RESEARCH ARTICLE

Microbial community composition along a 50 000-year lacustrine sediment sequence

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One sentence summary: Microbial community composition along a 50 000 year sediment sequence provides evidence for species selection of a deep lacustrine biosphere over geological time.

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ABSTRACT

For decades, microbial community composition in subseafloor sediments has been the focus of extensive studies. In deep lacustrine sediments, however, the taxonomic composition of microbial communities remains undercharacterized. Greater knowledge on microbial diversity in lacustrine sediments would improve our understanding of how environmental factors, and resulting selective pressures, shape subsurface biospheres in marine and freshwater sediments. Using high-throughput sequencing of 16S rRNA genes across high-resolution climate intervals covering the last 50 000 years in Laguna Potrok Aike, Argentina, we identified changes in microbial populations in response to both past environmental conditions and geochemical changes of the sediment during burial. Microbial communities in Holocene sediments were most diverse, reflecting a layering of taxa linked to electron acceptors availability. In deeper intervals, the data show that salinity, organic matter and the depositional conditions over the Last Glacial-interglacial cycle were all selective pressures in the deep lacustrine assemblage resulting in a genetically distinct biosphere from the surface dominated primarily by Bathyarchaeota and Atribacteria groups. However, similar to marine sediments, some dominant taxa in the shallow subsurface persisted into the subsurface as minor fraction of the community. The subsequent establishment of a deep subsurface community likely results from a combination of paleoenvironmental factors that have shaped the pool of available substrates, together with substrate depletion and/or reworking of organic matter with depth.

Keywords: lake sediment; deep biosphere; microbial communities; species selection; 16S rRNA gene high-throughput sequencing; Laguna Potrok Aike

INTRODUCTION

Microorganisms are essential components of aquatic ecosystems because they control important biogeochemical cycles in the water column and sediments. Global distribution of microbial populations in the marine realm is known to mainly derive from climatic control on primary production (Lozupone and Knight 2007), while sinking organic matter (OM) defines cell abundances in the subsurface according to sedimentation rate (Raes *et al.* 2011; Kallmeyer *et al.* 2012). As sediments accumulate over geological time, buried microbial life persists at decreasing growth rates due to the depletion of respiratory electron acceptors and the lesser reactivity of substrates with depth (Jørgensen and Boetius 2007; Hoehler and Jørgensen 2013; Jørgensen and Marshall 2016). This decrease in activity and density of microbial populations results in the assembly of the so-called deep biosphere (Walsh *et al.* 2016; Starnawski *et al.* 2017). Although this deep biosphere has been the subject of considerable study in recent years with the exploration of multiple environments (Orcutt *et al.* 2011; Edwards *et al.* 2012; Colwell and D'Hondt 2013), there is a clear scarcity of comparable studies in lacustrine sediments. Despite the correspondence observed between the depositional environmental and paleoceanographic characteristics with resident microbial populations in marine environments (Inagaki *et al.* 2003, 2006, 2015; Orsi *et al.* 2017), little is known about the relationship between subsurface communities and the depositional environment in lakes. However, geochemical evolution of the sediment and the establishment of microbial communities in their lacustrine counterparts have been reported (Vuillemin *et al.* 2016).

Considering global patterns of subsurface microbial biogeography, community compositions appear to primarily arise from dispersal of microbial seed banks (Lennon and Jones 2011) over the oceans and selection in sediments is attributed to the community present in the water column and shallow sediment during burial (Nemergut *et al.* 2013; Walsh *et al.* 2016). Microorganisms constituting the deep biosphere are apparently those displaying an enhanced ability to enter or reverse dormancy as they have to cope with environmental variability and energy limitation during burial (Starnawski *et al.* 2017). Although species interactions and selection are often considered the predominant forces driving vertical community profiles in the sediment column (Petro *et al.* 2017), there is debate as to whether microorganisms exhibit any biogeographic patterns (Hughes Martiny *et al.* 2006). Moreover, our understanding of the taxonomic compilation of lacustrine communities is presently limited to surface water (Newton *et al.* 2011) and shallow sediments (Yang *et al.* 2017), thus impeding our present understanding of subsurface biosphere composition in terms of environmental factors and processes (Macalady *et al.* 2013). The rate of sedimentation being generally higher in lakes than in oceans, lacustrine systems also react faster to changes in climatic conditions and input sources (Ariztegui *et al.* 2015). Because their sedimentary sequences represent repositories of past environmental conditions, they allow for tracking subsurface communities through a variety of ecological niches and assessing the link between stratigraphic deposition of the sediment and the distribution and activity of extant microbial populations (Vuillemin *et al.* 2014a; Kallmeyer *et al.* 2015).

At Laguna Potrok Aike, Argentina (Fig. 1), the sedimentation regime over the last 50 ka was mainly dependent on climatic

variations and river inflows due to the influence of the Westerly winds moving towards or away from the site, causing a strong rain shadow effect with increased evaporation or vice versa (Stine and Stine 1990; Hein *et al.* 2010). During dry conditions, water level fluctuations gave way to regression phases with shore erosion and reworking of the catchment (Kastner *et al.* 2010; Coronato *et al.* 2013). On the contrary, wetter conditions promoted transgression phases and pelagic conditions (Haberzettl *et al.* 2007; Gebhardt *et al.* 2012). The relationship that we could establish between paleoenvironmental conditions and the development of the subsurface biosphere is that wetter and warmer periods promoted higher initial colonization of the corresponding sediments by microorganisms (Vuillemin *et al.* 2013a), whereas erosive events disrupted microbial communities in shallow sediments (Vuillemin *et al.* 2013b). Lacustrine conditions at the time of sediment deposition exerted initial control on the sediment geochemistry and organic substrates (Vuillemin *et al.* 2016), and thereby determined the sustainability of microbial activity after isolation from the surface (Vuillemin *et al.* 2014a). Thus, we hypothesize that changes in climatic conditions and the related changes in the depositional environment lead to the development of different microbial communities in the corresponding lacustrine intervals. This is a logic hypothesis, given that selection after isolation from the surface would be expected to be driven by the initial OM substrates available for growth, the composition of which in sediments is controlled to a large extent by the depositional environment (Coolen *et al.* 2002; Orsi *et al.* 2017). To address this, we used high-throughput sequencing of partial 16S rRNA gene amplicons from environmental DNA extracted from a drill core from Laguna Potrok Aike, Argentina, reaching from the sediment surface down to 93 m depth (Gebhardt *et al.* 2012), and trace structural changes in microbial communities during burial. Our study takes advantage of previous paleoclimatic reconstructions (Kliem *et al.* 2013; Ohlendorf *et al.* 2013; Zolitschka *et al.* 2013) and geochemical investigations (Vuillemin *et al.* 2014b, 2016) as an existing framework that provide valuable context for the vertical profiles of microbial community stratigraphy. We investigate variations in bacterial and archaeal populations in response to both past environmental conditions and geochemical evolution of the sediment over the last 50 000 years and explore how the deep lacustrine biosphere is selected over geological time in order to compare it to its marine analogue.

METHODS

Study site and field sampling

Laguna Potrok Aike is a maar lake located in Southern Patagonia, Argentina (Fig. 1) within the Pali Aike volcanic field (Coronato *et al.* 2013). Because the Westerly winds and ice cap distribution in the Andes regulate the regional climate of Patagonia (Waldmann *et al.* 2009), environmental variations directly impact the lake's hydrological balance and mixing (Mayr *et al.* 2013; Ohlendorf *et al.* 2013). The steep morphology of the maar results in a depositional regime oscillating between pelagic and hemipelagic with frequent occurrence of mass movement events (Gebhardt *et al.* 2012; Kliem *et al.* 2013). The basin is presently endorheic with a maximum depth of 100 m (Fig. 1B). Due to the persistent influence of the Westerly winds on the study site, the lake is polymictic (Mayr *et al.* 2007) and the water
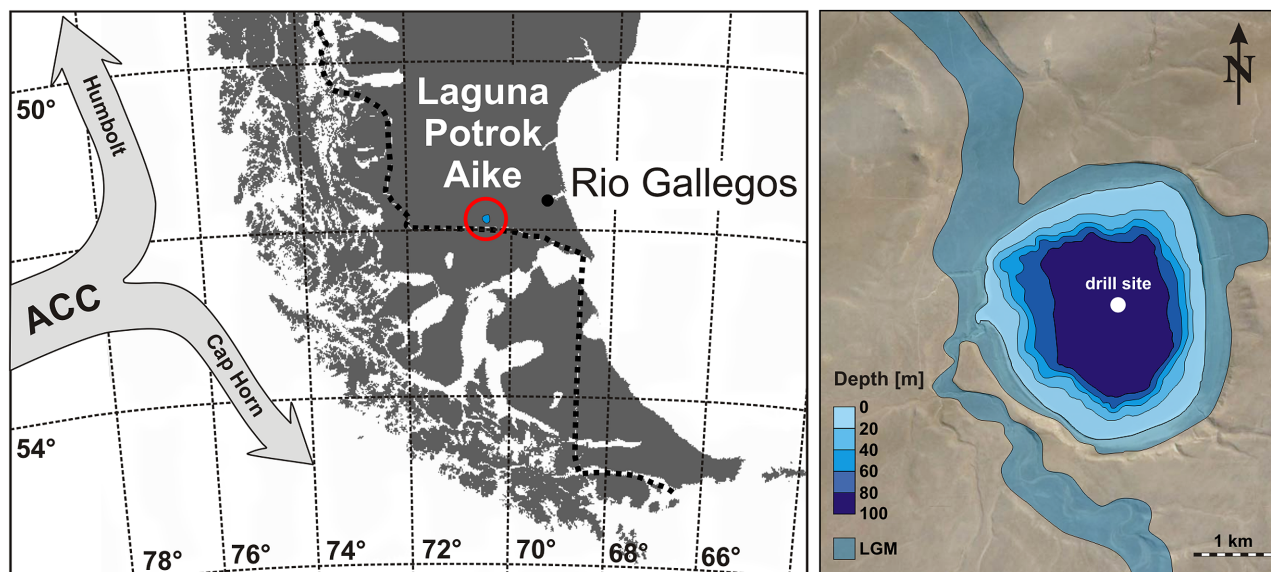
Figure 1. Location of the study site and bathymetric map of the lake. Left: The geographic location of Laguna Potrok Aike is under the permanent influence of the Westerly winds and Antarctic Circumpolar Current. Right: Bathymetric map of the lake merged with a satellite image from Google Earth (right). The highest lake-level had active overflow conditions to the north during the Last Glacial Maximum (after Zolitschka *et al.* 2013).

column not stratified in any season, displaying mean annual temperatures between 4°C and 10°C, with oxic conditions down to the water–sediment interface (Zolitschka *et al.* 2006). Oxygen penetration in surface sediment is nevertheless restricted to the uppermost few millimeters (Vuillemin *et al.* 2013a).

The sedimentary sequence, which was obtained in November 2008 during the International Continental Scientific Drilling Program (ICDP) field operations of the Potrok Aike maar lake Sediment Archive Drilling prOject (PASADO), represents an environmental and climatic archive covering the last 51 000 years (Zolitschka *et al.* 2009, 2013). A 93 m long hydraulic piston core (no. 5022-1D) was collected and sampled on site for detailed geochemistry and geomicrobiology of the sediment. In addition, high-resolution sampling was performed on a 1 m long gravity core (no. 5022-1J) retrieved from the same site (Vuillemin *et al.* 2013a). The size and configuration of the drilling platform prevented the installation of a laboratory with sufficiently clean conditions; therefore, retrieved cores were transported every 90 min from the platform back to the field laboratory where they were sampled under controlled sterile conditions. Two cm long and three cm wide sampling windows cut into the side of the core liner allowed a quick retrieval and processing of sediment samples. For the gravity core, windows were precut in the liner every 5 cm and subsequently closed with strong adhesive tape prior to coring. We used autoclaved end-cut syringes to extract 5 mL of sediment, removed the oxidized capping and distributed this volume into separate aliquots for DNA extraction, 4′, 6-diamidino-2-phenylindole (DAPI) cell counts, and on-site adenosine triphosphate (ATP) assays. For DNA extraction, 1 mL of sediment was transferred to an Eppendorf tube and kept frozen on site until extraction in the home laboratory. For total cell counts, 1 mL of sediment was fixed in the field with formaldehyde (final concentration: 2%) and stored until use in the home laboratory. Rapid ATP detections were performed on a Uni-Lite NG luminometer (3M Clean-Trace, St. Paul, United States) with Aqua-Trace water testers (3M Clean-Trace, St. Paul, United States) and

served as an assessment of in situ microbial activity within sediments (Nakamura and Takaya 2003). Background values measured on micropure H_2O ranged between 25 and 30 relative luminescence units (RLU). Thus, a value of 30 was systematically subtracted from the readings for background correction (Vuillemin *et al.* 2010). After each sampling window, spatulas were systematically cleaned with alcohol and burned to increase the grade of disinfection.

Bulk OM, pore water geochemistry and cell counts

For total organic carbon (TOC), samples were freeze-dried prior to analysis. Carbonate minerals were removed by treating the samples with HCl (5%) at 50°C, overnight sonication, repeated rinsing with deionized water, centrifugation to discard water and another round of freeze-drying. TOC of the homogenized bulk organic fraction was analyzed on decalcified samples using an elemental analyzer (EuroVector, EuroEA, Pavia, Italy). TOC was calculated according to the yield of CO_2 after sample combustion in the elemental analyzer. Analytical precision was ± 3% (1σ) and TOC of the decarbonized sample back-calculated to the whole sample to present results in weight percent.

Fifty mg of freeze-dried sediment were used for the successive measurement of inorganic phosphorus (IP) and organic phosphorus (OP) (Vuillemin *et al.* 2013b). Samples for IP were mixed with 5 mL HCl (1N), sonicated overnight, centrifuged and supernatants retrieved. The remaining sediment pellets were rinsed with 3 mL micropure H_2O and dried in stove overnight prior to OP extractions. Dried sediments were combusted to gradually reach 550°C, mixed with 5 mL HCl (1N), sonicated overnight, centrifuged and supernatants retrieved. Dilutions were performed with an autosampler dilutor Gilson 401. Aliquots of OP were diluted 10 times in micropure H_2O to reach a final volume of 5 mL. 200 μL ascorbic acid molybdate blue were added as the colorimetric reagent and absorbance was measured at 875 mm with a U-VIS λ25 photometer(PerkinElmer, Waltham, United-States).

Pore water samples were obtained from core no. 5022-1A using soil moisture samplers (Rhizon Eijkelkamp, Giesbeek, The Netherlands) inserted into sediments through small holes drilled into the core liners. Fluids were extracted using syringes connected to the Rhizons and maintained under low pressure. To avoid shifts in water chemistry, recovered samples were immediately flushed with helium gas. Transfer of pore water samples into sealed vials was performed under a N_2 atmosphere inside a glove bag. Anions were analyzed by ion-chromatography (Sykam, Fürstenfeldbruck, Germany) at the Geobio-Center, Munich. Based on the respective signal-to-noise (S/N) ratio of 3, detection limits were as follows: NO_2^-/NO_3^- (0.17 mg $\times$ L^{-1}), PO_4^{3-} (0.41 mg $\times$ L^{-1}) and SO_4^{2-} (0.19 mg $\times$ L^{-1}). Samples were measured in triplicates, the reproducibility was always better than 3% for each anion.

For total cell counts, sample slurries were diluted in 10 mL 1 $\times$ hexametaphosphate to desorb cells from sediments, centrifuged, and aliquots of 2 mL were retrieved in sterile 15 mL Eppendorf tubes and centrifuged down. The supernatant was discarded and aliquots were rinsed twice with 1 $\times$ PBS, centrifuged and suspended in 1 mL 1 $\times$ PBS-ethanol (1:1). For DAPI staining, aliquots were mixed to a solution of 2 mL 1 $\times$ PBS and 20 μL DAPI and incubated in a filtration column in a dark room for 7 min. Samples were filtered onto a 0.2 μm pore-size black polycarbonate filter (Millipore Ø 25 mm) backed by a supporting filter (BA85, Ø 25mm, 0.45 μm pore-size) (Whatman Schleicher and Schuell, Maidstone, United Kingdom) and rinsed with 2 mL 1 $\times$ PBS. Dry filters were mounted on glass slides with a non-fluorescent immersion oil and pictured under epifluorescent microscopy using a digital camera. Grids were digitally added to the images and cells counted up to a minimum of 300.

All protocols for lithostratigraphic and biogeochemical analyses related to bulk sediment composition, pore water geochemistry and cell count procedures have been published elsewhere (Vuillemin *et al.* 2013a,b). Complete data sets are available at http://doi.pangaea.de under accession number #811523 and #811524.

PCR amplification for high-throughput sequencing

Sampling windows from the gravity core 5022-1J were regularly spaced from 5 to 95 cm sediment depth, whereas a total of 60 windows were sampled throughout the core 5022-1D for an overall sediment depth of 93 m. Because coarse-grained sediments are more prompt to infiltration by modern lake water during drilling operations, we focused on pelagic sediments only and selected 11 samples from the gravity core 5022-1J and 32 samples from the hydraulic piston core 5022-1D for high-throughput sequencing. Total DNA was extracted from 1 mL of sediment using the DNA PowerSoil Isolation kit (MoBio Qiagen, Hilden, Germany). The methodology was applied as recommended in the manufacturer's instructions. Prior to PCR procedures, DNA extracts were purified using a High Pure PCR Cleanup Micro Kit (Roche Applied Science, Penzberg, Germany) and eluted in a final volume of 100 μL. PCR was performed on DNA extracts using the universal bacterial and archaeal primer pair 515F (5′- GTG CCA GCM GCC GCG GTA A -3′) and 806R (5′- GGA CTA CHV GGG TWT CTA AT -3′) with individual tags composed of eight nucleotides at each primer 5′-end to enable multiplexing of all PCR products in a single library. We acknowledge that this primer pair does not include the adaptation for increased coverage of archaea (Parada *et al.* 2016). PCR reactions were performed according to previously published mixtures and conditions (Pawlowski *et al.* 2014) with undiluted DNA templates. Negative and positive controls

were added to all PCR sets using 2.5 μL of molecular grade water and 2.5 μL of *E. coli* DNA (0.1 ng μL^{-1}), respectively. For each sample, 116 μL of PCR product were pooled, purified and eluted in a final volume of 25 μL. Concentrations were quantified by the fluorometric method and normalized to 32 ng for each sample. Volume of pooled samples was reduced to 120 μL using a Savant SpeedVac High Capacity Concentrator (Thermo Fisher Scientific, Munich, Germany).

Illumina library preparation, sequencing and data analysis

We used 60 μL of pooled PCR products (ca. 800 ng DNA) for the construction of an Illumina MiSeq library using an Illumina TruSeq DNA PCR-Free L Kit (Illumina, San Diego, United States) following the manufacturer's instructions. The library was validated by qPCR using the KAPA Library Quantification Kit (Kapa Biosystems, Wilmington, United States) following the manufacturer's manual. Final concentration was quantified by the fluorometric method. A MiSeq Reagent Nano kit v2, with 500 cycles with nano-flow cells was used to run the library on the Illumina MiSeq Sequencing System. Two 250 cycles were used for an expected output of 500 Mb and an expected number of 7 million reads (Vuillemin *et al.* 2017).

Quality of the raw data was checked using FastQC (www.bioinformatics.babraham.ac.uk). Demultiplexing was performed using in house scripts based on cutadapt (Martin 2011). Read pairs were merged using PEAR [Q 25; p 0.0001; v 20] (Zhang *et al.* 2014). Sequences were trimmed using Trimmomatic (Bolger *et al.* 2014). Chimeras were detected and removed using usearch61 and the ChimeraSlayer reference database (Edgar 2010) as it is implemented in the QIIME-pipeline (Caporaso *et al.* 2010). Operational taxonomic units (OTUs) were picked using the QIIME script (pick_open_reference.py), sequences were clustered and taxonomies assigned based on the SILVA release 119 database 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 each sample. Sequencing data after demultiplexing were submitted to the European Nucleotide Archive (www.ebi.ac.uk/ena) under study accession number PRJEB22477.

Statistical and phylogenetic analysis

Non-metric multidimensional scaling (NMDS) was performed using the Past 3.10 software applying the Bray–Curtis dissimilarity index (Hammer, Harper and Ryan 2001). The NMDS included all 43 samples from the gravity and hydraulic piston core. Statistical indices of alpha diversity (i.e. Taxa S, Shannon, and Evenness) were calculated based on the OTU table using the Past 3.10 software.

Representative sequences were then extracted for all OTUs. The SINA online v.1.2.11 aligner (Pruesse *et al.* 2007; Pruesse *et al.* 2012) was used to align our sequences. Phylogenetic analysis was performed with the ARB software package (Ludwig *et al.* 2004) based on the upload sequence alignments against the SILVA 16S rRNA SSU NR99 128 reference database release 07_09_2016 (Quast *et al.* 2013). Their closest environmental sequences and cultured species were selected as taxonomic references and used to calculate a bacterial and archaeal phylogenetic tree with nearly full-length sequences ($>$1400 bp) using the implemented bacterial and archaeal filter and the Maximum Likelihood algorithm RAxML with advanced bootstrap refinement of bootstrap tree using 100 replicates (Stamatakis 2006). Sample partial sequences were added to the trees using the

maximum parsimony algorithm without allowing changes of tree topology. Each phylogenetic tree included representative sequences from Laguna Potrok Aike and their respective reference sequences for Archaea and Bacteria (Figs S1–S3, Supporting Information).

A heat map analysis was conducted in R (www.r-project.org). Calculation was based on all OTUs corresponding to Bathyarchaeota, Atribacteria and Aminicenantes present in at least five samples to provide statistical comparison between surface and deep samples. Dendrograms were computed using unweighted pair grouping by arithmetic mean (UPGMA). Reference OTUs were checked for their environmental provenances.

RESULTS

Bulk sediment organic content and pore water geochemistry

The stratigraphy of Laguna Potrok Aike is composed of four main lithological units (Fig. 2), which correspond to the late to mid Holocene (present to 8.3 cal. ka BP), early Holocene to Late Glacial (8.3 to 17.2 cal. ka BP), Last Glacial Maximum (LGM) and Last Glacial period (17.2 to 51.2 cal. ka BP). The lithology documents distinct changes in sedimentation patterns from pelagic sediments (i.e. constant settling) at the top to frequent mass movement deposits at the base (Kliem *et al.* 2013). Because it is dominated by gravels, the fifth unit covering the lowermost 13 m of core 5022-1D is not displayed. Present results only focus on pelagic sediments from which DNA sequences were retrieved. Complete description of the core lithology and stratigraphy is published elsewhere (Vuillemin *et al.* 2013b, 2014a).

The average TOC content is very low in sediments of the deep glacial record (Fig. 2), often with values below 0.3% dry weight. However, TOC values display some minor peaks around 35 m, whereas the highest TOC content (2%) is observed around 10 m depth, corresponding to the Late Glacial-Holocene transition and an important redeposition event (Anselmetti *et al.* 2009). Overall, TOC content is higher in the Holocene (0.5 to 1.0%) than the Last Glacial record (<0.3%). OP displays a similar trend as TOC with minor peaks around 35 m depth in the deep glacial record (Fig. 2) and values increasing within the Late Glacial transition and fluctuations in the Holocene record. Maximum values for OP are found in the uppermost sediments.

For pore water concentrations (Fig. 2), nitrate/nitrite exhibits values lower than 0.4 mg L^{-1} without any relevant maxima or minima. Dissolved phosphate concentrations are mostly zero in the lower part of the record, showing only minor increases at 55 and 45 m, whereas concentrations range between 5 and 10 mg L^{-1} in Holocene sediments. Sulfate concentrations display three sharp peaks (ca. 1500, 1200 and 900 mg L^{-1}) around 50, 40 and 28 m depth, respectively, that correspond with sandy basaltic layers. Mafic volcanics reworked from basaltic tephra in the catchment act as main supply of sulfur during mass movements (Ross *et al.* 2010), which can presently explain the three sulfate peaks. Concentrations for the rest of the sediment column average 300 mg L^{-1} with particularly low values in the interval covering the Late Glacial and early Holocene. Values then increase toward the surface. For comparison, lake surface water samples measured from 2002 to 2004 display mean values of 1.6 mg L^{-1} for nitrate/nitrite, 2384 mg L^{-1} for orthophosphate and 26.9 mg L^{-1} for sulfate, and thus correspond to N-limiting conditions (Zolitschka *et al.* 2006).

ATP assays, total cell counts and alpha diversity

In situ ATP measurements (Fig. 2) show small peaks of activity in specific layers (ca. 34–29 m depth) of the LGM, with potentially residual activity at the base of the record. Along the Late Glacial-Holocene transition, ATP values display a rapid increase from ca. 30 to 110 RLU at 5 m depth and then increase towards the surface to a maximal value of 150 RLU at 0.5 m depth.

DAPI cell counts (Fig. 2) show that cell densities are lowest in the deepest part of the glacial record (10^7 cells g^{-1}), decreasing two orders of magnitude from ca. 10^9 cells g^{-1} at the surface. Minimal ($10^{7.5}$ cells g^{-1}) and maximal ($10^{9.4}$ cells g^{-1}) cell densities are, respectively, found at 70.8 and 0.3 m depths. Cell counts decrease slightly within the Late Glacial transition and reach an order of magnitude higher within the Holocene record (10^9 cells g^{-1}). From 40 to 60 m depth, cell counts decrease by an order of magnitude and remain constant throughout the LGM (ca. 10^8 cells g^{-1}). However, one has to take into account that DAPI staining does not allow the distinction between active, inert, dead and damaged cells that possibly accumulated in the sediment.

We normalized cell densities by the relative abundances of total Archaea and Bacteria. The two resulting profiles (Fig. 2) show that bacterial populations are generally denser than the archaeal ones, notably in Holocene sediments at ca. 5 m depth and within the uppermost meter. Below 10 m depth, both profiles run in parallel, displaying very close values. We applied the same normalization for the main taxonomic groups identified (i.e. Atribacteria, Aminicenantes, Chloroflexi, Deltaproteobacteria, Bathyarchaeota and Thermoplasmata) and compare them with alpha diversity profiles (Supporting Information).

Relative abundances of taxa

Sequencing depths used to plot relative abundances of taxa are all above 12 000 reads after trimming and chimera removal, and often reach more than 110 000 reads (Fig. S1, Supporting Information). Bar charts plotted for the uppermost meter of sediment (Fig. 3) display a depth trend corresponding to increasing relative abundances of Thermoplasmata, Dehalococcoidia, Aminicenantes and Atribacteria, and decreasing abundances of Anaerolineae and Deltaproteobacteria. Relative abundances of Acetothermia display a marked increase between 0.6 and 0.8 m depth, whereas the fractions of Planctomycetes, Alphaproteobacteria Actinobacteria and candidate SC4 remain rather constant with depth. Other groups of taxa represent minor fractions but, nevertheless, display an overall decrease in relative abundances of Thaumarchaeota, Acidobacteria, Bacteroidetes, Spirochaetes and Latescibacteria and even the disappearance of Chlorobi, Gemmatimonadetes, Nitrospirae and Omnitrophica with depth.

Down through the mid-Holocene and early Holocene record, relative abundances of Hadesarchaea, Dehalococcoidia, Clostridia, Elusimicrobia and Atribacteria significantly increase with depth. Aminicenantes and Planctomycetes become relatively less abundant, whereas Thermoplasmata, Deltaproteobacteria are rarely present minor and Alphaproteobacteria are even absent at these depths (5 to 10 m).

At the Late Glacial boundary, Bathyarchaeota become rapidly the dominant taxa, reaching up to 40% of all taxa below 10 m depth. Below the Late Glacial sediments, patterns of relative abundances do not vary consequently anymore. Throughout the glacial record, relative abundances are largely dominated
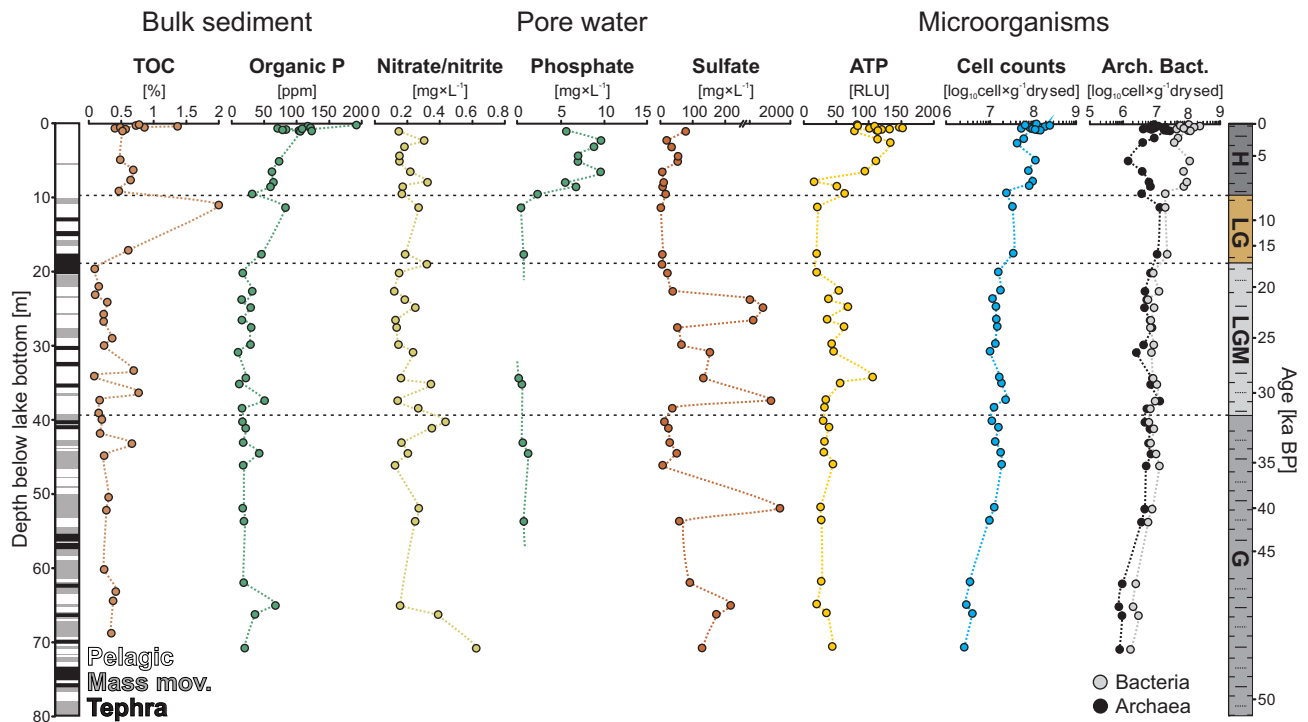
Figure 2. Multiple downcore profiles. From left to right: Lithology sequence of Laguna Potrok Aike [m] describing pelagic, mass movement and tephra sediments (after Kliem *et al.* 2013); total organic carbon [% weight] and organic phosphorus [ppm] contents in bulk sediment; pore water concentrations for nitrate/nitrite, phosphate and sulfate [mg × L^{-1}]; ATP assays in RLU; DAPI-stained total cell counts [log$_{10}$ cells × g^{-1} dry sed]; and total cell counts normalized to the relative abundances of Archaea and Bacteria [log$_{10}$ cells × g^{-1} dry sed]; stratigraphic units [ka BP] corresponding to climatic periods of the Holocene (H), Late Glacial (LG), (LGM), and Last Glacial (G).

by Bathyarchaeota, Hadesarchaea, Chloroflexi, Elusimicrobia, Aminicenantes, Atribacteria and Planctomycetes. Groups of taxa that are constantly present at low abundances include Acidobacteria, Actinobacteria, Deltaproteobacteria and candidate SC4, whereas Clostridia disappear in LGM sediments. Minor fluctuations can be noticed in the relative abundances of candidates AC1 and LD1, Bacteroidetes and Latescibacteria.

Statistical analyses, OTU distribution with age and phylogenetic trees

The Taxa S index (Fig.S2, Supporting Information), or richness reported as total number of OTUs, fluctuates throughout most the deep glacial record, with highest values (>100) located at 46, 31 and 18 m depth. Overall, the richness of microbial populations is systematically higher in Holocene sediments, with maximal values located around the late glacial transition (118) and in the uppermost sediment (161). The Shannon and Evenness indices display similar profiles to the Taxa S one, with some scatter of values in the LGM interval. Four intervals can be observed with transitions occurring at ca. 45, 18 and 0.5 m depth. These transitions mirror shifts in population densities related to the main taxonomic groups (Fig. S2, Supporting Information).

The NMDS calculation is based on 789 OTUs corresponding to 389 OTUs from gravity core no. 5022-1J and 538 OTUs for hydraulic piston core no. 5022-1D, with 138 OTUs common to both cores. The NMDS results were plotted in a two-dimensional space with coordinates 1 and 2 respectively corresponding to 79.4% and 25.2% and a resulting stress value of 0.1145 (Fig. 4). According to coordinate 1, the NMDS plot shows that sample distribution is strongly depth-dependent. Surface sediments are

located at the top of the plot, whereas deep samples stand out on the bottom part. The deep samples display a concentric distribution with increasing depth, accounting for similar OTU compositions with an overall diversity decreasing from the late glacial down to the LGM and Last Glacial sediment.

The heat map (Fig. 4) shows that populations of Bathyarchaeota, Atribacteria and Aminicenantes are clearly distinct in sediments corresponding to the Holocene and Last Glacial period. The diminishing or increasing presence of certain OTUs points at a selection process with depth and over time (horizontal axis) as minor elements of the community detectable at the surface become major components at depth, resulting in a nested community (Baselga 2010). In addition, normalized cell density profiles clearly show that populations of Atribacteria and Aminicenantes increase within mid-Holocene sediments (ca. 5 m) and subsequently decrease in concomitance with the increase of Bathyarchaeota at ca. 10 m depth (Fig S2, Supporting Information). This transition occurs in sediments of the Late Glacial and is visible on the heat map as well, suggesting that certain populations thrive better in specific stratigraphic intervals, but that the loss or replacement of taxa in terms of spatial turnover is limited. Otherwise, the provenance of the reference OTUs (i.e. closest match on the SILVA database) does not appear to play a significant role in the clustering of sequences since those initially identified from marine, freshwater, groundwater and soil environments are found together (vertical axis).

Extraction of representative sequences resulted in 593 reference OTUs among Bacteria and 77 reference OTUs among Archaea. Separate bacterial (Fig. S3, Supporting Information) and archaeal (Fig. S4, Supporting Information) phylogenetic trees allowed for detailed sequence clustering of classes and
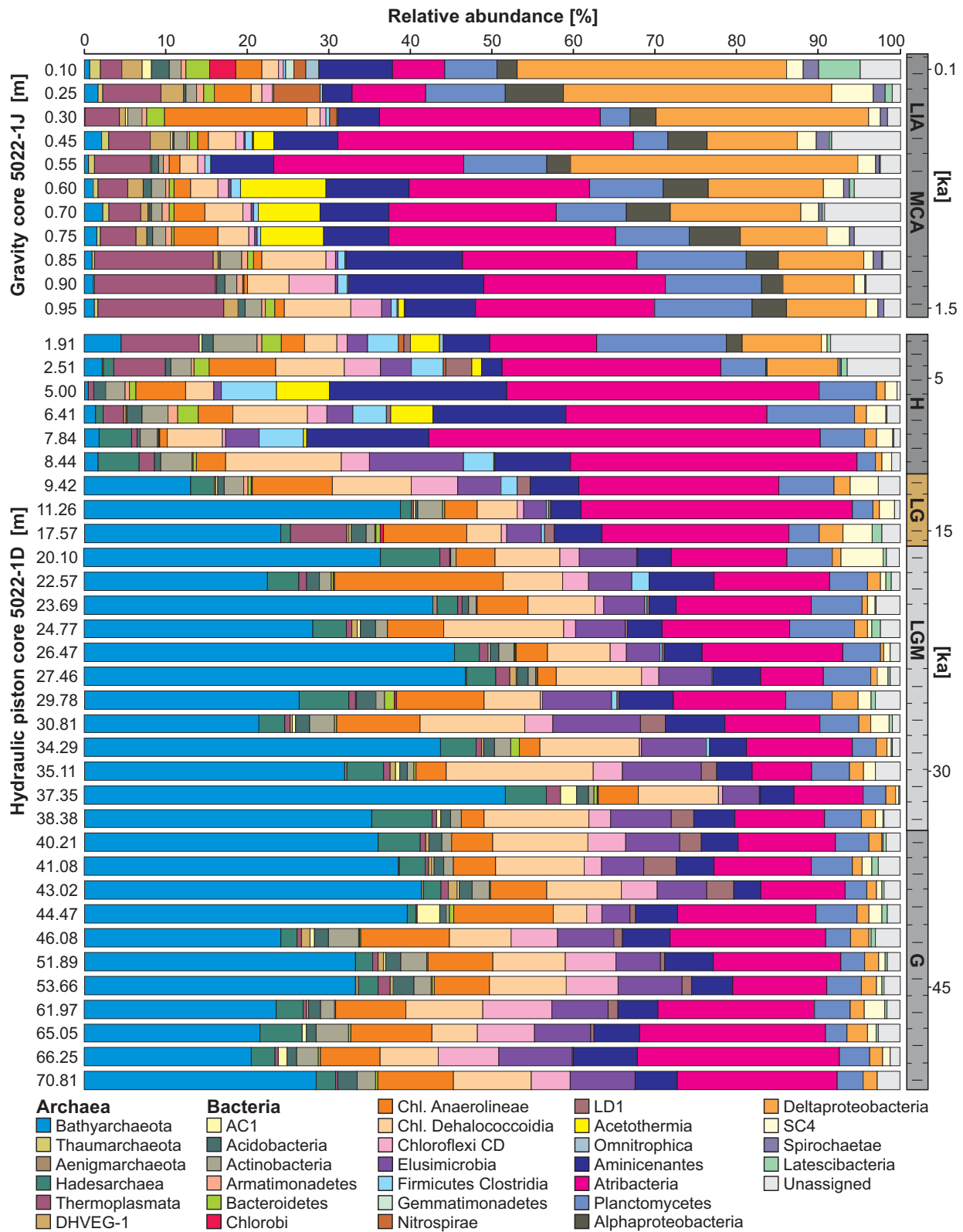
Figure 3. Bar charts for relative abundances of taxa with depth. Top: Bar charts for gravity core 5022-1J indicate that most abundant taxa are related to Thermoplasmata, Aminicenantes, Atribacteria, which abundances increase with depth, whereas those of Planctomycetes and Deltaproteobacteria decrease with depth. Bottom: Bar charts for hydraulic piston core 5022-1D display a clear shift in microbial populations at the Holocene-Late Glacial transition, corresponding with an increase in abundances of Bathyarchaeota and Chloroflexi. In LGM and Last Glacial sediment, patterns of microbial abundances remain fairly constant.
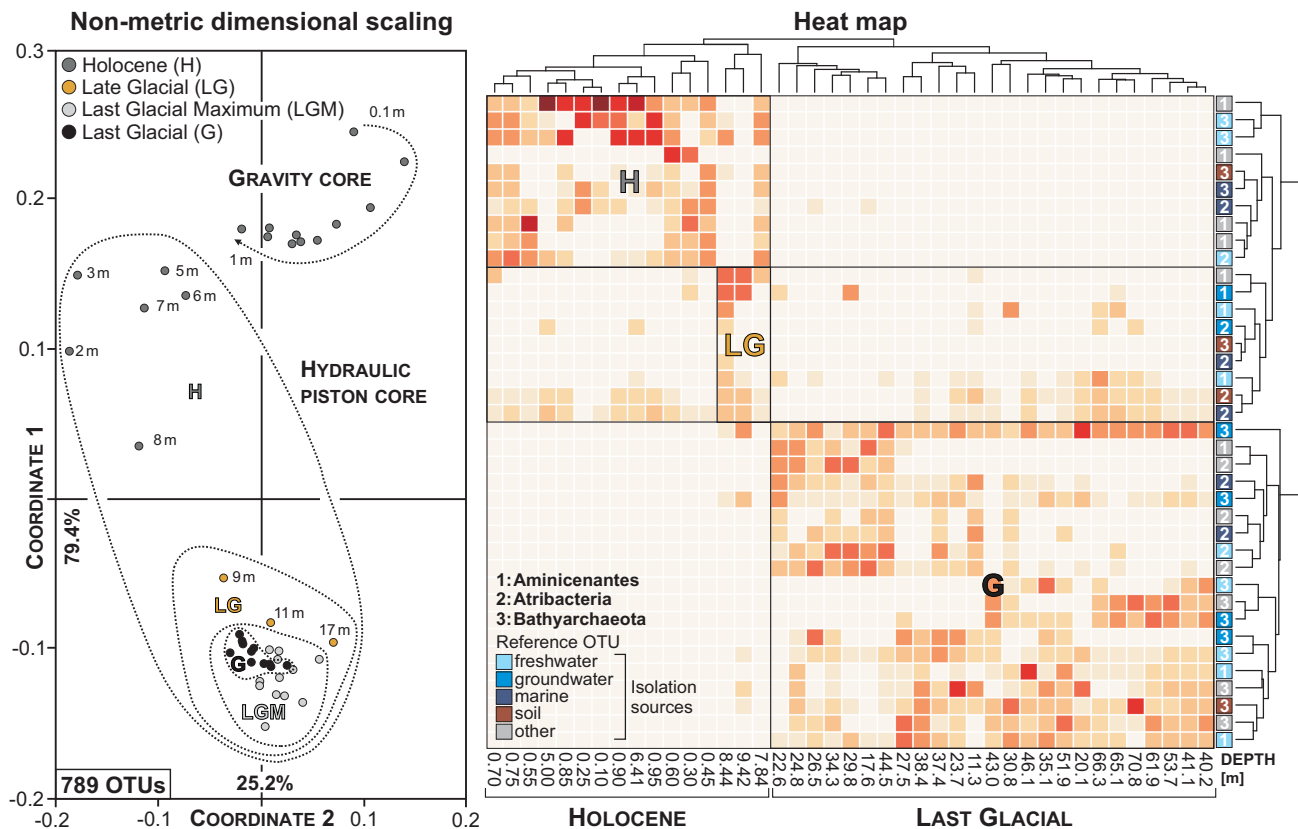
Figure 4. Non-metric multidimensional scaling plot and heat map. Left: The NMDS plot shows that sample distribution is strongly depth dependent. Surface sediments are located on the top of the plot, whereas deep samples stand out at the bottom. They display a concentric distribution with increasing depth, accounting for similar OTU distributions of an overall decreasing diversity. Right: The heat map obtained for the Bathyarchaeota, Atribacteria and Aminicenantes groups shows that the main OTUs present in Holocene sediments are selected against with depth, resulting in a very different population in sediments of the Last Glacial record. Dendrograms were computed using UPGMA. The color code (right) indicates the isolation source of the reference OTUs.

candidate divisions. Most diverse and represented groups among *Bacteria* in decreasing order are as follows: Chloroflexi, Planctomycetes, Deltaproteobacteria, Aminicenantes and Acidobacteria (Fig. S5, Supporting Information). These groups are found at all depths of the sediment sequence. In comparison, Alphaproteobacteria are only present in the Holocene record, while Clostridia disappear around 20 m depth. Although abundant and persisting with depth, the Atribacteria display a low diversity, whereas the presence of Bacteroidetes, Latescibacteria, Omnitrophica and Spirochaetae appears rather sketchy throughout the record (Fig. 3). The remaining representative bacterial sequences were resolved in 23 different single clusters (Figs S3 and S5, Supporting Information). Most diverse archaeal groups are the Bathyarchaeota and Thermoplasmata, which are more abundant in the Last Glacial and Holocene record, respectively (Fig. S2, Supporting Information).

The distribution of taxa with sediment age (Figs 2 and 5) reveals an overall decrease of richness and diversity towards the oldest sediments. In surface sediments, the dominant groups of taxa in terms of OTU number are first the Deltaproteobacteria but shift to Chloroflexi candidates in lowermost sediments, corresponding to ca. 1.5 ka years at the bottom of the gravity core (Vuillemin *et al.* 2013a). In the mid-Holocene sediment, Dehalococcoidia and Planctomycetes dominate the taxa distribution along with an increasing presence and diversity of Anaerolineae and Atribacteria, whereas Deltaproteobacteria clearly diminish. In Last Glacial sediments, Bathyarchaeota, Planctomycetes and the diverse Chloroflexi become dominant taxa, whereas Atribacteria tend to recede. The number of OTUs that are common to samples from different depths is systematically higher between samples that are closest, whereas it decreases exponentially in relation to surface samples (Fig. S6, Supporting Information). Altogether, all samples located within the Holocene and Last Glacial sequences are, respectively, more similar to each other. A clear transition occurs within the Late Glacial interval as already emphasized by the relative abundances of taxa (Fig. 3), the NMDS plot and heat map (Fig. 4).

DISCUSSION

Organic sources and geochemical conditions related to lake hydrology

Microbial communities in Holocene sediments were most diverse, reflecting a layering of taxa linked to electron acceptors and OM availability. In deeper intervals, environmental features over the Last Glacial-interglacial cycle appeared as selective pressures in the deep lacustrine assemblage, resulting in a genetically distinct biosphere from the surface (Figs 2 and 3). Because the establishment of a deep subsurface community likely results from a combination of paleoenvironmental factors and substrate depletion with depth, we proceed below to describe past climatic conditions that have shaped the pool of
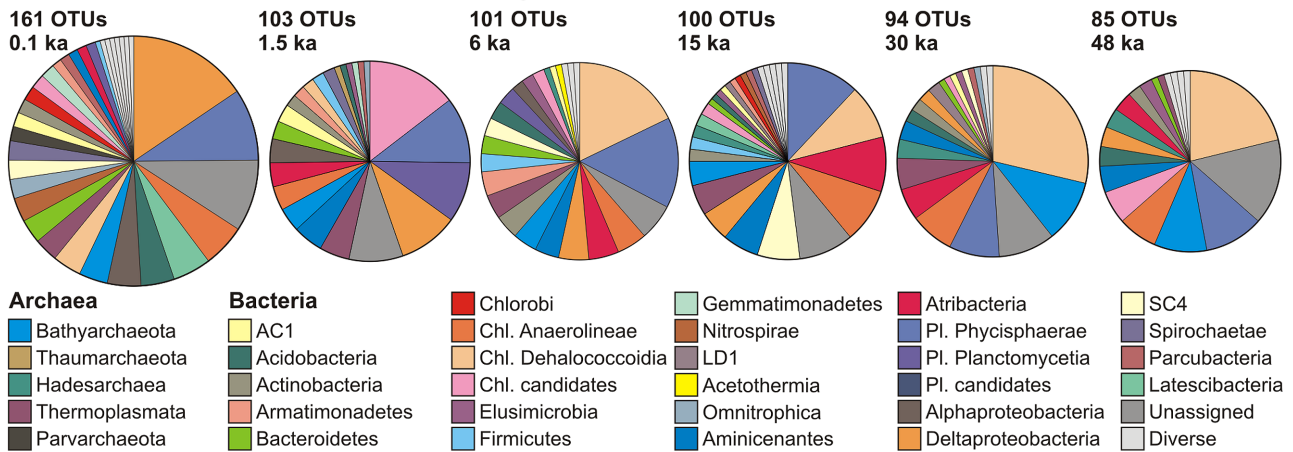
Taxa distribution with sediment age

Figure 5. Pie charts proportional to the number of taxonomic operational units with sediment age. The distribution of total number of OTUs at 0.1, 1.5, 6, 15, 30 and 45 ka years before present (left to right) shows that the overall richness of microbial populations decreases with depth. Taxonomic groups displaying highest OTU numbers are successively represented by Deltaproteobacteria, Chloroflexi, Planctomycetes and Bathyarchaeota and reflect the selection of a deep biosphere over time. Unassigned taxa correspond to recent candidate divisions that were resolved in two phylogenetic trees (Figs S3 and S4, Supporting Information).

available substrates and link them to the taxonomic composition of benthic microbial communities in the successive stratigraphic intervals.

Paleoreconstructions showed that the lake level of Laguna Potrok Aike was high from 51 ka until the early Holocene (i.e. 80–10 m sediment depth) when the Westerly winds were located further to the north (Zolitschka *et al.* 2013), which resulted in less evaporation and lower global temperatures in Southern Patagonia (Waldmann *et al.* 2009) and, thus, a positive water balance of the lake (Ohlendorf *et al.* 2013). Due to the presence of permafrost and reduced vegetation in the catchment (Haberzettl *et al.* 2009; Hahn *et al.* 2013), this period of active hydrology (Kliem *et al.* 2013), with both overflow and inflows into the lake (Fig. 1), promoted reworking and dispersal of soils and tephra layers to the lake (Ross *et al.* 2010). The water column reflected freshwater conditions with low primary productivity with, nevertheless, short productivity events and punctual increases of sulfate in pore water due to the alteration of mafic volcanics (Fig. 2). Last Glacial sediments thus coincide with a very low organic content and frequent external inputs, which can comprise genomic elements reworked from the catchment, such as resting stages and extracellular DNA, which can be considered allochthonous to the lake system. The OM of this interval mostly consisting of eroded soils can be characterized as poorly reactive and, thus, more resistant to microbial utilization (Glombitza *et al.* 2013). Moreover, the extremely low concentrations of nitrate/nitrite and phosphate in pore water (Fig. 2) are expected to limit organic-fuelled respiration rates after burial (D'Hondt *et al.* 2015), whereas the available sulfate can fuel metabolic processes related sulfur cycling and potentially lithotrophy over time. The respective community assemblage is dominated by Bathyarchaeota and Chloroflexi (Fig. 3) but also includes persisting elements which have no apparent link to the subsurface biosphere (e.g. Acidobacteria, Actinobacteria, Bacteroidetes and Latescibacteria).

During the Late Glacial to the early Holocene (20-10 m sediment depth), the position of the Westerly winds shifted to the location of the lake (Pollock and Bush 2013). This initiated the driest period of the record at Laguna Potrok Aike (9.4 ka BP) with elevated wind evaporation and overall positive temperature excursion in South Patagonia (Waldmann *et al.* 2010). The

resulting negative water balance caused a pronounced lake level decrease of more than 50 m (Zolitschka *et al.* 2013) with a concomitant salinity increase in the water column (Vuillemin *et al.* 2013b). As the basin became endhorreic, nutrient exhaustion occurred in the water column and lacustrine conditions became nitrogen-limited (Mayr *et al.* 2009) with brief periods of stratification (Zolitschka *et al.* 2013). This sediment sequence thus represents a highly isolated system under brackish and N-limiting conditions (Recasens *et al.* 2015) with massive reworking of the surrounding sediments due to important mass movements (Anselmetti *et al.* 2009). Such increased sedimentation rates disrupted microbial communities in surface sediment (Vuillemin *et al.* 2014a) and resulted in organic-rich sediments but poorly active microbial communities (Fig. 2). This climatic transition is clearly reflected in the pattern of community assemblages (Figs 3 and 4), which from this point includes mostly bacteria (e.g. Firmicutes, Elusimicrobia, Aminicenantes and Atribacteria).

Two millennia later (7 ka BP; 8 m sediment depth), the Westerly winds diminished in intensity. The water balance became positive again and lake level rose gradually to a subsequent maximum during the Little Ice Age (0.3 m sediment depth). Inflows of nutrient stimulated primary productivity by algae and cyanobacteria under constant pelagic sedimentation, which promoted microbial colonization of the sediment by organotrophs (Vuillemin *et al.* 2014b). Different contributions of autochthonous and allochthonous OM to the lake sediment were observed according to rapid hydrological changes and short-term lake-level fluctuations (Mayr *et al.* 2009; Hockun *et al.* 2016). This Holocene interval corresponds to fine-grained sediments and elevated rates of OM deposition, leading to higher microbial cell densities and activity in this part of the record (Fig. 2). The increase of phosphate and depletion of nitrate/nitrite concentrations in pore water (Fig. 2) attest of important OM degradation processes (Villar *et al.* 1999), as only phosphate is released from labile OM into pore water and efficiently stimulates microbial growth (Smith and Prairie 2004). In lowermost Holocene sediments, sulfate is depleted, whereas CO_2 becomes largely available as a result of OM fermentation and can be reduced via syntrophic associations during acetogenesis and methanogenesis (Wüst *et al.* 2009; Morris *et al.* 2013).

This is supported by the decreasing abundance of Deltaproteobacteria with depth, followed by an increase in Thermoplasmata that are further supplanted by Aminicenantes and Atribacteria deeper in Holocene sediments (Fig. 3; Fig. S2, Supporting Information).

Selection of microbial communities during burial

Regardless of their geographic location, microbial communities in the subsurface are hypothesized to derive from dispersal of sedimentary microorganisms in the aquatic environment, with elements inherited from the photic and subphotic zone, and selection from within the community present in shallow sediment (Walsh *et al.* 2016). The dominant subsurface community is subsequently brought on by environmental selection due to vertical depletion of respiratory electron acceptors during burial (Froehlich *et al.* 1979). As a result, the taxonomic richness is generally highest in the bottom water and lowest in deep sediments as only a small fraction of the surface community persists and becomes predominant in the subsurface (Petro *et al.* 2017). These persisting taxa are expected to display metabolic characteristics related to energy limitation (e.g. versatility in substrate fermentation, carbon storage, DNA repair, sporulation) to be successful in the subsurface (Starnawski *et al.* 2017; Petro *et al.* 2017). However, some taxa present in deep biosphere communities have also been reported to reflect past depositional conditions, suggesting they have experienced weak selection after burial (Orsi *et al.* 2017).

Although deep biosphere activities can be resolved via transcriptomics (Orsi *et al.* 2013), present taxonomic assemblages of Laguna Potrok Aike are only interpreted in the light of recent findings from single-cell genomics (Rinke *et al.* 2013). The increasing abundance of certain taxonomic groups with depth (Fig. 3) and their predominance in the oldest recovered sediments (Fig. 5) is thought to result from their potential to cope with substrate limitation in syntrophic associations (Kappler and Bryce 2017). For instance, Bathyarchaeota have considerable ecophysiological divergence that includes protein fermentation, homoacetogenesis and methane production/oxidation (Solden *et al.* 2016). Hadesarchaea have genes involved in CO and H_2 production/oxidation potentially coupled to nitrogen cycling (Baker *et al.* 2016). Dehalococcoidia display important metabolic versatility in the oxidation of organic substrates with implication in sulfur cycling (Wasmund *et al.* 2014, 2016). Elusimicrobia are anaerobic fermenters with metabolic capacities involving nitrogenases (Zheng *et al.* 2016). Atribacteria are widespread primary and secondary fermenters adapted to syntrophic catabolism, energy conservation and carbon storage (Nobu *et al.* 2015). These inferred metabolisms are also consistent with the substrate characteristics described for the Last Glacial record (i.e. available sulfate, poorly reactive OM). These genera are already well known from deep marine environments (Rinke *et al.* 2013), thus arguing for a similar selection mechanism for microbial community composition in these ancient lacustrine sediments (Fig. 4).

In addition, taxa that seemed to enter dormancy after a phase of activity were identified by relative abundances increasing and subsequently decreasing with depth (Fig. 3). They include Thermoplasmata (i.e. candidates CCA47, TMEG and ASC21), Acetothermia, Clostridia, Nitrospirae, Aminicenantes and Deltaproteobacteria. Clostridia are well known spore formers (Yutin and Galperin 2013), while Deltaproteobacteria and Aminicenantes have energy conservative metabolisms that allow them to persist at depth as minor elements (Sharon *et al.* 2015). All these taxa are dominant in Holocene sediments and

are involved in heterotrophic associations during OM degradation. Dormancy and mortality being substrate-dependent (Ayati 2012; Kamagata 2015), their respective decrease in abundance seems to be function of the availability of electron acceptors (i.e. nitrate/nitrite, sulfate) and OM reactivity with depth (Fig. 2). Stratification of these species is indeed consistent with a transition from sulfate to CO_2 reduction and a stepwise degradation of labile OM (Vuillemin *et al.* 2014b). Substrate evolution along the Holocene record thus selects successive metabolisms, bringing with depth dormancy or disappearance of taxa not adapted to energy conservation and thereby selecting against them in the deep assemblages. Although lacustrine conditions during the Holocene and Last Glacial were clearly distinct, provenance and dispersal do not appear to significantly shape microbial communities as reference taxa for Bathyarchaeota, Atribacteria and Aminicenantes, all depths combined, relate to different environmental sources (e.g. marine, freshwater, groundwater, soil) (Fig. 4). Taxa whose relative abundances do not increase during burial (e.g. Planctomycetes and Actinobacteria) were thought to either reflect the presence of inactive cells preferentially preserved as resting stages, or to derive from extracellular DNA after cell lysis. Finally, those taxa that already make up only a minor fraction in surface sediment and rapidly disappear with depth could be linked to microbial communities typically associated with the water column. They were identified as Thaumarchaeota, Deep-Sea Hydrothermal Vent Group 1 (DHVEG-1), Chlorobi, Omnitrophica and potentially some Alphaproteobacteria.

Deep biosphere and allochthonous sources of DNA

Overall, microbial populations displayed an apparent trend reflecting the receding activity and slow death of microorganisms (Vuillemin *et al.* 2016) with a net decrease in cell abundance with depth (Fig. 2) during which the Bathyarchaeota and Chloroflexi became more abundant in the 16S datasets (Figs 3 and 5). ATP values in the Holocene sediment indicated ongoing microbial processes, whereas they pointed at a sustained but considerably lower level of microbial activity in sediments of the LGM (Fig. 2). Thus, these two indices show that the major taxa constituting the deep assemblage do not necessarily grow faster than the other bacteria, but rather are more adept to subsist in the deeper layers while the other bacteria slowly die off or are selected against as they face substrate limitation (Petro *et al.* 2017).

Surface samples indicated the presence of layered microbial communities (Nealson and Stahl 1997) as a result of depletion of respiratory electron acceptors and degradation of organic substrates. This layering is visible in variations of relative abundances related to known denitrifiers (i.e. Alphaproteobacteria), sulfate reducers (i.e. Deltaproteobacteria) and methanogens (i.e. Thermoplasmata) with depth (Fig. 3; Fig S2, Supporting Information). In parallel, relative abundances of primary and secondary fermenters increase with depth (i.e. Clostridia, Acetothermia, Aminicenantes, Atribacteria). Taxa that appear to be most active in early Holocene sediments are the Aminicenantes and Atribacteria, which become outnumbered by the Bathyarchaeota and Chloroflexi in sediments of the Last Glacial as cell density and activity decrease with depth (Fig. S2, Supporting Information). The rapid establishment of a deep biosphere community between 10 and 20 m depth matches the lake transition from a pelagic regime with high OM deposition to freshwater and oligotrophic conditions, corresponding to Last Glacial sediments in which substrate limitation appears to be the main factor of

selection (Starnawski *et al.* 2017). Most elements of this deep community assemblage (e.g. Bathyarchaeota, Hadesarchaea and Dehaloccoidia) are readily detectable as minor elements of the microbial community at the surface but tend to become more abundant with increasing depth, indicating that the deep biosphere represents a nested community selected from the surface (Baselga 2010). In contrast, only few taxa initially predominant in the shallow subsurface clearly persisted at this depth (Fig. 4).

Because DNA can be preserved in dead cells, resting stages or as an extracellular fraction (Vuillemin *et al.* 2017), establishing the taxonomy of microbial populations that are metabolically active in the sediment would require transcriptomes of ribosomal RNA (Amann and Ludwig 2000). Genomic elements derived from terrestrial, aquatic and sediment sources can indeed persist at different degrees of preservation to constitute a dominant fraction of environmental DNA in sedimentary systems (Vuillemin *et al.* 2017). As such, they represent ancient DNA, which sometimes reflect allochthonous sources reworked from the catchment (e.g. soil bacteria) or past communities from the water column (e.g. primary producers). For instance, Acidobacteria, Actinobacteria and Bacteroidetes, which are present throughout the sediment record, are major components of the soil microbiome and known to produce resting stages (Jansen *et al.* 2002). Such persisting cells can easily be reworked from terrestrial soils and transported to the lake during periods of higher precipitation and inflows, potentially reflecting hydrological conditions in the catchment. Since these cells do not resuscitate in the sediment, they undergo little selection during burial (Fig. S7, Supporting Information). Sequence screening for preserved phototrophs revealed a very low number of Cyanobacteria affiliated with chloroplasts and few Chlorobi in the uppermost sediment, taxa that rapidly disappeared from the record. Because Chlorobi are mostly obligate anaerobic phototrophs, their presence could be indicative of past stratification events (Coolen and Overmann 2007) but are hardly detectable in the present record. Our interpretation is that sequences from planktonic species are degraded at a very early stage, even starting in the water column during particle settling. Besides, it was suggested that Planctomycetes (e.g. *Phycisphaera, Pirellula*) act as scavengers after algal blooms, resulting in the rapid overprint of phototrophic by heterotrophic sequences (Lage and Bondoso 2014; Jeske *et al.* 2016). The transition from oxic to anoxic conditions at the water-sediment interface also highly selects against the main elements of the water column, and thus reduces the paleoenvironmental role of overlying sources of water (Orsi *et al.* 2017) in the establishment of the deep lacustrine biosphere over geological time. Finally, the role of extracellular DNA as a key trophic resource in deep ecosystem functioning (Dell'Anno and Danovaro 2005) would not allow for its long-term persistence in ancient lacustrine sediments (Vuillemin *et al.* 2017).

CONCLUSIONS

Altogether, we could observe the establishment of a genetically distinct deep subsurface community in lacustrine sediments resulting from the depletion of electron acceptors and lesser reactivity of organic substrates with depth, the persistence of dormant species and preservation of ancient resting stages. Although not in direct link with environmental sources, shifts in community composition matched the sediment stratigraphy and reflected the drastic change of lake regime during the Last Glacial-interglacial transition. Due to climatic variations, hydrology of the catchment of Laguna Potrok Aike appeared to

highly influence sediment geochemistry and organic substrates, which in turn determined the degree of microbial colonization at the time of deposition. Inflows to the lake promoted transport of resting stages from reworked surrounding terrestrial soils and the river bed, which were then preserved in the sediment, adding an allochthonous component to subsurface microbial community composition. Changes in physical and chemical conditions in the lake, like salinity, productivity and sedimentation regime, were main factors of selection after burial in that they controlled supply of the various nutrients for microbial life over geological time.

Microbial communities at the surface were most diverse and reflected a layering of heterotrophic taxa linked to the availability of respiratory electron acceptors and organic substrates. In contrast, planktonic species were rapidly degraded and disappeared from the record at an early stage. In 6 ka old organic-rich Holocene sediments, the community composition was dominated by fermentative heterotrophs, while main elements from the surface already became minor in the assemblage, thus correlating a period of increased primary productivity followed by gradual degradation of labile OM during burial. In 15 ka old glacial sediments, microbial communities shifted radically to taxa adapted for energy conservation, thus matching the important climate-related transition in the lake to freshwater and oligotrophic conditions. Most elements of this deep community assemblage were readily detectable at the surface but minor (e.g. Bathyarchaeota, Hadesarchaea, Chloroflexi, Elusimicrobia), while some taxa initially predominant in the shallow subsurface clearly persisted with depth either as a substantial (e.g. Aminicenantes, Atribacteria, Planctomycetes) or minor fraction (e.g. Thermoplasmata, Deltaproteobacteria), thus evidencing environmental selection of a deep subsurface community. Similarly to the marine environment, initial conditions associated with the lake's sedimentation rate and primary productivity were selective pressures that shaped the pool of available substrates over the Last Glacial-interglacial cycle and resulted in genetically distinct subsurface biospheres.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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