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Interactive comment on "Soil bacterial community and functional shifts in response to thermal insulation in moist acidic tundra of Northern Alaska" *by* M. P. Ricketts et al.

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Interactive comment on "Soil bacterial community and functional shifts in response to thermal insulation in moist acidic tundra of Northern Alaska" by M. P. Ricketts et al.

Anonymous Referee #3

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The manuscript by Ricketts et al. addresses the effect of climate change predictions on the soil bacterial communities in Arctic tundra soils which are important global C sinks. The experiment was carried out in a long-term experimental field site offering a snow depth gradient from 25% lower to 100% more snow than the surrounding (control). Due

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to increasing snow depth significant changes in abiotic soil parameters (e.g. active layer thaw depth, T, C/N ratio) were observed as well as a shift in the bacterial community structure. The taxonomic information from 16S rRNA amplicon sequence data was further used to estimate the functional gene abundance. These results indicate a decreased SOM decomposition potential under predicted climate change conditions which might help to further optimize current climate models. Therefore the study is of interest for readers of SOIL journal. Authors give a good overview about the current literature and make the aim of the study clear. In general, the manuscript is well-written and follows a logic flow. However, there are some points which were not clear to me and should be addressed before publication.

Response: We thank the reviewer for the summary and positive comments on the aim and approach of the study. We hope to address each of the comments in the revision to produce the best paper possible.

The prediction of microbial functional composition from phylogeny is really advanced and delivers new insights for studies where only 16S rRNA genes were sequenced. However, I would have liked to have first more information on taxonomic composition of bacterial communities in soil treatments and not only tests on six dominant phyla. The response of bacterial taxa belonging to the same phylum might be completely different. I recommend adding a table of significantly treatment-responding genera (some were already mentioned, p.12 section 4.1) or a heatmap showing relative abundance of dominant OTUs or genera across all samples.

Response: We will provide additional supplementary material to address this concern. There were 462 treatment responding Genera.

Furthermore, it should be emphasized in the discussion that all functional gene abundances are based only on predictions not taking into account horizontal gene transfer that might decouple function from phylogeny. Furthermore, there might be a lot of unknown functions due to poorly characterized taxa in Tundra soils or not yet known links

between taxon and function which should be discussed.

Response: This is a great comment and has been incorporated into the discussion of the revision on Page 16 lines 4-8 as follows: "While the use of PICRUSt and ancestral state reconstruction does not provide direct measurements of gene abundance (e.g., does not account for horizontal gene transfer or unknown functional / taxonomic linkages that may exist in the sampled tundra soils), it does offer valuable insights into the functional capacities of bacterial communities (Langille et al., 2013)."

By the way, it would be also interesting to know the amount of unclassified bacteria in your samples.

Response: Due to the nature of closed OTU picking (which is required for PICRUSt analysis), there were no unclassified bacteria in our samples. As per the QIIME website (http://qiime.org/tutorials/otu_picking.html), "In a closed-reference OTU picking process, reads are clustered against a reference sequence collection and any reads which do not hit a sequence in the reference sequence collection are excluded from downstream analyses." To clarify this in the revision we added, "Any read that did not match a sequence in the reference database was discarded." to Page 8 line 5. To satisfy your curiosity, we also did run open OTU picking, in which "reads are clustered against a reference sequence collection and any reads which do not hit the reference sequence collection are subsequently clustered de novo." (QIIME website). This resulted in 1.2% of unassigned bacteria. We did not use or include this method in the study as to maintain consistency between the bacterial abundance results and the PICRUSt functional gene abundance results.

In this respect, I am also wondering whether you tested first for availability of nearby genome representatives for your dataset before using PICRUSt prediction (NSTI index)? Furthermore, PICRUSt outputs a gene potential and it remains unknown to which extent these genes are expressed in the end.

Response: Please see above comment, and discussion on Page 18 lines 3-13.

As far as I understood, there are no treatment repetitions at the experimental site available and per treatment only 3 pseudo-replicates were taken. This makes it difficult to exclude the effect of natural variation in the bacterial community composition between sampling points. Thus conclusions can be drawn only very carefully and you should avoid to speculate too much in the Discussion section.

Response: We acknowledge the natural variation in bacterial communities at the microscale, and our limited replication. Through our revisions, we hope that our discussion and conclusions do not overstep their bounds.

These are my specific comments: Abstract: p.2, l.4 – Does "more or less snow" mean that predictions on amount of precipitation are not sure yet? Please consider rephrasing.

Response: We omitted "or less" on Page 2 line 4 in the revision.

p.2, l.11 – I recommend writing "Microbial community DNA was extracted from soil".

Response: We added "Soil microbial community" to Page 2 line 11-12 in the revision.

p.2, l. 15 – Taxonomic names should be written in italics (throughout the manuscript).

Response: We kindly disagree. As a rule, only genus and species taxonomic levels are italicized. All names of taxonomic levels above genus are capitalized, but not italicized.

Introduction: p.3, l.3 – Do you refer to belowground "microbial" community structure?

Response: This is meant to be a general statement. While there is evidence in the literature suggesting belowground macro community shifts, the citations provided primarily refer to microbial community shifts.

p.5, l.1 + l.16 – Please change microbial into bacterial community.

Response: These have been changed in the revision as suggested.

p.5, l.14 – bacterial functions (plural).

Response: This has been changed in the revision as suggested.

Methods: p.6, l.1 – How far away from the snow fence was the CTL sampled?

Response: We added ">30m" to Page 6 line 4 in the revision.

p.6, l.9 – What was the distance between the replicates per treatment?

Response: We added "approximately 15-20m apart" to Page 6 line 12 in the revision.

p.7, l.4 – I couldn't find the results of the bacterial communities from the transition zone; and was DNA extracted from all three replicated cores per treatment?

Response: Only phyla from the organic and mineral horizons were reported for bacterial abundance and gene abundance, while all samples (including transition) were included in the distance matrices, NMDS ordinations, and accompanying statistics. To clarify, we added, "Samples were analysed separately by soil horizon (Organic and Mineral only, not Transition)." to Page 9 lines 4-5 in the revision. Also we added "analysed between all samples, and organic and mineral horizons separately" on Page 9 line 18 of the revision. Yes, DNA was extracted from all three cores per treatment and per soil horizon. To clarify, we added "of each soil core," to Page 7 line 7 in the revision.

p.7, l.16- Could you please add the reference for the primers?

Response: Yes! We have added the appropriate reference in the revision.

p.7, l.17 delete – in Mi-Seq but add to p.8, l.7 Bray- Curtis.

Response: Thank you! We have made these correction in the revision.

p.8, l.6- Why did you determine adequate sampling depth? – I could not find that result later on.

Response: We removed, "rarefaction curves to determine adequate sampling depth," from Page 8 line 12 of the revision.

p.7, l.25/26 (and following pages)– "enzyme gene abundance" does not exist - please

consider rephrasing, e.g. " relative abundance of bacterial 16S rRNA and functional genes".

Response: We have reworded this in the revision as suggested.

p.8, l.27 – later there is also a significance threshold of p<0.1 used.

Response: Yes. We used a higher threshold in the figures to acknowledge and indicate where "marginal" significance was found.

Results: I assume that the main focus here is the comparison of each treatment (LOW, INT, DEEP) to the control plot. However, sometimes this is not clear to me from the type of statistical tests you did and from the description of the results. I recommend in general spending some more sentences to explain your results. You often miss to look at the n-fold change of rel. abundance to the control or the comparison between organic and mineral layer. If there is a significant difference to the control- was it observed in all treatments? Is it the same change (decrease/increase) in all treatments? e.g. p.10, l.3 – Instead of saying that the "C/N ratios became more similar" I would point out the %N trend is opposed in the organic and mineral layer. Furthermore I would suggest to add that differences in treatments are in general smaller in the mineral layer compared to organic layer.

Response: We have hopefully addressed these issues throughout the results in the revision. N-fold changes were added to the results. We deleted "and became more similar between treatments" from Page 10 line 15 of the revision. We added "although changes were less drastic than in the organic layers," to Page 10 lines 14-15.

p.10, l.6 – delete "and".

Response: This has been reworded as follows in the revision: "...all relative abundance of bacterial 16S rRNA and functional genes were analyzed by individual horizon." on Page 10 lines 17-18.

p.10, section 3.2. – Please point out the n-fold change of relative abundances. For

instance, the change in sequences affiliated to Chloroflexi is much stronger than for Actinobacteria.

Response: We have added n-fold changes to each reported result!

p.10, l.11 According to the text p=0.011 for Chloroflexi but there is only one * in Fig. 3.

Response: Thank you so much for catching that! We have fixed Fig. 3 in the revision.

p.10, l.13 Please consider rephrasing. I guess this is what you want to say: "acidobacterial abundance was in all treatments (DEEP, INT, LOW) lower than in the control".

Response: We have clarified this in the revision by adding, "while Acidobacteria showed decreased abundance in all treatments relative to the CTL (CTL/DEEP - p=0.055; Fig. 3)." to Page 10 lines 24-25.

p.10, l.19 please check p-value for Actinobacteria text vs. Fig. S4.

Response: Yes! Thank you. p-value corrected to "p<0.001" on Page 11 line 1 of the revision.

p.11, l.6/7 Were lignin, pectin and xylan degradation not significantly different to the CTL?

Response: No, they were only marginally significant. DEEP to CTL: lignin – p=0.119, pectin – p=0.100, and xylan – p=0.119.

p.11, l.14 Please rephrase – I agree it is also a decrease in genes coding for lignin degradative enzymes over the gradient but the scale differs and both LOW and INT have higher gene abundances compared to CTL in mineral layer in contrast to the organic layer.

Response: To acknowledge this observation, we added the following sentence at the end of the paragraph, Page 12 lines 1-2 in the revision: "However, relative to the CTL, both INT and LOW lignin-degrading genes exhibited much greater abundances than

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they did in the organic horizon (Fig. 4)."

p.11, l.18 – Are Figs. S7-S9, S11 needed since they are not mentioned in the text?

Response: We believe that while these results are not significant, it is still important to include them in the supplementary information in order to maintain transparency.

p.11, l.23-25 - Please move this sentence to discussion and refer to Table S1.

Response: The Table S1 reference has been added to Page 12 line 10 in the revision. Also, the indicated sentence has been deleted from the results and the following sentence has been altered/added to the discussion, Page 16 line 9-11, "...and laccases (which are primarily associated with the degradation of lignin and other complex plant compounds) suggests that bacterial communities either preferentially degrade microbial biomass and polysaccharide polymers, or..."

Discussion: p.12, l.11 Please explain from which results the conclusion of "reduced SOM decomposition" was derived from!

Response: To clarify this, we added the following sentence to the revision Page 12 lines 25-27: "Our results indicate that increased snow pack reduced the abundance of genes associated with SOM decomposition in the organic soil horizon, suggesting a reduced SOM decomposition potential.."

p.12, l/12/13 – I don't agree with explanation 1) since you did not find differences in soil moisture along the gradient.

Response: Respectfully, there are many other factors besides moisture that contribute to O2 diffusion into the soil. In the organic horizons near the soil surface, compaction likely plays a role as well, especially in the DEEP zone where there is more snow pack. Also, no moisture differences were found because all measurements were close to 100% saturation, which may not reflect differences between submerged vs. non-submerged soil conditions. We have tried to clarify this in the revision.

p.12, l.30 – Actinomycetales are a bacterial order containing several taxa, thus please use plural.

Response: Good observation! We have changed the sentence to reflect the plural in the revision. "has" has been replace with "have" in Page 13 line 14 of the revision.

p.13, l.1 – Is the increase in Actinomycetales, that are linked to degradation of recalcitrant compounds, contradicting to your conclusions from functional predictions? Please discuss.

Response: The "stimulation of . . . recalcitrant C degradation" was meant to be linked to the "stimulation of ectomycorrhizal growth. . .". To clarify this, we added ". . .which degrade. . ." and removed ". . .degradation" on Page 13 line 15 in the revision.

p.13, l.19- I suggest to delete Koyama et al. reference here because this is a totally different experiment. Instead cite Fierer et al. 2007 who tried an ecological classification of soil bacteria.

Response: We appreciate your suggestion. However, we feel this study provides a good example of oligotrophic vs copiotrophic competition in a nutrient limited vs non-limited environments. The Fierer et al. 2007 study is discussed in the following paragraph, Page 14 lines 17-22.

p.14, l.9 and following- I suggest to delete R2 and p-values from the discussion.

Response: We removed the R2 and p-values as suggested in the revision.

p.14, l.25 and following- I recommend to transfer results of Fig.2 to Results section. Regarding the replicate size of this study I suggest to be more careful here with conclusions.

Response: The sentence referred to was replaced by "The NMDS plot (Fig. 2). . ." on Page 15 line 5 of the revision, and was revised and moved to the Results, Page 11 lines 9-12 of the revision as follows: "visualized by non-metric multidimensional

scaling (NMDS) plots of Bray-Curtis dissimilarity indices constructed from community matrices (Stress=0.090, Shepard plot non-metric R^2=0.992; Fig. 2) revealed significant differences in community structure associated with winter snow pack"

p.15, l.14-27- The statements about fungi and tannic compounds are too speculative since this can not be supported by data from your study. Instead I would like to have a discussion of PICRUSt limitations here. Why is there only a decrease in rel. abundance of functional genes- which genes might be increased?

Response: The role of fungi and tannic compounds, while outside the scope of the data collected in this study, may help explain our results. Therefore, we feel that this paragraph should remain. The limitations of PICRUSt are acknowledged on Page 16 lines 4-8.

Tables & Figures: Table 1 I don't really understand the number of replicates you refer to here. n=4 are technical replicates? Significance was tested between treatments for each layer separately?

Response: We have altered the table caption to clarify. Page 31 lines 1-6 of the revision. We have also added specifics on the number of replicates throughout the Methods section.

According to Methods part you measured temperature at 4 different depths but not at 12 cm.

Response: This was our mistake and has been corrected in the revision Page 6 line 31 – Page 7 line 1.

Was there no post-hoc test done for %N, C/N, and pH?

Response: While the post-hoc test was done, they were not significantly different. Therefore, the subscripts were not used, as indicated by the revised caption "Results are indicated by a,b,c only where $p<0.05$." on Page 31 line 6 of the revision.

Figure 2 Difficult to understand. Microbial communities from how many replicates and layers are plotted here? Please use "CTL" as abbreviation for control (similar to the text).

Response: We altered the caption Page 33 lines 3-5 in the revision to clarify as follows: "Each point represents the bacterial community structure within one of the 41 total samples used for DNA extraction from a variety of soil depths (Organic, Transition, and Mineral)."

Figure 3 Please indicate for which significant effect you tested here. I don't understand, why there was no post-hoc test performed for Acidobacteria or is there only a significant difference to the control and not between treatments. The same applies for Fig. 4.

Response: You are correct in interpreting the significant difference to be to the control and not between treatments. This is indicated at the end of each caption by the phrase "except where significant differences were to the control."

Supplement Fig.S1-S15 From your Methods section it is not clear to me whether you analyzed abiotic soil parameters in the same soil sample (depth) as the bacterial community composition.

Response: To clarify this, we added the following sentence to Page 9 lines 11-12 of the revision. "To ensure accurate comparisons, soil chemical properties were measured from the same samples that DNA was extracted from."

References (typos): p.22, l13 Gonzalez-Meler

Response: Thank you! This has been fixed in the revision.

p.23, l34 mcrA in italics

Response: Thank you! This has been fixed in the revision.

p.27, l13 CO2

Response: Thank you! This has been fixed in the revision.

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