Relevant changes made in the manuscript

- The introduction addresses sedimentary DNA as a proxy for microbial processes and the challenge that post-depositional modifications can represent.
- The method part provides detailed information on DNA procedures.
- Result sections are combined and summarized.
- We provide a new MiSeq Illumina dataset to complement clone libraries and DGGE:
    - ➔ it supports quantitatively our clone libraries
    - ➔ it provides a reference for the initial DNA assemblages in surface sediment.
    - ➔ it shows that phototrophic DNA sequences are rapidly erased.
- DNA quality is discussed in terms of preservation/degradation of different fragment sizes.
- We refocused the discussion on direct comparison between pigment and DNA assemblages.
- Conclusions and abstract are modified accordingly.

Comments of all four reviewers are listed here after. We provide point-by-point responses, which are followed by the revised manuscript. The corresponding modifications are highlighted in red font.

Referee no.1 (technical revisions)

Minor comments:

- P18354, L2: Consider to use words instead of numbers at the beginning of a sentence.

Answer: *This has been verified throughout the manuscript and modified accordingly.*

- P18353, L27: Include definition of OTU.

Answer: *The definition of this abbreviation, i.e. operational taxonomic units, is now mentioned in the manuscript.*

- P18384: The footnotes of Figure 2 are a little bit confusing to identify the different parts of the figure.

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

Referee no.2 (major revisions)

General comments:

The strategy applied in the study does not allow to conclude clearly about the post-depositional diagenetic processes. The authors do not know what the initial assemblages were (bacterial DNA at the moment of deposition) and cannot discriminate them from those arising from ancient sedimentary DNA. Consequently most of the conclusions regarding the role of post depositional alterations are impossible to verify. I would advise to moderate the conclusion regarding diagenesis processes.

Answer: *To counter this issue, we have decided to provide the reviewers with some latest MiSeq data from three different depths, namely at 0.3, 4.9 and 29.8 m depth. They allow comparison between recent sediment DNA and the one extracted from horizon A and B. Additional bar charts based on an Illumina MiSeq sequencing run (iTag primers 515F-806R) come to complement Figure 6 as supplementary material. The method part, as signified in some comments below, contains detailed information on DNA extraction, PCR primers, DNA quality and targeted fragment sizes. Altogether, it allows a more quantitative approach of the microbial assemblages. Part of the discussion has been refocused on DNA fragment sizes (clones: 1400-800 bp; MiSeq: 290 bp; and DGGE: 150 bp) and their relevance in terms of post-depositional changes of sedimentary DNA and resulting assemblages. Phototrophic sequences from the MiSeq run are listed in a new table in the supplementary material.*

Major comments

- In order to discriminate the active Bacteria/Archaea from the ancient DNA, the sampling strategy could have included both DNA and RNA (transcripts) as a solution to consolidate the observations.

Answer*: At the time of the field campaign (November 2008), we did not have any RNAlater solution with us. A metatranscriptomic approach could still have been possible if we had sampled higher amounts of sediments on the field and conditioned them in alu-foil bags under protective atmosphere. However, after returning from the filed campaign, the rest of this core was sampled and used for OSL-dating. We can only agree on your comment and regret not to have foreseen this issue.*

- The sequencing depth is very low and these inventories are probably far from revealing the real diversity. *e.g.* differences between bacterial DGGE band and OTU numbers. This has to be taken into account in the interpretation of results. Consequently, the Shannon index is hazardous.

Answer*: We have added bar charts from a very recent MiSeq run in order to provide more quantitative insights into the microbial assemblages. Numbers of calculated OTUs are shown in parallel for clone libraries, MiSeq samples and DGGE bands (Supplementary material). The interpretation of the results and course of the discussion have been carefully checked according to the reviewer's point of view.*

- The Material & Methods section has to be very precise regarding all DNA analyses, even if previous publications exist. Some points have to be clarified earlier in the manuscript, *i.e.* the type of extracted and targeted DNA, the length of DNA fragments amplified by PCR before cloning. Indeed, long fragments should come mainly from intact inactive cells and active taxa, while ancient DNA should be mainly found as short fragments.

Answer: *The Material & Methods section has been incremented as required by the referee to mention the exact extraction kit protocol, primers used during cloning, MiSeq and DGGE procedures. It now clearly states the respective final sizes of targeted DNA.*

Specific comments:

- P18349, L6-9: Clarify the sentence and what is meant by "modification of bacterial assemblages"' whether be it active bacteria or the global bacterial signal of both extant and past bacterial communities.

Answer: *This sentence has been modified as follows: "…and alter extant bacterial populations to lead to selective preservation of prior sedimentary assemblages (Miskin et al., 1998; Boere et al., 2011a, 2011b)."*

- P18350, L8-10: The sequenced DNA can come either from active or past inactive Archaea. Without criteria of differentiation, I suggest the modification of this sentence.

Answer: *This sentence has been modified as follows: "…to evaluate the recording of population changes with depth and during early diagenesis."*

- P18351, L4: Provide detailed information about the method of extraction (see comment above).

Answer: *The Material & Methods chapter has been complemented to list all these information (section 2.4).*

- P18352, L22: Diatom pigments do not represent the whole amount of eukaryotic pigments (e.g. chlorophytes, cryptophytes, dinophytes, etc.). Consequently, I would advise not to use "eukaryotic" if only diatoms are quantified. Check here and throughout the manuscript (for instance, P18856, L10).

Answer: *The Eukaryotes / Prokariotes ratio was calculated as follows: Total Eukaryotes = diatoxanthin + alloxanthin + phaeophytin b + fucoxanthin + chlorophyll a + chlorophyll b + β carotene + lutein-zeaxanthin / Total Prokaryotes = echinenone + canthaxanthin + isorenieratene + okenone. We have added the list of these pigments to the method part and kept the term "eukaryotic" throughout the manuscript.*

- P18352, L25: The authors have to mention which type of DNA extraction they used, the targeted DNA region and especially their lengths for both Bacteria and Archaea. This is of high importance since short DNA fragments might include more sequences associated with inactive taxa (fragmented DNA) while long DNA fragments (>500bp) should lead to the detection of less of these ancient taxa and proportionally to more active taxa (see comment above).

Answer: *The Material & Methods chapter has been complemented to list all these information (section 2.4). The different sizes of DNA fragments are now mentioned earlier in the Material & Methods as well as in the discussion. The discussion has been complemented to clarify the relationship between sequence lengths and the potential detection of active, inactive and ancient taxa.*

- P18353, L8-10: Indicate clearly how many clones per sediment layer were analyzed. The information is available on Figure 6, but could be mentioned here already.

Answer: *This information has been added accordingly.*

- P18358, L2: DGGE results provide evidence for the presence of amplifiable DNA sequences only and DAPI is not appropriate to discriminate dead or active cells. I would suggest to remove this sentence.

Answer: *We have decided to modify this sentence for the following one: "Regardless, taken together, these various indices provided evidence for the presence of amplifiable DNA related to microbial populations in decline at depth."*

- P18258, L12: It is not clear what is meant by ancient and recent sequences, whether it refers to the dating of the sediment or the discrimination between active taxa in upper deposits vs inactive in deeper

deposits.

Answer: *This sentence has been removed from the results. We have searched throughout the manuscript for similar phrasings in order to clarify them in terms of post-depositional changes of sediment DNA with depth and over time. Sequences preservation is considered based on their respective fragment lengths.*

- Parts 3.2.3 and 3.2.4: The sequencing effort is insufficient to conclude on the microbial composition of the sedimentary DNA. The comments regarding the presence / absence of bacterial and archaeal groups in the different sediment layers have to be reported and discussed with cautions.

Answer: *We have provided a complementary MiSeq dataset (Supplementary material) to support quantitatively our clone libraries. These results show that the global pattern of sequence affiliation is preserved, confirming that our clone libraries are constituted of the major components of the microbial assemblages. Part 3.2.3 and 3.2.4 have been combined and rephrased taking into account the limitations of the clone sequencing depth. Besides, we admit that one main taxon (6 %) remained missing for the horizon A, respectively the Acetothermia (former OP1). Also the obscure candidate division Bacteria SC4 could be identified (1 %) but not interpreted.*

- P18361, Part 4.2: This section can be shortened, avoiding repetitions of results and references to figures.

Answer: *We have removed references to figures and rephrased most of this section to avoid repetition of results. The second part of this section dealing with unspecific nucleic sequences has been combined with section 4.3 to address sedimentary DNA preservation and post depositional changes in parallel with fossil pigments.*

- P18364, L 24-28: The information on sequence lengths (*Archaea* = 900 bp and *Bacteria* = 1400 bp) is extremely important and has to be stated earlier (see Material & Methods comment).

Answer: *The Material & Methods chapter has been complemented to list this information (see response above). The different sizes of DNA fragments are now mentioned earlier in the discussion in order to address post-depositional changes of sedimentary DNA and environmental interpretation of the resulting assemblages.*

- P18365, L1-2: The DGGE band length (150bp) has to be clarified and stated earlier in the manuscript.

Answer: *This information is now discussed much earlier in the manuscript, starting in the Material & Methods chapter to emphasize size difference of targeted DNA in the cloning, MiSeq and DGGE procedures. In the Results chapter, we briefly discuss issues related to DNA quality and exemplify them with images of clone screening (Supplementary material), showing that most of the LGM clones did not match the expected length (i.e. 800-600 bp instead of 1400 bp). We also provide a short summary table of the OTUs with respect to DNA fragment sizes (Supplementary material). The issue of DNA quality and fragment sizes is now mentioned in section 4.2 in parallel with the degree of microbial activity and density. Unspecific nucleic acids from microbial assemblages are discussed in section 4.3 in relation to the fossil pigment record.*

- P18366: I would suggest to moderate the discussion /conclusion in view of the low sequencing depth applied in this study.

Answer: *We have removed sentences dealing with pigment production from bacteria that the present clone libraries could not support and refocused the discussion on the respective preservation of sedimentary DNA and fossil pigment. We have also screened our recent MiSeq results for preserved phototrophic sequences and listed them for some samples in a new table (Supplementary material).*

- P18367: Since the strategy applied in this study does not allow to define the magnitude of post depositional alterations, I suggest to moderate the conclusion/discussion on this specific point.

Answer: *We think that the additional information (i.e. gel screening, MiSeq sequences, OTUs in link to fragments sizes, table of phototrophic sequences) provide many additional hints on the sedimentary diagenetic issue. Section 4.4 has been rewritten accordingly. We have also added a short paragraph to deal with post-depositional alteration of sedimentary DNA in terms of fragment sizes. Finally we address the difficulty to accurately estimate time difference between microbial assemblages and surrounding sediments.*

- P18368, L21-22**:** This is actually a very strong limitation.

Answer: *We hope that our reviews have consolidated our approach. We have partly rephrased the conclusions and clearly signified the limitations of having to work on total DNA.*

Referee no.3 (minor revisions)

General comments

- Given the low number of analyzed clones in general, the relative estimates of abundance might be biased (supplementary material).

- In general, a more recent 16S rDNA community composition analysis from sediments would be useful to compare and to better characterize the community from the water column.

- In spite of the interesting and high descriptive value of the manuscript, much of the conclusions is hypothetical.

***Answer**: We admit that running statistics on a few hundred of clones cannot provide robust index results. To answer the issue about recent communities in surficial sediments and confirm quantitatively our assemblages, we have decided to add 3 samples from our more recent MiSeq results (i.e. shallow sediment, horizons A and B). Concerning the conclusions, other referees also pointed at the lack of supporting data for post-depositional alteration of sedimentary DNA and its interpretation in terms of diagenesis. We have incremented and clarified the discussion on extant and ancient assemblages in terms of DNA quality, preservation and fragment sizes. Most of the conclusions have been rephrased.*

Specific comments:

- P18352, L2: Have the pigment measurements been replicated?

***Answer**: Because of the expense of core collection and the high number of proxies analyses, only limited amount of sediment was available (see Ohlendorf et al., 2011). Rather than replicate the 2500 samples (at a normal cost of $350,000 USD), emphasis was placed on maximizing the temporal resolution of phototroph analysis. Fortunately, reproducibility of true analytical replicates is typically better than 20% standard deviation of the mean (Leavitt and Hodgson 2001), particularly when there are few changes in preservation environment, as recorded herein. Finally, strong agreement between multiple proxies shows that the results of pigment concentration are robust.*

- P18358: Given the abundance of cyanobacteria and diatom pigments during the more recent YD, cyanobacterial and chloroplast sequences should be amplified with the universal primers 27F and 1492R. Why are they not detected in the clone library?

***Answer**: As a general answer, specific primers for cyanobacteria often target very short fragments from 300 down to 150 bp (e.g. Pal et al., 2015, Journal of Paleolimnology). Nested PCR can be applied with limited bias after universal primers. In our present study, the length of the targeted DNA fragment probably led to hide fragmented phototroph sequences by heterotroph sequences. Phototrophic sequences may still be present among DGGE bands. Thus, we searched for preserved phototrophic sequences in the recent MiSeq results (iTag primers 515F-806R), which revealed only very limited numbers of Cyanobacteria and Chlorobi sequences (290 bp). We have listed them in a new table available as supplementary material. Our interpretation is that particulate organic matter containing the DNA from planktonic phototrophs is colonized and degraded quickly by heterotrophs during sinking. In contrast, Planctomycetes are better preserved due to more resistant cell membranes. Also the potential habitat (see below) may be an important factor since biomats developing on the steep flanks of the maar can be rapidly sedimented and buried during gravity events. These two aspects (i.e. planktonic vs benthonic bacteria / decantation vs gravity) are now part of the discussion.*

- P18263, L1: It is unclear whether direct measurements of methane have been performed. Currently conclusions are drawn from the abundance of genotypes in clone library and ATP measurements.

Answer: *Methane measurements were performed and published with $\delta^{13}C$ compositions of methane gas and fatty acids (Vuillemin et al., 2013 and 2014, Journal of Paleolimnology, Geomicrobiology Journal, respectively).*

- P18363, L8: One should read 29 ka old LGM.

Answer: *This has been corrected accordingly.*

- P18366, L3: The mismatch between isorenieratene pigment composition and DNA genotype analysis is discussed for green sulfur bacteria. Perhaps it would be helpful to speculate on the potential habitat of green sulfur bacteria in this ecosystem. In contrast to cyanobacteria (and diatoms) living in the epilimnetic water, sulfur bacteria might be able to live on the sediment surface.

Answer: *Thank you for this interesting point that we have added to the discussion. Biomats can probably grow on the flanks of the maar and be quickly sedimented during gravity events, whereas planktonic production has to sink through the whole water column. In addition, strong mixing due to the influence of Westerly Winds prevents quick sinking of particles as it leads to OM resuspension in the water column. Thus, the residence time of such particulate OM in the water column is rather high at present in the maar lake.*

Specific question:

- What is the situation at present? Is there evidence of (intermittent) lake stratification? In general the preservation of the DNA depends on the degree of deoxygenation of the water column. With a meromictic hypolimnion, cyanobacterial DNA can be recovered from relatively recent sediments (Savichtcheva, et al. 2011. Appl. Environ. Microbiol. 77(24), 8744-8753).

Answer: *This point was already mentioned in the study site section. However, we have decided to repeat it in the discussion to complement to the two comments above.*

Referee no.4 (short comments by F. Pick)

Major comments:

- This paper is not an easy read, is too long for the take home message and is somewhat confusing in its discussion of findings. While the work is essentially descriptive, it purports to test a hypothesis that makes no clear predictions (P18349, L15-19). Some recasting and explanation of limitations is in order.

- Diagenesis is part of the title yet there is not much evidence for these processes affecting specifically the DNA results. The question of how microbial community structure changes within sediments after deposition is really important, but the multiple complications due to erasure of pre-diagenesis and surface sediment lake signals were not clearly resolved in this study.

- The discussion on the diagenetic issue may show some overlap with previous publications.

Answer: *Taking into account all the comments of the reviewer, we have reoriented and rephrased the introduction and hypothesis around the potential uses and issues of sedimentary DNA. We have highlighted the difficulty to interpret environmental DNA due to post-depositional changes, while still considering its potential as a climatic recorder. We have added a preliminary MiSeq dataset for one surface sample and for the Holocene and LGM horizons (Supplementary material). The surface sample provides a reference for sedimentary DNA with limited post-depositional influence and help tracing elements from the early assemblage potentially preserved in the Holocene and LGM samples. It also brings quantitative confirmation of sequence affiliations, which otherwise could only be considered qualitative on the base of clone libraries. Fragment sizes (clone: 1400-900 bp / MiSeq: 291 bp / DGGE: 150 bp) are discussed in terms of DNA quality, extracellular DNA accumulation and degradation. MiSeq results were also checked for preserved phototrophic sequences (found to be very limited) and listed in a table (Supplementary material). These new data allow clear discussion of findings, addressing sedimentary DNA preservation and turnover and metabolic processes of the recorded assemblages. The overlap with previous publications is averted by discussing environmental DNA diagenesis specifically and showing that identified assemblages support and complement previous findings on carbon fractionation and diagenetic concretions associated with heterotrophic and lithotrophic processes (Vuillemin et al., 2013a and 2014a). Answering the specific comments below, we did our best to streamline the results and avoid repetitions of the paleoenvironmental context. We have brought fossil pigments and DNA assemblages in direct comparison by combining former section 4.2 with 4.3. We think that the take home message has been clarified and reinforced although the manuscript remains rather long.*

Specific comments:

- P18347: The abstract should describe more clearly the specific DNA findings and how these relate to what is known of the lake conditions during those time periods. The first paragraph of the abstract provides information more suitable for the introduction and could be summarized. Similarly the last paragraph could be one sentence.

Answer: *We have summarized the first and last paragraphs. We have highlighted the findings on microbial assemblages and how they relate to specific climatic contexts. Comparing pigment compositions and aged sediment assemblages, we conclude on post-depositional changes and genetic preservation of climatic and diagenetic information.*

- P18348, L9-14: Since some of the problems are not fully resolved, the referee suggests positioning the paper differently and revise the hypothesis section. The introduction could start with the first sentence, then move on to DNA and the challenges of interpreting sediment DNA as articulated in the second paragraph.

Answer: *The order of the introduction has been reviewed as proposed. The difficulties of interpreting environmental DNA due to post-depositional modifications are clearly stated. The hypothesis is reorganized around the use of sedimentary DNA as a proxy for microbial processes potentially recording in turn climate and diagenesis.*

- P18349, L25-26: Results should not be stated in the introduction unless this statement refers to a previous study.

Answer*: This sentence has been modified as follows: "Finally, we established six archaeal clone libraries at regular intervals throughout the microbially-active sediments of the Holocene period to evaluate the recording of population changes with depth and during early diagenesis.*"

- P18353: The method section 2.3 can be shortened as these are well established methods. Explain what ratios are used and, for example, that cyanobacteria are represented as the sum of 3 pigments.

Answer: *This protocol is now summarized. Specific ratios are explained and the different pigments used in their calculation are clearly listed.*

- P18353: Why was high-throughput 16S rDNA amplicon sequencing not performed? A few hundred clones for all samples combined are not sufficient to assess and compare microbial community structures (Fig. 6). These comparisons should be viewed as largely preliminary.

Answer: *We recently performed an Illumina MiSeq sequencing (iTag primers 515F-806R) on the same DNA extractions. We have decided to produce part of this data in the Supplementary material in order to confirm in a quantitative way the major elements of the assemblages identified in our clone libraries. For horizon A and B, all taxa above 1 % sequence affiliation were already present among clones, with the exception of one important taxon missing in the horizon A (Acetothermia, former OP1) and a rather limited presence of Bacteria SC4. We have also summed up in a short table the numbers of OTUs as calculated for clone libraries, MiSeq samples and DGGE bands. We think that this additional dataset confirms our clone libraries as qualitatively robust. The surface sediment sample (25 cm depth) also demonstrates that some elements of the assemblages are kept constant with depth and can represent initially preserved sequences as presently argued in our manuscript (i.e. Planctomycetes, Chloroflexi and Bacteroidetes).*

- P18353, L26: The method section mentions Mothur, but not the metrics that were used to compare community structures. Were the authors able to run some UNIFRAC tests?

Answer: *We extracted the phylip distance matrices from the two Maximum Likelihood trees and exported them to the Mothur software. We did not run any UNIFRAC tests. A UNIFRAC test is not fully consistent for a set of two populations only. Moreover, if the structure of the communities cannot be considered fully resolved as mentioned by the reviewer, the result of UNIFRAC should be considered hazardous as well. We admit that the size of our set of populations is not well adapted to statistical treatment.*

- P18355-6: Combine some of these sections and focus on the new and novel analyses.

Answer*: Sections that have been combined are: Organic matter (3.1.4) and pore water chemistry (3.1.2); microbial proxies (3.2.1) and sedimentary DNA (3.2.2), bacterial clone libraries (3.2.3) and archaeal clone libraries (3.2.4). Results have been summarized.*

- P18356: Pore water chemistry has been retrieved where sediments must be highly compacted. How useful is this information for the purpose of this paper and is it already available?

Answer: *We did not face any problem extracting pore water from deep sediments as silt concentrations are often around 60 %. This dataset is available elsewhere (Vuillemin et al. 2013, Journal of Paleolimnology),*

but we considered important to repeat it in Figure 3 to inform the reader on the sediment local geochemistry and emphasize its direct link to the detected assemblages.

- P18354: Section 3.1.1 is a summary of previous findings in other publications and should be moved either to the introduction or the study site section. Move also Figure 2 to the study site section as this pulls together information in previous studies on the lake.

<u>Answer:</u> *We have moved and shortened this section to an earlier part of the manuscript as suggested. It presently corresponds to section 2.2 "Sedimentary features of selected horizons" and follows the description of the study site. Subsequent sections have been renumbered accordingly.*

- P18356, L3-5. Explain this ratio and move its interpretation to the discussion part.

<u>Answer:</u> *This ratio is now explained in the Material & Methods chapter. Any further interpretation has been moved from the results to the discussion.*

- P18357, L5-8: The referee expresses some reserve on the use of ATP tester as a measure of microbial activity. The information on ATP should appear in the method part.

<u>Answer:</u> *We have moved this information to section 2.3 "On-site sampling and procedures". It is clearly mentioned that they are field assays obtained with a luminometer device.*

- P18359, L9-11: Rephrase "gradual evolution" for "brought on by environmental selection" or similar wording. This is repeated in P18361, L9.

<u>Answer:</u> *This phrasing has been changed for: "Despite potential cell migration in soft methane-saturated clays, archaeal sequences obtained from the Holocene record provided evidence for an environmental selection of assemblages with depth in the sedimentary profile (Figs. 5 and 6)" in P18359. In P18361, it has been modified as follows "…indicated a layering of the assemblages with depth likely related to environmental selection during early diagenesis."*

- P18360: Section 4.1 revisits much of the information on the known history of the lake. This could be summarized as a comparison between the 2 time periods.

<u>Answer:</u> *This has been done accordingly. We have summarized the general climatic background of the Last Glacial transition to bring direct comparison between the Holocene and LGM horizon.*

- P18364, L15-30: The quality of the DNA is an important point for interpretation of the results and should perhaps appear earlier in the discussion so that this point is dealt with. The information here is sufficiently convincing.

<u>Answer:</u> *This information is now discussed much earlier in the manuscript, starting in the Material & Methods chapter to emphasize the size difference of targeted DNA between clones, MiSeq and DGGE bands. In the Results chapter, we briefly discuss issues related to DNA quality and provide some gel screening images as example (Supplementary material) to show that most LGM clones did not match the expected length (i.e. 800-600 bp instead of 1400 bp). We have added a short summary table of OTUs (Supplementary material) in relation to the respective fragment sizes. Although a direct comparison the three different sets (clone libraries, MiSeq, DGGE bands) is a bit equivocal, the relative number of OTUs associated with long and short fragments appears to decrease and increase with depth, respectively. The issue of DNA quality and fragment sizes is then mentioned earlier in the discussion and put into parallel with the degree of microbial activity and density in order to address turnover and preservation potential and post-depositional alteration of sedimentary DNA. Theoretically, ancient DNA should represent short fragments*

compared to the extant microbial assemblages and should have better chances to accumulate with depth under declining microbial activity.

Moreover, to address the major issue raised by the reviewer (i.e. resolving erasure of pre-diagenesis and surface sediment signals), we have included a surface sediment sample (from 0.3 cm sediment depth) in the MiSeq dataset (Supplementary material) as reference, considering minimal exposure of its sedimentary DNA to post-depositional alteration.

- P18365. Some of the information in section 4.3 is repetitive. This section should be combined with the one before to integrate the lines of evidence on both DNA and pigment.

Answer: *We have combined this section with some aspects of the previous section 4.2. We have also summarized paleoclimatic interpretations of the pigment record by focusing them on the LGM and Holocene. We have removed sentences dealing with potential pigment production from bacteria that the present clone libraries could not support and refocused the rest of this section on the preservation potential of sedimentary DNA with respect to fossil pigments. We have also checked our recent MiSeq results for preserved phototrophic sequences and listed them in one final table (Supplementary material).*

- P18366, L3: This phrase is not supported by the following sentences, which seems somewhat circular.

Answer: *As already mentioned we have added MiSeq results for a sample at 0.3 m depth and screened them for preserved phototrophic sequences. These information are available in the Supplementary material. We have also reviewed this section and moderated our conclusions on post-depositional effects as the magnitude of such modifications remains hard to assess. A brief paragraph has been added to clearly state diagenesis of sedimentary DNA and the difficulty to accurately estimate time difference between surrounding sediments and preserved microbial assemblages.*

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

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Abstract

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

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

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

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

1 Introduction

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

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

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

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

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

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

15

16 ## 2 Material and methods

17 ### 2.1 Study site

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

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

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

2.2 Sedimentary features of selected horizons

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

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

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

2.3 On-site sampling and procedures

Sediment sampling protocols were optimized to avoid potential sources of microbial contamination (Kallmeyer et al., 2006; Vuillemin et al., 2010). The size and configuration of the drilling platform prevented an on-site laboratory with sufficient conditions of asepsis, therefore retrieved cores were transported every 90 min from the platform back to the field laboratory where a detailed protocol was applied to retrieve sediments under the most sterile conditions possible. The aperture of sampling windows allowed a quick retrieval and conditioning of sediments for DNA extraction, 4',6-diamidino-2-phenylindole (DAPI) cell counts, and on-site adenosine-5'-triphosphate (ATP) assays. Rapid ATP detections were performed on a Uni-Lite NG luminometer (BioTrace) with Aqua-Trace water testers and used 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 RLU. Thus, a value of 30 was systematically subtracted from the readings for background correction. Pore water was retrieved from small holes drilled in the liners using 0.15 µm pores soil moisture samplers (Rhizon Eijkelkamp). 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, 2013b). Complete datasets are available at http://doi.pangaea.de under accession numbers 10.1594/PANGAEA.811521 to 811524.

2.4 Pigment analysis

All extraction, isolation and quantification followed the standard procedures detailed elsewhere (Leavitt and Hodgson, 2002). In brief, carotenoid, chlorophyll (Chl) and derivative pigments were extracted from 2,500 freeze-dried sediment samples into degassed mixtures of organic solvents (i.e. acetone, methanol) and water under an inert N_2 atmosphere and filtered through 0.45-µm pore membrane filters. Extracts were injected into a Hewlett Packard model 1100 high performance liquid chromatographic (HPLC) system fitted with a reversed-phase C18 column, photo-diode array detector, and fluorescence detector for quantification. Peaks were identified and calibrated using authentic pigment standards (U.S. Environmental Protection Agency and DHI Lab Products, Denmark), unialgal cultures, and reference stocks

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

2.5 Clone library and phylogenetic analysis

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

For the cloning procedure, PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics SA), measured with a Nanodrop ND-1000

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

To provide a quantitative confirmation of the major elements identified in the clone libraries, a preliminary run of Illumina MiSeq sequencing was performed on the same DNA extracts for horizon A and B. In addition, one surface sample (0.25 m depth) was included to provide a reference for the initial microbial assemblages, assuming that it experienced minimal

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

3 Results

3.1 Geochemical analysis of bulk sediment

3.1.1 Organic matter and pore water chemistry

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

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

3.1.2 Pigment concentrations

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

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

19 3.2 Microbial characteristics

20 3.2.1 Microbial activity, density and diversity

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

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

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

16 **3.2.2 Bacterial and archaeal clone libraries**

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

1 (Figs. 2 and 4), although their respective sequences still formed separate clusters (Figs. 4 and
2 6).
3 Despite potential cell migration in soft methane-saturated clays, archaeal sequences obtained
4 from the Holocene record provided evidence for an environmental selection of assemblages
5 with depth in the sedimentary profile (Figs. 5 and 6). Main groups successively identified
6 with depth were affiliated with the Marine Group 1 and *Lokiarchaeota* (i.e. former Marine
7 Benthic Group B) within the first meter, *Methanomicrobia* and *Bathyarchaeota* (i.e. former
8 Miscellaneous Crenarchaeotal Group) plus Marine Benthic Group D within the next 4 m of
9 sediment, and candidate phyla *Hadesarchaea* (i.e. former South African Gold Mine Group;
10 Baker et al., 2016) and *Bathyarchaeota* below 5 m depth (Fig. 6). Methanogen sequences
11 corresponded with depth to *Methanolinea*, *Methanosarcina*, *Methanoregula* and uncultured
12 *Methanomicrobiaceace*. Finally, *Bathyarchaeota* sequences were present throughout
13 Holocene sediments forming clusters associated with their respective sampling intervals (Fig.
14 5). Direct comparison between the LGM and Holocene horizon (Figs. 5 and 6) revealed
15 archaeal assemblages mainly consisting of *Methanoregula* and Marine Benthic Group D in
16 the Holocene, and mostly *Hadesarchaea* sequences in the LGM.

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

27

4 Discussion

4.1 Holocene and LGM paleoclimatic and geochemical conditions

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

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

4.2 Interpretation of sedimentary DNA

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

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

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

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

these assemblages suggest that sedimentary microorganisms first degraded the labile OM from algae before generating fermentative H_2 and CO_2 that served as substrates for methane production by *Methanomicrobiales*. Such substrate evolution during prolonged OM diagenesis could promote the recycling of end products and syntrophic hydrogen consumption, as presently observed with autotrophic methanogenesis and homoacetogenesis (Wüst et al., 2009). Such a pattern also suggests that the final Holocene microbial assemblages arose from metabolic complementarities, reinforcing our previous study on their role in the degradation and geochemical cycling of OM (Vuillemin et al., 2014b).

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

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

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

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

4.3. Sedimentary DNA and fossil pigment preservation

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

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

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

4.4 A model for ancient and extant microbial assemblages

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

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

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

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

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

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

13

14 ## 5 Conclusions

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

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

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

Author contribution

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

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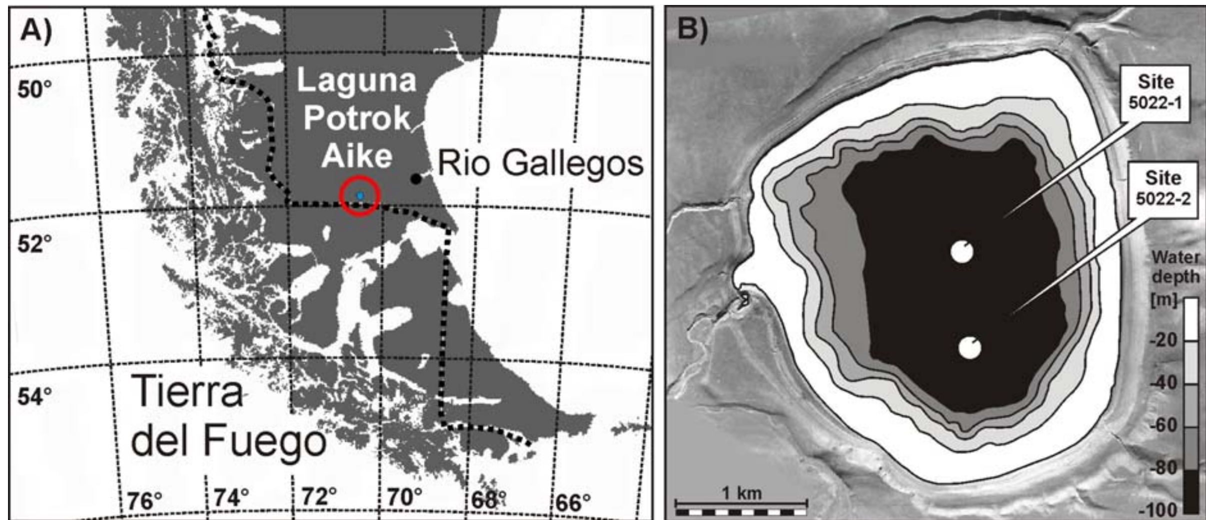

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3 Figure 1. Map of Southern Argentina displaying the location (**A**) and bathymetric map (**B**) of
4 Laguna Potrok Aike showing the two drilling sites (Zolitschka et al., 2006). Pore water and
5 geomicrobiological samples were retrieved from cores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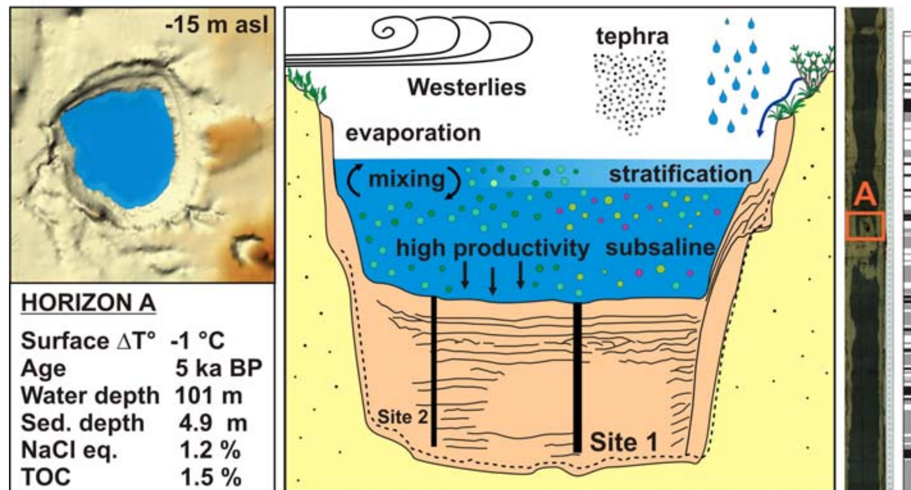

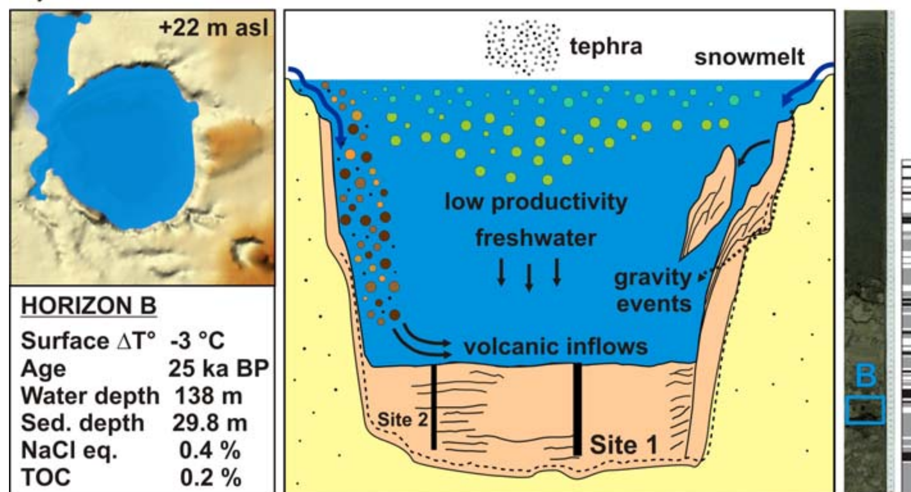

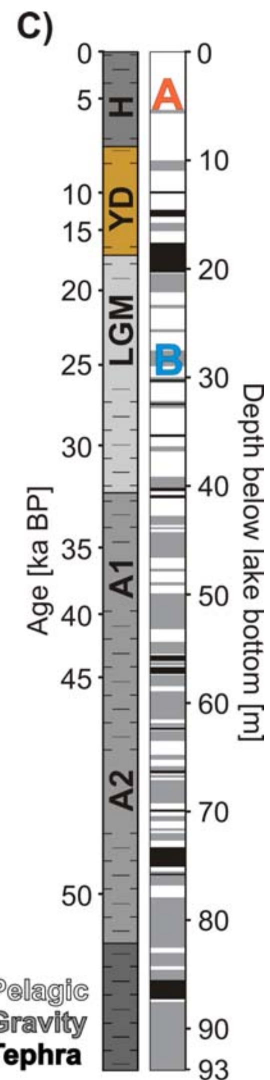

Figure 2. Paleoenvironmental conditions at Laguna Potrok Aike during the Holocene (**A**) and LGM times (**B**), with from left to right: Climatic and lacustrine parameters, sagittal views of the basin and respective core sections locating the 16S rRNA samples. Holocene times correspond with active Westerly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

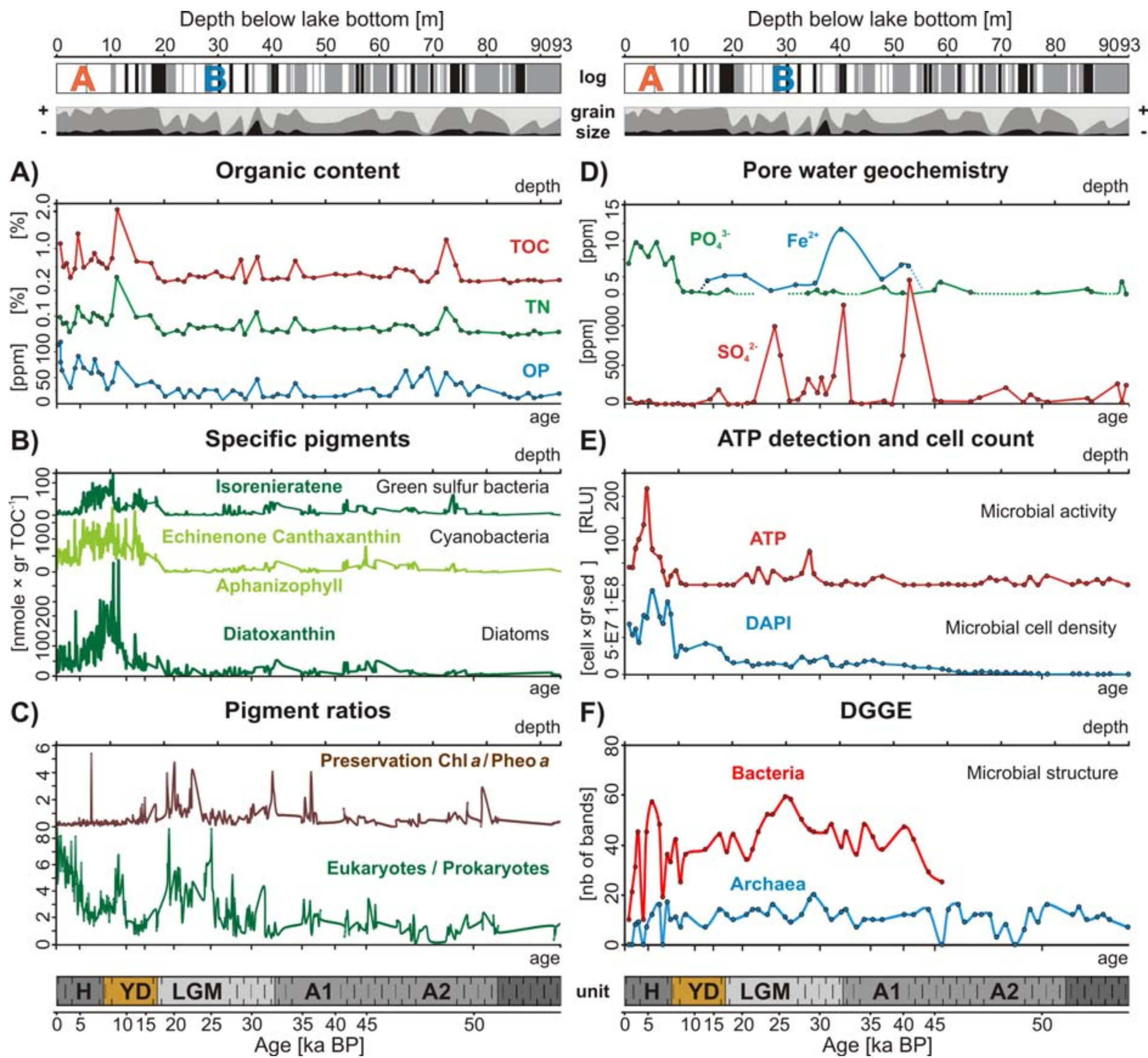

Figure 3. Paleoclimatic and geomicrobiological multiproxy. **Top)** Stratigraphic sequence of Laguna Potrok Aike, followed by grain size with clay (black), silt (dark grey) and sand (light grey). **A)** Total organic carbon (TOC), total nitrogen (TN) and organic phosphorus (OP) from bulk sediment. **B)**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

1 structural shifts in bacterial and archaeal communities. **Bottom**) Lithology log displaying the

2 five units established by Kliem et al. (2013) and their corresponding climatic intervals.

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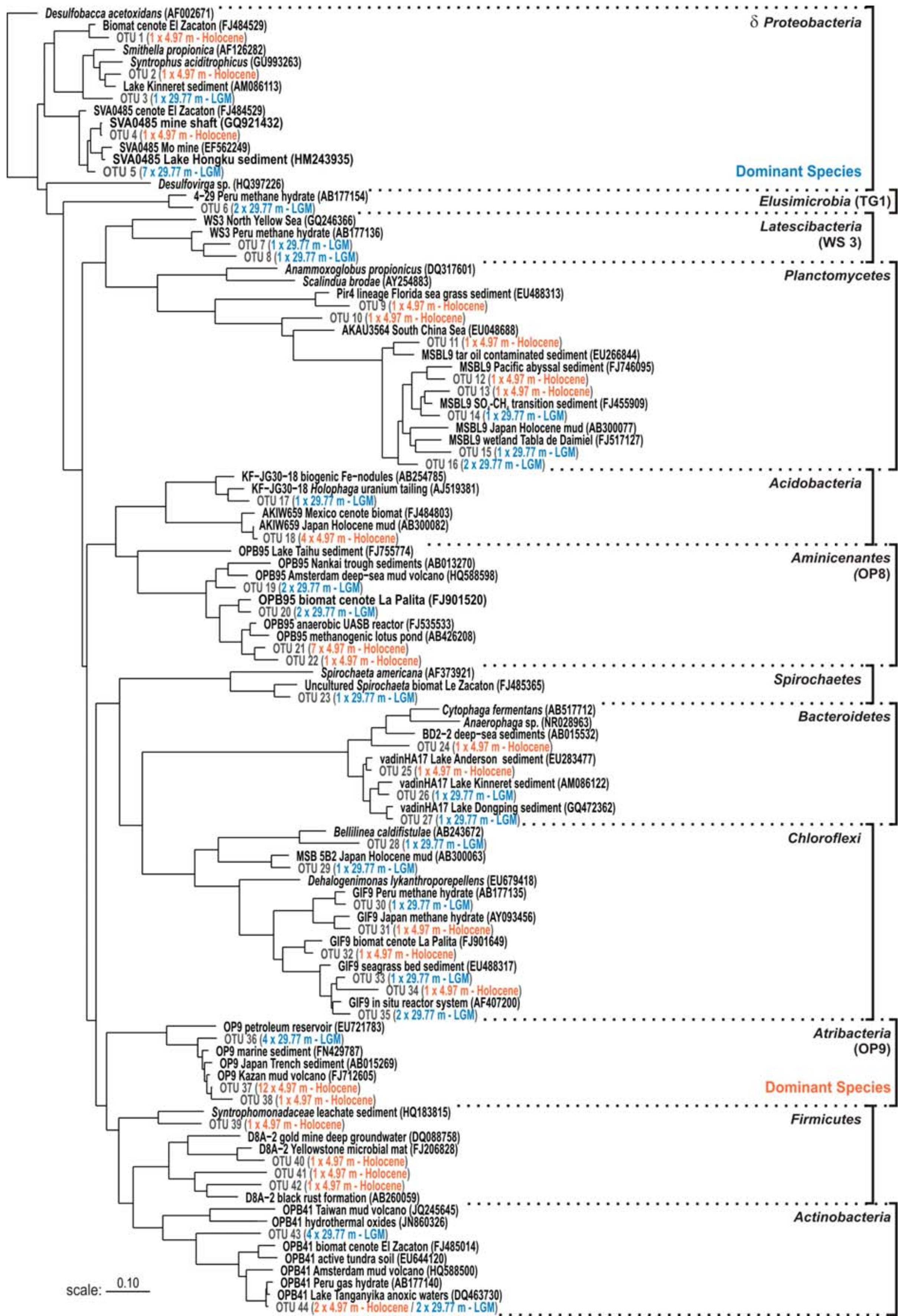

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2 Figure 4. Maximum likelihood phylogenetic tree of bacterial 16S rRNA gene sequences
3 (1400 bp) recovered at 4.97 and 29.77 m depth from Holocene (orange types) and LGM
4 (blue types) sediments. *Atribacteria* and *Aminicenantes* are the main taxa encountered in the
5 Holocene organic-rich pelagic sediments, whereas sulphate reducers are dominant in the
6 LGM horizon composed of intercalated volcanic mafic sands and hemipelagic sediments.
7 Boldface types signify database references with sequence accession numbers in parentheses.
8

1

1

2 Figure 5. Maximum likelihood phylogenetic tree of archaeal 16S rRNA gene sequences (900
3 bp) recovered at 0.25, 0.55, 1.90, 2.51, 4.97, 7.81, 9.37 and 29.77 m sediment depth. Clone
4 series established throughout the Holocene record (dark grey types) indicate a depth-related
5 evolution of the assemblages, with a general trend from marine groups to methanogens
6 ending with *Hadesarchaea* (i.e. SAGMEG) sequences. Comparatively, the Holocene
7 archaeal assemblage at 4.97 m depth (orange types) is mainly composed of
8 *Methanomicrobiales* and *Bathyarchaeota* (i.e. MCG), whereas the LGM archaeal assemblage
9 at 29.77 m depth (blue types) is restricted to *Hadesarchaea* and *Bathyarchaeota* divisions.
10 Boldface types signify database references with sequence accession numbers in parentheses.
11

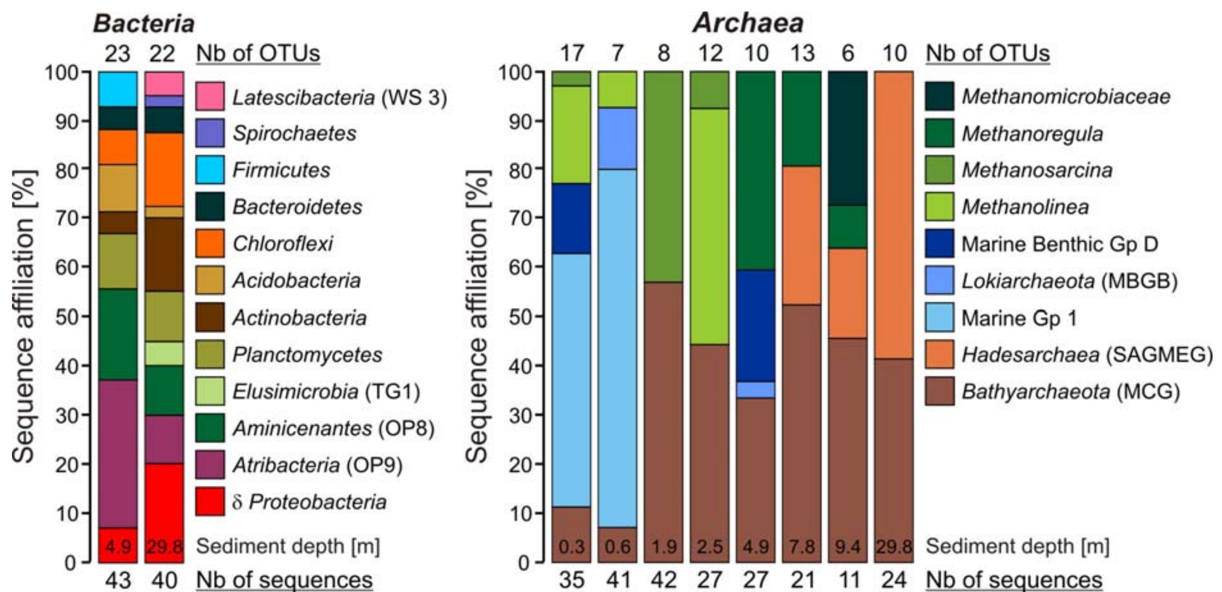

Figure 6. Histograms of identified phylotypes displayed in relative %, with OTU and sequence numbers at the top and bottom, respectively. **Left**) Several bacterial phylotypes are shared by the Holocene and LGM horizons (i.e. *Chloroflexi*, *Planctomycetes*, *Bacteroidetes*) as they are known ubiquists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

Supplementary material

1) Diversity indices (page 1)
2) Rarefaction curves (page 1)
3) Sedimentary features in Holocene and LGM horizons (page 2)
4) Geochemistry of pore water and lake surface waters (page 2)
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6) Illumina MiSeq bar charts for quantitative assessment of microbial assemblages (page 4)
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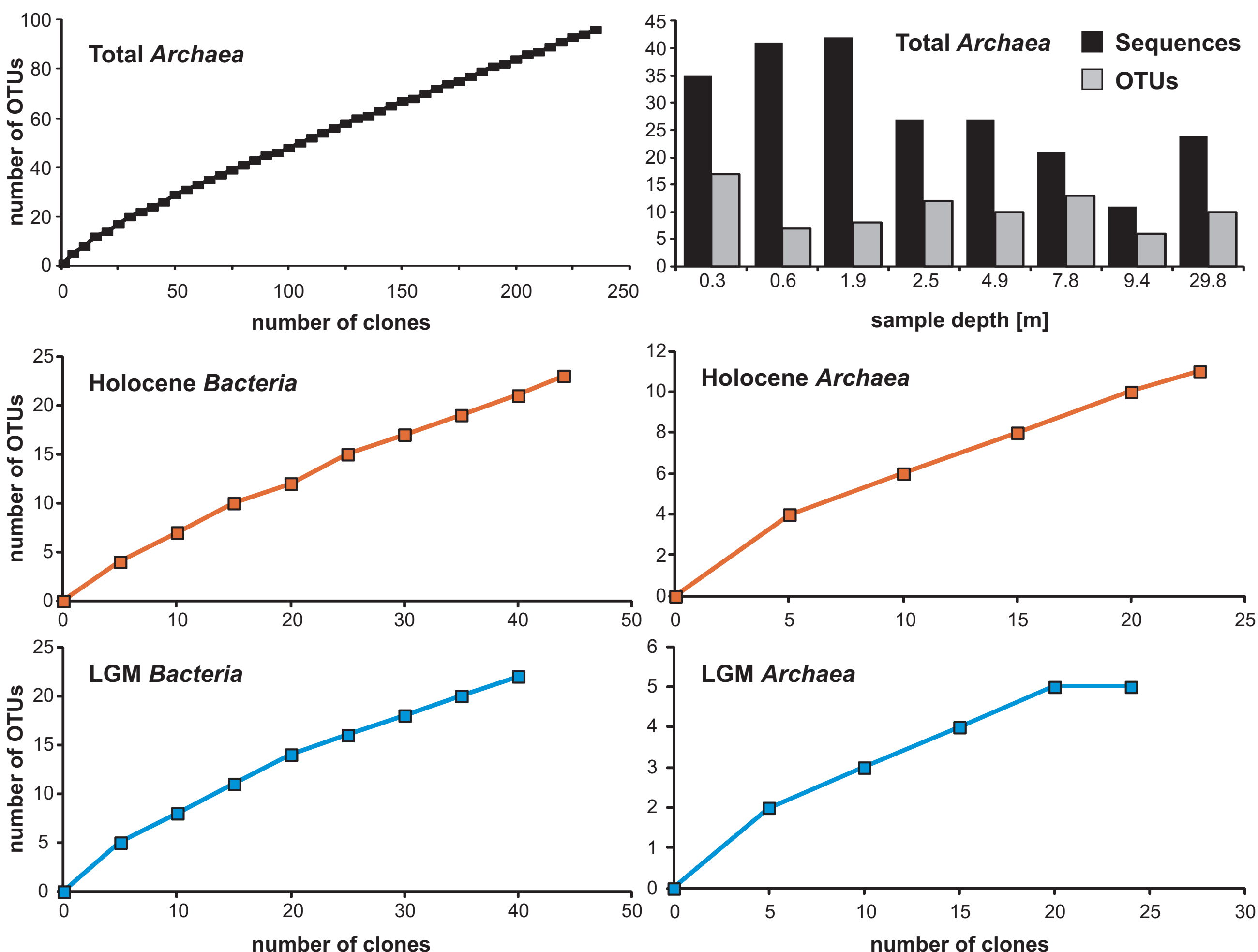
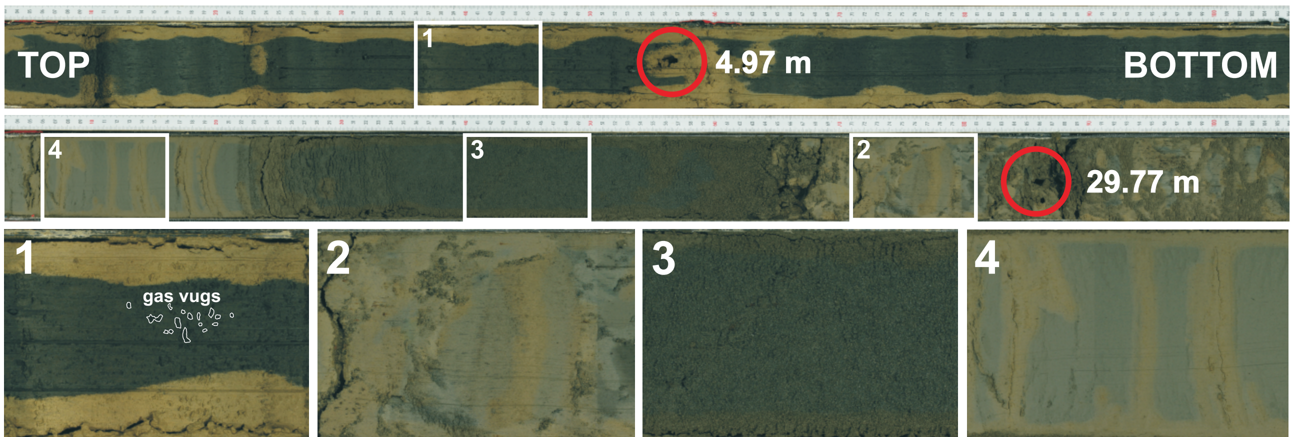
Bacteria	Sequence number	Cut-off	OTUs	Chao	Shannon	Dominant species
Total	84	3 %	44	126.67	3.40	*Atribacteria*
Holocene	44	3 %	23	108.50	2.64	*Atribacteria*
Glacial	40	3 %	22	33.14	2.86	*δ Proteobacteria*
Archaea						
Total	235	3 %	96	886.00	3.71	Marine Group 1
Holocene	23	3 %	11	21.50	2.10	*Methanomicrobiales*
Glacial	24	3 %	5	8.00	1.10	*Hadesarchaea*

1) Table displaying phylogenetic indices: OTUs were calculated for a 97 % sequence identity cut-off value.

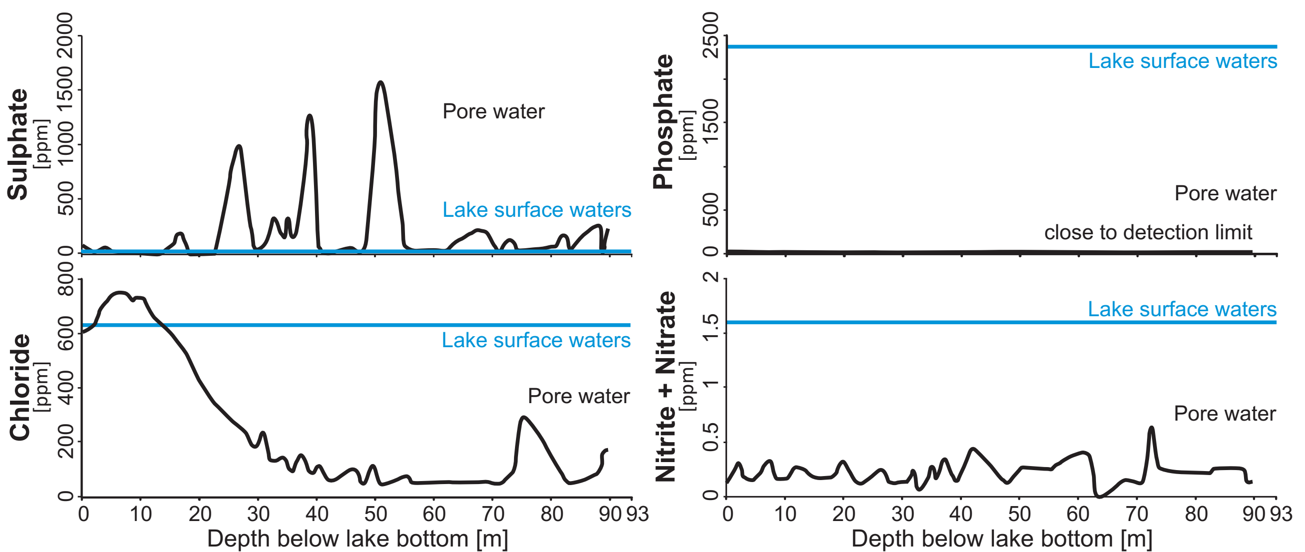


2) Rarefaction curves: OTUs were calculated for a 97 % sequence identity cut-off value.

page 1

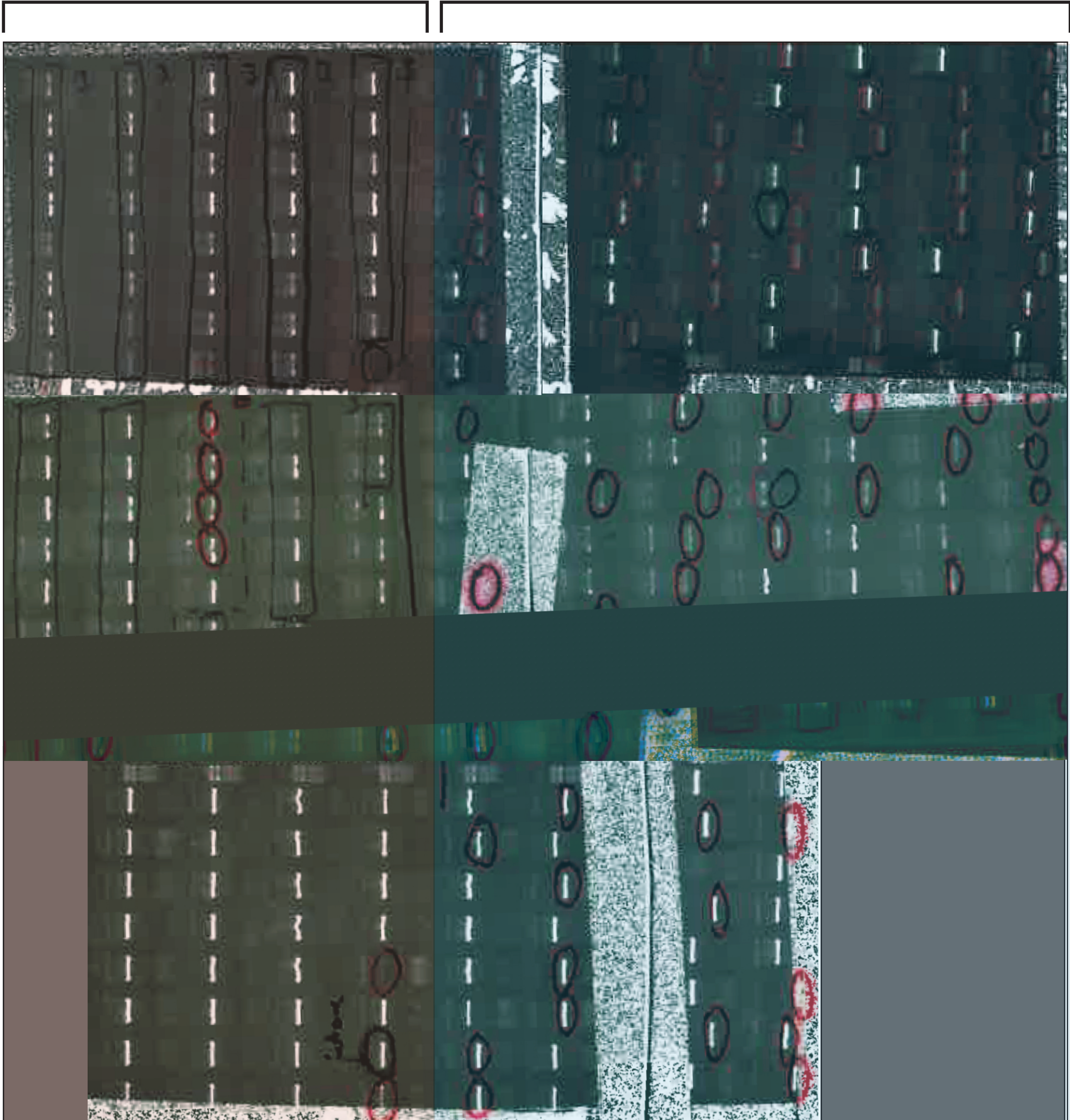


3) Comparison between Holocene and LGM sedimentary features in sections sampled for clone libraries: The Holocene sequence can be characterized as pelagic to hemipelagic, black and soft, anoxic gas-saturated sediments (**1**). Oxidized rims occurred during storage after the aperture of sampling windows. The LGM sequence first reflects a pelagic to hemipelagic regime with structures of fluid escapes (**2**). Then, fine mafic sands (**3**) could be associated with a gravity event that triggered gas escapes due to sudden loading on the underlying sediment (Vuillemin et al., 2013a). The top of the section shows a return to pelagic sedimentation (**4**). In general, the last glacial record displays multiple intercalations of volcanic detritus.

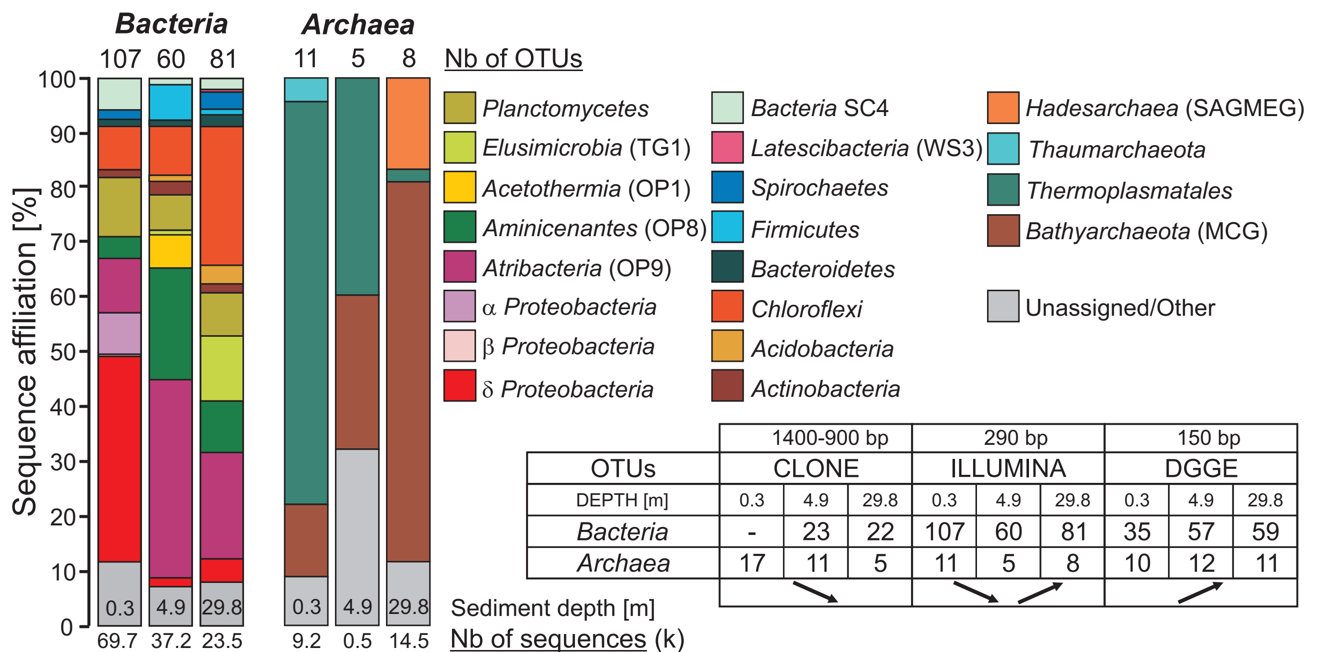


4) Geochemical comparison between pore water and lake surface waters: The present comparison provides evidence for the absence of sediment contamination by the hydraulic system during drilling operations. Results from pore water analysis can thus be used as indicators of paleoconditions (i.e. chloride) and geochemical changes within sediments (i.e. sulphate, phosphate) (Vuillemin et al., 2014b).

Holocene LGM



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



	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

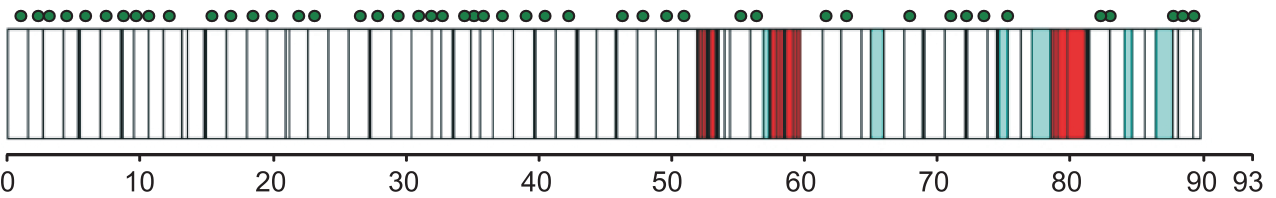
6) Preliminary results of Illumina MiSeq sequencing: Bar charts were established for horizon A (Holocene, 4.9 m depth) and B (LGM, 29.8 m depth) in order to provide quantitative comparison with the main elements identified in the clone libraries. One surface sample (0.3 m depth) was added as reference,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

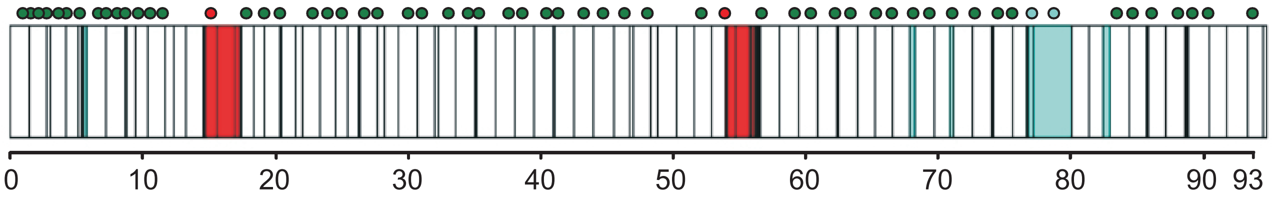
The surface sample reveals a majority of *Proteobacteria* potentially related to layered microbial communities. We note the absence of phototrophic sequences related to *Cyanobacteria*, *Chlorobi* or even chloroplasts. Another important point is that the presence of *Planctomycetes*, *Chloroflexi*, *Actinobacteria*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

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

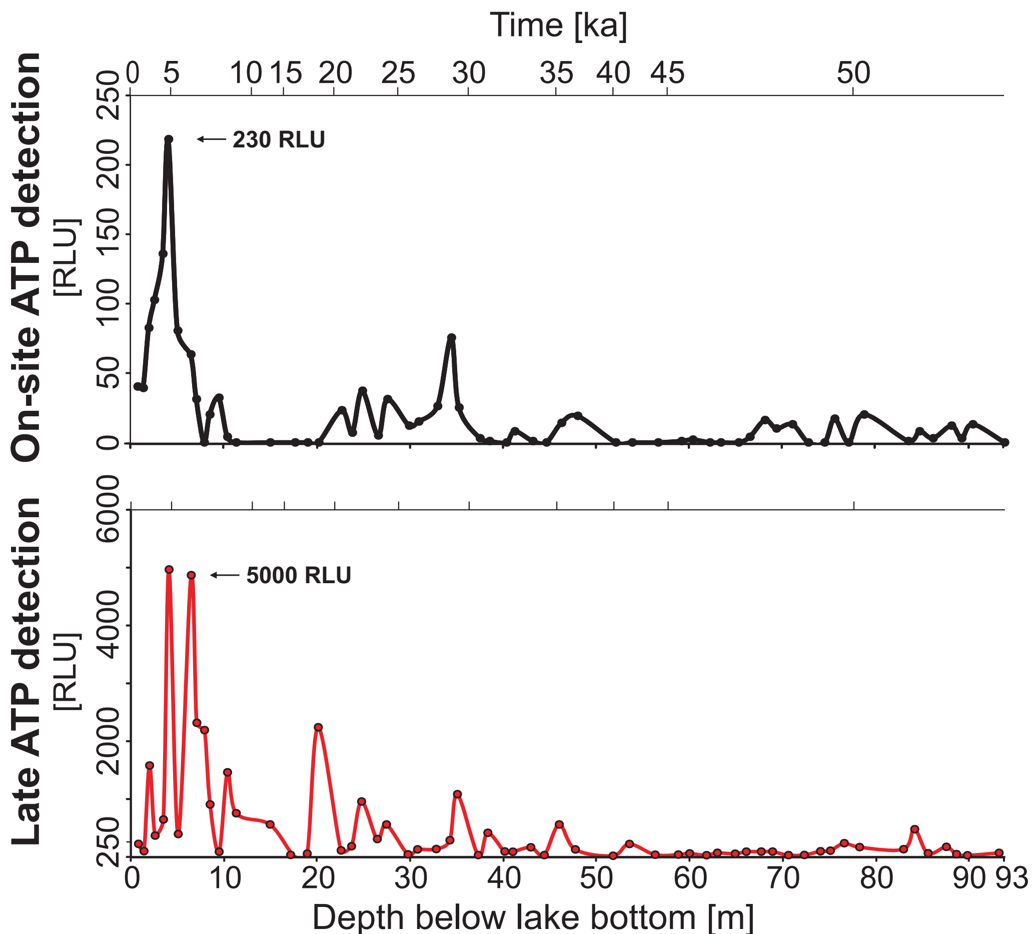
Method: We used bar code universal primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') to cover 291 bp of the bacterial and archaeal subunit 16S rRNA gene. Individual tags were composed of 8 nucleotides attached at each primer 5'-extremity. 32 ng of DNA per amplicon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

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





7) Core sections: Possible drilling artifacts (Ohlendorf et al., 2011) were put in parallel with sample locations in order to validate the genuine use and interpretation of their results in the absence of any established composite depth at site 1 (Gebhardt et al., 2012). Drilling artifacts were mostly related to coarse layers and gravity events (Kliem et al., 2013).



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

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

9) Screening Illumina MiSeq results for preserved phototrophic sequences: *Planctomycetes* related to *Phycisphaera* and *Pirellula ar*e 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.