Biogeosciences Discuss., 12, C9770–C9777, 2016
www.biogeosciences-discuss.net/12/C9770/2016/

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Recording of climate and diagenesis through fossil pigments and sedimentary DNA at Laguna Potrok Aike, Argentina" *by* A. Vuillemin et al.

A. Vuillemin et al.

aurele.vuillemin@gfz-potsdam.de

Received and published: 16 February 2016

All modifications in the manuscript are highlighted in red font. The supplementary material has been incremented with new data and bound into one single pdf (see supplement.pdf). This document covers answers to the four referees.

Referee no.4

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

Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

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

BGD
12, C9770–C9777, 2016

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: To avoid stating results in the introduction, this sentence has been modified as

BGD
12, C9770–C9777, 2016

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

BGD
12, C9770–C9777, 2016

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.

Answer: We have moved and shortened this section to an earlier part of the manuscript

BGD

12, C9770–C9777, 2016

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.

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

- 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.

Answer: 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.

Answer: This phrasing has been changed for: “...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.

Answer: 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

BGD

12, C9770–C9777, 2016

results and should perhaps appear earlier in the discussion so that this point is dealt with. The information here is sufficiently convincing.

Answer: 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 preservation potential, turnover 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

BGD

12, C9770–C9777, 2016

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.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C9770/2016/bgd-12-C9770-2016-supplement.pdf

---

Interactive comment on Biogeosciences Discuss., 12, 18345, 2015.