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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 #1 Received and published: 7 February 2016

In this manuscript, the authors describe the changes in bacterial community composition as a result of increased snow cover in a moist arctic Tundra. The study shows that increased snow cover led to changes in bacterial community composition along changes in soil chemistry and the plant community. The authors conclude that the observed changes in bacterial community composition and function might lead to re-

duced decomposition of SOM in these arctic systems. The manuscript is well written and structured and the story is for the most part easy to follow. After careful revisions the manuscript should be of great interest to the readership of SOIL. However there are some issues that need to be addressed or discussed in more detail to improve the manuscript.

Response: We would first like to thank the reviewer for the positive comments above and the constructive criticisms below. We will do our best to address them and are confident they will make the manuscript better.

1. Soil depth: As the authors point out, that there is a huge difference in edaphic factors between organic and mineral horizons in this study. Such depth related differences have been shown to potentially influence microbial community structure and function and the potential controls on those (Eilers 2012 SBB, Schnecker 2015 SBB). The authors should also test the effects of depth as well as treatment and potential interactions on the individual bacterial groups, their relations to soil factors and beta diversity using Adonis and perform the mantel tests with the edaphic factors separately for organic and mineral horizons.

Response: Samples were analysed separately by soil horizon / depth (Organic vs. Mineral) for most factors included in this manuscript (%C, %N, C:N, pH, bacterial abundance, predicted gene abundance). We have added Table 2 to the revision to report statistical differences in beta diversity, including separate analyses for each soil depth. We also did analyse statistical differences between soil depths / horizons for each of the six most abundant phyla and for each enzyme gene abundance. However, our primary goal for this paper was to address the effects of increasing snow pack on soil bacterial communities regardless of soil depth. Therefore, we chose to highlight the treatment effect over the depth effect.

2. Vegetation and decomposition: The authors state that an increased snow cover ultimately leads to reduced decomposition and C loss from the system since NPP is

increased and might offset potential losses of C. While their results show a reduced potential for decomposition in the bacterial community and other studies have found increased NPP in shrubby tundra compared to tussock tundra, the C contents in organic and mineral horizons decreased significantly. This huge loss could have either happened during the transition from tussock to shrubby vegetation, which would mean that NPP did not offset decomposition or during the transition into a sedge dominated fen, which would indicate that decomposition was not reduced despite the reduction of the bacterial potential for decomposition.

Response: Carbon content is not a good indicator of carbon-stock. Degrading permafrost often results in soil consolidation (loss of ice collapses soils) with associated changes in bulk density and depth redistribution of soil and C. The C-stock profile change as a result of the snow fence treatment is part of another paper. Carbon stock over the soil profile to the average active layer equivalent depth was 7% higher for the intermediate than for the control. Here we have used %C in our analyses because most of the C (if not all) is accessible to microbes (these acidic tundra soils have little to no physical aggregation, JD Jastrow personal communication). Therefore the factors affecting organic matter readiness to microbial decomposition is likely the chemistry/quality of the organic matter (%C, C/N) in addition to temperature and moisture. We hope this is made more clear in the revised manuscript. Also, we acknowledge that the use of the word "content" when referring to our %C data may have been misleading. To clarify, we have changed the phrase "C (or N) content" (i.e. C stock) to "C (or N) concentration" throughout the manuscript. Specifically at the following locations: Page 2 line 11, Page 6 line 22, Page 10 lines 7&8, and Page 14 line 16.

3. Fungi and oxidative enzymes: The authors should more strongly point out that this study is focused on bacterial community composition and function throughout the text and that fungi might play an important part especially in the production of oxidative enzymes which have been found in arctic soils (Tveit 2012 ISMEJ).

Response: We changed "microbial" to "bacterial", or "microorganisms" to "bacteria" in

the following locations in the revision: Page 5 lines 1, 6, 10, 14, 18 Page 12 lines 11, 24 Page 13 line 22 Page 15 line 18 Page 16 line 23 Page 17 line 9, Page 33, Figure 2, line 3 The role of fungi, while not highlighted in this study, is acknowledged and discussed on Page 16 lines 8-11. We also added "by specializing in the production of oxidative enzymes" to Page 16 lines 9-10.

4. The authors should be more careful with the interpretation of the ancestral state reconstruction, since these results are strictly based on the sequencing results of the bacterial community. Changes in the so obtained functions can only be interpreted as changes in the bacterial community composition. Any statements concerning enzyme kinetics, enzyme transcription, activity or even in situ functional gene copy number can only be speculated on and should be clearly marked as speculation (especially Page 17 Lines 1-19)

Response: This is well noted and care was taken to revise the manuscript with it in mind, including the following: We have added the following sentence to Page 15 line 30 – Page 16 lines 1-4: "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)." We have removed "found" and replaced it with "predicted" on Page 16 line 18. We have added "predicted" to Page 17 lines 17 & 27.

5. The authors should consider that any changes in the bacterial community composition could be independent of SOM properties and be a result of changes in temperature, moisture vegetation length and so on and could vary with depth (Schnecker 2014 Plos One, Gittel 2013 ISMEJ)

Response: We agree. However, untangling what is driving bacterial community shifts in this system requires isolation of these different factors in a laboratory setting, which

is outside the scope of this paper, however we are planning some of these experiments. The very nature of our experiment, altered snow pack over a long period of time, changes a variety of factors that may contribute to bacterial community change (O2 diffusion via moisture or compaction, temperature, plant community, etc.).

Detailed comments: Title: Since multiple environmental factors are changed with increased snow cover, “thermal insulation” should be replaced with “altered snow cover” or similar.

Response: Agreed! We replaced “thermal insulation” to “altered snow pack” in the revision.

Introduction: Page 3 Lines 17-23: Since there is another paragraph on SOM in the arctic this one could be omitted. Especially since the numbers for global C storage here and in the paragraph on arctic C storage are not the same.

Response: While we appreciate the comment, it is important to recognize that the paragraph being referred to highlights the important role that microorganisms play in C cycling on a global scale versus the later one that specifically describes the significance of C in Arctic ecosystems. We have modified the sentence to highlight global vs. arctic C dynamics. We added “on a global scale, releasing nutrients. . .” on Page 3 lines 15-16.

Page 4 Line 1-2: The nutrient limitation of Arctic soils has recently been challenged (Wild 2015 GBC, Melle 2015 SBB).

Response: We have removed the sentence from the manuscript. However, while this may be the case in more recent studies, as rising temperatures in the Arctic may be initially accelerating SOM decomposition and releasing more nutrients, historically the Arctic has been observed to be a nutrient limited ecosystem (Hobbie et al., 2002; Jonasson et al., 1999; Mack et al., 2004; Shaver and Chapin, 1980, 1986; Sistla et al., 2012).

Page 5 Lines 5-18: The Authors should consider using testable hypotheses instead. Structure and O2 availability were not measured in this study. The change in plant species composition might not be a consequence of increased nutrient availability but the result of changed water status. With the experimental setup it cannot be distinguished between substrate effects and environmental effects.

Response: This is a valid point. Changes in plant species (and underlying causes), while proposed as a contributing factor to bacterial shifts, was not the focus of this study, and therefore we chose not to test for causes of vegetation shifts within our study site.

Material and Methods: Please mention which program was used to perform the statistical analyses. As mentioned before the measured parameters, including beta diversity should be tested for depth effects and interactions of depth and treatment. All correlative tests should also be performed separately for organic and mineral horizons.

Response: We added “. . .in the R statistical software package. . .” on Page 9 lines 2-3 of the revision. We added Table 2 to report beta-diversity statistics, both for all samples (All layers), and separated by soil depth (organic and mineral) in the revision.

Results: Page 10 Lines 26- Page 11 Line 2: These results should be presented in a separate table.

Response: These results are included in Table 2 of the revision.

Page 11 Lines 10-11: The reported p-values are not significant.

Response: While the p-values are not significant, we felt it was still important to acknowledge notable trends with p-values <0.1 as long as the p-values are reported.

Page 11 Lines 24-25: This interpretation should be moved in the Discussion section of the manuscript and “microbial communities” should be replaced with “bacterial communities”.

Response: “Microbial” will be replaced with “bacterial” 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 lines 4-9, The overall absence of bacterial genes encoding for peroxides, phenol oxidases, 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 that the decomposition of more recalcitrant forms of C in Arctic soils is performed by other microorganisms such as fungi.”

Discussion: Page 12 Line 11: As stated before, while the bacterial functional potential might indicate reduced SOM decomposition, the decrease in C content from control to DEEP suggests otherwise.

Response: Please see response to general comment 2 above.

Page 12 Lines 12-17: An alternative explanation might be that the microbial community composition is shaped by the environmental factors and less so by SOM properties.

Response: This is a good point. However, this paragraph was meant to discuss the functional shifts as opposed to the phylogenetic shifts. To clarify, we will add the word “functional” to Page 12 line 23 of the revision.

Page 12 Line 16: Blanc-Bates et al. 2015 is missing in the Reference list. Is this the same that is listed as submitted in Page 16 Line 12. If this is the case and if this study was conducted at the same site, mentioning this and a short description of the findings would help the reader understanding the author’s arguments about changes in SOC dynamics.

Response: This citation has been removed.

Page 13 Line 26: The strong correlation of Acidobactera with pH and the non-significant correlation with C:N questions that statement.

Response: This sentence highlights competitive interactions between bacterial phyla.

Any correlation with abiotic factors in the context of this sentence would be indirect.

Page 13 Lines 28-30: This sentence can be omitted.

Response: We appreciate your opinion and will omit this sentence in the revision.

Page 14 Line 28: This could be a depth effect and not a result of the altered snow cover.

Response: The effects of the snow accumulation treatment are statistically significant in the organic horizon alone (p=0.001), the mineral horizon alone (p=0.003), and within all samples (p=0.017; please see Table 2 in the revision). Therefore, we are conservative in reporting that snow accumulation affects bacterial community structure with a p-value of 0.017.

Page 15 Lines 7-11: Is there any indication that increased tanning occurred at the studied site?

Response: We did not test for tannin concentration in this study, however the encroaching shrub species at the site are known to produce them (DeMarco et al., 2014; Schimel et al., 1996).

Page 15 Lines 23-27: Binding of enzymes to tannins could happen to any enzyme. Oxidative enzymes could actually degrade tannins and might thus be upregulated.

Response: This is a valid point. However, if the bacterial community does not possess the functional capacity to produce these oxidative enzymes (as suggested by our data), they will not be able to increase their production. As mentioned in Page 15 lines 16-21, fungi may perform this role in this system, and thus would not show up in our 16S rRNA gene analysis.

Page16 Line 13: Sistla et al 2013 did not use a snowfence study.

Response: We added “. . .warming and. . .” to Page 17 line 2 of the revision.

Page 16 Lines 13-28: While this study explains to some extent some of the author's statements, it is over represented for its current publication status.

Response: This citation and description of the study has been removed from the revised manuscript.

Page 17 Lines 1-19: see general comments above.

Response: Please see response to general comment 4 above.

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