Editor's review (minor revisions)

Modifications in the manuscript are highlighted in red font (.pdf).

Abstract

1) Remove paragraphs
Authors: *Done accordingly (P1-2, L18-24)*

2) P1, L1: Define climatic intervals
Authors: *We added the following information to define the two intervals: "Two climatic intervals (Mid-Holocene 5 ka BP; Last Glacial Maximum 25 ka BP)…" (P2, L1-2)*

3) P2, L5: Acronym OM not defined
Authors: *We replaced this acronym by organic matter, since abbreviation should not be used in the abstract. (P2, L6)*

Introduction

1) P3, L10: OM should refer to organic matter, not inorganic matter
Authors: *Organic and inorganic were indeed cited in the wrong order. We modified this part of the sentence by "…erosion and export of mineral soil and organic matter (OM) to lakes…" (P3, L10)*

2) P4, L7: Use "these" instead of "their". Use "also" instead of "as well" at the beginning of a sentence
Authors: *Modified accordingly (P4, L7)*

Material and Methods

1) P4, L20: Use "in the area" instead of "on the the area"
Authors: *Modified accordingly (P4, L20)*

2) P4, L23: "Dysoxic" is not really well defined term ($<1\mu M$ O_2?). Rather use "microoxic" and give a definition. Also, if the water column is never stratified as indicated before (which I presume you didn't intend), then low-O_2 bottom water is somewhat strange (though the presence of anoxygenic phototrophs attests to reduced conditions.) Thus, clarify the mixing regime of the lake.
Authors: *We confirmed the in situ measurments for dissolved oxygen published by Zolitschka et al. (2006) and added them to the manuscript. Bottom waters remain oxic but to a lesser extent than in the overlying water. The abrupt diminution of oxygen concentrations in the lowermost part of the water column is explained by the very steep morphology of the maar and currents in the profundal zone.*

This part of the text has been modified as follows: "The water column is fully oxic (220 μM) down to 80 m depth, where oxygen concentrations rapidly decrease to 60 μM in the last 20 m. Conditions are thus oxic but become microoxic at the water-sediment interface (Zolitschka et al., 2006), likely due to the steep morphology of the maar and currents in the profundal zone (Kastner et al., 2010)." (P4, L22-26)

3) P4, L24: O_2 penetration into sediments is usually very limited (µm to mm scale)
Authors: We modified the sentence as follows: "Oxygen penetration within surface sediment is likely restricted to the first mm (Vuillemin et al., 2013b)." (P4, L27)

4) P5, L20: Rephrase "freshwater water column lake level highstand"
Authors: Rephrased as follows: " …a lake level highstand with freshwater conditions," (P5, L22)

Results

1) P9, L15: Acronym YD not explained
Authors: This acronym is defined earlier in the manuscript (P5, L29)

2) P10, L17: Ratio of prokaryote/eukaryote is not really clear. Please, define.
Authors: Pigments used in the calculation of this ratio are listed in the method part (P7, L5-7). Some confusion comes from the fact that the term "algae" is nonspecific and therefore we preferred the use of the adjectives eukaryotic and prokaryotic to specify the type. To help the reader understand which algae are included in each designation, we modified the narrative as follows: "Analysis of the ratio of eukaryotic (e.g. diatoms, green algae, cryptophyte algae) to prokaryotic (e.g. cyanobacteria, green and purple sulfur bacteria) pigments…" (P10, L17-19)

3) P11, L8: Erase "regardless", it's not clear which of the information before are not taken into account.
Authors: Deleted

Discussion

1) Sometimes past tense is used (eg. P14, L18, sequences provided evidence). This needs to be changed to present tense. Change accordingly
Authors: We have checked the Discussion and corrected verbs to the present tense whenever we are referring to the significance and interpretation of the results.

2) A clear statement is needed to distinguish what is ancient from modern "fossilized".
Authors: Since the composition of sedimentary DNA is built up over time, the term fossilized does not apply well to total DNA extractions from sediments of differing ages. Thus, we modified the manuscript and now only use the term fossil in reference to pigments, whereas DNA is described as

either environmental or sedimentary in nature. In the literature, the term "environmental DNA" refers to multiple sources (outside of cells), whereas "ancient DNA" typically describes nucleic acids from much older sedimentary eukaryotic and microbial sources. To improve reader understanding, we use the terms as follows: environmental DNA for sequences of the same age as the sediment persisting from the catchment, water column and initial microbial assemblages; sedimentary DNA for the total DNA extracted from the sediment including the former and extant subsurface biosphere. We checked the whole manuscript to apply this wording consistently. As a result, we avoided the term "ancient DNA".

3) Comparison to other stratified systems with bottom water anoxia (eg. Lake Cadagno, Wirth et al., 2013) should be strengthened.
Authors: Although geology of the catchment and water depth differ considerably between these two lakes, we have added some contrasts. Specifically, we have modified the text to read : "During the YD and Holocene, reduced okenone and isorenieratene concentrations indicate two brief periods of stratification associated with lake-level lowstands (Zolitscka et al., 2013). Endorheic conditions resulted in nitrate limitation and may have favoured Cyanobacteria in comparison to other primary producers (Mayr et al., 2009; Zhu et al., 2013). Reflooding of the maar could then explain shifts in planktonic assemblages (Wirth et al., 2013) as increased lake level should improve conditions for planktonic production by eukaryotes." (P17, L3-9)

4) P13, L8: "Position of the Westerlies moved to the site" not clear
Authors: Modified to "…the position of the Westerlies shifted to lower latitudes and the location of the lake…" (P13, L8-9)

5) P14/15, L32-3: This is textbook knowledge. Also, Methanomicrobiales can use substrates other than H_2/CO_2.
Authors: Agreed. We modified this sentence to read: "These assemblages reflect the initial degradation of labile OM from algae and the generation of fermentative byproducts, such as acetate, H_2 and CO_2, which served as substrates for methane production by Methanomicrobiales." (P15, L1-4)

6) P17, L17: It seems unlikely that isorenieratane is a diagenetic product arising from other pigments, particularly in light that you found okenone as well.
Authors: Agreed. Sentence modified to read: "…that can be altered to complex derivatives in sedimentary environments (Sinninghe Damsté and Koopmans, 1997; Brocks and Schaeffer, 2008)." (P17, L22-23)

Figures

1) Use µM or the like for concentrations
Authors: Units have been changed accordingly from [ppm] to [μM] for phosphate, iron and sulphate (Figure 3, plot D). Same in the Results (P9, L18-28)

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

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Abstract

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

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

1   Introduction

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

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   DNA from ancient sediments has already been successfully employed to study the succession

5   of species as a result of environmental changes in lacustrine settings (Coolen and Gibson,

6   2009). For example, wet and warm climates result in high bacterial abundance and diversity

7   in the sediment, whereas cold and dry climates favour lower abundance and diversity of

8   microbes (Dong et al., 2010; Vuillemin et al., 2013a). Similarly, changes in terrestrial plant

9   cover along climate-related environmental gradients influence sedimentary microbes via

10   variations in erosion and export of mineral soil and organic matter (OM) to lakes (Clark and

11   Hirsch, 2008). Shifts in lake salinity, as well as modifications of the water column regime,

12   further induce large changes in bacterial populations (Coolen et al., 2006; Coolen et al.,

13   2008), while differences in the age and composition (lability) of sedimentary OM can also

14   create distinct bacterial niches (Nelson et al., 2007). Despite the fact that the composition of

15   sedimentary microorganisms shows a strong correspondence to geological and geochemical

16   conditions at the time of deposition in marine environments (Inagaki et al., 2003), little is

17   known about the relative influence of extant environmental conditions and post-depositional

18   sedimentary processes as controls of microbial assemblage composition in deep lacustrine

19   sedimentary settings (Vuillemin et al., 2013b). Moreover, persistent activity of microbes in

20   sediments following burial can further modify geochemical conditions via diagenesis

21   (Inagaki et al., 2006) and alter extant bacterial populations to lead to selective preservation of

22   prior sedimentary assemblages (Miskin et al., 1998; Boere et al., 2011a, 2011b). Therefore,

23   the 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 the sedimentary DNA assemblage potentially records

28   climatic in-lake processes, sedimentary environments and post-depositional alterations

29   associated with subsurface microbial communities. We compare phylogenetic signatures with

30   pigment data reflecting planktonic production by algae and phototrophic bacteria in an

31   unproductive glacial environment (ca. 25,000 years ago) to those characteristic of the

32   productive Holocene (ca. 5,000 years ago). Moreover, the detection of in situ microbial

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

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

2   Material and methods

2.1   Study site

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

(Fig. 2) such as mixing and hydrological balance (Mayr et al., 2007 and 2013; Ohlendorf et al., 2013). During wetter periods, elevated nutrient influx enhances lake primary productivity in the lake (Recasens et al., 2012), as well as colonization of the sediments by microbes (Vuillemin et al., 2013a).

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

2.2 Sedimentary features of selected horizons

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

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

1   Aike stratigraphy showed that these five lithological units correspond to specific climatic

2   periods, namely the Last Glacial, Antarctic events A2 and A1, LGM, Younger Dryas (YD)

3   and Holocene times (Buylaert et al., 2013; Kliem et al., 2013).

2.3   On-site sampling and procedures

5   Sediment sampling protocols were optimized to avoid potential sources of microbial

6   contamination (Kallmeyer et al., 2006; Vuillemin et al., 2010). The size and configuration of

7   the drilling platform prevented use of an on-site laboratory with sufficient conditions of

8   asepsis, therefore retrieved cores were transported every 90 min from the platform back to

9   the field laboratory where a detailed protocol was applied to retrieve sediments under the

10   most sterile conditions possible. The aperture of sampling windows allowed a quick retrieval

11   and conditioning of sediments for DNA extraction, 4',6-diamidino-2-phenylindole (DAPI)

12   cell counts, and on-site adenosine-5'-triphosphate (ATP) assays. Rapid ATP detections were

13   performed on a Uni-Lite NG luminometer (BioTrace) with Aqua-Trace water testers and

14   used as an assessment of in situ microbial activity within sediments (Nakamura and Takaya,

15   2003). Background values measured on micropure H_2O ranged between 25 and 30 RLU.

16   Thus, a value of 30 was systematically subtracted from the readings for background

17   correction. Pore water was retrieved from small holes drilled in the liners using 0.15 μm

18   pores soil moisture samplers (Rhizon Eijkelkamp). All protocols for lithostratigraphic and

19   biogeochemical analyses related to bulk sediment composition, pore water geochemistry and

20   cell count procedures have been published elsewhere (Vuillemin et al., 2013a, 2013b).

21   Complete datasets are available at http://doi.pangaea.de under accession numbers

22   10.1594/PANGAEA.811521 to 811524.

2.4   Pigment analysis

24   All extraction, isolation and quantification followed the standard procedures detailed

25   elsewhere (Leavitt and Hodgson, 2002). In brief, carotenoid, chlorophyll (Chl) and derivative

26   pigments were extracted from 2,500 freeze-dried sediment samples into degassed mixtures of

27   organic solvents (i.e. acetone, methanol) and water under an inert N_2 atmosphere and filtered

28   through 0.45-μm pore membrane filters. Extracts were injected into a Hewlett Packard model

29   1100 high performance liquid chromatographic (HPLC) system fitted with a reversed-phase

30   C18 column, photo-diode array detector, and fluorescence detector for quantification. Peaks

31   were identified and calibrated using authentic pigment standards (U.S. Environmental

1 Protection Agency and DHI Lab Products, Denmark), unialgal cultures, and reference stocks

2 of sedimentary pigments. Biomarker concentrations (nmol pigment g^{-1} total organic carbon)

3 were calculated for pigments characteristic of green sulphur bacteria (isorenieratene), total

4 *Cyanobacteria* represented by the sum of three pigments (echinenone, canthaxanthin,

5 aphanizophyll), purple bacteria (okenone) and mainly diatoms (diatoxanthin). Preservation

6 index was calculated from the ratio of chlorophyll *a* to its degradation product pheophytin *a*,

7 two pigments indicative of total algal abundance (Leavitt et al., 1997). Shifts in productivity

8 associated with lacustrine conditions were estimated from the ratio of total eukaryotic

9 pigments (alloxanthin, β-carotene, chlorophyll-*a*, chlorophyll-*b*, diatoxanthin, fucoxanthin,

10 lutein, phaeophytin-*b*, zeaxanthin) to total prokaryotic pigments (canthaxanthin, echinenone,

11 isorenieratene, okenone).

2.5   Clone library and phylogenetic analysis

13 Detailed procedures for DNA extraction, PCR amplification and denaturing gradient gel

14 electrophoresis (DGGE) were published elsewhere (Vuillemin et al., 2013a and 2014b). In

15 brief, total DNA was extracted from sediment samples using the commercial Mobio

16 PowerSoil Isolation kit. Amplifications of the small subunit 16S rRNA gene were performed

17 with the bacterial universal primer pair 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and

18 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). For archaeal gene amplifications, a nested

19 PCR approach was selected to avoid an enrichment step by cultures. The primer pair 4F (5'-

20 TCY GGT TGA TCC TGC CRG-3') and Univ1492R (5'-CGGTTA CCT TGT TAC GAC

21 TT-3') was used in the first place, followed by the overlapping forward primer 3F (5'-TTC

22 CGG TTG ATC CTG CCG GA-3') and reverse primer 9R (5'-CCC GCC AAT TCC TTT

23 AAG TTT C-3'). PCR amplifications resulted in DNA fragments of 1400 and 900 base pairs

24 (bp) for *Bacteria* and *Archaea,* respectively. These PCR products were used subsequently to

25 establish clone libraries. For DGGE, a final nested PCR round was performed on both

26 bacterial and archaeal products to fix the GC clam (5'- CGC CCG CCG CGC GCG GCG

27 GGC GGG GCG GGG GCA CGG GGG G -30) and shorten sequences to 150 bp to allow a

28 better denaturation in the gradient gel. Primers 357F-GC (GC clam + 5'-CCT ACG GGA

29 GGC AGC AG-3') with 518R (5'-ATT ACG CGC GCT GCT GG-3') were used for

30 *Bacteria* and A344F-GC (GC clam + 5'-ACG GGG AGC AGC AGG CGC GA-3') with

31 W31 (5'-TTA CCG CGC TGC TGG CAC-3') for *Archaea.*

For the cloning procedure, PCR products were purified using the High Pure PCR Product

Purification Kit (Roche Diagnostics SA), measured with a Nanodrop ND-1000

Spectrophotometer (Witec AG), and diluted to 10 ng/μL. Two μL of PCR products were

ligated to the pCR4-TOPO vector (Invitrogen by life technologies) and cloned into

competent *Escherichia coli* cells. Cloning procedure was performed using the TOPO TA

Cloning Kit (Invitrogen by life technologies) following the manufacturer's recommendations.

Transformed cells were incubated at 37°C for 20 hours on a LB medium containing 1g L$^-$

^{1}NaCl, 1 g L^{-1} Bactotryptone, 0.5 L^{-1} Bactoyeast, 1.5 g L^{-1}Bactoagar and 2 mL L^{-1} ampicillin.

To constitute libraries, 86 bacterial clones were selected from samples at 4.97 (43) and 29.77

(40) m sediment depth, and 228 archaeal clones from samples at 0.25 (35), 0.55 (41), 1.90

(42), 2.51 (27), 4.97 (27), 7.81 (21), 9.37 (11), and 29.77 (24) m sediment depth. Sequencing

cycles were performed using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied

BioSystems) with universal primers 27F and 1492R for *Bacteria* and vector primers D4 and

R5 from the BigDye sequencing kit for *Archaea*. Sequencing was performed on an

ABIPRISM 3130xl Genetic Analyzer (Applied BioSystems, Hitachi). Sequences were

assembled with CodonCode Aligner v.3.7.1 (CodonCode Corporation), aligned on Seaview

v.4.3.0 (Gouy et al., 2010) with ClustalW2. Primers were selectively cut off. Chimeras were

detected using the online program Bellerophon (Huber et al., 2004). 16S rRNA gene

sequences were identified using the megx Geographic-BLAST (http://www.megx.net) and

SILVA comprehensive ribosomal RNA databases (Pruesse et al., 2007). The SINA online

v.1.2.11 (Pruesse et al., 2012) was used to align, search and classify sequences and their

closest matches downloaded from the SILVA database as taxonomic references. All

sequences were uploaded on the ARB platform (http://www.arb-home.de/) and phylogenetic

trees established with the Maximum Likelihood method using the RAxML algorithm with

advanced bootstrap refinement of bootstrap tree using 100 replicates (Ludwig et al., 2004).

Phylip distance matrices were extracted from phylogenetic trees and exported to the Mothur$^®$

v. 1.32.1 software (Schloss et al., 2009) and number of operational taxonomic units (OTUs),

rarefaction curves, Chao, Shannon and Dominance-D indices were calculated at 97 %

sequence identity cut-off value (Supplementary material). All our sequences have been

deposited in the GenBank database under accession numbers JX272064 to JX272122,

JX472282 to JX472399 and KT381303 to KT381433.

To provide a quantitative confirmation of the major elements identified in the clone libraries,

a preliminary run of Illumina MiSeq sequencing was performed on the same DNA extracts

for horizon A and B. In addition, one surface sample (0.25 m depth) was included to provide a reference for the initial microbial assemblages, assuming that it experienced minimal degradation of its DNA following deposition. We used bar code universal primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') to cover 291 bp of the bacterial and archaeal subunit 16S rRNA gene. (Supplementary material).

3 Results

3.1 Geochemical analysis of bulk sediment

3.1.1 Organic matter and pore water chemistry

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

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

3.1.2 Pigment concentrations

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

3.2   Microbial characteristics

3.2.1 Microbial activity, density and diversity

Maximal ATP values (>100) were recorded in the Holocene sediment in between 8 and 4 m burial depth, indicating ongoing microbial processes. In contrast, only small peaks of ATP (>50) were observed in LGM sediments (ca. 40 to 20 m depth), pointing to a sustained but considerably lower level of microbial activity in discrete horizons. Analysis of DAPI cell counts (Fig. 3E) suggested that microbial populations were densest in Holocene sediments (ca. 5 m core depth), but that total cell abundance decreased gradually from the YD down through LGM sediments, with minimal values in the deepest glacial record. At present, we

1   cannot distinguish between active, inert or dead cells based on DAPI staining. Instead,

2   analyses of DGGE gel features were used to assess microbial community changes. Here, the

3   number of DGGE bands (Fig. 3F) for *Bacteria* was maximal at 5 and 30 m depth, which

4   corresponds with the two intervals where microbial populations appeared active based on

5   ATP levels. The *Bacteria* signal disappeared below 60 m sediment depth in horizons

6   potentially corresponding with increased gravity events and early reflooding of the maar

7   (Gebhardt et al., 2012; Kliem et al., 2013). Similarly, the *Archaea* profile displayed a reduced

8   but stable number of DGGE bands along the entire sedimentary record, with maximal values

9   located around 8 and 35 m depth (Fig. 3F). In general, the DGGE bands represented short

10   sequences (150 bp) which could not be used to distinguish between DNA arising from active

11   taxa, intact dead cells and fragmented extracellular DNA (Corinaldesi et al., 2011). Taken

12   together, these various indices provided evidence for the presence of amplifiable DNA

13   related to microbial populations in decline at depth.

14   Two sedimentary horizons appeared to be preferentially colonized by microbes and were thus

15   selected within the Holocene and LGM records to establish comparative clone libraries.

16   During gel screening, bacterial clones obtained from the Holocene sample all matched the

17   expected size of the targeted DNA fragment (1400 bp), whereas more than 50 % of the clonal

18   sequences isolated from the LGM sample were shorter (800-600 bp), indicating lower DNA

19   quality in aged sediment, were discarded from further analysis (Supplementary material).

20   ### 3.2.2 Bacterial and archaeal clone libraries

21   16S rRNA gene sequences from ca. 5 ka old Holocene sediments showed that *Atribacteria*

22   and *Aminicenantes*, respectively former candidate divisions OP9 and OP8 (Rinke et al.,

23   2014), were major phyla of the sedimentary microbial assemblage (Fig. 4). Additional

24   representative *Bacteria* identified from Holocene deposits were affiliated to *Acidobacteria*

25   (Barns et al., 1999), *Clostridia* and *δ Proteobacteria* partly related to syntrophic species

26   (Jackson et al., 1999; Liu et al., 1999 and 2011). In contrast, the microbial assemblage from

27   the ca. 25 ka old LGM interval revealed the significant presence of *δ Proteobacteria* (Fig. 4)

28   belonging to the SVA0485 candidate division likely involved in sulphate reduction (Bar-Or

29   et al., 2015). Remarkably, one *Acidobacteria* sequence was affiliated with known iron

30   reducers (Liesack et al., 1994). Other sequences specific to the LGM horizon clustered with

31   *Spirochaetes*, *Elusimicrobia* and *Latescibacteria*, respectively former candidate division

Termite Gut Group 1 and WS3 (Herlemann et al., 2009; Rinke et al., 2014; Youssef et al., 2015). Finally, sequences related to *Planctomycetes*, *Chloroflexi*, *Bacteroidetes* and *Actinobacteria* could not be uniquely associated with either the Holocene or LGM horizon (Figs. 2 and 4), although their respective sequences still formed separate clusters (Figs. 4 and 6).

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

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

4 Discussion

4.1 Holocene and LGM paleoclimatic and geochemical conditions

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

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

4.2 Interpretation of sedimentary DNA

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

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

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

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

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

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

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

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

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

4.3. Sedimentary DNA and fossil pigment preservation

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

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

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

1 sinking, while early colonization of algal mats after deposition would result in selective

2 recycling of bacteria (Antibus et al., 2012).

4.4 A model for ancient and extant microbial assemblages

4 Taken together, data collected herein and by the complementary studies of the ICDP-

5 PASADO project suggest that climate regulates the influx of organic and inorganic material

6 to the lake basin, which in turn determines water column chemistry, algal productivity and

7 sedimentation of particulate material. Water column conditions (e.g. salinity) and sediment

8 lithology then interact to determine final geochemistry of the sediment. Thus, environmental

9 and geochemical parameters arising from prevailing climatic conditions can exert the initial

10 control on microbial substrates, defining the degree of colonization at the time of deposition

11 (Vuillemin et al., 2013a and 2014a), and subsequently dominant subsurface assemblages

12 brought on by environmental selection during diagenesis. Results presented herein advance

13 this model by characterizing the main elements recorded in the sedimentary DNA and by

14 elucidating the metabolic pathways involved in post-depositional alterations.

15 During the Holocene interval, elevated rates of OM deposition under pelagic regime led to

16 increased pigment concentrations in the sediment. Sequences potentially derived from

17 ancient assemblages (i.e. *Planctomycetes*, *Actinobacteria* and *Bacteroidetes*) may have

18 emerged from the early degradation of algae and microbial biofilms. Seemingly, these

19 heterotrophic species actively grew at the expense of phototrophic species (Antibus et al.,

20 2012; Cole et al., 2014), leaving intact only their respective pigments although very few

21 sequences of *Cyanobacteria* and *Chlorobi* could still be identified in surface sediments

22 (Supplementary material). Phylogenetic sequences representing the main elements of the

23 subsurface biosphere are characteristic of those exhibiting solely anaerobic heterotrophic

24 metabolism, with *Atribacter* and *Methanomicrobiales* as the dominant taxa. They reflect the

25 sediment surrounding geochemical conditions and are indicative of advanced OM

26 degradation during early diagenesis, showing how long-term persistence and activity of

27 microorganisms can imprint organic proxies (Vuillemin et al., 2014b).

28 During the LGM period, limited nutrient inputs to the water column and volcanic inflows

29 engendered low primary production mainly by bacteria, presumably in the form of microbial

30 mats reworked to the basin during gravity events. Sequences issued from ancient

31 assemblages seem to refer to complex autotroph-heterotroph interactions (Cole et al., 2014)

32 and likely include *Elusimicrobia* 4-29 (Herlemann et al., 2009; Febria et al., 2015) and

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

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

5   Conclusions

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

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

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

Author contribution

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

Acknowledgements

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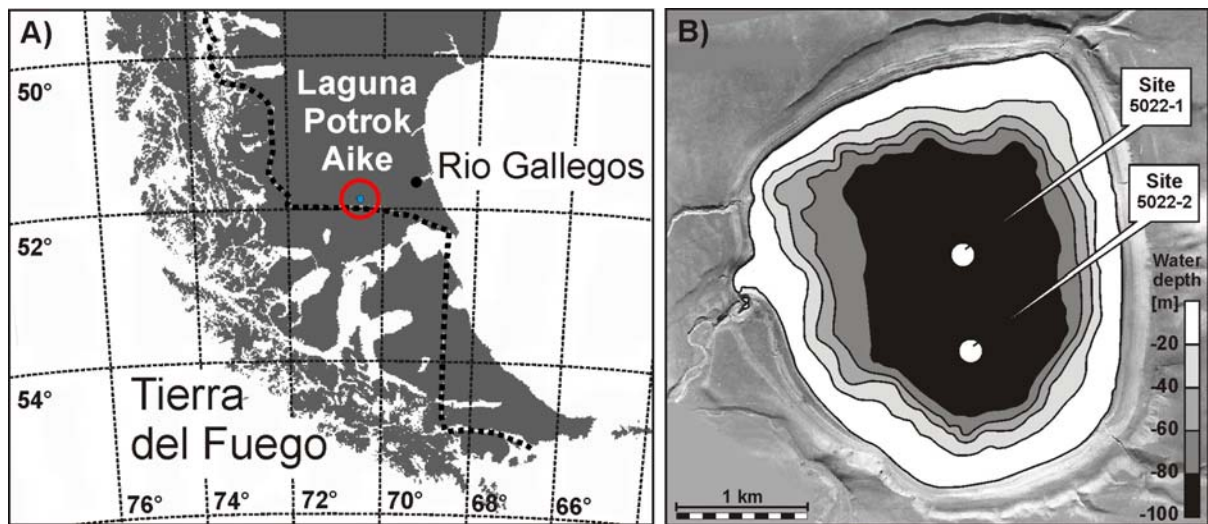25 25, 1063-1075, 2010.

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Figure 1. Map of Southern Argentina displaying the location (**A**) and bathymetric map (**B**) of Laguna Potrok Aike showing the two drilling sites (Zolitschka et al., 2006). Pore water and geomicrobiological samples were retrieved from cores at site 5022-1, whereas sediments for pigment analysis were obtained from cores at site 5022-2.
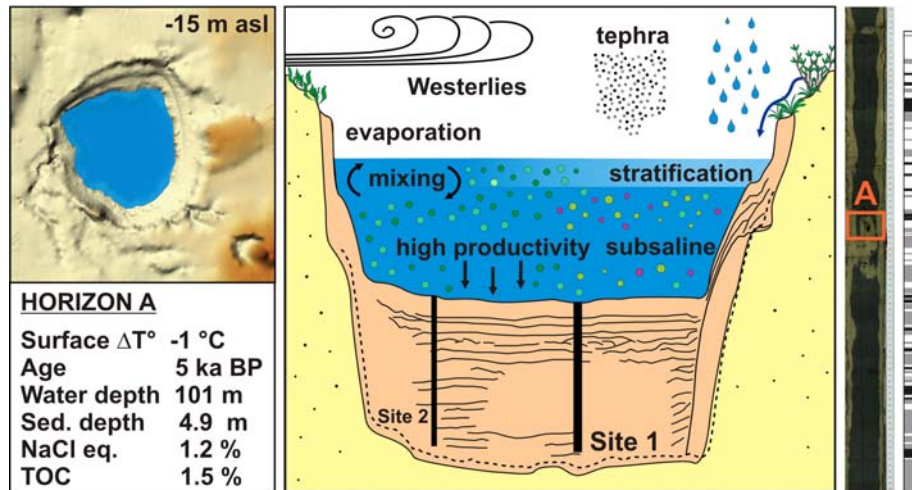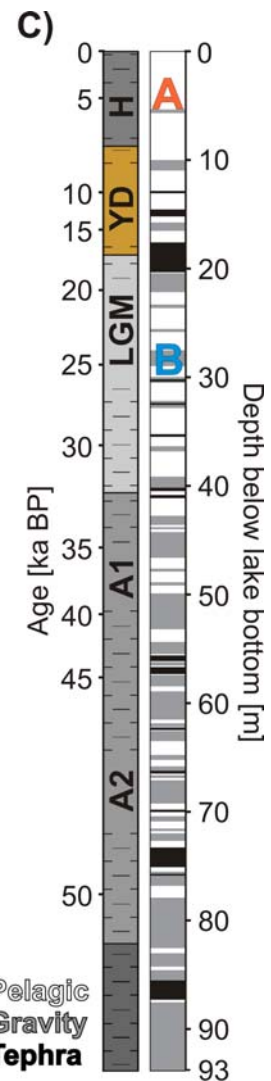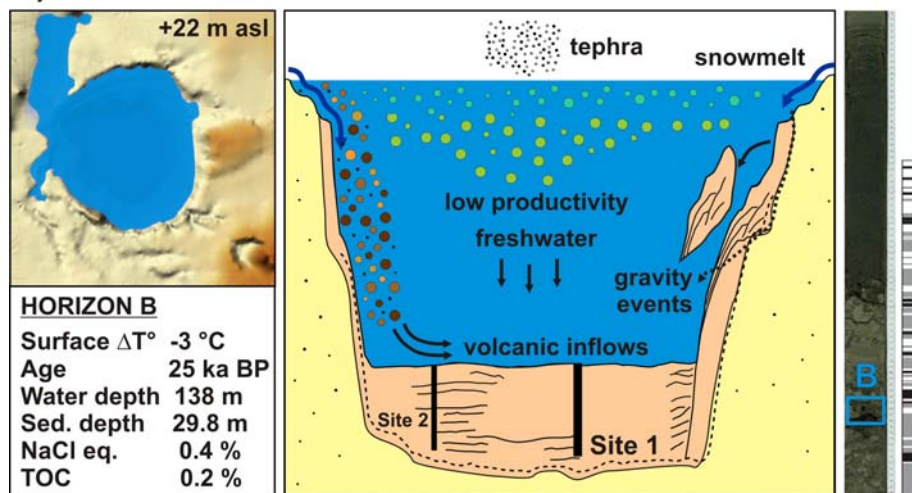
Figure 2. Paleoenvironmental conditions at Laguna Potrok Aike during the Holocene (**A**) and LGM times (**B**), with from left to right: Climatic and lacustrine parameters, sagittal views of the basin and respective core sections locating the 16S rRNA samples. Holocene times correspond with active Westerly winds, lake lowstand, subsaline conditions and high primary productivity in the basin and catchment, whereas LGM times are characterized by lake highstand and active overflow, freshwater conditions, low primary productivity in the basin and inflows restricted to runoff from the volcanic catchment. The whole lacustrine sequence (**C**) is displayed as stratigraphic units in age scale and lithology log in meter scale (after Kliem et al. 2013). The sedimentation can be defined as pelagic (white), gravity (grey) and tephra (black) layers. Time abbreviations stand for Holocene (H), Younger Dryas (YD), Last Glacial Maximum (LGM), Antarctic events 1 (A1) and 2 (A2).
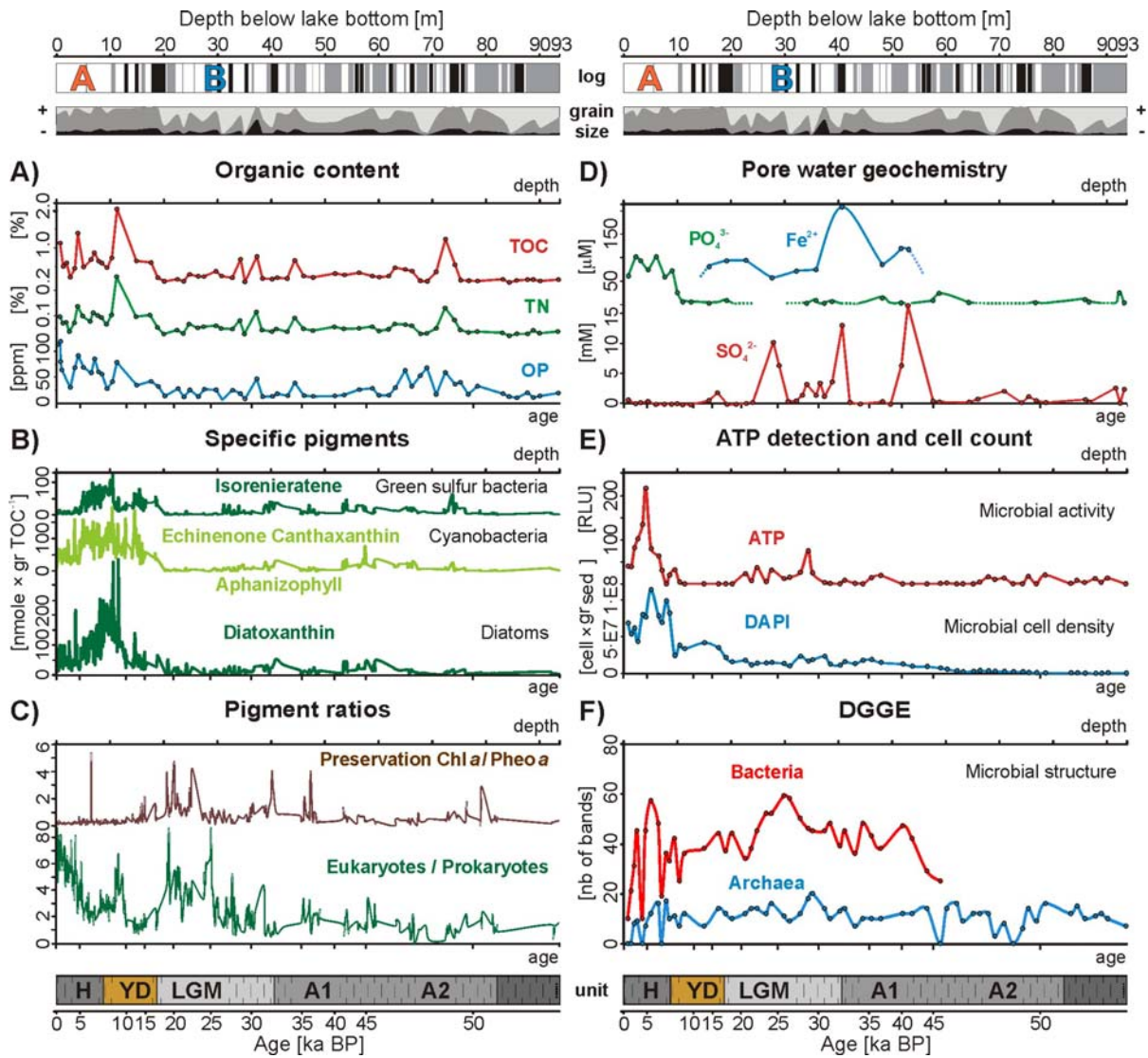
Figure 3. Paleoclimatic and geomicrobiological multiproxy. **Top)** Stratigraphic sequence of Laguna Potrok Aike, followed by grain size with clay (black), silt (dark grey) and sand (light grey). **A)** Total organic carbon (TOC), total nitrogen (TN) and organic phosphorus (OP) from bulk sediment. **B)** Specific pigments usually accounting for green sulphur bacteria (isorenieratene), cyanobacteria (echinenone, canthaxanthin, aphanizophyll) and diatoms (diatoxanthin). **C)** Preservation index based on the ratio of chlorophyll *a* to pheophytin *a*, with peaks indicative of increased preservation associated with high sedimentation rates, and ratio of eukaryotic to prokaryotic pigments. **D)** Pore water concentrations for phosphate, iron and sulphate. **E)** On-site adenosine triphosphate (ATP) detections and 4',6-diamidino-2-phenylindole (DAPI) cell counts respectively used as indices of microbial activity and population density. **F)** Number of bands from DGGE gels is used as relative index of

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.

3

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41
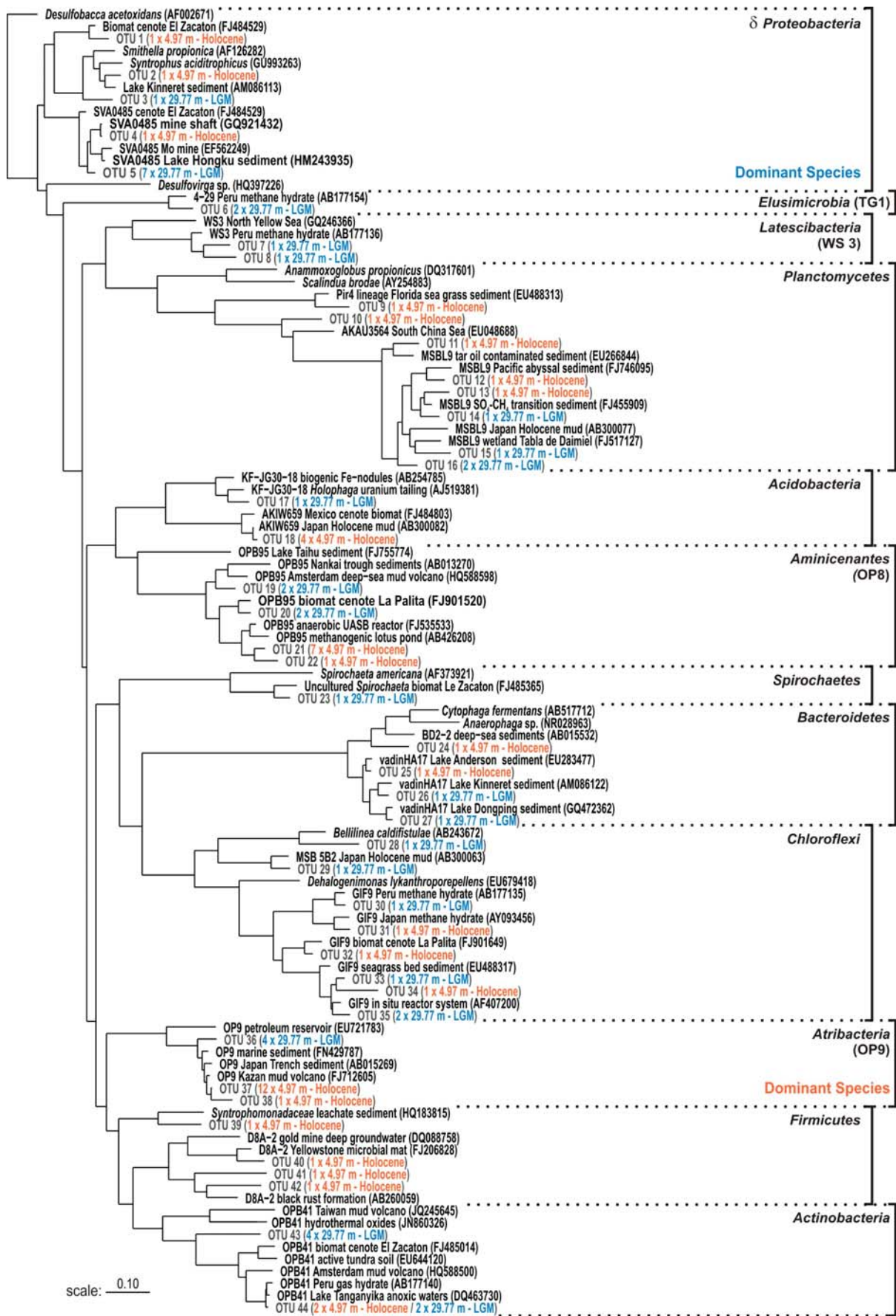
1

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

Iceland giant submarine smectite (AF411230)

Thermoprotei

China estuarine sediment (JQ245927)
Salt marsh sediment (EU284658)
OTU 1 (1 x 0.25 m / 1 x 7.81 m)
OTU 2 (1 x 0.25 m)
OTU 3 (1 x 0.25 m)
OTU 4 (1 x 7.81 m)
OTU 5 (1 x 4.97 m - Holocene)
OTU 6 (1 x 2.51 m)
Serpentinization alkaline groundwater (FN690972)
OTU 7 (3 x 2.51 m)
OTU 8 (1 x 0.25 m)
OTU 9 (1 x 2.51 m)
OTU 10 (1 x 2.51 m)
OTU 11 (1 x 2.51 m)
Spain freshwater sulfurous lake (AM076830)
Petroleum contaminated soil (AB161328)
Lake Hongku middle sediment (HM244135)
OTU 12 (1 x 9.37 m)
Finland boreal forest lake (AJ131315)
Ohio-Mississipi confluence (GQ906595)
OTU 13 (4 x 7.81 m)
OTU 14 (1 x 7.81)
OTU 15 (1 x 7.81)
OTU 16 (1 x 7.81)
OTU 17 (1 x 7.81)
OTU 18 (1 x 7.81)
Russian caldera spring (GQ328203)
Hong Kong harbour sediment (EF203585)
Holocene mud sediment (AB300143)
OTU 19 (2 x 9.37 / 1 x 29.77 - LGM)
Waikato coalfields sediment (AM883005)
Lake Baikal methane seep (KJ922116)
OTU 20 (3 x 29.77 m - LGM)
OTU 21 (2 x 9.37 m / 3 x 29.77 m - LGM)
OTU 22 (1 x 29.77 m - LGM)
OTU 23 (1 x 29.77 m - LGM)
OTU 24 (1 x 29.77 m - LGM)
Submarine permafrost (FJ982763)
Oyster shell (EU370093)
OTU 25 (1 x 0.55 m)
OTU 26 (1 x 4.97 m- Holocene)
OTU 27 (1 x 4.97 m- Holocene)
Mongolia groundwater (JX196210)
Japan Holocene mud (AB300142)
OTU 28 (1 x 0.55 m / 15 x 1.90 m / 3 x 2.51 m / 2 x 4.97 m - Holocene)
OTU 29 (3 x 1.90 m / 1 x 4.97 m - Holocene)
OTU 30 (2 x 1.90 m)
OTU 31 (1 x 2.51 m)
Tibet hypersaline lake (EF201709)
OTU 32 (1 x 2.51 m)
OTU 33 (1 x 0.55 m)
OTU 34 (1 x 4.97 m - Holocene)
OTU 35 (1 x 1.90 m)
OTU 36 (1 x 1.90 m)
OTU 37 (1 x 4.97 m- Holocene)
OTU 38 (1 x 1.90 m)

Bathyarchaeota
(MCG)

Cascadia margin methane hydrate (AB177287)
Okhotsk methane seep (FJ404091)
OTU 39 (1 x 4.97 m- Holocene)
OTU 40 (4 x 0.55 m)
OTU 41 (1 x 0.55 m)

Lokiarchaeota
(MBGB)

Mt Kunlun permafrost soil (JQ684417)
Pearl River estuary sediment (JX870390)
Germany marsh sulfidic spring (AM055707)
OTU 42 (14 x 0.25 m / 31 x 0.55 m)
OTU 43 (1 x 0.25 m)
OTU 44 (1 x 0.25 m)

Marine
Gp 1

Eel River methanic sediment (GQ356893)
Rich minerotrophic fen (EU155985)
Sulfur-rich sinkhole sediment (EU910622)
Alpine lake sediment (AM851082)
OTU 45 (2 x 0.25 m / 4 x 4.97 m- Holocene)
OTU 46 (1 x 0.25 m)
OTU 47 (1 x 0.25 m)
OTU 48 (1 x 0.25 m)

Marine Benthic
Gp D

Methanosarcina acetivorans (HI592326)
Methanosarcina lacustris (AY260430)
Methanosarcinales permafrost (FJ982666)
Methanosarcinales alpine lake (AM851080)
OTU 49 (1 x 0.25 m / 17 x 1.90 m)
OTU 50 (1 x 1.90 m / 1 x 2.51 m)
OTU 51 (1 x 2.51 m)

Methanomicrobiales

Lake Wivenhoe sediment (HQ330676)
Lake Hongku upper sediment (HM244093)
OTU 52 (3 x 9.37 m)
Methanoplanus petrolearius (AY196681)
Methanoculleus marisnigri (AF028693)
Methanosphaerula palustris (EU156000)
Methanoregula sp. acidic fen (EU155976)
Methanoregula boonei (EU887810)
Methanoregula formicicum (AB479390)
Methanoregula sp. acidic bog lake (AF481343)
OTU 53 (11 x 4.97 m - Holocene / 2 x 7.81 m / 1 x 9.37 m)
OTU 54 (1 x 7.81 m)
OTU 55 (1 x 7.81 m)
Methanospirillum hungatei (AB517987)
Methanolinea tarda (AB162774)
Methanolinea Lake Hongku (HM244180)
OTU 56 (4 x 0.25 m / 4 x 0.55 m / 12 x 2.51 m)
OTU 57 (1 x 0.25 m)
OTU 58 (1 x 2.51 m)
Salar de Huasco (EU481619)
OTU 59 (1 x 0.25 m)
OTU 60 (1 x 0.25 m)

Dominant Species

Nirano mud volcano (HQ654860)
OTU 61 (2 x 7.81 m)
South Africa deep gold mine water (AB050223)
Obsidian pool prime community (AY861953)
South China subseafloor sediment (EU385847)
Spain freshwater sulfurous lake (EU683399)
OTU 62 (4 x 7.81 m / 2 x 9.37 m / 11 x 29.77 m - LGM)
OTU 63 (1 x 29.77 m - LGM)
OTU 64 (1 x 29.77 m - LGM)
OTU 65 (1 x 29.77 m - LGM)
OTU 66 (1 x 7.81 m)

Hadesarchaea
(SAGMEG)

Dominant Species

scale: 0.10

1

43

1

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.

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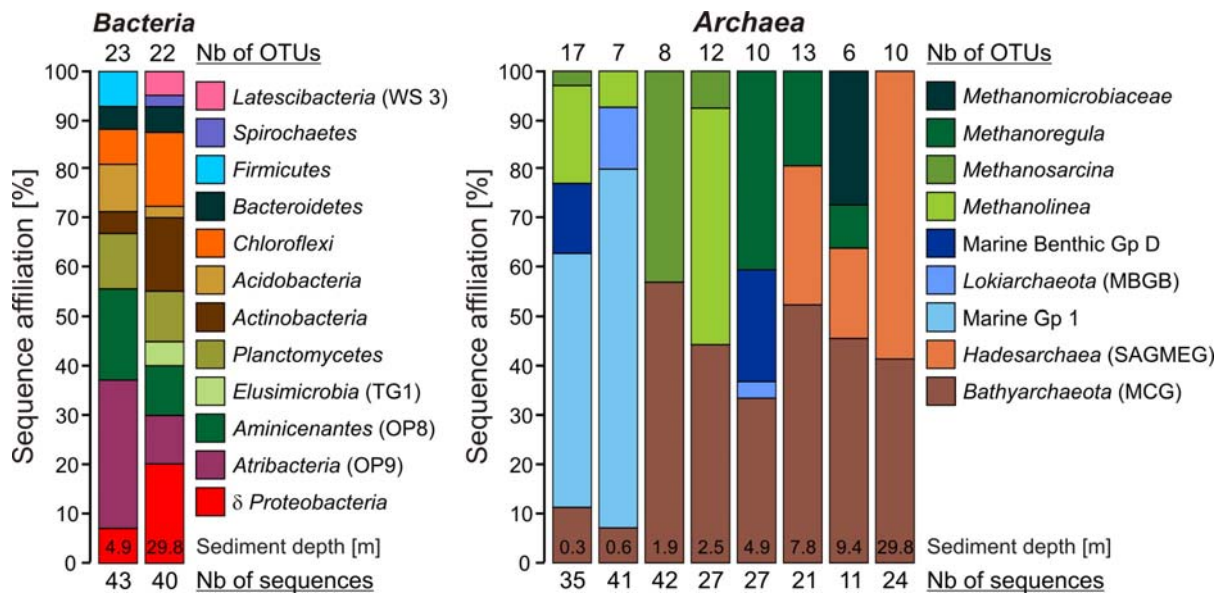
Figure 6. Histograms of identified phylotypes displayed in relative %, with OTU and sequence numbers at the top and bottom, respectively. **Left**) Several bacterial phylotypes are shared by the Holocene and LGM horizons (i.e. *Chloroflexi*, *Planctomycetes*, *Bacteroidetes*) as they are known ubiquists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

Supplementary material

| Bacteria	Sequence number	Cut-off	OTUs	Chao	Shannon	Dominant species
Total	84	3 %	44	126.67	3.40	*Atribacteria*
Holocene	44	3 %	23	108.50	2.64	*Atribacteria*
Glacial	40	3 %	22	33.14	2.86	δ *Proteobacteria*
Archaea						
Total	235	3 %	96	886.00	3.71	Marine Group 1
Holocene	23	3 %	11	21.50	2.10	*Methanomicrobiales*
Glacial	24	3 %	5	8.00	1.10	*Hadesarchaea*

1) Table displaying phylogenetic indices: OTUs were calculated for a 97 % sequence identity cut-off value.
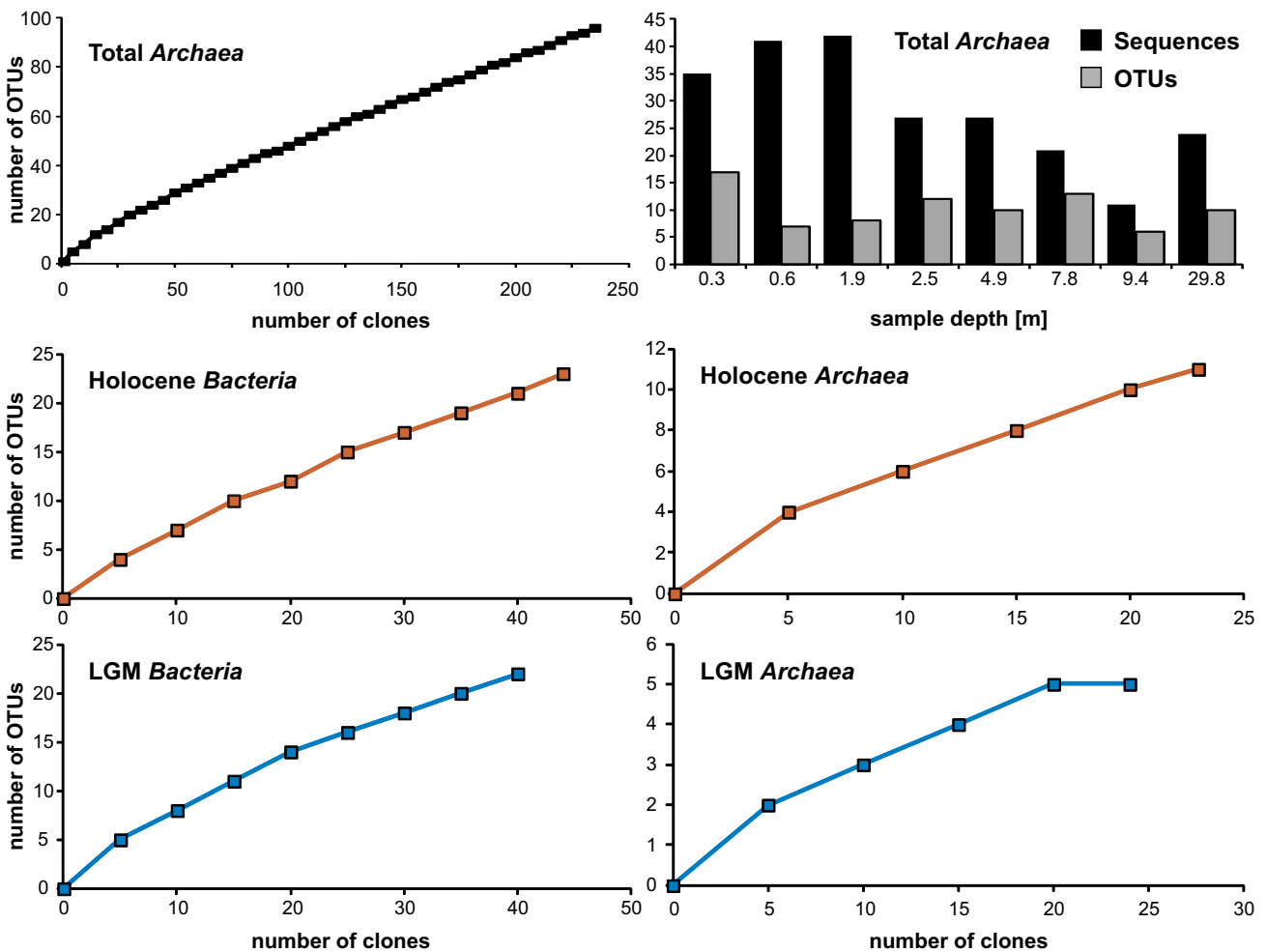
2) Rarefaction curves: OTUs were calculated for a 97 % sequence identity cut-off value.
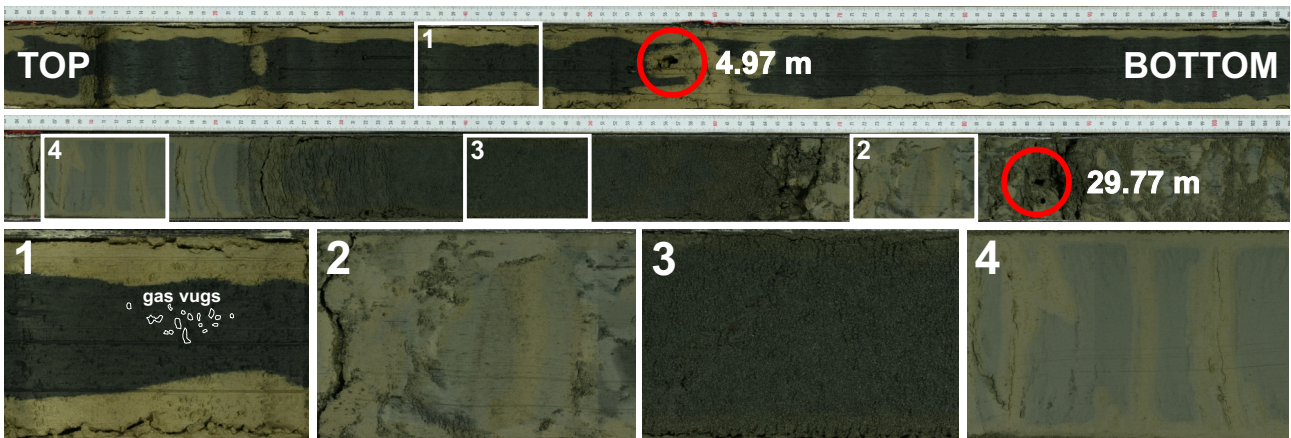
3) Comparison between Holocene and LGM sedimentary features in sections sampled for clone libraries: The Holocene sequence can be characterized as pelagic to hemipelagic, black and soft, anoxic gas-saturated sediments (**1**). Oxidized rims occurred during storage after the aperture of sampling windows. The LGM sequence first reflects a pelagic to hemipelagic regime with structures of fluid escapes (**2**). Then, fine mafic sands (**3**) could be associated with a gravity event that triggered gas escapes due to sudden loading on the underlying sediment (Vuillemin et al., 2013a). The top of the section shows a return to pelagic sedimentation (**4**). In general, the last glacial record displays multiple intercalations of volcanic detritus.
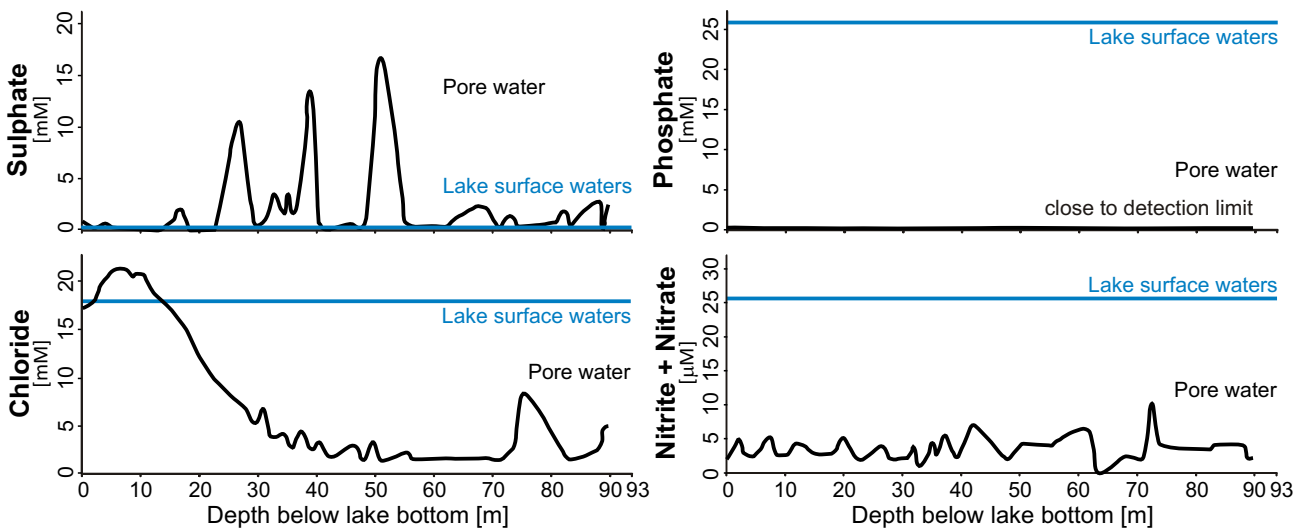
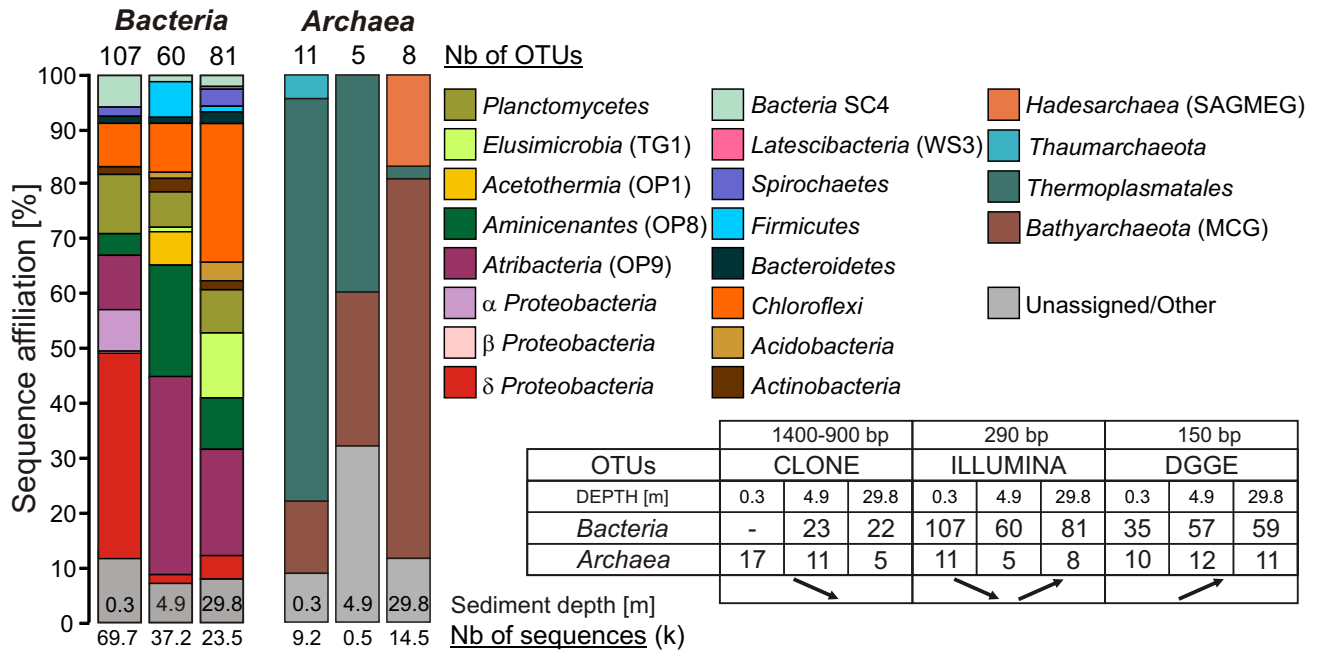**4) Geochemical comparison between pore water and lake surface waters:** The present comparison provides evidence for the absence of sediment contamination by the hydraulic system during drilling operations. Results from pore water analysis can thus be used as indicators of paleoconditions (i.e. chloride) and geochemical changes within sediments (i.e. sulphate, phosphate) (Vuillemin et al., 2014b).

Holocene   LGM
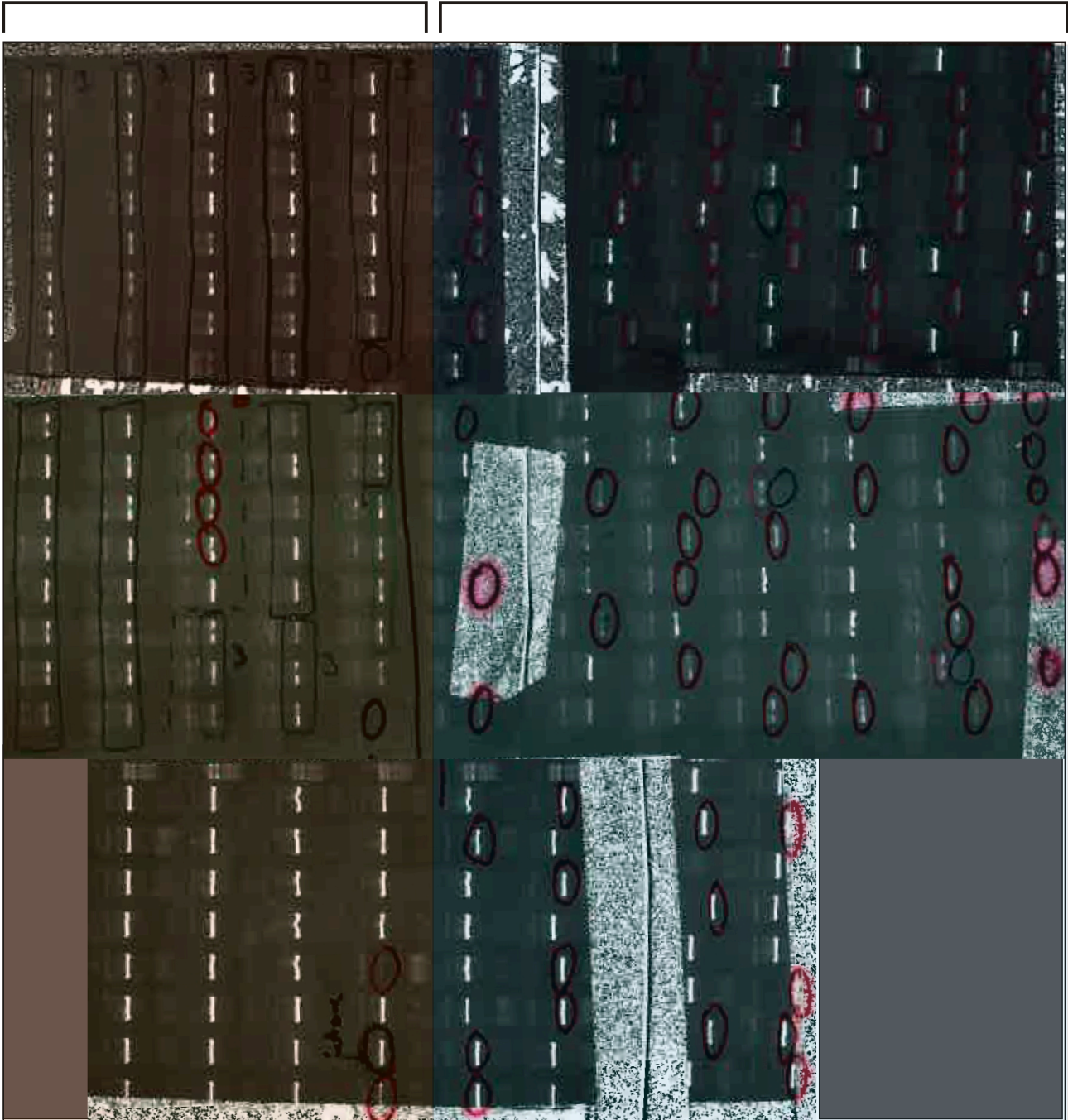
5) Clone screening: The targeted clonal DNA corresponds to 1400 bp long sequences. Holocene clones (left) all match the expected sequence length, whereas more than 50 % of the LGM clones are too short (ca. 800-600 bp). Such shorter sequences can arise from crosslinkage in the 16S rRNA gene upon release of extracellular DNA. This shows the lower quality of sedimentary DNA extracted from older sediments sheltering microbial communities maintaining low metabolic rates.

Bacteria 107 60 81 **Archaea** 11 5 8 Nb of OTUs

Legend:
- *Planctomycetes*
- *Elusimicrobia* (TG1)
- *Acetothermia* (OP1)
- *Aminicenantes* (OP8)
- *Atribacteria* (OP9)
- α *Proteobacteria*
- β *Proteobacteria*
- δ *Proteobacteria*
- *Bacteria* SC4
- *Latescibacteria* (WS3)
- *Spirochaetes*
- *Firmicutes*
- *Bacteroidetes*
- *Chloroflexi*
- *Acidobacteria*
- *Actinobacteria*
- *Hadesarchaea* (SAGMEG)
- *Thaumarchaeota*
- *Thermoplasmatales*
- *Bathyarchaeota* (MCG)
- Unassigned/Other

Sequence affiliation [%] (y-axis 0–100)

Sediment depth [m]: 0.3 4.9 29.8 | 0.3 4.9 29.8

Nb of sequences (k): 69.7 37.2 23.5 | 9.2 0.5 14.5

OTUs	1400-900 bp			290 bp			150 bp		
	CLONE			ILLUMINA			DGGE		
DEPTH [m]	0.3	4.9	29.8	0.3	4.9	29.8	0.3	4.9	29.8
Bacteria	-	23	22	107	60	81	35	57	59
Archaea	17	11	5	11	5	8	10	12	11

6) Preliminary results of Illumina MiSeq sequencing: Bar charts were established for horizon A (Holocene, 4.9 m depth) and B (LGM, 29.8 m depth) in order to provide quantitative comparison with the main elements identified in the clone libraries. One surface sample (0.3 m depth) was added as reference, considering minimal exposure of its sedimentary DNA to post depositional alteration. These results show that global patterns are preserved with similar assemblages as those of Figure 6. It confirms the qualitative aspect of our libraries and allows their interpretation in terms of sediment populations and infer some related diagenetic processes. We note that one main taxon (6 %) remained missing in the assemblage of horizon A, respectively the *Acetothermia* (former OP1). Also the obscure candidate division *Bacteria* SC4 could be identified (1 %).

The surface sample reveals a majority of *Proteobacteria* potentially related to layered microbial communities. We note the absence of phototrophic sequences related to *Cyanobacteria*, *Chlorobi* or even chloroplasts. Another important point is that the presence of *Planctomycetes*, *Chloroflexi*, *Actinobacteria* and *Bacteroidetes* appears to be kept constant with depth.

A rapid comparison between clone libraries, MiSeq results and DGGE bands shows that the relative number of OTUs associated with long fragments decreases with depth, apparently following microbial population decline in activity and density, whereas OTUs associated with short fragments increase. This may account for an accumulation of fragmented extracellular DNA due o turnover rates decreasing with depth.

Method: We used bar code universal primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') to cover 291 bp of the bacterial and archaeal subunit 16S rRNA gene. Individual tags were composed of 8 nucleotides attached at each primer 5'-extremity. 32 ng of DNA per amplicon sample were pooled and the mixture volume lowered to 120 μL using a Savant SpeedVac High Capacity Concentrator. 60 μL of pooled amplicons were used for the Illumina library preparation.

Libraries were prepared folowing the manufacturer instructions. Illumina PCR-free libraries were validated by qPCR using the KAPA Library Quantification Kit (Kapa Biosystems), following the manufacturer instructions. Final concentrations of each library were quantified by a fluorometric method using a QuBit HS dsDNA kit (Invitrogen). A MiSeq Reagent Nano kit v2, with 500 cycles with nano (2 tiles) flow cells was used to run libraries on the illumina MiSeq Sequencing System. Two 250 cycles were used for an expected output of 500 Mb. Quality of the raw data was checked using FastQC (http://www.bioinformatics.babraham.ac.uk). Demultiplexing was performed using own scripts based on cutadapt (Martin et al., 2011). No errors in barcodes were allowed with phred-Score above Q25. Read pairs were merged using pear (Zhang et al., 2014). Sequences were trimmed using trimmomatic (Bolger et al. 2014). Chimeras were detected and removed using usearch61 using the ChimeraSlayer reference database (Edgar et al., 2010) as it is implemented in the QIIME-pipeline (Caporaso et al., 2010). Script of this pipeline was used to cluster the sequences and assign taxonomies based on the Greengenes and SILVA databases at 97 % identity cut-off value (DeSantis et al., 2006). The resulting OTU table was filtered by removing all OTUs with abundance below 0.1% within the sample.

Core 5022-1A sampled for pore water
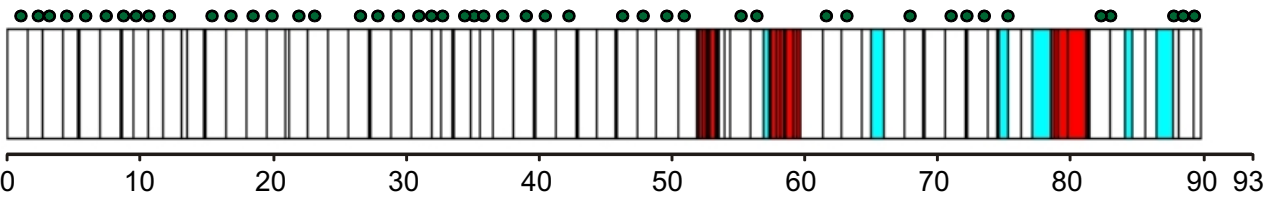
Core 5022-1D sampled for geomicrobiology
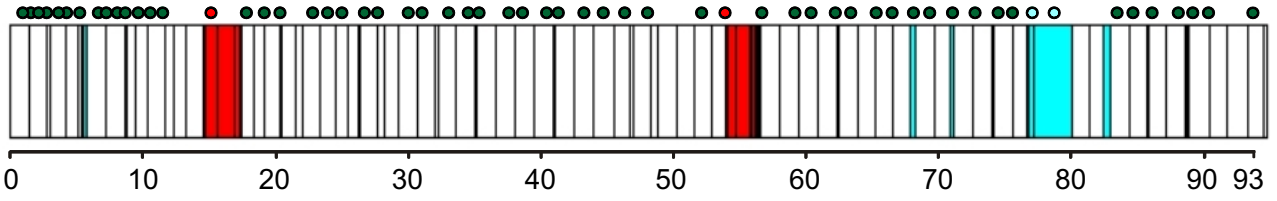
Depth below lake bottom [m]

7) Core sections: Possible drilling artifacts (Ohlendorf et al., 2011) were put in parallel with sample locations in order to validate the genuine use and interpretation of their results in the absence of any established composite depth at site 1 (Gebhardt et al., 2012). Drilling artifacts were mostly related to coarse layers and gravity events (Kliem et al., 2013).
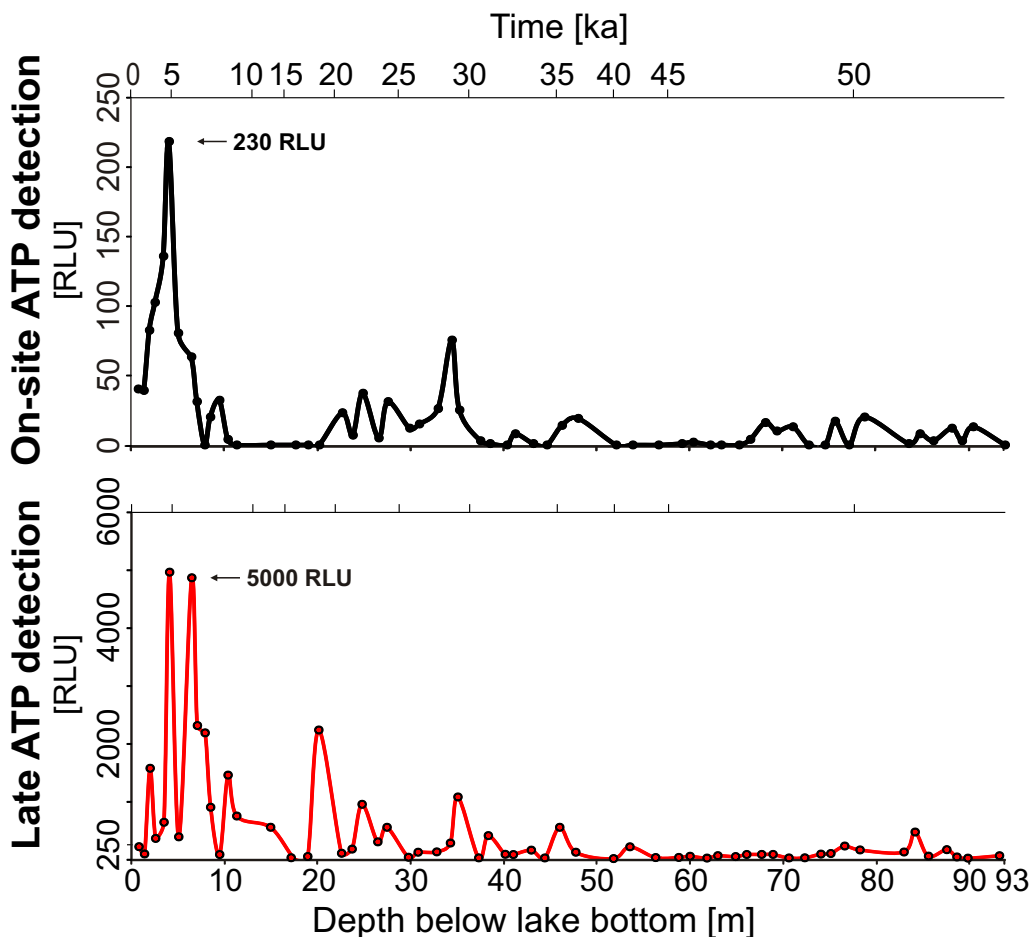
8) Comparison between on-site and late ATP measurements: A second round of ATP assays measured with a hand-held device showed that microbial colonization of sediments that were initially inactive did not occur during long-term storage. This fact emphasizes the habitability of specific horizons, such as those corresponding to the Holocene and LGM times. It also argues against the possible reworking of modern active microbes into underlying sediments during drilling operations.

DEPTH [m]	0.1	0.2	0.3	0.8	0.9	4.9	29.8
Chlorobi --> Ignavibacteria	1400	0	0	0	0	0	0
Chlorobi --> SJA-28	89	0	0	0	0	0	181
Cyanobacteria --> Chloroplast: Trebouxiophyceae	0	0	0	0	0	73	0
Cyanobacteria --> Chloroplast: Stramenopiles	0	0	0	55	121	0	0
Cyanobacteria --> Chloroplast: Streptophyta	0	0	251	0	0	0	0
Planctomycetes --> Phycisphaerae: AKAU3564	824	450	712	653	1509	1179	475
Planctomycetes --> Phycisphaerae: CCM11a	416	331	289	0	0	0	0
Planctomycetes --> Phycisphaerae: MSBL9	855	3807	1750	285	1280	194	2652
Planctomycetes --> Phycisphaerae: ODP1230B3009	128	1003	0	309	756	244	0
Planctomycetes --> Phycisphaerae: SHA-43	94	0	0	0	0	0	0
Planctomycetes --> Phycisphaerae: mle1-8	0	269	0	41	0	0	0
Planctomycetes --> Pirellulaceae	392	1471	190	2082	3108	57	0

9) Screening Illumina MiSeq results for preserved phototrophic sequences: *Planctomycetes* related to *Phycisphaera* and *Pirellula* are dominant among identified phototrophs. Such preferential preservation likely arises from their specific cell membranes. Sequences of *Cyanobacteria* are present in very low number and are all affiliated with chloroplasts. *Chlorobi* sequences are maximal in uppermost sediments and appear to be quickly degraded. Our interpretation is that sequences from planktonic species are partially degraded, or even erased from the record, at a very early stage, starting in the water column during particle settling. Overprint by heterotrophs then occurs during OM diagenesis. Further investigations will require primers specific to these taxa.