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

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

Table S1: Phylogenetic indices of OTUs calculated at 97 % sequence identity cut-off value.

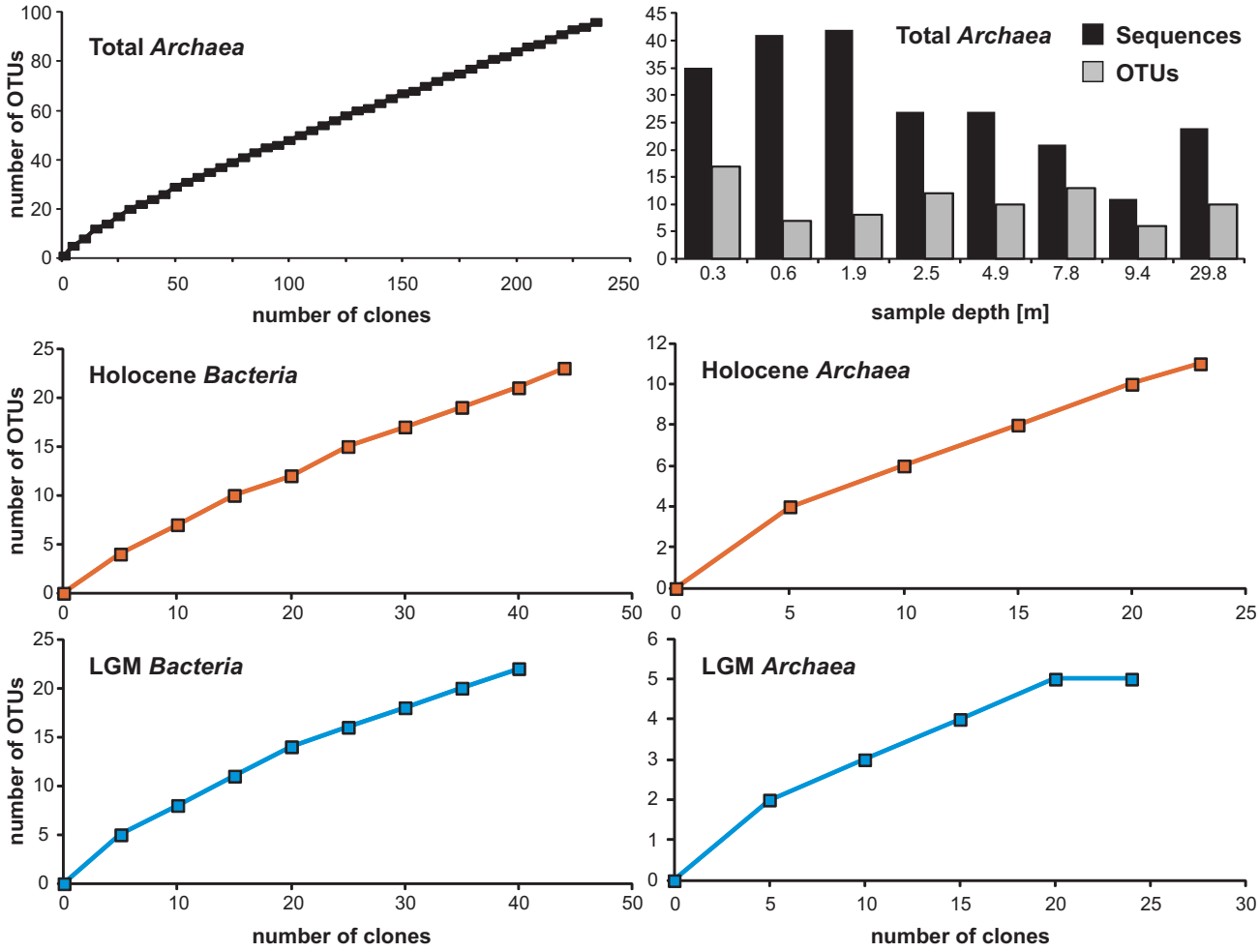


Figure S1: OTU rarefaction curves calculated at 97 % sequence identity cut-off value.

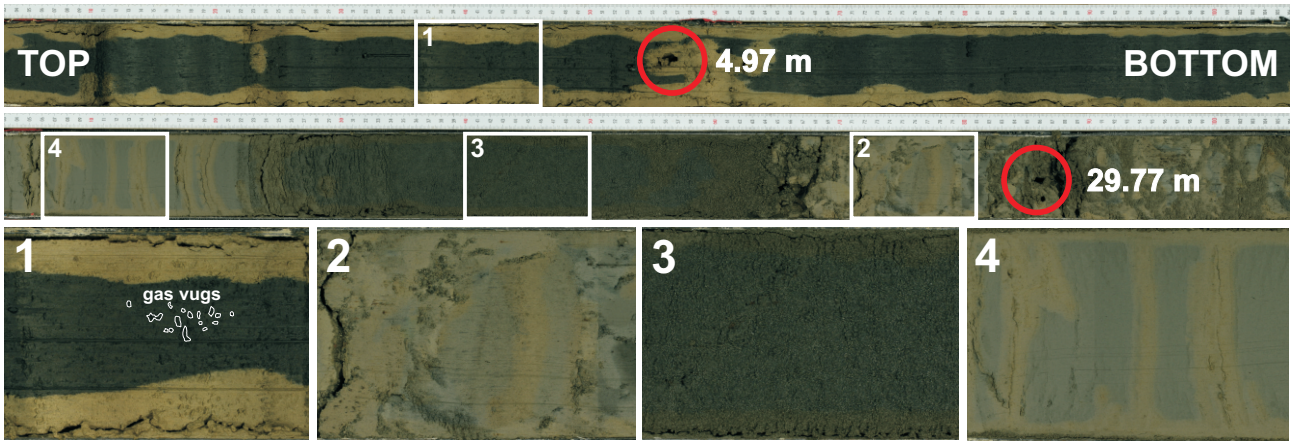


Figure S2: Comparison between Holocene and LGM sedimentary features in sections sampled for clone libraries. The Holocene sequence can be characterized as pelagic to hemipelagic, black and soft, anoxic gas-saturated sediments (1). Oxidized rims occurred during storage after the aperture of sampling windows. The LGM sequence first reflects a pelagic to hemipelagic regime with structures of fluid escapes (2). Then, fine mafic sands (3) could be associated with a gravity event that triggered gas escapes due to sudden loading on the underlying sediment. The top of the section shows a return to pelagic sedimentation (4). In general, the last glacial record displays multiple intercalations of volcanic detritus.

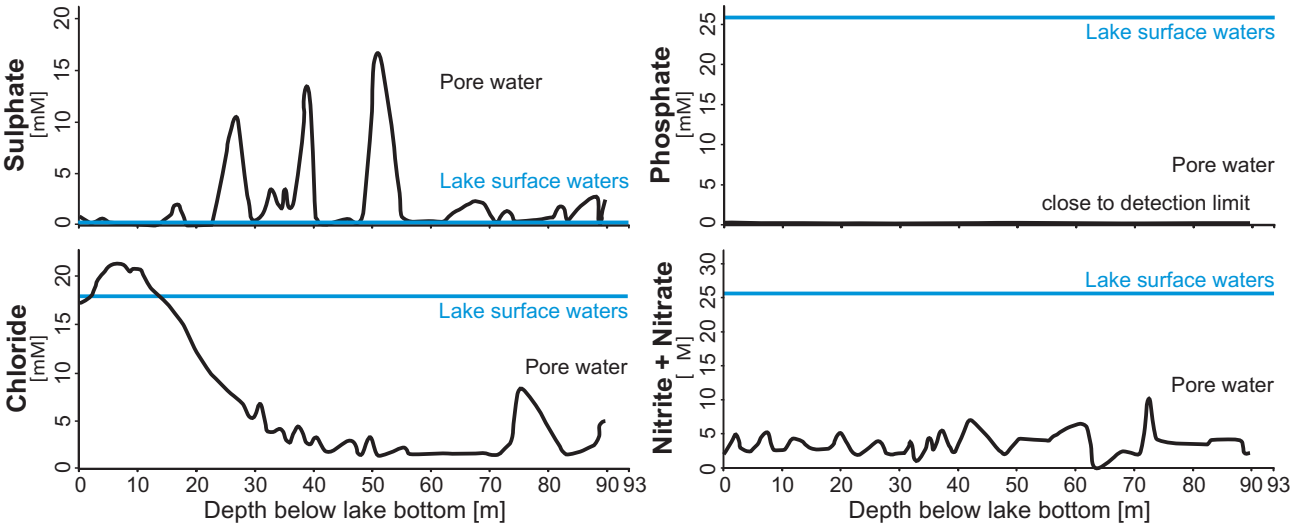


Figure S3: Geochemical comparison between pore water and lake surface waters, providing evidence for the absence of sediment contamination by the hydraulic system during drilling operations. Results from pore water analysis can thus be used as indicators of paleoconditions (i.e. chloride) and geochemical changes within sediments (i.e. sulphate, phosphate).

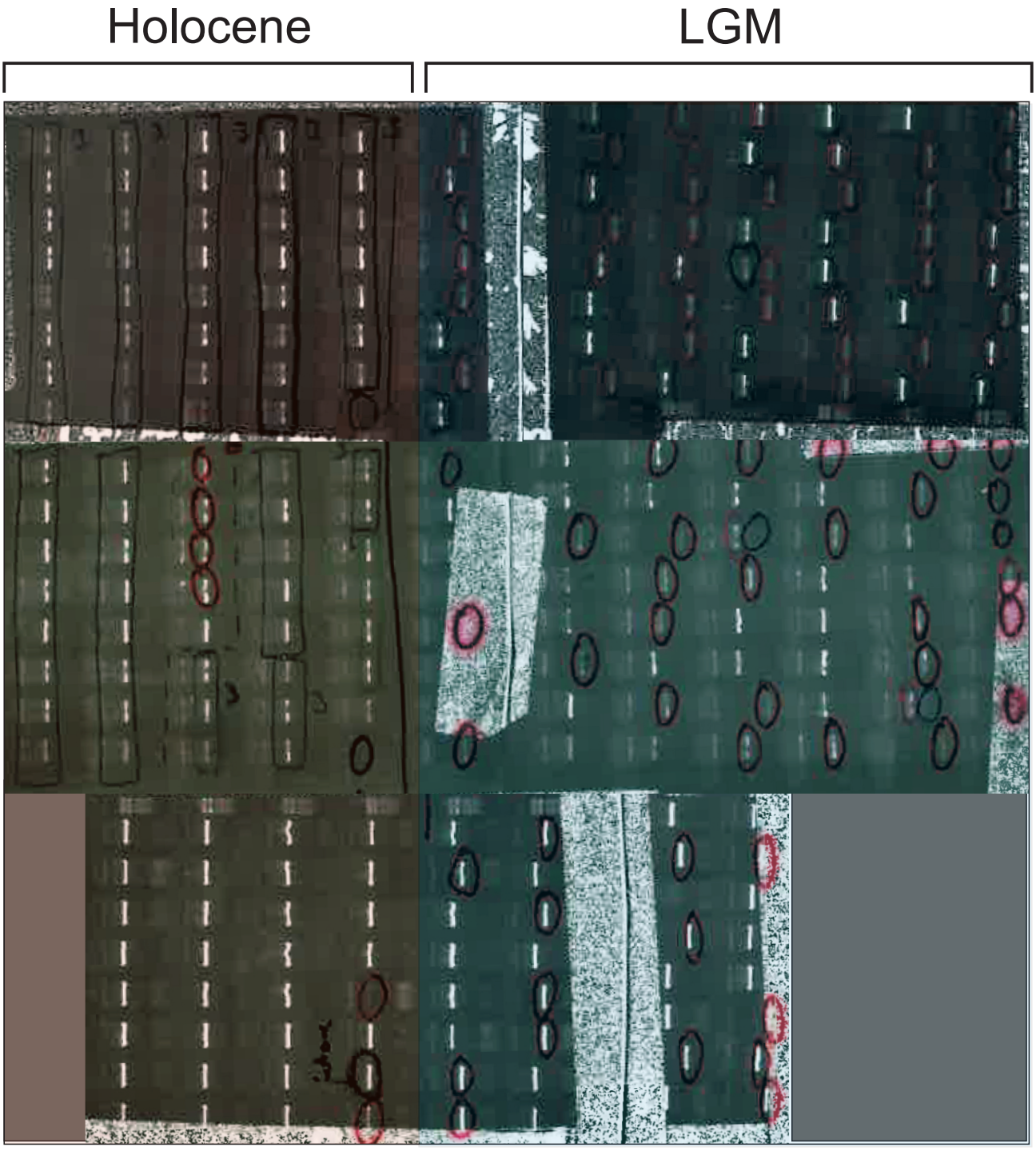
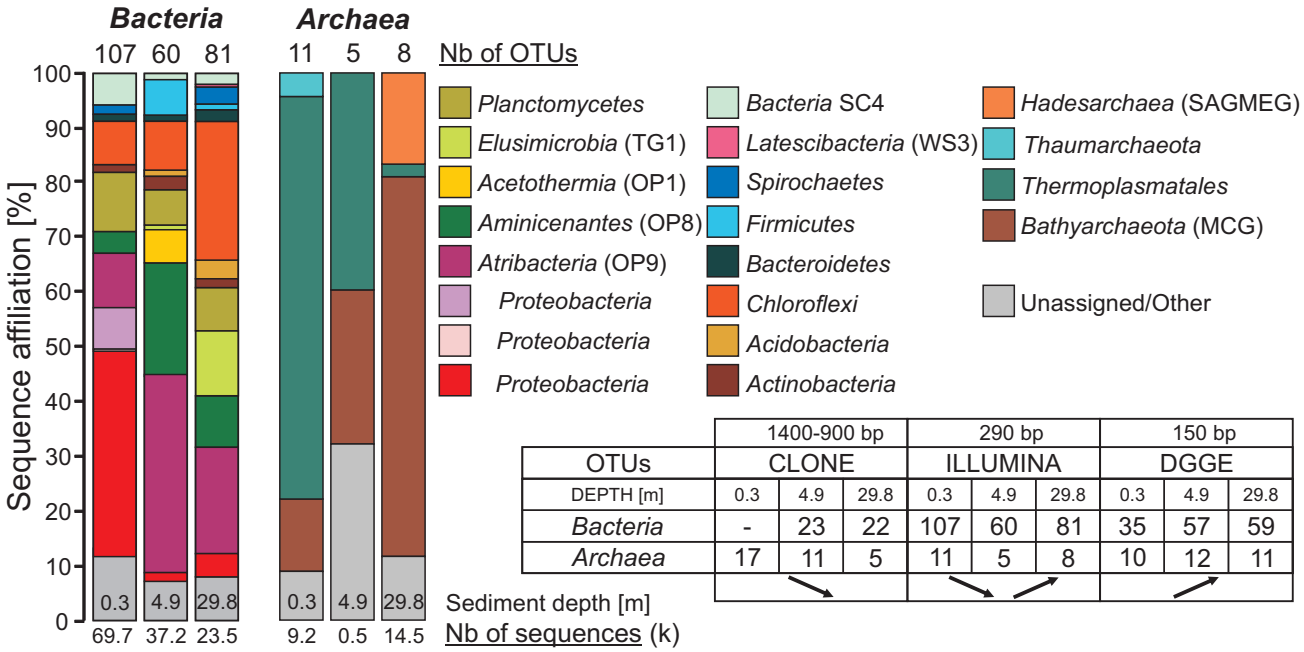


Figure S4: Clone screening of the targeted clonal DNA corresponds to 1400 bp long sequences. Holocene clones (left) all match the expected sequence length, whereas more than 50 % of the LGM clones are too short (ca. 800-600 bp). Such shorter sequences can arise from crosslinkage in the 16S rRNA gene upon release of extracellular DNA. This shows the lower quality of sedimentary DNA extracted from older sediments sheltering microbial communities maintaining low metabolic rates.



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

Figure S5: Illumina MiSeq sequencing was processed for horizon A (Holocene, 4.9 m depth) and B (LGM, 29.8 m depth) in order to provide quantitative comparison with the main elements identified in the clone libraries. One surface sample (0.3 m depth) was added as reference, considering minimal exposure of its sedimentary DNA to post-depositional alteration. These results show that global patterns are preserved with similar assemblages as those of Figure 6. It confirms the qualitative aspect of our libraries and allows their interpretation in terms of sediment populations and infer some related diagenetic processes. We note that one main taxon (6 %) remained missing in the assemblage of horizon A, respectively the *Acetothermia* (former OP1). Also the obscure candidate division *Bacteria* SC4 could be identified (1 %).

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

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

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

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

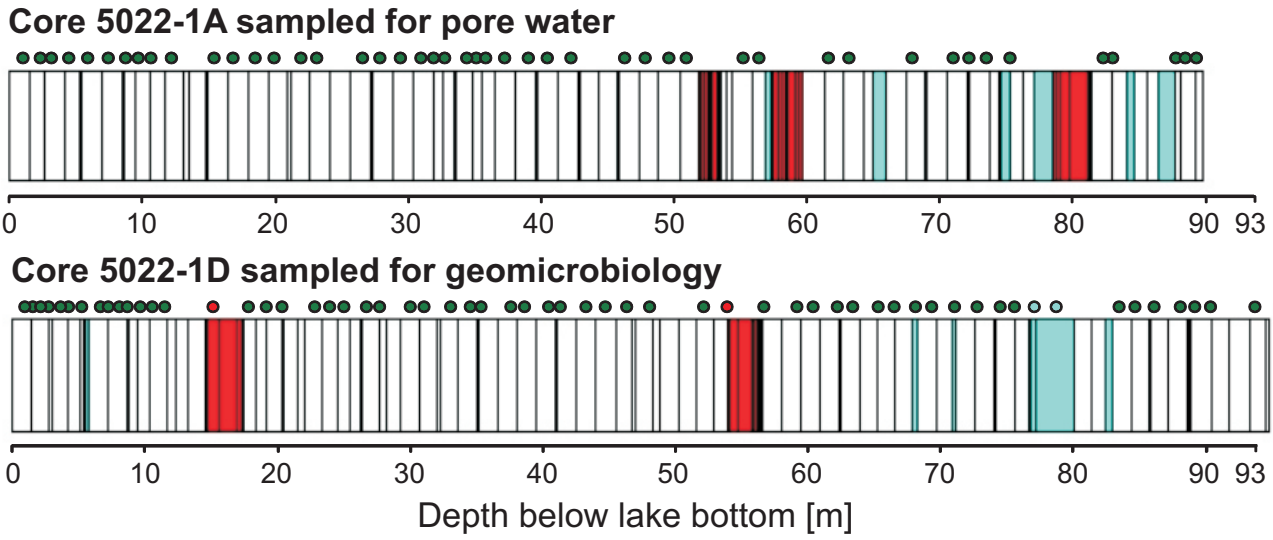


Figure S6: Core sections and possible drilling artifacts in parallel with sample locations in order to validate the genuine use and interpretation of their results in the absence of any established composite depth at site 1. Drilling artifacts were mostly related to coarse layers and gravity events.

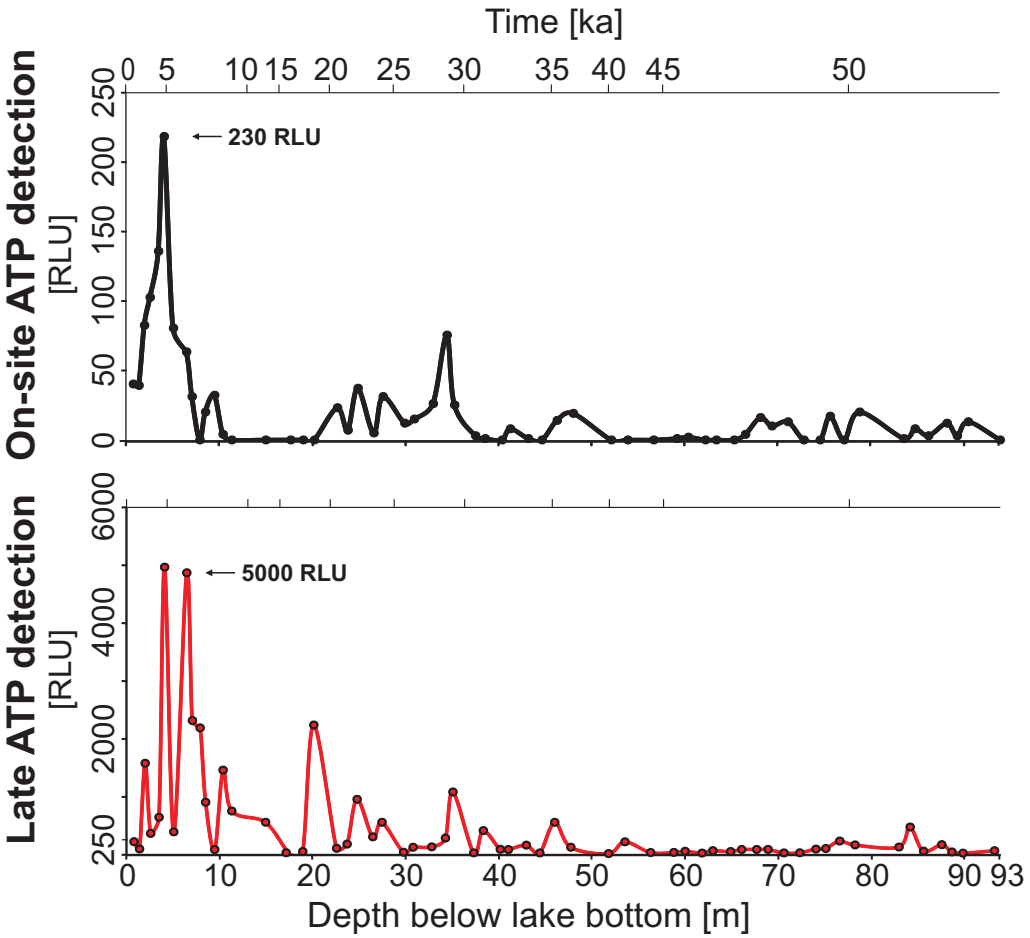


Figure S7: Comparison between on-site and late ATP detections measured with a hand-held device. The second round of measurements showed that microbial colonization of sediments that were initially inactive did not occur during long-term storage. This fact emphasizes the habitability of specific horizons, such as those corresponding to the Holocene and LGM times. It also argues against the possible reworking of modern active microbes into underlying sediments during drilling operations.

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

Table S2: Illumina MiSeq results were screened to look for preserved phototrophic sequences. *Planctomycetes* related to *Phycisphaera* and *Pirellula* are dominant among identified phototrophs. Such preferential preservation likely arises from their specific cell membranes. Sequences of *Cyanobacteria* are present in very low number and are all affiliated with chloroplasts. *Chlorobi* sequences are maximal in uppermost sediments and appear to be quickly degraded. Our interpretation is that sequences from planktonic species are partially degraded, or even erased from the record, at a very early stage, starting in the water column during particle settling. Overprint by heterotrophs then occurs during OM diagenesis. Further investigations will require primers specific to these taxa.