SOILD

Interactive
comment

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

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

Anonymous Referee #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. To clarify, we added, "While snowfall varied from year to year, the wind drift from the fence provided consistent relative snow accumulation at similar distances from the fence every winter." to Page 5 lines 29-30 – Page 6 line 1.

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 $p < 0.05$.

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.

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 contradictive. Omit "or less" or add "increased or decreased winter insulation".

Response: Agreed! 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.

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! 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.

C3

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).

Introduction Page 3 L 1: How can the stability of the structure be threatened? I suggest to change to more ecological terminology.

Response: 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.

L11 Needs reference

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

L12 Needs reference

Response: 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: We have omitted "...activity and..." from Page 4 line 16 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: We have removed, "...is unable...", and replaced it with "...results in a reduced ability..." on Page 5 lines 10-11 of the revision.

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?

Response: Each of these of these questions have been addressed separately. Please see below: -We did not collect detailed vegetation surveys for each plot. -Replicate distance = I estimate ~15-20m. We added "approximately 15-20m apart" to Page 6 line 12. -Number of cores/compiled – Added "replicate" and "and analyzed separately" to Page 6 lines 12-13. Added "of each soil core" and deleted "soil" to Page 7 line 7. -Control – Added ">30m" to Page 6 line 4. We also 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 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.

L29-30 Soil Survey 2015 is not listed in references

Response: 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 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.

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

Response: Yes. 3 – CTL, 3 – DEEP, 3 – INT, 3 – LOW = 12 cores. We added "(for a total of 12 soil cores)" to Page 6 line 13. We also made alteration throughout the Methods section and figures to be more transparent on sample sizes.

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. To clarify, we added "To maintain consistency, only these samples were used to analyse %C, %N, and pH relationships." to Page 7 lines 16-17 of the revision. Also, we added "To ensure accurate comparisons, soil chemical properties were measured from the same samples that DNA was extracted from." to Page 9 lines 10-11 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: Changed "...and was more variable (ranging from 15-36cm soil depth) due the varying depths of transition." to "(mean soil depth = 25.1cm $\pm$1.7cm)" in Page 7 lines 11-12. Added "(mean soil depth = 14.8$\pm$1.8cm)." to Page 7 line 10. Changed ",typically between 0-6cm soil depth, except in one case where the top 10cm was

primarily plant tissue" to "(mean soil depth = 5.6±1.3cm)" in Page 7 lines 8-9.

L22 Caporaso et al is not included in the reference list

Response: We added this to the references in the revision.

Page 8 L 4: "six most abundant phyla" this requires a quantitative documentation for the six phyla.

Response: We will add ",comprising 82% - 96% of total detected phyla per sample," to Page 8 lines 10-11.

L 18-20 needs reference(s)

Response: Added "(Sinsabaugh et al., 2008; Waldrop et al., 2010;. . ."to Page 8 line 25 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 horizon samples were analysed independently as suggested. We have removed the lower case letters "c" from the %C column, since that analysis was not part of the organic horizon analysis, and there were no statistical differences in the mineral horizon. Also, 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 the Table description, Page 31 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 the Table description, Page 31 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. We will clarify this by replacing, "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 16. The ordination of the Bray-Curtis distance matrices using NMDS yielded two convergent solutions found after four tries, resulting in 2 dimensions.

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. To clarify, we altered the sentence on Page 9 line 13, 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. We have corrected this in the revision. Page 9 lines 28-29. Sample sizes were added to clarify effect size on Page 9 lines 28-29 of the revision. Also, "(n=12 per treatment)" was added to Page 6 lines 30-31 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. Therefore to address this comment, we added "only slightly" to Page 10 line 11 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. 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: 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 also added n-fold changes to the Results in the revision.

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. We will add, "in the DEEP zone" to Page 11 line 20 and separate the sentence into two to clarify the difference.

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

Response: 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 an increase in genes necessary for N mobilization (CTL/DEEP – p=0.14) and a decrease in genes necessary for phosphate mobilization (CTL/DEEP – p=0.39)." on Page 11 lines 24-26.

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. We altered this in the revision as suggested.

L25: omit "simple cellulosic and"

Response: We deleted this in the revision as recommended.

C10

Page 12 L10: only moderate changes and in many cases they observed only trends. Please, focus on significant results p<0.05.

Response: To address this, we replaced the word "phylogeny" with the phrase, "community structure" in Page 12 line 21. 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 is 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.

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

Response: Thank you for your comment. We added, "...bacterial communities may be forced to use more labile C sources such as microbial biomass or root exudates, ultimately causing SOC to increase over time." to the introduction, Page 5 line 12-13.

L13: What is "SOM enzyme activity"?

Response: We replaced " decreased SOM enzyme activity" with "a decreased abundance of genes associated with SOM decomposition" in Page 12 line 29.

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 18 lines 3-13. We also added the reference "(Rocca et al., 2014)" to Page 13 line 1.

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: We have change the wording as recommended. Blanc-Betes reference

has been removed from this sentence in the revision.

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: This sentence was removed from the revision. Carbon content is not a good indicator of carbon-stock. Degrading permafrost often results in soil consolidation (loss of ice collapses soils) with associated changes in bulk density and depth redistribution of soil and C. The C-stock profile change as a result of the snow fence treatment is part of another paper. Carbon stock over the soil profile to the average active layer equivalent depth was 7% higher for the intermediate than for the control. Here we have used %C in our analyses because most of the C (if not all) is accessible to microbes (these acidic tundra soils have little to no physical aggregation, JD Jastrow personal communication). Therefore the factors affecting organic matter readiness to microbial decomposition is likely the chemistry/quality of the organic matter (%C, C/N) in addition to temperature and moisture. We hope this is made more clear in the revised manuscript. Also, we acknowledge that the use of the word "content" when referring to our %C data may have been misleading. To clarify, we have changed the phrase "C (or N) content" (i.e. C stock) to "C (or N) concentration" throughout the manuscript. Specifically at the following locations: Page 2 line 11, Page 6 line 24, Page 10 lines 10-11, and Page 14 line 22.

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.

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. We have added some of these cross-references throughout the discussion.

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.

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.

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

Response: We have added the citation to the reference list.

L28-30: Omit.

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

Page 14 L12-13: Results and no discussion.

Response: R2 and p-values have been removed in the revision. 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 29-31 and Page 15 lines 1-6.

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.

L28/29 Results and no discussion.

Response: R2 and p-values have been removed in the revision. These results high-

light 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 10-20.

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: 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 decomposers." on Page 15 lines 10-16.

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, we have removed the concept of increasing N from the sentence.

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" on Page 15 line 26 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 that 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 peroxides, phenol oxidases, and laccases in our data.

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

Response: We have added the reference "(Rocca et al., 2014)" to Page 16 line 21 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 comment for Page 12 L18/19. We will examine how normalizing the gene abundances by %C effects our results.

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

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

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. 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 3-4 and to the References section.

%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 17 line 22 – Page 18 line 3.

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

Response: We removed this citation from the revision. However, it does occur throughout the manuscript, so we have added the citation to the reference list.

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. For clarification, the sentence on Page 17 lines 12-14 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."

L21 change to "genes encoding enzymes involved in organic N degradation"

Response: This change has been made in the revision on Page 17 line 15 as follows:" genes encoding for enzymes involved in N mobilization,".

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

C16

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. We have modified the text on Page 17 lines 14-21 to reflect these interactions referring to SOM in general rather than microbial biomass decomposition.

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: This citation has been added as suggested.

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! The text has been updated to clarify these points on Page 17 lines 26-30 – Page 18 lines 1-3.

L26: needs reference

Response: This sentence has been removed from the revision.

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: We added "From the results of our study" to Page 18 line 17. We added "in the organic soil horizon" to Page 18 line 19.

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

Response: We moved the citation in the revision as suggested.

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: We altered Page 7 lines 10-17 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±1.3cm; 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±1.8cm; 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±1.7cm; 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 also added sample sizes to Table 1 in the revision We altered Page 10 lines 10-11 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 13 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: Please see response above. Alterations were made in the Methods of the revision to clarify replicates and sample sizes. We also altered the Figure 2 caption Page 33 lines 3-5 as follows "Each point represents the bacterial community structure within one of the 41 total samples used for DNA extraction from a variety of soil depths (Organic, Transition, and Mineral)." We have decided to keep the ellipses to better visualize the separation (or lack thereof) between the treatments.

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. We added the phrase "snow accumulation treatment" to the caption of both Figures. âĂČ

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