

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

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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.

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

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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? 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.

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

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". L8 "context of ecosystem response to climate change." Please change to "context of expected ecosystem response to ...". L15 "most abundant phyla" requires a value about the contribution of these phyla on total abundance. "20% or 80% of total detected phyla?" L26 The authors did not study the temperature sensitivity of extracellular enzymes (*sensu str.*) they are requested to omit any statement/conclusion about this.

Introduction Page 3 L 1: How can the stability of the structure be threatened? I suggest to change to more ecological terminology. L8 Anisimov and Vaughan must be changed to Anisimov et al. or the respective reference must be added to reference list. L11

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needs reference L12 needs reference P4 L16 omit activity or kinetics – I prefer the use of the term “kinetics”. P5 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”.

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? P5 L24 “strategically” needs further explanation. P5 L29-30 Soil Survey 2015 is not listed in references P6 L1 “regime”: the tested climate change scenario is: variable precipitation (that may induce differences in soil temperature) but constant air temperature. P6 treatment/factor levels: -25% vs. +50% (vs. +100%) are not equally selected. This might be problematic for ANOVA. Please check. P6 L9: n=3? Total number of sampled cores = 12? P6 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? P7 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. L22 Caporaso et al is not included in the reference list P8 L 4: “six most abundant phyla” this requires a quantitative documentation for the six phyla. P8 L 18-20 needs reference(s) P8 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

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estimates (and consistent) of the central tendency and variation of the data than mean and SD. 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. P9 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? P9 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?

Results P9 L18ff 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. P9 L30: p=0.32 I would not consider this as a significant difference. P10 L1: p=0.14 I would not consider this as a significant difference. L2 p=0.06 indicates a tendency L11 Verrucomicrobia and Actinobacteria p-values indicate tendencies. Section 3.2. in many cases the order of magnitude of the relationships were rather low. P11 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? L10 “N mobilization genes” Do the genes mobilize N? Please, correct. L12-13 It might be more meaningful to write as follows: “... included an increase in genes encoding enzymes involved in ...”.see P15 L16-17 L25: omit “simple cellulosic and”

Discussion P12 L10: only moderate changes and in many cases they observed only trends. Please, focus on significant results p<0.05. L12: “towards more labile sources” if it is an important “pathway” then the term/concept “labile” requires definition in the introduction section. L13: What is “SOM enzyme activity”? L14: The positive rela-

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tionship between gene copies and enzyme machinery requires a reference. 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 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? L22/23 Significant changes were observed only for a few groups. L28/29: What are the possible links to enzyme production and functioning? L29: Sangwan et al. is not included within the reference list. P13 L7 “cold saturated soil” Did you mean “cold, water-saturated soil”? L7 Costello and Schmidt . is not included within the reference list. L28-30: Omit. P14 L12-13: Results and no discussion. L26 omit “(Stress . . .) L28/29 Results and no discussion. P15 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. L7-8: Is this a logic relationship: increases in tannins and increases in N availability? L9 The decrease in C% might indicate exactly the opposite of the mechanism described above. 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?) L23-27: Does an interaction between tannins and extracellular enzymes necessarily induce shifts in gene abundances? Statement requires a reference. P16 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? L4-5 Sullivan 2008 is not included within 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)? L12 Blanc-Betes et al. Submitted: not included in reference list L19: Which alternate energetic pathway? L21 change to “genes encoding enzymes involved in organic N degradation” L22: Microbial biomass was frequently reported to be positively related to enzyme production and decomposition. Please, describe more precisely under which circumstances an

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increase in N availability and microbial biomass results in a decrease in decomposition rate. P17 L6 needs a reference - for example: Razavi et al. Front. Microbiol., 14 October 2015 | <http://dx.doi.org/10.3389/fmicb.2015.01126> L7-8 Speculative. If in-situ substrate availability is low, V_{max} will not be reached and enzyme functioning is controlled by K_m (Michaelis-Menten constant). Alternatively, authors may change to “reach the same catalytic rate”. L26: needs reference 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”).

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

Table 1: Describe in Methods how many repetitions and analytical replicates were used for the data. $a > b$, 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? 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). 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.

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