Biogeosciences
Discussions

Interactive comment on "Distinct microbial composition and functions in an underground high-temperature hot spring at different depths" *by* Shijie Bai and Xiaotong Peng

Anonymous Referee #2

Received and published: 16 January 2020

Overall comments:

The manuscript describes the taxonomy and functional potentials of microbial communities at three depths of hot springs. Based on the introduction of this study, it provides new data on microbes from the subsurface biosphere, but more analyses and thinking on these datasets are needed. The manuscript also needs to be rewritten in terms of the language.

Major criticisms:

1. The main conclusion of this study is that the microbial communities were different at different depths. Most figures were used to indicate how similar or different the

three samples were. This conclusion is too vague to deliver useful information from these valuable datasets. More thinking about the results are needed to obtain more specific findings. There was some discussion on specific microbes found in extreme environments from previous studies in the 'discussion' section. However, searching the 16S data in this study for microbial species which were expected in hot springs or analyzing the most abundant species to see whether they were previously recognized as hot spring specialists could make the discussion more solid. Based on functional genes, discuss what metabolic potentials may help the dominant species adapt to the extreme environment. If there was any nutrient data, could nutrients explain the functional potentials of dominant microbes? Solid conclusions are missing in the current manuscript.

2. Many statistical analyses were applied to the data, but explanations and justifications on these analyses are needed. For example, Figs 5 & 6: please justify why both PCoA and DCA are needed and explain what different information could be gained from them in the 'materials and methods' section. If only one is necessary, please update figures and text accordingly. Line 286: this conclusion was drawn from DCA (Fig 6B), but PCoA result (Fig 6A) did not support it. Please explain why the PCoA was not considered. Line 288-290: different depths harboring distinct microbial communities was indicated by PCoA and DCA analyses (Figs 5 & 6), what could Figs 7 & 8 tell us besides communities were different?

3. Microbial function potential was inferred from both 16S data via FAPROTAX and GeoChip 5.0. There was not any comparison between results from these two methods in the manuscript. Why two different methods were used? Did they tell the same story? If not, what is the reason? Is there any information we could get from one but not the other?

Detailed comments:

Abstract: went straight into the methods without any background information. Please

start with the motivation of the study.

Line 200-201 and Line 360-363: the logic seems to be that the environmental conditions at three depths were similar, but microbial communities were different. You cannot say that the environmental conditions were similar based only on two parameters (pH and temperature). How about other important parameters such as oxygen and nutrient concentrations?

Line 239-240: based on Fig 4B, the most abundant purple bar was labeled as Candidatus Nitrosocaldus, and nothing was labeled as 'Uncultured Desulfurococcales archaeon'.

Line 241-242: this was not consistent with Fig 4B either.

Fig 4B: is 'uncultured archaeon' in the legend supposed to be 'uncultured Desulfurococcales archaeon' as mentioned in the text? The text kept saying uncultured Desulfurococcales archaeon, but it does not even exist in the legend of Fig 4B. If 'uncultured archaeon' is actually 'uncultured Desulfurococcales archaeon' which doesn't belong to Thaumarchaeota, how could it be possible that more than 90% of archaea were from Thaumarchaeota phylum at 19 m (Fig 4A) and more than 50% of archaea in the same sample were uncultured Desulfurococcales archaeon which does not belong to Thaumarchaeota (Fig 4B)? Please check the taxonomic classification carefully.

Figs 9 & 10: (1). Need more explanations. For example, what is 'mean of relative abundance'? Should the numbers on the x-axis be in percentage such as 'relative abundance' in Figs 3 & 4? Do all the numbers of the metabolic potential categories listed in the Figs add up to 1 or 100% for each depth? (2) The metabolic potential categories should be carefully labeled. For example, 'aerobic_chemoheterotrophy' and 'chemoheterotrophy' were both listed in Fig 9. Was the latter 'anaerobic_chemoheterotrophy' or all chemoheterotrophy? Why were 'nitrite oxidation', 'ammonia oxidation' and 'nitrification' all listed while nitrification includes ammonia oxidation and nitrite oxidation? What does 'nitrous_oxide_denitrification' mean? Does it mean the production or con-

sumption of nitrous oxide via denitrification?

Line 296-298: (1) the expression is weird. (2) how could you tell that all archaea were involved? In Fig 10, none of the bars for ammonia oxidation or nitrification reached 1 or 100%.

Line 313-314: this sounds like that the absolute abundance of nitrogen cycling genes was the lowest. Wasn't it the relative abundance of nitrogen cycling genes among all the genes on GeoChip?

Fig 11 & 12: please correct the fond for genes. For example, narb should be narB.

Line 314-323: the heatmaps were interesting, but most of the text here was how similar or different the three depths were. I hope to see more detailed insights about the heatmaps.

Line 385-393: why Sphingobium and Bacillus were the most abundant genera at 19 m was discussed here. It would be better if the authors could dig further into their data related to the two genera. For example, what was the most abundant species in Bacillus at 19 m? Does this species have enzymes that could remain active at high temperatures?

Line 414-419: I could not follow the logic here: Some studies suggested archaea were rare, but other studies suggested that archaea and bacteria coexisted. Then I expected to hear about whether archaea in this study is rare or not. The data in this study could only indicate that Thaumarchaeota was the most abundant phylum among archaea but archaea could still be rare (i.e. much fewer than bacteria) in hot springs.

Some methods were in the results: The subtitle of the first part of the results 'sampling' sounds like a method section, and the entire section was only one paragraph describing pH and temperature. It could be easily moved to the beginning of another section in the results as one sentence.

Line 216-217: 'random resampling was conducted for further analyses' should be re-

moved.

Line 223: 'with a similarity of 97% for OTU classification', Line 225 'at a 97% similarity level.', and line 237 'at a 97% similarity level'. The criterium for OTU classification does need to be mentioned but once is enough.

Unclear, redundant or awkward expressions (some examples): Line 17-18: 'in response to the depths, ...shifts over the depth profile'. Line 87: 'community functional potential altered by the depth'. I think you wanted to say that the composition of microbial communities depended on the depth.

Line 112: 'three duplicate samples'. Duplicates mean two replicates. Three duplicates mean six samples. I think here you meant 'triplicates'.

Line 200-201: 'According to the temperatures and pH, there were no significant differences between the samples.' The authors may mean that the temperatures and pH of the samples were similar, but the sentence was saying that the samples were considered similar only based on two environmental variables.

Line 216:' For the microbial diversity, the composition and structure of each sample could be compared;' What do you mean? This sentence needs to be re-written.

Line 225-227: 'The bacterial groups at 0 m with the highest relative abundances at the phylum level were members of Aquificae, Gamma-proteobacteria, and Deinococcus-Thermus.' This sentence needs to be re-written and the Gamma-proteobacteria is not a phylum.

Line 231: awkward expression: 'At the genus level, the OTUs were distributed. . .'

Line 283-285: the second half of the sentence said the same thing as the first half.

C5