

Biogeosciences Discuss., author comment AC2
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Reply on RC2

Tzu-Hsuan Tu et al.

Author comment on "The biogeographic pattern of microbial communities inhabiting terrestrial mud volcanoes across the Eurasian continent" by Tzu-Hsuan Tu et al.,
Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-103-AC2>, 2021

- The stochastic process mentioned in the title is one of the two important processes in community assembly. Why is it only proved by DDR and HAR? Is there any other evidence, such as the use of β NTI and NST (Normalized stochasticity ratio).

Thanks for the suggestion. We have calculated the NST index by categorizing all samples into "bubbling fluid", "surface sediments", and "within site sediment". These sample categories represent source communities (for "bubbling fluid"), source communities subject to minor surface impact (for "surface sediments"), and communities potentially altered by localized geochemical context (for "within site sediment"). The results demonstrate a high stochastic control on "bubbling fluid" (NST = 1.00) and "surface sediment" (NST = 0.70) and a relatively strong control of deterministic process on "within site sediment" (12 out of 15 sites with NSTs < 0.5 and the remaining 3 with NSTs between 0.54 and 0.61), a data pattern consistent with the results obtained from existing DDR, NMDS, and CCA analyses. Similar approach has been also applied to the calculation of pNST, which is a measure of stochasticity based on phylogenetic distance between communities and represents an analogy of β NTI. The results demonstrate a pattern similar to that from NST. High stochastic control has been observed for "bubbling fluid" (pNST = 0.65) and "surface sediment" (pNST = 0.73), whereas mild deterministic control has been inferred for "within site sediment" (8 out of 15 sites with pNSTs < 0.5 and the remaining 7 with pNSTs between 0.51 and 0.92). Compared with ASV abundance dissimilarity, phylogenetic dissimilarity leads to a pattern with the stochastic control (pNST > 0.5) on more sites. This could be exemplified with sites SI and SH where relatively small numbers of ASVs were recovered. The pNSTs for these two sites are much higher than the corresponding NSTs (0.44-0.51 versus \sim 0.01), suggesting that contrast proportions of phylogenetically distant ASVs between communities lead to a higher degree of random dissimilarity. While all of our existing analyses are based on the ASV abundance dissimilarity (e.g., Brady-Curtis distance for NMDS and DDR), we will present the NST results only in order to maintain the consistency.

- The article has sequenced the prokaryotic communities, including bacteria and archaea, but why didn't the bacteria and archaea communities be analyzed separately during the analysis? Because the physiological and biochemical characteristics of the two are very

different.

Thanks for the comment. The proportions of bacteria and archaea varied considerably among the samples and sites. In some cases, the prokaryotic communities were almost composed of bacteria. If bacterial and archaeal communities are analyzed separately, community pattern could have been distorted. Furthermore, some groups of bacteria and archaea interact in different ways under specific geochemical context (e.g., symbiotic partnerships between ANME and SRB at the transition of sulfate to methane). Analyses by merging bacteria and archaeal communities could provide a better assessment on the co-occurrence of different groups and their distribution pattern with geochemical context. Therefore, we did not analyze bacteria and archaea separately.

- Line 25: This sentence has no real meaning, please omit or revised.

Thanks for the suggestion. We have revised the sentence to specifically state that chloride is the most influential parameter for community composition.

- Line 74: I may have missed this, but where the specific colonists you defined are mentioned?

We identified ASVs prevalently distributed or confined within a single site (detailed ASV table could be obtained upon request). The most widely distributed ASVs were present in 9 MVs (Fig. 3f; this figure will be modified to magnify the low percentage of prevalently distributed ASVs.) and affiliated with genus of Desulfuromonadaceae or *Desulfotignum*. In contrast, ~88% of ASVs (28,928 in total) were distributed only at one site (Fig. 3f). To reduce the complexity of data presentation, we focused on the discussion of these ASVs related to methane and sulfur metabolisms. In particular, the distribution of most abundant or widespread ASVs affiliated with ANME, Desulfobacterales, Methylococcales, and *Thiobacillus* were presented (Fig. S11). Nevertheless, we will add a supplementary table that comprises the proportion, taxonomic, and site information for 2 most widespread ASVs (across 9 sites) and 10 most abundant ASVs restricted at any individual site.

- Line 96-99: Please add the specific parameters used in the sequence data analysis.

The specific parameters were described in the Supplementary Information.

- Line 103: As far as I know, only dada2 and deblur softwares can achieve 100% clustering and get the ASV, but you use mothur, how is it achieved?

Thanks for the comment. We actually used DADA2 to denoise and identify ASVs first. The representative sequences of all ASVs were subsequently aligned and classified by Mothur. Detail analysis procedures were described in the Supplementary Information.

- Line 118: What is the parameter "a" meaning?

Thanks for the reminder. "a" is the intercept for the regression. We have revised the description.

- Line 177: I cannot draw the conclusion from Figure 3f that the most widely distributed ASV were presented in 9 MVs. In addition, the information expressed in the Figure 3 is not fully explained in the text, please revised.

Thanks for the comments and correction. We identified a total of 28,928 ASVs across 16 cores distributed at 15 sites. Only 2 of them were the most widespread and distributed at 9 sites, whereas other ASVs were recovered from 1 to 6 sites. The 2 ASVs comprised a fraction of 6.9×10^{-5} of the total ASVs. We have revised the figure (Fig. 3f) to magnify the proportions of ASVs distributed at 5 to 9 sites. We have revised the description about the proportions of different taxonomic units.

- Line 219: The result of Mantel test is negative, is this correct?

Thanks for the correction. The value should be positive. We have revised the value.