

## ***Interactive comment on “Microbial community composition and abundance after millennia of submarine permafrost warming” by Julia Mitzscherling et al.***

### **Anonymous Referee #1**

Received and published: 11 May 2019

Mitzscherling et al present an interesting study on the microbial communities living in permafrost underneath seawater on the continental shelf. The sampling campaign is quite impressive and extensive, four different drill cores were analyzed in an on shore to offshore transect. Multiple depths from the previously deposited permafrost layer were taken from each core for a comparison based on qPCR, cell counts, 16S rRNA gene sequencing, and various geochemical proxies. The size and integrated nature of the data make this certainly at a level that should be published in Biogeosciences. My main comments are related to methods details that need to be added, additional suggestions for figures (putting the qPCR and cell count data for every sample on an x-y plot), and more discussion of the very interesting findings. I think that the authors are sitting on a

C1

one-of-a-kind dataset, and the discussion as it is reads a bit general and does not do the data justice. With a bit more detailed discussion, the authors could possibly make some interesting links of the microbial groups to past paleo-ecological conditions. For example, I think they should discuss more about what their data mean for the assembly (or lack thereof) of deep biosphere communities in subseafloor sediments. I think that after a minor revision the paper should be suitable for publication.

Specific comments

Abstract:

line 29: Not clear what you mean by "...DOC content was least" (please also define DOC on first use).

line 32: Stable isotopes of what? Carbon?

line 34: Any Fungi?

Methods:

page 3, lines 18 - 26: Did you perform any contamination controls for the drilling? Or is this not necessary because no drill fluid was used? Please explain in the text.

page 4, lines 18-21: What depths do these sections correspond to?

page 4, lines 24-25: Where did you sub sample the core? In a laminar flow clean hood or just on the bench? Are qPCR values high enough that major contamination issues are not a concern? This seems to be the case since you are around  $10^7$ . If yes, please state in the text.

page 5, lines 18-20: Do these primers also target archaea? please specify in the text.

page 6, lines 5-6: These are rather unconventional primers for microbiome studies. Why did you chose them over say the Earth Microbiome primers (515F/806R)? Please explain whether your primers also targeted archaea or not.

C2

page 6, lines 9-10: Please explain in more detail your pipeline for picking 16S OTUs. OTUs are not clustered using the SILVA database, just taxonomy assigned. More information is needed here on how you processed the data, quality control, clustering methods, etc.

page 9, line 6: Which isotopes?  $^{18}\text{O}$ ? The community is not formed by the isotopes, but probably reflects something else that the isotopes are a proxy for. What is the proxy the water isotopes are showing? Paleo temperature? I thought this was supposed to be related to temperature? But below you say diversity is not related to temperature. Kind of confusing.

page 9, line 14: Again, please explain what the  $^{18}\text{O}$  and delta D isotopes are proxies for.

page 9, line 16-17: Since the samples all derive from different depths (at least this is what I gather looking at figure 2), how do you know that temperature is explaining the difference. Do all the depths have the same temperature? Or do the depths from each site have their own unique temperature range? This needs a lot of clarification in the text.

page 9, lines 25-30: Maybe I missed this, but what is physical state of the subsurface samples you acquired via drilling. Is it hard ice, or more slushy? e.g., has it thawed since being overlain with seawater? And, the samples from the terrestrial site are presumably colder, and harder, than those overlain with warmer seawater? If you have any photographs of the cores themselves showing these differences I suggest including them as a figure in the main text. This has important implications for preservation of organics as discussed here.

page 10, lines 9-13: Here, and throughout the text, when you refer to qPCR data can you please actually state in the text what the number of gene copies is? Instead of saying "Low gene copies...". I don't know what you mean by the word "low".

### C3

page 10, lines 24-25: Please also cite some of the recent studies showing an influence of paleoclimate on microbial abundance and diversity in sediments ([doi.org/10.1038/s41598-017-05590-9](https://doi.org/10.1038/s41598-017-05590-9) and [doi.org/10.1093/femsec/fiy029](https://doi.org/10.1093/femsec/fiy029)). This supports your findings here, which is very interesting.

page 12, lines 3-5: Do your qPCR and cell count data correlate? What is the strength of the correlation? Please add this to the results and show this on an X-Y plot (cell counts vs. qPCR values for all samples) as a new main figure in the text. In the X-Y plot you can give the different points different colors showing which core they derive from. This will be highly interesting and informative !!!

Figures:

Figure 4: Please add the x-y plot I have suggested above. All qPCR and cell count data per sample (it looks like you have a lot!) should be plotted against one another on an x y plot. All individual datapoint should be shown so that readers can see the spread in the data. This will be a major benefit to the paper, improving its strength.

Figure 5: This is a great figure and shows some remarkable patterns. For example, the Atribacteria seem restricted to C3. This was only superficially discussed in the text. What is known about Atribacteria and their ecology, that can explain this? They apparently dominate the entire community in C3. You could discuss this, in the context of the recent review on their metabolism and ecology in the subseafloor ([doi: 10.1038/s41579-018-0046-8](https://doi.org/10.1038/s41579-018-0046-8)).

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### C4