Point to Point response to Interactive comment and comments of two referees:

(Answers in *bold* and actual changes in the manuscript marked in *red*)

Interactive Comment:
Dear Christopher, Páll and Bjarni
It was a pleasure to me to find a manuscript which deals with a very similar fractionation approach and which presents an Icelandic study. Hence, it is very similar to my research which I did in Iceland some years ago. My memories from the southern part of Iceland came back... Therefore, I read the manuscript of your study and would like to give you my comments.

1) It is ingenious to use a natural heat source and the resulting warming gradient to study ecosystem changes. This is likely possible on a volcanic island like Iceland. But on volcanic islands, soil properties differ from other soil types of non-volcanic regions in the boreal ecosystem. How much are your results applicable to the rest of the boreal ecosystem? What could be the limitations?

Answer: Each case study has its limitations and should not be extrapolated to whole biomes. This is true when talking about absolute rates of change, while the direction of change or certain response mechanisms might well be more generic. The soil of this study is a quite specific one, and we agree that we have not accounted for this enough in the previous version. We now mention the word Andosol in the title to clarify the focus.

2) You mentioned that the worst scenario in the IPCC report predicts a temperature increase of 11°C for the region North of 60°N. Your temperature sequence ends at +17.5°C. Can you link your results to the different IPCC scenarios with regards to the maximum predicted temperature increases in the IPCC report? Can you make any assumption, how the SOC change would look like at +11°C (IPCC report) based on your temperature sequence (5.8°C, 17.5°C). By the way, does the referenced temperature increase (+11°C) corresponds to the air temperature? In this case, how do you link the increase of air temperature to the increase of soil temperature? Does soil temperature increase in the same way and with the same slope gradient in the future and therefore does your soil warming gradient corresponds to the assumed soil temperature increase in the future for boreal ecosystems? Please clarify this in the introduction part.

Answer: This comment includes a few important remarks at the same time. Of course, soil warming is not equal to air warming, and the source of warming may have a strong effect on the ecosystem response. The difference between soil temperature and air temperature is very site specific and we think that it is hard to predict how much air temperature increase is necessary to increase the average soil temperature e.g. by 5.8°C at that specific site. However, based on the most pessimistic IPCC extreme scenarios, we think that at least a warming of 5.8°C is within the realistic range. We have now been clearer in the discussion when we talk about warming if we refer to air temperature or soil temperature (also because one reviewer requested that). However, the introduction is not the place to discuss shortcomings of geothermal warming and also not how we can link our results with any IPCC scenario or how soil temperature will increase in the future. There is a lot of uncertainty involved, especially regarding the long-term effects of soil warming. And this is the reason why we mention (in the introduction) that long-term warming experiments are needed. Also, *the warming response of bulk SOC was linear, so it is easy to derive a number for any of the suggested warming scenarios, if you wish so - with high uncertainties: only one soil, soil warming not air warming, warming from below, very abrupt warming vs. long term gradual warming… Thus, we do not want to speculate too much and go too far into this direction and see the strength of this experiment more in the fact that we have strong and long soil warming to study mechanisms.*

3) Based on Comment 2, could you make any statements about the impact of temperature increase on SOC regarding the different IPCC scenarios (e.g. SOC change according to the smallest temperature increase).

Answer: For mentioned reasons (in answers 1 and 2): No. Recent papers (e.g. Crowther et al.) tried to predict changes in SOC until the end of the century or until 2050 based on short-term warming experiments. We think that this is problematic for several reasons and do not dare to directly conclude from our still relatively short-term warming experiments what will happen under global change in the long run.

4) In your study the temperature increased within 10 years. The modelled increase of air temperature will however change within several decades. What would you think, is the different time scale irrelevant concerning the change of SOC and the soil processes which control the SOC?

Answer: No, it is surely not irrelevant. We might see an overshoot-reaction here, or things might level off soon. We have indications for both temporal dynamics for many parameters measured at the ForHot Sites, and there will be a Nature Ecology and Evolution paper on that exact aspect soon (Walker et al.). The main message is: Be careful with linear extrapolations of short-term warming experiments. The doubts that you mention in your last 4 comments are all correct, but I think at the same time it is pretty clear that our experiment cannot solve the equation for all the boreal zone, all IPCC scenarios and until the end of the century. To clarify this, we added the following sentence to the discussion (p8, l.37ff: "However, the transferability of the results in this study to the SOC response to global warming is still rather limited and can only slightly reduce given uncertainties: i) we studied soil temperature, not air temperature increase, ii) the warming occurred abruptly and not gradually, iii) we studied an Andosol. Extrapolations to larger areas or longer time periods should thus be done carefully and were not intended with this study."

5) Is the vegetation (grassland or forest ecosystem) also changing during the warming within these 10 years? If there is also a change in the vegetation composition or the supply of OM to the soil phases, might these changes also be responsible for the changes in your SOC results? In this case, the increase of the temperature is not the only independent parameter that changes.

Answer: Yes, there were some changes in vegetation, while those were most pronounced in the most extreme warming intensities >15°C: For example, in the forest many trees died after this extreme and abrupt soil warming event. However, the changes in SOC we see are very gradual (along the temperature rise, so this is not reflected in the SOC data). Also, there is more understorey herbaceous vegetation now in the warmest treatment, so the input of C might not even be smaller. However, for both ecosystems we actually detected a decrease in root biomass and (although less pronounced) also in litter biomass. It is thus quite realistic, that changes in SOC are also partly driven by decreased C input, although biomass turnover *is not comprehensively measured yet. So yes, temperature increase is the independent parameter that changed in the very beginning, but then there is a whole chain of mechanisms that could lead to changes in SOC and SOC fraction distribution. We don't go into too much detail here, because that was not the focus of the study and data availability is partly scattered. However, we added the following sentences (in section 4.1): "In fact, root biomass in 0-10 cm decreased in both ecosystems (data not shown), leading to weak positive correlations (R^2=0.37 for forest and R^2=0.29 for grasslands) of SOC and root biomass. Also aboveground plant litter tended to decline in both ecosystems. This suggests that SOC losses were partly driven by decreasing C input with warming and not by increased microbial activity alone. However, a clear picture on absolute C inputs in the experimental plots is not available yet, since it needs to consider NPP and biomass turnover at the same time."*

6) Discussing the change of soil structure and SOC content (within SOC fraction), you might also need to have a look at the soil mineralogy. Did you analyse the volcanic clay minerals? Volcanic clay minerals and the abundance or change of metal-humus complexes, allophane content, ferrihydrite content can also explain the changes within more resistant SOC fractions.

Answer: This would surely be an interesting thing to do, especially for a better characterization of the soil, but was not done in this study since it might not be too relevant to explain the observed results. We think that i) temperature changes were not high enough to change soil mineralogy and ii) mineralogy is more related to SOC stabilization mechanisms in the finest fractions (including microaggregates), while here we observe the strongest changes in the larger fractions. We believe that the response that we observe (i.e. a break-down of large micro- and macroaggregates) is more controlled by biotic than by abiotic drivers. Organo-mineral interactions and aggregates in Andosols are known to be particularly strong and almost impossible to disintegrate, but what we see here is a break-down of aggregates due to warming. It seems unrealistic, that this related to the specific mineralogical features of an Andosol.

7) What do you suggest to use as a further analysis technique to characterize the stable SOC fraction (rSOC) or the <63 microns fraction? I ask you, because I used the same fractionation technique and later, however, read that the wet oxidation step is questioned. Spontaneously, I would measure the SOC and the volcanic clay minerals in the <63 microns fraction to get an idea about the characteristics between the SOC and the soil mineralogy. Do you have any further ideas which approaches can be used when the SOC of volcanic soils is fractioned and characterized?

Answer: There are different ways of wet oxidation and the one used in this study (with NaOCl) was found to be related (in previous studies) to the amount of extractable Fe and Al in the soil, so directly to soil mineralogy, which makes sense. It seems to be those organo-metal complexes that resist that oxidation step and that form the most stable SOC. The derived fraction rSOC is mostly found to be the one that shows the least response to any change or novelty, therefore we think that wet oxidation (especially with NaOCl) gives meaningful results to some extent. But, indeed it remains a mystery why this is not the case for warming (or at least in this experiment): rSOC shows pretty much the same response as the silt and clay fraction as a whole. If this is related to the type of soil or the type of treatment (warming) remains open. Adding mineralogy data to fraction data could always be helpful, but not sure if it will help to solve that specific question. Thermo-stability, as assessed using techniques like Rock-Eval can also be promising to determine the amount of persistent SOC-with its own methodological difficulties. We have slightly changed the section on this fraction *in section 4.1: "This has been observed before and questions the notion that this oxidation-resistant pool can be linked to a centennially persistent or even inert SOC pool (Lutfalla et al., 2014;Poeplau et al., 2019;Poeplau et al., 2017;Zimmermann et al., 2007). At the same time, NaOCl-resistant SOC has often been described as substantially older and thus slower cycling as bulk SOC (Helfrich et al., 2007) and was also found to correlate to the abundance of Al and Fe-oxides in the soil (Mikutta et al., 2005). Thus, the strong warming response of this fraction is somewhat in contrast to the slow responses observed to other treatments, such as C3-C4 vegetation changes (Poeplau et al. 2018)."*

Statistics 8) How many replicates do you have per category (e.g. Fig. 4)? Is it n=5? Can you mention the number of samples per category and the number of samples within the two ecosystem datasets in section 2? It might be also useful to mention it in the capture of Table 1.

Answer: It is 5 and we have now entered another sentence on that in the soil sampling section (although it was already mentioned before in the experimental design section). Also in the table capture of Tab.1 we included it now, thank you.

9) Mention also p-values in the text when giving the regression values (e.g. page 6 line 31, Figure 6). I remember the reviews of my manuscript

Answer: Done.

10) In Figure 4 and Figure 5, what does the intervals along the regression lines indicate? Is it the 95% range of the regression value or the 95% range of the modelled value?

Answer: It is the 95% confidence interval of the regression model- this information was added to all Figure captions.

11) Changes of ecosystem processes are not always linear. Figure 4 and Figure 5 show distributions of values which could be modelled more accurate with a non-linear function. For example, the patterns of the contents of bulk SOC, SA, POM or rSOC show different slopes along the temperature gradient and asymptotic properties. Did you tested other types of functions to explain the patterns of SOC changes? There might be also a non-linear correlation in Figure 6 D.

Answer: We admit that it was a bit oversimplified to assume linearity in all cases. We now tried logarithmic fits as a second option and indeed, it did fit better in several cases. Figure 4 and 5 were adjusted and so was the Statistics section. However, in Figure 6, a linear fit was much better – the optical non-linearity might originate from the three points at the lower left corner, while there is a wealth of observations much higher than those. We therefore did not adjust Figure 6.

12) In the case of a non-linear pattern, is it useful to show only one absolute change value (g C kg-1 fraction°C-1)?

Answer: We agree that using this one value is again oversimplified in case of a clear non-linear response. However, i) the values used in the abstract (and directly in the text) refer to linear responses of topsoil and subsoil bulk SOC and ii) the values given in table 1 (for the *fractions) are used as measures to directly compare the response of different fractions. In this sense, we think it is useful to give also these values. The only other chance we see is to use the difference between the unwarmed reference and the highest warming, but we think that a linear fit is a more fair comparison, since it does not overemphasize those two values. We have now added the following sentence to the table heading of table 1: "Although this was not the best model in all cases, we used this value as a proxy to compare the warming response among fractions."*

13) In the case of a non-linear pattern, when do you expect the highest rates of change? Is this in the beginning of the warming or at the end? What does this mean for the change of the boreal ecosystem and at what time do you expect the highest changes (in the next 10-20 years or in 80-100 years)?

Answer: This is a different question: We looked at the response to a temperature gradient; you are now referring to the temporal pattern. This is a different story and very uncertain (as mentioned earlier). The dataset investigated here cannot be used to answer this important question, but we might have indications that the loss actually levels off after the first years (mentioned in the discussion).

14) I offer you to read the reviews of my manuscript. The study deals also about SOC fractions in volcanic soils in Iceland and some comments might be also useful for the revision of your manuscript. https://www.soil-journal.net/5/223/2019/soil-5-223-2019-discussion.html

Answer: Thank you.

15) In this journal, square brackets are not used to note any units (e.g. [°C]). It uses parenthesis (°C).

Answer: Thank you, we have changed it everywhere.

16) I guess that Figure 5 shows the scatterplots for the SOC contents in the subsoil (20-30 cm). Please change the title in the Y-axis.

Answer: This is correct and was changed accordingly.

Reviewer #1

In large this is a good and interesting paper. However, there are some shortcomings with regard to these types of studies; The authors themselves have discussed problems connected to interpretation of results from experiments using geothermically warmed soils in a global warming / climate change context, concerns that I also share. Though I am not convinced that the study is bringing us much forward in questions regarding the fate of carbon in subarctic forest soils in future warmer climates, I still see the relevance with regard to the fate of soil carbon in geothermically warmed Andesitic soil. I believe the most important results in this study are the relative changes in proportion of C in the different fraction with increasing temperature and this should be more in focus than the loss of C and deterioration of soil structure. I also think that the differences between the two ecosystems (grassland and forest) should be better communicated in the tittle.

Answer: We thank the reviewer for the valuable comments and suggestions. The title has been adjusted to i) narrow the focus to Andosols and ii) include grasslands. Regarding the focus, we see that loss of C, fraction distribution and soil structure are closely coupled in this case and we tried to make exactly this point in the study.

More specific comments

1. Does the paper address relevant scientific questions within the scope of SOIL?
yes

2.Does the paper present novel concepts, ideas, tools, or data?
the paper presents new date, but is not particularly novel in concept, ideas and tools.

3. Does the paper address soils within a multidisciplinary context?
Yes, in a using geothermic warming of soils as a proxy for warming of soils in climate change scenario. But does not address the ecosystem changes/ vegetation as much it ought

4. Is the paper of broad international interest?
Yes, but not as broad as the title suggests, these are Andesitic soil and thermal warming of soils do have some limitations with regard to interpretation in a global change context.

5. Are clear objectives and/or hypotheses put forward?
Yes, three objectives are stated.
1.advance our understanding of the temperature response of different SOC fractions representing kinetic pools
2.assess the role of the ecosystem type in the temperature response of SOC
3.investigate potential links between SOC loss and so
il structure changes.

6.Are the scientific methods valid and clear outlined to be reproduced?
I have some questions with regard to sampling and interpretation of the term soil structure, see below.

7.Is the soil type/classification adequately described?
fairly, general information on soil type/classification at the experimental site is given. but I cannot see that the information that the soil type/classification provides is actually used in the interpretation of the results. Though only the upper 30 cm is used in this study it would have given valuable information if this was related to soil horizons.

Answer: The whole study design was not related to genetic horizons, but to fixed depth increments, which is a standard e.g. when different ecosystems are compared.

8.Are analyses and assumptions valid?
see comments below

9.Are the presented results sufficient to support the interpretations and associated

discussion?
see comments below

10.Is the discussion relevant and backed up?
see comments below

11.Are accurate conclusions reached based on the presented results and discussion?
Yes

12.Do the authors give proper credit to related and relevant work and clearly indicate their own original contribution?
Yes

13.Does the title clearly reflect the contents of the paper and is it informative?
See comments below

14.Does the abstract provide a concise and complete summary, including quantitative results?
Yes

15.
Is the overall presentation well structured?
yes

16.Is the paper written concisely and to the point?
OK

17.Is the language fluent, precise, and grammatically correct?
long cumbersome sentences make particularly the discussion, but also elsewhere. You are really not making it easy for the reader when you write sentences like this e.g.: Page 9 lines 30 to 33 " Thereby, topsoil and subsoil samples scattered approximately around the same regression line, indicating that especially the amount of young SOC, may it be driven by warming intensity or the location within the soil profile, was a very good predictor for the amount of stable aggregates in the soil." Or page 11 line 24-26: "Together with the fact that also more labile SOC was present in the forest topsoil, which responded more sensitive to warming than the soil, it seems likely that amount and fraction distribution of SOC drove the ecosystem specific warming response in the topsoil."

Answer: We agree that some sentences, especially in the discussion, were too long and reworked those. The mentioned sentences now read as follows: "Topsoil and subsoil samples scattered approximately around the same regression line. This indicates that the abundance of young and coarse SOC per se, rather than the degree of soil warming, is driving the amount of stable aggregates in the soil." And "Therefore it seems likely that amount and fraction distribution of SOC drove the ecosystem specific warming response in the topsoil." We further changed the sentence "In consequence of SOC loss, total pore space decreased strongly as indicated by poured bulk density used as a proxy for bulk density in undisturbed samples. The latter was unfortunately not determined in the present study." Into "In consequence of SOC loss, total pore space decreased strongly as indicated by poured bulk density. Poured bulk density was used as a proxy for in situ bulk density in the undisturbed soil, which was unfortunately not determined in the present study." And "While the authors did not explicitly link that to changes in soil structure, an increase in bulk density with *associated decrease in pore space might have fostered that change." Into "An increase in bulk density with associated decrease in pore space might have fostered this physiological response, although this was not explicitly mentioned by the authors."*

18.Are the figures and tables useful and all necessary? Yes (see point 20)

19.Are mathematical formulae, symbols, abbreviations, and units correctly defined and used according to the author guidelines?
I believe so

20.Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated?
1.most of the text is relevant and to the point, but I am unsure if the "poured bulk density - soil structure" part contributes in a meaningful way.D eleting it and giving more focus to the warming effects on fractionation -ecosystem comparisons stable soil C would benefit the paper.

Answer: We do not necessarily agree on that point, and for example reviewer 2 was especially in favour of that part. We therefore decided to keep the focus as it is, although we clearly acknowledge that the proxies used are not enough to fully understand the SOC effect on soil structure in the studied soil.

2.Most of the figures are nice and useful. Figure 5 must have the wrong Y-axis tittle – subsoil not topsoil SOC. However, I wonder if it would be possible to add the warming (some sort of colour code) to Figure6. It would be interesting to see if there were any systematic also with regard to temperature.

Answer: Figure 5 has been changed. In Figure 6, we think that giving the different warming intensities would over-complicate the figure. It already consists of 4 panels and thus a high information density. In the plots before, we show how warming and SOC are correlated, so this would be redundant information to some degree.

3.I miss a table showing general soil properties such as pH, oxide extraction of some sort (Fe, Al, Si), sand silt clay%. I am not too keen on table 2 and 3 which only show summary of statistics, they are much more valuable when they are connected to measured properties, merge or delete?

Answer: Soil pH, texture and initial SOC contents are given in the text describing the experiment. Fe, Al and Si contents were not measured. Also, some more information, e.g. on soil temperature profiles, are given in the mentioned publication which is a pure site description publication. Therefore, we think that an additional table is not necessary. We also think that both, table 2 and 3 are necessary and would like to keep them.

21.Are the number and quality of references appropriate?
Yes

22.
Is the amount and quality of supplementary material appropriate and of added value?
as far as I can see yes

I have a couple of more specific comments that I think the authors need to address to improve the paper

1)I think the use of the term "soil structure" is used wrong. Soil structure has not been investigated in this study. What has been studied is stable aggregate (SA) (63-2000µm) from the fractionation procedure and the carbon (C) connected to this fraction. The natural soil bulk density (BD) was not measured – which could have given some indication of soil structure and the "poured bulk density" does not replace the measurement of the natural BD, though it does give a relative difference between dried soil material in the fraction less than 2 mm. I therefore suggest a change in tittle. e.g.:
« Strong warming of subarctic forest soil reduces stabilisation of carbon in soil aggregates – Indications from organic matter fractionation".
I would also suggest including" subarctic andesitic forest soil" in the tittle as these soils normally show both chemical and physical properties that are markedly different from "subarctic forest soils" formed on other parent materials.

Answer: We agree that using the word soil structure in the title is misleading, or leads to different expectations. We also agree that the title was too brought, since we investigated a very specific soil type. Therefore we changed the title to: 'Strong warming of a subarctic Andosol depleted soil carbon and aggregation under forest and grassland cover'.

2)
Sampling method and comparisons, sampling should be better explained, and it would also be nice to know how many samples (N=) were behind each temperature/location?
In studies like this sampling method and a good description of these are crucial.
Many sophisticated analyses in the laboratory will never compensate for errors, flaws and inaccuracy in sampling– or description of sampling. I am afraid that the lack attention to the sampling procedure maymake the results of this study none reproducible. Personal experience with similar sampling schemes suggests that a soil of this nature (Silandic Andosol with a silt loam texture, 60 % silt) is easily compressed during sampling. How were the depth intervals determined? If sampling was done as I anticipate by extracting a 30 cm cylindrical core and then splitting it into 0-10, 10-20 and 20-30 samplesthis could be a real challenge. The comparisons between the different layers could be based on pure artefacts
- please convince me of the opposite

Answer: The amount of samples has been introduced in the section 2.1, which was renamed from study site to study site and experimental design. However, we also added the number of warming intensities and replicates to the section 2.2 and the sentence now reads as follows: 'In late April 2018, i.e. almost exactly 10 years after the warming was initiated, mineral soils of all permanent forest plots (six warming intensities, five replicates each) were sampled.'
Regarding the sampling and potential artefacts: We used a thin auger that is not necessarily suited for volumetric sampling, but has the advantage that soil compaction is relatively low. However, as the reviewer mentioned correctly, this kind of soil is easily compressed. This is especially true for the warmer treatments that are less stabile due to less SOC and less aggregates. Changes in soil structure and structural stability were thus already noticed during sampling. The procedure was the following: The auger was always driven into the soil to a depth of 30cm. When extracting the auger with the respective soil core, the soil core was several centimeters shorter than that ~27 cm. In this case we split the core into intervals of

9cm, assuming that compaction happened linearly. In this way, we avoided strong artefacts related to compaction, because 0-9, 9-18 and 18-27 cm in a compacted core should come relatively close to 0-10, 10-20 and 20-30 cm in an uncompacted core. To clarify this, we added the following sentences: 'In case of soil compaction within the auger, the increment depth was adjusted linearly. For example, a compaction of three cm over the whole soil core resulted in a sampling of 0-9, 9-18 and 18-27 cm increments.'

This brings me to my next point – if the warming of the soil has caused changes in the soil density particularly in the "top soil" this would cause the sampling at the warmer place to go deeper into the subsoil extracting soils that naturally (before the shift in geothermal flow) had a lower content of C. This would then be compared to the lower layer of the "none" warmed soil and we would wrongly conclude that the warming has caused loss of carbon?

Answer: Again, we agree with the reviewer that this is a problem to a certain extent: Shifts in SOC contents lead to shifts in bulk density, which should –in an optimal world- be considered before sampling. However, the difficulty is that such changes in bulk density are usually not known before sampling, so how can depth increments be properly adjusted? The only way is then to use a defined volume (e.g. a metal frame) and conduct already the sampling by equivalent soil mass (the approach that is mentioned below). Accounting for equivalent soil mass in the field is extremely elaborate and therefore rarely done. More importantly, it is based on fine soil (<2mm) of course, because this is where the carbon is. In this young, volcanic soil, the rock fragment fraction is extremely variable and partly very high (especially in the grassland soil >10cm). A sampling based on equivalent FINE soil mass is therefore not possible in this soil, and also bulk density values (that have been measured before) are hard to interpret. Nevertheless, the reviewer brought up an important point that is ignored in many studies measuring gradients in SOC contents (or even stocks). We have one argument that the observed loss of SOC is mostly really related to the warming effect: Along the soil profile of grassland and forest soils, the strongest gradients in SOC content with depth occur in the upper cm. The deeper in the profile, the less steep the gradient. This means, that a bulk density related sampling bias would show up most extreme in the topsoil (i.e. the relative loss of SOC should be higher in 0-10cm than in 20-30cm). However, in our case the opposite was the case: Relative losses were more pronounced in the subsoil.
We now address this issue in the following sentences in the discussion: "Also, those structural changes did most likely lead to a certain sampling bias and thus a slight overestimation of SOC losses: A sampling of fixed depth increments ignores the fact that depth increments change with changes in bulk density. Therefore, the depth increments sampled in the higher warming intensities do not exactly match the depth increments sampled in the lower warming intensities. However, this effect is expected to be more pronounced in the topsoil, were the SOC depth gradient is largest and thus a shift in reference soil depth would have the strongest impact on bulk SOC content. However, relative losses in SOC were even more pronounced in the subsoil, indicating that the sampling bias was might have been small. However, it should be mentioned that a mass-based instead of a depth-based sampling (Don et al. 2020) or at least an a-posteriori soil mass correction (Ellert and Bettany et al. 1993) would be indispensable to accurately estimate SOC stock changes."

Bringing me to my third point –as one of the main objectives of the study clearly is to assess losses of C and also quantify the losses, we need to be sure we that what we compare are comparable. Studies like this should be done by comparing equivalent mass (See Ellert, B. H. and J. R. Bettany (1995). "Calculation of organic matter and nutrients stored in soils under contrasting management regimes." Canadian Journal of Soil Science 75(4): 529-538 or others more recent paper). Also the 10-20 cm was not analysed, understandable from a resource point of view (many time-consuming and expensive analysis), but a simple analysis of SOC + weighing of the total dry sample would have added valuable information particularly for interpretation in a climate change context.

Answer: It is correct that for calculating SOC stocks in 0-30 cm, we would need the full picture: bulk densities and SOC contents in all three depth increments. We could then also apply the mass correction as suggested by Ellert and Bettany. However, the study was not designed to measure stocks, which has its own difficulties in this specific soil, but contents. Rock fragments were not determined, total soil mass was not measured. We also think that relative loss in SOC content comes close to a relative loss of soil mass corrected SOC stock (because the differences in bulk density and total fine soil mass will be vanished by mass correction).

The most important results in this study is the relative change in proportion of C in the different fraction with increasing temperature and this should be more in focus than the loss of C

Answer: The manuscript has three clearly formulated objectives and we think that each of these aspects is important. Also, in the discussion we argue that the change in C fraction distribution and structure change (i.e. aggregate breakdown) is directly related to C loss, it is thus hard not to focus on C loss as such, although it is only based on contents.

All analysis were done on the fraction < 2mm, but it would have been interesting to know the proportion of the coarse fraction.

Answer: This is correct and we think the same, therefore a comprehensive aggregate fractionation (including aggregates >2mm) is ongoing, at least for the grassland. Here, we used the chance to apply the very same fractionation procedure as used in an earlier publication in the grassland soil also to the forest soil. Only in this way, a direct ecosystem comparison is possible.

3) The study appears to focus on loss of C from the soil with warming, however there is little information in change in input of C to the system. Warming of the soil may have had an influence on the forest growth/productivity and litterinput. Some more information on the vegetation would have been appreciated. In this study the litter /O horizonis removed – it would have been nice to at least know how thick it was at the different locations? Several papers have been written with data from this experimental area–surly some information could have been extracted from these not just giving references and leaving the reader to find out for themselves. Additional information on mineralogy/alternatively selective extraction of different oxideswould also aidthe discussion on C stabilization mechanism. Also, DOC and pH normally are correlated – it would have been nice to have some pH measurements to go with the top and subsoil samples. As this warming is by geothermal heating, I am naturally curious to how this is affects the the top- and subsoil, are there any effects on soil moisture, any gradient between the two layers.

Answer: Yes, there was also a vegetation response, which could partly drive SOC losses. However, estimating C inputs to the soil is extremely complicated in this setting, because not only productivity, but also biomass turnover needs to be estimated. Concerning this, we have added the following sentences, using unpublished data from the database (section 4.1): "In fact, root biomass in 0-10 cm decreased in both ecosystems (data not shown), leading to weak *positive correlations (R^2=0.37 for forest and R^2=0.29 for grasslands) of SOC and root biomass. Also aboveground plant litter tended to decline in both ecosystems. This suggests that SOC losses were partly driven by decreasing C input with warming and not by increased microbial activity alone. However, a clear picture on absolute C inputs in the experimental plots is not available yet, since it needs to consider NPP and biomass turnover at the same time."* *Much of the data that is needed to understand the whole picture better is still in the pipeline and unpublished. As for mineralogy and oxides, we must admit that those have never been measured so far. Surely this would be interesting, but in our view there will be only minor changes in mineralogy with warming, so it might not be the most important parameter to explain the responses in this study.*

Regarding soil pH: We agree that an increased pH could also explain that observation. We have now added the following sentence: "However, also soil pH is acknowledged to affect DOC formation (Kalbitz et al., 2000), which might be another possible explanation for the observed increase in the proportion of DOC: in both ecosystems, soil pH increased by up to 0.5 units in the highest warming intensity (Sigurdsson et al. 2016).".

Soil moisture (only in 0-5 cm) has been measured in both ecosystem between April and August 2016. Interestingly, the difference between treatments was small and not related to warming. Data are presented in Sigurdsson et al. 2016 Journal of Icelandic agricultural sicences. We do not have information on a potential depth gradient.

4) The use of the term "topsoil" and "sub soil" when this refers to 0-10cm (topsoil) and 20 -30 (sub soil) is ill-conceived. I normal soil terminology both these layers refer to topsoil. Readers with an interest in C subsoil – non surface layers will perhaps be misled. Why not simply us "Upper" and "Lower" or even better were there any genetic differences A horizon – B horizon?

Answer: We agree that the term subsoil is potentially misleading when talking about soil within the top 30 cm of soil. However, the investigated soils, especially in the grassland, were very shallow and of course there are soils with shallow subsoils. We think that this is a matter of definition and define top and subsoil already in the abstract. Therefore we do not see that a change from topsoil to subsoil into "Upper soil" and "Lower soil" or "Upper" and "Lowe" would improve the manuscript. Also, we did not sample along genetic horizons, especially not representatively, therefore we cannot change the fixed depth increments into pedogenetic horizons.

5)Ecosystem comparison: I believe there should have been more focus on differences in input of C. You observe differences between the ecosystems only in the topsoil – ascribing this to the fact that the forest was planted on former unmanaged grassland. However, you also find that the forest soil has a more pronounced depletion of Cin the subsoil. Could also part of the explanation also be due to the fact that warming was geothermal – from beneath. In a situation where global warming (air warming) is the case the differences in the topsoil would be equally reflected in the subsoil.

Answer: Warming is coming from below, that is correct, and it might to some extent explain the relatively stronger response. This was already part of the discussion (section 4.1). But why or how could it explain that there is a distinct ecosystem response in the topsoil but not in the subsoil? For us, it is more likely that this is due to the fact that forest and grassland subsoils were generally very similar (in SOC and fraction distribution) and so responded also similarly to warming.

Reviewer #2:

The manuscript by Poeplau et al., investigates the effects of long-term soil warming on SOC fractions. Their carbon content as well as their relative distribution in response to warming are being discussed for two different soil depths and ecosystems. As I myself am working on warming effects on soil microbial communities in connection to biogeochemical cycles, I was very pleased to read about warming effects on the abiotic components of soil. Especially long-term in-situ warming experiments are rare and extremely valuable to study the mechanisms and concepts behind various warming effects. I completely agree with the authors that strong systematic gradients in SOC content (in the same soil) can provide an important framework to improve our understanding of SOC dynamics. I believe that this study adds some very valuable aspects the research field of soil warming. This paper and some there presented ideas could also provide the basis for hypotheses that could be targeted in other future mechanistic studies. The manuscript is well written and structured. Most parts of it are easy to follow, however I hereby want to suggest some minor and detailed revisions in order to improve the manuscript in terms of its readability and understandability.

Answer: We thank the reviewer for these very positive statements and many helpful comments that helped to improve the manuscript.

Personal comments:
Out of personal interest, I would like to ask if you have a suggestion to why both ecosystem types approach to more or less the same SOC content in response to long-term warming (Fig. 4 and 5)? The levelling off of (absolute) SOC losses from different soil types (with different native C contents) to a sort of threshold level is very interesting to me.

Answer: Indeed, this is interesting, but we have to keep in mind that the soil type is not that different, it is the land cover that differs. We added the following sentences to section 4.4: 'Finally, SOC contents in both ecosystems approach a similar baseline in the highest warming intensity. This might indicate that the specific amount of biogeochemical persistent SOC does not depend on land cover or vegetation type, but is rather controlled by mineralogy.'

I also like the idea of decreasing pore space with warming (due to aggregation loss). I myself often observed a decrease in microbial biomass with warming, which I now start considering to link to the suggested decrease in pore space and microhabitats. Therefore, one can see the need of better exploring structural soil changes upon warming in order to better interpret biotic responses.

Answer: Yes, we fully agree and hope to go into more detail regarding soil structure (of undisturbed soil columns) in the future.

Out of curiosity I also want to ask if you observed any changes in the vegetation cover after several years of soil warming? And if yes, if there is data on plant communities available or a paper covering that aspect? As far as I know, changes in vegetation with warming are quite often observed and I wonder about associated impacts on SOC contents and fraction distribution. E.g. if observed effects like the loss of aggregation could be associated with a change in the vegetation structure? Out of personal interest, is the light ultrasonic treatment more effective and more representative for natural conditions than the slacking treatment?

Answer: There is one paper out on phenology in the grassland (Leblans, Niki, Bjarni D Sigurdsson, Sara Vicca, Yongshuo Fu, Josep Penuelas, Ivan Janssens (2017). Phenological responses of Icelandic subarctic grasslands to short-term and long-term natural soil warming. Global Change Biology 23(11), 4932-4945. doi: 10.1111/gcb.13749) and the consortium has gathered quite some data on vegetation dynamics and biomass, much of it is however still unpublished or not available in a form that it can be properly used. However, we agree that vegetation responses should be mentioned to some extent, because they are likely to drive SOC dynamics. We now added the following sentences to section 4.1: "In fact, root biomass in 0-10 cm decreased in both ecosystems (data not shown), leading to weak positive correlations (R^2=0.37 for forest and R^2=0.29 for grasslands) of SOC and root biomass. Also aboveground plant litter tended to decline in both ecosystems. This suggests that SOC losses were partly driven by decreasing C input with warming and not by increased microbial activity alone. However, a clear picture on absolute C inputs in the experimental plots is not available yet, since it needs to consider NPP and biomass turnover at the same time."

Regarding the slacking: This is a very good question and slacking is usually done in the context of aggregate size fractionation or any questions related to aggregate stability. So in our case, slacking and an aggregate size fractionation would have been interesting as well (and is currently done by other groups working on the ForHot experiment). However, this was not clear before we started the fractionation work in the grassland and the beauty of this study is that we could repeat the exact same method for the forest.

Specific comments:

Short comment to the title: I am not sure if you can really call it a "deteriorated" soil structure. I would rather use the term "changed" or "affected" because it does not imply a judgement. Also. The authors sometimes refer to boreal systems throughout their manuscript. However, I much better like the term subarctic in the context of the study site. Throughout the manuscript, I found an inconsistency in terms of SOC terminology as it is stated on page four and also within the use of associated units. In order to improve the readability and the understandability of the text, I would also welcome to be more specific and explicit when writing about SOC changes e.g. SOC contents, concentrations, fractions, mass et cetera. For detailed comments about alternative phrasing suggestions see below.

Answer: We changed the title of the manuscript. Following suggestions also of the other reviewer, we now removed soil structure from the title, mentioned the very specific soil type (Andosol) and included that two different ecosystems were evaluated: "Strong warming of a subarctic Andosol depleted soil carbon and aggregation under forest and grassland cover". Regarding the word 'boreal', we have replaced it by subarctic or deleted it where appropriate. The suggestion on being more specific about the use of SOC was also realized (see below).

Page 1:

Line 14: Five different SOC fractions were isolated and their re-distribution as well as the amount of stable aggregates was assessed to link SOC to soil structure changes.

Answer: We agree and used the suggested sentence in the abstract.

Line 16: Soil warming had depleted SOC concentrations in forest bulk soil by...

Answer: We agree but used contents instead of concentrations.

Line 24:...indicating an indirect protective effect of SOC on aggregates...

Answer: Here, the rationale was actually the other way around. We now slightly changed the sentence to clarify this: '...indicating an indirect protective effect of aggregates >63 µm on SOC.'

Line 25: Topsoil changes in total SOC content and fraction distribution...

Answer: Changed accordingly.

Line 27:...in the response of subsoil SOC content and fraction distribution...

Answer: Changed accordingly.

The authors write in the abstract that no ecosystem effect was observed. However, this was confusing to me, as in my understanding Tab. 3 shows significant effects.

Answer: In the abstract, we are referring to the interactive effect of warming and ecosystem, not to the ecosystem effect (interaction effect is also given in the table). The question was, if the two ecosystems responded differently to warming. We modified the sentence to clarify that: 'However, no ecosystem effect on the warming response of subsoil SOC content and fraction distribution was observed.'

Line 32: Could you please specify if the stated temperature increase refers to air or soil temperature?

Answer: This is air temperature and we added that information.

Page3
Line 3: I believe that the statement about permafrost soils is out of place here as no permafrost is occurring at the investigated site.

Answer: We agree and deleted this part of the sentence. It now reads: 'The highest SOC stocks are located in high northern ecosystems (Tarnocai et al., 2009).'

Line 32: It was a bit hard to understand to how many samples you refer in your manuscript. Could you please state the explicit number of samples taken? Also, are all mentioned five transects situated in the investigated forest? Please also indicate the number of samples in your graphs.

Answer: The information was given above, when introducing the design of the experiment. However, it seamed a bit hidden because also the other reviewer did not see that. We therefore added that information again when describing the sampling procedure. The sentence (L30) read as follows: 'In late April 2018, i.e. almost exactly 10 years after the warming was initiated, mineral soils of all permanent forest plots (six warming intensities, five replicates each) were sampled.'

Page 4

Line 6: Distinct responses to warming were thus expected. Could you make an explicit statement what you were expecting?

Answer: Instead of going into more details here and setting up hypotheses in the middle of materials and methods, we decided to delete this small sentence. The method description reads more fluent now.

Line 15: Unit is missing for SPT (1.8 g cm3 -1)

Answer: Changed accordingly.

Line 33: Out of personal interest **-** what is the variability to the average mass recovery?

Answer: We added standard deviations to the given recoveries: 'Average mass recovery was 97±2%, average C recovery was 99±21%'. Of course, mass recovery was less variable.

Page 5

Line 11: Would you assume a positive or negative correlation between the poured bulk density and SOC in the SA fraction?

Answer: We changed the sentence slightly into: '...and hypothesized that ρ_i *would be negatively correlated to SOC content in the SA fraction in particular.'*

Line 31: Do you think that absolute SOC losses are higher in topsoil because of a higher "native" C concentration?

Answer: This is likely because relative SOC losses were similar (or even higher in the subsoil). We however do not speculate about this here, because this is not the discussion part.

Page 6

Line 1: Please state here that you talk about SOC contents in bulk soil.

Answer: Done.

Line 11: The depletion of SOC content lead to a changed relative distribution...

Answer: Done.

Line 12: The ANOSIM revealed... Please state here that you talk about topsoil findings.

Answer: Done: 'The ANOSIM revealed that a warming intensities of 5.8 and 17.5°C were necessary to significantly change topsoil SOC distribution (Tab. 2).'

Line 13:...fraction distribution was only significant from the unwarmed reference at a warming intensity of 5.8C. I was wondering about the + 2.7°C treatment (see Tab. 2)?

Answer: That is correct, in the subsoil significant differences were also found for 2.7°C. This was now included.

Line 15:...SOC in the POM and SA fractions, which were strongly depleted with warming (Fig 1). Please mention here, that this was the case in both depth increments.

Answer: In fact, in this specific sentence we are talking about the topsoil only. We therefore started the sentence now with 'In the topsoil…'.

Line 28:...the relative mass proportion of rSOC was expected to increase...

Answer: Done.

Line 30: Could you please give the p-value for the mentioned regression between rSOC and total SOC in the SC fraction?

Answer: Done. It was $p<0.001$.

Line 36: Could you please state the p-value for the significant negative relationship between the proportion of SOC in SA and the proportion of SOC in DOC

Answer: Done. It was $p=0.002$.

Page 7
Line 5: You mention similar SOC contents for subsoil forest and grassland soils. Is there more information about that e.g. an ANOVA?

Answer: We agree that similar is a vague term and actually the ANOVA (as presented in Tab.3 gave significant differences between ecosystems also in the subsoil. We have now rephrased the sentence to be more precise. It now reads as follows:'Also, the difference between ecosystems in subsoil SOC contents was less pronounced than in the topsoil.'

Line 14: You mention that POM in forest soils responded more negatively to warming than POM in grassland soils. Was this normalized to their respective C contents?

Answer: It is inferred from the slope of the regressions in Fig.4, which shows that forests start at a higher level than grasslands and both end up with a similar amount. So yes, it is true in absolute and relative terms.

Line 17: I would have appreciated it, if you mentioned earlier on that the warming in the grassland soils was only 6 years compared to the 10 years of warming in the forest.

Answer: This was mentioned in material and methods. Now on page 5, lines 12-14.

Line 20:...we found a strong negative correlation of bulk soil SOC content and poured BD. Please also give the R2 here.

Answer: Done.

Line 34: According to Tab. 1 the relative change in topsoil SOC content is -3.6% not -2.7% as stated.

Answer: This is correct. Numbers were mixed up here. Changed accordingly.

Line 36: Do you think that the + 5.8°C is a realistic warming intensity for soil or air temperatures?

Answer: To be clear about this difference, we modified the section, which now reads as follows: 'Considering that an air temperature increase of up to 11°C until the end of the century is within the possible range of IPCC climate change projections (IPCC 2013), we assume that a soil warming intensity of up to 5.8°C can be considered realistic. For example, Zhang et al. (2005) showed that soil temperature increase (+ 0.6°C) generally followed the air temperature increase (+ 1°C) in Canada during the 20th century. At a warming intensity of 5.8 °C, the investigated soil lost 29 % (topsoil) and 37 % (subsoil) SOC in ten years.'

Page 8

Line 17: You write, that the present study did not reveal tipping points. However, if I look at e.g. Fig 1. it seems to me that +5.8◦C causes some abrupt changes in SOC contents and SOC proportions in fractions? Please also specify "tipping points for SOC" here (e.g. SOC contents).

Answer: This is correct, it seems like the aggregate break-down kicks-in only after 2.7°C. However, in the mentioned section we discuss bulk SOC content only. We clarified this and also added a sentence on page 9 line 24-25: 'A tipping point for aggregate-breakdown appears to be located between the warming intensities of 2.7 and 5.8°C.'

Line 22: You write that climate change is likely to strongly affect SOC stocks of boreal forests. Generally, I strongly agree to that statement. However, would question the comparability of the relatively young investigated forest on volcanic bedrock material to the biome of naturally old grown boreal forests. Upscaling to the regional or global context might be a slight over interpretation.

Answer: We conducted a soil warming experiment in a subarctic forest and find strong losses of SOC. We do not think that it is an overinterpretation to infer that climate change is likely to affect subarctic forest SOC elsewhere or as a whole. We don't state that this will happen at the same rate as we found, so we think it is ok to leave that statement in the text.

Page 9

Line 17: In the unwarmed reference soil, it accounted for the highest proportion of soil mass and SOC content.

Answer: changed accordingly.

Line 29:...we found a very strong positive correlation of SOC mass and...

Answer: changed accordingly.

Page 10

Line 2: I did not understand the context of the sentence about carbon desorption from the mineral phase. It seemed a bit out of place to me.

Answer: We changed the sentence slightly: 'Desorption of carbon compounds from the mineral phase is likely to be fostered by increased surface area, which is the case when aggregates disintegrate.' – and hope that it is understandable now: break-down of aggregates increases the surface area that is exposed to water.

Line 8: According to your definition on page 4 the unit of SOC content should be (g C kg -1).

Answer: Done

Page 11
Line 7: You might rephrase the sentence to: Changes in SOC concentrations and the relative distribution of fraction masses in the grassland soils have been previously investigated.

Answer: Done.

Line 8 to Line 11: The fact that there is no difference in subsoil SOC dynamics... might indicate that the same mechanisms of SOC depletion were involved in both ecosystems.

Answer: Done.

Line 23: The sentence is very long, maybe you could split it apart.

Answer: We shortended the sentence into: 'Therefore it seems likely that amount and fraction distribution of SOC drove the ecosystem specific warming response in the topsoil.'

Line 37: You might rephrase the sentence to: Differences in the relative distribution of SOC fractions and their respective SOC concentration in response to warming have only been found in the topsoils of both examined ecosystems.

Answer: We rephrased the sentence as follows: 'Differences in the warming response of bulk SOC and SOC fractions between ecosystems have only been found in the topsoil,…'

Specific comments about graphs and tables:

1) Tab. 2: In the table description you write about testing "differences in SOC fraction distribution". Do you mean the relative mass distribution of SOC fractions?

Answer: Well, it is about how SOC is distributed in different fractions (reformulated to 'testing differences in the distribution of SOC in investigated fractions'). Fraction mass is not correct here, because in this paper fractions mass refers to the total soil mass in a specific fraction, not carbon.

2) Fig.1: is missing the a)b)c)d) notation in the individual graphs. Also the unit of the x-axis of a) and b) should be changed to SOC content (g C kg-1).

Answer: Done.

3) Fig.3: For me, the graph would be easier to understand if the title of the x and y axis would be changed to "percentage of total SOC in SA" and "percentage of total SOC in DOC". Also a p-value is missing in the graph as what is the depicted error range (95%

confidence interval?).

Answer: Changed accordingly.

4) Fig 4: I am a bit confused if the scatter plots show SOC masses or SOC contents of the fractions in response to warming. What is the depicted error range? Moreover, some relationships seem rather curvilinear to me than linear.

Answer: It is the carbon content as defined in m&m section, which you might understand as carbon mass actually (g C in the fraction per kg soil). It is confusing, because you can look at the data from different angles and at another point, we are talking about SOC concentration, which refers to g C in the fraction per kg fraction. The error is the 95% confidence interval and that was now added to the caption. Also, we admit that it was a bit oversimplified to use a linear fit in all cases. We now selected the best fit for each case using AIC, deciding between linear and logarithmic fits and also adjusted the statistics section.

5) Fig 5: Please change the title of the the y axis to subsoil instead of topsoil. I more-over have the same small issues with the graphs as mentioned for Fig. 4.

Answer: Changed accordingly.

6) Fig. 6 shows regression models. Please indicate the p-values here. 6a) The unit of SOC content should be (g C kg soil -1). 6b) The unit of SOC concentration should be (g C kg fraction -1). 6c) Please change the title of the x axis to " soil mass in stable aggregates". 6d) The shown relationship looks more curvilinear than linear to me.

Answer: We have added the p-value of <0.001 in the caption once, because this applied to all four regressions. We have changed the axes accordingly and regarding 6D: In fact, the linear fit was the best. The optical impression is a bit misleading, because of three slightly outlying points in the subsoil.

Questions provided by SOIL:
1) Does the paper address relevant scientific questions within the scope of SOIL? Yes, I think so.
2) Does the paper present novel concepts, ideas, tools, or data? The paper shows new interesting data on physical soil structure changes in responses to warming. However, to my state of knowledge, no new tools were involved. The manuscript provides some new concepts and ideas e.g. the proposed mechanism of warming leading to SOC loss (via enhanced microbial activity) which then results in the loss of stable aggregates. I also very much appreciate the proposed idea that the slope of the regression line between SOC and bulk density might be a useful indicator for aggregation affinity in unmanaged soils.

3) Does the paper address soils within a multidisciplinary context? n.a.

4) Is the paper of broad international interest? The scope of physical soil fractions and their response to warming seems of broad interest. It represents a framework of many biological responses to higher temperatures.

5) Are clear objectives and/or hypotheses put forward?

Three objectives are stated clearly on page three and then also addressed in the results and discussion of the paper.

6) Are the scientific methods valid and clear outlined to be reproduced?
Yes. Especially the SOC fractionation protocol is described in great detail and could be repeated in ourlab too.

7) Is the soil type/classification adequately described? Yes, most of the general information on soil types is given in the text.

8) Are analyses and assumptions valid? Yes.

9) Are the presented results sufficient to support the interpretations and associated discussion? Yes.

10) Is the discussion relevant and backed up?
In general yes. For detailed comments see above.

11) Are accurate conclusions reached based on the presented results and discussion?
Yes.

12) Do the authors give proper credit to related and relevant work and clearly indicate their own original contribution? Yes.

13) Does the title clearly reflect the contents of the paper and is it informative? Yes.
The title clearly reflects the later proposed mechanism of aggregate break-down which follows SOC loss that was caused by warming. I especially like that the title includes the term “subarctic” and not “boreal”.

14) Does the abstract provide a concise and complete summary, including quantitative results? Yes.

15) Is the overall presentation well structured?
Yes, I like the tripartite structure of the paper (1-warming effects on forest SOC and its fractions, 2-forest vs. soil SOC in response to warming, 3- soil structural changes). The focus on those three topics can be found in the introduction, results and discussion part.

16) Is the paper written concisely and to the point?
To my understanding the manuscript is mostly concise. However, sometimes the sentences were hard to follow (too long) and not precise enough to understand what the authors meant. This holds especially for “SOC-terminology and SOC units” – see detailed comments above).

17) Is the language fluent, precise, and grammatically correct? Mostly yes. However, some sentences are relatively long and thus hard to follow. This is especially the case in the discussion part.

18) Are the figures and tables useful and all necessary? The figures are nice and useful. For detailed suggestions see comments above.

19) Are mathematical formulae, symbols, abbreviations, and units correctly defined

and used according to the author guidelines?
Yes. In the context of units, please see detailed comments above.

20) Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated?
I think that the sampling procedure (amount of taken samples and analyzed) could be described in more detail.

21) Are the number and quality of references appropriate?
Yes.

22) Is the amount and quality of supplementary material appropriate and of added value?
Yes

Strong warming of a subarctic ~~forest~~ Andosol depleted soil ~~deteriorated soil structure via~~ carbon ~~loss – Indications from organic matter fractionation~~and aggregation under forest and grassland cover

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Keywords: soil warming, macroaggregates, temperature sensitivity, bulk density, soil organic matter

Abstract. Net loss of soil organic carbon (SOC) from terrestrial ecosystems is a likely consequence of global warming and this may affect key soil functions. Strongest changes in temperature are expected to occur at high northern latitudes, with ~~boreal~~ forest and tundra as prevailing land-cover types. However, specific ecosystem responses to warming are understudied. We used a natural geothermal soil warming gradient in an Icelandic spruce forest (0-17.5 °C warming intensity) to assess changes in SOC content in 0-10 cm (topsoil) and 20-30 cm (subsoil) after 10 years of soil warming. Five different SOC fractions were isolated and their re-distribution as well as the amount of stable aggregates ~~(63-2000 µm)~~ was assessed to link SOC to soil structure changes. Results were compared to an adjacent, previously investigated warmed grassland. Soil warming had depleted SOC contents in the forest soil by -2.7 g kg^{-1} °C^{-1} (-3.6 % °C^{-1}) in the topsoil and -1.6 g kg^{-1} °C^{-1} (-4.5 % °C^{-1}) in the subsoil. Distribution of SOC in different fractions was significantly altered, with particulate organic matter and SOC in sand and stable aggregates being relatively depleted and SOC attached to silt and clay being relatively enriched in warmed soils. The major reason for this shift was aggregate break-down: topsoil aggregate mass proportion was reduced from 60.7±2.2 % in the unwarmed reference to 28.9±4.6 % in the most warmed soil. Across both depths, loss of one unit SOC caused a depletion of 4.5 units aggregated soil, which strongly affected bulk density (R^2=0.91, $p<0.001$ when correlated to SOC and R^2=0.51, $p<0.001$ when correlated to soil mass in stable aggregates). The proportion of water extractable carbon increased with decreasing aggregation, ~~indicating~~which might indicate an indirect ~~SOC~~ protective effect of aggregates >63 µm on SOC. Topsoil changes in total SOC content and fraction distribution were more pronounced in the forest than in the adjacent warmed grassland soils, due to higher and more labile initial SOC. However, no ecosystem effect ~~was observed in~~on the warming response of subsoil SOC content and fraction distribution was observed. Whole profile differences across ecosystems might thus be small. Changes in soil structure upon warming should be studied more deeply and taken into consideration when interpreting or modelling biotic responses to warming.

1 Introduction

Global warming is inexorably progressing, with largest expected changes to occur in high northern latitudes (Diffenbaugh and Giorgi, 2012). The IPCC worst case scenario (RCP 8.5), predicts ~~a~~an air temperature increase of up to 11°C in areas North of 60° latitude until the end of this century (IPCC, 2013). This will lead to strong responses of ecosystems, one of which being increased microbial activity and thus oxidation of carbon (Melillo et al., 2002). Predicted alterations in soil organic carbon (SOC), as the largest terrestrial carbon (C) pool (Scharlemann et al., 2014), are inducing a positive climate- carbon cycle feedback loop. The highest SOC stocks~~, partly associated with thawing permafrost,~~ are located in high northern ecosystems (Tarnocai et al., 2009). This spatial coherence of the strongest warming and the highest SOC stocks is expected to turn the vast land masses in high northern latitudes into a major C source. Simple extrapolations of short-term soil warming experiments predicted a global SOC loss of up to 203±161 Pg C with 1 °C warming until 2050 (Crowther et al., 2016), which equals one fourth of the current atmospheric C pool. More conservative estimates of the same authors still predicted losses of 55±50 Pg C. This range in possible SOC changes, as well as the large standard errors associated to each of the estimates points towards the high uncertainty of potential changes in carbon fluxes from terrestrial ecosystems to the atmosphere (van Gestel et al., 2018).

One of the major uncertainties in predicting SOC responses to warming is due to an incomplete mechanistic understanding of the temperature sensitivity of different functional SOC pools. For example, owing to different methodological approaches and partly also misinterpretations (Conant et al., 2011), slow-cycling SOC is found to be more (Lefevre et al., 2014) or equally (Fang et al., 2005) sensitive to warming than fast-cycling SOC. In consequence, SOC models frequently use the same temperature sensitivity for all SOC functional pools. However, it has been suggested lately that the implementation of carbon turnover and stabilization in many models is outdated (Bradford et al., 2016) and that more wholistic experimental knowledge on warming-induced mechanisms related to carbon turnover in soils is necessary (Conant et al., 2011). Isolated quantifications of CO_2 fluxes, bulk SOC or even SOC fractions might thus not yield enough insights to understand and predict SOC dynamics under global warming. Furthermore, individual soil warming experiments are mostly restricted to one ecosystem type and differ strongly in methodology, i.e. type and degree of warming. Comparisons across ecosystems are thus hampered (Crowther et al., 2016), but might be critically important to i) foster the understanding of underlying processes driving SOC responses to warming and ii) inform land-surface models to increase their accuracy.

Apart from its significant role in the global carbon cycle, soil organic matter has numerous functions related to soil fertility and soil health: It is an important food source for soil biota (Barrios, 2007), contains and binds major plant nutrients and trace elements, has a large water storage capacity and is directly linked to soil structure, i.e. the three-dimensional arrangement of soil particles and pore space (Larsbo et al., 2016). Soil structure drives water and gaseous fluxes through the soil matrix, root growth and nutrient uptake as well as soils susceptibility of soils to compaction and erosion (Johnston et al., 2009;Chepil, 1951;Horn et al., 1994). In addition to the enrichment of atmospheric CO_2, soil carbon loss upon warming might thus also deteriorate soil quality, with potential consequences for net primary production. To date, such effects, and involved mechanisms, have been little-noticed, which might be related to the fact that most warming experiments were only run for a relatively short period of time and with moderate warming treatments (Rustad, 2001;Conant et al., 2011).

In essence, long-term multi-ecosystem warming studies with strong soil warming gradients that might even exceed realistic temperature changes are ideal for advancing our understanding of carbon cycling and related changes in soil functions under global change (Kreyling et al., 2014). Such an experiment has been established in southern Iceland, where an earthquake in 2008 shifted geothermal channels within the bedrock, resulting in strong gradients in soil warming (up to ~80°C) in previously unwarmed grassland and forest soils. A growing community of scientists is investigating warming effects in permanent monitoring plots on virtually all ecosystem aspects since 2013 (www.forhot.is). In a previous study, Poeplau et al. (2017) quantified the effect of soil warming on bulk SOC and five different SOC fractions with distinct turnover rates in the unmanaged grassland soil. The authors found a strong decline of soil mass and C in the stable aggregate fraction, indicating that either i) warming-induced SOC depletion led to a destabilization of aggregates or ii) warming-induced aggregate break-down led to a destabilization of SOC.

In this study, we isolated the identical SOC fractions from an equally warmed adjacent forest soil to i) advance our understanding of the temperature response of different SOC fractions representing kinetic pools, ii) assess the role of the ecosystem type in the temperature response of SOC and iii) investigate potential links between SOC loss and soil structure changes.

2 Materials and methods

2.1 Study site and experimental design

In May 2008, a major earthquake in southern Iceland affected geothermal channels close to its epicenter (Halldórsson et al., 2009). Thereby, a geothermal system in Reykir, close to the village of Hveragerði (64.008°N, 21.178°W) was moved to a previously unwarmed area, which is now constantly warmed in strong temperature gradients of up to ~80°C (O'Gorman et al., 2014). This recently warmed area is coverd by a Sitka spruce forest (*Picea sitchensis* (Bong.) Carr.) that was planted in 1966 and an adjacent unmanaged treeless grasslands dominated by common bent (*Agrostis capillaris*, L.). Those two ecosystems are located on a southwest sloping hill-slope (83-163 m a.s.l.). Mean annual temperature and precipitation between 2003 and 2015, as measured at the closest weather station, were 5.2 °C and 1457 mm respectively (Sigurdsson et al., 2016). According to the world reference base, the soil is characterized as a Silandic Andosol with a silt loam texture (clay:silt:sand:ratio of 8:61:31 in the forest and 6:53:41 in the grassland) (Sigurdsson et al., 2016). Soil pH is slightly acidic (5.3 in the forest and 5.7 in the grassland) and average SOC contents in 0-10 cm soil depth in the unwarmed soils are 75 g C kg^{-1} in the forest (present study) and 54 g C kg^{-1} in the grassland (Poeplau et al., 2017). Between autumn 2012 and spring 2014, a total of 30 permanent plots were installed in each ecosystem, comprising six different degrees of warming along five different transects. In 2014, the permanently monitored average soil temperature changes due to geothermal warming were 0, 1.0, 1.9, 2.7, 5.8 and 17.5°C in the forest and 0, 0.5, 2.1, 3.9, 10.5 and 17.3°C in the grassland (Sigurdsson et al., 2016).

2.2 Soil sampling, fractionation and analysis

In late April 2018, i.e. almost exactly 10 years after the warming was initiated, mineral soils of all permanent ~~plots in the~~ forest plots (six warming intensities, five replicates each) were sampled. Before sampling, the litter layer was carefully removed. Sampling was done with a thin auger (3 cm diameter) to a depth of 30 cm in direct proximity of the plot. For each plot, three individual soil cores were taken, split into 0-10, 10-20 and 20-30 cm depth increments and pooled per depth. In case of soil compaction within the auger, the increment depth was adjusted linearly. For example, a compaction of three cm over the whole soil core resulted in a sampling of 0-9, 9-18 and 18-27 cm increments. For this study, only 0-10 cm and 20-30 cm depth increments were used, which will hereafter be referred to as topsoil and subsoil. After sampling, soils were oven dried at 40°C and sieved to <2 mm.

Fractionation of SOC was performed as initially described by Zimmermann et al. (2007) and refined by Poeplau et al. (2013). A scheme can be found at https://www.somfractionation.org/combined-meth/part-dens-oxid-zimmermann/. The procedure involves chemical (oxidation) and physical (size and density separation) fractionation steps, based on current understanding of prevailing SOC stabilization mechanisms in soils. In a recent comprehensive method comparison, this method was among the most efficient to isolate SOC fractions with varying turnover rates (Poeplau et al., 2018). ~~Distinct responses to warming were thus expected.~~ In brief, 20 g of sieved soil were suspended in 150 ml deionised water and subjected to a light ultrasonic treatment of 21 J ml^{-1} at 30 W to disperse the most instable aggregates and associations. Subsequently, soil was wet sieved with a fixed amount of water over 63 µm to separate silt and clay-sized particles from sand-sized particles. Several ~~pretests~~pre-tests with the most extreme warming treatments and the unwarmed reference revealed that 1400 ml of deionized water was sufficient for a complete separation of coarse (>63 µm) and fine fraction (<63 µm) particles, as indicated by clear rinsing water. The fine fraction containing silt and clay-sized particles (SC) was centrifuged for 15 minutes at 1000 g and an aliquot of the supernatant was filtered over 0.45 µm to derive the dissolved organic carbon fraction (DOC). Fine and coarse fractions were oven-dried at 40°C and weighed. Sodium polytungstate (SPT) with a density of 1.8 g cm^{-3} was used to separate the coarse light fraction, i.e. particulate organic matter (POM), from the coarse heavy fraction, i.e. the sand and stable aggregates fraction (SA). To do that, about 40 ml SPT was added to the coarse fraction in a centrifuge tube and stirred gently. Stirred samples were left standing for several hours in room temperature so that particles could float or sink and subsequently centrifuged for 15 minutes at 1000 g for complete separation of light and heavy fractions. The supernatant was decanted into a sieve bag of 50 µm mesh size. The density fractionation procedure was repeated once to ensure complete separation of light and heavy fractions. After the second SPT treatment, the remaining heavy fraction was transferred to a sieve bag of 50 µm mesh size and both heavy and light fractions were washed thoroughly to remove all SPT, dried at 40°C and weighed. Based on this procedure, we use the term aggregates in the following for the 63-2000 µm aggregate size fraction, which comprises larger microaggregates as well as macroaggregates (Totsche et al., 2018). Finally, the SC fraction was subjected to sodium hypochlorite (NaOCl) oxidation, which is done to mimic strong enzymatic decay and isolate an oxidation-resistant SOC fraction (rSOC). To do so, NaOCl with 6 % Cl was first adjusted to pH 8 using concentrated HCl. A 1 g aliquot of the SC fraction was then mixed with 40 ml NaOCl. After 17 hours reaction time, samples were centrifuged, decanted and washed once with deionised water. The whole procedure was repeated twice to ensure complete oxidation of NaOCl-oxidizable SOC (SC-rSOC). Thereafter, soil was dried at 40°C and weighed to determine the mass loss caused by oxidation. All solid fractions and the bulk soil were ball-milled and measured for C and N contents via dry combustion (LECO-TruMac, St Joseph, MI, USA). The DOC fraction was measured using a liquid analyser (DIMATOC, Dimatec, Essen, Germany). Average mass recovery was 97±2%, average C recovery was 99±21%. In the following, two different measures of SOC in the isolated fractions will be used, depending on the context:

i) SOC concentration, which indicates the amount of SOC in each fraction per fraction mass [g C kg fraction^{-1}], and ii) SOC content, which indicates the amount of SOC in each fraction per bulk soil mass [g C kg soil^{-1}].

To determine the total amount of soil in stable aggregates, i.e. to separate the SA fraction into sand and stable aggregates, another 4 g of each bulk soil sample was used posterior. Instead of the soft ultrasonic treatment of 21 J ml^{-1}, we applied 500 J ml^{-1} at a high amplitude (70%) to completly disperse all aggregates (Schmidt et al., 1999). After subsequent wet sieving, the mass proportion of the coarse fraction (>63 µm) containing POM and pure sand grains was determined and subtracted from the earlier coarse fraction to determine the mass proportion of stable aggregates.

To evaluate the effect of bulk SOC and SOC fractions on soil structure, we determined the poured bulk density in the bulk soil as well as the coarse (SA+POM) and fine (SC) fractions of each sample. Poured bulk density is also known as aerated bulk density and is a measure of structural strength of loose material (Abdullah and Geldart, 1999). This was done by pouring the material of known weight into a scaled cylindric flask to measure the volume of the sample. Poured bulk density of each individual sample (ρ_i, g cm^{-3}) was then calculated as:

$$\rho_i = \frac{mass_i}{volume_i} \quad \text{(Eq. 1)},$$

where $Mass_i$ is the total soil mass of the individual fraction [g] and $Volume_i$ is the volume of the individual fraction [cm^{-3}]. We assumed that a higher poured bulk density would indicate less structure and hypothesized that ρ_i would be ~~particularly~~negatively correlated to SOC content in the SA fraction in particular.

Soil sampling of the adjacent grassland SOC (data from previous study) was done in December 2014, six years after the ~~the~~ warming was initiated, and involved the same experimental design and analyses as ~~was done~~ on the forest soil (Poeplau et al., 2017).

2.3 Statistics

The balanced design of the experiment, i.e. six warming intensities, five transects (replicates) and two different sampling depths, allowed the use of analysis of variance (ANOVA) to test differences between warming intensities in bulk SOC and SOC fractions for significance. Also, non-parametric analysis of similarity (ANOSIM) as implemented in the R package vegan (Oksanen et al., 2019) was used to test if warming significantly altered SOC composition, i.e. its distribution in different fractions. Finally, analysis of covariance was used to assess, whether forest SOC (data from this study) and grassland SOC (data from previous study) would differ in their response to soil warming. This was done using ~~linear regression models~~ ANOVA including ecosystem, warming intensity and their interaction. Linear ~~regressions~~ or logarithmic regression models were ~~also~~ used to describe the warming response of bulk SOC and SOC fractions. The Akaike Information Criterion (AIC) was used to select the most suitable model for each individual case. Despite the fact that some temperature responses were non-linear, we used linear regressions to derive absolute and relative changes in SOC ~~content~~concentration per °C as a proxy to compare the different fractions. Whenever necessary, data was log-transformed to approximate normal distribution, which was visually assessed using histograms. Significance was assessed at a level of p<0.05. All statistical tests and plots were done in R (R Development Core Team, 2010). For plots, the package ggplot2 was used (Wickham, 2016).

3 Results

3.1 Warming induced changes in forest soil organic carbon

After ten years of soil warming, bulk SOC content in the forest soil had dropped severely in all investigated warming treatments. ~~Warming~~In the forest soil, warming induced SOC losses increased linearly with degree of warming (Fig.1A, 1B, Tab. 1) in both depth increments. Absolute losses in the topsoil (-2.7 g kg^{-1} °C^{-1}, Tab. 1) were more pronounced than absolute losses in the subsoil (-1.6 g kg^{-1} °C^{-1}). In the topsoil, SOC dropped from 75.1 g kg^{-1} in the unwarmed soil to 26.5 g kg^{-1} in the most warmed soil; in the subsoil it dropped from 36.2 g kg^{-1} to 4.0 g kg^{-1}. Relative losses were thus even more pronounced in the subsoil (-4.5 % SOC °C^{-1}) as compared to the topsoil (-3.6 % SOC °C^{-1}). Despite these strong linear trends, SOC contents in the bulk soil were only significantly different from the unwarmed reference at a warming intensity of 5.8°C and 17.5°C (topsoil) as well as 17.5°C (subsoil) (Tab. 1). The same was true for SOC ~~concentrations~~contents in SA and POM, while for SC and rSOC only a warming intensity of 17.5 °C was enough to significantly decrease SOC ~~concentration~~content in both depths after 10 years. For DOC, significant changes with warming were only observed in the subsoil. In the topsoil, relative changes in SOC content were in the order POM > SA > bulk soil > DOC > SC > rSOC, which is in agreement with the concept of the fractionation method, i.e. a stronger decline in the most labile fractions and a slower decline in the more stable fractions. However, this was not the case for the subsoil, in which the order of relative SOC changes almost reversed to rSOC > SC > POM > bulk soil > SA>DOC (Tab. 1). The strong changes in rSOC and SC were however mainly driven by the 17.5°C warming intensity.

~~Depletion~~The depletion of bulk SOC ~~led~~content lead to ~~a changed~~altered relative ~~distribution~~distributions of SOC in the isolated fractions ~~with warming level~~ (Fig. 1C, 1D). The ANOSIM revealed that ~~a~~ warming intensities of 5.8 and 17.5°C were necessary to significantly change topsoil SOC distribution (Tab. 2). In the subsoil, fraction distribution was ~~only~~ significantly different from the unwarmed reference at a warming intensity of 2.7 °C and 5.8°C. ~~The~~In the topsoil, the unwarmed reference soil was strongly dominated by SOC in the POM and SA fractions (together ~90 %), which were strongly depleted with warming (Fig. 1). This led to a relative increase of SOC stored in the fine fractions (SC-rSOC and rSOC). In the topsoil, even an absolute increase of SOC in these fractions was observed upon warming (Fig. 1A), which strongly indicated a redistribution of fraction masses. Indeed, the soil mass of the SA fraction decreased with warming, while the mass of the SC fraction increased (Fig. 2). This was true for both investigated soil depths, with the mass distribution of the subsoil at 17.5°C warming intensity being an exception. As expected, the second ultrasonic step revealed that within the SA fraction, only the aggregates depleted, while the proportion of sand sized mineral particles remained stable across warming levels (Fig. 2). Thereby, aggregate mass proportion in the topsoil decreased from 60.7±2.2 % in the unwarmed reference to 28.9±4.6 % in the 17.5°C warmed soil. In the subsoil, it decreased from 43.7±3.8 % in the unwarmed reference to 17.7±2.9 % in the 5.8°C warmed soil, while at a warming intensity of 17.5°C the mass proportion of aggregates amounted to 32.9±4.9 %. The average sand content of 28 % determined after the second ultrasonic treatment (Fig. 2) was well in line with the 31 % sand content of the texture analysis.

Within the fine fraction, the relative mass proportion of rSOC was expected to increase with warming, due to its proposed higher biogeochemical stability as compared to the NaOCl-oxidised part of the SC fraction. This was however not the case: Across all warming intensities and both soil depths, we found a significant linear correlation between rSOC and total SOC in the SC fraction (y=0.319x, R^2=0.92, $p<0.001$). Thus, the NaOCl treatment did constantly oxidize two thirds of the SC fraction across all warming intensities, indicating that no relative accumulation of rSOC within the silt and clay sized soil fraction ~~occured~~occurred.

Interestingly, the proportion of SOC that was water soluble (DOC), tended to increase with warming in both investigated depth increments (Fig. 1C and D), which was not significant. However, for the topsoil, we detected a significantly negative relationship of the ~~propotion~~percentage of total SOC in SA and the ~~proportion~~percentage of total SOC in DOC (Fig. 3), which might point towards the SOC stabilizing function of aggregates.

3.2 Forest vs. grassland soil carbon responses to warming

The observed changes in bulk and fraction SOC in the forest soil were generally comparable to those in the adjacent grassland soils (Figure 4 and 5). Especially in the subsoil, the interaction effect of ecosystem and warming on SOC was not significant for four out of five fractions and the bulk soil, indicating the same SOC response to warming in both ecosystem types (Fig. 5, Tab. 3). Also, ~~SOC contents in~~ the ~~subsoil were similar for both~~difference between ecosystems in subsoil SOC contents was less pronounced than in the topsoil. This might partly be related to the fact that the forest was planted on an unmanaged grassland and that the forest subsoil SOC was still grassland-derived to a high extend. However, for the topsoil we found significant interactive effects of ecosystem and warming for four out of five fractions and the bulk soil (Tab. 3). The forest soil, which had ~~a~~ considerably higher bulk SOC ~~content~~contents in the unwarmed reference than the grassland, showed a stronger response to warming. The predominant SOC fraction in the forest topsoil was the SA fraction, which responded strongest to warming (Fig. 1). This was generally observed in both ecosystems. However, the stronger redistribution of soil mass across fractions in the forest soil as compared to the grassland soil led to very distinct responses of SC-rSOC and rSOC, with stronger warming induced increases of these fractions in the forest soil (Fig. 4). Also the POM fraction of the forest soil responded more negatively to warming than that in the grassland soil. Only for the warming response of DOC, we did not detect any differences between ecosystems in the topsoil. Interestingly, despite differences in initial SOC and warming duration, i.e. ten years for the forest and six years for the grassland, SOC in both ecosystems approached an almost equal SOC content in the most extreme warming intensities (Fig. 4).

3.3 Structural changes following soil carbon loss

As expected, we found a strong negative correlation of SOC content and poured bulk density (Fig. 6A, R^2=0.92, $p<0.001$). A very similar relationship with identical slope was observed for the coarse (>63 µm) soil fraction, comprising SA and POM (Fig. 6B). In contrast to that, we did not detect any correlation of SOC content and poured bulk density in the silt and clay fraction (data not shown). A direct link of poured bulk density and aggregates is given in Fig. 6C. Finally, in agreement with the strong decline of SOC and soil mass in the SA fraction with warming intensity (Fig. 1, 2), we found a strong positive correlation of SOC mass and soil mass in the coarse soil fraction comprising SA and POM (Fig. 6D). The slope of the regression was 4.5, indicating that one unit SOC was causing the aggregation of 4.5 units of soil. The effects of SOC on soil structure were equally observed in topsoil and subsoil. ~~Furtherhmore~~Furthermore, for all structure-related parameters shown in Figure 6, observations of both investigated soil depths scattered approximately around the same regression line. This might indicate that SOC depletion as such, rather than soil warming, induced the break-down of aggregates.

4 Discussion

4.1 Warming effects on forest soil organic carbon and its fractions

Ten years of forest soil warming caused a strong decline in SOC content. Along the temperature gradient, SOC changes followed a linear response, with -~~2.7~~3.6 % and -4.5 % change per °C in topsoil and subsoil, respectively. In the most extreme warming intensity of ~~+~~17.5°C, SOC was thus depleted by 65 and 89 %. ~~With~~Considering that an air temperature increase of up to 11°C until the end of the century is within the possible range of IPCC climate change projections (IPCC 2013), we assume that a soil warming intensity of up to 5.8°C~~, which~~ can be considered ~~a~~ realistic. For example, Zhang et al. (2005) showed that soil temperature increase (+ 0.6°C) generally followed the air temperature increase (+ 1°C) in ~~high northern latitudes until~~Canada during the ~~end of the~~20th century. At a warming intensity of 5.8 °C, the investigated soil lost 29 % (topsoil) and 37 % (subsoil) SOC in ten years. This is in line with other studies, which also reported significant losses of SOC upon warming (Crowther et al., 2016 and papers cited therein). In the investigated experiment, there is no doubt that potential warming-induced changes in net primary productivity (NPP; Sigurdsson et al., 2014) did not offset increased soil microbial activity. In fact, root biomass in 0-10 cm decreased in both ecosystems (data not shown), leading to weak positive correlations (R^2=0.37 for forest and R^2=0.29 for grasslands) of SOC and root biomass. Also aboveground plant litter tended to decline in both ecosystems. This suggests that SOC losses were partly driven by decreasing C input with warming and not by increased microbial activity alone. However, a clear picture on absolute C inputs in the experimental plots is not available yet, since it needs to consider NPP and biomass turnover at the same time.

Similar or relatively even more pronounced losses of SOC from the subsoil as compared to the topsoil are confirmed by results of a recent whole profile forest soil warming study, concluding that subsoils will be an important source of CO_2 under climate change (Hicks Pries et al., 2017). Higher relative losses of SOC in the subsoil could potentially be driven by warming-induced changes in C input patterns. Indeed especially fine root production and turnover of trees in the boreal zone was previously found to increase with moderate warming (Leppälammi-Kujansuu et al., 2014;Majdi & Öhrvik, 2004), and fine roots are primarily located in the uppermost cm of forest soils (Hansson et al., 2013;Leppälammi-Kujansuu et al., 2013). ~~but~~However, at the investigated site the amount of fine roots and mycorrhizal production has been found to decrease at the more extreme warming levels (Parts et al., 2014;Rosenstock et al., 2019). ~~Further, the fine roots are primarily located in the uppermost cm of forest soils (Hansson et al., 2013;Leppälammi-Kujansuu et al., 2013). Losses via SOC mineralization might have thus been buffered to a higher extent by C input in the topsoil as compared to the subsoil.~~ In addition, in this geothermal warming experiment, heat was coming from below, leading to slightly more intense soil warming in the subsoil. This is likely to explain the stronger relative SOC depletion in the subsoil~~, since soils are heated from below, even if~~ to a certain extent. Except for the highest warming level, the vertical gradients within the top 30 cm of soil were however not substantial~~ except in the highest warming level~~ (Sigurdsson et al., 2016).

A major strength of a warming gradient approach is the identification of potential tipping points, which may mark abrupt changes in ecosystem functionality (Kreyling et al., 2014). However, the present study did not reveal such tipping points for bulk SOC content, which changed surprisingly linear with increasing temperature in both investigated depth increments. Despite certain methodological drawbacks of the geothermal (or any other manipulated) soil warming experiment, such as very abrupt initial temperature changes, ~~as well as~~and soil warming from below instead of whole ecosystem warming from above, it can be inferred that climate change is likely to strongly affect SOC stocks of ~~boreal~~subarctic forests. The latter cover an area of approximately 15 mio km^2 or one third of the global forest area (Bonan, 2008). The analysis of the soil warming gradient also revealed detection limits for warming effects on SOC that is per se very heterogeneous in space and responds slowly to environmental change (Smith, 2004): Even after ten years of chronic soil warming, changes in topsoil SOC were only significant at a warming intensity of at least 5.8°C, when assessed using the ANOVA approach. The latter, instead of a regression analysis, needs to be used when only one warming treatment is investigated (e.g. Schnecker et al. 2016). If this treatment is relatively mild, e.g. below 4°C, changes might easily be undetectable against the background heterogeneity of SOC. This is an important insight considering the ongoing debate if SOC is lost upon warming or not (Crowther et al., 2016;van Gestel et al., 2018). The majority of currently available datasets are based on such experiments with relatively short, mild and singular warming treatments (van Gestel et al., 2018). However, the transferability of the results in this study to the SOC response to global warming is still rather limited and can only slightly reduce given uncertainties: i) we studied soil temperature, not air temperature increase, ii) the warming occurred abruptly and not gradually, iii) we studied an Andosol. Extrapolations to larger areas or longer time periods should thus be done carefully and were not intended with this study.

The fractionation method used in this study isolates SOC pools of different biogeochemical stabilities (Zimmermann et al., 2007). Turnover rates are estimated to range from several years in the POM fraction to centuries in the oxidation resistant rSOC fraction that is associated to silt and clay particles (von Lützow et al., 2007). Such differences are mainly related to different degrees of physico-chemical stabilization in the soil, such as the interaction with the mineral phase or occlusion into aggregates (von Lützow et al., 2007). Due to differences in composition and bioavailability of these SOC fractions, distinct responses to warming were expected in the order POM > DOC > SA > bulk soil > SC-rSOC > rSOC. Indeed the average relative decrease in SOC content, which might be the best indicator to describe a fraction‘s sensitivity to warming, was observed to follow a similar order in the topsoil: POM > SA > bulk soil > DOC > SC-rSOC > rSOC. This is well in line with the sensitivity of these fractions to land-use change as observed across different land-use changes by Poeplau and Don (2013). The difference in warming response between SC-rSOC (-2.14 % °C^{-1}) and rSOC (-2.05 % °C^{-1}) was however negligible, which was also reflected in the stable proportion of rSOC in the total SC fraction throughout the warming gradient. This indicated that NaOCl-oxidation did not yield a meaningful fraction with regard to biogeochemical ~~resistence~~resistance. This has been observed before and questions the ~~use of chemical~~ notion that this oxidation-resistant pool can be linked to ~~mimic biological oxidation (~~a centennially persistent or even inert SOC pool (Lutfalla et al., 2014;Poeplau et al., 2019;Poeplau et al., 2017~~). Paradoxically, many authors describe~~;Zimmermann et al., 2007). At the same time, NaOCl-resistant SOC has often been described as substantially older and thus slower cycling as bulk SOC (Helfrich et al., ~~2007;~~2007) and was also found to correlate to the abundance of Al and Fe-oxides in the soil (Mikutta et al., 2005). ~~In any case~~Thus, the ~~proposed function~~strong warming response of ~~rSOC~~this fraction is somewhat in contrast to the slow responses observed to other treatments, such as ~~centenially persistent or even inert SOC pool (Zimmermann et al., 2007) could repeatedly not be confirmed in this study.~~C3-C4 vegetation changes (Poeplau et al. 2018). In the subsoil, the average relative depletion in rSOC was even strongest across all fractions and the bulk soil. This was however related to the very low carbon content of the highest warming intensity (17.5 °C), driving the slope of the regression. Only when the highest warming intensity was excluded, the sensitivity of fractions followed the observed order in the topsoil, with DOC being an exception: POM > SA > bulk soil > SC-rSOC > rSOC > DOC.

4.2 Aggregate break-down induced by soil organic carbon losses or vice versa?

The most significant warming effect on the distribution of SOC in the isolated fractions was the strong decrease of SA. In the unwarmed reference soil, it accounted for the highest proportion of soil mass and SOC content. However, with warming, aggregates collapsed, leading to strong mass increases in the fine SC fractions, which even increased in carbon mass upon warming. The second ultrasonic step, which was used to distinguish sand from aggregates in the SA fraction, provided evidence that the investigated aggregate size fraction (63-2000 µm) was strongly reduced. A tipping point for aggregate-breakdown appears to be located between the warming intensities of 2.7 and 5.8°C. The same mechanism, yet less pronounced, was observed for the adjacent grassland (Poeplau et al., 2017). Observing SOC depletion and aggregate break-down at the same time ~~rises~~raises the question of cause and effect: Aggregates – at least micro-aggregates < 250 µm - are acknowledged to protect organic matter from microbial decomposition (Six et al., 2002). At the same time, organic matter, especially mucilage, polysaccharides and fungal hyphae acts as aggregate binding agent (Tisdall and Oades, 1982). Answering the question whether warming per se has fostered aggregate break-down through changes in biotic and abiotic environmental conditions might be of critical importance for conceptualizing and ~~modeling~~modelling warming effects on SOC dynamics. However, results of the present study suggest that the major cause of aggregate break-down was not necessarily warming, but could be well described with loss of SOC: we found a very strong positive correlation of SOC mass and total soil mass in the coarse soil fraction (comprising POM and SA) – one 1 g kg^{-1} of SOC was keeping 4.5 g kg^{-1} soil aggregated. ~~Thereby, topsoil~~Topsoil and subsoil samples scattered approximately around the same regression line~~, indicating~~. This indicates that ~~especially~~ the ~~amount~~abundance of young and coarse SOC~~, may it be driven by warming intensity or~~ per se, rather than the ~~location within the~~ degree of soil ~~profile, was a very good predictor for~~warming, is driving the amount of stable aggregates in the soil. This is well known and thus in accordance with the literature (Franzluebbers, 2002;Oades, 1984;Shepherd et al., 2002). Another reason to doubt that warming-induced aggregate break-down caused destabilization of SOC is the fact that the SOC protection capacity of macro-aggregtaes is debatable (Six et al., 2004). For example, Bischoff et al. (2017) found higher heterotrophic respiration in uncrushed soil as compared to the same soil with crushed macro-aggregates. To some extent, a positive feedback loop, i.e. SOC depletion causing aggregate break-down which in turn causes mineralization of then ~~accesible~~accessible C might indeed be possible. The fact that the proportion of water soluble SOC in the topsoil increased with decreasing aggregation, points in this direction. Desorption of carbon compounds from the mineral phase is likely to be fostered by increased surface area~~.~~, which is the case when aggregates disintegrate. However, also soil pH is acknowledged to affect DOC formation (Kalbitz et al., 2000), which might be another possible explanation for the observed increase in the proportion of DOC: in both ecosystems, soil pH increased by up to 0.5 units in the highest warming intensity (Sigurdsson et al., 2016).

4.3 Linking losses in soil organic carbon to changes in soil structure

In consequence of SOC loss, total pore space decreased strongly as indicated by poured bulk density. Poured bulk density was used as a proxy for ~~bulk density~~ in situ bulk density in the undisturbed ~~samples. The latter~~soil, which was unfortunately not determined in the present study. However, the relationship of SOC and poured bulk density was in the range of established pedotransfer functions (PTF) for field bulk density estimation using SOC content. In a literature review comparing different PTF (De Vos et al., 2005), slopes of the regressions model using SOC content ~~[~~(g C kg^{-1}~~]~~) to predict soil bulk density ~~[~~(g cm^{-3}~~]~~) ranged from -0.003 to -0.011, while the slope in the present study was -0.005 for both the bulk soil and the SA fraction. The negative correlation is due to ~~i)~~ a much lower specific gravity of organic matter as compared to mineral particles, but also due to the effect of organic matter on aggregation (De Vos et al., 2005). The variation in slopes, i.e. effect of SOC on bulk density, is most likely related to the soil‘s capability to form aggregates. In very sandy soils with a single grain ~~stucture~~structure, even high organic matter contents do not lead to considerable formation of aggregates so that the organic matter effect on bulk density is mainly restricted to a gravity effect. Using a two-pool mixing model of mineral particles with a density of 2.5 g cm^{-3} and soil organic matter with a density of 1, i.e. ignoring the structural effect of organic matter, we found a slope of -0.0026. Accordingly, Callesen et al. (2003) reported a PTF for sandy forest soils with a slope of approximately -0.0028 in the range between 0-80 g SOC kg^{-1} (non-linear function). The slope of -0.005 found in this study might thus indicate that approximately 50 % of the SOC effect on poured bulk density can be assigned to a structural effect. Indeed, we also found a strong negative correlation of the soil mass stored in aggregates and the poured bulk density. To conclude, the slope of the regression between SOC and bulk density, at least in unmanaged soils, might be a good indicator for the aggregation affinity of a soil. Surely, poured bulk density of disturbed and sieved soil can only express a potential and should be treated as such. On the other hand, factors like position in the soil profile that strongly influence the packing density of the soil are ~~canceled~~cancelled out, enabling a direct comparison of topsoil and subsoil samples.

Strong systematic gradients in SOC content in the same soil, as have been created by the soil warming in our study, are rare and extremely valuable to improve our understanding on organic matter functions. Larsbo et al. (2016) used a natural SOC gradient to evaluate its effect on pore networks, influencing solute and gaseous transport in the soil. Changes in soil structure as induced by the large SOC loss might also affect other key ecosystem properties, such as NPP (Oldfield et al., 2019), microbial biomass (Walker et al., 2018) or other soil biota. For example, in the adjacent warmed grassland plots, Holmstrup et al. (2018) detected a warming-induced shift in collembola species abundance towards species with smaller body size. ~~While the authors did not explicitly link that to changes in soil structure, an~~An increase in bulk density with associated decrease in pore space might have fostered ~~that change~~this physiological response, although this was not explicitly mentioned by the authors. Also, a positive correlation of pore volume and microbial and nematode biomass was found by Hassink et al. (1993). In the present study, aggregation and poured bulk density were assessed on sieved soils, which provided valuable first information on warming-induced changes in basic soil structural parameters. For two major reasons, a follow-up study should investigate soil structure and other physical parameters in undisturbed soil samples: i) the gradient in SOC content is unique and can be used to improve the general understanding of the link between organic matter and soil functions; ii) the warming responses of many ecosystem aspects are studied along the investigated warming gradients and knowledge on changes in soil physical properties might be central to interpret such responses. Also, those structural changes did most likely lead to a certain sampling bias and thus a slight overestimation of SOC losses: A sampling of fixed depth increments ignores the fact that depth increments change with changes in bulk density. Therefore, the depth increments sampled in the higher warming intensities do not exactly match the depth increments sampled in the lower warming intensities. However, this effect is expected to be more pronounced in the topsoil, were the SOC depth gradient is largest and thus a shift in reference soil depth would have the strongest impact on bulk SOC content. However, relative losses in SOC were even more pronounced in the subsoil, indicating that the sampling bias was might have been small. However, it should be mentioned that a mass-based instead of a depth-based sampling (Don et al. 2020) or at least an a-posteriori soil mass correction (Ellert and Bettany, 1995) would be indispensable to accurately estimate SOC stock changes.

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4.4 Comparing forest and grassland soil carbon responses to warming

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To date, warming experiments have mostly focused on one single type of ecosystem. However, the warming response could be ecosystem specific (Shaver et al., 2000), which can only be investigated in a paired ecosystem approach. In the present study, we investigated a small stretch of forest located directly adjacent to a similarly warmed grassland. Changes in SOC contents and ~~SOC fractions~~the relative distribution of fraction masses in the grassland soils have been previously investigated~~ previously~~ (Poeplau et al., 2017). Both ecosystems showed a similarly strong response to warming. The fact that no difference in subsoil SOC dynamics in the bulk soil or any isolated fractions were observed might indicate that the same mechanisms of SOC depletion were involved~~.~~ in both ecosystems. For example, aggregate break-down as well as equal decrease in rSOC and SC-rSOC were also observed in the grassland. However, the initial SOC content and fraction distribution in the topsoil differed across ecosystems, leading to distinct responses to warming: The unwarmed forest had about 50 % more SOC in the topsoil as compared to the grassland, and about 150 % more SOC was stored in the SA fraction. Also the POM fraction was almost doubled in the forest, with proportionally less SOC stored in more stable fractions. The shift in fraction mass distribution, i.e. aggregtae break-down, was more pronounced in the forest topsoil, leading to the increase in fine fraction SOC with warming, which was not observed in the grassland. Crowther et al. (2016) reported that SOC loss upon warming is a function of initial SOC – the present study confirms that. In fact, to some extent the explanation for that might be the higher proportion of labile SOC in soils with higher SOC stocks (Besnard et al., 1996). It has been reported previously that forest SOC is more labile than grassland SOC (Poeplau and Don, 2013). The forest was sampled after ten years of warming, the grassland after six years. However, i) subsoils showed an almost identical response to warming and ii) there are indications that at least the grassland reached a new steady state in SOC already after six years of warming (Walker et al., 2018). ~~Together with the fact that also more labile SOC was present in the forest topsoil, which responded more sensitive to warming than the bulk soil,~~Therefore it seems likely that amount and fraction distribution of SOC drove the ecosystem specific warming response in the topsoil. The difference in topsoil SOC and fraction distribution was found before and is related to the different sources and qualities of fresh organic matter inputs (Poeplau and Don, 2013;Huang et al., 2011). Especially needle litter is acknowledged to decompose slowly (Prescott et al., 2000). Differences in POM as well as total SOC stocks are observed to level off with increasing soil depth (Davis and Condron, 2002;Poeplau and Don, 2013). This might also be true for the response to warming, as indicated in the present study. Finally, SOC contents in both ecosystems approach a similar baseline in the highest warming intensity. This might indicate that the specific amount of biogeochemical persistent SOC does not depend on land cover or vegetation type, but is rather controlled by mineralogy.

5 Conclusion

Using a strong geothermal warming gradient, we highlighted the critical role of SOC for soil structure. Ten years of soil warming created a steep gradient in SOC contents that is rare and should be used to study the links of organic matter to soil structure and soil functions more deeply. Results of the present study reveal that the effects of warming on biogeochemical cycles are most likely not restricted to direct effects on biotic processes, but that changes in the soil abiotic environment should be considered. Those are likely to exert a strong indirect influence on any biotic response. Differences in the warming response of bulk SOC and SOC fractions ~~responses to warming across~~between ecosystems have only been found in the topsoil~~ only~~, which might however be related to the fact that the forest was planted on unmanaged grassland half a century ago. In the forest, depletion of SOC was more pronounced in the subsoil, which calls for more whole soil profile warming studies.

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Data availiability

The dataset is stored in the repository of the center for open science and available via DOI 10.17605/OSF.IO/SGUZ2.

Author contribution

CP designed the study, carried out parts of the lab work and prepared the manuscript with contributions from all co-authors. PS sampled the soils and ~~BS~~BDS initiated the entire field experiment.

Competing Interests

None.

Acknowledgements

We thank Tatjana Saevici for conducting the fractionation work. This work contributes to the Nordic CAR-ES and the ForHot (www.forhot.is) network projects and the Icelandic Research Fund project 163272-053.

References

Abdullah, E. C., and Geldart, D~~. J. P. t~~.: The use of bulk density measurements as flowability indicators, Powder Technology, 102, 151-165, 1999.

Barrios, E. ~~J. E. e.: ~~:Soil biota, ecosystem services and land productivity, Ecological Economics, 64, 269-285, 2007.

Besnard, E., Chenu, C., Balesdent, J., Puget, P., and Arrouays, D~~. J. E. J. o. S. S~~.: Fate of particulate organic matter in soil aggregates during cultivation, European Journal of Soil Science, 47, 495-503, 1996.

Bischoff, N., Mikutta, R., Shibistova, O., Puzanov, A., Silanteva, M., Grebennikova, A., Fuß, R., and Guggenberger, G~~. J. B., Nr. 10:~~.: Limited protection of macro-aggregate-occluded organic carbon in Siberian steppe soils, Biogeosciences, 14, 2627-2640, 2017.

Bonan, G. B~~. J. s~~.: Forests and climate change: forcings, feedbacks, and the climate benefits of forests, Science, 320, 1444-1449, 2008.

Bradford, M. A., Wieder, W. R., Bonan, G. B., Fierer, N., Raymond, P. A., and Crowther, T. W.: Managing uncertainty in soil carbon feedbacks to climate change, Nature Climate Change, 6, 751, 10.1038/nclimate3071, 2016.

Callesen, I., Liski, J., Raulund-Rasmussen, K., Olsson, M., Tau-Strand, L., Vesterdal, L., and Westman, C~~. J. G. c. b~~.: Soil carbon stores in Nordic well-drained forest soils—Relationships with climate and texture class, Global Change Biology, 9, 358-370, 2003.

Chepil, W~~. J. S. S~~.: Properties of soil which influence wind erosion: IV. State of dry aggregate structure, Soil Science, 72, 387-402, 1951.

Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., Evans, S. E., Frey, S. D., Giardina, C. P., and Hopkins, F. M.: Temperature and soil organic matter decomposition rates–synthesis of current knowledge and a way forward, Global Change Biology, 17, 3392-3404, 2011.

Crowther, T. W., Todd-Brown, K. E., Rowe, C. W., Wieder, W. R., Carey, J. C., Machmuller, M. B., Snoek, B., Fang, S., Zhou, G., and Allison, S. D~~. J. N~~.: Quantifying global soil carbon losses in response to warming, Nature, 540, 104, 2016.

Davis, M., and Condron, L~~. J. S. R~~.: Impact of grassland afforestation on soil carbon in New Zealand: a review of paired-site studies, Soil Research, 40, 675-690, 2002.

De Vos, B., Van Meirvenne, M., Quataert, P., Deckers, J., and Muys, B~~. J. S. S. S. o. A. J~~.: Predictive quality of pedotransfer functions for estimating bulk density of forest soils, Soil Science Society of America Jorunal, 69, 500-510, 2005.

Diffenbaugh, N. S., and Giorgi, F~~. J. C. C~~.: Climate change hotspots in the CMIP5 global climate model ensemble, Climatic Change, 114, 813-822, ~~10.1007/s10584-012-0570-x,~~ 2012.

Don, A., Hagen, C., Grüneberg, E., and Vos, C.: Simulated wild boar bioturbation increases the stability of forest soil carbon, Biogeosciences, 16, 4145-4155, 10.5194/bg-16-4145-2019, 2019.

Ellert, B. H., and Bettany, J. R.: Calculation of organic matter and nutrients stored in soils under contrasting management regimes, Canadian Journal of Soil Science, 75, 529-538, 1995.

Fang, C., Smith, P., Moncrieff, J. B., and Smith, J. U.: Similar response of labile and resistant soil organic matter pools to changes in temperature, Nature, 433, 57, 10.1038/nature03138, 2005

Franzluebbers, A. J.: Water infiltration and soil structure related to organic matter and its stratification with depth, Soil and Tillage Research, 66, 197-205, https://doi.org/10.1016/S0167-1987(02)00027-2, 2002.

Halldórsson, B., Sigbjörnsson, R~~. J. S. D., and Engineering, E.:~~.: The Mw6. 3 Ölfus earthquake at 15: 45 UTC on 29 May 2008 in South Iceland: ICEARRAY strong-motion recordings, Soil Dynamics and Earthquake Engineering, 29, 1073-1083, 2009.

Hansson, K., Fröberg, M., Helmisaari, H.-S., Kleja, D. B., Olsson, B. A., Olsson, M., & Persson, T.: Carbon and nitrogen pools and fluxes above and below ground in spruce, pine and birch stands in southern Sweden. Forest Ecology and Management, 309, 28–35, 2013.

Hassink, J., Bouwman, L., Zwart, K., Brussaard, L~~. J. S. B., and Biochemistry:~~.: Relationships between habitable pore space, soil biota and mineralization rates in grassland soils, Soil Biology and Biochemistry, 25, 47-55, 1993.

Helfrich, M., Flessa, H., Mikutta, R., Dreves, A., and Ludwig, B.: Comparison of chemical fractionation methods for isolating stable soil organic carbon pools, European Journal of Soil Science, 58, 1316-1329, 10.1111/j.1365-2389.2007.00926.x, 2007.

Hicks Pries, C. E., Castanha, C., Porras, R. C., and Torn, M. S.: The whole-soil carbon flux in response to warming, Science, 355, 1420-1423~~, 10.1126/science.aal1319 %J Science~~, 2017.

Holmstrup, M., Ehlers, B. K., Slotsbo, S., Ilieva-Makulec, K., Sigurdsson, B. D., Leblans, N. I. W., Ellers, J., and Berg, M. P.: Functional diversity of Collembola is reduced in soils subjected to short-term, but not long-term, geothermal warming, Functional Ecology, 32, 1304-1316~~, 10.1111/1365-2435.13058~~, 2018.

Horn, R., Taubner, H., Wuttke, M., Baumgartl, T~~. J. S., and Research, T~~.: Soil physical properties related to soil structure, Soil and Tillage Research, 30, 187-216, 1994.

Huang, Z., Davis, M. R., Condron, L. M., and Clinton, P. W.: Soil carbon pools, plant biomarkers and mean carbon residence time after afforestation of grassland with three tree species, Soil Biology and Biochemistry, 43, 1341-1349, ~~https://doi.org/10.1016/j.soilbio.2011.03.008, 2011.~~2011.

Johnston, A. E., Poulton, P. R., and Coleman, K~~. J. A. i. a~~.: Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes, Advances in Agronomy, 101, 1-57, 2009.

Kalbitz, K., Solinger, S., Park, J. H., Michalzik, B., and Matzner, E.: Controls on the dynamics of dissolved organic matter in soils: A review, Soil Science, 165, 277-304, 2000.

Kreyling, J., Jentsch, A., and Beier, C~~. J. E. L~~.: Beyond realism in climate change experiments: gradient approaches identify thresholds and tipping points, Ecology Letters, 17, 125-e121, 2014.

Larsbo, M., Koestel, J., Kätterer, T., and Jarvis, N.: Preferential Transport in Macropores is Reduced by Soil Organic Carbon, Vadose Zone Journal, 15, 2016.

Lefevre, R., Barre, P., Moyano, F. E., Christensen, B. T., Bardoux, G., Eglin, T., Girardin, C., Houot, S., Kaetterer, T., and Oort, F.: Higher temperature sensitivity for stable than for labile soil organic carbon–Evidence from incubations of long-term bare fallow soils, Global Change Biology, 20, 633-640, 2014.

Leppälammi-Kujansuu, Jaana, Salemaa, M., Kleja, D. B., Linder, S., & Helmisaari, H.-S.: Fine root turnover and litter production of Norway spruce in a long-term temperature and nutrient manipulation experiment. Plant and Soil, 374(1–2), 73–88, 2014.

Leppälammi-Kujansuu, J., Ostonen, I., Strömgren, M., Nilsson, L. O., Kleja, D. B., Sah, S. P., & Helmisaari, H.-S. (2013). Effects of long-term temperature and nutrient manipulation on Norway spruce fine roots and mycelia production. Plant and Soil, 366(1–2), 287–303, 2013.

Lutfalla, S., Chenu, C., and Barré, P.: Are chemical oxidation methods relevant to isolate a soil pool of centennial carbon?, Biogeochemistry, 118, 135-139, 2014.

Majdi, H., Ohrvik, J.: Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. Global Change Biology, 10(2), 182–188, 2004.

Melillo, J., Steudler, P., Aber, J., Newkirk, K., Lux, H., Bowles, F., Catricala, C., Magill, A., Ahrens, T., and Morrisseau, S. J. S.: Soil warming and carbon-cycle feedbacks to the climate system, 298, 2173-2176, 2002.

Mikutta, R., Kleber, M., Kaiser, K., and Jahn, R.: Review: Organic matter removal from soils using hydrogen peroxide, sodium hypochlorite, and disodium peroxodisulfate, Soil Science Society of America Journal, 69, 120-135, 2005.

O'Gorman, E. J., Benstead, J. P., Cross, W. F., Friberg, N., Hood, J. M., Johnson, P. W., Sigurdsson, B. D., and Woodward, G.: Climate change and geothermal ecosystems: natural laboratories, sentinel systems, and future refugia, Global ~~change biology~~Change Biology, 20, 3291-3299, 2014.

Oades, J. M.: Soil organic matter and structural stability: mechanisms and implications for management, in: Biological Processes and Soil Fertility, Springer, 319-337, 1984.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H.: vegan: Community ecology Package R package version 2.5-4, 2019.

Oldfield, E. E., Bradford, M. A., and Wood, S. A.: Global meta-analysis of the relationship between soil organic matter and crop yields, SOIL, 5, 15-32, 10.5194/soil-5-15-2019, 2019.

Parts, K., Tedersoo, L., Schindlbacher, A., Sigurdsson, B. D., Leblans, N. I. W., Oddsdóttir, E. S., Borken, W. Ostonen, I.: Acclimation of fine root systems to soil warming: comparison of an experimental setup and a natural soil temperature gradient. Ecosystems, 22(3), 457-472~~. doi:https://doi.org/10.1007/s10021-018-0280-y~~, 2019.

Poeplau, C., and Don, A.: Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe, Geoderma, 192, 189-201, 2013.

Poeplau, C., Don, A., Dondini, M., Leifeld, J., Nemo, R., Schumacher, J., Senapati, N., and Wiesmeier, M.: Reproducibility of a soil organic carbon fractionation method to derive RothC carbon pools, European Journal of Soil Science, 64, 735-746, ~~10.1111/ejss.12088,~~ 2013.

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

Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M. F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A., Macdonald, L. M., Soong, J., Trigalet, S., Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I., and Nieder, R.: Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – A comprehensive method comparison, Soil Biology and Biochemistry, 125, 10-26, ~~https://doi.org/10.1016/j.soilbio.2018.06.025, 2018.~~2018.

Poeplau, C., Barré, P., Cécillon, L., Baudin, F., and Sigurdsson, B. D.: Changes in the Rock-Eval signature of soil organic carbon upon extreme soil warming and chemical oxidation-A comparison, Geoderma, 337, 181-190, 2019.

Prescott, C. E., Zabek, L. M., Staley, C. L., and Kabzems, R.: Decomposition of broadleaf and needle litter in forests of British Columbia: influences of litter type, forest type, and litter mixtures, Canadian Journal of Forest Research, 30, 1742-1750, 10.1139/x00-097, 2000.

R Development Core Team: R: A language and environment for statistical computing. , R Foundation for Statistical Computing, Vienna, Austria, 2010.

Rosenstock, N., Ellström, M., Oddsdottir, E., Sigurdsson, B. D., Wallander, H.: Carbon sequestration and community composition of ectomycorrhizal fungi across a geothermal warming gradient in an Icelandic spruce forest. Fungal Ecology, 40, 32-42, 2019.

Rustad, L~~. J. N~~.: Global change: Matter of time on the prairie, Nature, 413, 578, 2001.

Formatiert: Schriftart: Nicht Kursiv

Scharlemann, J. P., Tanner, E. V., Hiederer, R., and Kapos, V.: Global soil carbon: understanding and managing the largest terrestrial carbon pool, Carbon Management, 5, 81-91, 2014.

Schmidt, M. W. I., Rumpel, C., and Kögel-Knabner, I.: Evaluation of an ultrasonic dispersion procedure to isolate primary organomineral complexes from soils, European Journal of Soil Science, 50, 87-94, ~~10.1046/j.1365-2389.1999.00211.x,~~ 1999.

Schnecker, J., Borken, W., Schindlbacher, A., & Wanek, W.: Little effects on soil organic matter chemistry of density fractions after seven years of forest soil warming. Soil Biology and Biochemistry, 103, 300-307, 2016.

Shaver, G. R., Canadell, J., Chapin, F. S., Gurevitch, J., Harte, J., Henry, G., Ineson, P., Jonasson, S., Melillo, J., and Pitelka, L~~. J. B~~.: Global Warming and Terrestrial Ecosystems: A Conceptual Framework for Analysis: Ecosystem responses to global warming will be complex and varied. Ecosystem warming experiments hold great potential for providing insights on ways terrestrial ecosystems will respond to upcoming decades of climate change. Documentation of initial conditions provides the context for understanding and predicting ecosystem responses, BioScience, 50, 871-882, 2000.

Shepherd, M., Harrison, R., Webb, J~~. J. S. u., and Management:~~.: Managing soil organic matter–implications for soil structure on organic farms, Soil Use and Management, 18, 284-292, 2002.

Sigurdsson, B. D., Leblans, N. I., Dauwe, S., Gudmundsdottir, E., Gundersen, P., Gunnarsdottir, G. E., Holmstrup, M., Ilieva-Makulec, K., Katterer, T., and Marteinsdottir, B.-S.: Geothermal ecosystems as natural climate change experiments: The ForHot research site in Iceland as a case study, Icelandic ~~agricultural sciences. Reykjavik, Akureyri~~Agricultural Sciences, 29, 53-71, 2016.

Sigurdsson, B. D., Leblans, N., Oddsdottir, E. S., Maljanen, M., Janssens, I. A.: Effects of geothermal soil warming on soil carbon and nutrient processes in a Sitka spruce plantation. Working Papers of the Finnish Forest Research Institute, 316, 11-13, 2014.

Six, J., Conant, R., Paul, E. A., and Paustian, K.: Stabilization mechanisms of soil organic matter: implications for C-saturation of soils, Plant and soil, 241, 155-176, 2002.

Six, J., Bossuyt, H., Degryze, S., Denef, K~~. J. S., and Research, T.:~~.: A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics, Soil and Tillage Research, 79, 7-31, 2004.

Smith, P. J. G. C. B.: How long before a change in soil organic carbon can be detected?, Global Change Biology, 10, 1878-1883, 2004.

Tarnocai, C., Canadell, J., Schuur, E. A., Kuhry, P., Mazhitova, G., and Zimov, S~~. J. G. b. c~~.: Soil organic carbon pools in the northern circumpolar permafrost region, Global Change Biology, 23, 2009.

Tisdall, J. M., and Oades, J. M.: Organic matter and water-stable aggregates in soils, European Journal of Soil Science, 33, 141-163, 1982.

Totsche, K. U., Amelung, W., Gerzabek, M. H., Guggenberger, G., Klumpp, E., Knief, C., Lehndorff, E., Mikutta, R., Peth, S., Prechtel, A~~. J. J. o. P. N., and Science, S~~.: Microaggregates in soils, Journal of Plant Nutrition and Soil Science, 181, 104-136, 2018.

van Gestel, N., Shi, Z., van Groenigen, K. J., Osenberg, C. W., Andresen, L. C., Dukes, J. S., Hovenden, M. J., Luo, Y., Michelsen, A., Pendall, E., Reich, P. B., Schuur, E. A. G., and Hungate, B. A.: Predicting soil carbon loss with warming, Nature, 554, E4, ~~10.1038/nature25745~~2018, ~~https://www.nature.com/articles/nature25745#supplementary-information, 2018.~~

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von Lützow, M., Kogel-Knabner, I., Ekschmittb, K., Flessa, H., Guggenberger, G., Matzner, E., and Marschner, B.: SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms, Soil Biology & Biochemistry, 39, 2183-2207, ~~10.1016/j.soilbio.2007.03.007,~~ 2007.

Walker, T. W. N., Kaiser, C., Strasser, F., Herbold, C. W., Leblans, N. I. W., Woebken, D., Janssens, I. A., Sigurdsson, B. D., Richter, A.: Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. Nature Climate Change, *8*(10), 885-889~~. doi:10.1038/s41558-018-0259-x~~, 2018.

Wickham, H.: ggplot2: elegant graphics for data analysis, Springer, 2016.

Zhang, Y., Chen, W., Smith, S. L., Riseborough, D. W., and Cihlar, J.: Soil temperature in Canada during the twentieth century: Complex responses to atmospheric climate change, Journal of Geophysical Research: Atmospheres, 110, 2005.

Zimmermann, M., Leifeld, J., Schmidt, M. W. I., Smith, P., and Fuhrer, J.: Measured soil organic matter fractions can be related to pools in the RothC model, European Journal of Soil Science, 58, 658-667, ~~10.1111/j.1365-2389.2006.00855.x,~~ 2007.

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Table 1: Average soil organic carbon (SOC) ~~concentrations~~contents (n=5) of all fractions and the bulk soil with standard errors and letters indicating significant differences (p<0.05) across warming intensities [°C] within one soil depth. Absolute and relative changes in SOC content as derived from linear regression models are also displayed for both investigated soil depths. Although this was not the best model in all cases, we used this value as a proxy to compare the warming response among fractions. Fractions were dissolved organic carbon (DOC), particulate organic matter (POM), SOC in sand and aggregates (SA), total silt- and clay-sized SOC (SC) and oxidation resistant silt- and clay-sized SOC (rSOC).

Depth	Warming intensity	Bulk soil	DOC	POM	SA	SC	rSOC
	°C	g C kg soil^{-1}	g C kg soil^{-1}	g C kg soil^{-1}	g C kg fraction^{-1}	g C kg fraction^{-1}	g C kg fraction^{-1}
Topsoil	0	75.1±5.5a	0.7±0.1a	11.8±2.6ab	6.8±0.5a	5.1±0.2a	1.6±0.1a
	1	71.5±4.0a	1.0±0.3a	21.6±4.4a	6.3±0.7a	4.9±0.3a	1.6±0.1a
	1.9	65.9±3.0a	0.7±0.1a	12.9±1.8abc	7.1±1.0a	5.4±0.2a	1.9±0.1a
	2.7	64.7±1.5ab	0.6±0.1a	16.0±3.5ab	5.8±0.4a	5.2±0.1a	1.7±0.1a

	5.8	53.1±3.2b	0.5±0.1a	6.0±1.5bc	5.2±0.6a	5.0±0.3a	1.8±0.1a
	17.5	26.5±1.9c	0.5±0.1a	0.4±2.6c	2.6±0.4b	3.3±0.2b	1.1±0.1b
Absolute change [g C kg^{-1} fraction °C^{-1}]		-2.71	-0.02	-0.84	-0.25	-0.11	-0.03
Relative change [% °C^{-1}]		-3.6	-2.49	-7.15	-3.63	-2.14	-2.05
Subsoil	0	36.2±4.3a	0.3±0.1ab	3.4±0.8a	2.9±0.7a	4.1±0.3a	1.3±0.1a
	1	28.6±4.2a	0.3±0.1ab	3.4±0.7a	1.7±0.4ab	3.8±0.3a	1.4±0.2a
	1.9	29.4±4.6a	0.3±0.0ab	2.0±0.3ab	1.5±0.4ab	3.8±0.5a	1.2±0.2a
	2.7	24.2±1.9a	0.2±0.0ab	2.1±0.7ab	1.2±0.1ab	3.4±0.2a	1.1±0.1a
	5.8	22.6±3.3a	0.3±0.0ab	0.8±0.2b	0.9±0.2b	3.1±0.4a	1.1±0.2a
	17.5	4.0±0.9b	0.2±0.0b	0.3±0.1b	0.2±0.0c	0.5±0.2b	0.2±0.1b
Absolute change [g C kg^{-1} fraction °C^{-1}]		-1.63	-0.01	-0.16	-0.11	-0.2	-0.07
Relative change [% °C^{-1}]		-4.52	-2.53	-4.79	-3.96	-4.95	-5.04

Table 2: Summary of the analysis of similarity (ANOSIM) testing differences in ~~SOC fraction~~the distribution of SOC in investigated fractions for all warming intensities tested against the unwarmed reference. P values <0.05 indicate significant differences, while n.s. indicates non~~-~~-significant differences. An R value close to 1 ~~suggest~~suggests dissimilarity between groups.

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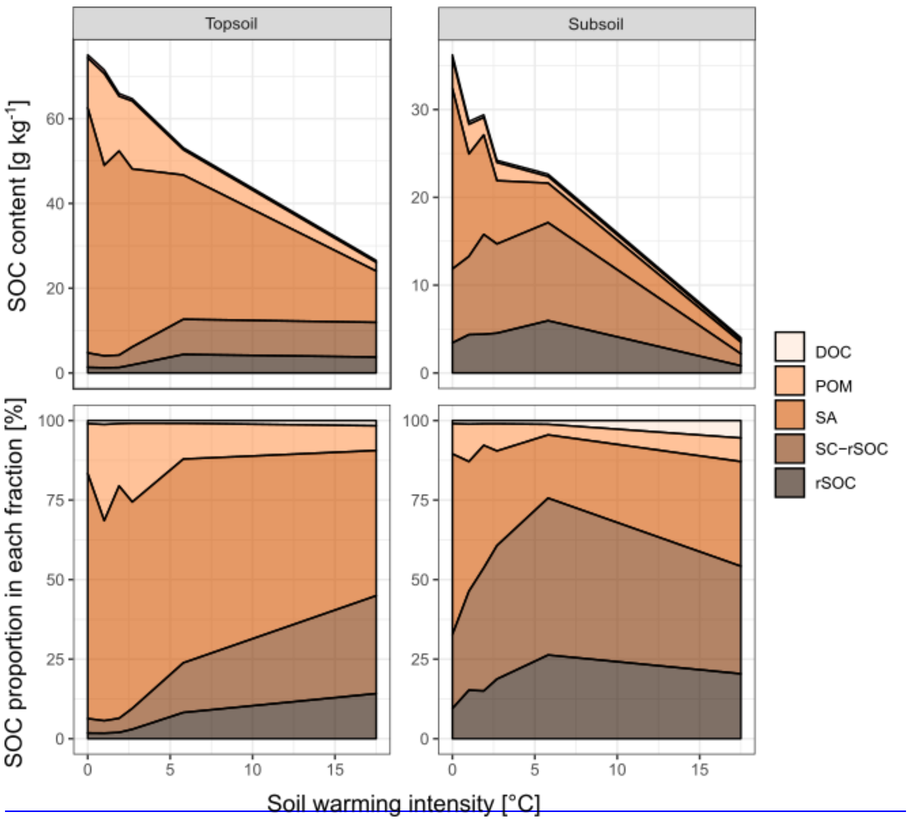
Warming	Topsoil		Subsoil	
[°C]	*R*	*p*	*R*	*p*
1	0.260	n.s.	0.040	n.s.
1.9	0.044	n.s.	0.168	n.s.
2.7	0.116	n.s.	0.380	0.044
5.8	0.272	0.036	0.840	0.011
17.5	0.868	0.005	0.196	n.s.

Table 3: Summary of the linear regression models (*p* values) assessing effects of warming, ecosystem (grassland vs. forest) and their interaction on soil organic carbon (SOC) for the bulk soil and all isolated fractions.

	Topsoil			Subsoil		
Fraction	Warming	Ecosystem	Interaction	Warming	Ecosystem	Interaction
Bulk soil	<0.001	<0.001	0.029	<0.001	0.038	n.s.
DOC	0.016	n.s.	n.s.	n.s.	0.001	n.s.
POM	<0.001	<0.001	0.002	<0.001	0.049	n.s.
SA	<0.001	<0.001	0.023	<0.001	<0.001	n.s.
SC-rSOC	<0.001	<0.001	0.001	<0.001	n.s.	n.s.
rSOC	<0.001	<0.001	0.002	<0.001	n.s.	0.042



Topsoil
Subsoil
SOC content [g kg-1]
SOC proportion in each fraction [%]
Soil warming intensity [°C]
DOC
POM
SA
SC−rSOC
rSOC

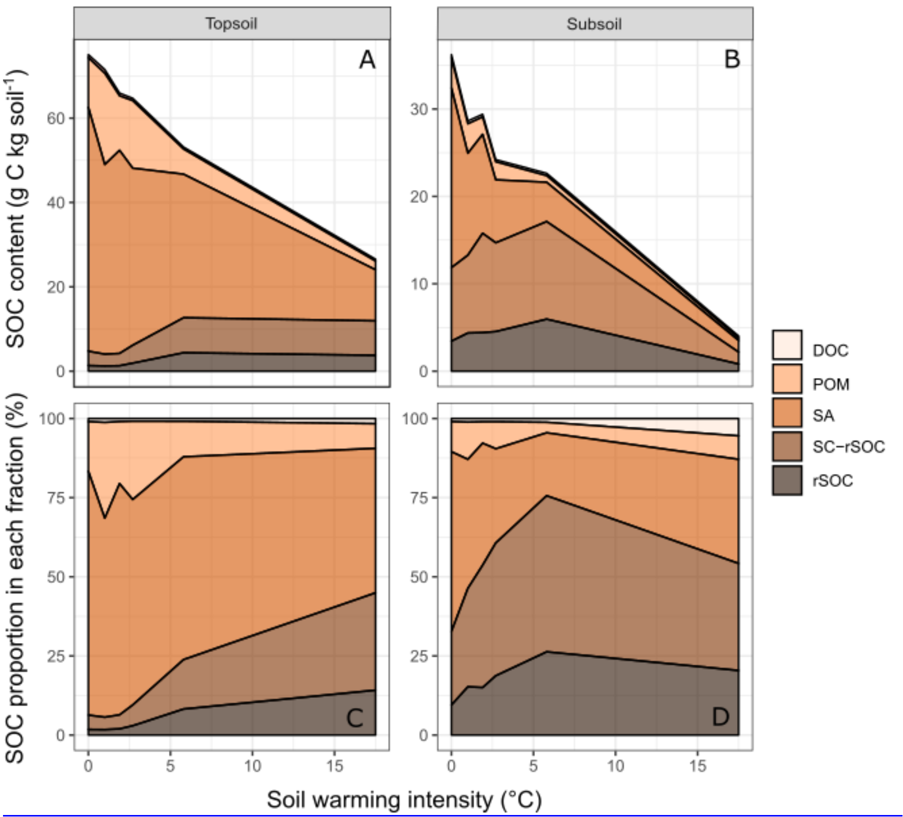
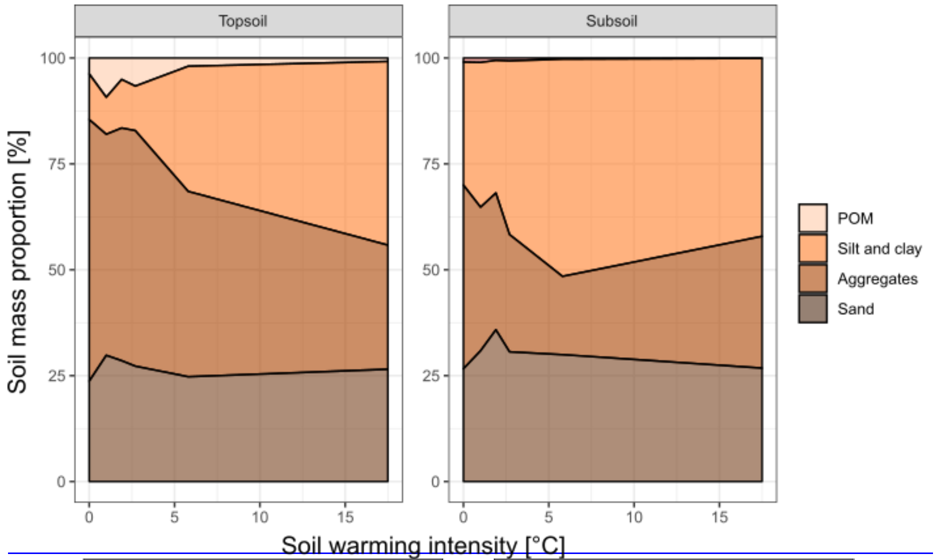


Figure 1: Areal plots of A) soil organic carbon (SOC) content in the topsoil and B) SOC content in the subsoil, C) SOC proportion in each fraction of the topsoil and D) SOC proportion in each fraction of the subsoil as a function of warming intensity. Fractions were dissolved organic carbon (DOC), particulate organic matter (POM), SOC in sand and aggregates (SA), non-oxidation resistant silt- and clay-sized SOC (SC-rSOC) and oxidation resistant silt- and clay-sized SOC (rSOC).



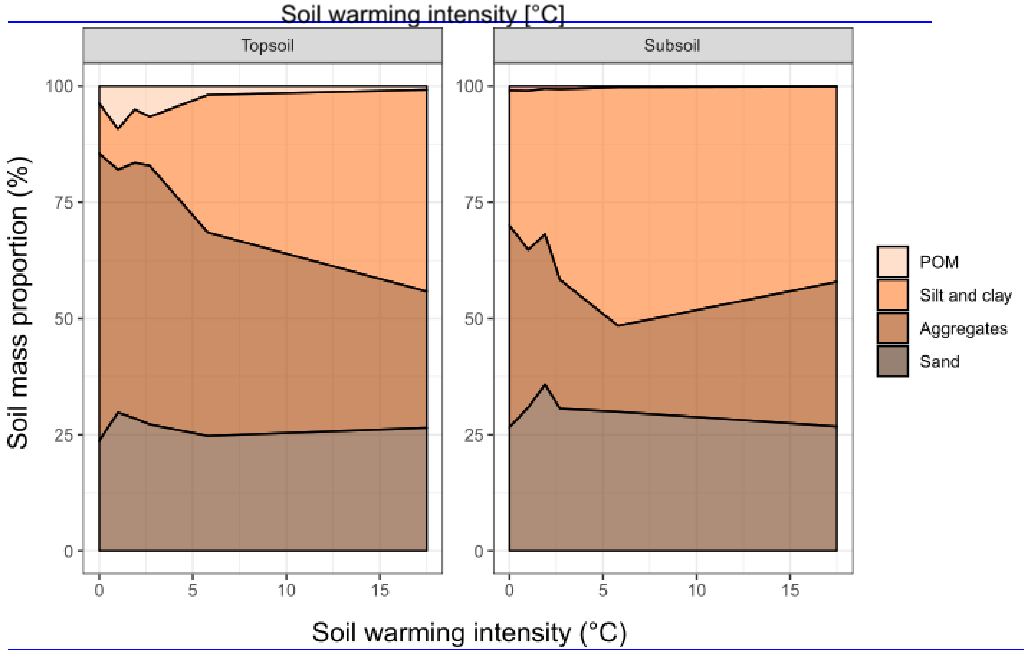
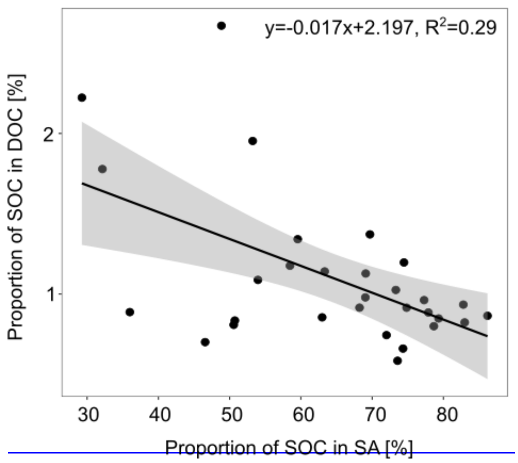


Figure 2: Areal plots of soil mass distribution in the fractions particulate organic matter (POM), sand and stable aggregates (SA) and silt and clay (SC) as a function of warming intensity.



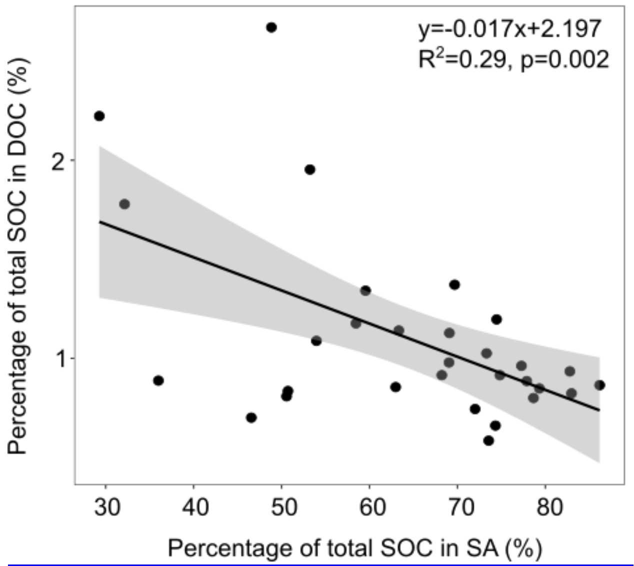
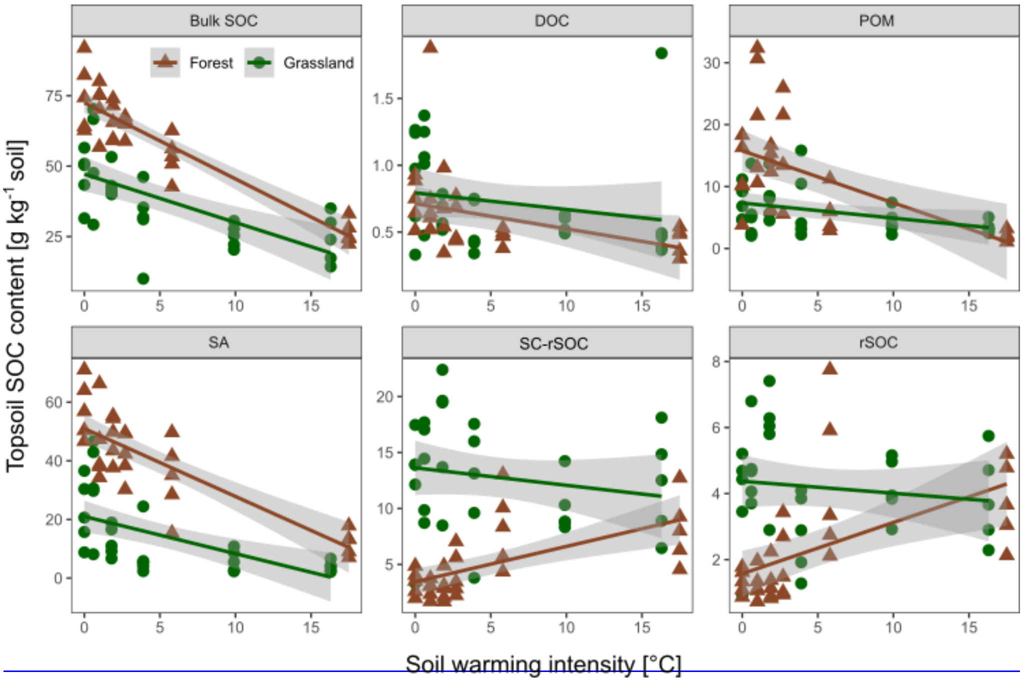


Figure 3: Correlation between the proportion of soil organic carbon (SOC) in the sand and aggregates (SA) and dissolved organic carbon (DOC) fractions with 95% confidence interval.



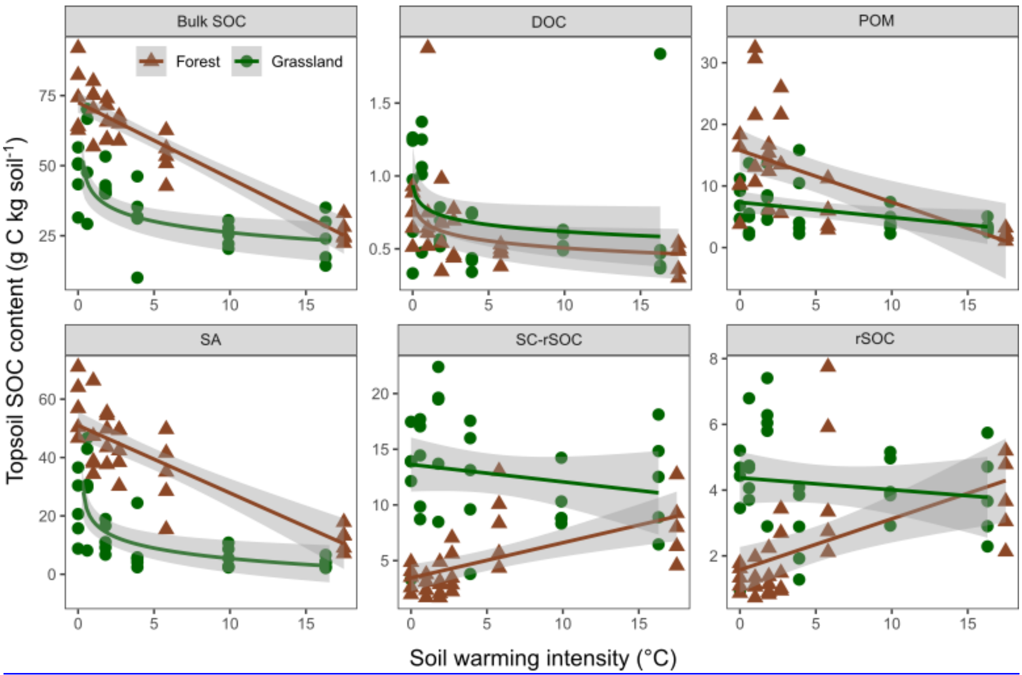
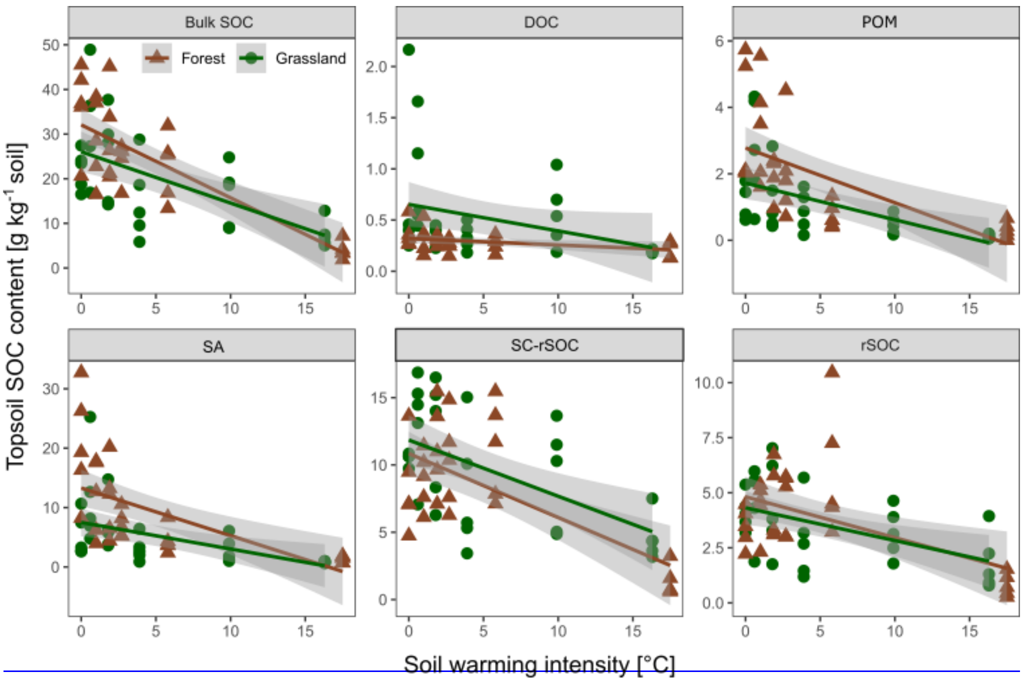


Figure 4: ~~Scatter plots showing soil~~Soil organic carbon (SOC) mass in bulk soil and fractions of the forest and grassland topsoils (0-10 cm) as a function of warming intensity~~.~~ with linear and logarithmic fits with 95% confidence intervals. Fractions were dissolved organic carbon (DOC), particulate organic matter (POM), SOC in

sand and aggregates (SA), non-oxidation resistant silt- and clay-sized SOC (SC-rSOC) and oxidation resistant silt- and clay-sized SOC (rSOC).



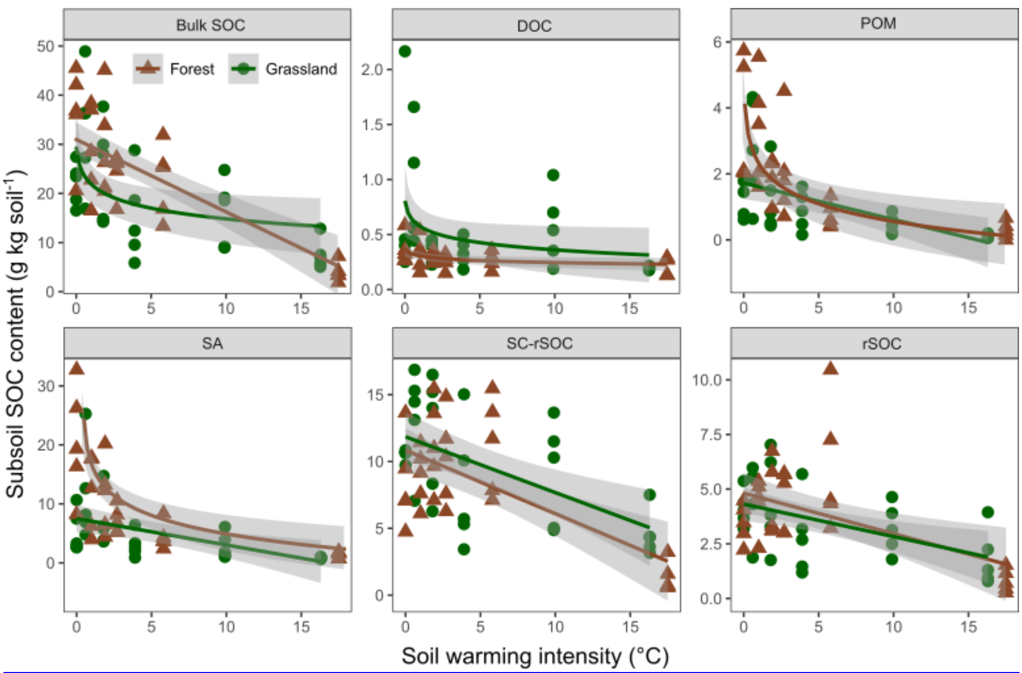
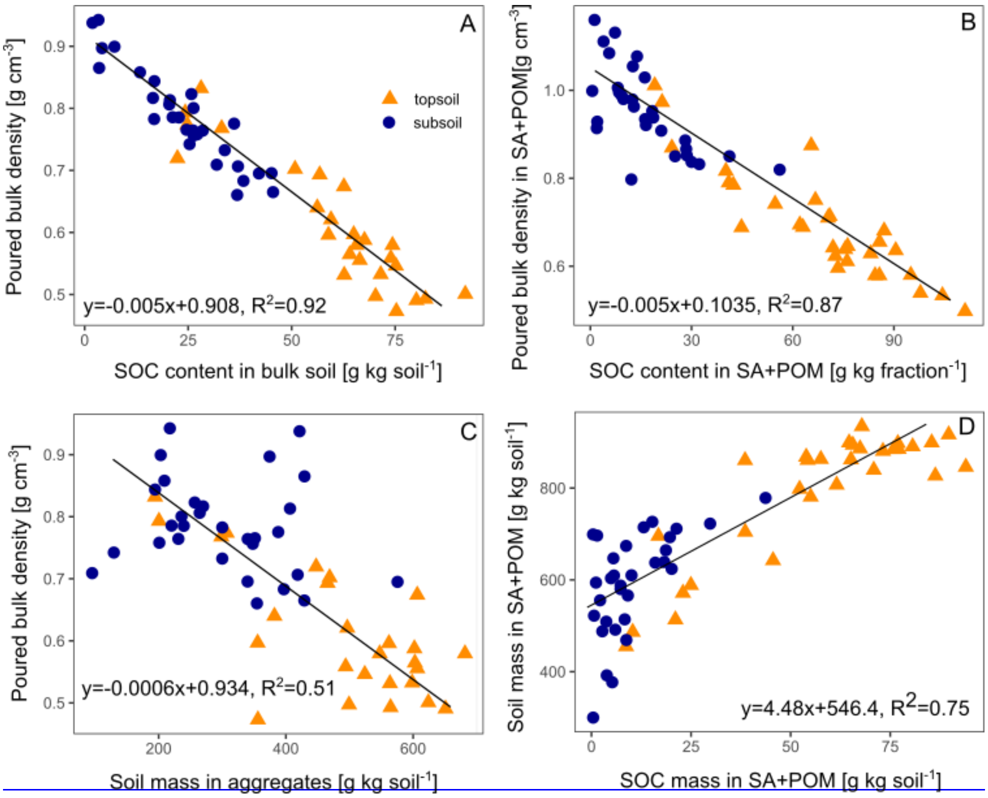


Figure 5: Scatter plots showing soil organic carbon (SOC) content in bulk soil and fractions of the forest and grassland subsoils (20-30 cm) as a function of warming intensity~~.~~ with linear and logarithmic fits with 95% confidence intervals. Fractions were dissolved organic carbon (DOC), particulate organic matter (POM), SOC in sand and aggregates (SA), non-oxidation resistant silt- and clay-sized SOC (SC-rSOC) and oxidation resistant silt- and clay-sized SOC (rSOC).



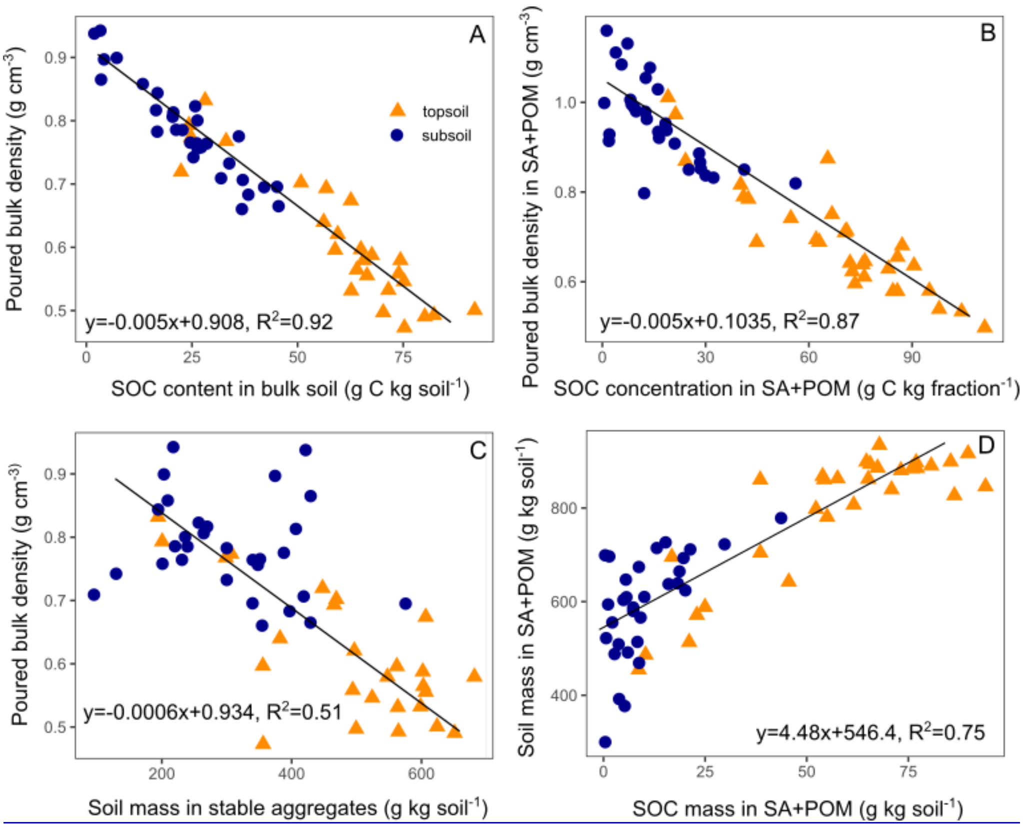


Figure 6: Poured bulk density as a function of soil organic carbon (SOC) content in A) the bulk soil and B) the coarse (>63 µm) soil fraction (sand and stable aggregates=SA and particulate organic matter=POM); C) poured bulk density as a function of soil mass in aggregates and D) soil mass in the coarse soil fraction as a function of SOC mass in the coarse soil fraction with regression models fitted to all observations~~.~~ ($p<0.001$ for all models).