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Discussions

Interactive comment on "Influence of microorganisms on initial soil formation along a glacier forefield on King George Island, maritime Antarctica" *by* Patryk Krauze et al.

Anonymous Referee #1

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The manuscript by Krauze et al. examined microbial communities found within recently deglaciated cryosols in Antarctica in order to couple dominant pedogenic processes with microbial community structure. This is an exciting topic within the scope of Biogeosciences and the authors have identified an interesting model system for their study. The manuscript was well written with clear language and was presented nicely as well. Unfortunately, serious flaws within the experimental design and methodology employed for assessing microbial communities have effectively prevented the authors from being able to draw any meaningful, scientifically robust conclusions regarding the microbiota within their system.

The first major problem is the experimental design. There was no experimental replication at any site and because the entire study consisted of four cores and four depths were examined for each core, there were only 16 samples in total. This would not be a problem in a simple system, but this is a complex system with many variables to account for. As soon as a simple factor is taken into account, depth for instance, the effective sample size decreases. Comparisons involving only the top layers, bottom layers or single cores are restricted to four observations. This number is important because regression typically requires five or more observations in order to be able to calculate significance values and a statistical evaluation of regression would allow statements such as: With depth, pH increased (paraphrased from lines 177-178), "The Shannon index showed a decreasing trend in diversity with depth" (line 197), "With depth, the relative abundances of Gemmatimonadetes and Actinobacteria increased, while the relative abundance of Bacteroidetes and Verrucomicrobia decreased" (lines 205-206), "the microbial communities became less diverse and more similar with increasing depth across all investigated soil profiles" (lines 258-259). Without statistics backing up these statements, they constitute opinions, not evidence. I will concede that it is typical to report a handful of opinions and observations, particularly when there is a visual indication of a relationship despite the lack of statistical support. However a close examination of this manuscript reveals that apparently no statements were evaluated using any sort of statistical test. This is not typical or acceptable. As a reader of this manuscript, I don't know what has been evaluated statistically and therefore I don't know what constitutes a scientific statement on the microbial community.

The second problem I identified involved specific methodological choices in the microbial community processing / analysis. I find it difficult to accept that 100% of the microbial community in any cryosol could be classified. This is likely a direct result of the OTU picking method, which involved mapping sequence data to a database. Results like this indicate that the entire community is not being reported. Instead the current analysis compares proportions of classified data, which may or may not represent the community accurately. To know for sure, it is necessary to know how much

of the microbial community is “missing” in the analysis because it did not map to the Silva database. It is often helpful to track the fate of raw data, which requires some additional accounting. The authors should list each dataset in a supplemental table. This table should include, for each individual dataset: SRA object ID, sample ID, number of reads obtained prior to any QC (raw data), number of reads that successfully merged, number of reads that remained after trimmomatic, reads that remained after chimera removal and finally, reads that were classified into OTUs which is the final number of reads that were used for analysis. Negative PCR controls should always be included in such a table.

Actually, I was surprised and somewhat concerned that there was no mention of negative controls. The method of Meier, 2019 used a total of 30 PCR cycles, which is quite high but understandable if the DNA yields are low, which apparently was the case for some samples (lines 151-152). Given the combination of high number of PCR cycles and low biomass, negative controls are absolutely necessary. Table 2 indicates that the deeper soils have much less template for PCR and the authors also concluded that the deeper soils are more similar to one another (line 259). If the deeper samples resemble negative controls due to having a small amount of template, it should come as no surprise that they resemble one another. This very real possibility needs to be ruled out. In addition, it would be quite helpful to include information on DNA extracted per gram of soil for each sample (and replicate) and which samples were extracted three times. The methods just say “samples with low yields”. As a side note, I don’t understand the point of pooling, since the method of Meier 2019 used a fixed volume of genomic DNA.

Finally, the microbial resesarch community is largely moving beyond OTU-based analysis in favor of amplicon sequence variant (ASV) analysis. The authors may consider switching over to this high-resolution approach that in some cases can provide deep insights into microbial community structure: (https://www.bioconductor.org/packages/devel/bioc/vignettes/dada2/inst/doc/dada2-

intro.html).

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