Microbial community composition along a 50,000 year lacustrine sediment sequence.

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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 preservation, 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 a 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, and 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 Martini 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 to 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 column not stratified in any season, displaying mean annual temperatures between 4 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 (BioTrace) with Aqua-Trace water testers 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 organic matter, pore water geochemistry, 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). 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 %.

50 mg of freeze-dried sediment were used for the successive measurement of inorganic (IP) and organic (OP) phosphorus (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 Perkin Elmer U-VIS λ25 photometer.

Pore water samples were obtained from core no. 5022-1A using soil moisture samplers (Rhizon, Eijkelkamp) 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) at the Geobio-Center, Munich. Based on a 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 × 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 × PBS, centrifuged and suspended in 1 mL 1×PBS-ethanol (1:1). For DAPI staining, aliquots were mixed to a solution of 2 mL 1 × 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 (Schleicher and Schuell BA85, Ø 25mm, 0.45 µm pore-size), and rinsed with 2 mL 1 × 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 1mL of sediment using the DNA PowerSoil Isolation kit (MoBio). 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) 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 8 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 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).

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 following the manufacturer's instructions. The library was validated by qPCR using the KAPA Library Quantification Kit (Kapa Biosystems) following the manufacturer's manual. Final concentration was quantified by 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 et al. 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 et al., 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 et al. 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, 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 et al. 2005). 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 (Supplementary Figs. S1-S3).

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 5 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, 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 %). Organic phosphorus 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, alpha diversity

In situ ATP measurements (Fig. 2) show small peaks of activity in specific layers (ca. 34–29 m depth) of the Last Glacial Maximum (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 respectively. 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, Thermoplasmata) and compare them with alpha diversity profiles (Supplementary material).

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 (Supplementary Fig. S1). 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 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 (Supplementary Fig. S2), 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 (Supplementary Fig. S2).

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 (Supplementary Fig S2). 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 (Supplementary Fig. S3) and archaeal (Supplementary Fig. S4) phylogenetic trees allowed for detailed sequence clustering of classes and candidate divisions. Most diverse and represented groups among *Bacteria* in decreasing order are: Chloroflexi, Planctomycetes, Deltaproteobacteria, Aminicenantes and Acidobacteria (Supplementary Fig. S5). 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 (Supplementary Figs. S3 and S5). Most diverse archaeal groups are the Bathyarchaeota and Thermoplasmata, which are more abundant in the Last Glacial and Holocene record, respectively (Supplementary Fig. S2).

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 (Supplementary Fig. S6). 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 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. 2010) 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, 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, 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; Supplementary Fig. S2).

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 which 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. 2016). 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, 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; Supplementary Figs. S2). 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 (Supplementary Fig. S2). 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 (Starnawsky et al. 2017). Most elements of this deep community assemblage (e.g. Bathyarchaeota, Hadesarchaea, 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 (Supplementary Fig. S7). Sequence screening for preserved phototrophs revealed a very low number of Cyanobacteria affiliated with chloroplasts and few Chlorobi in the uppermost sediment, taxa which 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.

Funding

This work was supported by funds from the following institutions: International Continent Scientific Drilling Program; University of Geneva and Swiss National Science Foundation, Switzerland (grant no. 200020-119931/2 to D. Ariztegui; grant no. P2GEP2_148621 to A. Vuillemin); University of Bremen and Deutsche Forschungsgemeinschaft, Germany (DFG Project OR 417/1-1 to W. Orsi; grant no. VU 94/1-1 to A. Vuillemin); Natural Sciences and Engineering Research Council, Canada; University of Buenos Aires and the Argentinean Research Council (CONICET), Argentina; the Vetenskapsrädet of Sweden; and the GFZ German Research Centre for Geosciences, Potsdam, Germany.

Acknowledgements

The supervision during Illumina MiSeq procedure of J. Pawlowski, M. Holzmann, L. Perret-Gentil, E. Reo and their research partners at the University of Geneva (Switzerland) is kindly acknowledged. We also thank C. Mayr and S. Becker for performing pore water sampling and analysis, A. Lücke and H. Wissel for carrying out the TOC measurements, D. Wagner for administrative and financial support, and M. Winkel for his help on the use of ARB platform.

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References

Amann R, Ludwig W. Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. FEMS Microbiol Rev 2000; 24: 555-565. doi: 10.1111/j.1574-6976.2000.tb00557.x

Anselmetti F, Ariztegui D, De Batist M, Gebhardt C, Haberzettl T, Niessen F, Ohlendorf C, Zolitschka, B. Environmental history of southern Patagonia unraveled by the seismic stratigraphy of Laguna Potrok Aike. *Sedimentology* 2009; **56**: 873–92. doi: 10.1111/j.1365-3091.2008.01002.x

Ariztegui D, Thomas C, Vuillemin A. Present and future of subsurface studies in lacustrine sediments through scientific drilling. *Int J Earth Sci* 2015; **104**: 1655–65. doi: 10.1007/s00531-015-1148-4

Ayati BP. Microbial dormancy in batch cultures as a function of substrate-dependent mortality. *J Theor Biol* 2012; **293**: 34-40. doi:10.1016/j.jtbi.2011.10.003

Baker BJ, Saw JH, Lind AE, Lazar CS, Hinrichs K-U, Teske AP, Ettema TJG. Genomic inference of the metabolism of cosmopolitan subsurface Archaea, Hadesarchaea. *Nat Microbiol* 2016; **1**: 16002. doi:10.1038/nmicrobiol.2016.2

Baselga A. Partitioning the turnover and nestedness components of beta diversity. *Global Ecol Biogeogr* 2010; **19**: 134-143. doi: 10.1111/j.1466-8238.2009.00490.x

Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics* 2014; **30**: 2114-20. doi: 10.1093/bioinformatics/btu170

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Gonzalez Peña A, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; **7**: 335-6. doi: 10.1038/nmeth.f.303

Colwell FS, D'Hondt S. Nature and extent of the deep biosphere. *Rev Mineral Geochem* 2013; **75**: 547-74. doi: 10.2138/rmg.2013.75.17

Coolen MJ, Cypionka H, Sass AM, Sass H, Overmann J. Ongoing modification of Mediterranean Pleistocene sapropels mediated by prokaryotes. *Science* 2002; **296**; 2407-10. doi: 10.1126/science.1071893

Coolen MJ, Overmann J. 217 000-year-old DNA sequences of green sulfur bacteria in Mediterranean sapropels and their implications for the reconstruction of the paleoenvironment. *Environ Microbiol* 2007; **9**: 238-249. doi: 10.1111/j.1462-2920.2006.01134.x

Coronato A, Ercolano B, Corbella H, Tiberi P. Glacial, fluvial and volcanic landscape evolution in the Laguna Potrok Aike maar area, Southern Patagonia, Argentina. *Quaternary Sci Rev* 2013; **71**: 13–26. doi: 10.1016/j.quascirev.2012.06.019

Dell'Anno A, Danovaro R. Extracellular DNA plays a key role in deep-sea ecosystem functioning. *Science* 2005; **309**: 2179. doi: 10.1126/science.1117475

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microb* 2006; **72**: 5069-72. doi: 10.1128/AEM.03006-05

D'Hondt S, Inagaki F, Alvarez Zarikian C, Abrams LJ, Dubois N, Engelhardt T, Evans H, Ferdelman T, Gribsholt B, Harris RN, Hoppie BW, Hyun J-H, Kallmeyer J, Kim J, Lynch JE, McKinley CC, Mitsunobu S, Morono Y, Murray RW, Pockalny R, Sauvage J, Shimono T, Shiraishi F, Smith DC, Smith-Duque CE, Spivack AJ, Steinsbu BO, Suzuki Y, Szpak M, Toffin L, Uramoto G, Yamaguchi YT, Zhang Gl, Zhang X-H, Ziebis W. Presence of oxygen and aerobic communities from sea floor to basement in deep-sea sediments. *Nat Geoscience* 2015; **8**: 299-304. doi: 10.1038/NGEO2387

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010; **26**: 2460-61. doi: 10.1093/bioinformatics/btq461

Edwards KJ, Becker K, Colwell F. The Deep, dark energy biosphere: Intraterrestrial life on Earth. *Nat Rev Microbiol* 2012; **40**: 551-68. doi: 10.1146/annurev-earth-042711-105500

Froelich PN, Klinkhammer GP, Bender ML, Luedtke NA, Heath GR, Cullen D, Dauphin P, Hammond D, Hartman B, Maynard V. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim Cosmochim Acta* 1979; **43**: 1075–90.

Gebhardt AC, Ohlendorf C, Niessen F, De Batist M, Anselmetti FS, Ariztegui D, Kliem P, Wastegård S, Zolitschka B. Seismic evidence of up to 200 m lake-level change in Southern Patagonia since MIS 4. *Sedimentology* 2012; **59**: 1087–100. doi: 10.1111/j.1365-3091.2011.01296.x

Glombitza C, Stockhecke M, Schubert CJ, Vetter A, Kallmeyer J. Sulfate reduction controlled by organic matter availability in deep sediment cores from the saline, alkaline Lake Van (Eastern Anatolia,Turkey). *Front Microbiol* 2013; **4**: 12. doi: 10.3389/fmicb.2013.00209

Haberzettl T, Mayr C,Wille M, Zolitschka B. Linkages between southern hemisphere Westerlies and hydrological changes in semi-arid Patagonia during the last 16,000 years. *PAGES News* 2007; **15**: 22–3.

Haberzettl T, Anselmetti FS, Bowen SW, Fey M, Mayr C, Zolitschka B, Ariztegui D, Mauz B, Ohlendorf C, Kastner S, Lücke A, Schäbitz F, Wille M. Late Pleistocene dust deposition in the Patagonian steppe – extending and refining the paleoenvironmental and tephrochronological record from Laguna Potrok Aike back to 55 ka. *Quaternary Sci Rev* 2009; **28**: 2927–39. doi: 10.1016/j.quascirev.2009.07.021

Hammer Ø, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol Electron* 2001; **4**: 1-9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hein AS, Hulton NRJ, Dunai TJ, Sugden DE, Kaplan MR, Xu S. The chronology of the Last Glacial Maximum and deglacial events in central Argentine Patagonia. *Quaternary Sci Rev* 2010; **29**: 1212–27. doi: 10.1016/j.quascirev.2010.01.020

Hoehler TM, Jørgensen BB. Microbial life under extreme energy limitation. *Nat Rev Microbiol* 2013; **11**: 83-94. doi: 10.1038/nrmicro2939

Hockun K, Mollenhauer G, Ho SL, Hefter J, Ohlendorf C, Zolitschka B, Mayr C, Lücke A, Schefuß E. Using distributions and compound-specific isotopes of n-alkanes to disentangle organic matter contributions to sediments of Laguna Potrok Aike, Argentina. *Org Geochem* 2016; **102**: 110-119. doi: 10.1016/j.orggeochem.2016.10.001

Hughes Martiny JB, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Adams Krumins J, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach A-L, Smith VH, Staley JT. Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol* 2006; **4**: 102-12. doi:10.1038/nrmicro1341

Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K, Nealson KH, Horikoshi K. Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Appl Environ Microb* 2003; **69**: 7224–35. doi: 10.1128/AEM.69.12.7224-7235.2003

Inagaki F, Nunoura T, Nagakawa S, Teske A, Lever M, Lauer A, Suzuki M, Takai K, Delwiche M, Colwell FS, Nealson KH, Horikoshi K, D'Hondt S, Jørgensen BB. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *P. Natl. Acad. Sci. USA* 2006; **103**: 2815–20. doi: 10.1073/pnas.0511033103

Inagaki F, Hinrichs K-U, Kubo Y, Bowles MW, Heuer VB, Hong W-L, Hoshino T, Ijiri A, Imachi H. Exploring deep microbial life in coal-bearing sediment down to ~2.5 km below the ocean floor. *Science* 2015; **349**: 420-4. doi:10.1126/science.aaa6882

Janssen PH, Yates PS, Grinton BE, Taylor PM, Sait M. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. *Appl Environm Microbiol* 2002; **68**: 2391–96. doi: 10.1128/AEM.68.5.2391–2396.2002

Jeske O, Surup F, Ketteniß M, Rast P, Förster B, Jogler M, Wink J, Jogler C. Developing techniques for the utilization of Planctomycetes as producers of bioactive molecules. *Front Microbiol* 2016; **7**: 1242. doi: 10.3389/fmicb.2016.01242

Jørgensen BB, Boetius A. Feast and famine - microbial life in the deep-sea bed. *Nat Rev Microbiol* 2007; **5**: 770-81. doi: 10.1038/nrmicro1745

Jørgensen BB, Marshall IP. Slow Microbial Life in the Seabed. *Annu Rev Mar Sci* 2016; **8**: 311-32. doi: 10.1146/annurev-marine-010814-015535

Kallmeyer J, Pockalny R, Adhikari RR, Smith DC, D'Hondt S. Global distribution of microbial abundance and biomass in subseafloor sediment. *P Natl Acad Sci USA* 2012; **109**: 16213-6. doi: 10.1073/pnas.1203849109

Kallmeyer J, Grewe S, Glombitza C, Kitte JA. Microbial abundance in lacustrine sediments: A case study from Lake Van, Turkey. *Int J Earth Sci* 2015; **104**: 1667–77. doi: 10.1007/s00531-015-1219-6

Kamagata Y. Keys to cultivating uncultured microbes: elaborate enrichment strategies and resuscitation of dormant cells. *Microbes Environ* 2015; **30**: 289–90. doi: 10.1264/jsme2.ME3004rh

Kappler A, Bryce C. Cryptic biogeochemical cycles: Unravelling hidden redox reactions. *Environ Microbiol* 2017; **19**: 842-6. doi: 10.1111/1462-2920.13687

Kastner S, Ohlendorf C, Haberzettl T, Lücke A, Mayr C, Maidana N I, Schäbitz F, Zolitschka B. Southern hemispheric westerlies control the spatial distribution of modern sediments in Laguna Potrok Aike, Argentina. *J Paleolimnol* 2010; **44**: 887–902. doi: 10.1007/s10933-010-9462-0

Kliem P, Enters D, Hahn A, Ohlendorf C, Lisé-Pronovost A, St-Onge G, Wastegård S, Zolitschka B, PASADO Science Team. Lithology, radiocarbon chronology and sedimentological interpretation of the lacustrine record from Laguna Potrok Aike, southern Patagonia. *Quaternary Sci Rev* 2013; **71**: 54–69. doi: 10.1016/j.quascirev.2012.07.019

Lage OM, Bondoso J. Planctomycetes and macroalgae, a striking association. *Front Microbiol* 2014; **5**: 1–9. doi: 10.3389/fmicb.2014.00267

Lennon JT, Jones SE. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat Rev Microbiol* 2011; **9**: 119-30. doi: 10.1038/nrmicro2504

Loizeau J-L, Span D, Coppee V, Dominik J. Evolution of the trophic state of Lake Annecy (eastern France) since the last glaciation as indicated by iron, manganese and phosphorus speciation. *J Paleolimnol* 2001; **25**: 205-14. doi: 10.1023/A:1008100432461

Lozupone CA, Knight R. Global patterns in bacterial diversity. *P Natl Acad Sci USA* 2007; **104**: 11436-40. doi: 10.1073/pnas.0611525104

Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar, Buchner A, Lai T, Steppi S, Jobb G, Förster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, König A, Liss T, Lüssmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A,

Schleifer KH. ARB: a software environment for sequence data. *Nucleic Acids Res* 2004; **32**: 1363-71. doi: 10.1093/nar/gkh293

Macalady JL, Hamilton TL, Grettenberger CL, Jones DS, Tsao LE, Burgos WD. Energy, ecology and the distribution of microbial life. *Philos Trans R Soc Lon B Biol Sci* 2013; **368**: 20120383. doi: 10.1098/rstb.2012.0383

Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 2011; **17**: 10-2. doi: 10.14806/ej.17.1.200

Mayr C, Wille M, Haberzettl T, Fey M, Janssen S, Lücke A, Ohlendorf C, Oliva G, Schäbitz F, Schleser GH, Zolitschka B. Holocene variability of the Southern Hemisphere westerlies in Argentinean Patagonia (52° S). *Quaternary Sci Rev* 2007; **26**: 579–84. doi: 10.1016/j.quascirev.2006.11.013

Mayr C, Lücke A, Maidana NI, Wille M, Haberzettl T, Corbella H, Ohlendorf C, Schäbitz F, Fey M, Janssen S, Zolitschka B. Isotopic fingerprints on lacustrine organic matter from Laguna Potrok Aike (southern Patagonia, Argentina) reflect environmental changes during the last 16,000 years. *J Paleolimnol* 2009; **42**: 81-102. doi: 10.1007/s10933-008-9249-8

Mayr C, Lücke A, Wagner S, Wissel H, Ohlendorf C, Haberzettl T, Oehlerich M, Schäbitz F,Wille M, Zhu J, Zolitschka B. Intensified Southern Hemisphere Westerlies regulated atmospheric CO_2 during the last deglaciation. *Geology* 2013; **41**: 831–4. doi: 10.1130/G34335.1

Morris BE, Henneberger R, Huber H, Moissl-Eichinger C. Microbial syntrophy: interaction for the common good. *FEMS Microbiol Rev* 2013; **37**: 384-406. doi: 10.1111/1574-6976.12019

Nakamura K, Takaya C. Assay of phosphatase activity and ATP biomass in tideland sediments and classification of the intertidal area using chemical values. *Mar Pollut Bull* 2003; **47**: 5–9. doi: 10.1016/S0025-326X(02)00471-X

Nealson KH, Stahl DA. Microorganisms and biogeochemical cycles: what can we learn from layered microbial communities? *Rev Mineral* 1997; **35**: 5-34. ISSN: 1529-6466

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, Ferrenberg S. Patterns and processes of microbial community assembly. *Microbiol Mol Biol R* 2013; **77**: 342–56. doi: 10.1128/MMBR.00051-12

Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S. A guide to the natural history of freshwater lake bacteria. *Microbiol. Mol Biol Rev* 2011; **75**: 14–49. doi: 10.1128/MMBR.00028-10.

Nobu MK, Dodsworth JA, Murugapiran SK, Rinke C, Gies EA, Webster G, Schwientek P, Kille P, Parkes RJ, Sass H, Jørgensen BB, Weightman AJ, Liu W-T, Hallam SJ, Tsiamis G, Woyke T, Hedlund BP. Phylogeny and physiology of candidate phylum ''Atribacteria'' (OP9/JS1) inferred from cultivation-independent genomics. *ISME J* 2015; **10**: 273–86. doi: 10.1038/ismej.2015.97

Ohlendorf C, Fey M, Gebhardt C, Haberzettl T, Lücke A, Mayr C, Schäbitz F, Wille M, Zolitschka B. Mechanisms of lake-level change at Laguna Potrok Aike (Argentina) – insights from hydrological balance calculations. *Quaternary Sci Rev* 2013; **71**: 27–45. doi: 10.1016/j.quascirev.2012.10.040

Orcutt BN, Sylvan JB, Knab NJ, Edwards KJ. Microbial Ecology of the Dark Ocean above, at, and below the Seafloor. *Microbiol Mol Biol R* 2011; **75**: 361-422. doi: 10.1128/MMBR.00039-10

Orsi WD, Edgcomb VP, Christman GD, Biddle JF. Gene expression in the deep biosphere. *Nature* 2013; **499**: 205–8. doi: 10.1038/nature12230

Orsi WD, Coolen MJL, Wuchter C, He L, More KD, Irigoien X, Chust G, Johnson C, Hemingway JD, Lee M, Galy V, Giosan L. Climate oscillations reflected within the microbiome of Arabian Sea sediments. *Sci Rep-UK* 2017; **7**: 6040. doi:10.1038/s41598-017-05590-9

Parada AE, Needham DM, Fuhrman JA. Every base matters:assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol* 2016; **18**; 1403-14. doi: 10.1111/1462-2920.13023

Pawlowski J, Esling P, Lejzerowicz F, Cedhagen T, Wilding TA. Environmental monitoring through protist next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic foraminifera communities. *Mol Ecol Resour* 2014; **14**: 1129–40. doi: 10.1111/1755-0998.12261

Petro C, Starnawski P, Schramm A, Kjeldsen KU. Microbial community assembly in marine sediments. *Aquat Microb Ecol* 2017; **79**: 177–95. doi:10.3354/ame01826

Pollock EW, Bush ABG. Atmospheric simulations of southern South America's climate since the last glacial maximum. *Quaternary Sci Rev* 2013; **71**: 218–28. doi: 10.1016/j.quascirev.2012.09.019

Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012; **28**: 1823-9. doi: 10.1093/bioinformatics/bts252

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* 2007; **35**: 7188-7196. doi: 10.1093/nar/gkm864

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013; **41**: D590–D596. doi: 10.1093/nar/gks1219

Raes J, Letunic I, Yamada T, Jensen LJ, Bork P. Toward molecular trait-based ecology through integration of biogeochemical, geographical and metagenomic data. *Mol Syst Biol* 2011; **7**: 1-9. doi: 10.1038/msb.2011.6

Recasens C, Ariztegui D, Gebhardt C, Gogorza C, Haberzettl T, Hahn A, Kliem P, Lisé-Pronovost A, Lücke A, Maidana NI, Mayr C, Ohlendorf C, Schäbitz F, St-Onge G, Wille M, Zolitschka B, PASADO Science Team. New insights into paleoenvironmental changes in Laguna Potrok Aike, Southern Patagonia, since the Late Pleistocene: the PASADO multiproxy record. *Holocene* 2012; **22**: 1323–35. doi: 10.1177/0959683611429833

Recasens C, Ariztegui D, Maidana NI, Zolitschka B, PASADO Science Team. Diatoms as indicators of hydrological and climatic changes in Laguna Potrok Aike (Patagonia) since the Late Pleistocene. *Palaeogeogr Palaeocl* 2015; **417**: 309-19. doi: 10.1016/j.palaeo.2014.09.021

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng J-F, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu W-T, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T. Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 2013; **499**: 431-7. doi:10.1038/nature12352

Ross PS, Delpit S, Haller MJ, Németh K, Corbella H. Influence of the substrate on maar-diatreme volcanoes—an example of a mixed setting from the Pali Aike volcanic field, Argentina. *J Volcanol Geoth Res* 2010; **201**: 253-71. doi: 10.1016/j.jvolgeores.2010.07.018

Sharon I, Kertesz M, Hug LA, Pushkarev D, Blauwkamp TA, Castelle CJ, Amirebrahimi M, Thomas BC, Burstein D, Tringe SG, Williams KH, Banfield JF. Accurate, multi-kb reads resolve complex populations and detect rare microorganisms. *Genome Res* 2015; **25**: 534–43. doi: 10.1101/gr.183012.114

Smith EM, Prairie YT. Bacterial metabolism and growth efficiency in lakes: the importance of phosphorus availability. *Limnol Oceanogr* 2004; **49**: 137–47. doi: 10.4319/lo.2004.49.1.0137

Solden L, Lloyd K, Wrighton K. The bright side of microbial dark matter: lessons learned from the uncultivated majority. *Curr Opin Microbiol* 2016; **31**: 217–26. doi: 10.1016/j.mib.2016.04.020

Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 2006; **22**: 2688-90. doi: 10.1093/bioinformatics/btl446

Starnawski P, Bataillon T, Ettema TJG, Jochum LM, Schreiber L, Chen X, Lever MA, Polz MF, Jørgensen BB, Schramm A, Kjeldsen KU. Microbial community assembly and evolution in subseafloor sediment. *P. Natl. Acad. Sci. USA* 2017; **114**: 2940–5. doi: 10.1073/pnas.1614190114

Stine S, Stine M. A record from Lake Cardiel of climate change in southern South America. *Nature* 1990; **345**: 705-8. doi:10.1038/345705a0

Villar C, de Cabo L, Vaithiyanathan P, Bonetto C. Pore water N and P concentration in a floodplain marsh of the Lower Paraná River. *Hydrobiologia* 1999; **392**: 65–71. doi: 10.1023/A:1003505424569

Vuillemin A, Ariztegui D, Vasconcelos C, PASADO Scientific Drilling Party. Establishing sampling procedures in lake cores for subsurface biosphere studies: assessing in situ microbial activity. *Sci Dri* 2010; **10**: 35–9. doi:10.2204/iodp.sd.10.04.2010

Vuillemin A, Ariztegui D, PASADO Science Team. Geomicrobiological investigations in subsaline maar lake sediments over the last 1500 years. *Quaternary Sci Rev* 2013a; **71**: 119–30. doi: 10.1016/j.quascirev.2012.04.011

Vuillemin A, Ariztegui D, De Coninck AS, Lücke A, Mayr C, Schubert CJ, PASADO Science Team. Origin and significance of diagenetic concretions in sediments of Laguna Potrok Aike, southern Patagonia. *J Paleolimnol* 2013b; **50**: 275–91. doi: 10.1007/s10933-013-9723-9

Vuillemin A, Ariztegui D, Lücke A, Mayr C, PASADO Science Team. Paleoenvironmental conditions define current sustainability of microbial populations in Laguna Potrok Aike sediments, Argentina. *Aquat Sci* 2014a; **76**: 101-14. doi: 10.1007/s00027-013-0317-4

Vuillemin A, Ariztegui D, Nobbe G, Schubert CJ, PASADO Science Team. Influence of methanogenic populations in Holocene lacustrine sediments revealed by clone libraries and fatty acid biogeochemistry. *Geomicrobiol J* 2014b; **31**: 285-96. doi: 10.1080/01490451.2013.824050

Vuillemin A, Ariztegui D, Leavitt PR, Bunting L, The PASADO Science Team. Recording of climate and diagenesis through sedimentary DNA and fossil pigments at Laguna Potrok Aike, Argentina. *Biogeosciences* 2016; **13**: 2475–92. doi:10.5194/bg-13-2475-2016

Vuillemin A, Horn F, Alawi M, Henny C, Wagner D, Crowe S, Kallmeyer J. Preservation and significance of extracellular DNA in ferruginous sediments from Lake Towuti, Indonesia. *Front Microbiol* 2017; **8**: 1440. doi: 10.3389/fmicb.2017.01440

Waldmann N, Ariztegui D, Anselmetti FS, Austin JAJr, Moy CM, Stern C, Recasens C, Dunbar RB. Holocene climatic fluctuations and positioning of the Southern Hemisphere westerlies in Tierra del Fuego (54° S), Patagonia. *J Quaternary Sci* 2009; **25**: 1063–75. doi: 10.1002/jqs.1263

Walsh EA, Kirkpatrick JB, Rutherford SD, Smith DC, Sogin M, D'Hondt S. Bacterial diversity and community composition from seasurface to subseafloor. *ISME J* 2016; **10**: 979–89. doi: 10.1038/ismej.2015.175

Wasmund K, Cooper M, Schreiber L, Lloyd KG, Baker BJ, Petersen DG, Jørgensen BB, Stepanauskas R, Reinhardt R, Schramm A, Loy A, Adrian L. Single-cell genome and group-specific dsrAB sequencing implicate marine members of the class Dehalococcoidia (phylum Chloroflexi) in sulfur cycling. *Curr Opin Microbiol* 2016; **31**: 217–26. doi: 10.1016/j.mib.2016.04.020

Wasmund K, Schreiber L, Lloyd KG, Petersen D, Schramm A, Stepanauskas R, Jørgensen BB, Lorenz A: Genome sequencing of a single cell of the widely distributed marine subsurface Dehalococcoidia, phylum Chloroflexi. *ISME J* 2014; **8**: 383–97. doi: 10.1038/ismej.2013.143

Wüst PK, Horn MA, Drake HL. Trophic links between fermenters and methanogens in a moderately acidic fen soil. *Environ Microbiol* 2009; **11**: 1395–409. doi: 10.1111/j.1462-2920.2009.01867.x.

Yang J, Jiang H, Wu G, Liu W, Zhang G. Distinct factors shape aquatic and sedimentary microbial community structures in the lakes of Western China. *Front Microbiol* 2017; **7**: e1782. doi: 10.3389/fmicb.2016.01782

Yutin N, Galperin MY. A genomic update on clostridial phylogeny: Gram-negative spore formers and other misplaced clostridia. *Environ Microbiol* 2013; **15**: 2631-41. doi: 10.1111/1462-2920.12173

Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 2014; **30**: 614-20. doi: 10.1093/bioinformatics/btt593

Zheng H, Dietrich C, Radek R, Brune A. *Endomicrobium proavitum*, the first isolate of Endomicrobia class. nov. (phylum Elusimicrobia)–an ultramicrobacterium with an unusual cell cycle that fixes nitrogen with a Group IV nitrogenase. *Environ Microbiol* 2016; **18**: 191–204. doi:10. 1111/1462-2920.12960

Zolitschka B, Schäbitz F, Lücke A, Corbella H, Ercolano B, Fey M, Haberzettl T, Janssen S, Maidana N, Mayr C, Ohlendorf C, Oliva G, Paez MM, Schleser GH, Soto J, Tiberi P, Wille M. Crater lakes of the Pali Aike Volcanic Field as key sites for paleoclimatic and paleoecological reconstructions in southern Patagonia, Argentina. *J S Am Earth Sci* 2006; **21**: 294–309. doi: 10.1016/j.jsames.2006.04.001

Zolitschka B, Anselmetti F, Ariztegui D, Corbella H, Francus P, Ohlendorf C, Schäbitz F, the PASADO Scientific Drilling Team. The Laguna Potrok Aike scientific drilling project PASADO (ICDP expedition 5022). *Sci Dri* 2009; **8**: 29-34. doi:10.2204/iodp.sd.8.04.2009

Zolitschka B, Anselmetti F, Ariztegui D, Corbella H, Francus P, Lücke A, Maidana NI, Ohlendorf C, Schäbitz F,

Wastegård S. Environment and climate of the last 51,000 years - new insights from the Potrok Aike maar lake Sediment Archive prOject (PASADO). *Quaternary Sci Rev* 2013; **71**: 1–12. doi: 10.1016/j.quascirev.2012.11.024

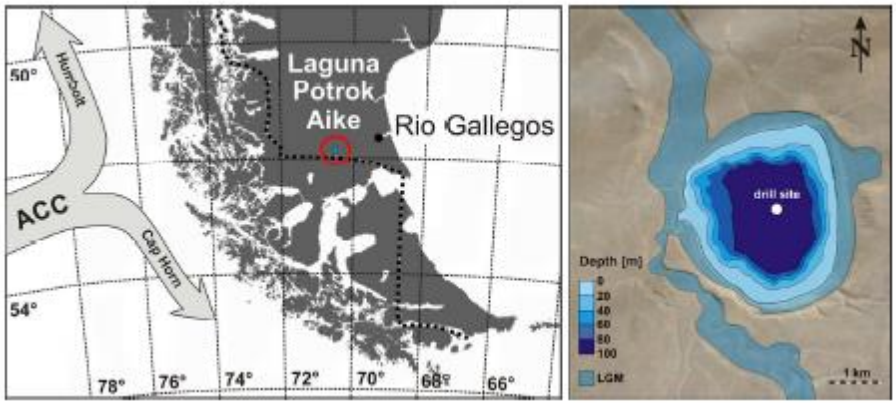

Figure 1 | Location of the study site and bathymetric map of the lake. Left: The geographic location of Laguna Potrok Aike is under the permanent influence of the Westerly winds and Antarctic Circumpolar Current (ACC). Right: Bathymetric map of the lake merged with a satellite image from Google Earth (right). The highest lake-level had active overflow conditions to the north during the Last Glacial Maximum (after Zolitschka et al. 2013).

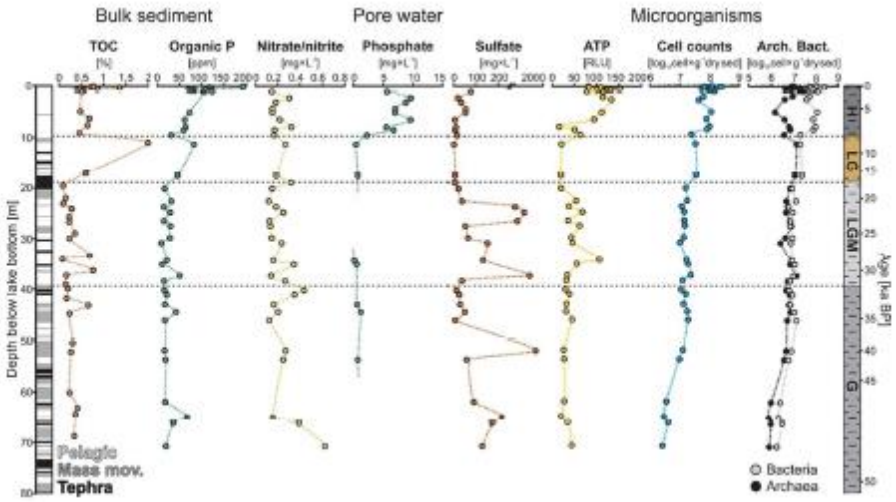

Figure 2 | Multiple downcore profiles. From left to right: Lithology sequence of Laguna Potrok Aike [m] describing pelagic, mass movement and tephra sediments (after Kliem et al. 2013); total organic carbon [% weight] and organic phosphorus [ppm] contents in bulk sediment; pore water concentrations for nitrate/nitrite, phosphate and sulfate [mg × L^{-1}]; adenosine triphosphate (ATP) assays in relative luminescence unit [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), Last Glacial Maximum (LGM), and Last Glacial (G).

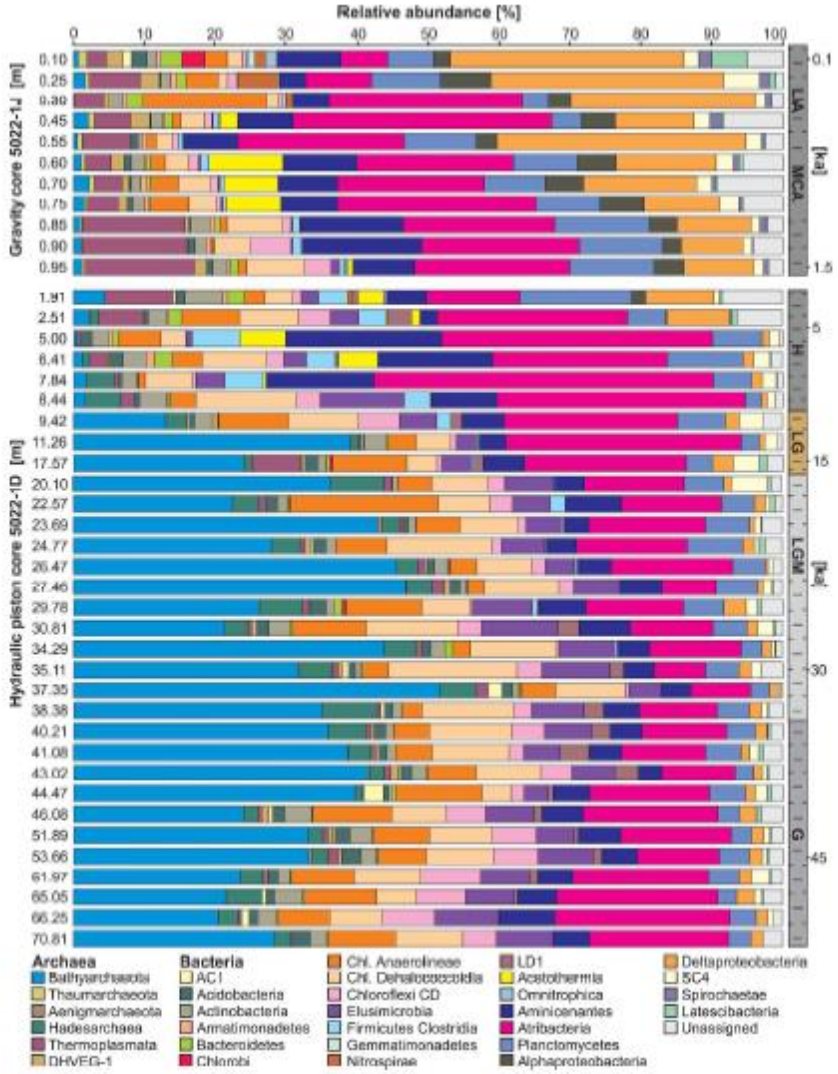

Figure 3 | Bar charts for relative abundances of taxa with depth. Top: Bar charts for gravity core 5022-1J indicate that most abundant taxa are related to Thermoplasmata, Aminicenantes, Atribacteria, which abundances increase with depth, whereas those of Planctomycetes and Deltaproteobacteria decrease 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.

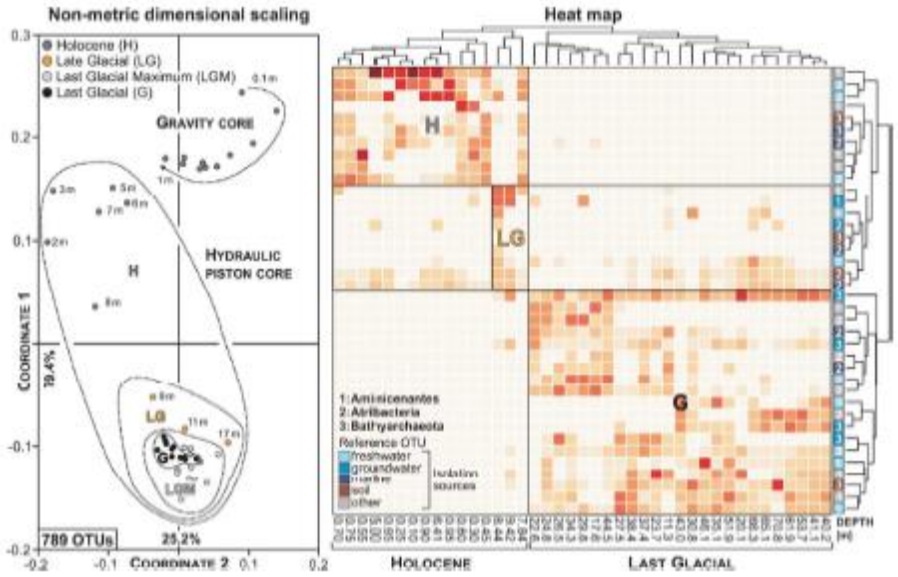

Figure 4 | Non-metric multidimensional scaling plot and heat map. Left: The NMDS plot shows that sample distribution is strongly depth-dependent. Surface sediments are located on the top of the plot, whereas deep samples stand out at the bottom. They display a concentric distribution with increasing 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 unweighted pair grouping by arithmetic mean (UPGMA). The color code (right) indicates the isolation source of the reference OTUs.

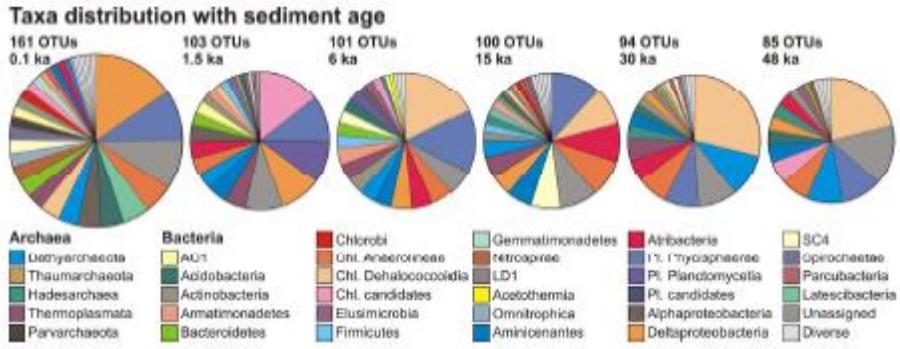

Figure 5 | Pie charts proportional to the number of taxonomic operational units with sediment age. The distribution of total number of OTUs at 0.1, 1.5, 6, 15, 30 and 45 ka years before present (left to right) shows that the overall richness of microbial populations decreases with depth. Taxonomic groups 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 (Supplementary Figs. S3-S4).