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

A. Vuillemin[1], D. Ariztegui[2], P. R. Leavitt[3,4], L. Bunting[3], and the PASADO Science Team[5,6]

[1]GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Sect. 4.5 Geomicrobiology, Telegrafenberg, 14473 Potsdam, Germany
[2]Department of Earth Sciences, University of Geneva, rue des Maraîchers 13, 1205 Geneva, Switzerland
[3]Limnology Laboratory, Department of Biology, University of Regina, Regina, Saskatchewan, Canada
[4]Institute of Environmental Change and Society, University of Regina, Regina, Saskatchewan, Canada
[5]GEOPOLAR, Institute of Geography, University of Bremen, Celsiusstr. FVG-M, 28359 Bremen, Germany
[6]GEOTOP Research Centre, Montréal, Canada

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Correspondence to: A. Vuillemin (aurele.vuillemin@gfz-potsdam.de)

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Abstract

Aquatic sediments record past climatic conditions while providing a wide range of ecological niches for microorganisms. Although marine sedimentary microbial assemblages are often defined by their surrounding geochemical conditions, the influence of environmental features upon microbial development and post-depositional survival remains largely unknown in the lacustrine realm. Due to long-term microbial activity, the composition of environmental DNA can be expected to evolve with sediment depth and over time and therefore should reflect both ancient and extant microbial populations, but this hypothesis has rarely been tested using a multiproxy approach.

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 well defined climatic intervals whose sediments harboured active microbial populations and measurable ATP were sampled for a comparative environmental study based on fossil pigments and 16S rRNA gene sequences. Bacterial and archaeal 16S rRNA gene sequences recovered from the Holocene record revealed a microbial community adapted to subsaline conditions actively producing methane during organic matter degradation. These characteristics were associated with sediments resulting from endorheic lake conditions with high evaporative stress and concomitant high algal productivity. Moreover, archaeal clone libraries established throughout the Holocene record indicate an age-related stratification of these populations, consistent with a gradual use of organic substrates after deposition. 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.

These patterns reveal that microbial assemblages identified from environmental DNA stemmed from a variety of sedimentary niches associated with climate-dependent factors (catchment inflows, water column conditions, productivity), but that initial assem-

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blages underwent structural changes and selective preservation during early diagenesis to result in the final composition entombed in the sediments. We conclude that environmental DNA obtained from lacustrine sediments provides essential genetic information to complement paleoenvironmental indicators and trace climate change and post-depositional diagenetic processes over tens of millennia.

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. 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., 2013a). In principle, climatic conditions should 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) will further influence the nature of sediments and associated microbial biota. Finally, evolution of sediment environments during early diagenesis is expected to influence the final composition of entombed microbial consortia (Nelson et al., 2007; Zhao et al., 2008).

Ancient DNA 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., 2013b). Similarly, changes in terrestrial plant cover along climate-related environmental gradients influence sedi-

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mentary microbes via variations in erosion and export of organic and inorganic 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, 2008), while differences in the age and composition (lability) of sedimentary OM can also create distinct bacterial niches (Nelson et al., 2007). Finally, the long-term persistence and activity of microbes in sediments following burial can further modify geochemical conditions via diagenesis (Inagaki et al., 2006), leading to selective preservation and modification of bacterial assemblages (Miskin et al., 1998; Boere et al., 2011a, b). Therefore, in principle the composition of microbial communities deep sedimentary environments arises from a combination of the climatic and catchment conditions at the time of deposition, the nature and provenance of the sediments, diagenetic modifications following burial, and the metabolic activity, distribution and diversity of microbial populations (Ariztegui et al., 2015; Kallmeyer et al., 2015). To date, these hypotheses have not been clearly tested using multiple proxies.

This paper tests the hypothesis that climate, in-lake processes, sedimentary environments and post-depositional alterations should all be reflected in the final microbial composition of environmental DNA by comparing the phylogenetic signatures of microbial assemblages in an unproductive glacial environment (ca. 25 000 years ago) to those characteristic of the productive Holocene (ca. 5000 years ago). Moreover, the detection of in situ microbial activity within sediments from the Holocene and Last Glacial Maximum (LGM) periods provided the opportunity to assess the preservation of environmental DNA over time and discriminate nucleic acid sequences of 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 in the sediments (Freudenthal et al., 2001). Finally, evaluation of the Holocene record, which was characterized by high cell densities and homogeneous sediments, allowed preliminary evaluation of how the structure of active microbial populations varied with depth and over time.

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In this contribution, we take advantage of previous paleoclimatic reconstructions (Gebhardt et al., 2012; Kliem et al., 2013) and blend their results with new pigment data reflecting planktonic production by algae and phototrophic bacteria. As well, we complement geomicrobiological investigations (Vuillemin et al., 2013a, 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 DNA over the last 25 000 years in response to both past environmental conditions and geochemical evolution of the sediments. Finally, we established archaeal clone libraries at regular intervals throughout the microbially-active sediments of the Holocene period to provide structural criteria of differentiation between extant and ancient populations.

2 Material and methods

2.1 Study site

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). 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 on the area (Mayr et al., 2007), the lake is polymictic and the water column currently unstratified throughout the year. The basin has a maximum depth of 100 m (Fig. 1b), while mean annual temperatures range from 4 to 10 °C. Dissolved oxygen normally shifts from oxic to dysoxic conditions at the water-sediment interface (Zolitschka et al., 2006) and oxygen penetration within surface sediment is restricted (Vuillemin et al., 2013b). 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

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et al., 2007, 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., 2013b). We supplement these insights with a new 16S rRNA gene analysis of the total sedimentary DNA 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. Fossil pigment and sedimentary DNA extractions from the two climatic intervals also allows for a unique comparison between climatic and genetic records in the frame of well-established paleo-environmental reconstructions.

2.2 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 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. 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, cell count and denaturing gradient gel electrophoresis (DGGE) procedures have been published elsewhere (Vuillemin et al., 2013a, b). Complete datasets are available at http://doi.pangaea.de under accession numbers 10.1594/PANGAEA.811521 to 811524.

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2.3 Pigment analysis

Historical changes in algal abundance and gross community composition were quantified by analyzing fossil algal pigment concentrations in 2500 samples spanning ca. 50 000 ^{14}C years (Kliem et al., 2013). Sediments for pigment analysis were frozen immediately after core collection (Ohlendorf et al., 2011) and stored in the dark until isolation and quantification of carotenoid, chlorophyll (Chl) and derivative pigments using a Hewlett Packard model 1100 high performance liquid chromatographic (HPLC) system. All extraction, isolation and quantification followed the standard procedures detailed elsewhere (Leavitt and Hodgson, 2002). In all cases, pigments were extracted from freeze-dried sediments into degassed mixtures of organic solvents (i.e. acetone, methanol) and water under an inert N_2 atmosphere, filtered through 0.45 µm pore membrane filters, and injected into HPLC system fitted with a reversed-phase C18 column, photo-diode array detector, and fluorescence detector. HPLC systems were calibrated and peaks identified using authentic pigment standards (US Environmental Protection Agency and DHI Lab Products, Denmark), unialgal cultures, and reference stocks of sedimentary pigments. Biomarker concentrations (nmol pigment × gr total organic carbon^{-1}) were calculated for pigments usually characteristic of green sulphur bacteria (isorenieratene), total cyanobacteria (echinenone), Nostocales and potentially N_2-fixing cyanobacteria (canthaxanthin, aphanizophyll), purple bacteria (okenone) and mainly diatoms (diatoxanthin). Preservation index was calculated from the ratio of chlorophyll *a* to pheophytin *a*, pigments normally 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 to total prokaryotic pigments.

2.4 Clone library and phylogenetic analysis

Detailed procedures for DNA extraction, PCR amplification and DGGE are described elsewhere (Vuillemin et al., 2013b, 2014a). The same DNA extracts were used to establish clone libraries. PCR products were purified using the High Pure PCR Product

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Purification Kit (Roche Diagnostics SA), measured with a Nanodrop ND-1000 Spectrophotometer (Witec AG), and diluted to 10 ng μL^{-1}. 2 µL PCR product 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 h on a LB medium containing 1 g L^{-1} NaCl, 1 g L^{-1} Bactotryptone, 0.5 g L^{-1} Bactoyeast, 1.5 g L^{-1} Bactoagar and 2 mL L^{-1} ampicillin. To constitute libraries, 83 bacterial clones and 228 archaeal clones were respectively selected from samples at 4.97 and 29.77 m sediment depth and samples at 0.25, 0.55, 1.90, 2.51, 4.97, 7.81, 9.37 and 29.77 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). Distance matrices were exported to the Mothur® v.1.32.1 software (Schloss et al., 2009) and number of OTUs, rarefaction curves, Chao, Shannon and Dominance-D indices were calculated at 97 % sequence identity cut-off value (Supplement). All our sequences have been de-

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posited in the GenBank database under accession numbers JX272064 to JX272122, JX472282 to JX472399 and KT381303 to KT381433.

3 Results

3.1 Holocene and LGM environmental features

3.1.1 Sedimentary features

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 freshwater water column lake level highstand, 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). An important sedimentary transition was identified between lithological

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units 5 and 4 (Fig. 2c) that corresponded with increased percentage of mass movement deposits, some events exceeding 1 m in thickness and containing fine gravels (Kliem et al., 2013). These horizons were interpreted as paleo-shorelines following a desiccation stage of the maar dated around 53 ka BP (Gebhardt et al., 2012).

3.1.2 Organic matter

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.

3.1.3 Pigment concentrations

Analyses of bacterial and algal pigment concentrations provided clear indication for algal abundance related to biomass (i.e. assessed productivity) being lower and higher during the LGM and Holocene periods, respectively (Fig.3b). Specifically, elevated fossil concentrations of isorenieratene (100 nmol × gr TOC^{-1}) suggest 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 nmol × gr TOC^{-1} in Holocene sediments and close to detection limit in the glacial record. Taken together, concentrations of echinenone, canthaxanthin and aphanizophyll (Fig. 3b) revealed that cyanobacteria and N_2-fixing cyanobacteria contributed substantially to the labile OM during the YD and Holocene times, but are present only sporadiccally within the glacial interval. Finally,

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diatoxanthin showed that diatoms (Fig. 3b) were abundant during the late YD and early Holocene period (Recasens et al., 2015).

The pigment preservation index (chlorophyll *a*/pheophytin *a*; Fig. 3c) suggested that the main patterns of past production were robust to changes in lake morphometry and diagenesis (Leavitt, 1993). However, peaks of the index also correlated coarse grain sizes associated with increased sedimentation rates (Fig. 3, top and bottom), suggesting that there may have been intervals of poor preservation related to low OM content. Such a phenomenon may be common during the LGM and YD transition, a period of important lake level fluctuations (Kliem et al., 2013; Zolitschka et al., 2013). Analysis of the ratio of eukaryotic to prokaryotic pigments (Fig. 3c) revealed that the relative importance of eukaryotic algae increased during climatic transitions (late LGM, YD and early Holocene; Fig. 3, bottom), and that this pattern exhibited important similarities to published diatom counts and their link to warming events and tephra inputs (Recasens et al., 2015). Otherwise, baseline values oscillated around 2.0, indicating that prokaryotic biomass is considerably less abundant than the eukaryotic one during the glacial period. However, the water depth difference between the Holocene and LGM times (i.e. 37 m) likely promoted preservation of bacterial OM during lake lowstand (Figs. 2b and 3b).

3.1.4 Pore water chemistry

Chloride concentrations (Supplement) indicated a shift from freshwater (200 ppm) to subsaline (600 ppm) conditions during the YD. Nitrite + nitrate concentrations (Supplement) were always very low throughout the sedimentary sequence, with values in between 0.2 and 0.6 ppm. Phosphate concentrations (Fig. 3d) were ca. 10 ppm in Holocene sediments and most often close to detection limit (0.4 ppm) within the rest of the sedimentary sequence. Dissolved iron (Fe^{2+}) was often below detection limit (3.7 ppm), but was quantifiable from 55 to 15 m sediment depth, reaching concentrations between 5 and 15 ppm. The sulphate concentration profile (Fig. 3d) displays frequent variations with baseline values oscillating between 5 and 70 ppm. Extraordinary

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peaks were located at 49, 38 and 25 m sediment depth, reaching concentrations of ca. 1590, 1270 and 980 ppm, respectively, in concomitance with tephra layers (Fig. 3, top).

3.2 Microbial characteristics

3.2.1 Microbial activity, density and diversity

Rapid ATP detections (Fig. 3e) were used as an index 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. Maximal values (> 100) were recorded in the Holocene record in between 8 and 4 m burial depth, indicating significant 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 lower level of microbial activity in discrete horizons. Analysis of DAPI cell counts (Fig. 3e) showed 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 and one minor peak at 34 m depth.

Analyses of DGGE gel features were used to assess structural changes in microbial communities. Number of DGGE bands (Fig. 3f) for *Bacteria* were 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).

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3.2.2 Sedimentary DNA from LGM and Holocene horizons

Together ATP, DAPI and DGGE profiles (Fig. 3e and f) provided evidence for viable microbial assemblages in the Holocene and LGM sediments. These two horizons seemingly reflected specific habitats which promoted microbial assemblages and were selected to establish comparative clone libraries (Fig. 2a and b). That said, we acknowledge that DAPI staining does not distinguish between active, inert or dead cells and that the present DGGE bands represent short sequences (150 base pairs) that may include extracellular DNA preserved under anoxic conditions (Corinaldesi et al., 2011). Nevertheless, the detection of small amounts of ATP (Fig. 3e) suggested active metabolism in LGM sediments. Thus, the obtained 16S rRNA gene sequences (1400 and 900 bp) were considered to reflect the major components of former and currently-viable microorganism assemblages (Figs. 4 and 5). In addition, we established six additional archaeal clone libraries established throughout the Holocene record to provide criteria of discrimination between ancient and recent 16S rRNA sequences (see below; Figs. 5 and 6).

3.2.3 Bacterial 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), dominated the sedimentary microbial assemblage (Fig. 4). Additionally, representative *Bacteria* identified from Holocene deposits were mainly affiliated to *Acidobacteria* (Barns et al., 1999) and *Clostridia* that were partly related to syntrophic species (Liu et al., 2011). Similarly, some *δ Proteobacteria* sequences were affiliated with syntrophic species (Jackson et al., 1999; Liu et al., 1999).

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.,

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1994). Other sequences specific to the LGM horizon clustered with *Spirochaetes*, *Nitrospirales* 4-29 and *Latescibacteria*, former candidate division WS3 (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).

3.2.4 Archaeal clone libraries

Diffusion and cell migration can occur in soft methane-saturated clays and may rework layered microbial communities. Nevertheless, archaeal sequences obtained from the Holocene record provided evidence for gradual evolution of these assemblages with depth (Figs. 5 and 6). The uppermost meter of sediment mainly revealed the presence of phyla Marine Group1 and Marine Benthic Group B. The underlying two meters are dominated by sequences of *Methanomicrobiales* and *Bathyarchaeota,* formerly Miscellaneous Crenarchaeotal Group (Evans et al., 2015), while the same assemblage plus the phylum Marine Benthic Group D constituted the next meter. Below 5 m depth, *Methanomicrobiales* receded and gave way to the phylum South African Goldmine Group (SAGMEG). In detail, methanogen sequences correspond with depth to *Methanolinea*, *Methanosarcina*, *Methanoregula* and uncultured *Methanomicrobiaceace* (Fig. 6). Finally, *Bathyarchaeota* sequences were present throughout Holocene sediments and, while uniformly present in every sample below 2 m depth, formed clusters associated with the main sampling intervals (Fig. 5). Direct comparison of the LGM and Holocene horizons (Figs. 5 and 6) revealed that the Holocene archaeal assemblage mainly consisted of *Methanoregula* along with some sequences related to the Marine Benthic Group D, whereas the LGM assemblage was dominated by SAGMEG sequences.

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4 Discussion

4.1 Paleoclimatic and geochemical conditions revealed by sediments

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 (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). Pore water geochemistry reflected such historical changes and was interpreted in terms of paleoconditions and sedimentary processes. Lithological horizons concealing rhyolitic tephra material (Wastegård et al., 2013) and mafic sands contributed to punctual increases in iron and sulphate concentrations (Fig. 3d). Water column salinity rapidly shifted from freshwater to subsaline conditions during the YD as the position of the Westerlies moved to the site (Killian and Lamy, 2012; Pollock and Bush, 2013), resulting in elevated wind evaporation and lake level decline. At this time, the basin also became endorheic (Ohlendorf et al., 2013), resulting in nitrogen-limiting conditions (Zhu et al., 2013). Phosphate concentrations (Fig. 3d) within Holocene sediments, although potentially reflecting past trophic level of the basin, were instead considered representative of post-depositional OM degradation by microorganisms (Vuillemin et al., 2013a, 2014a).

In general, the LGM horizon (Fig. 2b) coincided with a period of active hydrology within the lake basin, with both overflow and active inflows into the lake (Haberzettl et al., 2007), but reduced vegetation in the catchment (Haberzettl et al., 2009). Throughout Patagonia, glaciers started waning from maximum extent to initial deglaciation phase (ca. 25–22 ka), promoting periglacial and wind-related erosion (Hein et al., 2010). Overall, the sedimentary record of Laguna Potrok Aike during this period appeared reliant on mass movements and tephra layers (Fig. 2c) triggering small-scale shifts in productivity (Hahn et al., 2013). In contrast, the Holocene horizon (Fig. 2a) corresponded to a period of lake level rise after an extreme lowstand (Anselmetti et al.,

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2009) due to strong Westerlies and positive temperature excursion in South Patagonia (Waldmann et al., 2010; Kilian and Lamy, 2012). Periods of lake level rise correspond with important nutrient fluxes into the closed-basin, elevated primary productivity (Recasens et al., 2015), and high microbial colonization of the sediment under pelagic conditions (Vuillemin et al., 2014a).

4.2 Preservation and interpretation of fossil DNA

Analysis of ATP detection and sedimentary cell densities showed that microbial populations were abundant and metabolically active in the sediment of the Holocene period (Fig. 3e). Archaeal phylotypes (Figs. 5 and 6) indicated gradual evolution of the assemblages within the uppermost 10 m of sediment. For example, the predominant methanogen varied with depth from *Methanolinea* to *Methanosarcina* and *Methanoregula* (Vuillemin et al., 2014b). Marine-related sequences also shifted from Group 1 to Benthic Group B and then Benthic Group D before disappearing below 5 m depth. SAGMEG sequences were only first identified at 7.8 m depth, but then dominated the assemblage at 29.8 m depth. *Bathyarchaeota*, although present in every sample, formed unique clusters according to sampling depths (Figs. 5 and 6). Similar changes in archaeal assemblages have also been identified in marine environments from 1 to 10 m below the seafloor (Vigneron et al., 2014). In this latter case, marine groups are expected to degrade complex organic matter, such as cellulose, proteins and aromatic compounds (Lloyd et al., 2013; Meng et al., 2013), and *Bathyarchaeota* methyl compounds (Evans et al., 2015). Thus, the present series of *Archaea* may reflect a dynamic transition in the subsurface biosphere during early diagenesis of OM.

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). However, within this trend, the mid-Holocene and LGM intervals (Fig. 2) appear to be preferentially colonized sedimentary horizons (Fig. 3e and f) for which genetic analyses showed distinct assemblages of microbial consortia (Figs. 4, 5 and 6) corresponding with their different geochemical surroundings (Fig. 3d).

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First, at 5 m depth (ca. 5 ka BP), 16S rRNA gene sequences revealed a predominantly active microbial assemblage capable of on-site methane production during OM degradation (Wüst et al., 2009; Vuillemin et al., 2014b) during an interval of subsaline and productive lake conditions (Fig. 2). In contrast, at 30 m depth (ca. 25 ka BP), retrieved sequences exhibited microbial populations characteristic of sulphate reduction and lithotrophy (Figs. 4 and 5) when an unproductive freshwater lake episode deposited clayey sediments overlain by fine basaltic material (Fig. 2).

In the organic-rich Holocene clays buried at 5 m depth, 16S rRNA gene sequences provided evidence for the presence of *Methanoregula* (Bräuer et al., 2011), a taxon which was identified in association with *Syntrophus* (Jackson et al., 1999) and *Syntrophomonadaceae* (Liu et al., 2011). Genes from alkalotolerant species, such as *Clostridia* (Nakagawa et al., 2006) and Marine Benthic *Archaea* (Jiang et al., 2008) were also detected (Figs. 4, 5 and 6). These organisms, 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). *Atribacteria* and *Aminicenantes* sequences (Rinke et al., 2013) dominated the Holocene horizon (Figs. 4 and 6). These microbes were initially described from hot springs (Hugenholtz et al., 1998), but are often abundant in anaerobic marine sediments (Inagaki et al., 2003). In addition, *Atribacteria* have been described recently as energy-conservative heterotrophic anaerobes which act either as primary fermenters of sugars or secondary fermenters of organic acids (Nobu et al., 2015) capable of syntrophic propionate catabolism (Sieber et al., 2012). Additionally, 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).

Taken together, these assemblages suggest that sedimentary microorganisms first degraded the labile OM from algae before generating fermentative H_2 and CO_2, and that in turn, these gases served as substrates for methane production by *Methanomicrobiales*. Such substrate evolution during prolonged OM diagenesis could promote

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the recycling of end products and syntrophic hydrogen consumption, as presently observed with autotrophic methanogenesis and homoacetogenesis (Inagaki et al., 2003; Wüst et al., 2009). Such a pattern also suggests that the final Holocene microbial assemblage arose from metabolic complementarities involved in the degradation and geochemical cycling of OM. Thus, microbial populations displayed an age-related stratification within the sedimentary environment made possible by a steady pelagic and productive regime (Fig. 6).

Microbial communities recovered from ca. 2 ka old LGM sediments also showed sustained metabolic activity, albeit at lower population densities (Fig. 3e). Genetic sequences from this LGM assemblage (Figs. 4, 5 and 6) recorded the important presence of sulphate-reducing *δ Proteobacteria* and SAGMEG *Archaea* (Takai et al., 2001). Substantial H_2S production during sulphate reduction was thought to promote the alteration of mafic minerals and provide molecular hydrogen, iron and sulphur to lithotrophic species (Johnson, 1998; Blanco et al., 2014) and eventually the formation of authigenic sulphides such as framboids (Vuillemin et al., 2013a). In general, conditions at such sedimentary interfaces (Fig. 2) would greatly limit any methane production (Schubert et al., 2011) and select for a microbial assemblage capable of sulphate and iron reduction instead. However, syntroph sequences were also identified among *δ Proteobacteria* and *Chloroflexi*, which is consistent with the degradation of secondary metabolites such as propionate (Liu et al., 1999; De Bok et al., 2001; Yamada et al., 2007). Otherwise, organotrophs capable of refractory OM degradation were represented by *Acidobacteria* (Liesack et al., 1994), *Spirochaeta* (Hoover et al., 2003), *Actinobacteria* (Pachiadaki et al., 2011) and *Bacteroidetes* (Fig. 6). This pattern of sequences was interpreted as arising from the intercalation of organic-poor clays with volcanic material that could act as sources of iron and sulphate (Fig. 3). 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), whereas *Nitrospirales* 4-29 have been shown to be abundant in temporary streams under dry conditions (Febria et al., 2015).

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Some sedimentary nucleic acids could not be unambiguously associated with a specific sedimentary interval. 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. Indeed, microbes 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 were 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 sedimentary settings, OM anaerobic metabolisms appeared 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), consistently with the present microbial assemblages although the methanogenesis zone overlies the sulphate reduction zone.

Several lines of evidence suggested 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 displayed only one single OTU in common (Fig. 4). Secondly, sedimentary ATP activity recorded less than two hours after core recovery showed the same pattern of ATP concentration than that measured substantially later, and was also coherent with more extensive laboratory analyses (Supplement). 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 (Supplement). Finally, 16S rRNA gene sequences of *Archaea* (900 bp) and *Bacteria* (1400 bp) analyzed in our phylogenies were considered long enough to derive from intact bacterial cells rather than arising from extracellular DNA. However, because DNA could have also been extracted from dead and dormant intact cells (Corinaldesi et al., 2011), we urge caution to avoid interpreting sediment populations in terms of bacterial growth and active metabolisms in the different intervals. In addition, the presence

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of extracellular DNA amidst DGGE band sequences (150 bp) may have increased the relative bacterial features at 30 m depth (Fig. 3f), with the restriction that the turnover rate of the related compounds depends on the maintenance of an activity by microbes in ancient sediments (Corinaldesi et al., 2007 and 2008).

4.3 Preservation and interpretation of fossil pigments

Analyses of bacterial and algal pigment concentrations indicated high primary productivity during the Holocene while oligotrophic conditions characterized the last glacial period (Fig. 3b). During the LGM, pigment concentrations were variable and exhibited short intervals of elevated productivity related to mass movement and tephra layer inflows. Profiles of preservation index (Fig. 3c) and organic macro remains (Hahn et al., 2013) suggest the presence of primary and reworked OM in pelagic and gravity-related sediments, respectively (Fig. 3b and c). Still, bacterial sources constituted an important fraction of the organic sedimentary record (Fig. 3a and b). During the late LGM and early YD, strengthened winds and nitrogen limitation appeared to be unfavourable for primary producers (Zhu et al., 2013). However, concentrations of pigments indicative of cyanobacteria clearly increased at this time (Fig. 3b), revealing a potential importance of nitrogen fixation processes in the basin (Mayr et al., 2009). During the late YD, deglacial warming and nutrients inflows associated with vegetation recovery along with a lake lowstand improved conditions for planktonic production by eukaryotes, as indicated by elevated concentrations of diatom pigments (diatoxanthin) and eukaryote : prokaryote pigment ratios (Fig. 3b and c). During the early Holocene, planktonic primary productivity appeared restrained (Fig. 3b), possibly due to increased intensity of the Westerlies and lasting nitrogen-limited conditions (Zhu et al., 2013, 2014). Changes in basin morphometry, light penetration, bottom water stratification can potentially bias the correlation between fossil and algal abundance through time (Leavitt, 1993; Leavitt and Hodgson, 2002). Primarily because of the maar morphology, these factors vary as a function of lake level and gravity events. Fortunately, interpretation of

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the pigment preservation index suggests that pelagic production is accurately recorded during intervals of elevated sedimentation (Fig. 3c).

Comparison of fossil pigment and sedimentary DNA assemblages suggested that the initial nucleic acid composition of sediments could be modified by microbial ontogeny following deposition. For example, although high concentrations of isorenieratene from brown varieties of green sulfur bacteria (Glaeser and Overmann, 2001) were recorded in the sediments throughout the Holocene, genetic markers of the relevant carotenoid-producing phototrophic taxa were rare in the mid-Holocene intervals subject to DNA analysis. Early research suggested that isorenieratene was unique to anaerobic taxa which used H_2S as an electron donor for photosynthesis, and so could be used as an index of the penetration of light to anoxic waters or sediments (e.g., Leavitt et al., 1989). However, more recent work suggests that isorenieratene-like compounds can be produced by different types of bacteria (Sinninghe Damsté and Hopmans, 2008; Krügel et al., 1999; Krubasik and Sandmann, 2000) and that genes involved in isorenieratene biosynthesis may be found in cyanobacteria as well (Phadwal, 2005). Therefore environmental 16S rRNA sequences may provide an alternative means to evaluate the composition of past bacterial phototrophic communities (Overmann et al., 1999; Coolen and Overmann; 2007). In this paper, *Planctomycetes*, *Actinobacteria* and *Bacteroidetes* were 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). Moreover, alteration of these compounds can form isorenieratane in sedimentary environments (Brocks and Pearson, 2005). Of interest is the observation that these heterotrophic taxa are common in ancient algal mats assemblages, 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). Present results of taxonomic blasts (Fig. 4) confirmed that these heterotrophic species are characteristic of anoxic aquatic and sedimentary lacustrine habitats (De Wever et al., 2005; Schwarz et al., 2007; Song et al., 2012). Such colonization of algal material after deposition would result in selective recycling of bacteria (Antibus et al., 2012), and suggest that

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sedimentary genetic assemblages may be altered while fossil pigment composition is better conserved.

4.4 A model for ancient and extant microbial assemblages

Together, our results suggest that prevailing climatic conditions exerted the initial control on microbial substrates at the time of deposition (Fig. 2), which subsequently defined the subsurface biosphere assemblages and set the pathway for further post-depositional alterations. In this model, 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 (Figs. 2 and 3) and dominant subsurface assemblage.

During the Holocene interval, elevated rates of OM deposition under pelagic regime led to increased pigment concentrations in the sediment (Figs. 2a and 3b). Sequences potentially derived from ancient assemblages (i.e. *Planctomycetes*, *Actinobacteria* and *Bacteroidetes*) may have also emerged from the early degradation of algae and microbial biofilms (see above). 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. Phylogenetic sequences representing the subsurface biosphere were characteristic of those exhibiting solely anaerobic heterotrophic metabolism, with *Atribacter* and *Methanomicrobiales* as the dominant taxa (Figs. 4, 5 and 6). They reflected the sediment surrounding geochemical conditions and were indicative of advanced OM degradation during early diagenesis.

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. Similarly, sequences issued from ancient assemblages seemed to refer to complex autotroph-heterotroph interactions (Cole et al., 2014) and likely included *Nitrospirales* 4-29 (Febria et al., 2015) and *Latescibacteria* (Youssef et al., 2015). Surrounding geochemical conditions asso-

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ciated with the formation of OM-poor but iron- and sulphur-rich sediments selected for a subsurface biosphere capable of sulphate reduction and lithotrophy, mainly including sequences affiliated to *δ Proteobacteria* and the SAGMEG group.

5 Conclusions

Climatic and lacustrine conditions at the time of sediment deposition appear to be the main factors defining sediment geochemistry and microbial substrates. Preferential preservation of microbial sources occurred during synsedimentary processes and after burial, leading to changes in the DNA assemblages with conserved pigment compositions. Sedimentary niches at the time of deposition exerted initial constraints on the development of the subsurface biosphere. Changing geochemical conditions associated with sustained metabolic activity performed a selection of viable microorganisms over time and defined the final microbial assemblages. Identified taxa were *in fine* characteristic of conditions associated with past environmental factors, with *Atribacteria* and methanogens, sulphate reducers and SAGMEG 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 fossil 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 (Hugenholtz et al., 1998; Nakagawa et al., 2006). Similarly, we also recognize that our analytical platform represents only an 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 (~ 500 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 environmental DNA.

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This study provides new evidence for mechanism underlying the preservation of sedimentary DNA sequences. We show clearly that fossil assemblages of nucleic acids differ among major historical climate zones and that some fossil elements even sustain activity for 25 000 years after burial. Moreover, the present results demonstrate that sedimentary DNA can help reconstructing 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.

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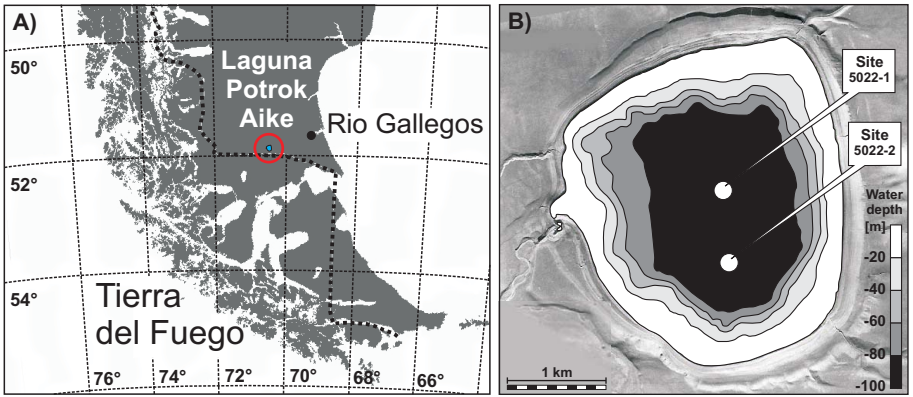

Figure 1. Map of Southern Argentina displaying the location **(a)** and bathymetric map **(b)** of Laguna Potrok Aike showing the two drilling sites (Zolitschka et al., 2006). Pore water and geomicrobiological samples were retrieved from cores at site 5022-1, whereas sediments for pigment analysis were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

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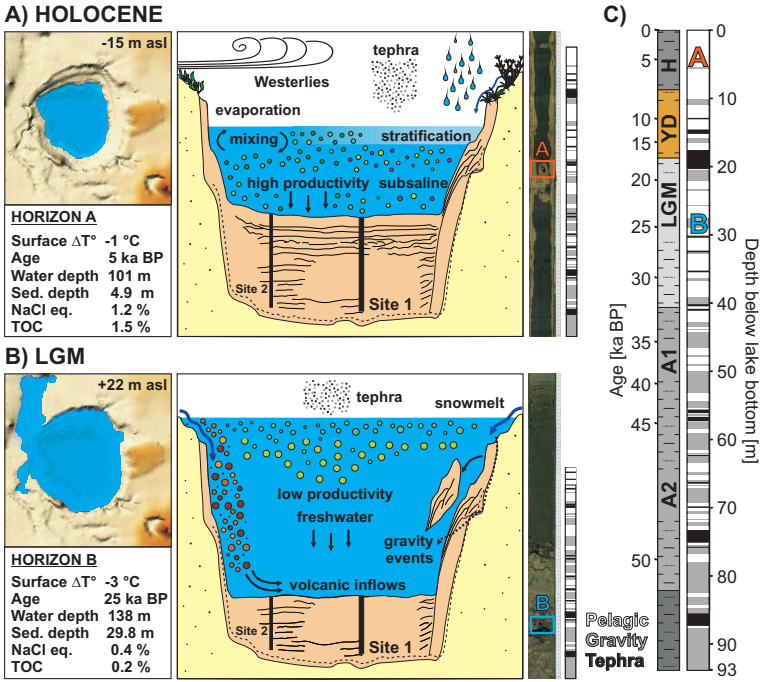

Figure 2. Sketches depicting paleoenvironmental conditions at Laguna Potrok Aike. From left to right: Climatic and lacustrine parameters; sagittal view of the basin; core picture with sample location of the 16S rRNA library; and stratigraphic sequence. **(a)** During Holocene times with active Westerly winds, lake lowstand, subsaline conditions and high primary productivity in the basin and catchment. **(b)** During LGM times with lake highstand and active overflow, freshwater conditions, low primary productivity in the basin and inflows restricted to runoff from the volcanic catchment. **(c)** Lithology log in age scale displaying the 5 units defined in Kliem et al. (2013), and stratigraphic sequence in meter scale displaying 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).

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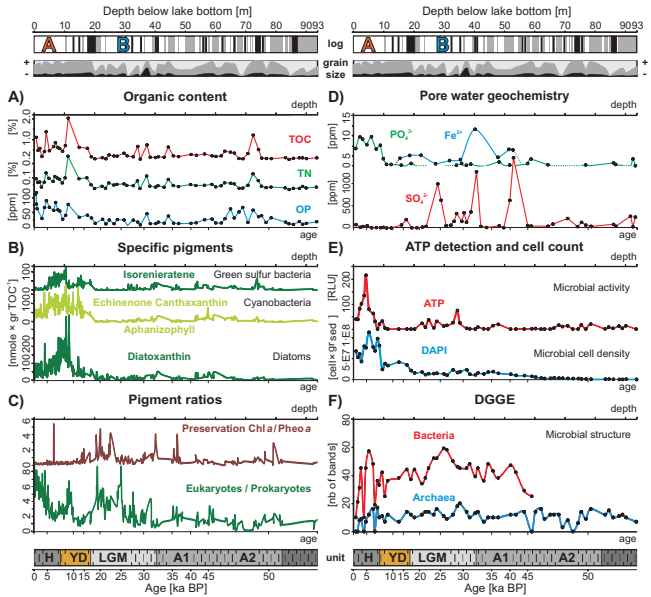

Figure 3. Paleoclimatic and geomicrobiological multiproxy. Top: Stratigraphic sequence of Laguna Potrok Aike, followed by grain size with clay (black), silt (dark grey) and sand (light grey). **(a)** Total organic carbon (TOC), total nitrogen (TN) and organic phosphorus (OP) from bulk sediment. **(b)** 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.

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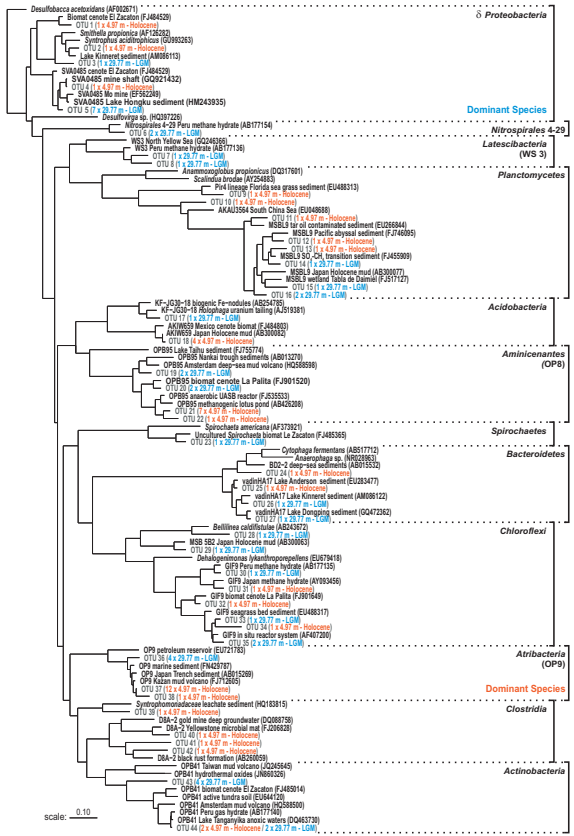

Figure 4. Maximum likelihood phylogenetic tree of bacterial 16S rRNA gene sequences (1400 bp) recovered at 4.97 and 29.77 m depth from Holocene (orange types) and LGM (blue types) sediments. *Atribacteria* and *Aminicenantes* are the main taxa encountered in the Holocene organic-rich pelagic sediments, 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.

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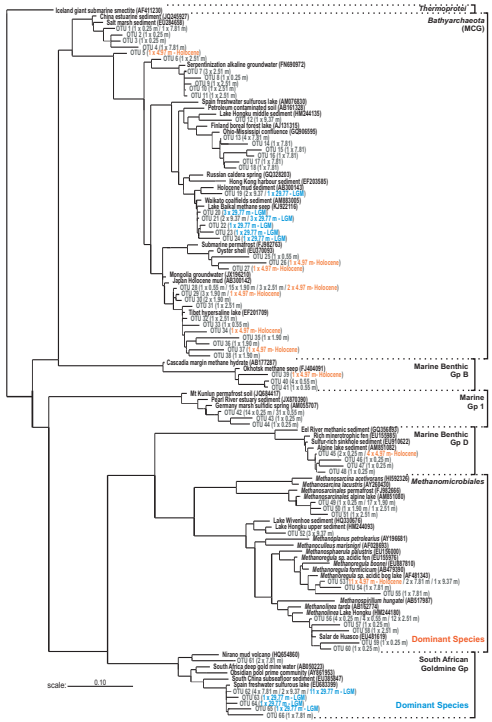

Figure 5. Maximum likelihood phylogenetic tree of archaeal 16S rRNA gene sequences (900 bp) recovered at 0.25, 0.55, 1.90, 2.51, 4.97, 7.81, 9.37 and 29.77 m sediment depth. Clone series established throughout the Holocene record (dark grey types) indicate a depth-related evolution of the assemblages,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

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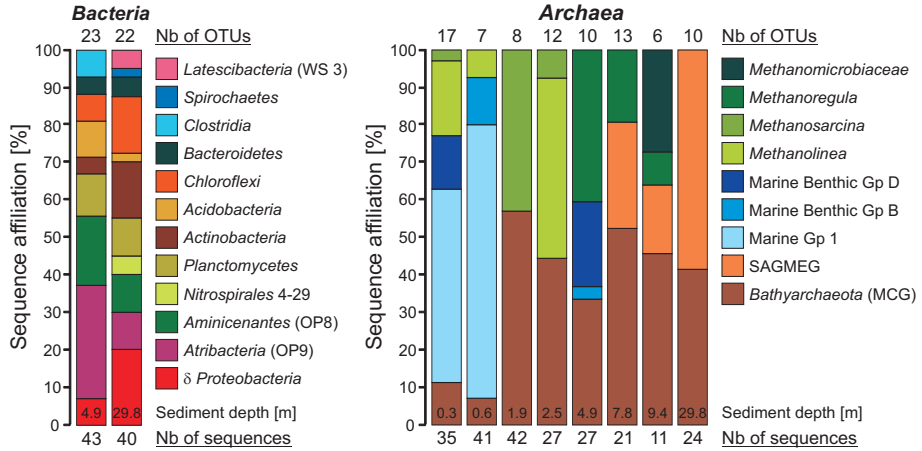

Figure 6. Histograms of identified phylotypes displayed in relative %, with OTU and sequence numbers at the top and bottom, respectively. Left: Several bacterial phylotypes are shared by the Holocene and LGM horizons (i.e. *Chloroflexi*, *Planctomycetes*, *Bacteroidetes*) as they are known ubiquists 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, Benthic Group B and Benthic Group D and disappear below 5 m depth; SAGMEG sequences are only identified from 7.8 m depth, but dominate the assemblages at 29.8 m depth.

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