

SOILD
Interactive
comment

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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 reduced 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 layer / 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 have also added Table S3 to the supplementary revision to report statistical differences in alpha diversity both between soil layers and between treatments. In addition, we did analyse statistical differences between soil depths / layers for each of the six most abundant phyla and for each enzyme gene abundance. We have added this data in Table S2 of the supplementary information. All new tables are briefly described and discussed in additions made within the text. While we believe these additions are beneficial to the manuscript, our primary goal for this paper was to address the effects of increasing snow pack on soil bacterial

communities regardless of soil depth. Therefore, in the text we chose to highlight the treatment effect over the depth effect.

Changes made to manuscript:

- *Text added to Page 8, lines 26-27.*
- *Text added to Page 8, line 29 – Page 9 line 2.*
- *Text added to Page 9, line 12.*
- *Text revised / added to Page 9, lines 14-18.*
- *Text revised / added to Page 11, lines 6-12.*
- *Text added to Page 11, lines 20-23.*
- *Text added to Page 13, lines 18-20.*
- *Text added to page 15, lines 23-24.*
- *Text added to page 15, lines 27-28.*
- *Text added to page 17, lines 15-16.*
- *Table 2 added to Page 33, lines 1-6*
- *Table S2 added to Supplementary Information Page 11.*
- *Table S3 added to Supplementary Information Page 12.*

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.

Changes made to manuscript:

- *Change made Page 2 line 11*
- *Change Page 6 line 19*
- *Change Page 10 lines 7-8*
- *Clarification and changes made to Page 15 lines 5-10.*

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: The role of fungi, while not highlighted in this study, is acknowledged and discussed on Page 16 lines 8-11.

We changed “microbial” to “bacterial”, or “microorganisms” to “bacteria” throughout the manuscript:

Changes made to manuscript:

- Page 4 line 30
- Page 5 lines 4, 8, 11
- Page 12 lines 26
- Page 15 lines 18 & 27
- Page 16 line 10
- Change to Page 17 line 21
- Change to Page 33, Figure 2, line 4

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:

Changes made to manuscript:

- Addition to Page 16 lines 21-31
- We have added “predicted” to Page 17 lines 15 and Page 18 line 16.

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.

Changes made to manuscript:

- Page 1 line 2

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.

Changes made to manuscript:

- 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).

Changes made to manuscript:

- Sentence removed from Page 4 lines 1-2.

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.

Changes made to manuscript:

- *None.*

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 / Changes made to manuscript:

- *We added "...in the R statistical software package..." on Page 8 lines 28-29 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) on Page 33 lines 1-6.*

Results:

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

Response / Changes made to manuscript:

- *These results are included in Table 2 of the revision Page 33 lines 1-6.*

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.*

Changes made to manuscript:

- *None.*

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.

Changes made to manuscript:

- *See comments for general comment # 3.*
- *The indicated sentence has been deleted and replaced on Page 17 lines 1-5.*

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. See below changes.

Changes made to manuscript:

- *Addition of the word "functional" to Page 13 line 9 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 / Changes made to manuscript:

- *This citation has been removed.*

Page 13 Line 26: The strong correlation of Acidobacteria 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.

Changes made to manuscript:

- *None.*

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

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

Changes made to manuscript:

- *This sentence has been omitted on Page 14 line 27.*

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.

Changes made to manuscript:

- *Table 2 added on Page 33 lines 1-6*

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).

Changes made to manuscript:

- None.

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.

Changes made to manuscript:

- None.

Page 16 Line 13: Sistla et al 2013 did not use a snowfence study.

Response / Changes made to manuscript:

- *We added "...warming and..." to Page 17 line 29 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.

Changes made to manuscript:

- *Major revisions have been made from Page 17 line 28 - Page 18 line 12.*

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

Response:

Please see response to general comment 4 above.

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 #2

Received and published: 15 February 2016

Ricketts et al. examined an interesting question: How do changes in snow cover affect soil bacterial community structure and function? They sampled and analyzed soil material from an interesting long-term snow depth manipulation experiment and applied up-to-date methods for bacterial community characterization. They claimed out that an increase in snow depth resulted in an increase in soil insulation that led to changes in bacterial community structure, to a decrease in enzyme encoding genes and in C%. The MS presents results of a relevant experiment and the topic is within the scope of the Journal.

Response:

We thank the reviewer for the encouraging summary and for the criticisms which are addressed below.

General comment #1- The Introduction is well written. However, in my opinion more information about the experimental set-up and the sampling design are required in the Methods section. How did they ensure that the snow depth was continuously increased for 100%, 50%, or decreased for 25% along the study period of 18 yrs? Did they continuously measure snow depth each year? Did they remove or add snow in cases with more or less than e.g. 100% than control? Or is the treatment rather a distribution of increased/decreased snow depth around e.g. 100% (+/- SD) than a fixed treatment level? How did they monitor the annual input of snow at each point? Was it equal for all years?

Response:

Snow treatments are caused by the wind drift distance from the snowfence. Snow fall varies from year to year but the drift caused the relative snow accumulation at similar distances from the fence every winter. Snow depth and density have been measured sporadically and reported in other papers which we cite.

Changes made to manuscript:

- *Addition to Page 5 lines 23-251.*

General comment #2 - The applied non-parametric statistics seem to be appropriate for the experiment and data set, however, re-analysis of the data might be necessary because organic and mineral soil samples seem to be included within one analysis (without accounting for differences in sampling depth). In my opinion, too many results were mentioned as significant effects even though the p values were above 0.05. This is problematic and partly lead to a rather speculative discussion and conclusion. To sum up, I recommend thorough revision of the manuscript in order to focus on the observed effects of snow depth on soil bacterial communities.

Response:

Thank you for this suggestion, one that has also been pointed out by other referees. We have included new stats and language to distinguish the soil depth analyses and added p

values to let the reader decide the statistical significance of results. We have noted results with marginal significance of $p < 0.1$ in addition to the $p < 0.05$.

Changes made to manuscript:

* Please see response and changes made to anonymous referee #1 general comment #1.

- Text added to Page 8, lines 26-27.
- Text added to Page 8, line 29 – Page 9 line 2.
- Text added to Page 9, line 12.
- Text revised / added to Page 9, lines 14-18.
- Text revised / added to Page 11, lines 6-12.
- Text added to Page 11, lines 20-23.
- Text added to Page 13, lines 18-20.
- Text added to page 15, lines 23-24.
- Text added to page 15, lines 27-28.
- Text added to page 17, lines 15-16.
- Table 2 added to Page 33, lines 1-6
- Table S2 added to Supplementary Information Page 11.
- Table S3 added to Supplementary Information Page 12.

At least four references cited in the text are not included in the reference list.

Response:

We have reviewed our citations and added references in the revision.

Changes made to manuscript:

- Please see additions / changes in References section, Pages 21 – 30.

Please, find my specific comments below:

Abstract

Line 4-5 “(i.e. more or less snow), resulting in increased winter insulation” This statement is partly contradictory. Omit “or less” or add “increased or decreased winter insulation”.

Response:

Agreed! Please see changes below.

Changes made to manuscript:

- We omitted “or less” on Page 2 line 4 in the revision.

L8 “context of ecosystem response to climate change.” Please change to “context of expected ecosystem response to : : :”

Response:

We think this is a valid point and have changed it in the revision, as recommended.

Changes made to manuscript:

- Revised as recommended on Page 2 line 8.

L15 “most abundant phyla” requires a value about the contribution of these phyla on total abundance. “20% or 80% of total detected phyla?”

Response:

This is a good idea! Please see changes below.

Changes made to manuscript:

- *We added "... (ranging from 82% to 96% of total detected phyla per sample)..." to Page 2 lines 15-16 in the revision.*

L26 The authors did not study the temperature sensitivity of extracellular enzymes (sensu str.) they are requested to omit any statement/conclusion about this.

Response:

Thank you for your comment. However, we feel that while this sentence is speculative, it is an important possible mechanism explaining our results and is supported by outside literature (Conant et al., 2011; Davidson and Janssens, 2006; Lützow and Kögel-Knabner, 2009).

Changes made to manuscript:

- *None.*

Introduction

Page 3

L 1: How can the stability of the structure be threatened? I suggest to change to more ecological terminology.

Response / Changes made to manuscript:

- *We have changed the wording of this sentence to "Broad and rapid environmental changes are driving both above- and belowground community shifts in the Arctic" on Page 3 lines 2-3 of the revision.*

L8 Anisimov and Vaughan must be changed to Anisimov et al. or the respective reference must be added to reference list.

Response:

Thank you! This was intended to be the same reference! We have fixed it in the revision.

Changes made to manuscript:

- *Fixed reference on Page 2 line 11 of the revision.*

L11 Needs reference

Response / Changes made to manuscript:

- *We have added (Anisimov et al., 2007; Liston and Hiemstra, 2011) to Page 3 line 11 of the revision.*

L12 Needs reference

Response / Changes made to manuscript:

- *We have added (Hugelius et al., 2013; Ping et al., 2008; Schuur et al., 2009; Tarnocai et al., 2009) to Page 3 lines 12-13 of the revision.*

Page 4

L16 omit activity or kinetics – I prefer the use of the term “kinetics”.

Response / Changes made to manuscript:

- *We have omitted “...activity and...” from Page 4 line 14 of the revision.*

Page 5

L11 Why should microbes be unable to degrade SOM from shrubs? Needs further explanation or changing in the sense of” the potential to degrade SOM might be reduced”.

Response / Changes made to manuscript:

- *This sentence has been deleted from the revised manuscript on Page 5 line 7.*

Methods

Are detailed vegetation surveys available for each plot? How far away were the replications at each treatment located from each other? Did they sample more than one soil core for each replicated plot and compiled composite samples or not? Figure 1 is important and helps to understand the experimental set-up. However, where is the control located?

Each of these of these questions have been addressed separately. Please see below:

Response:

We did not collect detailed vegetation surveys for each plot.

Changes made to manuscript:

- *None.*

Response:

Replicate distance = estimated ~15-20m.

Changes made to manuscript:

- *We added “approximately 15-20m apart” to Page 6 line 7.*

Response:

Clarification of number of cores/compiled

Changes made to manuscript:

- *Added “replicate” and “and analyzed separately” to Page 6 line 8. Added “of each soil core” and deleted “soil” to Page 7 line 2.*

Response:

For the location of the control, please see below.

Changes made to manuscript:

- *Added “>30m” to Page 5 line 28.*
- *Added “Three soil cores were obtained from each treatment zone, labelled Deep, Intermediate, and Low, and a Control zone located >30m outside the effect of the snowfence.” to the caption for Figure 1, Page 31 lines 3-5.*

Page 5

L24 “strategically” needs further explanation.

Response:

The word “strategically” refers to its orientation based on wind patterns and is described in the papers we referenced (Jones et al., 1998; Walker et al., 1999). Also, please see response to general comment #1 above.

Changes made to manuscript:

- *None.*

L29-30 Soil Survey 2015 is not listed in references

Response / Changes made to manuscript:

- *We added “Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at <http://websoilsurvey.nrcs.usda.gov/>. Accessed [11/17/2015].” to the references, Page 28 lines 27-29.*

Page 6

L1 “regime”: the tested climate change scenario is: variable precipitation (that may induce differences in soil temperature) but constant air temperature.

Response:

Please see response to general comment #1 above.

P6 treatment/factor levels: -25% vs. +50% (vs. +100%) are not equally selected. This might be problematic for ANOVA. Please check.

Response:

For our analysis, each treatment was defined as a categorical variable, thus avoiding any numerical percentage gradient. This is the more conservative approach.

Changes made to manuscript:

- *None.*

L9: n=3? Total number of sampled cores = 12?

Response:

Yes. 3 – CTL, 3 – DEEP, 3 – INT, 3 – LOW = 12 cores.

Changes made to manuscript:

**Alteration throughout the Methods section and figures were made to be more transparent on sample sizes.*

- *Page 6 line 25*
- *Page 7 lines 5-12*
- *Page 32 lines 5-7*
- *Page 33 lines 4-6*

L18: unclear, how did they use the 2 cm depth segments for further analysis since they presented data for “organic” and “mineral” soil only. Did they calculate the average value of C% etc. for

each of the two strata by considering the data of the single segments?

Response:

We only analysed %C, %N, and pH for the samples used for the DNA extractions.

Clarifications were made in the revised manuscript. See below.

Changes made to manuscript:

- *Added "To maintain consistency, only these samples were used to analyse %C, %N, and pH relationships." to Page 7 lines 11-12 of the revision.*
- *Added "To ensure accurate comparisons, soil chemical properties were measured from the same samples that DNA was extracted from." to Page 9 lines 7-9 of the revision.*

Page 7

L 11: Please provide the absolute sampling depths for each treatment (average value and variation) in the Methods section. Sampling depth might be considered as co-variable in non-parametric ANCOVA in order to account for any effect.

Response / Changes made to manuscript:

- *Changed "typically between 0-6cm soil depth, except in one case where the top 10cm was primarily plant tissue" to "(mean soil depth \pm standard error [S.E.] = 5.6 ± 1.3 cm)" in Page 7 lines 6-7.*
- *Changed to "(mean soil depth \pm S.E. = 14.8 ± 1.8 cm)." on Page 7 lines 8-9.*
- *Changed "...and was more variable (ranging from 15-36cm soil depth) due the varying depths of transition." to "(mean soil depth \pm S.E. = 25.1 ± 1.7 cm)" in Page 7 line 10.*

L22 Caporaso et al is not included in the reference list

Response / Changes made to manuscript:

- *We added this to the references in the revision, Page 21, lines 24-29.*

Page 8

L 4: "six most abundant phyla" this requires a quantitative documentation for the six phyla.

Response / Changes made to manuscript:

- *We added ",comprising 82% - 96% of total detected phyla per sample," to Page 8 lines 5-6.*

L 18-20 needs reference(s)

Response / Changes made to manuscript:

- *Added "(Sinsabaugh et al., 2008; Waldrop et al., 2010;..." to Page 8 line 20 of the revision.*

L 24 – P9 L 14 Selected types of statistical analysis seem to be appropriate for the experimental set-up and data. (Maybe non-parametric ANCOVA is required for the consideration of sampling depth as co-variable). However, P9 L1 the selection of linear regression analysis is inconsistent since the authors applied non-parametric tests for the data. Taken into account that the primary assumptions for parametric tests are not full-filled (they used the non-parametric tests) then the

use of linear regression seems not to be adequate. In addition, the use of median and median absolute deviation might be more robust estimates (and consistent) of the central tendency and variation of the data than mean and SD.

Response:

This is a valid point and we thank the reviewer for pointing it out. The linear regression analyses were initially performed simply to give us an idea of how the abiotic soil data affected individual bacterial phylum, and gene group abundances. While there are many interesting relationships, we ultimately decided to report the more appropriate non-parametric stats for the bulk of the analyses, but to still include the regressions in the supplemental material.

Table 1:

The lower case letters used indicate that organic and mineral soil material was included in one analysis. (?) I suggest comparing the treatment effects on the two strata (organic and mineral) independently of each other (for both KW-ANOVA and Nemenyi-test; performing the respective tests for the treatment effect on e.g., C% of “organic”). Add a brief description of the treatments (-25% of snow cover compared to control etc.) to the table description.

Response:

For clarification, the organic and mineral layer samples were analysed independently as suggested. See changes that were made below.

Changes made to manuscript:

- *We have removed the lower case letters “c” from the %C column in Table 1, Page 32 line 7, since that analysis was not part of the organic horizon analysis, and there were no statistical differences in the mineral horizon.*
- *We added, “Organic and mineral samples were analysed separately using the Nemenyi post hoc test. Results are indicated by _{a,b,c} only where $p < 0.05$.” to Table 1 description, Page 32 lines 5-6.*
- *We added, “(Low = ~25% less snow pack than the Control, Int = ~50% more snow pack than the Control, Deep = ~100% more snow pack than the Control).” to Table 1 description, Page 32 lines 1-3.*

Page 9

L7-8: two-sample t-test for the comparison of four groups? I do not understand. The selected measure of dissimilarity as well as the criteria for NMDS seems to be adequate / sufficient. How many dimensions were included / considered for NMDS?

Response:

The two-sample t-tests were used to perform pairwise comparisons of the treatment groups. The ordination of the Bray-Curtis distance matrices using NMDS yielded two convergent solutions found after four tries, resulting in 2 dimensions.

Changes made to manuscript:

- *We replaced, “The Shannon alpha diversity metric was compared across treatments using...” with, “Pairwise comparisons of the Shannon alpha diversity metrics from each treatment group were made using...” in Page 9 line 12-13.*

L10-11: Do I understand right? The analysis of associations between explanatory variables (i.e.,

soil chemical properties) and bacterial data were further analyzed by Mantel test? Which statistical software was used?

Response:

Yes. The QIIME script `compare_distance_matrices.py` was used with `--method=mantel` to compare the Bray-Curtis distance matrices to distance matrices created from the abiotic data using the QIIME script `distance_matrix_from_mapping.py`. The QIIME software was used to run these tests.

Changes made to manuscript:

- *We altered the sentence on Page 9 lines 10-12, as follows: “Bacterial diversity statistics were calculated using QIIME (Caporaso et al. 2010), specifically the `compare_alpha_diversity.py`, `compare_categories.py`, and `compare_distance_matrices.py` scripts.”*

Results

L18 I thought the test statistic of KW-ANOVA is the “H-value” and not Chi²? Please check. Please calculate the effect sizes for each tested factor and variable.

Response:

Yes, you are correct. Confusion arose because of the way the R package reports the values for `kruskal.test` (“Kruskal-Wallis chi-squared = 0.80317, df = 3, p-value = 0.8487”). After checking, we discovered that this value is indeed the Kruskal-Wallis test statistic, otherwise known as “H”, although it has been reported both ways.

Changes made to manuscript:

- *We have corrected this in the revision. Page 9 lines 26-27.*
- *Sample sizes were added to clarify effect size on Page 9 lines 26-27 of the revision.*
- *“(n=12 per treatment)” was added to Page 6 line 25 in the revision.*

L30: p=0.32 I would not consider this as a significant difference.

Response:

While not considered significant, the increasing trend does contribute to decreasing C:N.

Changes made to manuscript:

- *To address this comment, we added “only slightly” to Page 10 line 8 of the revision.*

Page 10

L1: p=0.14 I would not consider this as a significant difference.

Response:

While the result is insignificant, we feel that the overall trend contributes to the story.

Changes made to manuscript:

- *We have reworded the sentence as follows: “This resulted in a decreasing trend in C:N ratios across snow accumulation treatment zones and relative to the control (CTL/DEEP - p=0.14; Table 1).” on Page 10 lines 8-10 of the revision.*

L2 p=0.06 indicates a tendency

Response / Changes made to manuscript:

- *We added the phrase “tended to” to Page 10 line 10 of the revision.*

L11 Verrucomicrobia and Actinobacteria p-values indicate tendencies. Section 3.2. in many cases the order of magnitude of the relationships were rather low.

Response:

These comments are true, and as such, we attempted to phrase our results in a way that openly and accurately reports the data, without dismissing trends that may or may not be the result of the treatment or abiotic soil conditions. Hopefully, this will allow readers to be able to openly interpret the data. We have added the below changes to more accurately address the orders of magnitude of the relationships.

Changes made to manuscript:

- *We added n-fold changes to all appropriate results in the revision, from Page 10 line 19 –to- Page 12 line 11.*

Page 11

L5-8 p-values of which comparisons? CTL to DEEP or LOW to DEEP. Or do the p-values represent the results of the KW-ANOVAs?

Response:

Thank you for catching this confusion. The first two, cellulose and chitin, were comparing the DEEP zone relative to the CTL. The latter three were comparing across treatments from LOW to DEEP.

Changes made to manuscript:

- *We added, “in the DEEP zone” to Page 11 line 28.*
- *Altered and separated the sentence into two to clarify, Page 11 line 28 –to- Page 12 line 1.*

L10 “N mobilization genes” Do the genes mobilize N? Please, correct.

Response / Changes made to manuscript:

- *To clarify this, we have altered the sentence as follows: “Shifts along the snow accumulation gradient were also observed in gene groups involved in nutrient mobilization with a 1.18-fold increase in genes necessary for N mobilization ($p=0.14$) 1.12-fold decrease in genes necessary for phosphate mobilization ($p=0.39$) in the DEEP zone relative to the CTL.” on Page 12 lines 3-6.*

L12-13 It might be more meaningful to write as follows: “: : : included an increase in genes encoding enzymes involved in : : :”.see P15 L16-17

Response:

Great recommendation!

Changes made to manuscript:

- *Altered as recommended, Page 12 lines 7-8 of the revision.*

L25: omit “simple cellulosic and”

Response / Changes made to manuscript:

- *This entire sentence has been removed from the manuscript, Page 12 line 21..*

Page 12

L10: only moderate changes and in many cases they observed only trends. Please, focus on significant results $p < 0.05$.

Response:

The result from the adonis test ($p=0.017$) supports the claim that community structure is significantly affected by the snow addition treatment. Additionally, this sentence (while broad in scope), is not false. The significant results we do find still do indicate a “change”. The remainder of the discussion focuses on these significant results, occasionally using the trends in the data to speculate cause.

Changes made to manuscript:

- *To address this confusion, we replaced the word “phylogeny” with the phrase, “community structure” in Page 13 line 5.*

L12: “towards more labile sources” if it is an important “pathway” then the term/concept “labile” requires definition in the introduction section.

Response:

While the term “labile” may not be familiar to a broad audience, we feel that it does not require defining in the context of this journal.

L13: What is “SOM enzyme activity”?

Response / Changes made to manuscript:

- *We replaced “ decreased SOM enzyme activity” with “a decreased abundance of genes associated with SOM decomposition” in Page 13 line 11.*

L14: The positive relationship between gene copies and enzyme machinery requires a reference.

Response:

We’re glad you noticed that! We have considered this and it is acknowledged and discussed with references on Page 17 lines 24-26.

Changes made to manuscript:

- *We also added the reference “(Rocca et al., 2014)” to Page 13 line 12 and Page 17 line 12.*

L15/16 Which limitations of enzyme kinetics? Change “enzymatic decomposition reactions” to “enzyme functioning” . Blanc-Betes et al. is not included within the reference list

Response / Changes made to manuscript:

- *We have changed the wording as recommended on Page 13 line 14 in the revision.*
- *Blanc-Betes reference has been removed from this sentence, Page 13 line 14.*

L18-19 But in the present account a decrease in C% was observed. Discuss this issue in contrast to the results in the literature. Is the decrease only found in C% or also in C-stock?

Response:

Please see response to anonymous referee #1 general comment #2.

Changes made to manuscript:

- *This sentence was removed from the revision, Page 13 line 15.*
- *Please see changes from anonymous referee #1 general comment #2.*

L22/23 Significant changes were observed only for a few groups.

Response:

While this is true, the sentence refers to “bacterial community structure” (or beta diversity), which is significantly altered by the treatment, as per the adonis test ($p=0.017$). In other words, just because significant results were only obtained for a few of the most abundant phyla, the overall community structure (which takes into account ALL organisms/OTU's) does significantly change due to increased snow depth.

Changes made to manuscript:

- *None.*

L28/29: What are the possible links to enzyme production and functioning?

Response:

Enzymes involved in the utilization of plant biomass and SOM are described in the discussion, Page 16 lines 22-26.

Changes made to manuscript:

- *None.*

L29: Sangwan et al. is not included within the reference list.

Response:

Thank you for catching that. We have added the citation to the reference list.

Changes made to manuscript:

- *Citation added to Page 27 lines 18-21.*

Page 13

L7 “cold saturated soil” Did you mean “cold, water-saturated soil”?

Response:

Yes, we did! We have fixed it as recommended in the revision.

Changes made to manuscript:

- *Added “, water-saturated” to Page 14 line 3.*

L7 Costello and Schmidt . is not included within the reference list.

Response:

We have added the citation to the reference list.

Changes made to manuscript:

- *Citation added to Page 22 lines 8-10.*

L28-30: Omit.

Response:

We appreciate your opinion and have omitted this sentence in the revision.

Changes made to manuscript:

- *Sentence has been omitted from the manuscript, Page 14 line 27.*

Page 14

L12-13: Results and no discussion.

Response:

These results are meant to introduce the idea that while some individual abiotic soil factors may be correlated with specific bacterial phylum abundance, predictors may vary depending on the organism. This is discussed on Page 14 lines 28-30 and Page 15 lines 1-5.

Changes made to manuscript:

- *R² and p-values have been removed in the revision, Page 15 lines 8, 9, and 11.*

L26 omit“(Stress : : :)

Response:

It is the authors understanding that the stress value is a metric used to evaluate how well the ordination represents the original distances of the matrices, and should be reported when discussing the NMDS plot.

Changes made to manuscript:

- *None.*

L28/29 Results and no discussion.

Response:

These results highlight the relationship between the snow accumulation treatments and subsequent soil chemistry changes. A possible mechanism for shifting bacterial community structure is outlined on Page 15 lines 29-31 and Page 16 lines 1-14.

Changes made to manuscript:

- *None.*

Page 15

L1-2 Speculative. The authors did not measure enzyme kinetics. Omit “Rate” in “Rate of enzyme kinetics” since enzyme kinetics are the substrate-dependent rate of enzyme-substrate interaction.

Response / Changes made to manuscript:

- *We have modified the sentence as follows, “The initial effects of increased snow pack result in altered physical factors (greater active layer thaw depth and increased soil temperatures and moisture; Blanc-Betes et al., 2016) which may lead to increased SOM availability and faster enzyme activities with the potential to enhance SOM decomposition. Higher SOM mineralization may promote the documented shifts in aboveground plant communities and increased NPP (Natali et al., 2012; Sturm et al., 2005, Anderson-Smith 2013), and vegetation shifts to more shrubby species may alter the chemistry and quality of new litter inputs, ultimately affecting decomposer communities..” on Page 15 lines 29-31 and Page 16 lines 1-5.*

L7-8: Is this a logic relationship: increases in tannins and increases in N availability?

Response:

The increases in tannins and N availability are meant to be two separate possible causes of reduced microbial activity in the DEEP zone, each of which are supported with references. However, in light of the comment above regarding insignificant difference in %N concentration (Results L30), we have removed the concept of increasing N from the sentence.

Changes made to manuscript:

- *Alteration removing ‘increased N availability’ made to sentence, Page 16 line 10.*

L9 The decrease in C% might indicate exactly the opposite of the mechanism described above.

Response:

To avoid confusion, we removed “%C and C:N” and replaced it with “relative abundance of genes required for SOM decomposition”.

Changes made to manuscript:

- *Above change made on Page 16 lines 10-11 of the revision.*

L18: Are any data available about fungal biomass? Is the microbial biomass dominated by bacterial or by fungal biomass? (Did you investigate effects of snow depth on microbial biomass?)

Response:

Unfortunately, we did not collect fungal data. However, we felt it important to acknowledge the role that fungi play in SOM decomposition, and provide a reason why we did not find peroxidases, phenol oxidases, and laccases in our data. This is discussed on Page 17 lines 3-5.

Changes made to manuscript:

- *Slight alterations made to sentence, Page 17 lines 3-5.*

L23-27: Does an interaction between tannins and extracellular enzymes necessarily induce shifts in gene abundances? Statement requires a reference.

Response / Changes made to manuscript:

- *We have added the reference “(Rocca et al., 2014)” to Page 17 line 12 of the*

revision.

Page 16

L1-2: Does the decrease correspond to a decrease in C% or in C-stock or to a decrease in microbial biomass (as a factor for enzyme production)? Typically, enzyme activities are normalized with C%. Would this data treatment (gene abundances / C%) lead to a disappearance of treatment effects?

Response:

Please see response to anonymous referee #1 general comment #2, regarding differences between %C and C-stock.

Changes made to manuscript:

- *Please see changes from anonymous referee #1 general comment #2.*

L4-5 Sullivan 2008 is not included within the reference list

Response / Changes made to manuscript:

- *Thank you for catching that. We have added the citation to the reference list on Page 29 lines 1-3.*

L8-9: Is the system nutrient limited? Reference. N% increased in DEEP treatment - why did enzyme production not (that requires N and P)?

Response:

Yes. Historically, the Arctic has been shown to be a nutrient limited ecosystem. See below for changes.

%N concentration did not significantly increase in the DEEP treatment, possibly explaining lack of enzyme production. Also, the increase in temperature may lead to decreased enzyme gene copies without altering enzymatic capacity from decomposers as explained in discussion, Page 18 lines 13-24.

Changes made to manuscript:

- *We added the following references (Hobbie et al., 2002; Jonasson et al., 1999; Mack et al., 2004; Shaver and Chapin, 1980, 1986; Sistla et al., 2012) to Page 17 lines 25-26*
- *We added the following references to the References section, Page 24 lines 7-9, Page 24 lines 23-25, Page 25 lines 23-25, Page 28 lines 11-15, Page 28 lines 21-23.*

L12 Blanc-Betes et al. Submitted: not included in reference list

Response / Changes made to manuscript:

- *We removed this citation from the revision, Page 17 line 30.*
- *However, it does occur throughout the manuscript, so we have added the citation to the reference list, Page 21 lines 18-20.*

L19: Which alternate energetic pathway?

Response:

There are many possibilities, including fermentation, anaerobic respiration, and

chemolithotrophy. The main point behind this sentence is to establish the idea that alternate and less efficient forms of metabolism may be selected for under these conditions.

Changes made to manuscript:

- *For clarification, the sentence has been modified as follows, “may select for microorganisms that use anaerobic metabolic pathways such as methanogenesis (Blanc-Betes et al. 2016). These hypoxic soil conditions would limit aerobic decomposition.” on Page 18 lines 4-6.*

L21 change to “genes encoding enzymes involved in organic N degradation”

Response / Changes made to manuscript:

- *This change has been made in the revision as follows: “genes encoding for enzymes involved in N mobilization,” on Page 18 lines 6-7*

L22: Microbial biomass was frequently reported to be positively related to enzyme production and decomposition. Please, describe more precisely under which circumstances an in N availability and microbial biomass results in a decrease in decomposition rate.

Response:

Bacteria in a N limited system must decompose SOM to gain access to more N, potentially increasing decomposition rates. As enzyme production for SOM decomposition is energetically demanding, there is a threshold (high microbial biomass and alleviated N limitation) where bacteria may switch to alternate sources of N, such as microbial biomass, resulting in a decrease in SOM decomposition. This is supported by a theoretical C/N limitation model developed by Schimel, 2003. Also, In the Arctic, soil moisture is a confounding factor, as increased soil moisture may also decrease decomposition, as may be the case with the snow addition treatments.

Changes made to manuscript:

- *We have modified the text to better reflect these interactions on Page 18 lines 1-12.*

Page 17

L6 needs a reference - for example: Razavi et al. Front. Microbiol., 14 October 2015 | <http://dx.doi.org/10.3389/fmicb.2015.01126>

Response / Changes made to manuscript:

- *This citation has been added as suggested, Page 18 line 19.*
- *Citation added to Reference list, Page 27 lines 8-10.*

L7-8 Speculative. If insitu substrate availability is low, Vmax will not be reached and enzyme functioning is controlled by Km (Michaelis-Menten constant). Alternatively, authors may change to “reach the same catalytic rate”.

Response:

We agree. Thank you clarifying this!

Changes made to manuscript:

- *The text has been updated to clarify these points on Page 18 lines 17-24.*

L26: needs reference

Response / Changes made to manuscript:

- *This sentence has been removed from Page 18 line 28 of the revised manuscript.*

L26-27 This statement should be reformulated in order to account for the low number of observed effects (the low number of repetitions) and the revised results of the KW-ANOVA (separate data from “organic” from those of “mineral soil”).

Response / Changes made to manuscript:

- *We added “From the results of our study” to Page 18 line 28.*
- *We added “in the organic soil layers” to Page 18 line 30.*

References

IPCC should be shifted to I and not to W.(?)

Response / Changes made to manuscript:

- *We moved the citation in the revision as suggested, from Page 30 line 2 to Page 24 line 20.*

Table 1:

Describe in Methods how many repetitions and analytical replicates were used for the data. a> Or < b, please add information. No effect on N% - that differs from the description in the results section Why is there no effect on C:N in Table 1?

Response / Changes made to manuscript:

- *We altered Page 7 lines 5-12 of the revision as follows: “Organic samples were collected just below where plant tissue transitioned into dark brown/black soil (mean soil depth = 5.6 ± 1.3 cm; CTL n=4, DEEP n=4, INT n=3, LOW n=4), transitional samples were taken from the visual border between organic and mineral horizons based on change in soil colour (mean soil depth = 14.8 ± 1.8 cm; CTL n=3, DEEP n=3, INT n=4, LOW n=3), and mineral samples were collected 10cm below this transition (mean soil depth = 25.1 ± 1.7 cm; CTL n=3, DEEP n=4, INT n=3, LOW n=3), totalling 41 samples. To maintain consistency, only these samples were used to analyse %C, %N, and pH relationships.”*
- *We added sample sizes to Table 1 in the revision, Page 32 line 7.*
- *We altered Page 10 lines 7-8 of the revision as follows: “...while the %N concentration only slightly increased (LOW/DEEP - $p=0.32$).”*
- *While it might seem from the numbers that there was a significant treatment effect on C:N, the p-value was 0.14. Please see Page 10 line 8 of the revision.*

Figure 2:

remove the ellipses from the graph. I counted 11 triangles but 10 circles etc. Why? How many sample points were included within the NMDS? NMDS requires detailed description within the results section. What are the main gradients observed? The NMDS optimized the illustration of the dissimilarity in beta diversity data but not in explanatory variables. Therefore, the combined illustration is somehow misleading (but the Mantel test is the appropriate method of choice).

Response:

- *We have decided to keep the ellipses to better visualize the separation (or lack*

thereof) between the treatments.

Changes made to manuscript:

- *Please see response above for Table 1. Alterations were made in the Methods of the revision to clarify replicates and sample sizes on Page 7 lines 5-12, and Page 32 line 7.*
- *We altered the Figure 2 caption Page 34 lines 3-5 as follows “Each point represents the bacterial community structure within one of the 41 total samples used for DNA extraction from all soil depths (Organic, Transition, and Mineral).”*

Figure 3:

No clear effect on bacterial phyla in organic samples (only some tendencies). Mineral soil: effect on Verrucmicrobia.

Figs 3 and 4:

I would prefer to change the order of treatments within the graph (from low (left) to deep (right) and the use of the same scale (y-axis) for all panels.) Add information about the treatments to Figure captions. Fig 4 Superoxides +2 to -3% difference to control? This might be a very low effect.

Response:

While we appreciate your opinion, we have decided not to change the order of the treatments. Also, we initially attempted using the same scale on the y-axis for all panels, however it resulted in loss of visual interpretation. Significant differences and trends became unnoticeable. We decided not to change the y-axis scales.

Changes made to manuscript:

- *We added the phrase “snow accumulation treatment” to the caption of Figure 3, Page 35 line 3.*

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

Received and published: 18 February 2016

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 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:
There were 462 significant treatment responding Genera.*

*Changes made to manuscript:
- Heatmaps have been included in the supplementary information, Pages 13-17.*

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.

Changes made to manuscript:

- *Added, "While this method 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 using 16S rRNA data (Langille et al., 2013)." Page 16 lines 20-23 of the revision.*

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." However, to satisfy your curiosity, we 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.

Changes made to manuscript:

- *To clarify this in the revision we added, "Any read that did not match a sequence in the reference database was discarded." to Page 7 line 29.*

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 response directly above and discussion on Page 16 lines 24-28.

Changes made to manuscript:

- *Sentences revised and moved from Page 18 line 24 to Page 16 lines 24-28.*

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 micro-scale, 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, 1.4 – Does “more or less snow” mean that predictions on amount of precipitation are not sure yet? Please consider rephrasing.

Response / Changes made to manuscript:

- *We omitted “or less” on Page 2 line 4 in the revision.*

p.2, 1.11 – I recommend writing “Microbial community DNA was extracted from soil”.

Response / Changes made to manuscript:

- *We added “Soil microbial community” to Page 2 lines 11-12 in the revision.*

p.2, 1. 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.

Changes made to manuscript:

- *None.*

Introduction:

p.3, 1.3 – 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.

Changes made to manuscript:

- *None.*

p.5, 1.1 + 1.16 – Please change microbial into bacterial community.

Response:

The word “microbial” has been changed to “bacterial” where appropriate throughout the revision of the manuscript.

Changes made to manuscript:

- *Page 4 line 30*
- *Page 5 lines 4, 8, 11*
- *Page 12 lines 26*
- *Page 15 lines 18 & 27*
- *Page 16 line 10*
- *Change to Page 17 line 21*
- *Change to Page 33, Figure 2, line 4*

p.5, 1.14 – bacterial functions (plural).

Response / Changes made to manuscript:

- *This has been changed in the revision as suggested on Page 5 line 9.*

Methods:

p.6, 1.1 – How far away from the snow fence was the CTL sampled?

Response / Changes made to manuscript:

- *We added “>30m” to Page 5 line 28 in the revision.*

p.6, 1.9 – What was the distance between the replicates per treatment?

Response / Changes made to manuscript:

- *We added “approximately 15-20m apart” to Page 6 line 7 in the revision.*

p.7, 1.4 – 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.

Yes, DNA was extracted from all three cores per treatment and per soil horizon. We hope the changes below will clarify these points.

Changes made to manuscript:

- *Text was altered as follows, “Due to significant differences between soil layers (Table S2), each layer was analysed separately. Only organic and mineral layers are reported.” to Page 8 line 29 and Page 9 lines 1-2 in the revision.*
- *We significantly altered text from Page 9 lines 12-18 of the revision.*
- *We added “of each soil core,” to Page 7 line 2 in the revision.*

p.7, 1.16- Could you please add the reference for the primers?

Response / Changes made to manuscript:

- *Yes! We have added the appropriate reference to Page 7 line 16 of the revision.*
- *Added to reference list, Page 21 lines 30-33.*

p.7, 1.17 delete – in Mi-Seq but add to p.8, 1.7 Bray- Curtis.

Response:

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

Changes made to manuscript:

- *Deleted “-“ in MiSeq, Page 7 line 18.*
- *Added “-“ in Bray-Curtis, Page 8 lines 7-8.*

p.8, 1.6- Why did you determine adequate sampling depth? – I could not find that result later on.

Response / Changes made to manuscript:

- *We removed, “rarefaction curves to determine adequate sampling depth,” from Page 8 line 7 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 / Changes made to manuscript:

- *We have reworded this in the revision as suggested, Page 8 lines 27-28 and Page 9 lines 2-3.*

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.

Changes made to manuscript:

- *None.*

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.

Changes made to manuscript:

- *We added n-fold changes to all appropriate results in the revision, from Page 10 line 19 –to- Page 12 line 11.*
- *The manuscript was revised as follows, “The changes in the mineral soil layers were less pronounced than in the organic layers. C:N ratios again showed a decreasing trend as snow accumulation increased, while soil pH increased in the DEEP zone but did not show a trend along the treatment gradient (Table 1).” on Page 10 lines 12-15.*

p.10, l.6 – delete “and”.

Response / Changes made to manuscript:

- *This sentence has been removed from the manuscript, Page 10 line 15.*

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 / Changes made to manuscript:

- *We added n-fold changes to all appropriate results in the revision, from Page 10 line 19 –to- Page 12 line 11.*

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!

Changes made to manuscript:

- *We have fixed Fig. 3 in the revision, Page 35 line 1.*

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 / Changes made to manuscript:

- *We have clarified this in the revision by adding, “Acidobacteria showed decreased abundance in all treatments relative to the CTL, with the DEEP zone exhibiting the largest difference with a 1.98-fold decrease ($p=0.055$; Fig. 3).” to Page 10 lines 22-24.*

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

Response / Changes made to manuscript:

- *Yes! Thank you. p-value corrected to “ $p<0.001$ ” on Page 10 line 30 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 – $p=0.119$.

Changes made to manuscript:

- *None.*

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 / Changes made to manuscript:

- *To acknowledge this observation, we added the following sentence, “However, relative to the CTL, both INT and LOW lignin-degrading genes exhibited much greater abundances than they did in the organic horizon (Fig. 4).” on Page 12 lines 12-14 of the revision:*

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.

Changes made to manuscript:

- None.

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

Response / Changes made to manuscript:

- The "(Table S1)" reference has been added to Page 12 line 22 in the revision.
- The indicated sentence has been altered and moved to Page 17 lines 1-4.

Discussion:

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

Response / Changes made to manuscript:

- To clarify this, we added the following sentence, "Our results indicate that increased snow pack reduced the abundance of genes associated with SOM decomposition in the organic soil layers, suggesting a reduced SOM decomposition potential." to the revision Page 13 lines 8-10:

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.

Changes made to manuscript:

- None.

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.

Changes made to manuscript:

- We replaced "has" with "have" in Page 13 line 28 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...".

Changes made to manuscript:

- To clarify this, we added "...which degrade..." and removed "...degradation" on Page 13 line 29 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 / Changes made to manuscript:

- We removed the R2 and p-values as suggested in the revision, Page 15 lines 9-10, and 12.

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 / Changes made to manuscript:

- The sentence referred to was replaced by "The NMDS plot (Fig. 2)..." on Page 15 lines 26-27 of the revision.
- It was revised as follows: "visualized by a NMDS plot 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 between all samples (organic, transition, and mineral) associated with winter snow pack", and moved to the Results, Page 11 lines 14-17 of the revision.

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.

Changes made to manuscript:

- None.

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 / Changes made to manuscript:

- We have altered the table caption to clarify. Page 32 lines 1-6 of the revision

- *Added specifics on the number of replicates throughout the Methods section, Page 7 lines 6-12.*

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

Response / Changes made to manuscript:

- *This was our mistake and has been corrected in the revision, Page 6 line 26-27.*

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.

Changes made to manuscript:

- *We added, "Results are indicated by _{a,b,c} only where $p < 0.05$." on Page 32 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 / Changes made to manuscript:

- *We altered the caption as follows: "Each point represents the bacterial community structure within one of the 41 total samples used for DNA extraction from all soil depths (Organic, Transition, and Mineral).", on Page 34 lines 3-5 in the revision.*

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.", Page 35 line 7 and Page 36 line 8.

Changes made to manuscript:

- *None.*

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 / Changes made to manuscript:

- *To clarify this, we added the following sentence, "To ensure accurate comparisons, soil chemical properties were measured from the same samples that DNA was extracted from.", to Page 9 lines 8-9 of the revision.*

References

(typos):

p.22, 113 Gonzalez-Meler

Response / Changes made to manuscript:

- *Thank you! This has been fixed in the revision, Page 24 line 10.*

p.23, 134 mcrA in italics

Response / Changes made to manuscript:

- *Thank you! This has been fixed in the revision, Page 26 line 9.*

p.27, 113 CO2

Response / Changes made to manuscript:

- *Thank you! This has been fixed in the revision, Page 30 line 2.*

1 **Soil bacterial community and functional shifts in response to**
2 **altered snow pack in moist acidic tundra of Northern Alaska**

3 **M. P. Ricketts¹, R. S. Poretsky¹, J. M. Welker², and M. A. Gonzalez-Meler¹**

4 [1] {Biological Sciences Department, Ecology and Evolution, University of Illinois at Chicago,
5 Chicago, IL, USA}

6 [2] {Department of Biological Sciences, University of Alaska, Anchorage, AK, USA}

7 Correspondence to: Michael P Ricketts (rickett4@uic.edu)

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1 **Abstract**

2 Soil microbial communities play a central role in the cycling of carbon (C) in Arctic tundra
3 ecosystems, which contain a large portion of the global C pool. Climate change predictions for
4 Arctic regions include increased temperature and precipitation (i.e. more snow), resulting in
5 increased winter soil insulation, increased soil temperature and moisture, and shifting plant
6 community composition. We utilized an 18-year snowfence study site designed to examine the
7 effects of increased winter precipitation on Arctic tundra soil bacterial communities within the
8 context of expected ecosystem response to climate change. Soil was collected from three pre-
9 established treatment zones representing varying degrees of snow accumulation (DEEP, INT,
10 LOW), soil physical properties (temperature, moisture, active layer thaw depth) were measured,
11 and samples were analysed for C concentration, nitrogen (N) concentration, and pH. Soil
12 microbial community DNA was extracted and the 16S rRNA gene was sequenced to reveal
13 phylogenetic community differences between samples and determine how soil bacterial
14 communities might respond (structurally and functionally) to changes in winter precipitation and
15 soil chemistry. We analysed relative abundance changes of the six most abundant phyla (ranging
16 from 82-96% of total detected phyla per sample) and found four (Acidobacteria, Actinobacteria,
17 Verrucomicrobia, and Chloroflexi) responded to deepened snow. All six phyla correlated with at
18 least one of the soil chemical properties (%C, %N, C:N, pH), however a single predictor was not
19 identified suggesting that each bacterial phylum responds differently to soil characteristics.
20 Overall bacterial community structure (beta diversity) was found to be associated with snow
21 accumulation treatment and all soil chemical properties. Bacterial functional potential was
22 inferred using ancestral state reconstruction to approximate functional gene abundance, revealing
23 a decreased abundance of genes required for soil organic matter (SOM) decomposition in the
24 organic Jayers of the deep snow accumulation zones. These results suggest that predicted climate
25 change scenarios may result in altered soil bacterial community structure and function, and
26 indicate either a reduction in decomposition potential, or alleviated temperature limitations on
27 extracellular enzymatic efficiency, or both. The fate of stored C in Arctic soils ultimately
28 depends on the balance between these mechanisms.

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1 Introduction

Broad and rapid environmental changes are driving both above- and belowground community shifts in the Arctic (Elmendorf et al., 2012a, 2012b; Tape et al., 2006, 2012; Wallenstein et al., 2007). It is well established that soil microbial communities may alter their composition in response to changing environmental factors such as nutrient availability, moisture, pH, temperature, and aboveground vegetation shifts (Lauber et al., 2009; Morgado et al., 2015; Semanova et al., 2015), and ecological and climate induced changes to Arctic soil microbial community structure and function have important effects on ecosystem carbon (C) cycling and nutrient availability for plant growth (Deslippe et al., 2012; Graham et al., 2012; Waldrop et al., 2010; Zak and Kling, 2006). Because many of these environmental features are rapidly changing in Arctic tussock tundra ecosystems (Anisimov et al., 2007; Liston and Hiemstra, 2011), and because of the large amounts of C stored in Arctic soils (Hugelius et al., 2013; Ping et al., 2008; Schuur et al., 2009; Tarnocai et al., 2009), it is imperative to examine microbial responses in this system.

Soil microorganisms play a key role in the decomposition of soil organic matter (SOM) on a global scale, releasing nutrients into the soil and stored C into the atmosphere in the forms of CO₂ and CH₄, two major greenhouse gases that contribute to global warming (Anisimov et al., 2007). Decomposition of SOM by soil microorganisms amounts to at least half of the 80-90 Gt C released each year by soil respiration, the second largest terrestrial flux after gross primary productivity (GPP; Davidson and Janssens, 2006; Hopkins et al., 2013; Raich et al., 2002). Because global soils contain about 2,000 Gt of C, ~1,500 Gt of which is in the form of SOM (Batjes, 1996; IPCC, 2000), large scale changes in the rate of microbial decomposition will have an impact on the rate at which CO₂ accumulates in the atmosphere (Schimel and Schaeffer, 2012).

The decomposition rate of SOM, resulting in heterotrophic respiration from soils (R_h), has been shown to be sensitive to temperature and moisture (Davidson and Janssens, 2006; Frey et al., 2013; Hopkins et al., 2012, 2013; Xia et al., 2014). As the Arctic climate warms, increasing R_h may be capable of producing a positive feedback on the climate system as C stored in soils over millennia is released back to the atmosphere (Czimeczik and Welker, 2010; Jonasson et al., 1999; Lupascu et al., 2014b; Mack et al., 2004; Nowinski et al., 2010; Shaver and Chapin, 1980, 1986).

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1 Northern latitude permafrost soils may house over 50% of the world's soil organic C (SOC; the
2 C component of SOM), approximately twice the amount of C present in the atmosphere
3 (Hugelius et al., 2013; Ping et al., 2008; Schuur et al., 2009; Tarnocai et al., 2009). In addition,
4 Arctic ecosystems are more susceptible to the effects of climate change, warming at
5 approximately twice the rate as temperate zones and exhibiting increased winter precipitation
6 patterns (Anisimov et al., 2007; Liston and Hiemstra, 2011). Deeper snow has a suite of
7 cascading consequences in tundra ecosystems as snow acts to insulate soil from extreme winter
8 air temperatures resulting in soil temperatures under deeper snow pack up to 10°C warmer than
9 soils under ambient snow depths (Schimel et al., 2004). Altered soil conditions under deeper
10 snow may thus lead to increased SOM decomposition, causing changes in SOC stocks while also,
11 releasing nutrients for plant and microbial growth (Anisimov et al., 2007; Leffler and Welker,
12 2013; Rogers et al., 2011; Welker et al., 2005). The predicted increase in soil temperature as a
13 result of deeper winter snow accumulation should enhance the rate of SOM decomposition by: 1)
14 a direct temperature effect on enzyme kinetics, and 2) by increasing substrate availability to
15 decomposers as the active layer deepens and permafrost thaws (Lützow and Kögel-Knabner,
16 2009; Nowinski et al., 2010; Schuur et al., 2008). Therefore, warming and deeper snow in the
17 Arctic are likely to expose C stored over millennia to decomposers, resulting in a major source of
18 C to the atmosphere.

19 However, ecosystem C loss may be offset by increased soil moisture, causing hypoxic conditions
20 and limiting R_h (Blanc-Betes et al. 2016). Also, microbial mineralization of plant nutrients, such
21 as nitrogen (N) and phosphorus (P), from SOM decomposition are likely to contribute to
22 increased net primary productivity (NPP; Hinzman et al., 2005; Natali et al., 2012; Pattison and
23 Welker, 2014) and cause shifts in vegetation from herbaceous species (Cottongrass tussock-
24 *Eriophorum vaginatum*) towards woody species (Arctic shrubs – *Betula nana* and *Salix pulchra*)
25 that may produce a larger amount of plant litter compounds that are more resistant to
26 decomposition (Bret-Harte et al., 2001; Pearson et al., 2013; Sturm et al., 2005; Wahren, 2005).
27 The balance between these processes will determine the extent to which Arctic tundra
28 ecosystems feedback on the global climate, making the fate of this stored C unclear (Sistla et al.,
29 2013).

30 This study examined changes in soil bacterial community composition due to increased winter
31 snow accumulation and subsequent altered biotic and abiotic factors using a long-term snow

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1 fence manipulation experiment that mimics changes in winter precipitation by creating a gradient
2 of snow depths from much deeper than ambient to shallower than ambient levels (Jones et al.,
3 1998; Pattison and Welker, 2014; Welker et al., 2000). We postulated that increased soil thermal
4 insulation from deeper winter snow accumulation would elicit bacterial community response via:
5 1) altered soil physical characteristics such as soil temperature, moisture, or O₂ availability, and
6 2) altered soil chemistry produced by increased microbial mineralization of SOM resulting in
7 increased nutrient availability and changes in plant species composition and litter. Here we
8 evaluated phylum level shifts in bacterial community phylogeny using 16S rRNA gene analysis
9 and predicted bacterial functions using the program PICRUSt (Langille et al., 2013) to test
10 whether increased snow accumulation and associated changes in soil conditions (warmer
11 temperatures, altered plant inputs, and increased hypoxia) would cause shifts in bacterial
12 community structure and functional potential that reflect increased SOM decomposition and
13 nutrient mineralization.

14

15 2 Methods

16 2.1 Site description and sample collection

17 The study utilized a long-term snow depth manipulation experiment site (Jones et al., 1998;
18 Walker et al., 1999) established in 1994 in a moist acidic tundra ecosystem located near Toolik
19 Lake Field Station, Alaska (68°37'N, 149°32'W). It consists of a strategically placed snow fence
20 designed to simulate the increased precipitation patterns and continuous snow-cover episodes
21 predicted under global warming scenarios, resulting in a gradient of increasing snow
22 accumulation (and thus increasing soil thermal insulation, soil temperatures, and active layer
23 thaw depth/permafrost thaw) with proximity to the fence. While snowfall varied from year to
24 year, the wind drift from the fence provided consistent relative snow accumulation at similar
25 distances from the fence every winter (Fahnestock et al., 2000; Welker et al., 2005). The soil is
26 classified as Typic Aquiturbel, exhibiting characteristics of cryoturbation and poor drainage
27 (Ping et al., 1998; Soil Survey, 2015). Four experimental zones were identified according to their
28 snow accumulation regime: Control (CTL, taken >30m outside the effects of the snowfence),
29 Deep snow (DEEP ~ 100% increase in snow pack relative to the Control), Intermediate snow
30 (INT, ~50% increase in snow pack, relative to the Control), and Low snow (LOW, ~25%

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1 | decrease in snow pack relative to the Control; Fig. 1). The DEEP snow zone is unique in that it is
2 | waterlogged during thaw periods, and dominated not by Cottongrass tussock or woody shrub
3 | species (e.g. *Eriophorum vaginatum*, *Betula nana*, or *Salix pulchra*), but by a sedge species,
4 | *Carex bigelowii*. However, the vegetative history of this plot includes a transition from tussock
5 | cottongrass to woody shrub species, and finally to wet sedge species (Arft et al., 1999; Walker
6 | and Wahren, 2006).

7 | Three replicate soil cores were taken approximately 15-20m apart from each experimental snow
8 | zone (totalling 12 soil cores) in August of 2012 and analysed separately. All soil coring
9 | equipment was cleaned and sterilized in the field between each sample using water and 100%
10 | ethanol. The top 10cm representing the organic layers was taken first using a sharpened steel
11 | pipe (5.08cm diameter X 12cm length) and serrated knife to cut through surface vegetation and
12 | to minimize soil compaction. A slide hammer with 2x12" split soil core sampler (AMS Inc., ID,
13 | USA) was used to obtain the remainder of the active layer down to permafrost (~35–65cm soil
14 | depth), including mineral soil layers. The soil cores were stored in sterile Whirl-pak® bags,
15 | immediately frozen on site, and shipped to the Stable Isotope Laboratory at the University of
16 | Illinois at Chicago where they were sectioned horizontally into 2cm depth segments using a
17 | sterilized ice-core cutter, providing a 2cm resolution soil depth profile for each core. A portion of
18 | each segment was ground into a fine powder using a Spexmill mixer/mill 8000 (SPEX
19 | SamplePrep, NJ, USA) and analysed for C and N concentration, and stable isotopes using a
20 | Costech Elemental Analyser (Valencia, CA, USA) in line with a Finnigan Deltaplus XL IRMS
21 | (isotope ratio mass spectrometer, Bremen, Germany). Soil pH was measured from portions of the
22 | same segments by creating a soil slurry mixture (2ml H₂O:1g soil) and using an Accumet Basic
23 | AB15 pH meter with a calomel reference pH electrode (Thermo Fisher Scientific Inc., MA,
24 | USA). In addition, at the time of collection, soil temperature, soil moisture, and active layer thaw
25 | depth were measured and recorded at four points around each soil core hole (n=12 per treatment)
26 | to characterize the soil environment. Soil temperatures (°C) were measured using a 12cm Taylor
27 | TruTemp Digital Instant Read Probe Thermometer (Taylor Precision Products, Inc., NM, USA),
28 | surface (top 12cm) volumetric water content (%) was measured using an HS2 HydroSense II Soil
29 | Moisture Measurement System (Campbell Scientific Inc., UT, USA), and active layer thaw
30 | depths (cm) were measured by inserting a meter stick attached to a metal rod into the ground
31 | until it hit ice.

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2.2 DNA extraction, sequencing, and analysis

Samples from organic and mineral layers of each soil core, as well as the transition between the two, were selected for DNA extraction initially based on visual examination of each individual core section and further classified by %C in saturated soils as per the Soil Survey Division Staff, (1993; Organic \geq 12% SOC, Mineral: $<$ 12% SOC). Organic samples were collected just below where plant tissue transitioned into dark brown/black soil (mean soil depth \pm standard error [S.E.] = 5.6 ± 1.3 cm; CTL n=4, DEEP n=4, INT n=3, LOW n=4), transitional samples were taken from the visual border between organic and mineral layers based on change in soil colour (mean soil depth \pm S.E. = 14.8 ± 1.8 cm; CTL n=3, DEEP n=3, INT n=4, LOW n=3), and mineral samples were collected 10cm below this transition (mean soil depth \pm S.E. = 25.1 ± 1.7 cm; CTL n=3, DEEP n=4, INT n=3, LOW n=3), totalling 41 samples. To maintain consistency, only these samples were used to analyse %C, %N, and pH relationships. Samples were sent to Argonne National Laboratory for DNA extraction, amplification, and sequencing as per standards used by the Earth Microbiome Project (Gilbert et al., 2014). DNA extractions were performed using MoBio's PowerSoil[®]-htp 96 Well Soil DNA Isolation Kit as per protocol, the V4 region of the 16S rRNA gene was amplified using PCR primers 515F/806R (Caporaso et al., 2012), DNA quantification was performed using PicoGreen, and 2x150bp paired-end sequencing was performed using an Illumina MiSeq instrument.

Samples were barcoded prior to sequencing for downstream sample identification and paired-end assembly, demultiplexing, quality filtering, operational taxonomic unit (OTU) picking, and preliminary diversity analyses were performed using the QIIME software package version 1.8.0 (Caporaso et al. 2010). Forward and reverse reads were assembled using fastq-join (Aronesty, 2011) with 15bp overlap at 15% maximum difference. Quality filtering included removal of reads that didn't have at least 75% consecutive high quality (phred $>$ q20) base calls and truncation of reads with more than three consecutive low quality (phred $<$ q20) base calls. This resulted in an assembled-read median sequence length of 253bp.

To reveal phylogenetic abundance and relationships, sequence reads were assigned taxonomic identities using closed reference OTU picking that clusters and matches each read to a reference database. Any read that did not match a sequence in the reference database was discarded. All

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1 default QIIME parameters were used (reference database = Greengenes (13_8), OTU picking
2 method = uclust, and sequence similarity threshold = 97%). Because many organisms are known
3 to possess multiple copies of the 16S rRNA gene in their genome, the abundance assignments
4 were corrected based on known copy numbers using PICRUST's *normalize_by_copy_number.py*
5 script. The relative abundances of the six most abundant phyla, comprising 82% - 96% of total
6 detected phyla per sample, were analysed for treatment effects, and alpha and beta diversities
7 were examined using the Shannon diversity index to estimate within sample diversity, and Bray-
8 Curtis dissimilarity matrices to determine community structure differences.

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9 The genetic functional potential of bacterial communities was determined using the software
10 package PICRUST version 1.0.0 (Langille et al., 2013) which predicts functional gene copy
11 numbers in a community based on 16S rRNA sequencing results. Recent advances in sequencing
12 technologies and bioinformatics has greatly enhanced our current knowledge of the genetic
13 potential of soil microorganisms, allowing us to determine what genes a group of organisms is
14 likely to possess based on ancestral state reconstruction of metagenome assemblies from current
15 genomic databases (Langille et al., 2013; Martiny et al., 2013). PICRUST utilizes this knowledge,
16 revealing functional potential, in the form of gene abundance, associated with phylogenetic
17 community structure. For this study, we targeted Kyoto Encyclopedia of Gene and Genomes
18 (KEGG) ortholog assignments for enzymatic genes commonly associated with SOM
19 decomposition, nutrient (nitrogen and phosphate) mobilization, and environmental stress
20 responses (Sinsabaugh et al., 2008; Waldrop et al., 2010; full list in Table S1). These genes were
21 then grouped according to functional role, resulting in the following nine gene groups: 1) lignin
22 degradation, 2) chitin degradation, 3) cellulose degradation, 4) pectin degradation, 5) xylan
23 degradation, 6) arabinoside degradation, 7) nitrogen mobilization, 8) phosphate mobilization, and
24 9) superoxide dismutation.

25 2.3 Statistical analyses

26 Differences between soil layers (Organic, Transition, Mineral) and snow accumulation
27 treatments (CTL, DEEP, INT, LOW), including abiotic measurements and relative abundance of
28 bacterial 16S rRNA and functional genes, were determined using the Kruskal-Wallis test in the R
29 statistical software package with a significance threshold of $p < 0.05$. Due to significant

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1 differences between soil layers (Table S2), each layer was analysed separately. Only organic and
2 mineral layers are reported. All abiotic factors, phyla relative abundances, and relative
3 abundances of functional genes were analysed individually to elucidate the treatment effects for
4 each group, and pairwise comparisons were made to determine significant differences between
5 treatments using the Nemenyi post hoc test. In addition, linear regressions were performed to
6 determine relationships between soil chemical properties (%C, %N, C:N, and pH) and bacterial
7 abundance at the phylum level, as well as the gene abundances of SOM degrading enzymes,
8 (Supplementary Figs. S1-S15). To ensure accurate comparisons, soil chemical properties were
9 measured from the same samples that DNA was extracted from. Only R^2 values > 0.30 are
10 discussed.

11 Bacterial diversity statistics were calculated using QIIME (Caporaso et al. 2010), specifically the
12 `compare_alpha_diversity.py`, `compare_categories.py`, and `compare_distance_matrices.py`
13 scripts. Pairwise comparisons of the Shannon alpha diversity metrics from soil layer and each
14 treatment group were made, using non-parametric two-sample t-tests with 999 Monte Carlo
15 permutations. Beta diversity was analysed by comparing Bray-Curtis dissimilarity matrices of
16 bacterial abundance data from each sample to soil chemical properties, and between soil layers
17 and snow accumulation treatments using adonis tests with 999 permutations. Organic and
18 mineral layers were also analysed separately when comparing snow accumulation treatments and
19 soil chemical properties (Table 2). Analyses of soil chemical properties were further
20 substantiated by Mantel tests, again using 999 permutations. This data was visualized by creating
21 a non-metric multidimensional scaling (NMDS) plot (Stress=0.090, Shepard plot non-metric
22 $R^2=0.992$) in the R package `phyloseq` (McMurdie and Holmes, 2013) using the same Bray-Curtis
23 dissimilarity matrices (Fig. 2).

24

25 3 Results

26 3.1 Environmental changes

27 Significant differences in soil temperature ($n=12$, $H_0=33.29$, $df=3$, $p<0.001$), active layer thaw
28 depth ($n=12$, $H_0=21.35$, $df=3$, $p<0.001$), and organic layers %C ($n=4$, $H_0=9.74$, $df=3$, $p=0.021$) were
29 associated with the four different snow zones. Post hoc tests revealed higher temperatures in the
30 DEEP snow zone relative to the CTL ($p=0.009$), the INT ($p=0.001$), and the LOW snow zone

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1 (p<0.001; Table 1). Active layer depth data revealed similar results, increasing in the DEEP
2 snow accumulation zone and decreasing as snow pack was experimentally reduced. Only in the
3 DEEP zone was the active layer thaw depth significantly (p=0.020) deeper than the CTL zone.
4 However, along the snow accumulation gradient, thaw depth significantly increased from LOW
5 to DEEP plots (LOW/INT - p=0.021, LOW/DEEP - p<0.001; Table 1). Soil moisture was not
6 correlated with snow accumulation, possibly the result of surface hydrology at the site, which
7 was largely saturated throughout the growing season. In the organic soil layers, the %C
8 concentration of soil declined with increased snow accumulation (LOW/DEEP - p=0.03), while
9 the %N concentration only slightly increased (LOW/DEEP - p=0.32). This resulted in a
10 decreasing trend in C:N ratios across snow accumulation treatment zones and relative to the
11 control (CTL/DEEP - p=0.14; Table 1). Soil pH tended to increase (became more neutral) with
12 increased snow accumulation (LOW/DEEP - p=0.06). The changes in the mineral soil layers
13 were less pronounced than in the organic layers. C:N ratios again showed a decreasing trend as
14 snow accumulation increased, while soil pH increased in the DEEP zone but did not show a
15 trend along the treatment gradient (Table 1).

16 3.2 Bacterial community shifts

17 Some bacteria exhibited shifting trends in response to snow depth, both across treatments and
18 relative to the control, while other community shifts were either not significant or did not appear
19 to be the result of the snow depth treatments (Figs. 3 and S16-S20). Noticeable trends at the
20 phylum level included a 1.6-fold increased abundance in Verrucomicrobia (p=0.068), a 2.1-fold
21 increase in Actinobacteria (p=0.083), and a 329.0-fold increase in Chloroflexi (p=0.010) in the
22 organic layers from the LOW to DEEP snow zones. Acidobacteria showed decreased abundance
23 in all treatments relative to the CTL, with the DEEP zone exhibiting the largest difference with a
24 1.98-fold decrease (p=0.055; Fig. 3). In the mineral layers, significant increases in the phylum
25 Chloroflexi (7.18-fold increase; p=0.011) occurred from the CTL to DEEP zones, while
26 significant decreases (2.84-fold decrease; p=0.019) were observed from CTL to DEEP zones in
27 the phylum Verrucomicrobia (Fig. 3).

28 Bacterial abundance in each phylum correlated with at least one of the soil chemical properties
29 we measured (%C, %N, C:N, or pH). The best overall predictor was %C, correlating with four
30 out of the six phyla. It showed negative relationships with Actinobacteria ($R^2=0.38$, p<0.001;

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1 Fig. S4) and Chloroflexi ($R^2=0.34$, $p<0.001$; Fig. S6), and positive relationships with
2 Bacteroidetes ($R^2=0.33$, $p<0.001$; Fig. S5) and Proteobacteria ($R^2=0.32$, $p<0.001$; Fig. S2).
3 Actinobacteria was also negatively correlated with %N ($R^2=0.34$, $p<0.001$; Fig. S4), and
4 Chloroflexi, positively with soil pH ($R^2=0.34$, $p<0.001$; Fig. S6). The best and only predictor for
5 Acidobacteria abundance was soil pH, which correlated negatively ($R^2=0.46$, $p<0.001$; Fig. S1).
6 Verrucomicrobia abundance correlated positively with %N ($R^2=0.36$, $p<0.001$; Fig. S3).

7 Soil depth significantly affected bacterial relative abundance in all phyla except for
8 Acidobacteria (Table S2). The organic layers were more abundant in Proteobacteria (1.59-fold
9 difference; $p<0.001$), Verrucomicrobia (1.48-fold difference; $p<0.001$), and Bacteroidetes (2.27-
10 fold difference; $p=0.001$). Phyla that were more abundant in the mineral layers were
11 Actinobacteria (4.48-fold difference; $p<0.001$) and Chloroflexi (14.21-fold difference; $p<0.001$).
12 Alpha diversity, measured using the Shannon index, was found to differ between soil layers
13 (organic / mineral – $p=0.003$), but not between snow accumulation treatments (Table S3).
14 However, beta diversity of bacterial communities visualized by a NMDS plot of Bray-Curtis
15 dissimilarity indices constructed from community matrices (Stress=0.090, Shepard plot non-
16 metric $R^2=0.992$; Fig. 2) revealed significant differences in community structure between all
17 samples (organic, transition, and mineral) associated with winter snow pack (adonis $R^2=0.13$, $p =$
18 0.017), %C (adonis $R^2=0.24$, $p < 0.001$; Mantel r statistic=0.63, $p < 0.001$), %N (adonis $R^2=0.14$,
19 $p < 0.001$; Mantel r statistic=0.34, $p < 0.001$), C:N (adonis $R^2=0.19$, $p < 0.001$; Mantel r
20 statistic=0.42, $p < 0.001$), and pH (adonis $R^2=0.15$, $p < 0.001$; Mantel r statistic=0.49, $p < 0.001$).
21 In addition, analysis of each soil layer separately showed that soil chemical properties and snow
22 accumulation treatment affected bacterial community structure more in the organic layers than in
23 the mineral layers, and that in the organic layer, the snow pack treatment ($p<0.001$), %C
24 ($p=0.004$), and pH ($p<0.001$) are the main drivers of community shifts (Table 2).

25 3.3 PICRUST functional analysis

26 Of the functional gene groups examined, the most significant treatment effects occurred in the
27 organic soil layers. A 1.27-fold decrease in the abundance of genes involved in cellulose
28 degradation ($p=0.018$) and a 1.56-fold decrease in the abundance of genes involved in chitin
29 degradation ($p=0.029$) was observed in the DEEP zone relative to the CTL (Fig. 4). Also, across
30 treatments from LOW to DEEP, lignin degrading gene abundance decreased 12.29-fold

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1 (p=0.023), pectin degrading gene abundance decreased 1.41-fold (p=0.018), and xylan degrading
2 gene abundance decreased 1.63-fold (p=0.014; Fig. 4). A similar trend was observed in enzymes
3 responsible for the regulation of oxygen radicals with a 1.05-fold decrease in the DEEP zone
4 compared to the LOW (p=0.083). Shifts along the snow accumulation gradient were also
5 observed in gene groups involved in nutrient mobilization with a 1.18-fold increase in genes
6 necessary for N mobilization (p=0.14), and a 1.12-fold decrease in genes necessary for phosphate
7 mobilization, (p=0.39) in the DEEP zone relative to the CTL.

8 Trends in the mineral layers were less clear. Significant shifts included a 2.18-fold increase in
9 genes encoding for enzymes involved in arabinoside degradation (p=0.049) and a 1.23-fold
10 decrease in enzymes involved in N mobilization (p=0.019) in the DEEP zone relative to the CTL,
11 (Fig. 4). Genes for lignin-degrading enzymes again showed decreasing abundance along the
12 treatment gradient from LOW to DEEP (16.23-fold decrease; p=0.051). However, relative to the
13 CTL, lignin-degrading genes in both INT and LOW zones exhibited much greater abundances
14 than they did in the organic layers (Fig. 4).

15 All soil chemical properties were found to be poor predictors of gene abundance, with the
16 exception of genes associated with lignin degradation. Both %C and C:N showed positive
17 relationships ($R^2=0.32$, $p<0.001$ and $R^2=0.54$, $p<0.001$, respectively; Fig. S10), and soil pH
18 showed a negative relationship ($R^2=0.41$, $p<0.001$; Fig. S10).

19 While the analysis did reveal significant changes in enzyme gene abundance across the snow
20 zones, many of the KEGG ortholog groups of enzymes targeted in this study were either not
21 found in any of the samples or were found in very low quantities, including phenol oxidases,
22 peroxidases, and laccases (Table S1).

23

24 4 Discussion

25 This study documents changes in soil bacterial community structure in the active layer of moist
26 acidic tundra in response to long-term (18 year) experimental changes in winter precipitation.

27 We examined how changes in bacterial community functional potential as a result of climate
28 forcing factors might affect SOM degradation and alter the C balance of this Arctic tundra
29 ecosystem. Low temperatures in Arctic ecosystems limit soil C availability and decomposability

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1 (Conant et al., 2011; Davidson and Janssens, 2006). However, global warming-induced
2 permafrost thaw may partially alleviate this temperature limitation, potentially releasing large
3 amounts of C into the atmosphere via SOM decomposition and further increasing the rate of
4 global warming (Lupascu et al., 2013, 2014a; Lützow and Kögel-Knabner, 2009; Schuur et al.,
5 2008).

6 After 18 years of experimental winter snow addition, bacterial community structure, and
7 functional potential in Arctic moist acidic tundra changed under deeper winter snow
8 accumulation. Our results indicate that increased snow pack reduced the abundance of genes
9 associated with SOM decomposition in the organic soil layers, suggesting a reduced SOM
10 decomposition potential. Possible explanations for this functional shift may include: 1) altered
11 bacterial C substrate preferences towards more labile sources under lowered O₂ availability that
12 would result in a decreased abundance of genes associated with SOM decomposition, and 2) a
13 reduced amount of enzymatic machinery (and fewer gene copies; Rocca et al., 2014), necessary
14 to accomplish similar metabolic results, as increased soil temperatures under snow accumulation
15 may alleviate kinetic limitations of enzyme functioning (German et al., 2012; Sinsabaugh et al.,
16 2008).

17 4.1 Bacterial community shifts

18 Our results indicate that altered snow accumulation has a significant effect on soil bacterial
19 community structure in Arctic moist acidic tussock tundra ecosystems. While large differences in
20 relative abundances were found between soil layers (Table S2), the most notable effects of snow
21 accumulation occurred in the organic layers. For instance, we observed shifts in the relative
22 abundance in many of the most abundant phyla including Verrucomicrobia, Acidobacteria, and
23 Actinobacteria, particularly in the DEEP snow zone (Fig. 3). Shifts in Verrucomicrobia were
24 primarily driven by increases in the order Chthoniobacterales in the DEEP snow zones relative to
25 the LOW snow zones. This order contains facultative aerobic heterotrophs able to utilize
26 saccharide components of plant biomass, but unable to use amino acids or organic acids other
27 than pyruvate (Sangwan et al., 2004). Shifts in Actinobacteria were dominated by the order
28 Actinomycetales, gram-positive facultative bacteria that have been linked to the stimulation of
29 ectomycorrhizal growth which degrade recalcitrant C_v (Goodfellow and Williams, 1983; Maier et
30 al., 2004; Pridham and Gottlieb, 1948). While not as abundant, the phylum Chloroflexi also

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1 | responded to snow pack treatments, increasing in abundance from LOW to DEEP snow zones
2 | (Fig. 3). Shifts in Chloroflexi were the result of increasing abundance of the class Anaerolineae
3 | in the DEEP zone. Anaerolineae include green non-sulfur bacteria able to thrive in anaerobic
4 | environments and have previously been found in similar cold, water-saturated soils (Costello and
5 | Schmidt, 2006). These results appear consistent with the increased soil moisture and decreased
6 | partial pressure of O₂ documented under increased snow pack at the study site (Blanc-Betes et
7 | al., 2016).

8 | These shifts in bacterial phyla indicate that even at the coarsest level of phylogeny and a high
9 | degree of variance between samples, deeper snow in winter and associated changes in soil
10 | conditions may be driving changes in the belowground community. Bacterial community shifts
11 | may be resulting in potentially altered substrate use preference by decomposers, and different
12 | genetic functional activity. This is supported by other studies from Arctic soil and permafrost
13 | ecosystems that provide evidence of altered microbial community composition and rapid
14 | functional response to temperature manipulations, thawing soils, or fertilization treatments
15 | (Deslippe et al., 2012; Koyama et al., 2014; Mackelprang et al., 2011). For example,
16 | Actinobacteria abundance was found to increase in response to both increased temperature
17 | (Deslippe et al., 2012) and in freshly thawed permafrost soils (Mackelprang et al., 2011), similar
18 | to the response we observed in the DEEP zone (Fig. 3). Mackelprang et al. (2011) also reported
19 | varying shifts in a wide array of functional genes in response to permafrost thaw. In addition,
20 | Koyama et al. (2014) documented a decrease in the Acidobacteria phylum in response to
21 | fertilizer soil inputs which they attributed to be a direct result of competition with α -, β -, and γ -
22 | Proteobacteria (oligotrophic vs. copiotrophic bacteria, respectively) which increased in
23 | abundance with fertilizer treatment. While oligotrophic organisms such as Acidobacteria are
24 | adapted to survive in low nutrient environments, they are often outcompeted in more fertile soils
25 | by generalist copiotrophs (such as Proteobacteria) who are better equipped to harvest available
26 | nutrients. Our results did not show a clear pattern for Proteobacteria, but they do show that
27 | Acidobacteria abundance shifts associate negatively with Proteobacteria shifts in the DEEP zone
28 | where C:N soil values are lowest (most fertile; Table 1 and Fig. 3).

29 | Correlations between soil chemical characteristics (%C, %N, C:N, and pH) and bacterial phylum
30 | abundance partially support findings reported in Fierer et al. (2007). They identified C

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1 mineralization rates (a proxy for C availability) to be the best predictor of bacterial abundance in
2 the dominant phyla, including positive relationships with Bacteroidetes and β -Proteobacteria,
3 and a negative relationship with Acidobacteria (Fierer et al., 2007). We acknowledge that C
4 mineralization and availability differ from %C in that regardless of carbon concentration,
5 physical and chemical factors in the Arctic such as temperature limitations, and high tannin
6 concentrations may limit C mineralization (Davidson and Janssens, 2006; Schimel et al., 1996).
7 Physical protection of SOM by soil aggregates, also known to limit C mineralization, has not
8 been observed in Arctic soils to our knowledge. Regardless of these difference in C
9 measurement, our study did find weak positive relationships between %C and Proteobacteria
10 (Fig. S2) as well as Bacteroidetes (Fig. S5), similar to Fierer et al., 2007. Interestingly, although
11 N can be a limiting factor for microbial growth, %N only correlated to two phyla, positively with
12 Verrucomicrobia (Fig. S3) and negatively with Actinobacteria (Fig. S4). While identifying
13 individual abiotic factors that may predict bacterial abundance at the phylum level is informative,
14 it is important to recognize that often a variety of interacting factors determine microbial
15 community composition, and effects at the phylum scale may be too coarse for adequate
16 interpretation. Our results suggest that while C:N (a proxy for SOM quality) is a poor indicator
17 of individual bacterial phylum abundance, %C and %N (and in some cases soil pH) alone may
18 be more relevant in these acidic tundra soils. More detailed studies that address the relationships
19 between soil chemical/abiotic characteristics and microbial community composition at finer
20 phylogenetic scales are needed to adequately identify dependable predictors.

21 While the alpha diversity of soil bacterial communities via the Shannon index did differ between
22 soil layer, it did not differ between snow pack treatment zones. Also, it does not elucidate
23 community structural or functional differences between samples, and it fails to distinguish shifts
24 in genetic potential among treatments. In contrast, beta diversity analyses better revealed soil
25 bacterial community responses to snow accumulation. Bacterial community structure
26 significantly shifted between snow pack treatment zones at all soil depths / layers (Table 2). The
27 NMDS plot (Fig. 2) shows bacterial community structures to be associated with the snow
28 accumulation treatment as soil chemical properties changed (%C, %N, C:N, and pH), indicating
29 that bacterial β -diversity may respond to indirect changes in soil chemistry in response to winter
30 snow accumulation. The initial effects of increased snow pack result in altered physical factors
31 (greater active layer thaw depth and increased soil temperatures and moisture; Blanc-Betes et al.,

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1 2016) which may lead to increased SOM availability and faster enzyme activities with the
2 potential to enhance SOM decomposition. Higher SOM mineralization may promote the
3 documented shifts in aboveground plant communities and increased NPP (Natali et al., 2012;
4 Sturm et al., 2005, Anderson-Smith 2013), and vegetation shifts to more shrubby species may
5 alter the chemistry and quality of new litter inputs, ultimately affecting decomposer
6 communities. Moreover, soil moisture and compaction can reduce O₂ diffusion into the soil,
7 inhibiting aerobic SOM decomposition (Blanc-Betes et al., 2016; O'Brien et al., 2010), and
8 altering bacterial community composition by selecting for microorganisms that utilize simple C
9 substrates, leaving behind complex organic compounds and plant polymers. In addition, tannins
10 produced by expanding woody shrubs may act to inhibit microbial activity (Schimel et al., 1996),
11 further slowing decomposition. This is supported by the lower relative abundance of genes
12 required for SOM decomposition in the DEEP snow accumulation zone where we observed the
13 most significant shifts in bacterial community composition (Figs. 3&4). The balance between
14 these two competing processes, and the functional shifts associated with them, will ultimately
15 influence the C balance of the system.

16 4.2 Functional shifts

17 To examine the influence of shifting bacterial abundances on soil community functioning and the
18 C balance of Arctic ecosystems, we focused on the genetic potential of the bacterial community
19 to produce enzymes required for the degradation of various forms of SOM. We did this by using
20 PICRUSt software to estimate functional gene abundance via ancestral state reconstruction
21 (Langille et al., 2013). While this method does not provide direct measurements of gene
22 abundance (e.g. does not account for horizontal gene transfer or unknown functional / taxonomic
23 linkages that may exist in the sampled tundra soils), it does offer valuable insights into the
24 functional capacities of bacterial communities using 16S rRNA data (Langille et al., 2013).
25 Furthermore, gene abundance in itself is not a direct measurement of gene expression or enzyme
26 activity (Wood et al., 2015). However it does provide a measure of genetic potential and may be
27 positively correlated to enzyme activity and gene expression (Morris et al., 2014; Neufeld et al.,
28 2001; Rocca et al., 2014). To accurately measure enzymatic functional potential or gene
29 expression would require a targeted metagenomic and metatranscriptomic approach.

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1 Many bacterial genes encoding for enzymes associated with the degradation of lignin and other
2 complex plant compounds (such as peroxides, phenol oxidases, and laccases) were not detected
3 in this study. This suggests that bacterial communities preferentially degrade microbial biomass
4 and polysaccharide polymers, and that the decomposition of more recalcitrant forms of C in
5 Arctic soils is performed by other microorganisms such as fungi. Fungi typically play a key role
6 in the degradation of recalcitrant organic matter by specializing in the production of oxidative
7 enzymes (Deslippe et al., 2012; Morgado et al., 2015). The absence of bacterial genes that
8 encode for peroxides, phenol oxidases, and laccases, could also be due to the presence of tannins
9 in the soil, which are common in the Alaskan floodplain and are produced by encroaching shrub
10 species (DeMarco et al., 2014; Schimel et al., 1996). Tannic compounds have been shown to
11 inhibit microbial activity and decrease decomposition by binding to vital enzymes (Schimel et
12 al., 1996). If production of phenol oxidases and peroxides yield little to no benefit for bacteria in
13 this ecosystem due to competition with fungi and interference from tannins and other phenolic
14 compounds, genes encoding for these enzymes may be reduced (Rocca et al., 2014).

15 The PICRUSt predicted copies of genes for enzymes responsible for SOM decomposition, while
16 generally more abundant in the organic layers (Table S2), were less abundant in the organic
17 layers of the DEEP snow zone than in the CTL and LOW snow accumulation zones (Fig. 4). The
18 genes most affected encode enzymes required for the breakdown of plant derived litter, such as
19 cellulose, xylan, or pectin, all major constituents of plant cell walls. Xylans in particular are
20 common in woody plant tissues (Timell, 1967). The observed decrease of these genes in DEEP
21 snow pack suggests bacterial preference of readily available substrates, such as microbial
22 biomass or root exudates (Sullivan and Welker, 2005; Sullivan et al., 2007, 2008). Production of
23 these substrates, may have been stimulated by increased soil temperatures and NPP predicted
24 under a climate change scenario, and require less energetic investment in exo-enzyme production
25 (Schimel, 2003). The production of enzymes for the degradation of complex polysaccharides is
26 energetically demanding. Therefore, in an energy and nutrient limited ecosystem such as the
27 Arctic tundra (Hobbie et al., 2002; Jonasson et al., 1999; Mack et al., 2004; Shaver and Chapin,
28 1980, 1986; Sistla et al., 2012), more labile substrates are likely preferable, which may lead to
29 accumulation of SOM, and thus SOC (Lupascu et al., 2013, 2014a).

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1 Our results indicating reduced decomposition potential under deeper snow pack is consistent
2 with other long-term warming and snowfence studies from Arctic tundra ecosystems that report
3 zero net C loss (or even C gain) during the growing season (Natali et al., 2012, 2014; Sistla et al.,
4 2013). We speculate that initial soil conditions likely favour decomposer activity and
5 decomposition rates increase in response to increased temperatures, resulting in C loss. Over
6 time changing soil conditions (e.g. increased moisture, decreased O₂ availability, changes in
7 chemistry of litter inputs) may select for microorganisms that use anaerobic metabolic pathways
8 such as methanogenesis (Blanc-Betes et al. 2016). These hypoxic soil conditions would limit
9 aerobic decomposition. As bacterial communities increase the abundance of genes encoding for
10 enzymes involved in N mobilization, newly available N would enhance microbial biomass
11 production, plant NPP, leaf litter N content, and induce plant community shifts (Pattison and
12 Welker, 2014; Schimel, 2003; Welker et al., 2005). A decrease in SOM decomposition is
13 possibly supported by data from this study, which shows a decreased abundance of genes
14 involved in SOM decomposition in conjunction with trends suggesting increased abundance of N
15 mobilization genes in the organic Jayers as snow pack increases (Fig. 4).

16 Increased temperature may provide an alternate explanation to the decreased PICRUST predicted
17 abundance of genes associated with SOM decomposition in the organic Jayers of the DEEP snow
18 accumulation zone (Fig. 4). Enzyme activity is partially regulated by the rate of gene expression
19 as well as by post-transcriptional regulating factors, which include environmental factors (Gross
20 et al., 1989). Michaelis-Menten enzyme kinetics are sensitive to temperature (German et al.,
21 2012), increasing the maximum rate of enzyme activity (V_{max}) by increasing the catalytic
22 constant of the reaction (Razavi et al., 2015). Increased V_{max} may represent an excess potential
23 enzyme activity for the given substrate or growth conditions, resulting in a down regulation of
24 genes required for the enzyme (e.g. Gonzalez-Meler et al., 1999, 2001), because fewer enzymes
25 are needed to achieve similar V_{max} at higher temperatures. Therefore, increases in soil
26 temperature under deeper snow may partially explain the decrease in PICRUST predicted
27 abundance of genes required for SOM decomposition (Table 1 and Fig. 4).

28 4.3 Ecosystem response to snow accumulation

29 Whether bacterial communities are responding to changing plant inputs and corresponding
30 altered SOM quality (decreased C:N; Table 1) or whether they are directly altering SOM

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1 chemistry through selective decomposition remains unclear. From the results of our study, it is
2 clear that increased snow accumulation may lead to changes in both bacterial community
3 composition and SOM chemistry in the organic soil layers (Table 1 and Fig. 3). Unlike other
4 ecosystems where plants are the first responders to abiotic climate change factors, in the Arctic,
5 microbes are likely the first responders to changes in temperature by initially increasing nutrient
6 mineralization. These released nutrients facilitate plant community shifts and increase ecosystem
7 NPP (Chapin III et al., 1995). Over time, the combination of increased snow accumulation and
8 soil compaction may lead to hypoxic/anaerobic soil conditions (e.g., Blanc-Betes et al., 2016)
9 and further vegetative shifts to wet-sedge (*Carex*) species, limiting SOM decomposition. This in
10 combination with a recent history of more recalcitrant plant litter inputs could result in re-accrual
11 of SOC (e.g., Sistla et al., 2012), ultimately mitigating the positive feedback loop hypothesized
12 in current literature (Davidson and Janssens, 2006; Natali et al., 2014; Schuur et al., 2009; Sturm
13 et al., 2005).

14

15 5 Conclusions

16 The results presented here support the hypothesis that bacterial community structure and function
17 shift as a result of consistently deepened snowpack. Increases in soil hypoxia under deepened
18 snow may have resulted in an increased abundance of anaerobic or facultative bacteria, slowing
19 decomposition. Decreases in PICRUSt predicted gene copies suggest that SOM decomposition
20 may be slowed under accumulated snow, and bacterial community substrate preference may shift
21 to more labile compounds. Concentrations of C and N, as opposed to C:N, better explained
22 bacterial community responses to snow pack treatments. Together these results strongly suggest
23 that soil decomposers of moist acidic tundra are key in determining the direction and magnitude
24 of permafrost C feedbacks on the climate system.

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1 **Author Contributions**

2 M. P. Ricketts, J. M. Welker, and M. A. Gonzalez-Meler designed the experiment. R. S. Poretsky
3 provided expertise and insight into the bioinformatics and data analyses. M. P. Ricketts
4 performed all sample collections, lab work, and data analyses. M. P. Ricketts prepared the
5 manuscript with contributions from all co-authors.

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7 **Data Availability**

8 All data used in this report are publically accessible through two separate data repositories. The
9 16S rRNA gene sequences derived from Illumina Mi-Seq sequencing have been deposited in the
10 NCBI Sequence Read Archive (SRA) under accession number SRP068302. All computer script
11 text files used in QIIME and R packages, as well as BIOM and Excel files, are available via the
12 NSF Arctic Data Center, doi:10.18739/A2FS9J.

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13 **Acknowledgements**

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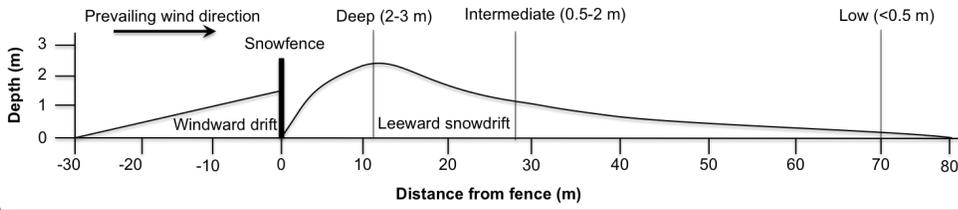
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2 Figure 1. Modified from Walker et al., 1999. Schematic of snow accumulation depth at moist
 3 acidic tundra site from snow fence manipulation. Three soil cores were obtained from each
 4 treatment zone (labeled Deep, Intermediate, and Low) and a Control zone located >30m outside
 5 the effect of the snowfence.

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1 Table 1. Abiotic characteristics of soil from snow accumulation treatments (Low = ~25% less
 2 snow pack than the Control, Int = ~50% more snow pack than the Control, Deep = ~100% more
 3 snow pack than the Control). Values are means ± standard errors. Soil chemical properties were
 4 obtained from samples used for DNA extraction, while temperature and thaw depth were
 5 measured *in situ* (n=12). Organic and mineral samples were analysed separately using the
 6 Nemenyi post hoc test. Results are indicated by a,b,c only where p<0.05.

Treatment	Soil Layers	%C	%N	C:N	pH	Temp @ 12cm (°C)	Thaw Depth (cm)
Control	Organic (n=4)	45.21±1.09 _{ab}	1.01±0.20	50.04±9.44	4.59±0.09	4.32±0.27 _b	59.17±1.23 _{bc}
	Mineral (n=3)	2.57±0.39 _▼	0.15±0.03	17.67±1.34	5.15±0.05 _{ab}		
Low	Organic (n=4)	46.63±0.73 _a	1.06±0.07	44.59±2.54	4.44±0.08	2.92±0.24 _b	50.92±3.20 _c
	Mineral (n=3)	4.18±1.92 _▼	0.22±0.11	19.42±0.65	5.16±0.20 _{ab}		
Int	Organic (n=3)	40.59±2.43 _{ab}	1.17±0.25	38.38±8.85	4.69±0.41	4.08±0.25 _b	61.88±1.19 _{ab}
	Mineral (n=3)	2.58±0.49 _▼	0.14±0.02	18.58±1.45	5.01±0.04 _a		
Deep	Organic (n=4)	36.51±4.27 _b	1.40±0.07	26.27±3.41	5.61±0.21	6.49±0.20 _a	65.42±1.49 _a
	Mineral (n=4)	1.65±0.19 _▼	0.10±0.01	16.41±0.56	5.83±0.17 _b		

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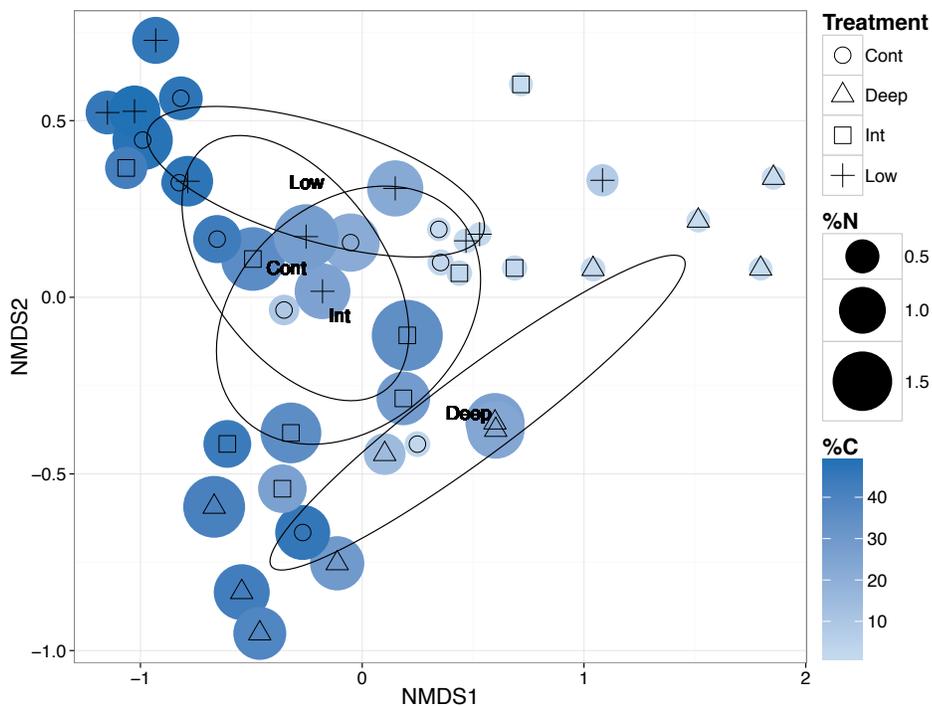
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1 Table 2. Statistical analysis of beta diversity using adonis and Mantel tests. Bray Curtis distance
 2 matrices of bacterial communities for each sample were compared between soil layers (Organic,
 3 Transition, Mineral) and snow accumulation treatments (CTL, DEEP, INT, LOW), and to soil
 4 chemical properties. Sample sizes were n=15 for “Organic”, n=13 for “Mineral”, and n=41 for
 5 “All layers”. Significance is indicated by asterisks (* = p<0.05, ** = p<0.01, *** = p<0.001).

	Samples	adonis			Mantel test	
		R ²	df	p-value	r statistic	p-value
Soil layers	All	0.320	2	<0.001***	NA	NA
Snow pack	All	0.126	3	0.017*	NA	NA
	Organic only	0.421	3	<0.001***	NA	NA
	Mineral only	0.485	3	0.003**	NA	NA
%C	All	0.239	1	<0.001***	0.633	<0.001***
	Organic only	0.212	1	0.004**	0.490	0.008**
	Mineral only	0.055	1	0.720	0.047	0.791
%N	All	0.141	1	<0.001***	0.341	<0.001***
	Organic only	0.111	1	0.131	-0.0245	0.883
	Mineral only	0.051	1	0.788	0.032	0.844
C:N	All	0.191	1	<0.001***	0.415	<0.001***
	Organic only	0.165	1	0.022*	0.180	0.269
	Mineral only	0.108	1	0.195	-0.063	0.629
pH	All	0.147	1	<0.001***	0.490	<0.001***
	Organic only	0.368	1	<0.001***	0.709	<0.001***
	Mineral only	0.297	1	0.004**	0.526	<0.001***

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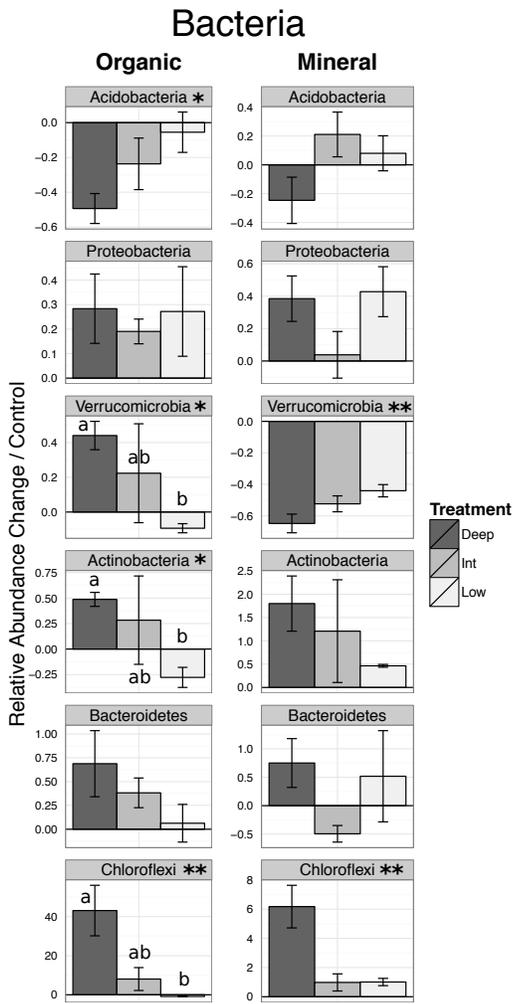


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2 Figure 2. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity
 3 matrices (Stress=0.090, Shepard plot non-metric $R^2=0.992$). Each point represents the
 4 bacterial community structure within one of the 41 total samples used for DNA extraction from
 5 all soil depths (Organic, Transition, and Mineral). Colours indicate %C ranging from 1.4% (light
 6 blue) to 48.6% (dark blue), bubble size indicates %N ranging from 0.09% (small) to 1.95%
 7 (large), and shapes indicate snow accumulation treatments (CTL, DEEP, INT, LOW). Ellipse
 8 centroids represent treatment group means while the shape is defined by the covariance within
 9 each group.

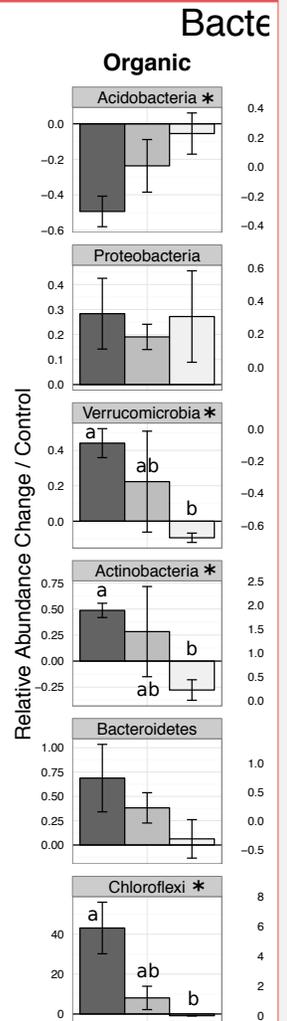
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 2 Figure 3. Averaged relative abundance of the six most abundant bacterial phylum relative to the
 3 control, separated by snow accumulation treatment, and in order of greatest abundance (top to
 4 bottom). Error bars represent standard error (standard error of controls ranged from 12.929 in
 5 Chloroflexi to 0.026 in Verrucomicrobia). Significance determined by Kruskal-Wallis tests is
 6 indicated by asterisks (* = $p < 0.1$, ** = $p < 0.05$), while post-hoc Nemenyi test results are indicated
 7 by “a, b, ab”, except where significant differences were to the control.

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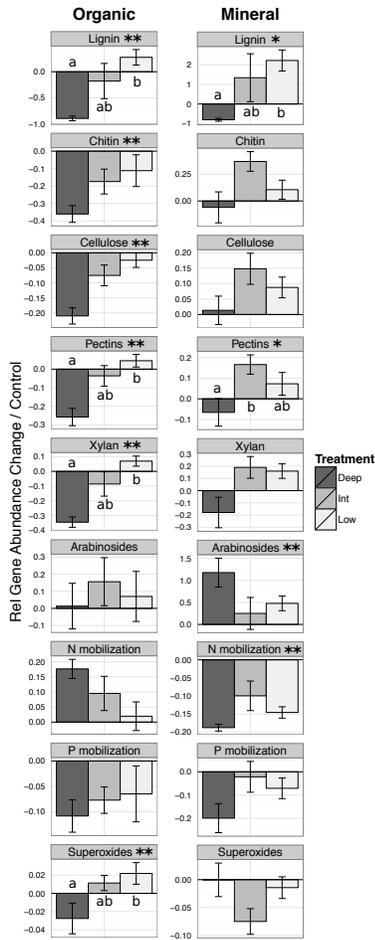
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Enzymes



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2 Figure 4. Averaged relative abundance of genes for enzyme functional groups relative to the
 3 control and separated by snow accumulation treatment. Functional groups involved in soil
 4 organic matter decomposition are ordered from recalcitrant to labile substrates (top to bottom).
 5 Error bars represent standard error (standard error of controls ranged from 1.220 in the lignin
 6 group to 0.008 in the superoxides group). Significance determined by Kruskal-Wallis tests is
 7 indicated by asterisks (* = $p < 0.1$, ** = $p < 0.05$), while post-hoc Nemenyi test results are indicated
 8 by “a, b, ab”, except where significant differences were to the control.

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Supplemental Information

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- 2
- 3 **Table S1 - List of targeted KEGG ortholog enzymes used in the PICRUSt analyses. For more information see materials and methods**
- 4 [in main text.](#)

Functional role	Enzyme name	EC number	KEGG orthology number
PRESENT IN SAMPLES			
Arabinoside degradation	arabinogalactan endo-beta-1,4-galactanase	3.2.1.89	K01224
	arabinan endo-1,5-alpha-L-arabinanase	3.2.1.99	K06113
Cellulose degradation	cellulase	3.2.1.4	K01179
	beta-glucosidase	3.2.1.21	K01188, K05349, K05350
	cellulose 1,4-beta-cellobiosidase (CBH)	3.2.1.91	K01225
Chitin degradation	chitinase (NAG)	3.2.1.14	K01183
	bifunctional chitinase/lysozyme	3.2.1.14 & 3.2.1.17	K13381
Nitrogen mobilization	leucyl aminopeptidase (LAP) urease	3.4.11.1 3.5.1.5	K01255 K01427, K01428, K01429, K01430, K14048
Lignin degradation	tyrosinase (phenol oxidase)	1.14.18.1	K00505
Pectin degradation	polygalacturonase	3.2.1.15	K01184
	alpha-L-rhamnosidase	3.2.1.40	K05989
Phosphate mobilization	alkaline phosphatase (AP) acid phosphatase (AP)	3.1.3.1 3.1.3.2	K01077, K01113 K01078, K01093, K09474, K03788, K14379
Superoxides	superoxide dismutase	1.15.1.1	K00518, K04564, K04565
	superoxide reductase	1.15.1.2	K05919
Xylan degradation	xylan 1,4-beta-xylosidase	3.2.1.37	K01198
NOT FOUND IN ANY SAMPLES			
	polyphenol oxidase	1.10.3.1	K00422

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peroxidase	1.11.1.7	K00430
fructan beta-fructosidase	3.2.1.80	K03332
laccase (oxidoreductase)	1.10.3.2	K05909
eosinophil peroxidase	1.11.1.7	K10788
cytosol aminopeptidase	3.4.11.1 & 3.4.11.5	K11142
peroxiredoxin 6, 1-Cys peroxiredoxin	1.11.1.7, 1.11.1.15, & 3.1.1.-	K11188
lactoperoxidase	1.11.1.7	K12550
low molecular weight phosphotyrosine protein phosphatase	3.1.3.2 3.1.3.48	K14394
lysophosphatidic acid phosphatase type 6	3.1.3.2	K14395
acid phosphatase	3.1.3.2	K14410
beta-D-xylosidase 4	3.2.1.37	K15920

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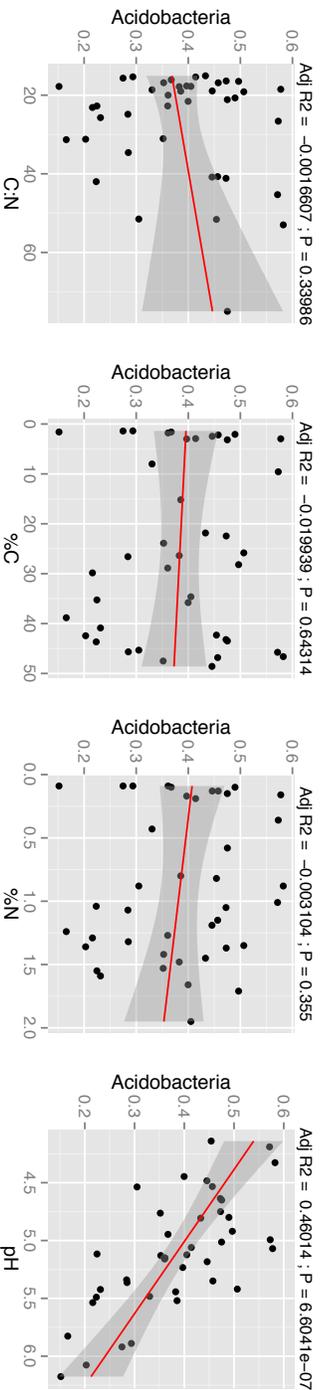
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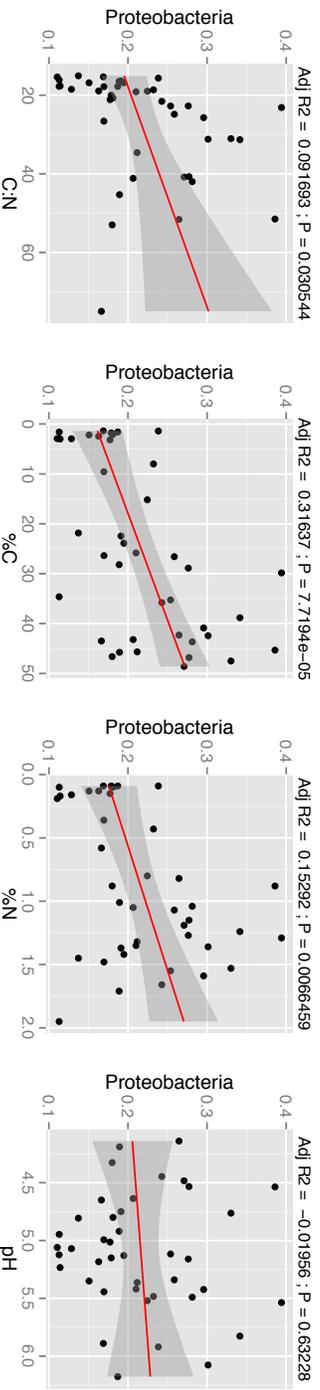
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1 **Figures S1-to-S6**. Linear fit regressions of bacterial phyla abundance with respect to soil properties such as soil carbon-to-nitrogen
 2 ratio (C/N), soil carbon mass concentration (%C), soil nitrogen mass concentration (%N), and soil pH across all snow treatment sites
 3 and all soil depths. The bacterial relative abundance is ordered from most abundant phylum (Fig. S1) to least abundant (Fig. S6). See
 4 table S2 for statistics and significance.



6 **Fig. S1** – Linear relationships between Acidobacteria relative abundance and soil C:N, C and N concentrations, and pH from all 41
 7 samples. Shaded areas indicate 95% confidence intervals.



10 **Fig. S2** – Linear relationships between Proteobacteria relative abundance and soil C:N, C and N concentrations, and pH from all 41
 11 samples. Shaded areas indicate 95% confidence intervals.

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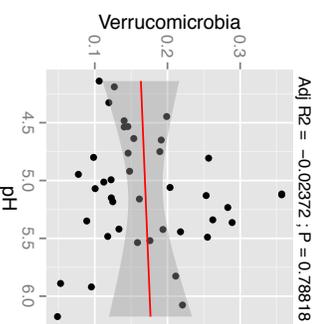
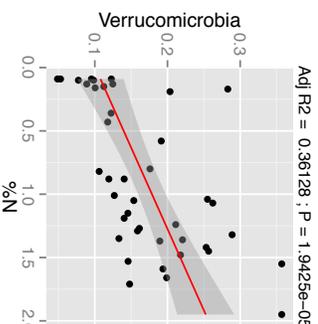
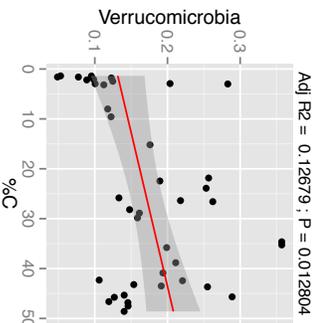
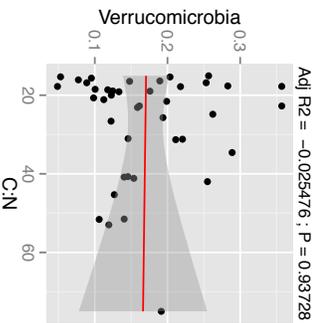


Fig. S3 – Linear relationships between Verrucomicrobia relative abundance and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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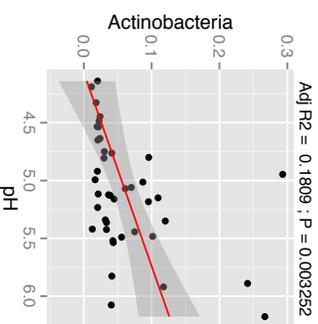
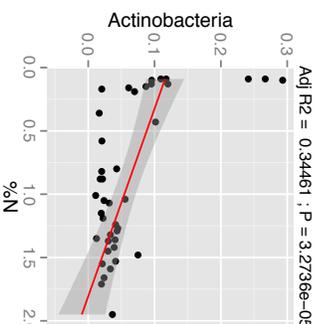
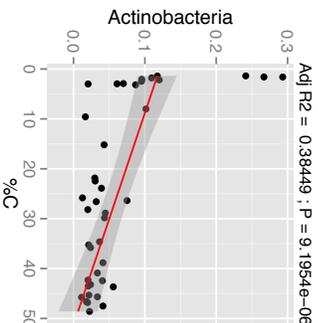
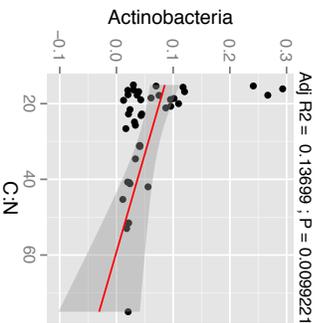


Fig. S4 – Linear relationships between Actinobacteria relative abundance and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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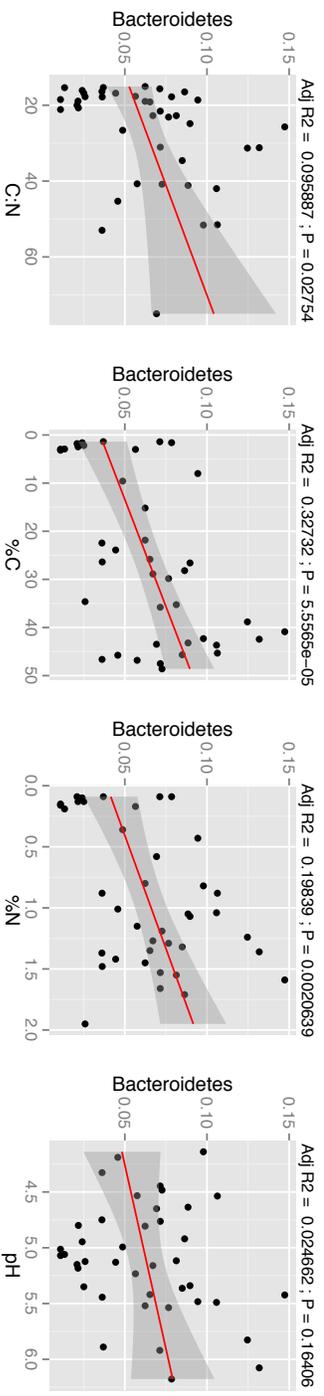
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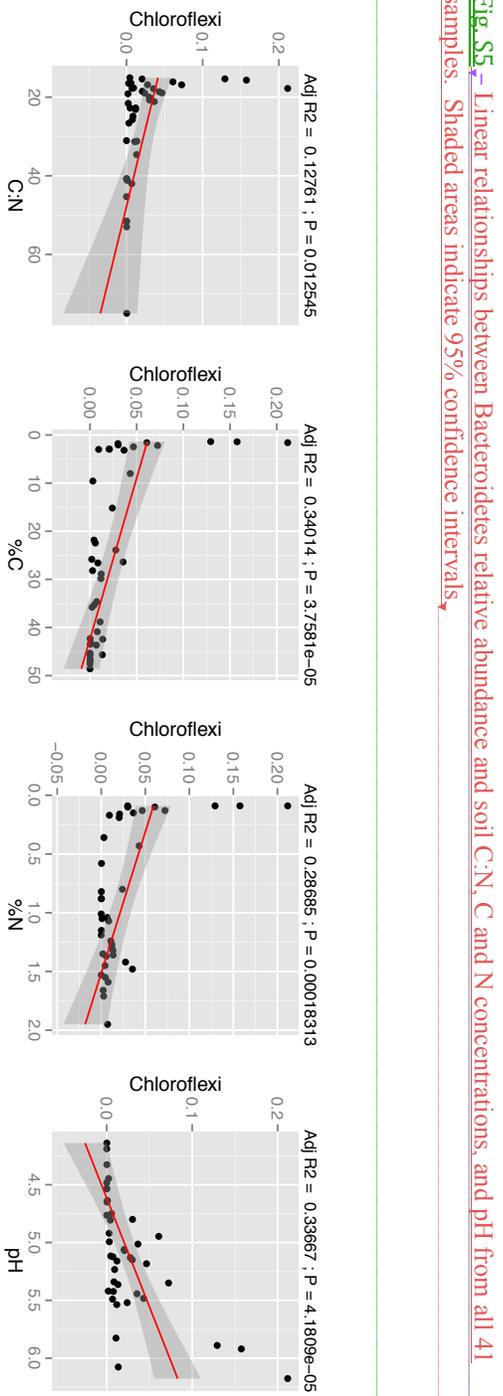
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Fig. S6 - Linear relationships between Chloroflexi relative abundance and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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Figures S7-to-S15. Linear fit regressions of relative gene abundance with respect to soil properties such as soil carbon-to-nitrogen ratio (C/N), soil carbon mass concentration (%C), soil nitrogen mass concentration (%N), and soil pH across all snow treatment sites and all soil depths. Enzymes were grouped by their general activities as explained in main text and they are shown on the Y-axis of the figures. See table S2 for statistics and significance.

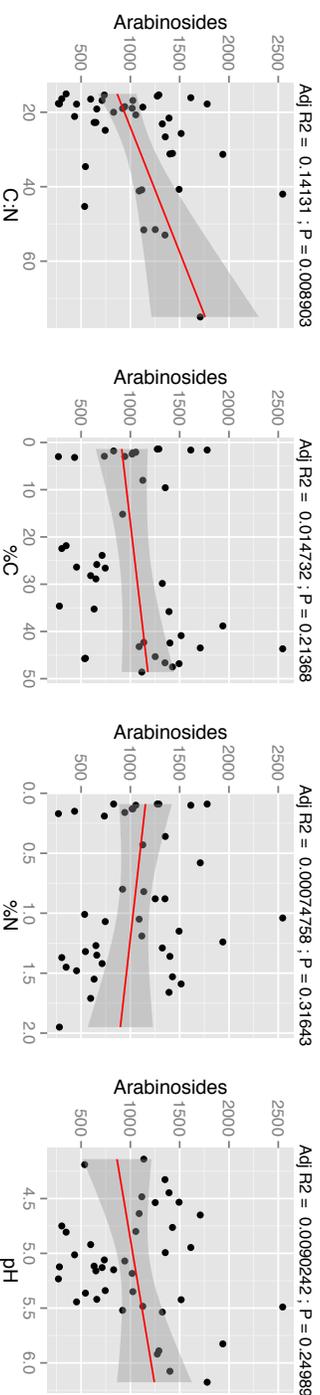


Fig. S7 – Linear relationships between the relative abundance of genes required for arabinoside degradation and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

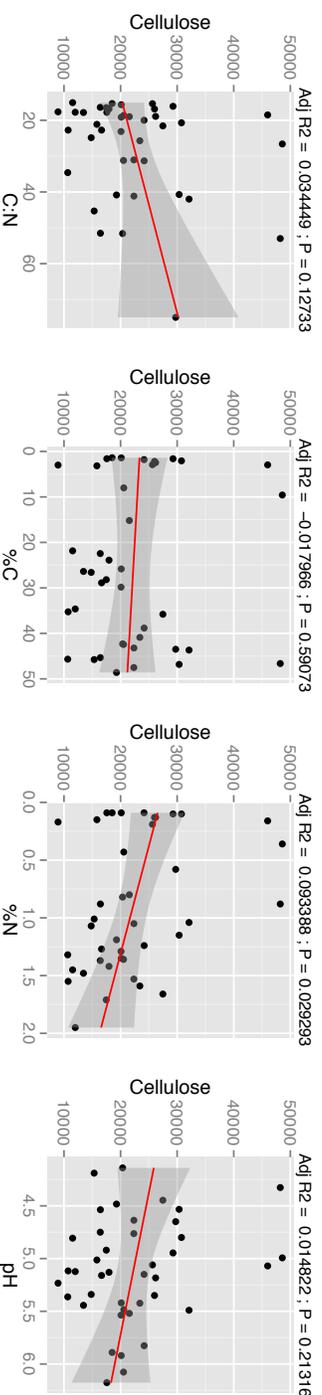


Fig. S8 – Linear relationships between the relative abundance of genes required for cellulose degradation and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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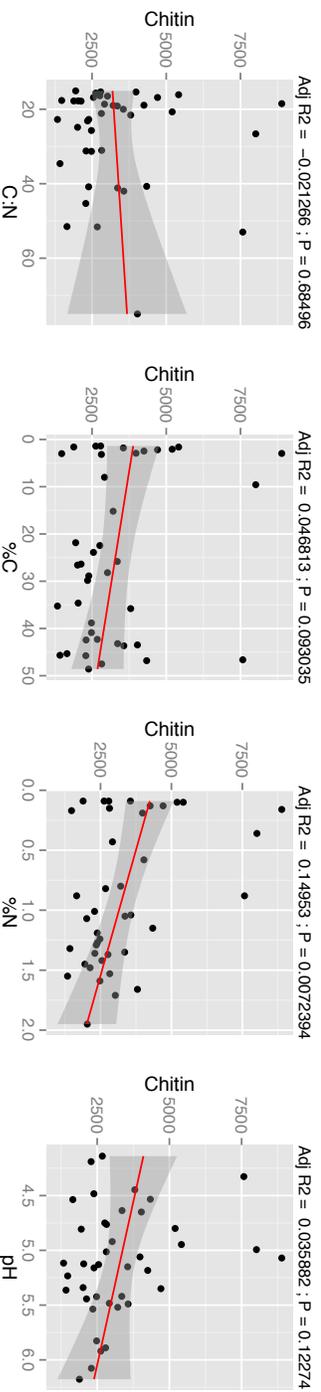


Fig. S9 – Linear relationships between the relative abundance of genes required for chitin degradation and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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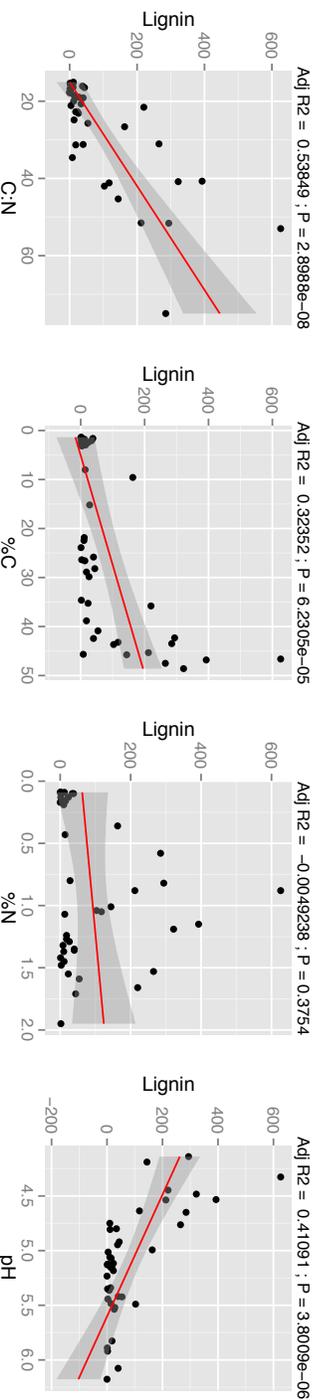


Fig. S10 – Linear relationships between the relative abundance of genes required for lignin degradation and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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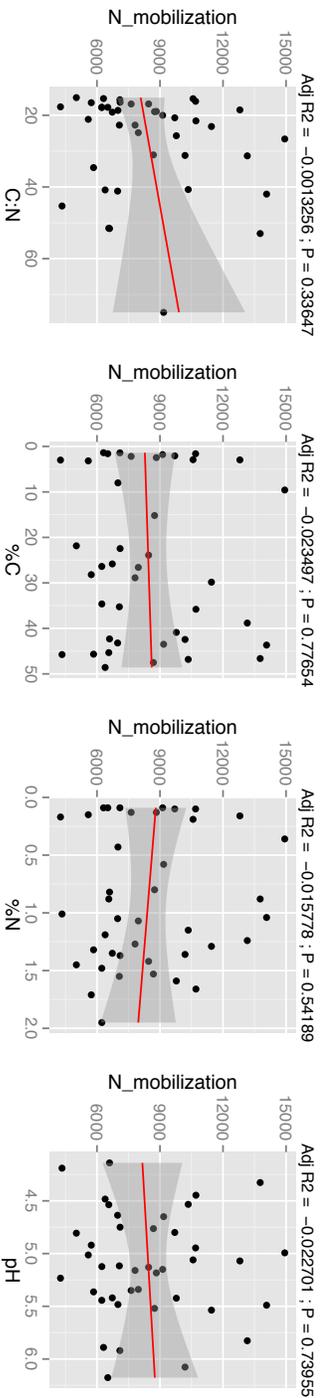


Fig. S11 – Linear relationships between the relative abundance of genes required for N mobilization and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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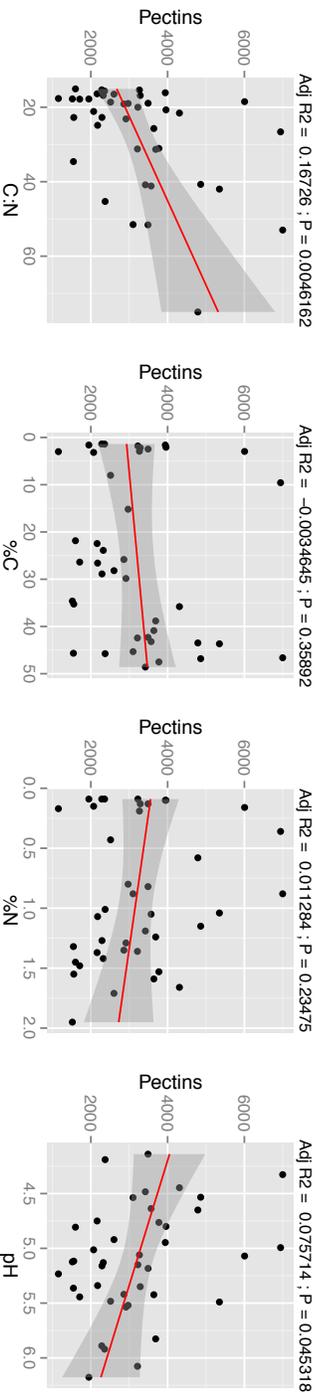


Fig. S12 – Linear relationships between the relative abundance of genes required for pectin degradation and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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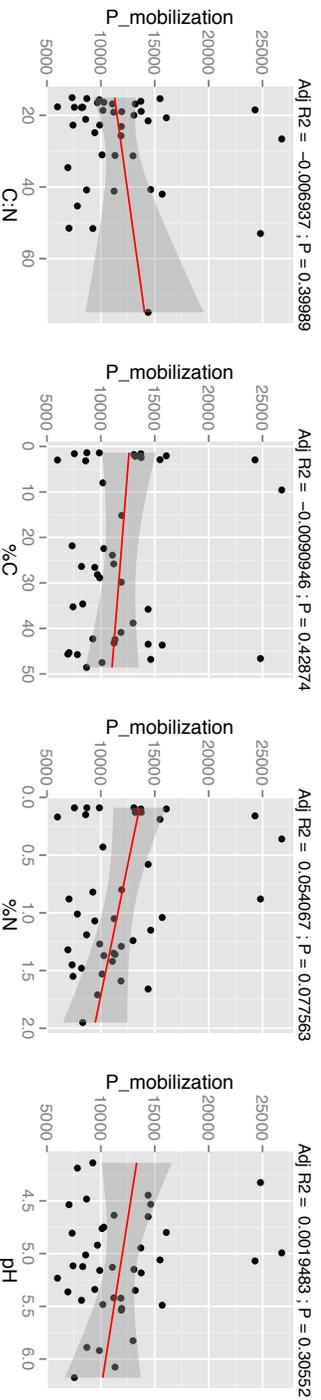
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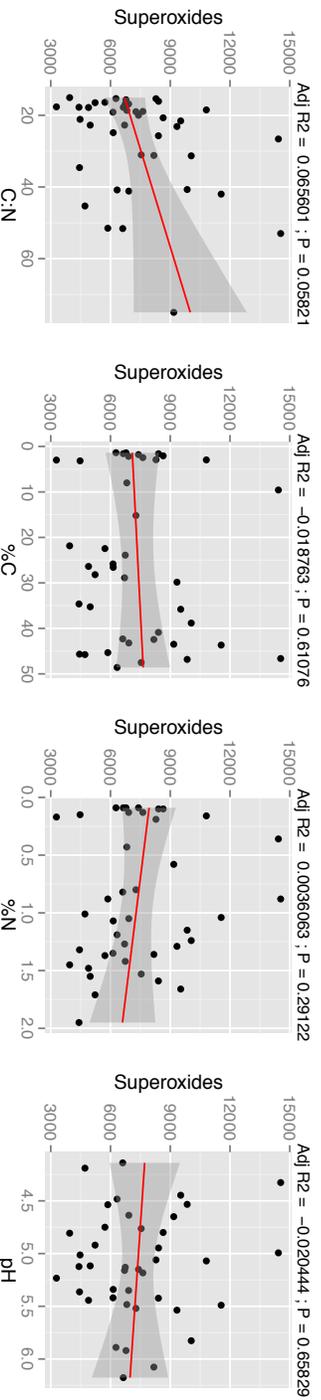
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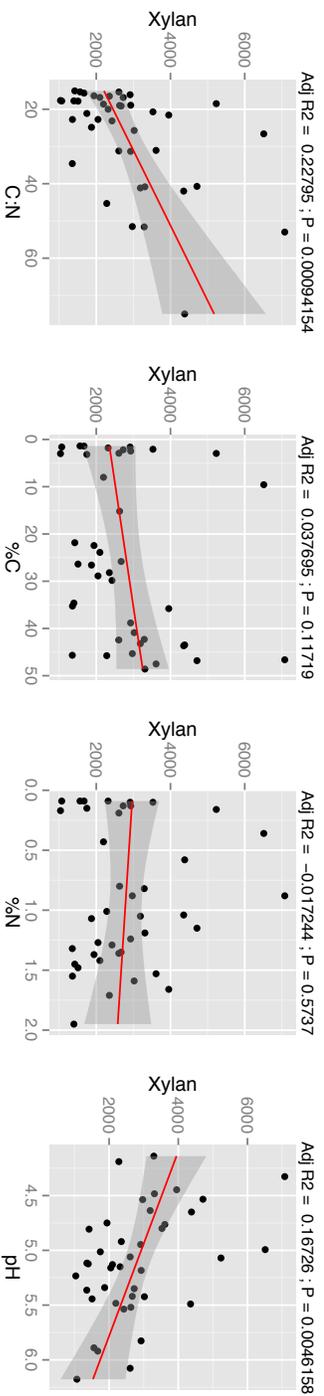
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Fig. S14 – Linear relationships between the relative abundance of genes required for superoxide regulation and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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Fig. S15 – Linear relationships between the relative abundance of genes required for xylan degradation and soil C:N, C and N concentrations, and pH from all 41 samples. Shaded areas indicate 95% confidence intervals.

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1 Table S2 – Effects of soil depth characteristics (Organic, Transition, Mineral) on soil
 2 chemistry, bacterial phylum relative abundance, and relative abundance of genes organized
 3 by functional groups, as determined by the Kruskal-Wallis test. Degrees of freedom = 2 for
 4 all analyses. * = p<0.05, ** = p<0.01, *** = p<0.001

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	H-statistic	p-value	Soil layer w/ higher value (Org vs Min)
<u>Soil Chemistry</u>			
<u>%C</u>	<u>32.32</u>	<u>9.57 x 10⁻⁸ ***</u>	<u>Organic</u>
<u>%N</u>	<u>26.53</u>	<u>1.74 x 10⁻⁶ ***</u>	<u>Organic</u>
<u>C:N</u>	<u>21.70</u>	<u>1.94 x 10⁻⁵ ***</u>	<u>Organic</u>
<u>pH</u>	<u>6.81</u>	<u>0.03 *</u>	<u>Mineral</u>
<u>Bacterial phylum abundance</u>			
<u>Acidobacteria</u>	<u>0.05</u>	<u>0.98</u>	<u>NA</u>
<u>Proteobacteria</u>	<u>14.78</u>	<u>6.17 x 10⁻⁴ ***</u>	<u>Organic</u>
<u>Verrucomicrobia</u>	<u>14.93</u>	<u>5.73 x 10⁻⁴ ***</u>	<u>Organic</u>
<u>Actinobacteria</u>	<u>20.16</u>	<u>4.12 x 10⁻⁵ ***</u>	<u>Mineral</u>
<u>Bacteroidetes</u>	<u>13.08</u>	<u>1.45 x 10⁻³ **</u>	<u>Organic</u>
<u>Chloroflexi</u>	<u>24.80</u>	<u>4.13 x 10⁻⁶ ***</u>	<u>Mineral</u>
<u>Enzyme gene abundance</u>			
<u>Lignin</u>	<u>20.17</u>	<u>4.17 x 10⁻⁵ ***</u>	<u>Organic</u>
<u>Chitin</u>	<u>3.00</u>	<u>0.22</u>	<u>NA</u>
<u>Cellulose</u>	<u>8.36</u>	<u>0.02 *</u>	<u>Organic</u>
<u>Pectin</u>	<u>17.25</u>	<u>1.80 x 10⁻⁴ ***</u>	<u>Organic</u>
<u>Xylan</u>	<u>15.79</u>	<u>3.72 x 10⁻⁴ ***</u>	<u>Organic</u>
<u>Arabinoside</u>	<u>13.62</u>	<u>1.10 x 10⁻³ **</u>	<u>Organic</u>
<u>N mobilization</u>	<u>11.67</u>	<u>2.92 x 10⁻³ **</u>	<u>Organic</u>
<u>P mobilization</u>	<u>3.30</u>	<u>0.19</u>	<u>NA</u>
<u>Superoxide</u>	<u>29.61</u>	<u>3.72 x 10⁻⁷ ***</u>	<u>Organic</u>

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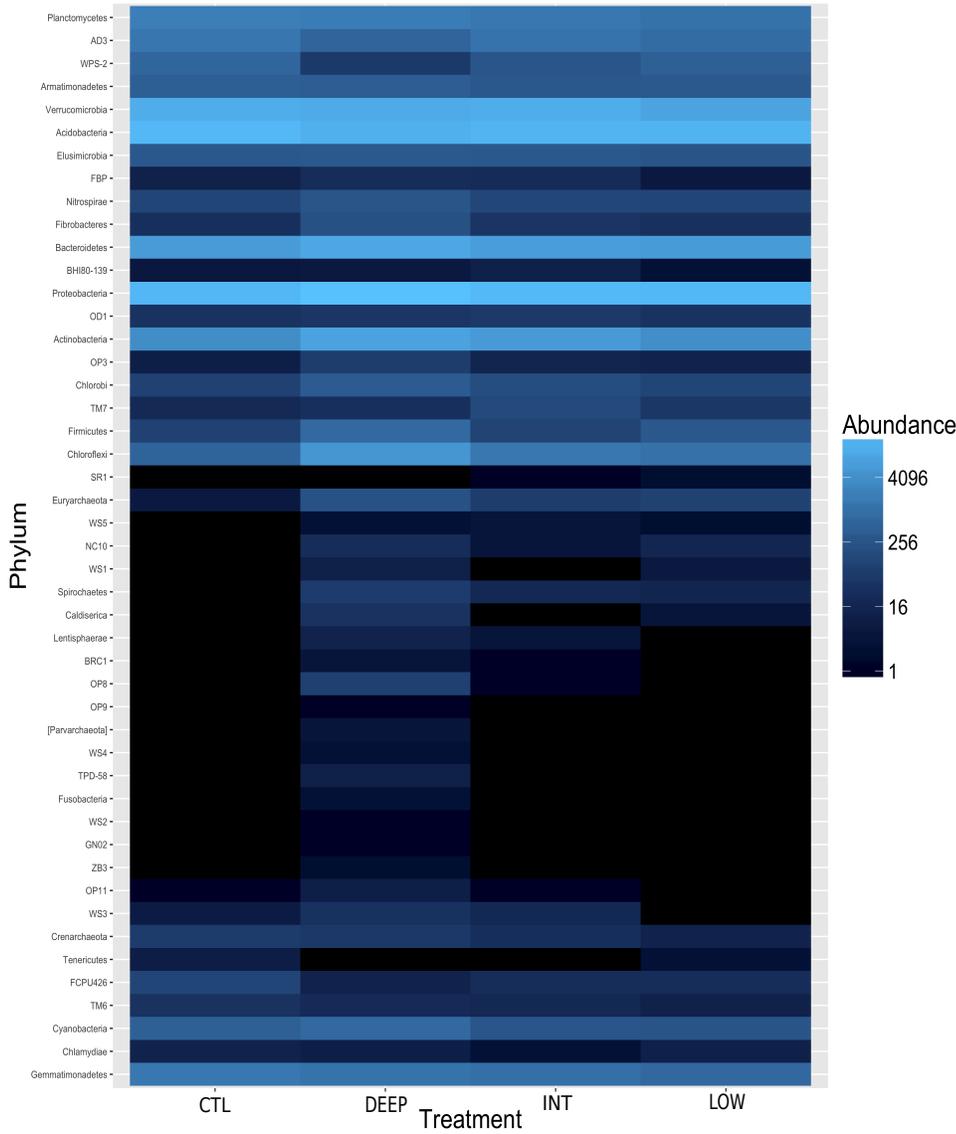
1 Table S3 – Statistical analyses of alpha diversity using non-parametric two-sample t-tests
 2 with 999 Monte Carlo permutations. Pairwise comparisons of Shannon diversity metrics
 3 from each sample were made between each soil layer (Organic, Transition, Mineral) and
 4 each snow accumulation treatment (Control, Deep, Intermediate, Low).
 5 * = p<0.05, ** = p<0.01, *** = p<0.001.
 6

<u>Two-sample t-tests</u>		
<u>Soil layers</u>	<u>t- statistic</u>	<u>p-value</u>
<u>Organic / Mineral</u>	<u>5.58</u>	<u>0.003 **</u>
<u>Trans / Organic</u>	<u>-0.26</u>	<u>1</u>
<u>Trans / Mineral</u>	<u>5.22</u>	<u>0.003**</u>
<u>Treatment</u>		
<u>Cont / Deep</u>	<u>0.30</u>	<u>1</u>
<u>Cont/ Int</u>	<u>-0.49</u>	<u>1</u>
<u>Cont / Low</u>	<u>1.70</u>	<u>0.6</u>
<u>Deep / Int</u>	<u>-4.0 x 10⁻⁴</u>	<u>1</u>
<u>Deep / Low</u>	<u>-.30</u>	<u>1</u>
<u>Int / Low</u>	<u>0.51</u>	<u>1</u>

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1 Figures S16-S20. Heatmaps showing the relative raw abundance of operational taxonomic
 2 units (OTU) at different taxonomic levels (phyla, class, family, individual OTU) for each of
 3 the snow treatments: control (CTL), 100% more snow accumulation (DEEP), 50% more
 4 snow (INT), and 25% less snow than control (LOW).



5 Fig. S16 – Heatmap of raw gene abundance (# of OTU's) for all detected Phyla. Columns
 6 represent snow accumulation treatment groups.
 7

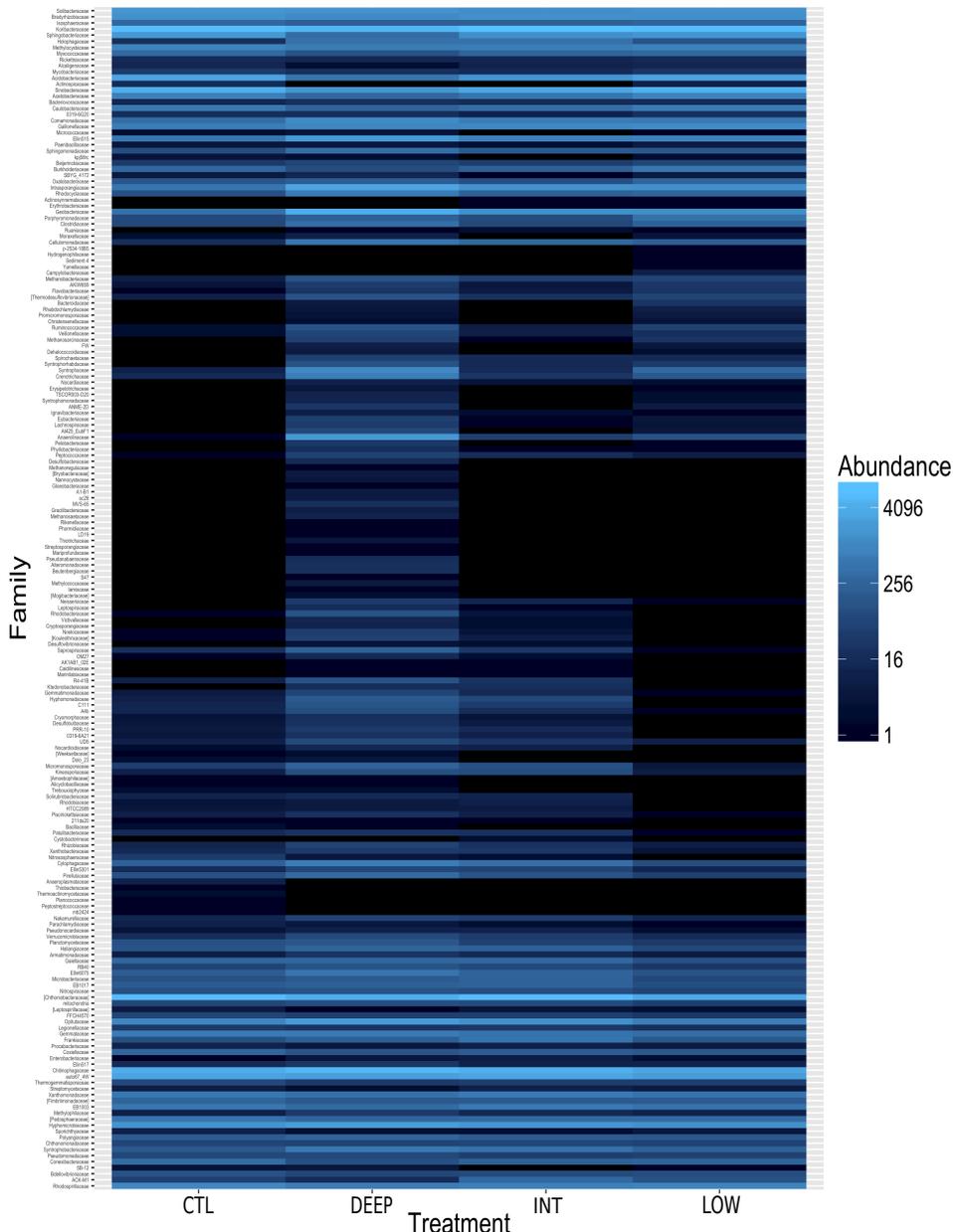
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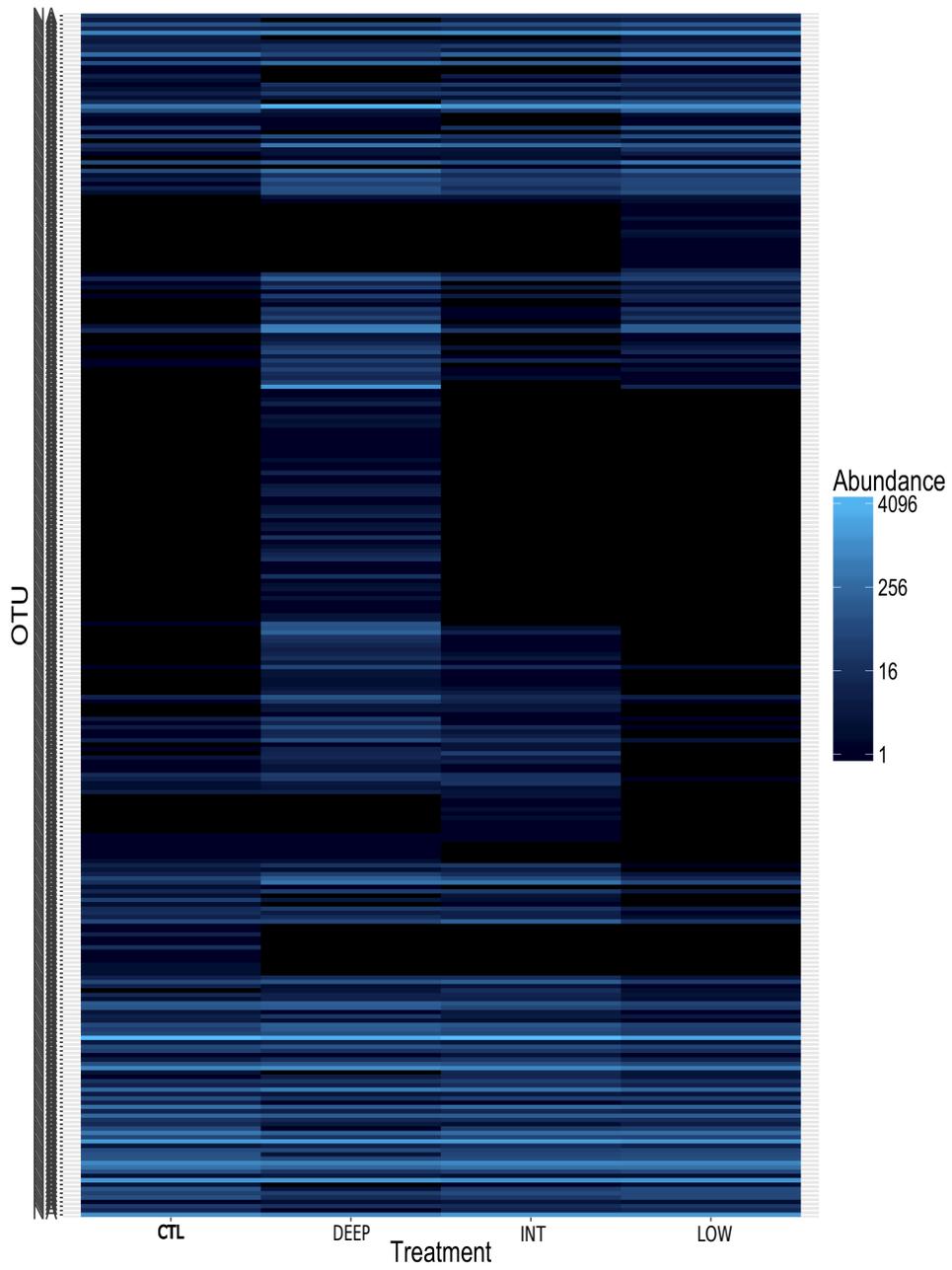


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Fig. S19 - Heatmap of raw gene abundance (# of OTU's) for all detected Families. Columns represent snow accumulation treatment groups.

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Fig. S20 - Heatmap of raw gene abundance (# of OTU's) for individual OTU assignments.
Columns represent snow accumulation treatment groups.

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