SOIL Discuss.,
doi:10.5194/soil-2015-89-RC3, 2016

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

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Discussion paper

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

These are my specific comments:

Abstract: p.2, l.4 – Does “more or less snow” mean that predictions on amount of

precipitation are not sure yet? Please consider rephrasing. p.2, l.11 – I recommend writing “Microbial community DNA was extracted from soil”. p.2, l. 15 - Taxonomic names should be written in italics (throughout the manuscript).

Introduction: p.3, l.3 – Do you refer to belowground “microbial” community structure? p.5, l.1 + l.16 – Please change microbial into bacterial community. p.5, l.14 – bacterial functions (plural).

Methods: p.6, l.1 – How far away from the snow fence was the CTL sampled? p.6, l.9 – What was the distance between the replicates per treatment? p.7, l.4 – I couldn’t find the results of the bacterial communities from the transition zone; and was DNA extracted from all three replicated cores per treatment? p.7, l.16- Could you please add the reference for the primers? p.7, l.17 delete – in Mi-Seq but add to p.8, l.7 Bray-Curtis. p.8, l.6- Why did you determine adequate sampling depth? – I could not find that result later on. 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”. p.8, l.27 – later there is also a significance threshold of $p<0.1$ used.

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. p.10, l.6 – delete “and”. 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. p.10, l.11 According to the text p=0.011 for Chloroflexi but there is only one * in Fig. 3. 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”. p.10, l.19 please check p-value for Actinobacteria text vs. Fig. S4. p.11, l.6/7 Were lignin, pectin and xylan degradation not significantly different to the CTL? 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. p.11, l.18 – Are Figs. S7-S9, S11 needed since they are not mentioned in the text? p.11, l.23-25 - Please move this sentence to discussion and refer to Table S1.

Discussion: p.12, l.11 Please explain from which results the conclusion of “reduced SOM decomposition” was derived from! p.12, l/12/13 – I don’t agree with explanation 1) since you did not find differences in soil moisture along the gradient. p.12, l.30 – Actinomycetales are a bacterial order containing several taxa, thus please use plural . 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. 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. p.14, l.9 and following- I suggest to delete R2 and p-values from the discussion. 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. 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?

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? According to Methods part you measured temperature at 4 different depths but not at 12 cm. Was there no post-hoc test done for %N, C/N, and pH? 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). 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. 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.

References (typos): p.22, l13 Gonzalez-Meler p.23, l34 mcrA in italics p.27, l13 CO2

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