

## ***Interactive comment on “The keystone species of Precambrian deep bedrock biosphere belong to Burkholderiales and Clostridiales” by L. Purkamo et al.***

**Anonymous Referee #2**

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General comments: Overall the manuscript is well written. The introduction clearly states the objectives, the results part is well organized and data was nicely described. The results obtained in this study are interesting and help understand microbial processes in the deep terrestrial biosphere. I have one main crucial problem though, and that is the use of metagenome predictions, specifically for quantitative analysis of certain pathways. I think the results based on this analysis should be toned down, and maybe focus more on possible absence/presence of some pathways. Also, I didn't find much use of these analyses in the discussion part, whereas the paragraph in the results part was quite substantial.

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Specific comments: The title is a bit misleading since it reads like the samples come from rock cores and not groundwater. Please address this.

Material & Methods P6\_L16: Why was DNase treatment not applied to all the RNA extracts? P8\_L11: What is the coverage of the archaeal 16S rRNA gene diversity with the primer pair used for the nested PCR? Different primer pairs are known to be biased against certain archaeal groups. See for example Teske et al. (2008). P8\_L20: What were the PCR conditions for the 16S rRNA genes?

Results P15\_L5: I see in figure 4 that there are some phototrophs in the RNA-based analysis from the 2260 m sample. How do the authors explain this? P15\_L17: There exists a recently published paper on a SAGMEG genome. These archaea have been renamed Hadesarchaea (Baker, Nature microbiology, 2016). Please change conclusions in the discussion part based on findings in this paper as well.

Discussion P20\_L16: (...) these organisms can prevail ‘in the same environment’ or ‘same environments’. P22\_L3: the authors don’t offer an explanation as to why the SAGMEG dominate the DNA-based analysis but methanogens dominate the RNA-based analysis at 967m. Is the number of reads obtained for each analysis significantly different? Please address this issue. P22\_L15: represented P22\_L16: this observation could be due to low coverage by the primer pair used for the nested PCR, and by the fact that 2 PCRs were carried out for the archaeal diversity analysis.

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