Biogeosciences Discuss., 12, C9759–C9766, 2016
www.biogeosciences-discuss.net/12/C9759/2016/

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.2

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 assem-

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

blages 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 reveal-

BGD
12, C9759–C9766, 2016

ing 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.

BGD
12, C9759–C9766, 2016

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 + ïĄć 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.

BGD

12, C9759–C9766, 2016

- 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

BGD
12, C9759–C9766, 2016

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.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C9759/2016/bgd-12-C9759-2016-supplement.pdf

BGD
12, C9759–C9766, 2016

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