Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2019-144-RC2, 2019

Biogeosciences Discussions

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

Anonymous Referee #2

Received and published: 12 July 2019

general comments

The manuscript of Mitzcherling et al. describes a field survey in the arctic, which tested the hypothesis that the effect of permafrost warming can be examined already before the thawing starts. Intriguingly, the experimental design was to use frozen sediment cores of diverging base temperatures ranging between -12° and -1.4°C, but from the approximately same age. This is a very clever setup, however, the implementation of this was limited by using only four sites (maybe because of the costs and logistics of such an expedition), which also limits the statistics and the final conclusions. The authors tried to compensate the limited sampling by taking 6-10 replicates (which could

be statistically interpreted as pseudoreplicates) per site from the targeted frozen period, but in the end could only see a moderate, and even negative effect of temperature on the microbial parameters. Furthermore, other factors such as depth and potential differences in the palaoenvironmental origin obscured the temperature signal, which the authors discussed, accordingly. In general, the study is cleverly designed and provides new research concepts for studying permafrost changes over long time scales. Although the results leave room for discussions due to the limited sampling sites, this work is an interesting study, and a good basis for future studies within the same setting.

specific comments

Page 4 – Age measurements: The authors estimated the age of the core profiles, but it wasn't included in the statistics (or at least I couldn't find it); I would assume that age could explain part of the variation in the microbial community composition.

Page 5 – DNA extraction: Could the authors maybe in the supplement provide a gel picture of the extracted DNA? I am asking this, since the fragmentation of the DNA can also be seen as an indicator for the presence of non-cellular ancient DNA (aDNA). In addition, could the authors please specifiy which size fraction was extracted from gel?

Page 6 – HTS: Please keep in mind that 35 PCR cycles is an unusual high cycle number for an amplicon based microbiome analysis, which will probably cause larger shifts in relative abundance values of microbial groups (this may become relevant when you try to implement some of the RC1 comments).

Page 7 – Multivariate Statistics: To me the authors used a suboptimal set of statistical methods for analysing the microbial community composition. While Mantel tests are good for testing the correlation of two matrices (e.g. the community matrix with the environmental matrix or a subset of parameters (e.g. a matrix of depth, temp, stable isotopes), it is rather uncommon to use it for testing single parameters. (As a side mark, the Mantel test can be performed rank-based or parametric based, this should be specified). Current alternatives to Mantel tests are PERMANOVA variants that are

suitable for continuous variables, or for a priori hypothesis such as the temperature hypothesis distance based redundancy analysis followed by an ANOVA test. However, since the authors start with an exploratory analysis, one of the most frequent used methods is to fit the variables into the ordination by regression/correlation. CCA may not be the best option in this case, but a PCoA or an NMDS would be the preferred method.

Page 7 – statistics: Isn't the Dunn's test the PostHoc test for non-parametric tests such as Kruskal-Wallice? For an ANOVA, I would have expected a Tukey-HSD. Please doublecheck.

Page 7 – General statistics: The authors will need to think about corrections for multiple comparisons. In particular in Table 1 or for the Mantel tests presented in the supplemental, which will both require p-value corrections (e.g. using Bonferroni). If these corrections are not done, this has to be stated explicitly.

Page 8 – Line 16: Please state the correlation values between DNA, copy numbers, and cell counts. This may be important to interpret Table 1.

Page 9 – curiosity comment: The authors took a vertical profile of each core, but this is not really implemented in the study. Out of curiosity: Do the points in e.g. the CCA or the PCA also structure according to the vertical profile? If so, this could be an interesting aspect that may also explain some of the variance observed, caused by differences in ages and/or the paleaoenvironment.

Page 9 – Discussion: The discussion is rather comprehensive and understandable. I think, I can agree with the arguments of the authors. Please include a brief discussion on the limited sampling design of only 4 sites. With such a high variation between the cores, it may require > 30 cores to really answer the hypothesis.

Figures:

Optional comment: Since DOC became important for the discussion of the cell counts

(and equivalents), maybe it could be worthwhile to include a figure on this in the main text. Please discuss this among yourselves.

technical corrections

Page 6 – please specifiy which Illumina MiSeq chemistry was used (2x 250 or 2x300 nt?)

Page 6 – brackets are falsely set in line 13 for Llobet-Brossa et al. 1998

Page 7 – line 19: Please indicate the PSU of the seawater in this area

Figures:

Figure 4: Please indicate the number of measurements for each box (n= . . .)

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2019-144, 2019.