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Interactive comment on "Arctic Gypsum Endoliths: a biogeochemical characterization of a viable and active microbial community" *by* L. A. Ziolkowski et al.

L. A. Ziolkowski et al.

lorized@gmail.com

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Response to reviewer #1 comments.

We thank the reviewer for their thoughtful comments. Most of the requested changes are focused on the methodology for the molecular analyses of the microbial community. Many of these details were purposely left out to maintain brevity in the manuscript. We have now included the details requested, as outlined below.

"There is no mention whatsoever to the number of sequences produced, what was the

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chemistry used (Titanium, I guess?), etc" → We have included the more specific details of the pyrosequencing methods and the numerical descriptions of the results.

"Furthermore, it is unclear if the authors carried out metagenomic sequencing of total DNA, as could be inferred from lines 23-24 page 2276 ("Total DNA was concentrated from multiple extractions ... and sent for pyrosequencing analyses..."), or if they sequenced SSU rDNA amplicons, as can be deduced from the description of primers used (lines 24 page 2276-line 2 page 2277) and lines 25-26 page 2279 ("Pyrosequencing of the 16S, 18S, and 23S plastid rRNA genes of..."). And if they pyrosequenced amplicons, did they mix them? How? How many libraries per run or line?" → Detailed descriptions addressing the above points have now been clarified in the Methods section including specifics of the DNA extractions, ribosomal subunit amplicon libraries (a total of 4 datasets), and reference to the sequencing protocols from the sequencing centre.

"When the authors say that only sequences of groups constituting more than 1% of the reads, how many are these? Later, "representative sequences" of what, how many and what was their proportion from the total? All the basic details are absent." → A more detailed description of the sequencing data and filtered datasets that were then used to create the results figures have now been described in detail in the methods and results. Also, the number of filtered (> 1%) reads that were used were already depicted in Figure 4. We have edited the figure caption to increase clarity.

"The methodology to treat the pyrosequences is also far from clear. Pyrosequencing provides massif amounts of reads that cannot be easily analyzed by manual BLAST, without some kind of clustering process earlier, which is not described. There are also very important concerns regarding sequence analyses. Pyrosequencing introduces many errors. How did the authors control for quality? How many sequences were eliminated? Also, depending on the clustering method used to group the sequences in operational taxonomic units (if this was at all done – because it is not described, though standard procedure), the diversity can be highly overestimated. Provide the

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total number of sequences, describe exactly how you treated them and clustered them (what cut-off values were used) and provide a final number of OTUs retained as true per phylum (e.g. in a table). –> Details of the pyrosequencing data quality processing and post-filtering analyses have been included (although standard when referring to a public pipeline such as the RDP). A summary table for the sequencing data has been added and can be included as part of supplemental information (Table S1)

“This is very important: Sequences must be deposited in a public database and made accessible to the scientific community. The authors should submit their sequences to the Sequence Read Archive (NCBI) and provide an accession number.” → The sequences read libraries have been deposited in the Sequence Read Archive under accession numbers SRA074427 and the revised text now includes the accession number.

“Phylogenetic analyses. In the methods section, the authors say that they select "representative" sequences and aligned them to generate a neighbor-joining phylogenetic tree. However, the figures show "maximum likelihood" trees. Please, precise. Also, indicate whether the ambiguously aligned positions were removed. Making phylogenetic trees with relatively few positions (pyrosequences) is dangerous, especially if sequences from disparate phyla are included. The tree in Figure 5 is an example. Alphaproteobacteria are not monophyletic. Cyanobacteria and chloroplasts do not cluster together. Likely, if chloroplast sequences were removed, a better resolution could be achieved for bacteria. “ → We have clarified how the phylogenetic alignments were generated. We generated both neighbour-joining and maximum likelihood phylogenetic trees and we chose the ML trees for publication although similar patterns were obtained with both models. We acknowledge that two clusters are not monophyletic as presented, however, the specific clusters with the closest matching sequences for each of our representative sequences are robust and we preferred showing the full diversity of the bacterial hits and the environmental affiliations from the closest matching sequences.

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"The benefit of using 23S rRNA gene markers to describe the diversity of algae is unclear, since in most cases, the environmental diversity of plastid markers is not yet linked to the diversity of the nuclear 28S rRNA genes. And even if it was the case, the database for 28S rRNA genes is much poorer than that of the most widely used 18S rRNA gene. I do not understand why the authors use fungal 18S rRNA primers and 23S rRNA plastid primers instead of using universal eukaryotic 18S rRNA primers, which would have led to the amplification of both, fungi and all kinds of algae, plus other protists. Indeed, here, if there are other protists associated to the endoliths, we are missing them, because they have not been targeted." → Several studies have shown that 454 primers for "universal" 18S markers are not entirely "universal". As we have microscopically observed an abundance of fungi and bacterial phototrophs in the samples, we wanted to target those specific groups primarily, with secondary datasets looking at green algae plastid phylotypes since these were the targets are most of the endolithic studies present in the literature (i.e. Wong et al., 2009). We agree that it might have been more comprehensive to employ a more universal 18S primer, though none was available at the sequencing centre we chose and the depth of community analyses conducted is greater than most of the endolithic profiles published to date.

"The authors use "Algae" as if algae constituted a taxon: it is not. Algae group a variety of photosynthetic eukaryotes belonging to very different phyla (green algae, red algae, diatoms, euglenas, etc). Therefore, the authors should use the corresponding taxon name. Also, vernacular names should not be written starting with a capital letter, only Latin names should." → We have clarified our taxon specification to "green algae/chlorophyta" and have corrected the letter case for all instances of vernacular names.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/10/C1734/2013/bgd-10-C1734-2013-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 10, 2269, 2013.