

Biogeosciences Discuss., author comment AC1
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Reply on RC1

Niel Verbrigghe et al.

Author comment on "Soil carbon loss in warmed subarctic grasslands is rapid and restricted to topsoil" by Niel Verbrigghe et al., Biogeosciences Discuss.,
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The manuscript presents a study of SOC stocks to 30-cm depth in a unique long-term soil warming study based on natural geothermal gradients. The manuscript presents differences in SOC stocks along warming gradients at sites that had been warmed for 5-10 years (medium-term) or 5-55 years (long-term) at the time of the study. The key finding is that C stocks in the topsoil declined with increasing temperature after 5-10 years of warming, but not after 50-55 years, and subsoil C stocks (long-term site only) did not differ with temperature.

I read the manuscript with great interest, as data on changes in subsoil C stocks are rare, as are such long-term climate change experiments. The topic is certainly appropriate for Biogeosciences and although the overarching research questions and hypotheses are interesting, I am not entirely convinced that the present study address them in full.

We thank the reviewer for the thorough reading of our manuscript and its critical evaluation. We have tried to answer the questions in the best possible way and edit the manuscript accordingly.

First, the chosen soil sampling depth needs to be justified, as many researchers would not consider 10-30 cm depth as 'subsoil'.

A justification of the chosen soil sampling depth for topsoil and subsoil is given in the material and methods section on lines 132-135. Differentiation between topsoil and subsoil is based on three strong differences between the two layers: lower soil C% (figure B2), lower fine root density (figure B5) and higher bulk density in subsoil (figure B7).

To increase clarity about how we differentiated between topsoil and subsoil, we added a reference to the justification in the introduction of the main text on line 34-35: "An elaboration on the choice of these two soil layer depths is provided in the material and methods section in appendix A."

Second the 10-30 cm depth was only sampled at the site with long-term warming. So it is entirely unknown whether the deeper soils experienced similar short-term effects to the topsoil.

We acknowledge the shortcoming of our experimental design, due to the shallow soil depth in the medium-term warmed grassland. In our manuscript however, we only report

about the long-term warming effects on subsoil. We believe the finding about stable subsoil subject to long-term warming is relevant, also without knowing the short- or medium-term warming effects.

In addition, there are a few key points that should be considered in the interests of clarity and scientific rigour:

First, the language of the present manuscript is somewhat misleading because 'SOC losses' are referred to throughout, but actual C loss was not measured. The space-for-time approach used in the study demonstrates differences in SOC stocks between plots that have been warmed to varying degrees for different lengths of time, which is not the same as measuring C losses. The language in the text should be edited to reflect this.

The reviewer is right that we did not measure the actual C losses, which occur through both leaching and respiration. For estimation of the C loss through respiration, eddy covariance would be needed, which is impossible to measure on these small scale warming gradients.

To make sure the used terminology corresponds with the actual processes measured, we changed 'SOC losses' to 'SOC stock losses' throughout the manuscript.

Second, the reasoning behind the hypothesis of long-term warming needs a justification – is 50 years sufficient for a new equilibrium to be reached? And why would you expect the new equilibrium to be reached at lower SOC content? Theoretically, maintenance of SOC stocks might also be predicted over the longer term if, e.g. acclimation of microbial communities and C turnover rates, increased plant productivity, or declining nutrient availability with long-term warming eventually compensate for initial losses... A rationale for this hypothesis could be provided by drawing on previous research from the site (currently discussed on lines 76-85).

We fully agree that the processes raised by the referee could lead to a maintenance of the SOC content, but at the same time many studies have suggested a larger temperature sensitivity of SOC decomposition than of primary productivity. We added a sentence on this to introduce the hypothesis on line 17-18: "However, soil warming could also be expected to result in increased and/or unaltered SOC stocks, if, e.g., there is rapid acclimation of microbial communities or if plant productivity increases strongly."

Additionally, we assume 50 years will suffice for the ecosystem to reach a new equilibrium. If not, the system should already be very close to equilibrium. Since any statement on this would be pure speculation, we did not change the text.

Third, the introduction states clearly that the processes involved in SOC formation and mineralisation are rarely studied below 20-30 cm depth, which sounds like a justification to study subsoils below 30-cm depth - so why was only 0-30 cm considered in this study? The justification for the split between 0-10 and 10-30 cm is given, but it does not explain why the 10-30 cm increment should be considered as subsoil, nor whether it is likely to be representative of subsoil at greater depths. Indeed, subsoils are often considered as being below 30-cm depth.

Indeed, subsoils are often regarded being below 30 cm depth. For the three reasons given before however, we believe the 10-30 cm depth can be considered as subsoil on our experimental site. We agree with the reviewer that studying soil layers deeper than 30 cm would also be extremely interesting. A new PhD student is currently studying deep soil SOM stocks, and the preliminary measurements in the LTW grassland did not reveal changes in SOC at greater depths.

Finally, I'm not entirely convinced by the argument that C inputs did not increase with warming. If I understand correctly, the evidence for the lack of changes in C inputs (presented in figure B3 and B5) is based on measurements and samples collected after the first 5 years of warming. So how do you know there were no short-term changes in C inputs during the first 5 years? The major losses in the first 5 years could be an artefact of the sudden increase in soil temperature – what precludes a similar short-term increase in plant inputs? At the long-term site, I also wondered whether gradual change in temperature and growing season length over the last 50 years could have partially compensated for early C losses?

From the start of the experiment, we continuously measured LAI and NDVI, and observed no increases in the seasonal maximum. Hence, we can preclude a short term increase in plant productivity and soil C inputs.

As the reviewer indicates, a short-term overshoot on many ecosystem parameters and a (partial) mitigation in response to long-term warming was documented in Walker et al. (2020). However, on SOC stock loss alone, there was no observable, statistically significant SOC increase after five years of warming. Hence, we did further not elaborate on a possible mitigation after five years of warming, but rather focussed on a rapid SOC stock loss in topsoil.

*Walker, T. W., Janssens, I. A., Weedon, J. T., Sigurdsson, B. D., Richter, A., Peñuelas, J., ... & Verbruggen, E. (2020). A systemic overreaction to years versus decades of warming in a subarctic grassland ecosystem. *Nature ecology & evolution*, 4(1), 101-108.*

Data analyses

The data analysis section should clarify how the data were handled and what was considered a replicate. In the methods section, the transects are referred to as replicates but the 6 plots per transects represent different temperatures. I therefore assume that the models are based on plot-level data. At the very least, transect or sampling plot should be included as a random effect in the models (ideally plot nested within transect to account for the experimental design). Including location in the models could help deal with the high variability. In addition, figure 1 shows regression lines but no regressions are described in the analyses.

We thank the reviewer for her input on the model regression analyses. The linear mixed model as used for analysing most of the data in the manuscript is described in the lines 203-214. We used sampling year as a random factor to account for sampling differences and interannual variabilities between the two sampling campaigns.

Using plot ID as a random factor would confound the model results, as soil warming level is inherent to the plot ID, and soil warming is included as a main factor. As the reviewer suggested, we included transect as a random factor, in combination with sampling year in a crossed random effects design. This adaptation did only slightly alter the results and did not affect the conclusions of the manuscript. The statistics were edited in the figures and throughout the main text.

I note that these issues do not detract from the interesting and potentially important findings on the differences between soil depths and long- vs. short-term warming. However, the presentation and discussion of the results should be revised to ensure the main messages are accurate and the limitations of the study are clear.

With kind regards

E.J. Sayer

Additional minor comments by line:

L15 – suggest replacing “lead to increased” with “increase”

The reviewer’s suggestion was implemented.

L17 – what is meant by “sign”? Do you mean whether the feedback is positive or negative? This could be rephrased to make it clearer.

We indeed meant whether the feedback is positive or negative. The paragraph was extended, and the word ‘sign’ replaced with ‘direction’ to clarify: ‘ However, soil warming could also be expected to result in increased and/or unaltered SOC stocks, if, e.g., there is rapid acclimation of microbial communities or if plant productivity increases strongly. This implies that the strength and even direction of this carbon cycle-climate feedback are, highly uncertain (Crowther et al., 2016; Todd-Brown et al., 2018; van Gestel et al., 2018).’

L20 – omit soil before SOC

‘Soil’ was omitted.

L22 – extrapolations of responses from, or model parametrisation based on, short-term experiments

A comma was included in the sentence.

L27: above it

The suggestion was implemented.

L57: lose SOC (not ‘loose’)

The error was corrected.

Line 67 states: “Even grasslands that had been warmed at least 55 years exhibited no larger SOC loss than that observed after 5 years of soil warming.” How were SOC losses over 55 years assessed without analysing samples from 55 years ago? Or does this instead mean that the SOC stocks in the long-term warmed transects were similar to those in the medium-term transects?

This does indeed mean that the SOC stocks in the long-term warmed transects were similar to those in the medium-term transects. Hence, the sentence was edited to match the observations exactly: “Even grasslands that had been warmed at least 55 years exhibited no larger SOC stocks than that observed after 5 years of soil warming.”

L81-83: you present no data for microbial communities in this study, so upon what basis do you infer there is no evidence for physiological adaptations or compositional shifts?

The basis for this claim is in the next sentence. To make the meaning of the paragraph more clear, we edited the paragraph as following: “Alternatively, ephemeral SOC stock loss under warming may have resulted from physiological adaptations (Allison et al., 2010; Bradford et al., 2019) or compositional shifts (Melillo et al., 2017) in the microbial community. However, previous research in these grasslands showed that soil microbial

carbon use efficiency (CUE) remained constant under short- and long-term warming (Walker et al., 2020), and microbial community composition was only affected by more intense (>9 °C) long-term warming (Radujković et al., 2018), meaning evidence for such mechanisms is lacking.”

L103-106: The differences to the other studies referred to here could be partly explained by differences in sampling depth, e.g. Lin et al. considered soils to 60 cm depth, Soong et al considered soils to 1-m depth and even Jia et al. considered 30-40 cm. In addition, I believe that all of these studies focussed on early or short-term changes in SOC, which were not considered for the subsoil in this work.

As the reviewer suggests, the short-term effect of the other studies might play a role here. However, Lin et al. and Soong et al., considered forests and Jia et al. found grass roots up to 85cm depth. Hence, all these cited studies have a deeper rooting zone than ours, which we think is a more important determinant.

L165 states that medium-term warmed soils were too shallow to sample deeper than 10 cm and yet lysimeters were placed at 30-40 cm depth (L177). If there were at least some sites with deeper soils in the medium-term transects, why were they not sampled?

We understand the concern of the reviewer. The diameter of the Prenart Super Quartz lysimeter was only 21 mm. Because of the small diameter, coring before installing the device was feasible up to 30-40 cm depth down between the rocks.

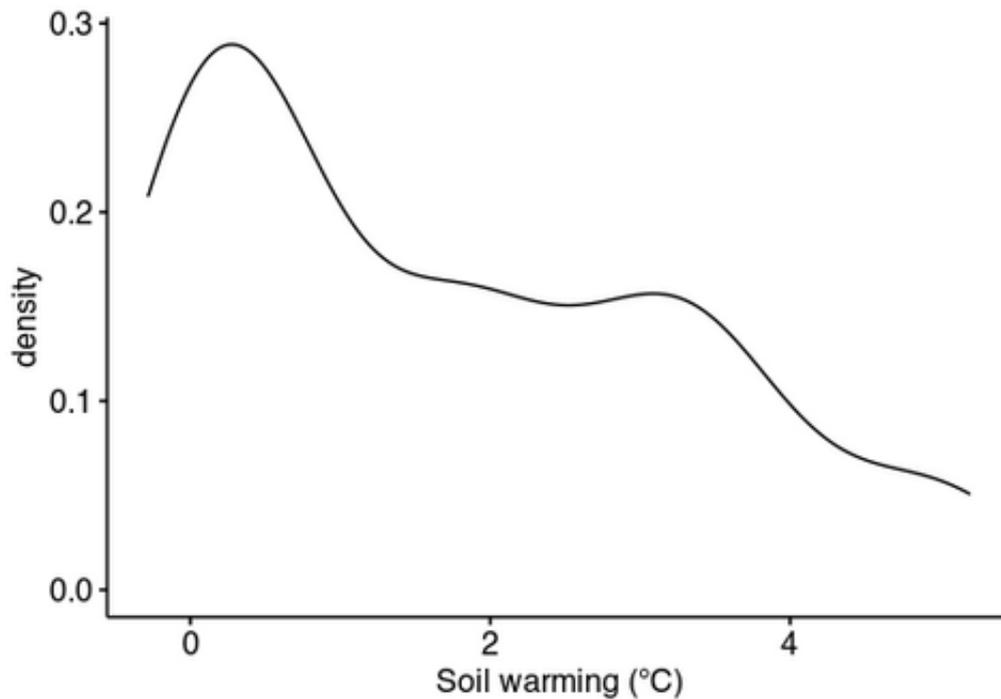
L130: incorrect spelling of Agrostis

We thank the reviewer for pointing out this error. It was corrected.

Figure 1: There seems to be a bias towards more plots at the lower end of the warming gradient, whereas at the hotter end of the gradient, it looks like there are only 3 plots – It would be useful to give an indication of the spread of the data along the warming gradient.

If most soil C loss occurred during the first 5 years of warming, why does the regression include data from both sampling times? It also looks like not all plots were sampled at both times – is this correct?

The reviewer is right that the density of our plots decreases with soil warming (see figure below). However, since there are no assumptions in linear model about the distribution of the independent variables, this should not be a problem for our statistical analysis. The residuals of the linear mixed model met the normality and homoscedasticity requirements.



The regression line as shown in figure 1 shows result of the linear mixed model. Hence, no separate regression lines were shown for different grasslands (MTW vs. LTW) or sampling times, since there was no statistically significant effect of them in our model.

All plots were sampled at the sampling campaigns in 2013 and 2018. Unfortunately, for some plots, the sample measurement failed or the data point was removed because it was an outlier (deviating more than 3 standard deviations from the mean of the data set). This was the case for 4 out of 122 data points.

Figure 2: The smoothing lines are misleading, because they imply “no change” between 10 and 50 years, for which there is no evidence

The reviewer is right that in our 'space-for-time' approach, we cannot infer that there would not be any change from 10 to 50 years of warming. The smoother lines in figure 2 show that we do not find a difference in soil warming response between 10 years of warming at the MTW grassland and 50 years of warming at the LTW grassland. As this conceptual graph is a representation of the rationale of our manuscript, we believe it can be left in as it is.

Figure 3: Are these the data for topsoil C fractions? Please clarify in the legend. Are there fractionation data for the subsoils?

Indeed only topsoil data was used for figure 3. This is clarified in the first sentence of the legend: “Relative mass, soil C % and absolute soil C amount of soil aggregate fractions originating from topsoil in the medium-term warmed (MTW) and long-term warmed (LTW) grassland.”

Unfortunately we do not have fractionation data for the subsoils. For analysis of the subsoil fractionation data we refer to Poeplau et al., 2017.

Poeplau, C., Kätterer, T., Leblans, N. I., & Sigurdsson, B. D. (2017). Sensitivity of soil carbon fractions and their specific stabilization mechanisms to extreme soil warming in a subarctic grassland. *Global Change Biology*, 23(3), 1316-1327.