Response to Reviewer Comments on Manuscript bg-2019-144: "Microbial community composition and abundance after millennia of submarine permafrost warming"

Comments of Reviewer #1:

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 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.

We are glad about the positive feedback of referee #1 and the constructive comments on our manuscript. The comments and suggestions contribute to an improvement of the manuscript and we are happy to implement them in the text.

Regarding the general comments, especially the wish for discussing "more about what [our] data mean for the assembly (or lack thereof) of deep biosphere communities in subseafloor sediments", we added the following paragraph to our discussion (page 11 line 11).

Our data suggest that the bacterial community in submarine permafrost sediments has experienced a weak selection after deposition and mostly reflects the paleo-environmental and climatic conditions. Thereby this study joins a number of other studies reporting on microbial groups that are referred to as "the paleome". Those studies found correlations between the microbial diversity and past depositional conditions (Lyra et al., 2013; Orsi et al., 2017; Vuillemin et al., 2016). Marine communities were found in terrestrial settings or soil communities in (sub)seafloor sediments (Ciobanu et al., 2012; Inagaki et al., 2015; Inagaki and Nealson, 2006). Like those, our study implies that the bacterial communities in permafrost soils under the seafloor underwent a weak selection pressure after burial either through dormancy or very low generation times under freezing conditions.

<u>Abstract:</u>

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

Century-scale permafrost warming is accompanied with decreasing microbial abundance i.e. total cell counts and 16S rRNA gene copies. This is expressed through decreasing abundance from the onshore permafrost core C1, over the offshore cores C4 to C3. In contrast, in the outermost core C2, which experienced warming not only for centuries but for ~2500 years, the abundance increased again. Looking at the dissolved organic carbon content in each of the cores draws a different picture. The lowest DOC values of all cores were found in C2, which experienced warming for millennia. Highest DOC contents were found in core C4, which had lowest 16S rRNA gene copies and TCC that were comparable to core C2.
To clarify this, we rephrased the sentence as follows:

"On time-scales of centuries, permafrost warming coincided with an overall decreasing microbial abundance **whereas** millennia after warming microbial abundance was similar to cold onshore permafrost. **In addition, the dissolved organic carbon** content **of all cores** was **lowest in submarine permafrost after millennia-scale warming**."

line 32: Stable isotopes of what? Carbon?
Meant are the stable isotopes of water, i.e. of hydrogen and oxygen – $\delta^{18}O$ and δD. For more clarity we rephrased the sentence as follows:
"Bacterial community composition correlated only weakly with temperature but strongly with porewater stable isotope**s $\delta^{18}O$ and δD,** and **with** depth. "

line 34: Any Fungi?

Only the bacterial community composition was investigated in this study. The community composition refers to the bacterial community mentioned in the sentence before. We rephrased the sentence as follows.
"Bacterial community composition correlated only weakly with temperature but strongly with porewater stable isotope signatures and depth. **The bacterial** community showed substantial spatial variation and an overall dominance of Actinobacteria, Chloroflexi, Firmicutes, Gemmatimonadetes and Proteobacteria which are amongst the microbial taxa that were also found to be active in other frozen permafrost environments."

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.

Drilling was performed by rotary drilling without using drilling mud as described in page 3 line 22.

Drilling was performed with a hydraulic rotary-pressure **system (Drilling Technologies Factory, St. Petersburg, Russia, Model URB-2A-2) and without the use of any drilling fluid.** All samples were frozen immediately after recovery and were kept at -22 °C until further processing.

Thus, a contamination control of drill fluid was not necessary. Nevertheless, a possible contamination on the rim of the core caused by the drilling equipment should be circumvented by taking subsamples from the center of the core. In order to explain this we added the following information to the text on page 4 lines 24-25:

"**In order to prevent** contamination **caused by the drilling equipment** we took the subsamples from the center of the core"

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

Detailed information about the depth location of each sample can be found in table S4. The approximate depth of each sample and of Unit II within each core is visualized in figure 2a. Numerical data on the depth of Unit II in each core and its extent can be found in table S1 describing each borehole location.

In order to give more details on the sample depths we will add the following information:

"For molecular analyses we took 6 replicate samples from each of the cores C1 (C1-1 – C1-6), C4 (C4-1 – C4-6) and C3 (C3-1 – C3-6) and 8 replicates from core C2 (C2-1, C2-2, C2-4, C2-5, C2-7, C2-8, C2-9, C2-10) (Fig. 2a). Those replicates were located at different depths within Unit II (Table S4). **Samples from C1 were located around 27 to 44 meters below surface, while samples from C4 were taken between 13 and 30 meters below the seafloor, samples from C3 between 9 and 25 m bsf, and samples from C2 between 40 and 58 m bsf**."

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 10ˆ7. If yes, please state in the text.

The core was subsampled in a climate chamber under freezing conditions and by using sterile tools. Thus, contamination can be excluded and are not of concern for downstream analyses like qPCR.

We added these information to the text (page 4 line 25):

Subsampling was performed in a climate chamber under freezing conditions by using sterile tools. Thus, a contamination of the samples can be excluded.

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

According to SILVA those primers do not target archaea when binding without mismatches. Allowing one mismatch only 0.6% of archaeal sequences are targeted.

"Quantitative PCR was performed using the CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) and the primers S-D-Bact-0341-b-S-17 and S-D-Bact-0517-a-A-18 **targeting** the bacterial 16S rRNA gene (Table S5)."

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.

Microbial biomass in extreme environments like permafrost is known to be low. This can be seen for example in the submarine permafrost core C3. The project from which this manuscript is part of aimed at studying the bacterial and archaeal communities (Mitzscherling et al., 2017; Winkel et al., 2018). However, the archaeal community in permafrost accounts for only a very low percentage of the total microbial community (Hoj et al., 2008; Kobabe et al., 2004). A combined primer pair like the universal 515F/806R may discriminate the amplification of archaeal sequences in presence of an overwhelming amount of bacterial sequences. Hence, we decided to amplify both communities separately. Thus, the primer pair used in this study to investigate the bacterial community was not aimed to target archaeal sequences.
According to SILVA this primer pair covers 0.1% of archaea when assuming no mismatches of the primers. With one mismatch those primers still cover only 1.6% of archaeal sequences.
We agree that the information about the primers target is missing and added the information to the text:
"**The sequencing** primers **that were used in this study only target bacteria and** comprised different combinations of barcodes (Table S6). PCR amplification was carried out with a T100™ Thermal Cycler (Bio-Rad Laboratories, CA, USA). The PCR mixtures (25 µl) contained 1.25 U of OptiTaq DNA Polymerase (Roboklon), 10x concentrate buffer C (Roboklon), 0.5 µM of the sequencing primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Table S5), dNTP 5 mix (0.2 mM each), additional 0.5 mM of $MgCl_2$ (Roboklon), PCR-grade water, and 2.5 µl of template DNA."

p**age 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.**

Thank you for this remark. Adding these information to the text instead of referencing them helps the reader to get the important information about the sequence analysis and bioinformatical tools right away. Due to the wish of reviewer 2 to specifiy which Illumina MiSeq chemistry was used, we furthermore added detailed information about the sequencing preparation and procedure as well. Thus, we rearranged the methods section as follows:

2.6 Total cell counts

…

2.7 High throughput Illumina16S rRNA gene sequencing

Sequencing of each sample was performed in two technical replicates. Primers comprised different combinations of barcodes (Table S6). PCR amplification was carried out with a T100™ Thermal Cycler (Bio-Rad Laboratories, CA, USA). The PCR mixtures (25 µl) contained 1.25 U of OptiTaq DNA

Polymerase (Roboklon), 10x concentrate buffer C (Roboklon), 0.5 µM of the sequencing primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Table S5), dNTP mix (0.2 mM each), additional 0.5 mM of $MgCl_2$ (Roboklon), PCR-grade water, and 2.5 µl of template DNA. **PCR conditions comprised an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (95°C for 30 s), annealing (56°C for 30 s) and elongation (72°C for 1 min), and a final extension step of 72°C for 10 min. The PCR products were purified from agarose gel with the HiYieldPCR Clean-Up and Gel-Extraction Kit (Südlabor, Gauting, Germany) and were quantified with the QBIT2 system (Invitrogen, HS-Quant DNA). They were mixed in equimolar amounts and sequenced from both directions (GATC Biotech, Konstanz) based on the Illumina MiSeq technology. The library was prepared with the MiSeq Reagent Kit V3 for 2× 300 bp paired-end reads. The 15% PhiX control v3 library was used for better performance due to different sequencing length.**

2.8 Sequence analysis and bioinformatics

The data analysis of raw bacterial sequences started with the quality control of the sequencing library by the tool FastQC (Quality Control tool for High Throughput Sequence Data http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ by S. Andrews). The tool CutAdapt [Martin, 2011] was used to demultiplex the sequence reads according to their barcodes and to subsequently remove the barcodes. Forward and reverse sequenced fragments with overlapping sequence regions were merged using PEAR [J. Zhang et al., 2014], and the nucleotide sequence orientation was standardized. Low-quality sequences were filtered and trimmed by Trimmomatic [Bolger et al., 2014], and chimeras were removed by Chimera. Slayer. Finally, the QIIME pipeline was used to cluster sequences into operational taxonomic units (OTUs) and to taxonomically assign them employing the SILVA database (release 123) with a cutoff value of 97% [Caporaso et al., 2010].

2.9 Statistics

…

page 9, line 6: Which isotopes? 18O? 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.

Those are valid questions and show that we did not explain this well enough. The interpretation of these parameters, however, is part of the discussion. The relationship to stable isotopes tells us about paleo-temperature at deposition; temperature of the sediment tells us about the evolution of the microbial environment today, subsequent to deposition. In this paragraph the term "temperature" refers to the temperature of the permafrost sediments at the time of drilling. We hope that this will become clear when adding the following information to the text.

"However, the community formation was stronger influenced by pore water stable isotopes **$\delta^{18}O$ and δD** ($p = 0.0001$, $R = 0.40$) and sample depth ($p = 0.0001$, $R = 0.36$), than by **permafrost** temperature."

page 9, line 14: Again, please explain what the 18O and delta D isotopes are proxies for.

We rephrased the sentence as follows:

Variance of samples from the bottom left to the top right was explained by rising **permafrost** temperature, while variance of samples from the top left to the bottom right are likely explained by decreasing values of the stable water isotopes $\delta^{18}O$ and δD**, a proxy for paleo-temperature and –climate.**

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.

Yes, each site has its own unique temperature range. The temperature differences within each core are smaller than across the cores. A description of the temperature range of each core can be found in the Results section (page 7, line 12-15) as well as in the supplementary table S2. Also figure 2b shows the vertical temperature profile of each core.

In order to make this clear we rephrased the sentence …

The variance between C1, C4 and C2 samples are explained by **the** temperature differences **of the permafrost across the cores (Fig. 2b).**

… and rearranged the "Study Site and Drilling" section on page 3 as follows:

The study area (~73°60'N, 117°18'E) is situated in the western part of the Laptev Sea, on the East Siberian Arctic Shelf (Fig. 1). Mean annual bottom water temperatures in the Laptev Sea range between -1.8 °C to -1 °C (Wegner et al., 2005) leading to sediment temperatures of -1.0 °C and -2.0 °C within the largest part of the shelf (Romanovskii et al., 2004). We investigated four cores (C1-C4, Fig. 2a) that were retrieved along an onshore-offshore transect in the coastal region of Cape Mamontov Klyk in 2005 (Overduin, 2007; Rachold et al., 2007**). Cores were named after the order of drilling and we kept this order (C1, C4, C3, C2) for better comparability with previous studies (Koch et al., 2009; Mitzscherling et al., 2017; Overduin et al., 2008; Winkel et al., 2018). From onshore to offshore all cores were characterized by an increase in water depth, in depth to the ice-bonded permafrost table (Fig. 2a, Table S1) and in permafrost temperature (Table S2) (Overduin, 2007; Rachold et al., 2007). The transect was characterized by a temperature gradient that covered an increment of more than 10 °C compared to the onshore permafrost. Thereby, each core displayed its own unique temperature range (Fig. 2b).**

Assuming a constant mean annual coastal erosion rate of 4.5 m yr^{-1} (Grigoriev, 2008) the drill site located furthest offshore (C2, 11.5 km off the coast) was inundated approximately 2500 years ago (Rachold et al., 2007). Accordingly, the drill sites C3 and C4, located 3 km and 1 km off the coast, were inundated around 660 and 220 years ago, respectively. More recent analysis based on remote sensing shows that 40-year coastal erosion rates for the same stretch of coastline between 1965 and 2007 were slower (about 2.9 m yr^{-1}) (Günther et al., 2013), which would translate into even longer inundation periods. However, in the present study we refer to Grigoriev (2008), which are based on direct observations of coastal erosion at the C1 coring site. **Drilling was performed with a hydraulic rotary-pressure system (Drilling Technologies Factory, St. Petersburg, Russia, Model URB-2A-2) and without the use of any drilling fluid. All samples were frozen immediately after recovery and were kept at -22 °C until further processing.** Temperature measurements at all sites were done using thermistors and infra-red sensors (Junker et al., 2008).

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.

As described in the section 2.2 samples were selected from Unit II. This "lithostratigraphic Unit II was identified in all cores (Fig. 2a) and was entirely located within the ice-bonded permafrost." Unit II has not been thawed since inundation; it was only warmed from about -12 °C (C1) to around -1 °C (C3 and C2). The colder onshore permafrost has less liquid water than the warmer submarine permafrost as a result. Terrestrial permafrost is therefore probably somewhat "harder", although this has not been measured and will depend on other factors as well, especially sediment characteristics.
Winterfeld et al., (2011) states that the cryostructure of Unit II in all cores was characterized mainly by pore ice cementing the sediment (gravimetric ice content 20–50 wt%) and also showed typical features of terrestrial permafrost such as segregated ice lenses, ice veins bordering on wood fragments, and composite sand–ice wedges. We added this information to the paragraph "2.2 Sample selection".
Photographs of Unit II from the terrestrial core C1 and the submarine core C2 were published by Winterfeld et al., (2011). We refrained from publishing them again but referred to them it in our text.

"Each of the four drill cores exhibited different sedimentological units. Lithostratigraphic Unit II was identified in all cores (Fig. 2a) and was entirely located within the ice-bonded permafrost. **Irrespective of the permafrost temperature Unit II sediments of all cores were cemented mainly by pore ice but were also characterized by terrestrial permafrost features like ice lenses, ice veins and ice-wedges. Photographs of (Winterfeld et al., 2011) show similar ice and sediment structures of the terrestrial core C1 and the outermost submarine core C2.** Depth location of Unit II within each core can be found in Table S1."

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".

We added the order of magnitude when cell counts or gene copy numbers were mentioned in the discussion as follows (page 10 line 30 – page 11 line 11):

The cores C3 and C4 had significantly lower TCC and bacterial gene copy numbers **(10^6 cells and 10^5 gene copies)** than the onshore core C1 and the C2 **(10^7 cells and 10^6 gene copies)** core furthest offshore. Thus, microbial activity and substrate utilization were likely low in C3 and C4. A negative influence of permafrost warming on microbial abundance is further challenged through some indication for microbial proliferation in core C2, which had experienced longest warming of all cores. In detail, TCC in C2 were higher than in the other submarine cores while DOC values were lower in C2, significantly different from C4 and C1 (Table S13). Permafrost warming for more than two millennia may have enabled microbial communities to adapt to the new temperature regime and sediment properties as suggested before (Mitzscherling et al., 2017). A direct effect of permafrost warming on microbial abundance was not evident; the effect of changing pore-water salinity is more plausible than that of permafrost warming. Rising salinity correlates significantly both with TCC and bacterial gene copy numbers. Also, bacterial 16S rRNA gene copy numbers were lowest in core C4

(10^5 gene copies), where pore-water salinities were elevated (electrical conductivity values >2000 µS cm^{-1}, Table S3). Low gene copy numbers **(10^5 gene copies)** may result from osmotic stress that limits microbial growth (Galinski, 1995; Rousk et al., 2011) and decreases microbial abundance in sediments (Jiang et al., 2007; Rath and Rousk, 2015; Rietz and Haynes, 2003; Wen et al., 2018).

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 and doi.org/10.1093/femsec/fiy029). This supports your findings here, which is very interesting.

Thank you for the great suggestion of literature, which perfectly substantiates of our findings. We added the following information at a later stage of the discussion on page 11 in line 4.

We suggest that microbial community composition like microbial abundance reflects the paleoclimate and sedimentation history and not a direct effect of permafrost warming. In detail, we observed a weak correlation between community composition with **permafrost** temperature and a strong correlation with stable water isotope values and depth, i.e. age. **This suggestion is supported by similar findings in sea sediments as well as in lacustrine sediments. Microbial taxa of Arabian Sea sediments reflected past depositional conditions and exhibited paleo-environmental selection (Orsi 2017), while the microbial population in sediments of Laguna Potrok Aike in Argentina changed in response to both past environmental conditions and geochemical changes during burial (Vuillemin, 2018)**.

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 !!!

As also reviewer 2 asked for the correlation values between DNA, copy numbers, and cell counts, we added detailed information about p-values and correlation coefficients to table S10 and to the table 1 showing the results of the rank-based Spearman's correlation.

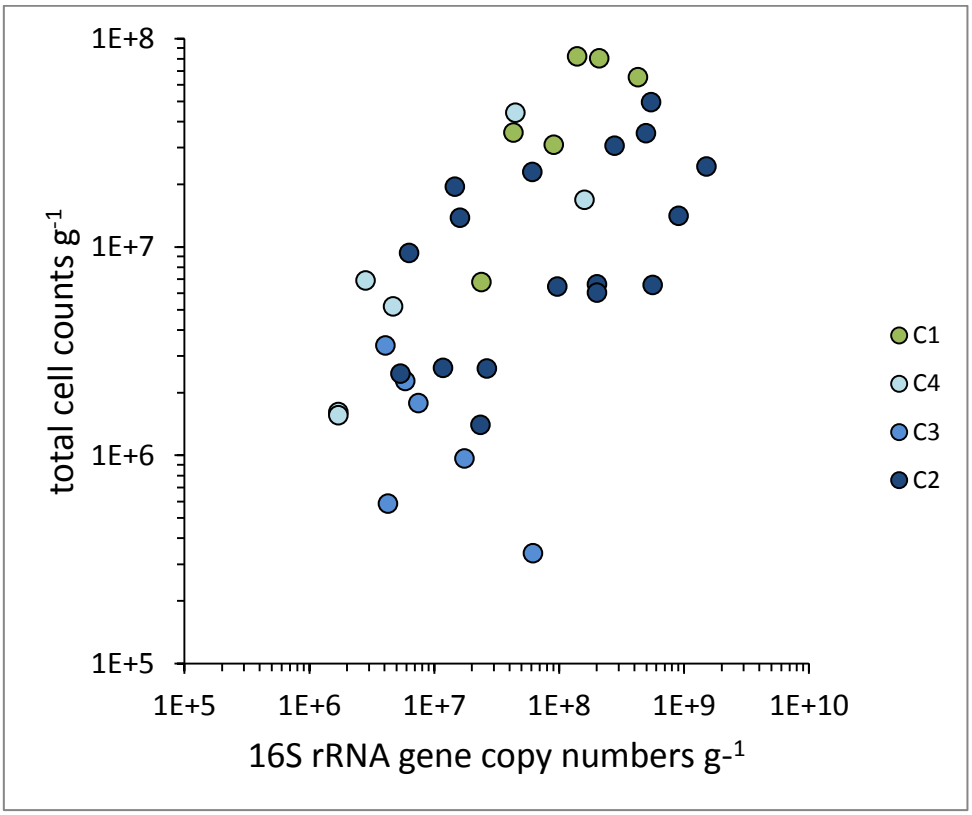
	16S Bacteria	16S/DNA	TCC	Temp	Salinity	Depth [mbsl]	Depth [mbs/ mbsf]	Ba^{2+}	Ca^{2+}	K^{+}	Mg^{2+}	Na^{+}	Si_{aq}	Cl^{-}	SO_4^{2-}	Br^{-}	NO_3^{-}	δ18O	δD	pH	TC	TN	TS	TOC	Clay	Silt	Sand	Grav. Water Content
p-value																											
DNA 16S	**>0.001**	**>0.001**	**>0.001**	**0.030**	**0.039**	0.813	0.076	0.658	0.604	**0.020**	0.061	**0.021**	0.872	**0.011**	0.410	**0.015**	0.593	**0.027**	0.055	**0.008**	**0.017**	0.329	0.175	**0.045**	0.307	0.111	0.130	**0.006**
Bacteria 16S /		**>0.001**	**>0.001**	0.173	**0.003**	0.164	**0.002**	0.860	0.248	**0.003**	**0.005**	**>0.001**	0.475	**>0.001**	0.128	**0.001**	0.587	**0.023**	0.054	**0.002**	**0.009**	0.175	0.056	**0.021**	0.821	0.886	0.926	**0.007**
DNA			**0.03**	0.503	**>0.001**	0.216	**0.004**	0.799	**0.005**	**0.001**	**>0.001**	**>0.001**	0.268	**>0.001**	**0.016**	**>0.001**	0.425	0.369	0.528	**0.001**	0.218	0.171	0.135	0.284	0.055	0.102	0.084	0.153
TCC				**>0.001**	**0.008**	0.465	0.138	**0.024**	0.193	**0.012**	**0.029**	**0.002**	0.262	**0.001**	0.593	**0.002**	0.890	**0.028**	**0.027**	0.097	0.749	0.759	0.233	0.429	0.184	0.572	0.524	0.369
correlation coefficient r_s																											
DNA	0.87	0.47	0.68	-0.37	-0.35	0.04	0.30	-0.08	-0.09	-0.39	-0.32	-0.39	-0.03	-0.43	-0.14	-0.41	0.09	-0.37	-0.33	-0.44	0.40	0.17	-0.23	0.34	0.18	0.27	-0.26	0.47
16S Bacteria		0.79	0.61	-0.24	-0.48	0.24	0.51	0.03	-0.20	-0.49	-0.46	-0.57	-0.12	-0.56	-0.26	-0.54	0.09	-0.38	-0.33	-0.52	0.44	0.23	-0.33	0.39	-0.04	0.03	-0.02	0.47
16S / DNA			0.36	-0.12	-0.63	0.21	0.47	0.04	-0.47	-0.55	-0.60	-0.71	-0.19	-0.67	-0.40	-0.66	-0.14	-0.16	-0.11	-0.54	0.21	0.24	-0.26	0.19	-0.33	-0.28	0.30	0.26
TCC				-0.64	-0.44	-0.13	0.26	-0.38	-0.23	-0.42	-0.37	-0.50	-0.19	-0.52	-0.09	-0.50	0.02	-0.37	-0.37	-0.28	0.06	0.05	-0.21	0.14	-0.23	-0.10	0.11	0.16

Table S10

	16S Bacteria	16S/DNA	TCC	Temp	Salinity	Depth [mbs/ mbsf]	Ba^{2+}	Ca^{2+}	K^{+}	Mg^{2+}	Na^{+}	Cl^{-}	SO_4^{2-}	Br^{-}	δ18O	δD	pH	TC	TOC	Grav. Water Content
DNA	**0.87**	**0.47**	**0.68**	**-0.37**	**-0.35**	0.30	-0.08	-0.09	**-0.39**	-0.32	**-0.39**	**-0.43**	-0.14	**-0.41**	**-0.37**	-0.33	**-0.44**	**0.40**	**0.34**	**0.47**
16S Bacteria		**0.79**	**0.61**	-0.24	**-0.48**	**0.51**	0.03	-0.20	**-0.49**	**-0.46**	**-0.57**	**-0.56**	-0.26	**-0.54**	**-0.38**	-0.33	**-0.52**	**0.44**	**0.39**	**0.47**
16S / DNA			**0.36**	-0.12	**-0.63**	**0.47**	0.04	**-0.47**	**-0.55**	**-0.60**	**-0.71**	**-0.67**	**-0.40**	**-0.66**	-0.16	-0.11	**-0.54**	0.21	0.19	0.26
TCC				**-0.64**	**-0.44**	0.26	**-0.38**	-0.23	**-0.42**	-0.37	**-0.50**	**-0.52**	-0.09	**-0.50**	**-0.37**	**-0.37**	-0.28	0.06	0.14	0.16

Table 1

We also added the suggested X-Y plot showing the total cell counts and versus gene copy numbers in different colors which indicate the core they derive from. Both axes are shown in log scale. We will integrate this figure in Figure 4.



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.

Please see the answer to the comment above.

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).

We thank the reviewer for raising those important questions and giving advice to the interesting literature that can perfectly add up information to the description of Atribacteria in the manuscript. We will add the following information to page 11 line 22-24:

"***Candidatus*** Atribacteria, which dominated in the core C3, was recently described to harbor functions for survival under extreme conditions like high salinities and cold temperatures (Glass et

al., 2019). **They are further one of the cosmopolitan groups in the subseafloor and dominate the bacterial community in deep anoxic sediments with low organic carbon contents (Orsi, 2018). This makes Atribacteria another candidate for activity under in situ conditions in submarine permafrost. Genome-based metabolic prediction shows that *Ca.* Atribacteria can ferment sugars and propionate producing H_2, which is a critical source of energy in anoxic settings, and they have the potential to polymerize carbohydrates and store them in shell proteins of bacterial microcompartments, thus increasing their fitness and leading to their selection (Orsi, 2018). Besides subseafloor sediments *Candidatus* Atribacteria were found to be abundant in lacustrine sediments in Argentina that were deposited under similar environmental conditions like C3, with permafrost and reduced vegetation in the catchment, an active hydrology reworking and dispersing the soils, and a very low organic carbon content. Also climatic conditions in the sedimentation period of the lacustrine sediments were similar to that of Unit II in C3, covering the driest period of the record and overall positive temperatures (Vuillemin et al., 2018).**

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