2010). We supplement these insights with a new 16S rRNA gene analysis of the sedimentary DNA assemblage extracted from the whole Holocene record and one deep ancient LGM horizon (Fig. 2B), as well as a full sequence analysis of key sedimentary carotenoids from eukaryotic and prokaryotic phototrophs, which preserve well for over 100,000 years (Hodgson et al. 2005). Fossil pigment and sedimentary DNA extractions from the two climatic intervals also allow for a unique comparison between climatic and genetic records in the frame of well-established paleoenvironmental reconstructions.

2.2 Sedimentary features of selected horizons

Lake basin conditions at the time of the Holocene horizon A (Fig. 2A) were defined as subsaline (1.2 % NaCl eq.) during a water-column lowstand (Ohlendorf et al., 2013). Annual mean surface atmospheric temperatures were slightly colder than those of the present day (-1°C; Pollock and Bush, 2013). Sedimentary features of horizon A consist of fine intercalations of laminated silts with soft methane-saturated black clays, reflecting a continuous pelagic to hemipelagic regime (Fig. 2A). In contrast, paleoconditions of the LGM horizon B (Fig. 2B) corresponded with a lake level highstand with freshwater conditions, and colder annual mean surface temperatures (-3°C; Pollock and Bush, 2013). Sedimentary features of horizon B mainly consist of compacted greyish clays with numerous intercalations of mafic sands associated with terrestrial events (Fig. 2B).

Previous sedimentary studies (Kliem et al., 2013; Gebhardt et al., 2012; Ohlendorf et al., 2013) defined five main lithological units throughout the record of Laguna Potrok Aike. These five units are based on stratigraphic features associated with the frequency of gravity inflows in response to climatic lake level fluctuations (Fig. 2C). Such fluctuations promoted important reworking of the catchment with influx of terrestrial and volcanic detritus to the center of the basin (Zolitschka et al., 2013). Furthermore, time calibration of Laguna Potrok Aike stratigraphy showed that these five lithological units correspond to specific climatic

periods, namely the Last Glacial, Antarctic events A2 and A1, LGM, Younger Dryas (YD) and Holocene times (Buylaert et al., 2013; Kliem et al., 2013).

2.3 On-site sampling and procedures

Sediment sampling protocols were optimized to avoid potential sources of microbial contamination (Kallmeyer et al., 2006; Vuillemin et al., 2010). The size and configuration of the drilling platform prevented use of an on-site laboratory with sufficient conditions of asepsis, therefore retrieved cores were transported every 90 min from the platform back to the field laboratory where a detailed protocol was applied to retrieve sediments under the most sterile conditions possible. The aperture of sampling windows allowed a quick retrieval and conditioning of sediments for DNA extraction, 4',6-diamidino-2-phenylindole (DAPI) cell counts, and on-site adenosine-5'-triphosphate (ATP) assays. Rapid ATP detections were performed on a Uni-Lite NG luminometer (BioTrace) with Aqua-Trace water testers and used as an assessment of in situ microbial activity within sediments (Nakamura and Takaya, 2003). Background values measured on micropure H_2O ranged between 25 and 30 RLU. Thus, a value of 30 was systematically subtracted from the readings for background correction. Pore water was retrieved from small holes drilled in the liners using 0.15 µm pores soil moisture samplers (Rhizon Eijkelkamp). All protocols for lithostratigraphic and biogeochemical analyses related to bulk sediment composition, pore water geochemistry and cell count procedures have been published elsewhere (Vuillemin et al., 2013a, 2013b). Complete datasets are available at http://doi.pangaea.de under accession numbers 10.1594/PANGAEA.811521 to 811524.

2.4 Pigment analysis

All extraction, isolation and quantification followed the standard procedures detailed elsewhere (Leavitt and Hodgson, 2002). In brief, carotenoid, chlorophyll (Chl) and derivative pigments were extracted from 2,500 freeze-dried sediment samples into degassed mixtures of organic solvents (i.e. acetone, methanol) and water under an inert N_2 atmosphere and filtered through 0.45-µm pore membrane filters. Extracts were injected into a Hewlett Packard model 1100 high performance liquid chromatographic (HPLC) system fitted with a reversed-phase C18 column, photo-diode array detector, and fluorescence detector for quantification. Peaks were identified and calibrated using authentic pigment standards (U.S. Environmental Protection Agency and DHI Lab Products, Denmark), unialgal cultures, and reference stocks

of sedimentary pigments. Biomarker concentrations (nmol pigment g^{-1} total organic carbon) were calculated for pigments characteristic of green sulphur bacteria (isorenieratene), total *Cyanobacteria* represented by the sum of three pigments (echinenone, canthaxanthin, aphanizophyll), purple bacteria (okenone) and mainly diatoms (diatoxanthin). Preservation index was calculated from the ratio of chlorophyll *a* to its degradation product pheophytin *a*, two pigments indicative of total algal abundance (Leavitt et al., 1997). Shifts in productivity associated with lacustrine conditions were estimated from the ratio of total eukaryotic pigments (alloxanthin, β-carotene, chlorophyll-*a*, chlorophyll-*b*, diatoxanthin, fucoxanthin, lutein, phaeophytin-*b*, zeaxanthin) to total prokaryotic pigments (canthaxanthin, echinenone, isorenieratene, okenone).

2.5 Clone library and phylogenetic analysis

Detailed procedures for DNA extraction, PCR amplification and denaturing gradient gel electrophoresis (DGGE) were published elsewhere (Vuillemin et al., 2013a and 2014b). In brief, total DNA was extracted from sediment samples using the commercial Mobio PowerSoil Isolation kit. Amplifications of the small subunit 16S rRNA gene were performed with the bacterial universal primer pair 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). For archaeal gene amplifications, a nested PCR approach was selected to avoid an enrichment step by cultures. The primer pair 4F (5'-TCY GGT TGA TCC TGC CRG-3') and Univ1492R (5'-CGGTTA CCT TGT TAC GAC TT-3') was used in the first place, followed by the overlapping forward primer 3F (5'-TTC CGG TTG ATC CTG CCG GA-3') and reverse primer 9R (5'-CCC GCC AAT TCC TTT AAG TTT C-3'). PCR amplifications resulted in DNA fragments of 1400 and 900 base pairs (bp) for *Bacteria* and *Archaea,* respectively. These PCR products were used subsequently to establish clone libraries. For DGGE, a final nested PCR round was performed on both bacterial and archaeal products to fix the GC clam (5'- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G -30) and shorten sequences to 150 bp to allow a better denaturation in the gradient gel. Primers 357F-GC (GC clam + 5'-CCT ACG GGA GGC AGC AG-3') with 518R (5'-ATT ACG GCG GCT GCT GG-3') were used for *Bacteria* and A344F-GC (GC clam + 5'-ACG GGG AGC AGC AGG CGC GA-3') with W31 (5'-TTA CCG CGC TGC TGG CAC-3') for *Archaea*.

For the cloning procedure, PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics SA), measured with a Nanodrop ND-1000

Spectrophotometer (Witec AG), and diluted to 10 ng/µL. Two µL of PCR products were ligated to the pCR4-TOPO vector (Invitrogen by life technologies) and cloned into competent *Escherichia coli* cells. Cloning procedure was performed using the TOPO TA Cloning Kit (Invitrogen by life technologies) following the manufacturer's recommendations. Transformed cells were incubated at 37°C for 20 hours on a LB medium containing 1g L^{-1}NaCl, 1 g L^{-1} Bactotryptone, 0.5 L^{-1} Bactoyeast, 1.5 g L^{-1}Bactoagar and 2 mL L^{-1} ampicillin. To constitute libraries, 86 bacterial clones were selected from samples at 4.97 (43) and 29.77 (40) m sediment depth, and 228 archaeal clones from samples at 0.25 (35), 0.55 (41), 1.90 (42), 2.51 (27), 4.97 (27), 7.81 (21), 9.37 (11), and 29.77 (24) m sediment depth. Sequencing cycles were performed using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied BioSystems) with universal primers 27F and 1492R for *Bacteria* and vector primers D4 and R5 from the BigDye sequencing kit for *Archaea*. Sequencing was performed on an ABIPRISM 3130xl Genetic Analyzer (Applied BioSystems, Hitachi). Sequences were assembled with CodonCode Aligner v.3.7.1 (CodonCode Corporation), aligned on Seaview v.4.3.0 (Gouy et al., 2010) with ClustalW2. Primers were selectively cut off. Chimeras were detected using the online program Bellerophon (Huber et al., 2004). 16S rRNA gene sequences were identified using the megx Geographic-BLAST (http://www.megx.net) and SILVA comprehensive ribosomal RNA databases (Pruesse et al., 2007). The SINA online v.1.2.11 (Pruesse et al., 2012) was used to align, search and classify sequences and their closest matches downloaded from the SILVA database as taxonomic references. All sequences were uploaded on the ARB platform (http://www.arb-home.de/) and phylogenetic trees established with the Maximum Likelihood method using the RAxML algorithm with advanced bootstrap refinement of bootstrap tree using 100 replicates (Ludwig et al., 2004). Phylip distance matrices were extracted from phylogenetic trees and exported to the Mothur® v. 1.32.1 software (Schloss et al., 2009) and number of operational taxonomic units (OTUs), rarefaction curves, Chao, Shannon and Dominance-D indices were calculated at 97 % sequence identity cut-off value (Supplementary material). All our sequences have been deposited in the GenBank database under accession numbers JX272064 to JX272122, JX472282 to JX472399 and KT381303 to KT381433.

To provide a quantitative confirmation of the major elements identified in the clone libraries, a preliminary run of Illumina MiSeq sequencing was performed on the same DNA extracts for horizon A and B. In addition, one surface sample (0.25 m depth) was included to provide a reference for the initial microbial assemblages, assuming that it experienced minimal

degradation of its DNA following deposition. We used bar code universal primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') to cover 291 bp of the bacterial and archaeal subunit 16S rRNA gene. (Supplementary material).

3 Results

3.1 Geochemical analysis of bulk sediment

3.1.1 Organic matter and pore water chemistry

Total organic carbon (TOC), total nitrogen (TN) and organic phosphorus (OP) displayed very similar stratigraphic variations, with all profiles covarying with grain size and the occurrence of gravity events (Fig. 3, top). Low OM contents were associated with coarse grain sizes and gravity events as they regularly occurred during the Last Glacial period. In contrast, four sediment intervals displayed increased OM values around 70, 40, 10 m depth and uppermost sediments (Fig. 3A). In context of the overall stratigraphy (Fig. 3, bottom), these intervals correspond to the Antarctic event A2, early LGM, YD and late Holocene times, respectively.

Chloride concentrations (Supplementary material) indicated a shift from freshwater (5.6 mM) to subsaline (16.9 mM) conditions during the YD. Nitrite + nitrate concentrations (Supplementary material) were always very low throughout the sedimentary sequence, with values in between 3.2 and 9.7 µM. Phosphate concentrations (Fig. 3D) were ca. 105 µM in Holocene sediments and most often close to detection limit (4 µM) within the rest of the sedimentary sequence. Dissolved iron (Fe^{2+}) was often below detection limit (65 µM), but was quantifiable from 55 to 15 m sediment depth, reaching concentrations between 89.5 and 268.6 µM. The sulphate concentration profile (Fig. 3D) displays frequent variations with baseline values oscillating between 52.0 and 728.7 µM. Extraordinary peaks were located at 49, 38 and 25 m sediment depth, reaching concentrations of ca. 16.6, 13.2 and 10.2 mM, respectively, in concomitance with tephra layers.

3.1.2 Pigment concentrations

Analyses of bacterial and algal pigment concentrations provided clear indication for algal abundance (i.e. total productivity) being lower and higher during the LGM and Holocene

periods, respectively (Fig. 3B). Specifically, elevated fossil concentrations of isorenieratene (100 nmole × gr TOC^{-1}) suggested that bacteria related to sulphur metabolism were an important component of the primary producer community during the late YD and early Holocene (Fig. 3B). Sporadic peaks in isorenieratene concentrations were also observed in the glacial record. In contrast, okenone concentrations (not shown) were always below 20 nmole × gr TOC^{-1} in Holocene sediments and close to detection limit in the glacial record. Total *Cyanobacteria* contributed substantially to the labile OM during the YD and Holocene times, but are present only sporadically within the glacial interval. Finally, diatoxanthin showed that diatoms (Fig. 3B) were abundant during the late YD and early Holocene period in agreement with diatom counts (Recasens et al., 2015). The pigment preservation index (Fig. 3C) displayed sporadic peaks correlating coarse grain sizes and increased sedimentation rates, notably during the LGM and YD transition, due to either degradation of chlorophyll *a* in coarse sediments or external inputs of pheophytin *a* reworked from the catchment. Analysis of the ratio of eukaryotic (e.g. diatoms, green algae, cryptophyte algae) to prokaryotic (e.g. cyanobacteria, green and purple sulfur bacteria) pigments (Fig. 3C) revealed that the relative importance of eukaryotic algae increased during climatic transitions (late LGM, YD and early Holocene). Otherwise, baseline values oscillated around 2.0, indicating that prokaryotic biomass is considerably less abundant than the eukaryotic one during the glacial period.

3.2 Microbial characteristics

3.2.1 Microbial activity, density and diversity

Maximal ATP values (>100) were recorded in the Holocene sediment in between 8 and 4 m burial depth, indicating ongoing microbial processes. In contrast, only small peaks of ATP (>50) were observed in LGM sediments (ca. 40 to 20 m depth), pointing to a sustained but considerably lower level of microbial activity in discrete horizons. Analysis of DAPI cell counts (Fig. 3E) suggested that microbial populations were densest in Holocene sediments (ca. 5 m core depth), but that total cell abundance decreased gradually from the YD down through LGM sediments, with minimal values in the deepest glacial record. At present, we cannot distinguish between active, inert or dead cells based on DAPI staining. Instead, analyses of DGGE gel features were used to assess microbial community changes. Here, the number of DGGE bands (Fig. 3F) for *Bacteria* was maximal at 5 and 30 m depth, which

corresponds with the two intervals where microbial populations appeared active based on ATP levels. The *Bacteria* signal disappeared below 60 m sediment depth in horizons potentially corresponding with increased gravity events and early reflooding of the maar (Gebhardt et al., 2012; Kliem et al., 2013). Similarly, the *Archaea* profile displayed a reduced but stable number of DGGE bands along the entire sedimentary record, with maximal values located around 8 and 35 m depth (Fig. 3F). In general, the DGGE bands represented short sequences (150 bp) which could not be used to distinguish between DNA arising from active taxa, intact dead cells and fragmented extracellular DNA (Corinaldesi et al., 2011). Taken together, these various indices provided evidence for the presence of amplifiable DNA related to microbial populations in decline at depth.

Two sedimentary horizons appeared to be preferentially colonized by microbes and were thus selected within the Holocene and LGM records to establish comparative clone libraries. During gel screening, bacterial clones obtained from the Holocene sample all matched the expected size of the targeted DNA fragment (1400 bp), whereas more than 50 % of the clonal sequences isolated from the LGM sample were shorter (800-600 bp), indicating lower DNA quality in aged sediment, were discarded from further analysis (Supplementary material).

3.2.2 Bacterial and archaeal clone libraries

16S rRNA gene sequences from ca. 5 ka old Holocene sediments showed that *Atribacteria* and *Aminicenantes*, respectively former candidate divisions OP9 and OP8 (Rinke et al., 2014), were major phyla of the sedimentary microbial assemblage (Fig. 4). Additional representative *Bacteria* identified from Holocene deposits were affiliated to *Acidobacteria* (Barns et al., 1999), *Clostridia* and δ*Proteobacteria* partly related to syntrophic species (Jackson et al., 1999; Liu et al., 1999 and 2011). In contrast, the microbial assemblage from the ca. 25 ka old LGM interval revealed the significant presence of δ*Proteobacteria* (Fig. 4) belonging to the SVA0485 candidate division likely involved in sulphate reduction (Bar-Or et al., 2015). Remarkably, one *Acidobacteria* sequence was affiliated with known iron reducers (Liesack et al., 1994). Other sequences specific to the LGM horizon clustered with *Spirochaetes*, *Elusimicrobia* and *Latescibacteria*, respectively former candidate division Termite Gut Group 1 and WS3 (Herlemann et al., 2009; Rinke et al., 2014; Youssef et al., 2015). Finally, sequences related to *Planctomycetes*, *Chloroflexi*, *Bacteroidetes* and *Actinobacteria* could not be uniquely associated with either the Holocene or LGM horizon

(Figs. 2 and 4), although their respective sequences still formed separate clusters (Figs. 4 and 6).

Despite potential cell migration in soft methane-saturated clays, archaeal sequences obtained from the Holocene record provided evidence for an environmental selection of assemblages with depth in the sedimentary profile (Figs. 5 and 6). Main groups successively identified with depth were affiliated with the Marine Group 1 and *Lokiarchaeota* (i.e. former Marine Benthic Group B) within the first meter, *Methanomicrobia* and *Bathyarchaeota* (i.e. former Miscellaneous Crenarchaeotal Group) plus Marine Benthic Group D within the next 4 m of sediment, and candidate phyla *Hadesarchaea* (i.e. former South African Gold Mine Group; Baker et al., 2016) and *Bathyarchaeota* below 5 m depth (Fig. 6). Methanogen sequences corresponded with depth to *Methanolinea*, *Methanosarcina*, *Methanoregula* and uncultured *Methanomicrobiaceace*. Finally, *Bathyarchaeota* sequences were present throughout Holocene sediments forming clusters associated with their respective sampling intervals (Fig. 5). Direct comparison between the LGM and Holocene horizon (Figs. 5 and 6) revealed archaeal assemblages mainly consisting of *Methanoregula* and Marine Benthic Group D in the Holocene, and mostly *Hadesarchaea* sequences in the LGM.

High-throughput 16S rRNA sequences supported the main taxa identified in clone libraries, although with different affiliation percentages (Supplementary material), allowing for general interpretation in terms of sediment populations and related processes. One main taxon (6 %) remained missing in the assemblage of horizon A, specifically the *Acetothermia* (i.e. former candidate division OP1). In the surface sample, *Proteobacteria* constituted about 50 % of the assemblage, followed by *Planctomycetes*, *Chloroflexi* and *Atribacteria*. In the surface sample, *Proteobacteria* constituted about 50 % of the assemblage, followed by *Planctomycetes*, *Chloroflexi* and *Atribacteria*. Checking results for the presence of phototrophs, we noted that sequences related to *Cyanobacteria*, *Chlorobi* and chloroplasts were minority and not uniformly present (Supplementary material).

4 Discussion

4.1 Holocene and LGM paleoclimatic and geochemical conditions

The sedimentation regime of Laguna Potrok Aike over the last 51 ka was mainly dependent on climatic variations and river inflows as water level fluctuations led to shore erosion and reworking of the catchment (Kastner et al., 2010; Coronato et al., 2013). Dry conditions during glacial times gave way to regression phases and multiple gravity events, whereas moister conditions promoted transgression phases and pelagic conditions (Haberzettl et al., 2007; Gebhardt et al., 2012; Ohlendorf et al., 2013). During the YD, the position of the Westerlies shifted to lower latitudes and the location of the lake (Killian and Lamy, 2012; Pollock and Bush, 2013), resulting in elevated wind evaporation and lake level decline along with a overall positive temperature excursion in South Patagonia (Waldmann et al., 2010; Kilian and Lamy 2012).

In general, the LGM horizon coincides with a period of active hydrology within the lake basin, with both overflow and active inflows into the lake (Haberzettl et al., 2007). Reduced vegetation in the catchment (Haberzettl et al., 2009) promoted periglacial and wind-related erosion (Hein et al., 2010). Tephra layers (Wastegård et al., 2013) with mafic sands reworked from the catchment triggered small-scale shifts in productivity (Hahn et al., 2013) and contributed to punctual increases of iron and sulphate in pore water (Fig. 3D). In contrast, the Holocene horizon corresponds to a period of lake level rise and endorheic phase (Anselmetti et al., 2009; Ohlendorf et al., 2013) with subsaline and nitrogen-limiting conditions in the water column (Zhu et al., 2013). Such lake level rise corresponds with important nutrient fluxes, elevated primary productivity (Recasens et al., 2015) and higher microbial colonization of the sediment under pelagic conditions (Vuillemin et al., 2014a).

4.2 Interpretation of sedimentary DNA

Overall, microbial populations were defined according to an apparently depth-dependent trend reflecting the receding activity and slow death of microorganisms (Vuillemin et al., 2014a). Subsequent to cell lysis, nucleic acids are released into the surrounding sediment where they can be actively degraded or sorbed to sediments (Corinaldesi et al., 2007 and 2011). Exposure of extracellular DNA to microbial processes then results in the turnover or preservation of sequences with depth (Corinaldesi et al., 2008). Theoretically, short

fragments are associated mainly with ancient and inactive taxa, whereas longer DNA fragments should better record changes in recent and active taxa. Therefore, clonal 16S rRNA gene sequences (1400 and 900 bp) are considered significant of some major components of formerly preserved and currently viable microbial assemblages, whereas DGGE bands (150 bp) is likely influenced by the accumulation of extracellular DNA.

Microbial populations were abundant and metabolically active in the sediment of the Holocene period. Archaeal phylotypes indicate a layering of these assemblages with depth likely related to environmental selection during diagenesis. While *Bathyarchaeota* are major elements of the archaeal assemblage throughout the sediment, predominant methanogens vary with depth from *Methanolinea* to *Methanosarcina* and *Methanoregula*. Marine-related sequences also shift from Group 1 to *Lokiarchaeota* (Spang et al., 2015) and Benthic Group D and are replaced by *Hadesarchaea* sequences below 5 m depth. Similar changes in archaeal assemblages have also been identified in marine subseafloor environments (Vigneron et al., 2014). In this latter case, *Bathyarchaeota* and marine groups are expected to degrade complex organic matter, such as cellulose, proteins and aromatic compounds (Lloyd et al., 2013; Meng et al., 2013). Thus, the present series of *Archaea* likely reflect an environmental selection of subsurface biosphere during early diagenesis of OM, with an age-related stratification made possible by a stable pelagic regime at that time.

16S rRNA gene sequences provide evidence for the presence of *Atribacteria* and *Aminicenantes* (Rinke et al., 2013) as dominant sequences of the assemblage within the organic-rich Holocene clays buried at 5 m depth (ca. 5 ka BP) (Fig. 6 + Supplementary material). These microbes, initially described from hot springs (Hugenholtz et al., 1998), are often abundant in anaerobic marine sediments (Inagaki et al., 2003). Recently, *Atribacteria* have been described as energy-conservative heterotrophic anaerobes which act either as primary or secondary fermenters (Nobu et al., 2015) capable of syntrophic catabolism (Sieber et al., 2012). *Methanoregula* (Bräuer et al., 2011) was detected in association with *Syntrophus* (Jackson et al., 1999) and *Syntrophomonadaceae* (Liu et al., 2011). GIF9 *Chloroflexi*, which are closely related to *Dehalogenimonas* (Moe et al., 2009) and widely abundant in organic-rich anoxic sediments, are presumably homoacetogenic fermenters (Hug et al., 2014). In addition, alkalotolerant species, such as *Clostridia* (Nakagawa et al., 2006) and Marine Benthic *Archaea* (Jiang et al., 2008), when active, mainly ferment labile organic compounds (Wüst et al., 2009), whereas cellulose and lignin are degradable by

Actinobacteria and *Bacteroidetes* equally present (Pachiadaki et al., 2011). These assemblages reflect the initial degradation of labile OM from algae and the generation of fermentative byproducts, such as acetate, H_2 and CO_2, which served as substrates for methane production by *Methanomicrobiales*. Such substrate evolution during prolonged OM diagenesis promotes the recycling of end products and syntrophic hydrogen consumption, as presently observed with autotrophic methanogenesis and homoacetogenesis (Wüst et al., 2009). Such a pattern also suggests that the final Holocene microbial assemblages arise from metabolic complementarities of component taxa, reinforcing our previous study on their role in the degradation and geochemical cycling of OM (Vuillemin et al., 2014b).

Microbial communities recovered from ca. 25 Ka old LGM sediments were not considered dormant or dead, but instead appear to subsist in a viable state at low metabolic rate (Hoelher and Jørgensen, 2013). This LGM assemblage records the intricate presence of organotrophs capable of refractory OM degradation with mostly *Atribacteria*, *Aminicenantes*, *Elusimicrobia* (Herlemann et al., 2009; Febria et al., 2015) and *Chloroflexi*, to which *Acidobacteria* (Liesack et al., 1994), *Spirochaeta* (Hoover et al., 2003), *Planctomycetes,* *Actinobacteria*, and *Bacteroidetes* are added. Syntroph sequences among δ*Proteobacteria* and *Chloroflexi* are consistent with the degradation of secondary metabolites such as propionate (Liu et al., 1999: De Bok et al., 2001; Yamada et al., 2007), while sulphate-reducing δ*Proteobacteria* and *Hadesarchaea* (Takai et al., 2001; Baker et al., 2016) are thought to reflect the specific sediment geochemistry. Finally, *Latescibacteria* have been recently presented as anaerobes mediating the turnover of multiple complex algal polymers in deep anoxic aquatic habitats (Youssef et al., 2015). This pattern of sequences is interpreted as arising from the intercalation of organic-poor clays with volcanic material that could act as sources of iron and sulphate. In general, conditions at such sedimentary interfaces would greatly limit any methane production (Schubert et al., 2011) and instead select for a microbial assemblage capable of sulphate and iron reduction. H_2S production during sulphate reduction likely promotes lithotrophic species via the alteration of mafic minerals (Johnson, 1998; Blanco et al., 2014) and act in the formation of authigenic minerals such as framboidal sulphides (Vuillemin et al., 2013b).

Heterogeneous sedimentation or prolonged exposure to diagenesis can obscure the interpretation of DNA sources. For example, consistent with their ubiquity noted in other studies (Kubo et al., 2012; Farag et al., 2014), *Bathyarchaeota* and *Aminicenantes* sequences

were not specifically associated with environmental or metabolic features of either the Holocene and LGM horizons, while sequence affiliation to *Planctomycetes*, *Chloroflexi*, *Actinobacteria* and *Bacteroidetes* appears to be kept constant with depth (Supplementary material). Indeed, some microorganisms easily tolerate different kinds of environmental change with high functional redundancy (Sunagawa et al., 2015). Global patterns of bacterial distribution in the environment have shown that the main drivers of community composition are temperature and primary production in the oceans (Raes et al., 2011) and salinity and substrate type in sedimentary environments (Lozupone and Knight, 2007). In deep sediment settings, OM anaerobic metabolisms appear as the dominant activities, with cell densities in link to pore-water sulphate concentrations (Orsi et al., 2013) and sedimentation rates (Kallmeyer et al., 2012). All these parameters are consistent with the present microbial assemblages although the Holocene methanogenesis zone overlies the LGM sulphate reduction zone.

Several lines of evidence suggest that patterns of microbial activity and composition did not arise from contamination of ancient sediments with modern microbes. Firstly, phylogenetic results from Holocene and LGM sediments display only one single OTU in common (Fig. 4). Secondly, sedimentary ATP activity recorded less than two hours after core recovery shows the same pattern of ATP concentration than that measured substantially later, and is also coherent with more extensive laboratory analyses (Supplementary material). Thirdly, deep sediments lacked any of the chemical or lithological characteristics of the younger sediments (Fig. 3), including framboidal iron sulphides, lower salinity, pigment composition, color of clays and absence of gas vugs (Supplementary material).

4.3. Sedimentary DNA and fossil pigment preservation

In addition to diagenesis, important lake level fluctuations can influence the sediment record due to changes in lake morphometry, light penetration and bottom water stratification (Leavitt, 1993; Leavitt and Hodgson, 2002). Complementary analyses of bacterial and algal pigment concentrations indicate high primary productivity during the Holocene while oligotrophic conditions characterized the last glacial period. Sporadically, the pigment preservation index suggests intervals of poor preservation related to low OM content as well as the presence of reworked OM in gravity-related sediments (Hahn et al., 2013). Fortunately, pelagic production can be considered accurately recorded. During the LGM,

short intervals of elevated productivity appear to correlate with warming events, tephra inputs and mass movements (Recasens et al., 2015). Still, bacterial sources constitute an important fraction of the organic sedimentary record. During the YD and Holocene, reduced okenone and isorenieratene concentrations indicate two brief periods of stratification associated with lake level lowstands (Zolitscka et al., 2013). Endorheic conditions resulted in nitrate limitation and may have favoured *Cyanobacteria* in comparison to other primary producers (Mayr et al., 2009; Zhu et al., 2013). Reflooding of the maar could explain shifts in planktonic assemblages (Wirth et al., 2013) and increased lake level should have improved conditions for primary production by eukaryotes. However, the water depth difference between the Holocene and LGM times (i.e. 37 m) likely promoted OM preservation during lowstand.

Comparison of fossil pigments with sedimentary DNA assemblages suggests that the initial nucleic acid composition of sediments could be rapidly modified by microbial ontogeny following deposition. For example, high concentrations of isorenieratene from brown varieties of green sulfur bacteria (Leavitt et al., 1989; Glaeser and Overmann, 2001) were recorded in the sediments throughout the Holocene, but genetic markers of the relevant carotenoid-producing phototrophic taxa were rare in the mid-Holocene intervals subject to DNA analysis. Similarly, despite high concentrations of cyanobacterial pigments in the Holocene record, related sequences were hardly detected in shallow sediments, even using high-throughput sequencing (Supplementary material). In this paper, *Planctomycetes*, *Actinobacteria* and *Bacteroidetes* are among the heterotrophs (Fig. 4) which can produce carotenoids pigments (Hahn et al., 2003; Warnecke et al., 2005; Fukunaga et al., 2009; Jehlička et al., 2013) that can be altered to complex derivatives in sedimentary environments (Sinninghe Damsté and Koopmans, 1997; Brocks and Schaeffer, 2008). Of interest is the observation that these heterotrophic taxa are characteristic of anoxic aquatic and sediment habitats and common in ancient algal mat assemblages (De Wever et al., 2005; Schwarz et al., 2007; Song et al., 2012), often persisting long after associated phototrophic bacterial species have been lost (Antibus et al., 2012; Cole et al., 2014; Lage and Bondoso, 2011 and 2015). Additionally, initial habitats may play an important role in the preservation of phototrophic sequences. Strong mixing due to Westerly Winds leads to particle resuspension in the water column, while biomats developing on the flanks of the maar and sediment surface can be rapidly buried during gravity events. Our interpretation is that particulate organic matter and planktonic sequences are quickly degraded by heterotrophs during

sinking, while early colonization of algal mats after deposition would result in selective recycling of bacteria (Antibus et al., 2012).

4.4 A model for ancient and extant microbial assemblages

Taken together, data collected herein and by the complementary studies of the ICDP-PASADO project suggest that climate regulates the influx of organic and inorganic material to the lake basin, which in turn determines water column chemistry, algal productivity and sedimentation of particulate material. Water column conditions (e.g. salinity) and sediment lithology then interact to determine final geochemistry of the sediment. Thus, environmental and geochemical parameters arising from prevailing climatic conditions can exert the initial control on microbial substrates, defining the degree of colonization at the time of deposition (Vuillemin et al., 2013a and 2014a), and subsequently dominant subsurface assemblages brought on by environmental selection during diagenesis. Results presented herein advance this model by characterizing the main elements recorded in the sedimentary DNA and by elucidating the metabolic pathways involved in post-depositional alterations.

During the Holocene interval, elevated rates of OM deposition under pelagic regime led to increased pigment concentrations in the sediment. Sequences potentially derived from ancient assemblages (i.e. *Planctomycetes*, *Actinobacteria* and *Bacteroidetes*) may have emerged from the early degradation of algae and microbial biofilms. Seemingly, these heterotrophic species actively grew at the expense of phototrophic species (Antibus et al., 2012; Cole et al., 2014), leaving intact only their respective pigments although very few sequences of *Cyanobacteria* and *Chlorobi* could still be identified in surface sediments (Supplementary material). Phylogenetic sequences representing the main elements of the subsurface biosphere are characteristic of those exhibiting solely anaerobic heterotrophic metabolism, with *Atribacter* and *Methanomicrobiales* as the dominant taxa. They reflect the sediment surrounding geochemical conditions and are indicative of advanced OM degradation during early diagenesis, showing how long-term persistence and activity of microorganisms can imprint organic proxies (Vuillemin et al., 2014b).

During the LGM period, limited nutrient inputs to the water column and volcanic inflows engendered low primary production mainly by bacteria, presumably in the form of microbial mats reworked to the basin during gravity events. Sequences issued from ancient assemblages seem to refer to complex autotroph-heterotroph interactions (Cole et al., 2014) and likely include *Elusimicrobia* 4-29 (Herlemann et al., 2009; Febria et al., 2015) and

Latescibacteria (Youssef et al., 2015). Surrounding geochemical conditions associated with the formation of OM-poor but iron- and sulphate-rich sediments selected for a subsurface biosphere capable of sulphate reduction and lithotrophy, mainly including sequences affiliated to δ *Proteobacteria* and *Hadesarchaea* (Baker et al., 2016). Related diagenetic processes resulted in the presence of authigenic concretions in LGM sediments (Vuillemin et al., 2013b).

Post-depositional diagenesis plays an important role in modifying the sequences of sedimentary DNA. Long sequences appear to derive from intact bacterial cells, whereas extracellular DNA released upon cell lysis gives way to an accumulation of short fragments in ancient sediments. Analysis of nucleic acid sequences reveals that phototrophic and pre-diagenetic assemblages are rapidly overprinted by subsurface heterotrophic communities. Taxa are then selected according to microbial substrates and geochemical conditions, resulting in the overall decline of microbial activity and density with depth and decreasing turnover of sedimentary DNA. However, despite these insights, further high-resolution research is needed to establish the time lag between deposition of the original microbial assemblages and establishment of the final composition of DNA in the sediments.

5 Conclusions

Climatic and lacustrine conditions at the time of sediment deposition appeared to be the main factors defining sediment geochemistry and microbial substrates. Preferential preservation of microbial sources already occurred during synsedimentary processes. Sedimentary niches at the time of deposition exerted initial constraints on the development of the subsurface biosphere. After burial, changing geochemical conditions associated with sustained metabolic activity performed a selection of viable microorganisms over time and defined the final microbial assemblages. Genetic information related to phototrophic communities was mostly erased by heterotrophic bacteria while conserving pigment compositions. Identified taxa were *in fine* characteristic of conditions associated with past environmental and present geochemical factors, with *Atribacteria* and methanogens, sulphate reducers and *Hadesarchaea* as dominant species in the Holocene and LGM sediment, respectively. Further research using a combination of DNA and other proxies will advance our understanding of the mechanisms forming sedimentary nucleic acid assemblages. For

example, at present, it is unclear whether microorganisms actively grew for centuries in past sedimentary environments or whether their sequences were merely entombed during the study period, leaving uncertainties concerning the temporal lag between original microbial deposition and establishment of the final composition of sedimentary DNA. Similarly, we also recognize that our analytical platform represent a preliminary insight into genetic variations of Laguna Potrok Aike sediments and that the length of the targeted sequence (1400 bp) likely prevented the detection of partially preserved phototrophic bacteria (<300 bp). However, the rapid development of single cell sequencing technologies and metatranscriptomic analysis will enable a refined view of deep biosphere activities, while massive parallel sequencing will provide extensive phylogeny of microbial DNA in lake deposits.

This study provides new evidence for mechanism underlying the preservation of sedimentary DNA sequences. We show clearly that sedimentary assemblages of nucleic acids differ among major historical climate zones and that some initial elements even sustain activity for 25,000 years after burial, albeit at low metabolic rates. Moreover, the present results demonstrate that sedimentary DNA could help reconstructing microbial diagenetic processes undergone by lacustrine sediments and favourably complement paleoreconstructions based on fossil pigments. Application of this approach to other lake sequences will improve interpretation of past climate proxies and eventually disentangle depositional from diagenetic signals.

Author contribution

A. V. carried out field sampling, 16S fingerprinting techniques and bulk sediment analyses. D. A. designed the research as principal investigator of the PASADO project and carried out field sampling. P. R. L. and L. B. performed pigment extractions and analyses. A.V. wrote the initial manuscript, and all authors edited and revised the paper.

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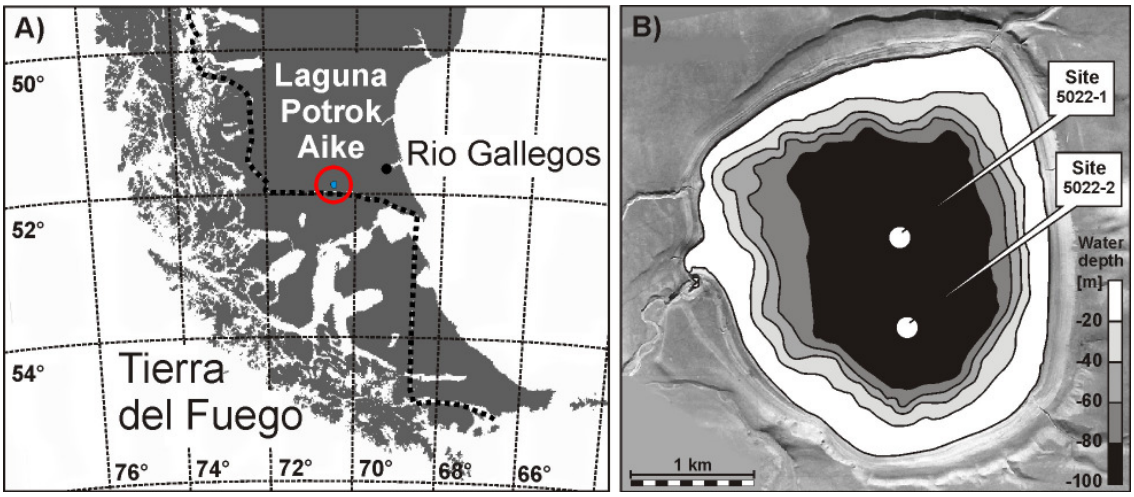

Figure 1. Map of Southern Argentina displaying the location (**A**) and bathymetric map (**B**) of Laguna Potrok Aike showing the two drilling sites (Zolitschka et al., 2006). Pore water and geomicrobiological samples were retrieved from cores at site 5022-1, whereas sediments for pigment analysis were obtained from cores at site 5022-2.

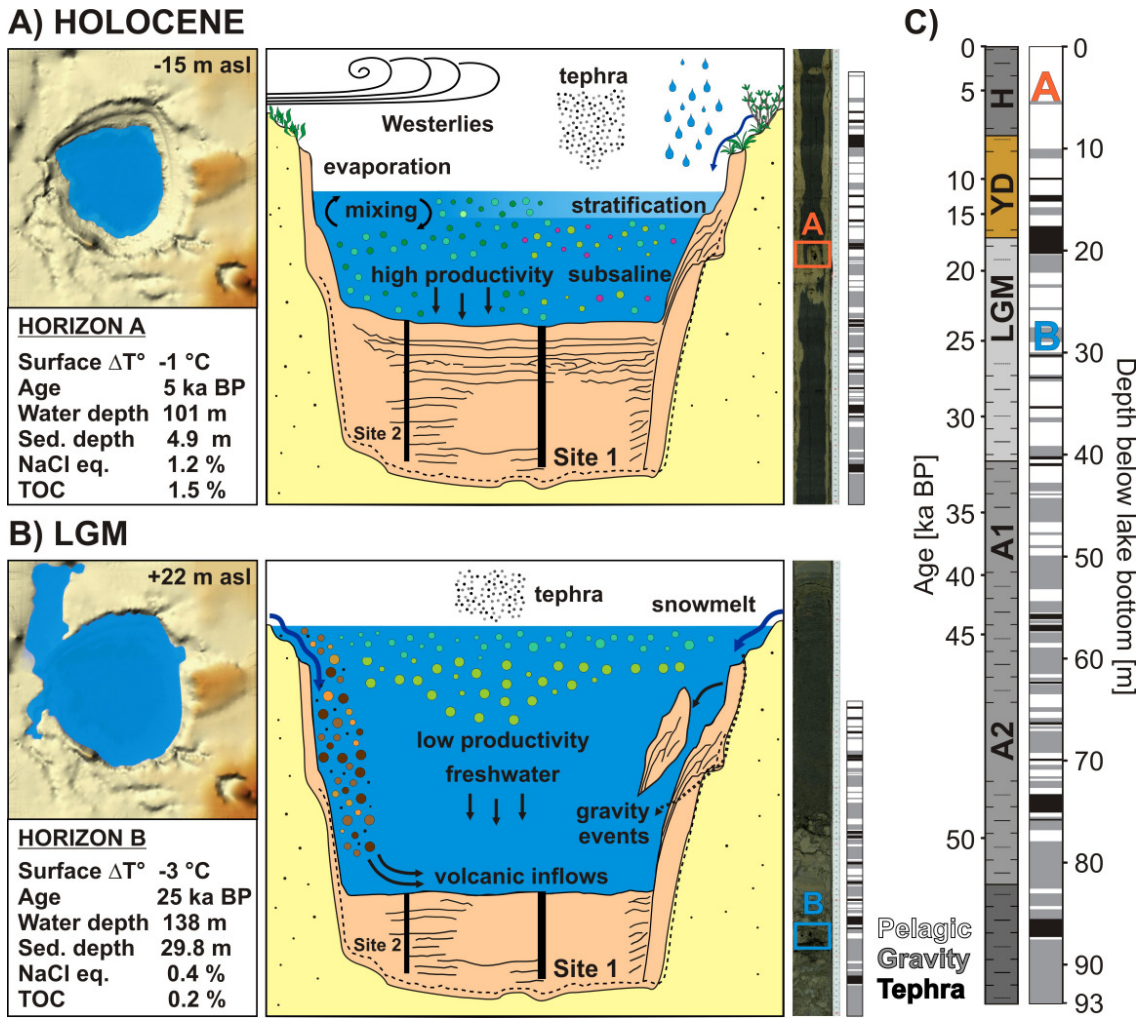

Figure 2. Paleoenvironmental conditions at Laguna Potrok Aike during the Holocene (**A**) and LGM times (**B**), with from left to right: Climatic and lacustrine parameters, sagittal views of the basin and respective core sections locating the 16S rRNA samples. Holocene times correspond with active Westerly winds, lake lowstand, subsaline conditions and high primary productivity in the basin and catchment, whereas LGM times are characterized by lake highstand and active overflow, freshwater conditions, low primary productivity in the basin and inflows restricted to runoff from the volcanic catchment. The whole lacustrine sequence (**C**) is displayed as stratigraphic units in age scale and lithology log in meter scale (after Kliem et al. 2013). The sedimentation can be defined as pelagic (white), gravity (grey) and tephra (black) layers. Time abbreviations stand for Holocene (H), Younger Dryas (YD), Last Glacial Maximum (LGM), Antarctic events 1 (A1) and 2 (A2).

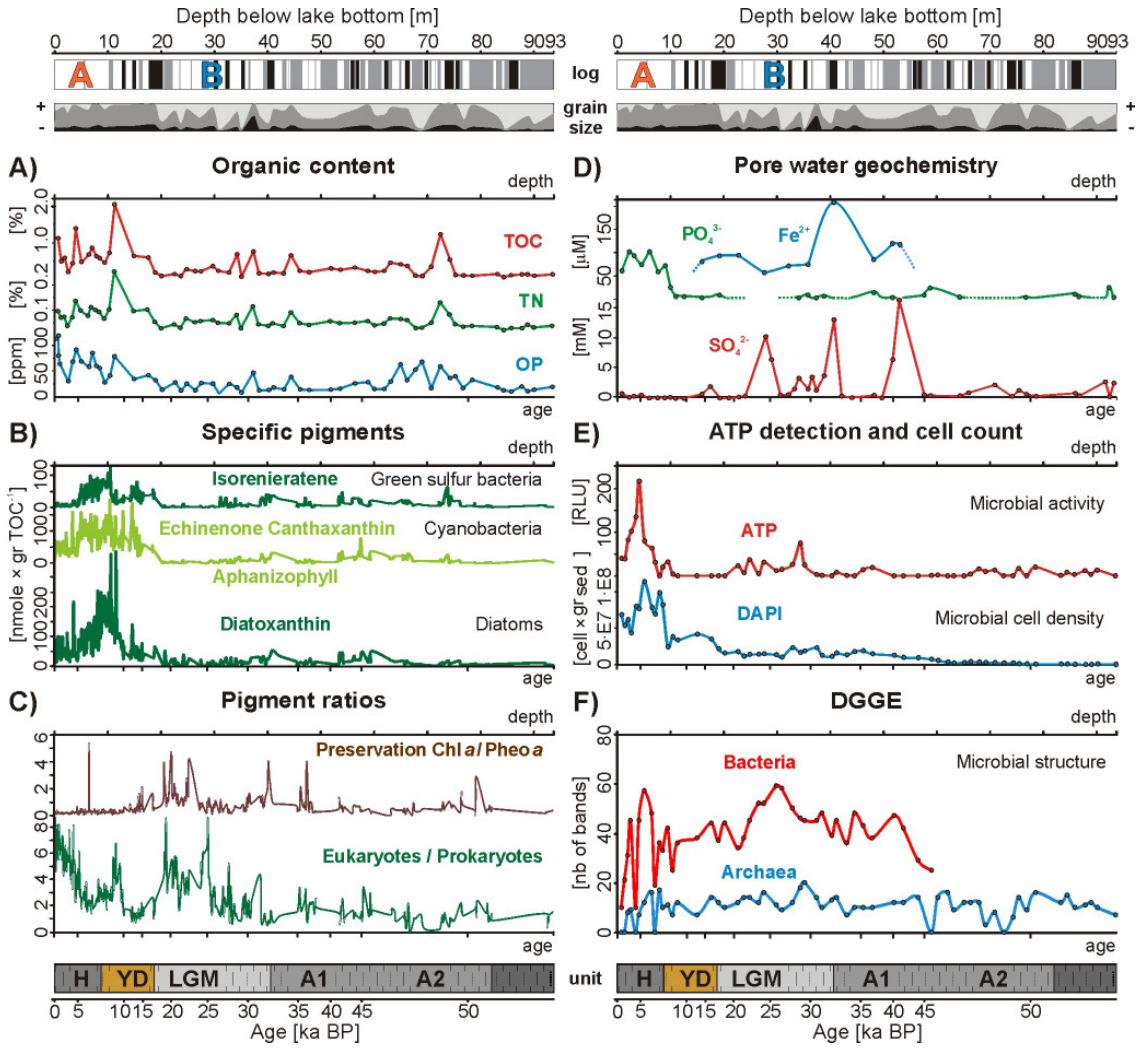

Figure 3. Paleoclimatic and geomicrobiological multiproxy. **Top)** Stratigraphic sequence of Laguna Potrok Aike, followed by grain size with clay (black), silt (dark grey) and sand (light grey). **A)** Total organic carbon (TOC), total nitrogen (TN) and organic phosphorus (OP) from bulk sediment. **B)** Specific pigments usually accounting for green sulphur bacteria (isorenieratene), cyanobacteria (echinenone, canthaxanthin, aphanizophyll) and diatoms (diatoxanthin). **C)** Preservation index based on the ratio of chlorophyll *a* to pheophytin *a*, with peaks indicative of increased preservation associated with high sedimentation rates, and ratio of eukaryotic to prokaryotic pigments. **D)** Pore water concentrations for phosphate, iron and sulphate. **E)** On-site adenosine triphosphate (ATP) detections and 4',6-diamidino-2-phenylindole (DAPI) cell counts respectively used as indices of microbial activity and population density. **F)** Number of bands from DGGE gels is used as relative index of

structural shifts in bacterial and archaeal communities. **Bottom**) Lithology log displaying the five units established by Kliem et al. (2013) and their corresponding climatic intervals.


Desulfobacca acetoxidans (AF002671)
Biomat cenote El Zacaton (FJ484529)
OTU 1 (1 x 4.97 m - Holocene)
Smithella propionica (AF126282)
Syntrophus aciditrophicus (GU993263)
OTU 2 (1 x 4.97 m - Holocene)
Lake Kinneret sediment (AM086113)
OTU 3 (1 x 29.77 m - LGM)
SVA0485 cenote El Zacaton (FJ484529)
SVA0485 mine shaft (GQ921432)
OTU 4 (1 x 4.97 m - Holocene)
SVA0485 Mo mine (EF562249)
SVA0485 Lake Hongku sediment (HM243935)
OTU 5 (7 x 29.77 m - LGM)
Desulfovirga sp. (HQ397226)
4-29 Peru methane hydrate (AB177154)
OTU 6 (2 x 29.77 m - LGM)
WS3 North Yellow Sea (GQ246366)
WS3 Peru methane hydrate (AB177136)
OTU 7 (1 x 29.77 m - LGM)
OTU 8 (1 x 29.77 m - LGM)
Anammoxoglobus propionicus (DQ317601)
Scalindua brodae (AY254883)
Pir4 lineage Florida sea grass sediment (EU488313)
OTU 9 (1 x 4.97 m - Holocene)
OTU 10 (1 x 4.97 m - Holocene)
AKAU3564 South China Sea (EU048688)
OTU 11 (1 x 4.97 m - Holocene)
MSBL9 tar oil contaminated sediment (EU266844)
MSBL9 Pacific abyssal sediment (FJ746095)
OTU 12 (1 x 4.97 m - Holocene)
OTU 13 (1 x 4.97 m - Holocene)
MSBL9 SO4-CH4 transition sediment (FJ455909)
OTU 14 (1 x 29.77 m - LGM)
MSBL9 Japan Holocene mud (AB300077)
MSBL9 wetland Tabla de Daimiel (FJ517127)
OTU 15 (1 x 29.77 m - LGM)
OTU 16 (2 x 29.77 m - LGM)
KF-JG30-18 biogenic Fe-nodules (AB254785)
KF-JG30-18 Holophaga uranium tailing (AJ519381)
OTU 17 (1 x 29.77 m - LGM)
AKIW659 Mexico cenote biomat (FJ484803)
AKIW659 Japan Holocene mud (AB300082)
OTU 18 (4 x 4.97 m - Holocene)
OPB95 Lake Taihu sediment (FJ755774)
OPB95 Nankai trough sediments (AB013270)
OPB95 Amsterdam deep-sea mud volcano (HQ588598)
OTU 19 (2 x 29.77 m - LGM)
OPB95 biomat cenote La Palita (FJ901520)
OTU 20 (2 x 29.77 m - LGM)
OPB95 anaerobic UASB reactor (FJ535533)
OPB95 methanogenic lotus pond (AB426208)
OTU 21 (7 x 4.97 m - Holocene)
OTU 22 (1 x 4.97 m - Holocene)
Spirochaeta americana (AF373921)
Uncultured Spirochaeta biomat Le Zacaton (FJ485365)
OTU 23 (1 x 29.77 m - LGM)
Cytophaga fermentans (AB517712)
Anaerophaga sp. (NR028963)
BD2-2 deep-sea sediments (AB015532)
OTU 24 (1 x 4.97 m - Holocene)
vadinHA17 Lake Anderson sediment (EU283477)
OTU 25 (1 x 4.97 m - Holocene)
vadinHA17 Lake Kinneret sediment (AM086122)
OTU 26 (1 x 29.77 m - LGM)
vadinHA17 Lake Dongping sediment (GQ472362)
OTU 27 (1 x 29.77 m - LGM)
Bellilinea caldifistulae (AB243672)
OTU 28 (1 x 29.77 m - LGM)
MSB 5B2 Japan Holocene mud (AB300063)
OTU 29 (1 x 29.77 m - LGM)
Dehalogenimonas lykanthroporepellens (EU679418)
GIF9 Peru methane hydrate (AB177135)
OTU 30 (1 x 29.77 m - LGM)
GIF9 Japan methane hydrate (AY093456)
OTU 31 (1 x 4.97 m - Holocene)
GIF9 biomat cenote La Palita (FJ901649)
OTU 32 (1 x 4.97 m - Holocene)
GIF9 seagrass bed sediment (EU488317)
OTU 33 (1 x 29.77 m - LGM)
OTU 34 (1 x 4.97 m - Holocene)
GIF9 in situ reactor system (AF407200)
OTU 35 (2 x 29.77 m - LGM)
OP9 petroleum reservoir (EU721783)
OTU 36 (4 x 29.77 m - LGM)
OP9 marine sediment (FN429787)
OP9 Japan Trench sediment (AB015269)
OP9 Kazan mud volcano (FJ712605)
OTU 37 (12 x 4.97 m - Holocene)
OTU 38 (1 x 4.97 m - Holocene)
Syntrophomonadaceae leachate sediment (HQ183815)
OTU 39 (1 x 4.97 m - Holocene)
D8A-2 gold mine deep groundwater (DQ088758)
D8A-2 Yellowstone microbial mat (FJ206828)
OTU 40 (1 x 4.97 m - Holocene)
OTU 41 (1 x 4.97 m - Holocene)
OTU 42 (1 x 4.97 m - Holocene)
D8A-2 black rust formation (AB260059)
OPB41 Taiwan mud volcano (JQ245645)
OPB41 hydrothermal oxides (JN860326)
OTU 43 (4 x 29.77 m - LGM)
OPB41 biomat cenote El Zacaton (FJ485014)
OPB41 active tundra soil (EU644120)
OPB41 Amsterdam mud volcano (HQ588500)
OPB41 Peru gas hydrate (AB177140)
OPB41 Lake Tanganyika anoxic waters (DQ463730)
OTU 44 (2 x 4.97 m - Holocene / 2 x 29.77 m - LGM)
scale: 0.10
δ Proteobacteria
Dominant Species
Elusimicrobia (TG1)
Latescibacteria (WS 3)
Planctomycetes
Acidobacteria
Aminicenantes (OP8)
Spirochaetes
Bacteroidetes
Chloroflexi
Atribacteria (OP9)
Dominant Species
Firmicutes
Actinobacteria

1

Figure 4. Maximum likelihood phylogenetic tree of bacterial 16S rRNA gene sequences (1400 bp) recovered at 4.97 and 29.77 m depth from Holocene (orange types) and LGM (blue types) sediments. *Atribacteria* and *Aminicenantes* are the main taxa encountered in the Holocene organic-rich pelagic sediments, whereas sulphate reducers are dominant in the LGM horizon composed of intercalated volcanic mafic sands and hemipelagic sediments. Boldface types signify database references with sequence accession numbers in parentheses.


Thermoprotei
Bathyarchaeota
(MCG)
Iceland giant submarine smectite (AF411230)
China estuarine sediment (JQ245927)
Salt marsh sediment (EU264658)
OTU 1 (1 x 0.25 m / 1 x 7.81 m)
OTU 2 (1 x 0.25 m)
OTU 3 (1 x 0.25 m)
OTU 4 (1 x 7.81 m)
OTU 5 (1 x 4.97 m - Holocene)
OTU 6 (1 x 2.51 m)
Serpentinization alkaline groundwater (FN690972)
OTU 7 (3 x 2.51 m)
OTU 8 (1 x 0.25 m)
OTU 9 (1 x 2.51 m)
OTU 10 (1 x 2.51 m)
OTU 11 (1 x 2.51 m)
Spain freshwater sulfurous lake (AM076830)
Petroleum contaminated soil (AB161328)
Lake Hongku middle sediment (HM244135)
OTU 12 (1 x 9.37 m)
Finland boreal forest lake (AJ131315)
Ohio-Mississipi confluence (GQ906595)
OTU 13 (4 x 7.81 m)
OTU 14 (1 x 7.81)
OTU 15 (1 x 7.81)
OTU 16 (1 x 7.81)
OTU 17 (1 x 7.81)
OTU 18 (1 x 7.81)
Russian caldera spring (GQ328203)
Hong Kong harbour sediment (EF203585)
Holocene mud sediment (AB300143)
OTU 19 (2 x 9.37 / 1 x 29.77 - LGM)
Waikato coalfields sediment (AM883005)
Lake Baikal methane seep (KJ922116)
OTU 20 (3 x 29.77 m - LGM)
OTU 21 (2 x 9.37 m / 3 x 29.77 m - LGM)
OTU 22 (1 x 29.77 m - LGM)
OTU 23 (1 x 29.77 m - LGM)
OTU 24 (1 x 29.77 m - LGM)
Submarine permafrost (FJ982763)
Oyster shell (EU370093)
OTU 25 (1 x 0.55 m)
OTU 26 (1 x 4.97 m- Holocene)
OTU 27 (1 x 4.97 m- Holocene)
Mongolia groundwater (JX196210)
Japan Holocene mud (AB300142)
OTU 28 (1 x 0.55 m / 15 x 1.90 m / 3 x 2.51 m / 2 x 4.97 m- Holocene)
OTU 29 (3 x 1.90 m / 1 x 4.97 m- Holocene)
OTU 30 (2 x 1.90 m)
OTU 31 (1 x 2.51 m)
Tibet hypersaline lake (EF201709)
OTU 32 (1 x 2.51 m)
OTU 33 (1 x 0.55 m)
OTU 34 (1 x 4.97 m- Holocene)
OTU 35 (1 x 1.90 m)
OTU 36 (1 x 1.90 m)
OTU 37 (1 x 4.97 m- Holocene)
OTU 38 (1 x 1.90 m)
Cascadia margin methane hydrate (AB177287)
Okhotsk methane seep (FJ404091)
OTU 39 (1 x 4.97 m- Holocene)
OTU 40 (4 x 0.55 m)
OTU 41 (1 x 0.55 m)
Lokiarchaeota
(MBGB)
Mt Kunlun permafrost soil (JQ684417)
Pearl River estuary sediment (JX870390)
Germany marsh sulfidic spring (AM055707)
OTU 42 (14 x 0.25 m / 31 x 0.55 m)
OTU 43 (1 x 0.25 m)
OTU 44 (1 x 0.25 m)
Marine
Gp 1
Eel River methanic sediment (GQ356893)
Rich minerotrophic fen (EU155985)
Sulfur-rich sinkhole sediment (EU910622)
Alpine lake sediment (AM851082)
OTU 45 (2 x 0.25 m / 4 x 4.97 m- Holocene)
OTU 46 (1 x 0.25 m)
OTU 47 (1 x 0.25 m)
OTU 48 (1 x 0.25 m)
Marine Benthic
Gp D
Methanosarcina acetivorans (HI592326)
Methanosarcina lacustris (AY260430)
Methanosarcinales permafrost (FJ982666)
Methanosarcinales alpine lake (AM851080)
OTU 49 (1 x 0.25 m / 17 x 1.90 m)
OTU 50 (1 x 1.90 m / 1 x 2.51 m)
OTU 51 (1 x 2.51 m)
Methanomicrobiales
Lake Wivenhoe sediment (HQ330676)
Lake Hongku upper sediment (HM244093)
OTU 52 (3 x 9.37 m)
Methanoplanus petrolearius (AY196681)
Methanoculleus marisnigri (AF028693)
Methanosphaerula palustris (EU156000)
Methanoregula sp. acidic fen (EU155976)
Methanoregula boonei (EU887810)
Methanoregula formicicum (AB479390)
Methanoregula sp. acidic bog lake (AF481343)
OTU 53 (11 x 4.97 m - Holocene / 2 x 7.81 m / 1 x 9.37 m)
OTU 54 (1 x 7.81 m)
OTU 55 (1 x 7.81 m)
Methanospirillum hungatei (AB517987)
Methanolinea tarda (AB162774)
Methanolinea Lake Hongku (HM244180)
OTU 56 (4 x 0.25 m / 4 x 0.55 m / 12 x 2.51 m)
OTU 57 (1 x 0.25 m)
OTU 58 (1 x 2.51 m)
Salar de Huasco (EU481619)
OTU 59 (1 x 0.25 m)
OTU 60 (1 x 0.25 m)
Dominant Species
Nirano mud volcano (HQ654860)
OTU 61 (2 x 7.81 m)
South Africa deep gold mine water (AB050223)
Obsidian pool prime community (AY861953)
South China subseafloor sediment (EU385847)
Spain freshwater sulfurous lake (EU683399)
OTU 62 (4 x 7.81 m / 2 x 9.37 m / 11 x 29.77 m - LGM)
OTU 63 (1 x 29.77 m - LGM)
OTU 64 (1 x 29.77 m - LGM)
OTU 65 (1 x 29.77 m - LGM)
OTU 66 (1 x 7.81 m)
Hadesarchaea
(SAGMEG)
Dominant Species
scale: 0.10

1

Figure 5. Maximum likelihood phylogenetic tree of archaeal 16S rRNA gene sequences (900 bp) recovered at 0.25, 0.55, 1.90, 2.51, 4.97, 7.81, 9.37 and 29.77 m sediment depth. Clone series established throughout the Holocene record (dark grey types) indicate a depth-related evolution of the assemblages, with a general trend from marine groups to methanogens ending with *Hadesarchaea* (i.e. SAGMEG) sequences. Comparatively, the Holocene archaeal assemblage at 4.97 m depth (orange types) is mainly composed of *Methanomicrobiales* and *Bathyarchaeota* (i.e. MCG), whereas the LGM archaeal assemblage at 29.77 m depth (blue types) is restricted to *Hadesarchaea* and *Bathyarchaeota* divisions. Boldface types signify database references with sequence accession numbers in parentheses.

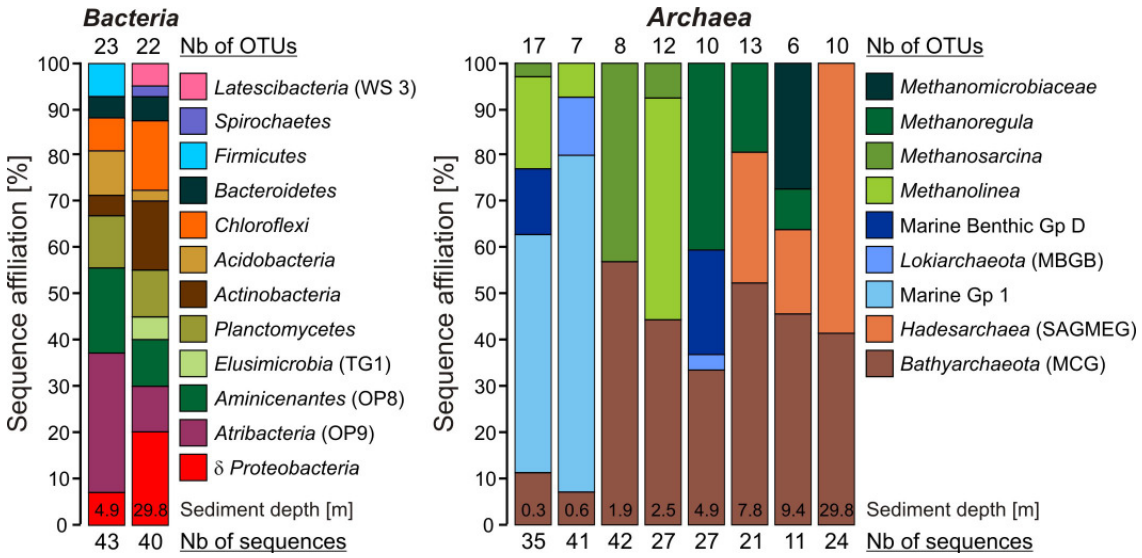

Figure 6. Histograms of identified phylotypes displayed in relative %, with OTU and sequence numbers at the top and bottom, respectively. **Left**) Several bacterial phylotypes are shared by the Holocene and LGM horizons (i.e. *Chloroflexi*, *Planctomycetes*, *Bacteroidetes*) as they are known ubiquists in aquatic environments. **Right)** Archaeal phylotypes indicate a gradual evolution with depth of the assemblages. Methanogens correspond in turn to *Methanolinea*, *Methanosarcina* and *Methanoregula*; marine-related sequences to Group 1, *Lokiarchaeota* and Benthic Group D and disappear below 5 m depth. *Hadesarchaea* sequences are only identified from 7.8 m depth, but dominate the assemblages at 29.8 m depth.