

1 **Recording of climate and sediment diagenesis through**  
2 **fossil DNA and pigments at Laguna Potrok Aike, Argentina**

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16

17 **Abstract**

18 Aquatic sediments record past climatic conditions while providing a wide range of ecological  
19 niches for microorganisms. In theory, benthic microbial community composition should  
20 depend on environmental features and geochemical conditions of surrounding sediments, as  
21 well as ontogeny of the subsurface environment as sediment degraded. In principle, residual  
22 environmental DNA in sediments should be composed of ancient and extant microbial  
23 elements at different degrees of preservation, although to date few studies have quantified the  
24 relative influence of each factor in regulating final composition of fossil DNA.

25 Here geomicrobiological and phylogenetic analyses of a Patagonian maar lake were used to  
26 indicate that the different sedimentary microbial assemblages derive from specific lacustrine

1 regimes during defined climatic periods. Two climatic intervals whose sediments harboured  
2 active microbial populations were sampled for a comparative environmental study based on  
3 fossil pigments and 16S rRNA gene sequences. The genetic assemblage recovered from the  
4 Holocene record revealed a microbial community displaying metabolic complementarities in  
5 the geochemical cycling of OM actively producing methane. The series of *Archaea* identified  
6 throughout the Holocene record indicated an age-related stratification of these populations  
7 brought on by environmental selection during early diagenesis. These characteristics were  
8 associated with sediments resulting from endorheic lake conditions and stable pelagic  
9 regime, high evaporative stress and concomitant high algal productivity. In contrast,  
10 sulphate-reducing bacteria and lithotrophic *Archaea* were predominant in sediments dated  
11 from the Last Glacial Maximum, in which pelagic clays alternated with gravity fine volcanic  
12 material characteristic of a lake level highstand and freshwater conditions, but reduced water  
13 column productivity.

14 Comparison of sedimentary DNA composition with that of fossil pigments suggested that  
15 post-depositional diagenesis resulted in a rapid loss of the initial nucleic acid composition  
16 and overprint of phototrophic communities by heterotrophic assemblages with preserved  
17 pigment compositions. Long sequences (1400-900 bp) appeared to derive from intact  
18 bacterial cells, whereas short fragments (290-150 bp) reflected extracellular DNA  
19 accumulation in ancient sediments. We conclude that environmental DNA obtained from  
20 lacustrine sediments provides essential genetic information to complement  
21 paleoenvironmental indicators and trace post-depositional diagenetic processes over tens of  
22 millennia. However, it remains difficult to estimate the time lag between original deposition  
23 of lacustrine sediments and establishment of the final environmental DNA composition.

## 24 **1 Introduction**

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

1 Finally, evolution of sediment environments during early diagenesis is expected to select for  
2 the final composition of entombed microbial consortia (Nelson et al., 2007; Zhao et al.,  
3 2008).

4 Ancient DNA has already been successfully employed to study the succession of species as a  
5 result of environmental changes in lacustrine settings (Coolen and Gibson, 2009). For  
6 example, wet and warm climates result in high bacterial abundance and diversity in the  
7 sediment, whereas cold and dry climates favour lower abundance and diversity of microbes  
8 (Dong et al., 2010; Vuillemin et al., 2013b). Similarly, changes in terrestrial plant cover  
9 along climate-related environmental gradients influence sedimentary microbes via variations  
10 in erosion and export of organic and inorganic matter (OM) to lakes (Clark and Hirsch,  
11 2008). Shifts in lake salinity, as well as modifications of the water column regime, further  
12 induce large changes in bacterial populations (Coolen et al., 2006; Coolen et al., 2008), while  
13 differences in the age and composition (lability) of sedimentary OM can also create distinct  
14 bacterial niches (Nelson et al., 2007). Despite the fact that the composition of sedimentary  
15 microorganisms shows a strong correspondence to geological and geochemical conditions at  
16 the time of deposition in marine environments (Inagaki et al., 2003), little is known about the  
17 relative influence of extant environmental conditions and post-depositional sedimentary  
18 processes as controls of microbial assemblage composition in deep lacustrine sedimentary  
19 settings (Vuillemin et al., 2013a). Moreover, persistent activity of microbes in sediments  
20 following burial can further modify geochemical conditions via diagenesis (Inagaki et al.,  
21 2006) and alter extant bacterial populations to lead to selective preservation of prior  
22 sedimentary assemblages (Miskin et al., 1998; Boere et al., 2011a, 2011b). Therefore, the  
23 composition of microbial communities in deep sedimentary environments arises from a  
24 combination of climatic conditions at the time of deposition, sediment provenance,  
25 diagenetic modifications and metabolic activity and distribution of microbial populations  
26 (Ariztegui et al., 2015; Kallmeyer et al., 2015).

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

1 from the Holocene and Last Glacial Maximum (LGM) provides a way to assess  
2 environmental DNA preservation over time and discriminate nucleic acid sequences of the  
3 initial microbial assemblages at the time of deposition (Anderson-Carpenter et al., 2011;  
4 Jørgensen et al., 2012) from those arising from diagenetic processes following entombment  
5 (Freudenthal et al., 2001).

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

15

## 16 **2 Material and methods**

### 17 **2.1 Study site**

18 Laguna Potrok Aike is a maar lake located in southern Patagonia, Argentina (Fig. 1A) within  
19 the Pali Aike volcanic field (Coronato et al., 2013). Due to the persistent influence of  
20 Westerly winds on the area (Mayr et al., 2007), the lake is polymictic and the water column  
21 currently unstratified throughout the year. The basin has a maximum depth of 100 m (Fig.  
22 1B), while mean annual temperatures range from 4 to 10 °C. Dissolved oxygen normally  
23 shifts from oxic to dysoxic conditions at the water-sediment interface (Zolitschka et al.,  
24 2006) and oxygen penetration within surface sediment is restricted (Vuillemin et al., 2013b).  
25 This hydrologically-closed basin contains a sedimentary record of the climatic regime in  
26 southernmost South America in which changes in the Westerly winds and ice cap distribution  
27 in the Andes regulate variations in regional environmental conditions and in-lake conditions  
28 (Fig. 2) such as mixing and hydrological balance (Mayr et al., 2007 and 2013; Ohlendorf et  
29 al., 2013). During wetter periods, elevated nutrient influx enhances lake primary productivity

1 in the lake (Recasens et al., 2012), as well as colonization of the sediments by microbes  
2 (Vuillemin et al., 2013b).

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

## 13 **2.2 Sedimentary features of selected horizons**

14 Lake basin conditions at the time of the Holocene horizon A (Fig. 2A) were defined as  
15 subsaline (1.2 % NaCl eq.) during a water-column lowstand (Ohlendorf et al., 2013). Annual  
16 mean surface atmospheric temperatures were slightly colder than those of the present day (-  
17 1°C; Pollock and Bush, 2013). Sedimentary features of horizon A consist of fine  
18 intercalations of laminated silts with soft methane-saturated black clays, reflecting a  
19 continuous pelagic to hemipelagic regime (Fig. 2A). In contrast, paleoconditions of the LGM  
20 horizon B (Fig. 2B) corresponded with a freshwater water column lake level highstand, and  
21 colder annual mean surface temperatures (-3°C; Pollock and Bush, 2013). Sedimentary  
22 features of horizon B mainly consist of compacted greyish clays with numerous  
23 intercalations of mafic sands associated with terrestrial events (Fig. 2B).

24 Previous sedimentary studies (Kliem et al., 2013; Gebhardt et al., 2012; Ohlendorf et al.,  
25 2013) defined five main lithological units throughout the record of Laguna Potrok Aike.  
26 These five units are based on stratigraphic features associated with the frequency of gravity  
27 inflows in response to climatic lake level fluctuations (Fig. 2C). Such fluctuations promoted  
28 important reworking of the catchment with influx of terrestrial and volcanic detritus to the  
29 center of the basin (Zolitschka et al., 2013). Furthermore, time calibration of Laguna Potrok  
30 Aike stratigraphy showed that these five lithological units correspond to specific climatic

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

### 3 **2.3 On-site sampling and procedures**

4 Sediment sampling protocols were optimized to avoid potential sources of microbial  
5 contamination (Kallmeyer et al., 2006; Vuillemin et al., 2010). The size and configuration of  
6 the drilling platform prevented an on-site laboratory with sufficient conditions of asepsis,  
7 therefore retrieved cores were transported every 90 min from the platform back to the field  
8 laboratory where a detailed protocol was applied to retrieve sediments under the most sterile  
9 conditions possible. The aperture of sampling windows allowed a quick retrieval and  
10 conditioning of sediments for DNA extraction, 4',6-diamidino-2-phenylindole (DAPI) cell  
11 counts, and on-site adenosine-5'-triphosphate (ATP) assays. Rapid ATP detections were  
12 performed on a Uni-Lite NG luminometer (BioTrace) with Aqua-Trace water testers and  
13 used as an assessment of in situ microbial activity within sediments (Nakamura and Takaya,  
14 2003). Background values measured on micropure H<sub>2</sub>O ranged between 25 and 30 RLU.  
15 Thus, a value of 30 was systematically subtracted from the readings for background  
16 correction. Pore water was retrieved from small holes drilled in the liners using 0.15 µm  
17 pores soil moisture samplers (Rhizon Eijkelkamp). All protocols for lithostratigraphic and  
18 biogeochemical analyses related to bulk sediment composition, pore water geochemistry and  
19 cell count procedures have been published elsewhere (Vuillemin et al., 2013a, 2013b).  
20 Complete datasets are available at <http://doi.pangaea.de> under accession numbers  
21 10.1594/PANGAEA.811521 to 811524.

### 22 **2.4 Pigment analysis**

23 All extraction, isolation and quantification followed the standard procedures detailed  
24 elsewhere (Leavitt and Hodgson, 2002). In brief, carotenoid, chlorophyll (Chl) and derivative  
25 pigments were extracted from 2,500 freeze-dried sediment samples into degassed mixtures of  
26 organic solvents (i.e. acetone, methanol) and water under an inert N<sub>2</sub> atmosphere and filtered  
27 through 0.45-µm pore membrane filters. Extracts were injected into a Hewlett Packard model  
28 1100 high performance liquid chromatographic (HPLC) system fitted with a reversed-phase  
29 C18 column, photo-diode array detector, and fluorescence detector for quantification. Peaks  
30 were identified and calibrated using authentic pigment standards (U.S. Environmental  
31 Protection Agency and DHI Lab Products, Denmark), unialgal cultures, and reference stocks

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

## 11 **2.5 Clone library and phylogenetic analysis**

12 Detailed procedures for DNA extraction, PCR amplification and denaturing gradient gel  
13 electrophoresis (DGGE) were published elsewhere (Vuillemin et al., 2013b and 2014b). In  
14 brief, total environmental DNA was extracted from sediment samples using the commercial  
15 Mobio PowerSoil Isolation kit. Amplifications of the small subunit 16S rRNA gene were  
16 performed with the bacterial universal primer pair 27F (5'-AGA GTT TGA TCC TGG CTC  
17 AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). For archaeal gene  
18 amplifications, a nested PCR approach was selected to avoid an enrichment step by cultures.  
19 The primer pair 4F (5'-TCY GGT TGA TCC TGC CRG-3') and Univ1492R (5'-CGGTTA  
20 CCT TGT TAC GAC TT-3') was used in the first place, followed by the overlapping  
21 forward primer 3F (5'-TTC CGG TTG ATC CTG CCG GA-3') and reverse primer 9R (5'-  
22 CCC GCC AAT TCC TTT AAG TTT C-3'). PCR amplifications resulted in DNA fragments  
23 of 1400 and 900 base pairs (bp) for *Bacteria* and *Archaea*, respectively. These PCR products  
24 were used subsequently to establish clone libraries. For DGGE, a final nested PCR round was  
25 performed on both bacterial and archaeal products to fix the GC clam (5'- CGC CCG CCG  
26 CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G -30) and shorten sequences to  
27 150 bp to allow a better denaturation in the gradient gel. Primers 357F-GC (GC clam + 5'-  
28 CCT ACG GGA GGC AGC AG-3') with 518R (5'-ATT ACG GCG GCT GCT GG-3') were  
29 used for *Bacteria* and A344F-GC (GC clam + 5'-ACG GGG AGC AGC AGG CGC GA-3')  
30 with W31 (5'-TTA CCG CGC TGC TGG CAC-3') for *Archaea*.  
31 For the cloning procedure, PCR products were purified using the High Pure PCR Product  
32 Purification Kit (Roche Diagnostics SA), measured with a Nanodrop ND-1000

1 Spectrophotometer (Witec AG), and diluted to 10 ng/ $\mu$ L. Two  $\mu$ L of PCR products were  
2 ligated to the pCR4-TOPO vector (Invitrogen by life technologies) and cloned into  
3 competent *Escherichia coli* cells. Cloning procedure was performed using the TOPO TA  
4 Cloning Kit (Invitrogen by life technologies) following the manufacturer's recommendations.  
5 Transformed cells were incubated at 37°C for 20 hours on a LB medium containing 1 g L<sup>-1</sup>  
6 <sup>1</sup>NaCl, 1 g L<sup>-1</sup> Bactotryptone, 0.5 L<sup>-1</sup> Bactoyeast, 1.5 g L<sup>-1</sup>Bactoagar and 2 mL L<sup>-1</sup> ampicillin.  
7 To constitute libraries, 86 bacterial clones were selected from samples at 4.97 (43) and 29.77  
8 (40) m sediment depth, and 228 archaeal clones from samples at 0.25 (35), 0.55 (41), 1.90  
9 (42), 2.51 (27), 4.97 (27), 7.81 (21), 9.37 (11), and 29.77 (24) m sediment depth. Sequencing  
10 cycles were performed using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied  
11 BioSystems) with universal primers 27F and 1492R for *Bacteria* and vector primers D4 and  
12 R5 from the BigDye sequencing kit for *Archaea*. Sequencing was performed on an  
13 ABIPRISM 3130xl Genetic Analyzer (Applied BioSystems, Hitachi). Sequences were  
14 assembled with CodonCode Aligner v.3.7.1 (CodonCode Corporation), aligned on Seaview  
15 v.4.3.0 (Gouy et al., 2010) with ClustalW2. Primers were selectively cut off. Chimeras were  
16 detected using the online program Bellerophon (Huber et al., 2004). 16S rRNA gene  
17 sequences were identified using the megx Geographic-BLAST (<http://www.megx.net>) and  
18 SILVA comprehensive ribosomal RNA databases (Pruesse et al., 2007). The SINA online  
19 v.1.2.11 (Pruesse et al., 2012) was used to align, search and classify sequences and their  
20 closest matches downloaded from the SILVA database as taxonomic references. All  
21 sequences were uploaded on the ARB platform (<http://www.arb-home.de/>) and phylogenetic  
22 trees established with the Maximum Likelihood method using the RAxML algorithm with  
23 advanced bootstrap refinement of bootstrap tree using 100 replicates (Ludwig et al., 2004).  
24 Phylip distance matrices were extracted from phylogenetic trees and exported to the Mothur<sup>®</sup>  
25 v. 1.32.1 software (Schloss et al., 2009) and number of operational taxonomic units (OTUs),  
26 rarefaction curves, Chao, Shannon and Dominance-D indices were calculated at 97 %  
27 sequence identity cut-off value (Supplementary material). All our sequences have been  
28 deposited in the GenBank database under accession numbers JX272064 to JX272122,  
29 JX472282 to JX472399 and KT381303 to KT381433.  
30 To provide a quantitative confirmation of the major elements identified in the clone libraries,  
31 a preliminary run of Illumina MiSeq sequencing was performed on the same DNA extracts  
32 for horizon A and B. In addition, one surface sample (0.25 m depth) was included to provide  
33 a reference for the initial microbial assemblages, assuming that it experienced minimal



1 degradation of sedimentary DNA following deposition. We used bar code universal primers  
2 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG  
3 TWT CTA AT-3') to cover 291 bp of the bacterial and archaeal subunit 16S rRNA gene.  
4 (Supplementary material).

5

## 6 **3 Results**

### 7 **3.1 Geochemical analysis of bulk sediment**

#### 8 **3.1.1 Organic matter and pore water chemistry**

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

16 Chloride concentrations (Supplementary material) indicated a shift from freshwater (200  
17 ppm) to subsaline (600 ppm) conditions during the YD. Nitrite + nitrate concentrations  
18 (Supplementary material) were always very low throughout the sedimentary sequence, with  
19 values in between 0.2 and 0.6 ppm. Phosphate concentrations (Fig. 3D) were ca. 10 ppm in  
20 Holocene sediments and most often close to detection limit (0.4 ppm) within the rest of the  
21 sedimentary sequence. Dissolved iron ( $\text{Fe}^{2+}$ ) was often below detection limit (3.7 ppm), but  
22 was quantifiable from 55 to 15 m sediment depth, reaching concentrations between 5 and 15  
23 ppm. The sulphate concentration profile (Fig. 3D) displays frequent variations with baseline  
24 values oscillating between 5 and 70 ppm. Extraordinary peaks were located at 49, 38 and 25  
25 m sediment depth, reaching concentrations of ca. 1590, 1270 and 980 ppm, respectively, in  
26 concomitance with tephra layers.

#### 27 **3.1.2 Pigment concentrations**

28 Analyses of bacterial and algal pigment concentrations provided clear indication for algal  
29 abundance related to biomass (i.e. assessed productivity) being lower and higher during the

1 LGM and Holocene periods, respectively (Fig. 3B). Specifically, elevated fossil  
2 concentrations of isorenieratene ( $100 \text{ nmole} \times \text{gr TOC}^{-1}$ ) suggested that bacteria related to  
3 sulphur metabolism were an important component of the primary producer community  
4 during the late YD and early Holocene (Fig. 3B). Sporadic peaks in isorenieratene  
5 concentrations were also observed in the glacial record. In contrast, okenone concentrations  
6 (not shown) were always below  $20 \text{ nmole} \times \text{gr TOC}^{-1}$  in Holocene sediments and close to  
7 detection limit in the glacial record. Total *Cyanobacteria* contributed substantially to the  
8 labile OM during the YD and Holocene times, but are present only sporadically within the  
9 glacial interval. Finally, diatoxanthin showed that diatoms (Fig. 3B) were abundant during  
10 the late YD and early Holocene period in agreement with diatom counts (Recasens et al.,  
11 2015). The pigment preservation index (Fig. 3C) displayed sporadic peaks correlating coarse  
12 grain sizes and increased sedimentation rates, notably during the LGM and YD transition,  
13 due to either degradation of chlorophyll *a* in coarse sediments or external inputs of  
14 pheophytin *a* reworked from the catchment. Analysis of the ratio of eukaryotic to prokaryotic  
15 pigments (Fig. 3C) revealed that the relative importance of eukaryotic algae increased during  
16 climatic transitions (late LGM, YD and early Holocene). Otherwise, baseline values  
17 oscillated around 2.0, indicating that prokaryotic biomass is considerably less abundant than  
18 the eukaryotic one during the glacial period.

## 19 **3.2 Microbial characteristics**

### 20 **3.2.1 Microbial activity, density and diversity**

21 Maximal ATP values ( $>100$ ) were recorded in the Holocene sediment in between 8 and 4 m  
22 burial depth, indicating ongoing microbial processes. In contrast, only small peaks of ATP  
23 ( $>50$ ) were observed in LGM sediments (ca. 40 to 20 m depth), pointing to a sustained but  
24 considerably lower level of microbial activity in discrete horizons. Analysis of DAPI cell  
25 counts (Fig. 3E) suggested that microbial populations were densest in Holocene sediments  
26 (ca. 5 m core depth), but that total cell abundance decreased gradually from the YD down  
27 through LGM sediments, with minimal values in the deepest glacial record. At present, we  
28 cannot distinguish between active, inert or dead cells based on DAPI staining. Instead,  
29 analyses of DGGE gel features were used to assess microbial community changes. Here, the  
30 number of DGGE bands (Fig. 3F) for *Bacteria* was maximal at 5 and 30 m depth, which  
31 corresponds with the two intervals where microbial populations appeared active based on

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

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

### 16 **3.2.2 Bacterial and archaeal clone libraries**

17 16S rRNA gene sequences from ca. 5 ka old Holocene sediments showed that *Atribacteria*  
18 and *Aminicenantes*, respectively former candidate divisions OP9 and OP8 (Rinke et al.,  
19 2014), were major phyla of the sedimentary microbial assemblage (Fig. 4). Additional  
20 representative *Bacteria* identified from Holocene deposits were affiliated to *Acidobacteria*  
21 (Barns et al., 1999), *Clostridia* and  $\delta$ *Proteobacteria* partly related to syntrophic species  
22 (Jackson et al., 1999; Liu et al., 1999 and 2011). In contrast, the microbial assemblage from  
23 the ca. 25 ka old LGM interval revealed the significant presence of  $\delta$ *Proteobacteria* (Fig. 4)  
24 belonging to the SVA0485 candidate division likely involved in sulphate reduction (Bar-Or  
25 et al., 2015). Remarkably, one *Acidobacteria* sequence was affiliated with known iron  
26 reducers (Liesack et al., 1994). Other sequences specific to the LGM horizon clustered with  
27 *Spirochaetes*, *Elusimicrobia* and *Latescibacteria*, respectively former candidate division  
28 Termite Gut Group 1 and WS3 (Herlemann et al., 2009; Rinke et al., 2014; Youssef et al.,  
29 2015). Finally, sequences related to *Planctomycetes*, *Chloroflexi*, *Bacteroidetes* and  
30 *Actinobacteria* could not be uniquely associated with either the Holocene or LGM horizon

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

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

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

27

## 1 **4 Discussion**

### 2 **4.1 Holocene and LGM paleoclimatic and geochemical conditions**

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

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

### 23 **4.2 Interpretation of sedimentary DNA**

24 Overall, microbial populations were defined according to an apparently depth-dependent  
25 trend reflecting the receding activity and slow death of microorganisms (Vuillemin et al.,  
26 2014a). Subsequent to cell lysis, nucleic acids are released into the surrounding sediment  
27 where they can be actively degraded or sorbed to sediments (Corinaldesi et al., 2007 and  
28 2011). Exposure of extracellular DNA to microbial processes then results in the turnover or  
29 preservation of sequences with depth (Corinaldesi et al., 2008). Theoretically, short  
30 fragments are associated mainly with ancient and inactive taxa, whereas longer DNA

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

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

18 16S rRNA gene sequences provided evidence for the presence of *Atribacteria* and  
19 *Aminicenantes* (Rinke et al., 2013) as dominant sequences of the assemblage within the  
20 organic-rich Holocene clays buried at 5 m depth (ca. 5 ka BP) (Fig. 6 + Supplementary  
21 material). These microbes, initially described from hot springs (Hugenholtz et al., 1998), are  
22 often abundant in anaerobic marine sediments (Inagaki et al., 2003). Recently, *Atribacteria*  
23 have been described as energy-conservative heterotrophic anaerobes which act either as  
24 primary or secondary fermenters (Nobu et al., 2015) capable of syntrophic catabolism  
25 (Sieber et al., 2012). *Methanoregula* (Bräuer et al., 2011) was detected in association with  
26 *Syntrophus* (Jackson et al., 1999) and *Syntrophomonadaceae* (Liu et al., 2011). GIF9  
27 *Chloroflexi*, which are closely related to *Dehalogenimonas* (Moe et al., 2009) and widely  
28 abundant in organic-rich anoxic sediments, are presumably homoacetogenic fermenters (Hug  
29 et al., 2014). In addition, alkalotolerant species, such as *Clostridia* (Nakagawa et al., 2006)  
30 and Marine Benthic *Archaea* (Jiang et al., 2008), when active, mainly ferment labile organic  
31 compounds (Wüst et al., 2009), whereas cellulose and lignin are degradable by  
32 *Actinobacteria* and *Bacteroidetes* equally present (Pachiadaki et al., 2011). Taken together,

1 these assemblages suggest that sedimentary microorganisms first degraded the labile OM  
2 from algae before generating fermentative H<sub>2</sub> and CO<sub>2</sub> that served as substrates for methane  
3 production by *Methanomicrobiales*. Such substrate evolution during prolonged OM  
4 diagenesis could promote the recycling of end products and syntrophic hydrogen  
5 consumption, as presently observed with autotrophic methanogenesis and homoacetogenesis  
6 (Wüst et al., 2009). Such a pattern also suggests that the final Holocene microbial  
7 assemblages arose from metabolic complementarities, reinforcing our previous study on their  
8 role in the degradation and geochemical cycling of OM (Vuillemin et al., 2014b).

9 Microbial communities recovered from ca. 25 Ka old LGM sediments were not considered  
10 dormant or dead, but instead appeared to subsist in a viable state at low metabolic rate  
11 (Hoelher and Jørgensen, 2013). This LGM assemblage recorded the intricate presence of  
12 organotrophs capable of refractory OM degradation with mostly *Atribacteria*, *Aminicenantes*,  
13 *Elusimicrobia* (Herlemann et al., 2009; Febria et al., 2015) and *Chloroflexi*, to which  
14 *Acidobacteria* (Liesack et al., 1994), *Spirochaeta* (Hoover et al., 2003), *Planctomycetes*,  
15 *Actinobacteria*, and *Bacteroidetes* were added. Syntroph sequences among *δProteobacteria*  
16 and *Chloroflexi* were consistent with the degradation of secondary metabolites such as  
17 propionate (Liu et al., 1999; De Bok et al., 2001; Yamada et al., 2007), while sulphate-  
18 reducing *δProteobacteria* and *Hadesarchaea* (Takai et al., 2001; Baker et al., 2016) were  
19 thought to reflect the specific sediment geochemistry. Finally, *Latescibacteria* have been  
20 recently presented as anaerobes mediating the turnover of multiple complex algal polymers  
21 in deep anoxic aquatic habitats (Youssef et al., 2015). This pattern of sequences was  
22 interpreted as arising from the intercalation of organic-poor clays with volcanic material that  
23 could act as sources of iron and sulphate. In general, conditions at such sedimentary  
24 interfaces would greatly limit any methane production (Schubert et al., 2011) and select for a  
25 microbial assemblage capable of sulphate and iron reduction instead. H<sub>2</sub>S production during  
26 sulphate reduction was thought to promote lithotrophic species via the alteration of mafic  
27 minerals (Johnson, 1998; Blanco et al., 2014) and act in the formation of authigenic minerals  
28 such as framboidal sulphides (Vuillemin et al., 2013a).

29 Heterogeneous sedimentation or prolonged exposure to diagenesis can obscure the  
30 interpretation of DNA sources. For example, consistent with their ubiquity noted in other  
31 studies (Kubo et al., 2012; Farag et al., 2014), *Bathyarchaeota* and *Aminicenantes* sequences  
32 were not specifically associated with environmental or metabolic features of either the

1 Holocene and LGM horizons, while sequence affiliation to *Planctomycetes*, *Chloroflexi*,  
2 *Actinobacteria* and *Bacteroidetes* appeared to be kept constant with depth (Supplementary  
3 material). Indeed, some microorganisms easily tolerate different kinds of environmental  
4 change with high functional redundancy (Sunagawa et al., 2015). Global patterns of bacterial  
5 distribution in the environment have shown that the main drivers of community composition  
6 were temperature and primary production in the oceans (Raes et al., 2011) and salinity and  
7 substrate type in sedimentary environments (Lozupone and Knight, 2007). In deep sediment  
8 settings, OM anaerobic metabolisms appeared as the dominant activities, with cell densities  
9 in link to pore-water sulphate concentrations (Orsi et al., 2013) and sedimentation rates  
10 (Kallmeyer et al., 2012). All these parameters are consistent with the present microbial  
11 assemblages although the Holocene methanogenesis zone overlies the LGM sulphate  
12 reduction zone.

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

#### 22 **4.3. Sedimentary DNA and fossil pigment preservation**

23 In addition to diagenesis, important lake level fluctuations can influence the sediment record  
24 due to changes in lake morphometry, light penetration and bottom water stratification  
25 (Leavitt, 1993; Leavitt and Hodgson, 2002). Complementary analyses of bacterial and algal  
26 pigment concentrations indicated high primary productivity during the Holocene while  
27 oligotrophic conditions characterized the last glacial period. Sporadically, the pigment  
28 preservation index suggested intervals of poor preservation related to low OM content as  
29 well as the presence of reworked OM in gravity-related sediments (Hahn et al., 2013).  
30 Fortunately, pelagic production could be considered accurately recorded. During the LGM,  
31 short intervals of elevated productivity correlated warming events, tephra inputs and mass



1 movements (Recasens et al., 2015). Still, bacterial sources constituted an important fraction  
2 of the organic sedimentary record. During the Holocene, nitrate limitation favoured  
3 *Cyanobacteria* in comparison to other primary producers (Mayr et al., 2009; Zhu et al.,  
4 2013). Lake level rise improved conditions for planktonic production by eukaryotes.  
5 However, the water depth difference between the Holocene and LGM times (i.e. 37 m) likely  
6 promoted OM preservation during lowstand.

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

#### 30 **4.4 A model for ancient and extant microbial assemblages**

31 Taken together, data collected herein and by the complementary studies of the ICDP-  
32 PASADO project suggest that climate regulates the influx of organic and inorganic material

1 to the lake basin, which in turn determines water column chemistry, algal productivity and  
2 sedimentation of particulate material. Water column conditions (e.g. salinity) and sediment  
3 lithology then interact to determine final geochemistry of the sediment. Thus, environmental  
4 and geochemical parameters arising from prevailing climatic conditions can exert the initial  
5 control on microbial substrates, defining the degree of colonization at the time of deposition  
6 (Vuillemin et al., 2013b and 2014a), and subsequently dominant subsurface assemblages  
7 brought on by environmental selection during diagenesis. Results presented herein advance  
8 this model by characterizing the main elements recorded in the environmental DNA and by  
9 elucidating the metabolic pathways involved in post-depositional alterations.

10 During the Holocene interval, elevated rates of OM deposition under pelagic regime led to  
11 increased pigment concentrations in the sediment. Sequences potentially derived from  
12 ancient assemblages (i.e. *Planctomycetes*, *Actinobacteria* and *Bacteroidetes*) may have  
13 emerged from the early degradation of algae and microbial biofilms. Seemingly, these  
14 heterotrophic species actively grew at the expense of phototrophic species (Antibus et al.,  
15 2012; Cole et al., 2014), leaving intact only their respective pigments although very few  
16 sequences of *Cyanobacteria* and *Chlorobi* could still be identified in surface sediments  
17 (Supplementary material). Phylogenetic sequences representing the main elements of the  
18 subsurface biosphere were characteristic of those exhibiting solely anaerobic heterotrophic  
19 metabolism, with *Atribacter* and *Methanomicrobiales* as the dominant taxa. They reflected  
20 the sediment surrounding geochemical conditions and were indicative of advanced OM  
21 degradation during early diagenesis, showing how long-term persistence and activity of  
22 microorganisms can imprint organic proxies (Vuillemin et al., 2014b).

23 During the LGM period, limited nutrient inputs to the water column and volcanic inflows  
24 engendered low primary production mainly by bacteria, presumably in the form of microbial  
25 mats reworked to the basin during gravity events. Sequences issued from ancient  
26 assemblages seemed to refer to complex autotroph-heterotroph interactions (Cole et al.,  
27 2014) and likely included *Elusimicrobia* 4-29 (Herlemann et al., 2009; Febria et al., 2015)  
28 and *Latescibacteria* (Youssef et al., 2015). Surrounding geochemical conditions associated  
29 with the formation of OM-poor but iron- and sulphate-rich sediments selected for a  
30 subsurface biosphere capable of sulphate reduction and lithotrophy, mainly including  
31 sequences affiliated to  $\delta$  *Proteobacteria* and *Hadesarchaea* (Baker et al., 2016). Related

1 diagenetic processes resulted in the presence of authigenic concretions in LGM sediments  
2 (Vuillemin et al., 2013a).

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

13

## 14 **5 Conclusions**

15 Climatic and lacustrine conditions at the time of sediment deposition appeared to be the main  
16 factors defining sediment geochemistry and microbial substrates. Preferential preservation of  
17 microbial sources already occurred during synsedimentary processes. Sedimentary niches at  
18 the time of deposition exerted initial constraints on the development of the subsurface  
19 biosphere. After burial, changing geochemical conditions associated with sustained  
20 metabolic activity performed a selection of viable microorganisms over time and defined the  
21 final microbial assemblages. Genetic information related to phototrophic communities were  
22 mostly erased by heterotrophic bacteria while conserving pigment compositions. Identified taxa  
23 were *in fine* characteristic of conditions associated with past environmental and present  
24 geochemical factors, with *Atribacteria* and methanogens, sulphate reducers and  
25 *Hadesarchaea* as dominant species in the Holocene and LGM sediment, respectively.  
26 Further research using a combination of DNA and other proxies will advance our  
27 understanding of the mechanisms forming fossil nucleic acid assemblages. For example, at  
28 present, it is unclear whether microorganisms actively grew for centuries in past sedimentary  
29 environments or whether their sequences were merely entombed during the study period,  
30 leaving uncertainties concerning the temporal lag between original microbial deposition and  
31 establishment of the final composition of environmental DNA. Similarly, we also recognize

1 that our analytical platform represent a preliminary insight into genetic variations of Laguna  
2 Potrok Aike sediments and that the length of the targeted sequence (1400 bp) likely  
3 prevented the detection of partially preserved phototrophic bacteria (<300 bp). However, the  
4 rapid development of single cell sequencing technologies and metatranscriptomic analysis  
5 will enable a refined view of deep biosphere activities, while massive parallel sequencing  
6 will provide extensive phylogeny of environmental DNA.

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

15

## 16 **Author contribution**

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

21

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6

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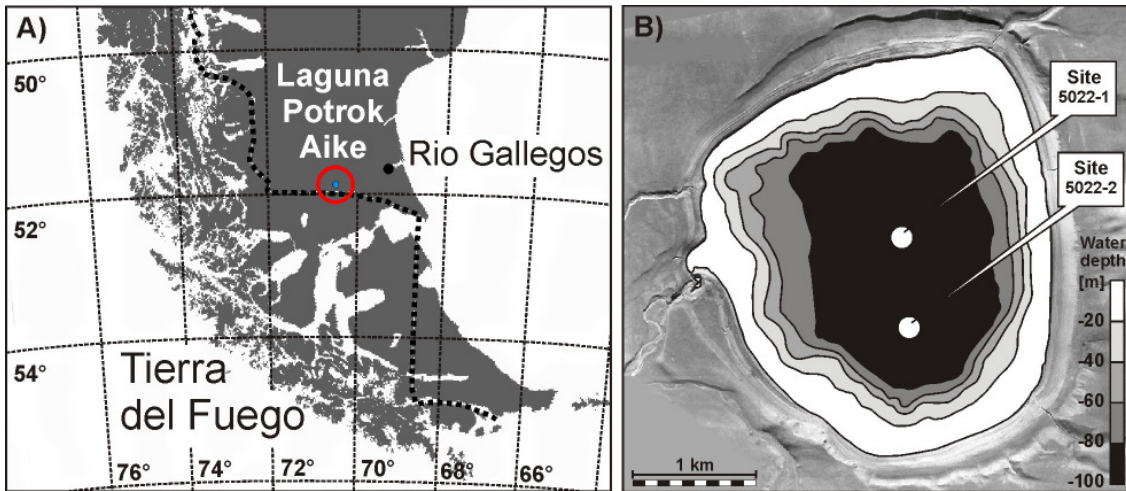
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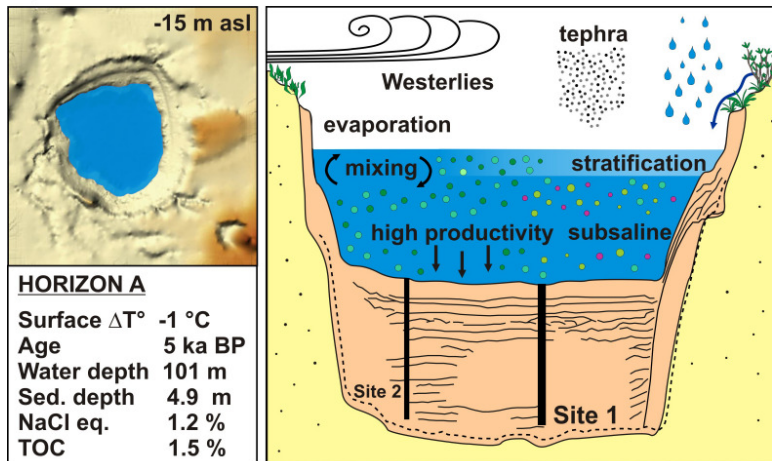
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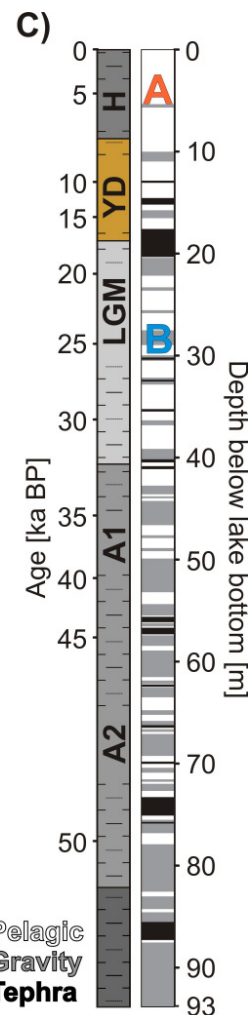
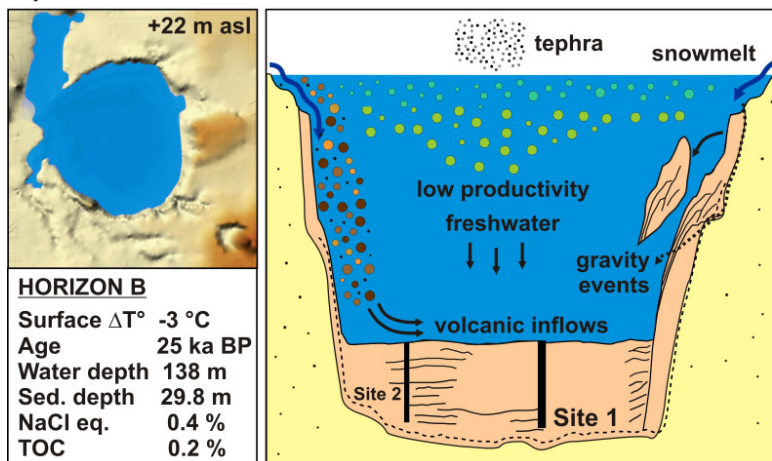
3 Figure 1. Map of Southern Argentina displaying the location (A) and bathymetric map (B) of  
4 Laguna Potrok Aike showing the two drilling sites (Zolitschka et al., 2006). Pore water and  
5 geomicrobiological samples were retrieved from cores at site 5022-1, whereas sediments for  
6 pigment analysis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

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## A) HOLOCENE

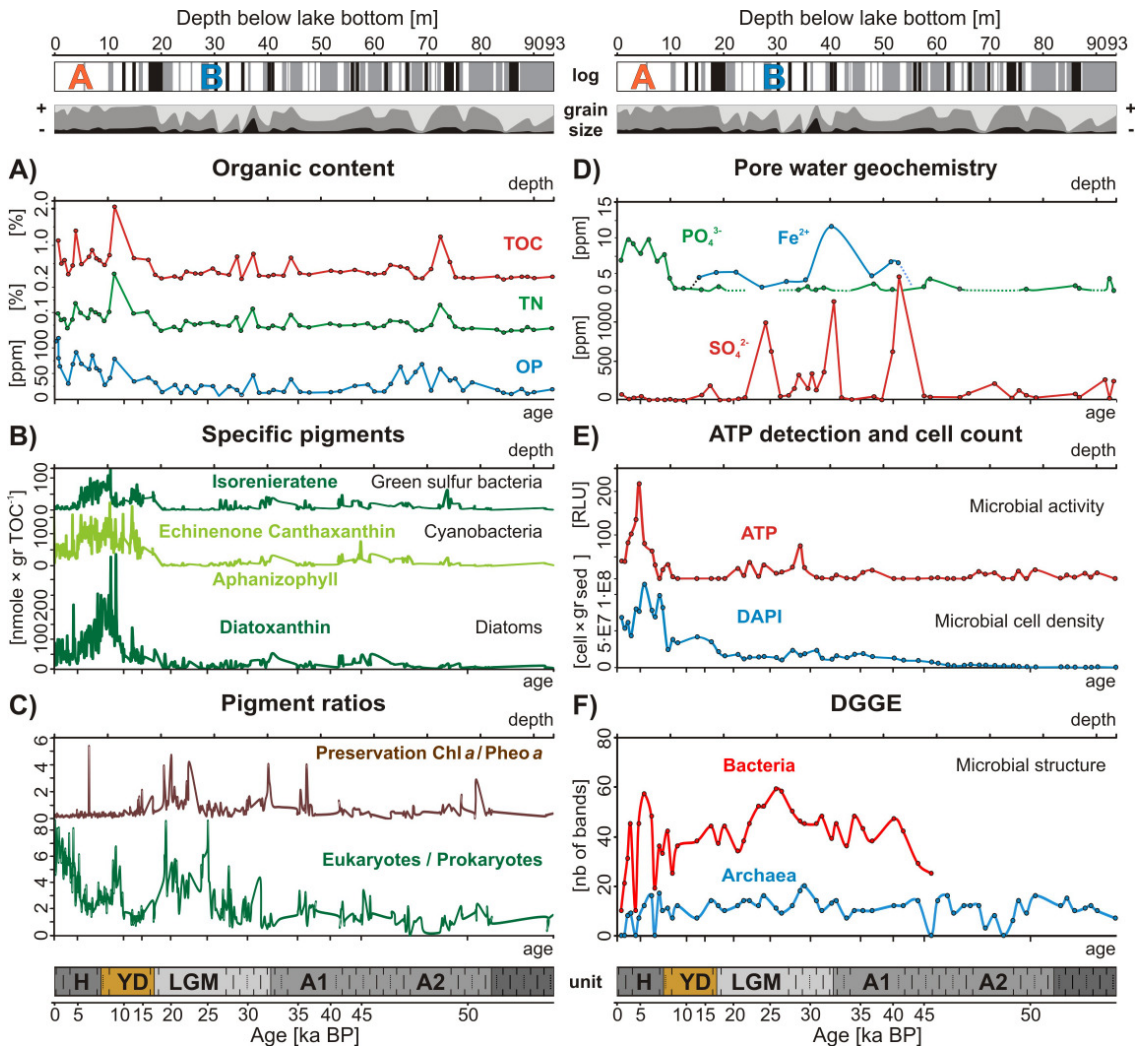


## B) LGM



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

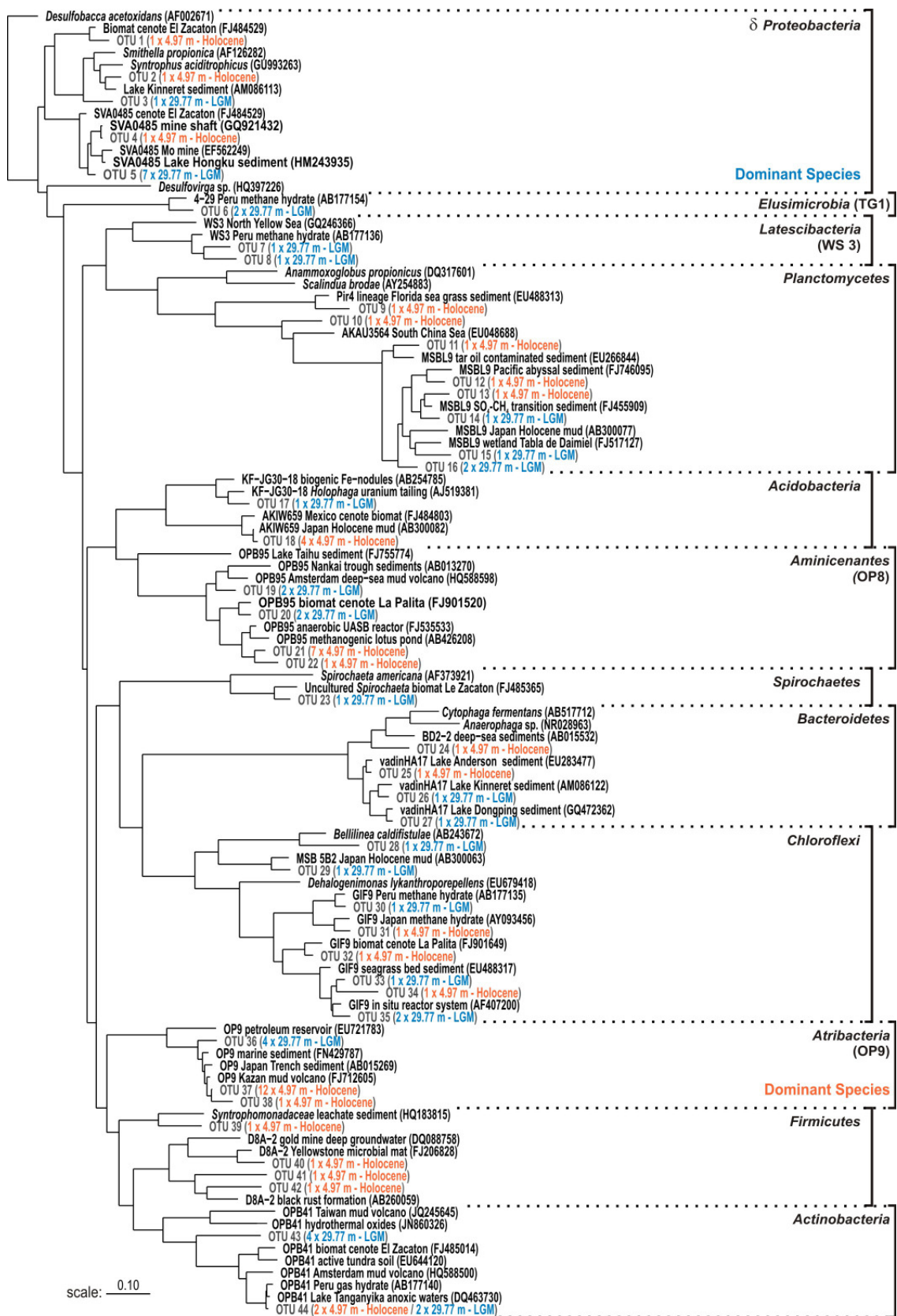


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3 Figure 3. Paleoclimatic and geomicrobiological multiproxy. **Top**) Stratigraphic sequence of  
 4 Laguna Potrok Aike, followed by grain size with clay (black), silt (dark grey) and sand (light  
 5 grey). **A)** Total organic carbon (TOC), total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

- 1 structural shifts in bacterial and archaeal communities. **Bottom)** Lithology log displaying the
- 2 five units established by Kliem et al. (2013) and their corresponding climatic intervals.
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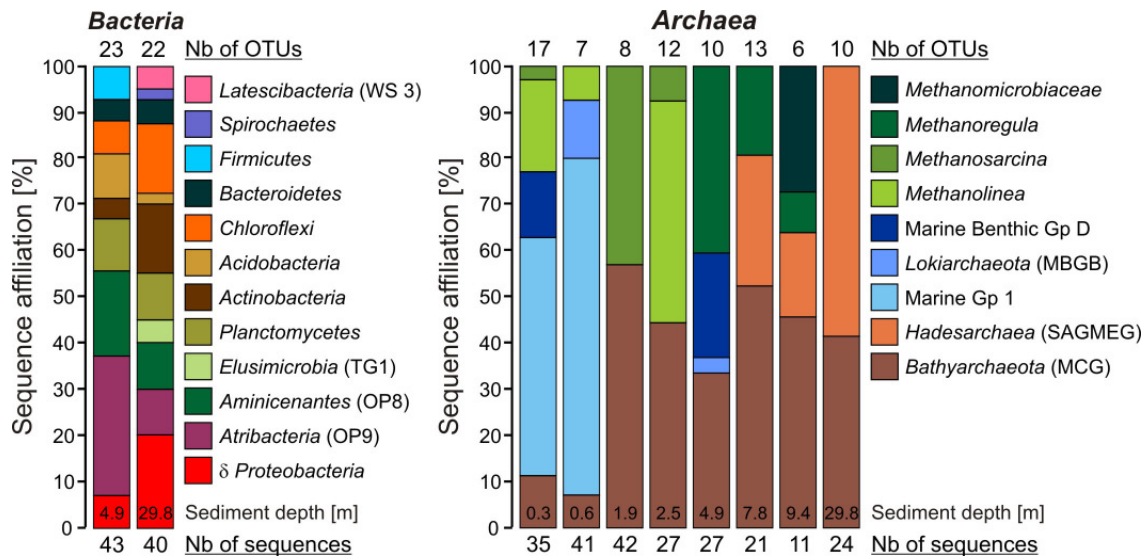
2 Figure 4. Maximum likelihood phylogenetic tree of bacterial 16S rRNA gene sequences  
3 (1400 bp) recovered at 4.97 and 29.77 m depth from Holocene (orange types) and LGM  
4 (blue types) sediments. *Atribacteria* and *Aminicenantes* are the main taxa encountered in the  
5 Holocene organic-rich pelagic sediments, whereas sulphate reducers are dominant in the  
6 LGM horizon composed of intercalated volcanic mafic sands and hemipelagic sediments.  
7 Boldface types signify database references with sequence accession numbers in parentheses.  
8



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

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