Biogeosciences Discuss., 12, C10227–C10230, 2016
www.biogeosciences-discuss.net/12/C10227/2016/

Biogeosciences Discussions
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EGU

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

L. Purkamo et al.

lotta.purkamo@gmail.com

Received and published: 17 March 2016

The authors thank the referee for constructive remarks and criticisms that will be helpful in improving this manuscript. Referee #1 first brings up a question about the reliability of metagenomic prediction with PICRUSt. As the PICRUSt analysis is depending on the partial 16S rRNA sequence data, this approach may only be used as a prediction of the properties of the metagenome. The high weighted NSTI (nearest sequenced taxon index) values ranging from 0.07 to 0.3 also indicate the uncertainty of the prediction. However, we do not infer major conclusions about the predicted metagenome although we feel that this analysis gives an overview of the top-level functionality of

the metagenome of this environment. Nevertheless, we consider omitting the results of the HUMANn analysis (p.18114, l.1-8 and p.18121, l. 20→p.18122, l. 4 and l. 27→p.18123, l. 2), where we have used these predicted metagenomes as starting material. As referee #1 points out, this analysis depends ultimately to the partial 16S rRNA sequences, so stating that certain pathways are complete may not be feasible. Furthermore, the dominance of heterotrophy in this ecosystem can be inferred from the taxonomy of the dominating OTUs, as the referee #1 states. Referee #1 also questions the reasons why we have named OTUs belonging to Burkholderiales and Clostridia as the keystone species. We have based this statement to the suggestion by Steele et al. 2011, who stated that the highly connected nodes would be analogous to microbial keystone species. As keystone species are described as organisms that have more significant role in the ecosystem that their abundance usually refers and if these organisms would be removed from the ecosystem, it would be subjected to dramatic changes. If we consider the possible role of species belonging Burkholderiales in the deep subsurface, they are most likely involved in hydrogen oxidation and thus are the potential primary producers in this oligotrophic environment. Clostridial species on the other hand could be occupying the niche for heterotrophic carbon assimilation, possibly providing H2 produced in fermentation processes. Burkholderiales and clostridial species have been found from other serpentinite-hosted deep subsurface aquifers (Brazelton et al. 2012, Tiago et al. 2012). These studies have suggested that Burkholderiales and clostridial species play a significant role in carbon and energy exchange between one another and other bacterial groups in these environments. We would be happy to implement this interpretation to the manuscript. What comes to the abundance of these species/OTUs, we argue that they are in fact quite abundant at least in all other total fracture communities except at 1820 m. However, we could tone down the heading of the paper towards more descriptive, such as ”Microbial co-occurence patterns in Precambrian deep bedrock biosphere”. The members of Staphylococcus as a keystone species was not expected. We believe that this result could be explained by contamination for two reasons: 1) staphylococci are common in human body, and 2)

BGD

12, C10227–C10230, 2016

the amount of sequences affiliating with Staphylococcus were low but they were discovered in all samples. However, as mentioned by the referee #1, we can't ascertain the physiology of an OTU from it's taxonomic affiliation, and it can be that these bacteria with Staphylococcus-like 16S rRNA may be true members of the deep biosphere with unknown metabolic properties. We can add this explanation to the discussion part of the manuscript.

Specific comments: The information about the samples coming from deep groundwater can be added to the beginning of the discussion part (p. 18124, l.4: The bacterial community structure in the groundwaters of Outokumpu fracture zones. . .), and also to the abstract (p.18104, l. 4 . . .intrinsic microbial communities from groundwater of six fracture zones..),. p. 18110, l. 16: Information about the E. coli plasmids containing 16S rRNA gene can be added. p. 18111, l. 4-19: First PCR was done with 30 cycles in both archaeal and mcrA –targeted PCR. As referee #1 suggests, the nested PCR approach is likely to introduce greater error to the subsequent sequencing analysis than the regular PCR. Nevertheless, as the number of archaea and especially methanogens have been shown to be low in comparison to bacteria both in previous studies (Purkamo et al. 2013, Nyyssönen et al. 2014) as well as in this study, it was imperative to choose the nested PCR approach in order to obtain community data of these microbial groups. p. 18112, l. 16: The length limit of 100 bp was chosen because of the relatively low amount of longer good-quality reads. This may affect the phylogenetic analysis of mcrA sequences towards less reliable. However this was necessary in order to obtain information about the methanogens in the fractures of Outokumpu. p. 18113, l. 2-9: We calculated average estimate of coverage (ACE) for the dsrB dataset, and overall the observed species values were ranging from 50 to 80% of the estimated number of species. However, lower coverage was detected from the samples, where the number of sequences were low (180 m DNA and RNA, 1820 m DNA) and where one OTU dominated the reads (500 m RNA). With the archaeal dataset, all rare species were singletons, so ACE could not be calculated. However, several archaeal samples contained less than 10 observed species (500 m, 2260 m and 2300 m), although the

BGD
12, C10227–C10230, 2016

amount of reads in these samples ranged from less than 300 to over 14000. Therefore, we estimate that the interpretation of archaeal community composition is likely correct. p. 18114, l. 21-23: Range of cell numbers can be added to the text as well. Table 1: There is an error in the Table 1 heading, concentrations of cations and anions should be in mg/L , not mg/ml. Also, alkalinity was measured in mmol/L, not mmol/ml. The naturally high salinity of the samples complicated the geochemical analysis, which reflects to the raised detection limit of several cations and anions. Table 2: The lower cell counts than 16S rRNA gene copy numbers in the upper samples may be explained by multiple 16S rRNA gene operons in one cell, but the inversed trend for the deeper samples may be for example due to lower DNA extraction yields in the deeper fractures, where salinity is higher. There is also an error in the heading of the table, last sentence should be ”Values are given mL-1.” (i.e. per mL).

Interactive comment on Biogeosciences Discuss., 12, 18103, 2015.