

General comments.

Broadly the interest in subsurface life has grown in recent years, and the linkage of 16S rRNA gene identified taxa distribution and geochemical parameters would be of broad interest to journal readers from a variety of backgrounds. Overall, the manuscript reads cleanly and is easy to follow. The authors carefully inferred from the data- however additional details on how certain analyses were conducted, or in the case of piecrust, more information on the potential error is necessary. The 4 figures are largely overshadowed by the massive information contained in tables, in general I don't think tables are as effective for visually reporting data, and think the authors could include more visual representation of the data. For instance rather than the table or in addition to the tables, some visual representation of community structure and change would be appreciated. Also the figures and tables in the text are very small and needs to be increased throughout.

Specific comments.

Abstract:

line 6: IN general avoid "great" in sequencing depth- as it is qualitative. Report number of reads and depth of sampling and let it stand for itself. Depth is dependent on structure of community and amount of reads.

I appreciated the "background" information being included in this manuscript so readers do not have to look up information in another paper to understand the system.

Why was V6 selected as the primer region? Might be nice to include a reference for this region from recent studies done on a similar sequencer (read length). Also please confirm sequencer and model- a HiSeq and include model, 2000 or GAI?

Results:

Line 15, in a few words define "occurring". Does this mean a single read, or a certain level of abundance (at least 1%) in all samples? Also define rare.

Figure 3. Can the authors provide some more information on the image- perhaps color and shape to represent the sample scores (dots) by depth and location more easily than text? As a reader I am trying to understand what is unique about each of the samples and the geochemistry. For instance is OL-K444 unusually deep or location wise distinct from the others.

Were all samples collected at the same time? Sorry if I missed this.

Please check language on top of 13831, I wasn't quite sure what was being said here. An introductory sentence could also help. *"Of the 651 bacterial and 81 archaeal genera (or equivalent groups) identified in this study 42 bacteria and 59 archaeal genera showed any significant correlation with other genera."* In general I am having a difficult time tracking this analyses- additional details would help simplify the reading of the manuscript. How was rare calculated and determined? Not clear.

From this section and figure 4.

- 1) Please in the text provide how are the "communities" 1-7 defined (analogous to figure 4- different clusters of microbial taxa)? I find use of "communities" somewhat confusing, and think it is sufficient to call them co-occurring clusters. For instance the piecrust was done on the level of the individual samples (also

communities) from different or the co-occurring cluster communities that were just defined two sentences before? Please refrain from using name community for the in silico identified clusters.

2) Figure 4.

- How was the chemistry overlaid on the groups, was this included into the analyses or done manually based on another analyses (I assume strong= statistically significant correlations, and if so state rather than strong).
- Do the circles represent OTU level designations- if so why is there differences in taxonomy- some are family level IDs others are genus? If different, why wasn't the same taxonomic unit selected for this analyses.
- Also please make figure 4 larger, it was difficult to read when printed out.

Abstract and again in results on Line 24. Is rough an euphemism for inaccurate? Can the authors give a scale for NSTI scale- I know 1 means no match, but for instance is 0.282 for Archaea considered too far diverged that the data is error prone "or rough"? I personally do not use Picrust for environmental systems, for the reasons the authors allude to (why not do metagenomics, there is a danger in inferring function from divergent 16S), but I am open to entertaining its use if necessary precautions are taken and quantified. So I think the readers would benefit from some authors providing some additional data here. For instance, typically for human microbiome samples the NSTI ranges from XX-XX, while other NSTI reported from environmental datasets have had a range of XX-XX. Provide precedence please for including seemingly high numbers, and thus more inaccuracy, in the analyses, as this will be good to incorporate in future studies/comparisons.

I presume inferred community metabolism change did not also with geochemistry if not depth? Table 9b is very difficult to read. After reading the picrust analyses in the results, I am really not sure what level of information it adds-The discussion was more clear and contained many details not included in the results.

"However, at specific depths (328, 423 m) the archaea may contribute with over 50 % of the estimated 16S rRNA gene pool (Table 1). The major archaeal group present at these depths were the ANME- 2D archaea indicating that nitrate-mediated anaerobic oxidation of methane may be 25 especially common (Haroon et al., 2013)." **Is this consistent with geochemistry from the site?**

The NSTI values for both the bacterial and well as the archaeal communities were great indicating 10 that no closely related species have yet been sequenced
The values were high not great.

This statement may not be correct, as many obligate fermenters are known to have and use ATP synthase using a variety of alternative proton pumping mechanisms outside of NADH dehydrogenase. What criteria used to determined oxidative phosphorylation? The presence of NADH dehydrogenase? A full ETC? cytochrome oxidase. Please qualify by noting what genes were detected in this category.

"Oxidative phosphorylation was one of the most prominent energy generating 15 metabolic pathways in the bacterial community. This indicates that ATP is generated by electron transfer to a terminal electron acceptor, such as oxygen, nitrate or sulphate."