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Sensitivity of soil carbon fractions and their specific stabilisation mechanisms to extreme soil warming in a subarctic grassland

Running head: Soil carbon loss after strong natural warming

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Abstract

Terrestrial carbon cycle feedbacks to global warming are major uncertainties in climate models. For in-depth understanding of changes in soil organic carbon (SOC) after soil warming, long-term responses of SOC stabilisation mechanisms such as aggregation, organo-mineral interactions and chemical recalcitrance need to be addressed. This study investigated the effect of six years of geothermal soil warming on different SOC fractions in an unmanaged grassland in Iceland. Along an extreme warming gradient of +0 to $\sim+40^\circ\text{C}$, we isolated five fractions of SOC that varied conceptually in turnover rate from active to passive in the following order: particulate organic matter (POM), dissolved organic carbon (DOC), SOC in sand and stable aggregates (SA), SOC in silt and clay (SC-rSOC) and resistant SOC (rSOC). Soil warming of 0.6°C increased bulk SOC by $22\pm 43\%$ (0-10 cm soil layer) and $27\pm 54\%$ (20-30 cm), while further warming led to exponential SOC depletion of up to $79\pm 14\%$ (0-10 cm) and $74\pm 8\%$ (20-30) in the most warmed plots ($\sim+40^\circ\text{C}$). Only the SA fraction was more sensitive than the bulk soil, with $93\pm 6\%$ (0-10 cm) and $86\pm 13\%$ (20-30

cm) SOC losses and the highest relative enrichment in ^{13}C as an indicator for the degree of decomposition ($+1.6\pm 1.5\text{‰}$ in 0-10 cm and $+1.3\pm 0.8\text{‰}$ in 20-30 cm). The SA fraction mass also declined along the warming gradient, while the SC fraction mass increased. This was explained by deactivation of aggregate-binding mechanisms. There was no difference between the responses of SC-rSOC (slow-cycling) and rSOC (passive) to warming, and ^{13}C enrichment in rSOC was equal to that in bulk soil. We concluded that the sensitivity of SOC to warming was not a function of age or chemical recalcitrance, but triggered by changes in bio-physical stabilisation mechanisms, such as aggregation.

Introduction

Soils contain about three-fold the amount of carbon stored in the atmosphere (Davidson *et al.*, 2000). The CO_2 efflux from soil as the end-product of soil organic carbon (SOC) mineralisation has been shown to increase with increasing temperature (Conant *et al.*, 2008, Knorr *et al.*, 2005). Terrestrial carbon cycle feedback to global warming is thus expected to significantly accelerate climate change. Ecosystems at high northerly latitudes, which have the highest SOC densities and stocks (Scharlemann *et al.*, 2014) and are predicted to be exposed to the strongest temperature rise (Stocker *et al.*, 2013), are likely to act as hotspots of biospheric CO_2 efflux. However, it has also been suggested that a global increase in air temperature can lead to higher SOC storage, due to stimulated net primary production (NPP) and thus higher carbon input to the soil (Melillo *et al.*, 2002, Shaver *et al.*, 2000). While the short-term temperature dependency of SOC mineralisation in disturbed, unvegetated and incubated soils is well established, the mid- to long-term effects of rising temperature on the carbon balance and SOC storage at ecosystem scale have not been well studied. This is especially true for soil warming exceeding 5°C , which might be related to the fact that *in situ*

soil temperature manipulation is complex and expensive and that naturally warmed sites, e.g. warmed by geothermal energy (O'Gorman *et al.*, 2014), are scarce. The resulting lack of complex mechanistic understanding constitutes a major uncertainty in climate models.

Previous multi-annual field studies have reported mixed responses of SOC to elevated temperature. Melillo *et al.* (2002) increased the soil temperature in a mid-latitude hardwood forest for one decade by 5°C and observed that CO₂ flux from the warmed soil was only significantly elevated in the first four years, while increased nitrogen (N) mineralisation stimulated plant growth in the warmed plots, leading to rapid compensation for SOC losses in the following six years. After two decades of a greenhouse experiment in the arctic tundra, which elevated soil temperatures by about 1-2°C, Sistla *et al.* (2013) found no differences in total SOC storage. In a tallgrass prairie, Xu *et al.* (2012) found depleted labile SOC stocks after 8 years of soil warming (2°C) but little effect on total SOC stocks, while Schaeffer *et al.* (2013) reported significant SOC losses after 7 years of soil warming (4°C) in a high arctic tundra soil. The direction of the response (loss or gain) of SOC to increased temperature could be modulated by the degree of warming, but this can only be investigated in experiments with gradients of soil warming.

The debate about the temperature sensitivity of different SOC pools is ongoing, with the old pool paradoxically concluded to be less (Knorr *et al.*, 2005, Lomander *et al.*, 1998), more (Conant *et al.*, 2008, Lefèvre *et al.*, 2014, Leifeld & Fuhrer, 2005) or equally (Conen *et al.*, 2006) sensitive than young SOC. Karhu *et al.* (2010) reported the intermediate SOC pool to be most temperature sensitive. The high variability in results is thereby most likely attributable to the high variability in experimental approaches. Due to missing evidence and for reasons of simplification, most SOC turnover models do not distinguish between the temperature sensitivity of young and old pools. Conant *et al.* (2011) argued that the debate should be refocused on factors controlling the decomposability of SOM. They listed several

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mechanisms responsible for SOC stabilisation such as protection by the soil matrix (aggregation, organo-mineral interactions) or chemical recalcitrance that should be studied in depth regarding their response to warming. Depolymerisation of complex compounds (Davidson *et al.*, 2006), microbial enzyme production (Cusack *et al.*, 2010) as well as adsorption/desorption (Ten Hulscher & Cornelissen, 1996) and aggregate turnover were identified processes that were likely to respond to temperature changes. They further stressed that long-term field-studies are necessary to study processes controlling the temperature sensitivity of the SOC pools with decadal to centennial turnover times, as the major components of SOC stocks (Conant *et al.*, 2011).

Zimmermann *et al.* (2007) published a SOC fractionation procedure which corresponds well to the model pools of the Rothamsted Carbon Model (RothC) (Coleman & Jenkinson, 1996), one of the most frequently used SOC models. The procedure is a combination of physical and chemical fractionation and yields five different fractions, forming a continuum from fast cycling to passive. Moreover, each of the isolated fractions is characterised by a dominant stabilization mechanism, ranging from no stabilisation to aggregate formation, organo-mineral interactions in the silt and clay fraction and chemical recalcitrance.

Natural abundance of the stable isotope ^{13}C increases with SOC age (Balesdent *et al.*, 1996, Menichetti *et al.*, 2015). The underlying processes of ^{13}C enrichment in SOC are diverse but predominantly related to biological fractionation. Isotopic fractionation occurs within the plant, during preferential decay of ^{13}C depleted organic compounds but also within decomposer cells during metabolism (Ehleringer *et al.*, 2000, Gleixner *et al.*, 1993). Boström *et al.* (2007) hypothesised a preferential retention of ^{13}C -enriched microbial necromass. The abundance of ^{13}C is therefore a mixed indicator for SOC origin (plant-derived compound), decomposability, degree of recycling and thus indirectly for SOC turnover time and age. Menichetti *et al.* (2015) used five long-term bare fallow experiments in temperate climates to

link shifts in $\delta^{13}\text{C}$ to SOC losses and to quantify the shift in ^{13}C abundance over time. They found a mean annual increase in $\delta^{13}\text{C}$ of 0.013‰ which can be used as a reference for the rate of ^{13}C enrichment along with SOC depletion.

The area around the village of Hveragerði in Iceland is located close to the Eurasian and North-American tectonic plate boundary that splits the country into two halves. The region is pervaded by shallow geothermal bedrock channels, one of which was shifted after a large earthquake in May 2008 (Halldorsson & Sigbjörnsson, 2009). Since then, a previously unwarmed hillslope has been warmed (O'Gorman *et al.*, 2014). A gradient of soil temperature increase from +0 to > +40°C has been formed, creating outstanding opportunities to conduct fundamental ecological and biogeochemical research on the effects of soil warming.

In this study, we used the natural soil warming gradient at Hveragerði to investigate the sensitivity of the five different SOC fractions to soil warming. Natural abundance of ^{13}C in the fractions of the unwarmed reference soil was used to test, whether the fractions differed initially in age or turnover time. Shifts in $\delta^{13}\text{C}$ along the warming gradient were used to assess whether this difference among fractions had any influence on their temperature response. We aimed to answer the question which SOC stabilisation mechanism is most sensitive to temperature changes and what might drive this sensitivity.

Materials and Methods

Study site and soil sampling

The study site is located on an unmanaged grassland hillslope close to the village of Hveragerði (64°00'01''N, 21°11'09''W, 85-145 m a.s.l) in the Hengil geothermal area, 40 km east of Reykjavik, Iceland. It is part of the ongoing FORHOT research project

(www.forhot.is). Mean monthly air temperature (1961-1990) at the site is -1.1°C in the coldest month (January) and 10.8°C in the warmest (July) and mean annual precipitation amounts to 1372 mm (Icelandic Meteorological Office; www.vedur.is). The unwarmed unmanaged grassland is dominated by common bent (*Agrostis capillaris* L.), common meadow grass (*Poa pratensis* L.), meadow horsetail (*Equisetum pratense* L.) and meadow buttercup (*Ranunculus acris* L.). The underlying soil, classified as Brown Andosol (Arnalds, 2015), has a fine texture (silt loam) and is on average 38 cm thick. It lies on top of a hillslope moraine and has rather high stoniness. In the topsoil layer (0-10 cm) of the unwarmed grassland soil, $\text{pH}_{(\text{H}_2\text{O})}$ is 5.7, $\text{pH}_{(\text{KCl})}$ is 4.2 and C:N ratio is 12.1.

The study site is located in an area warmed since 29 May 2008, when a large earthquake (Halldorsson & Sigbjörnsson, 2009) shifted geothermal systems under previously unwarmed soils. The soil warming is caused by heat conduction from the underlying bedrock, which is warmed from below by hot groundwater. Due to this heat conduction, a gradient in soil temperature with soil depth was expected, but was for the upper 30 cm only detected for the most heated plots ($+16.9^{\circ}\text{C}$ and $+40^{\circ}\text{C}$). Profiles of soil temperature changes have been measured at two points in time (data not shown). The soil warming, as constantly measured at 10 cm depth, is relatively stable throughout the year, resulting in normal seasonal fluctuations in soil temperature. No sign of soil contamination by geothermal water has been found within the soil along the warming gradient.

In 2013, five 50-80 m long transects were established along the temperature gradient, with seven 2 m x 2 m permanent plots in each transect, in which the warming was $+0^{\circ}$, $+0.6^{\circ}$, $+1.8^{\circ}$, $+3.9^{\circ}$, $+9.9^{\circ}$ and $+16.3^{\circ}\text{C}$ (mean for spring 2013-spring 2015, soil temperature measured hourly at 10 cm soil depth) and $+40^{\circ}\text{C}$ (measured only occasionally by hand). Depending on the position of the geothermal warming, three transects ran downhill (unwarmed reference on the upper slope) and two transects ran uphill (unwarmed reference

on the lower slope), which gave a certain natural randomisation of the five replicates (transects). Despite the strong gradients in temperature and the sloping terrain, the spatial variability in key soil parameters such as pH and water content was negligible below the +16.3°C warming plots (O'Gorman *et al.*, 2014). However, when soil warming exceeded 10°C, the plant species composition of the grassland changed gradually, with mosses becoming more and more abundant and the warmest plots investigated (+40°C) were only vegetated with mosses.

In December 2014, soil to a depth of 30 cm was sampled close to all permanent plots, in subplots that were established for destructive measurements. A thin auger ($\phi = 2$ cm) was used, due to the high stoniness of the soil. Five auger cores per plot were divided into three depth increments (layers), 0-10 cm, 10-20 cm and 20-30 cm, and pooled per layer. In this study, soil from only the uppermost (0-10 cm; hereafter “topsoil”) and lowermost (20-30 cm; hereafter “subsoil”) layers was fractionated and analysed.

Soil fractionation and analysis

Soil samples were dried at 60°C, visible fine roots were removed and the soil was sieved to 2 mm. A 20 g subsample was taken from each of a total of 70 samples (2 depth layers x 7 plots x 5 transects) and SOC fractionation was performed as initially described by (Zimmermann *et al.*, 2007) and modified by Poeplau *et al.* (2013). A schematic overview of the procedure is depicted in Figure 1. For this, 100 mL deionised water were added to the soil in a 250 mL glass beaker and dispersed with 22 J mL⁻¹, using an output power of 30 W. The dispersion was wet-sieved over a 63 µm sieve to separate the fine silt and clay (SC) fraction from a coarse fraction (>63 µm), using an aerosol pump spray to flush the sample with a fixed amount of 3000 mL water. Poeplau *et al.* (2013) recommends using 2000 mL to flush the

sample, but this was not sufficient for the Icelandic Andosol studied here to entirely separate into the two fractions. The suspension was centrifuged for 30 minutes at 1000 g. An aliquot of the decanted water (200 mL) was filtered through a 0.45 μm membrane filter and analysed for DOC (as one fraction) in a liquid analyser (TOC-VCPH, Shimadzu). The SC fraction and the coarse sieving residues were dried at 60°C and weighed. The coarse fraction (>63 μm) was further separated into a heavy sand and stable aggregates (SA) fraction and a light particulate organic matter (POM) fraction by density fractionation with sodium polytungstate (SPT) solution at a density of 1.6 g cm^{-3} . This is a slight modification from the original protocol, which recommends using a density of 1.8 g cm^{-3} . However, a pre-test revealed that a large amount of mineral soil aggregates floated up at a density of 1.8 g cm^{-3} , while the POM fraction should only consist of fairly undecomposed plant tissue. After adding the SPT to the coarse soil, the sample was centrifuged for 15 minutes at 1000 g and left to rest for several hours, until the POM fraction was entirely floating on the SPT. The POM was then carefully transferred to sieve bags. The density fractionation step was repeated once to ensure complete separation of SA and POM. Both fractions were carefully washed to remove all SPT and then dried and weighed. Finally, a chemically resistant SOC (rSOC) fraction was isolated from the fine SC fraction by oxidation with 6% NaOCl. The NaOCl solution was first adjusted to a pH of 8 using HCl, before 50 mL of the solution were added to a 1 g subsample of the SC fraction. After 18 hours of oxidation at room temperature, the sample was centrifuged (15 min, 1000 g) and decanted. This procedure was repeated twice and after each oxidation and decanting step the sample was washed once with 50 mL deionised water. To ensure complete oxidation and washing, a vortex shaker was used every time liquid was added to the soil. The NaOCl-resistant carbon (rSOC) in the 1g subsample of the SC fraction was multiplied with the total SC fraction mass to obtain the total amount of rSOC in the soil sample. Finally, we subtracted rSOC from the total carbon in the SC fraction to receive a fraction that is bound to

silt and clay particles but not resistant to NaOCl oxidation. This fraction is called SC-rSOC. In the following, when the term SC fraction is used, we refer to total mass of carbon or soil in the silt and clay fraction. The fractionation procedure thus yielded five fractions, two fast cycling (POM, DOC), one intermediate (SA), one slow cycling (SC-rSOC) and one passive (rSOC). All solid fractions, as well as a bulk soil subsample, were milled and successively analysed for total C and N and the abundances of stable isotopes ^{13}C in a continuous flow Isoprime isotope-ratio mass spectrometer (GV Instruments; Manchester, UK) coupled to an elemental analyser (EuroEA3024; Eurovector, Milan, Italy) at Lund University. The liquid fraction (DOC) was freeze dried and the residues were analysed for $\delta^{13}\text{C}$. Unfortunately, it was not possible to recover enough solid freeze-dried material for all samples. Therefore, the $\delta^{13}\text{C}$ dataset was incomplete (67%) and has to be interpreted with caution. The total fraction masses and SOC contents were used to calculate absolute and relative changes of each fraction with increasing soil temperature.

Statistics

Linear mixed effect models were applied to test the effect of temperature on bulk SOC and all fractions for significance. This was done for the variables SOC concentration, SOC mass and total mass in the case of the POM, SA and SC fractions. Transect was used as a random effect. To test whether soil temperature had a significant effect on the distribution of SOC in different fractions, the relative proportion of each fraction in the total SOC for each sample was calculated and a MANOVA was conducted with temperature as independent variable and the five fractions as dependent variables. Statistical analyses were conducted in R, while for mixed effect models the package lme4 was used (Bates *et al.*, 2007). Errors given in the text are standard deviations.

Results

Change in carbon concentration

Continuous soil warming of more than 3.9°C for 6.5 years had a significant effect on bulk soil SOC concentration (Table 1). Within 6.5 years of soil warming, SOC in the bulk soil had been depleted by 79±14% (topsoil) and 74±8% (subsoil) in the most warmed plots (Fig. 2a, 2b). The average SOC concentration of 46.5±9.6 g kg⁻¹ (topsoil) and 26.4±4.3 g kg⁻¹ (subsoil) in the unwarmed plots dropped to 9.7±6.9 g kg⁻¹ and 6.7±1.2 g kg⁻¹, respectively. The C concentration in the DOC, SC-rSOC, rSOC and SA fractions also declined significantly, but only the SA fraction responded more strongly than the bulk soil, with losses of 93±6% in the topsoil and 86±13% in the subsoil of the most warmed plots. No significant changes in C concentration with soil warming were found for the POM fraction, which was related to the fact that the density fractionation method employed here isolates an almost entirely organic fraction with a rather stable C content. Interestingly, soil warming of 0.6°C led to a pronounced increase in SOC concentration in bulk soil in both soil layers. Additional increases in soil temperature led to an exponential decline in SOC, with a new equilibrium, i.e. no further changes in SOC, being reached at roughly 16.6°C in the subsoil. Equilibrium was not reached in the topsoil, although between 9.9°C and 16.6°C no significant change in SOC was observed for any fraction or bulk soil. The equilibration in the subsoil at a lower temperature level as compared to the topsoil might be related to the more pronounced soil warming in the subsoil due to heat conduction from below.

Change in fraction mass

The fractionation procedure divided the soil into three different solid fractions of soil material: the fine fraction (SC), the heavy coarse fraction (SA) and the light coarse fraction (POM). Soil warming not only changed the C concentration within those fractions, but also significantly altered the distribution of the soil mass. While the unwarmed reference soil (topsoil) consisted on average of $64\pm 18\%$ SA and only $34\pm 18\%$ SC, topsoil in the most warmed soil ($+40^\circ\text{C}$) consisted of only $42\pm 11\%$ SA and $58\pm 11\%$ SC (Fig. 2c). A similar shift in fraction distribution, but less pronounced, was observed in the subsoil, where the SA fraction decreased from $63\pm 10\%$ to $54\pm 10\%$ and the SC fraction increased from $37\pm 10\%$ to $45\pm 10\%$ (Fig. 2d). Since the SA fraction consists of sand and stable aggregates, this shift must be related to decreasing aggregation with increasing temperature.

Change in carbon distribution in fractions

Despite the strong depletion of total SOC with increasing temperature (Fig. 3a, 3b), all fractions were present in all plots. In accordance with the previously described C concentration and soil mass dynamics within the fractions, the greatest losses of SOC occurred in the SA fraction. In the topsoil of the unwarmed reference, most SOC was stored in the coarse and rather labile fraction, while the most warmed soil consisted of $70\pm 9\%$ passive and slow cycling SOC (rSOC and SC-rSOC). The deeper soil layer was initially dominated by the stable fractions ($66\pm 11\%$), but showed the same pattern of relative enrichment of stable fractions with increasing soil temperature. The POM fraction was highly variable in the topsoil and was as sensitive to soil warming as the bulk soil; no relative enrichment or depletion was detected (Fig. 3c). In the lower layer, the POM fraction depleted more than the bulk soil. However, the additional SOC after soil warming with 0.6°C was

almost entirely found in the SA fraction, confirming the finding that this fraction is most sensitive to soil temperature change. DOC was only of minor absolute importance, representing less than 5% of total SOC. However, while this fraction is considered labile, we found relative enrichment of DOC with increasing temperature in both soil layers. The response of the chemically highly recalcitrant rSOC fraction to soil warming equalled the response of the SC-rSOC fraction. As can be seen in Figure 4, rSOC was a very constant proportion (25%) of the total SC fraction, irrespective of the degree of warming. The total shift in SOC distribution in different fractions along the warming gradient was highly significant for the topsoil ($p < 0.001$), but only marginally significant for the subsoil ($p = 0.056$).

Change in abundance of ^{13}C

In the bulk soil and in all fractions except POM, a significant increase in the abundance of ^{13}C was observed, expressed as $\delta^{13}\text{C}$ values in Figure 5. In the topsoil of the unwarmed reference, the $\delta^{13}\text{C}$ value ranged from -28.6 ± 0.2 in the POM fraction to -26.6 ± 0.2 in the rSOC fraction, indicating an age gradient within the fractions. Despite this gradient, the shift in ^{13}C abundance in response to warming was similar for all fractions except POM, which did not respond much to warming. On average, the change in $\delta^{13}\text{C}$ values in the topsoil at $+40^\circ\text{C}$ warming was in the order SA ($+1.6\text{‰}$) $>$ rSOC ($+1.3\text{‰}$) $>$ SC ($+1.1\text{‰}$) + bulk soil SOC ($+1\text{‰}$) $>$ POM ($+0.4\text{‰}$). Values and trends observed in the subsoil were similar to those in the upper layer, with a slightly less pronounced difference between the different fractions in the unwarmed soil and also slightly less pronounced differences in their responses to warming. The change in $\delta^{13}\text{C}$ values in the subsoil was in the order SA ($+1.3\text{‰}$) $>$ SC ($+1\text{‰}$) = Bulk SOC ($+1\text{‰}$) $>$ rSOC (0.6‰) $>$ POM (0.5‰). DOC was excluded from this

comparison, since the dataset was incomplete and measurements highly variable. The small increase in $\delta^{13}\text{C}$ in the POM fraction was related to the fact that it consists mainly of fresh plant material that has not undergone decomposition. In both soil layers studied, the $\delta^{13}\text{C}$ value for the SA fraction was lower than that in the bulk soil for unwarmed soil, but exceeded that in the bulk soil in soil with more than +16.6°C warming. The SA fraction thus showed the strongest relative enrichment in ^{13}C , which is in line with the high mass loss and C depletion in this fraction. For the subsoil, we found decreasing $\delta^{13}\text{C}$ values for 0.6°C soil warming in the bulk soil and in all fractions, which is in line with the observed increased total SOC storage. However, for the topsoil we did not observe this decrease in $\delta^{13}\text{C}$.

Discussion

Soil organic carbon response to soil warming

Bulk soil organic carbon

Studies on the response of SOC to soil warming in moderate climate change scenarios (1-4°C warming) investigated on an ecosystem scale show mixed results, ranging from slight losses to significant gains in SOC (Melillo *et al.*, 2002, Schaeffer *et al.*, 2013, Sistla *et al.*, 2013, Xu *et al.*, 2012). In the present study, we were able to investigate the SOC response of unmanaged grassland soil to low, moderate and extreme increases in soil temperature. Warming of 0.6°C for 6.5 years led to an increase in SOC, which could possibly be explained by relatively stronger stimulation of NPP due to higher N availability from increased N mineralisation (Melillo *et al.*, 2002), rather than the stimulating effect of the low soil warming on SOC mineralisation. A clear increase in above- or below-ground biomass

production at +0.6°C warming has however not been detected in the study plots (unpublished data, not shown). When soil temperature increased beyond +0.6°C, it led to exponential decline in SOC. This indicates that there might be a threshold between 0.6°C and 1.9°C below which soil warming has a positive effect on soil carbon at the investigated site. This threshold value, which is most likely ecosystem specific, is well in the range of projected global average air temperature changes for the next century (Stocker *et al.*, 2013), revealing the importance of our findings for carbon models. The specific peak in carbon storage at +0.6°C warming will be further investigated within the FORHOT project. The IPCC “worst case”-scenario (RCP8.5) predicts a global average temperature rise until the end of the century of $4\pm 1^\circ\text{C}$, and in high northern latitudes, of around 10°C (Pachauri *et al.*, 2014). Therefore, SOC losses that occurred at +3.9°C and +9.9°C might be most relevant for predicting potential SOC losses under climate change. At the investigated site, we found losses of 34% and 44% in the topsoil and of 36% and 40% in the subsoil for +3.9°C and +9.9°C of soil warming, respectively. However, soil warming from below cannot be compared with air temperature warming and enrichment of CO₂ in the atmosphere, which would both stimulate plant growth and thus counterbalance SOC losses to some degree. The warmest treatments lost as much as 79% (topsoil) and 74% of their initial SOC in 6.5 years. These losses exceed the 65% loss as observed in the Versailles experiment after 80 years of bare fallow (no carbon inputs) (Menichetti *et al.*, 2015), which illustrates the strong acceleration of SOC decomposition at elevated temperatures. The extreme warming treatments investigated in this study are unrealistic and might therefore not be meaningful for predicting terrestrial carbon cycle feedbacks to climate change. However, they can provide fundamental mechanistic insights into SOC dynamics. Furthermore, due to the absence of grass and herbaceous vegetation, the +40°C warming treatment can be regarded as a “no input”-treatment. Surprisingly, we did not observe large differences in SOC losses between

+16.6°C and +40°C. This might be related to the fact that especially root biomass was also significantly reduced with warming up to +16.6°C (Leblans *et al.*, in prep.), so that the difference in belowground carbon input was relatively small.

The stable isotope ^{13}C in SOC is discriminated during microbial breakdown and is therefore relatively enriched in more refractory SOC (Nadelhoffer & Fry, 1988). The average annual change in $\delta^{13}\text{C}$ in bare fallow experiments due to trophic fractionation reported (Menichetti *et al.*, 2015) was 0.013 ‰, while the annual change in $\delta^{13}\text{C}$ in the 3.9, 9.9, 16.6 and 40°C warmed plots in the present study was 3.6-, 3.7-, 10- and 12.8-fold higher, despite the partly sustained annual input of relatively ^{13}C poor plant material. This comparison shows that extreme soil warming >3.9°C may increase SOC decomposition by an order of magnitude. The difference in $\delta^{13}\text{C}$ between the youngest SOC (POM) and the oldest SOC (rSOC) in the soil was 2‰ and 3.1‰ for the top- and subsoils in the unwarmed control plots, which is a result of presumably several centuries of SOC recycling. Within 6.5 years of soil warming, the bulk soil $\delta^{13}\text{C}$ value changed by 1‰ in both soil layers in the most warmed plots, which accounted for 50% and 32% of the SOC recycling needed to turn POM into the most recalcitrant fraction (rSOC).

Particulate organic matter (POM)

The POM fraction is the youngest and most labile SOC fraction (Leifeld & Kögel-Knabner, 2005, Poeplau & Don, 2013) and might therefore be expected to respond strongly to soil warming (Xu *et al.*, 2012). However, this was not the case in the present study. Only in the subsoil it depleted more rapidly than in the bulk soil. This was most likely related to the fact that the POM pool was permanently restored to some degree by below-ground plant-derived C inputs. With increasing depth, the input of plant-derived SOC decreases (Jackson *et al.*,

1996), which might explain the greater depletion of POM after warming in the subsoil layer than in the topsoil layer. Unexpectedly, we were able to detect POM even in the grass-free +40°C treatment, which might indicate that i) some carbon input persisted even under a moss cover or that ii) a part of POM had very low decomposability.

Dissolved organic carbon (DOC)

The DOC fraction, which in the fractionation procedure applied here represents water-extractable SOC, is generally regarded as an active fraction with more rapid turnover than bulk soil (Zimmermann *et al.*, 2007). However, in this study we found relative enrichment of DOC with increasing temperature. This is in line with (Poeplau & Don, 2013), who found DOC to be less sensitive to land use change than bulk soil. In the case of warming, this finding could be related to decreased aggregation, which might have exposed relatively more organic matter particles to dissolution. Despite an incomplete dataset for $\delta^{13}\text{C}$ in DOC, we were able to show strong enrichment of ^{13}C with increasing soil warming (Fig. 4). Since the DOC measured in this study refers to carbon dissolved during wet sieving, this enrichment indicates that wet sieving dissolved older material in the more warmed soils. Under field conditions, this increasing potential of SOC to be dissolved and leached might have contributed to some degree to the overall losses of SOC.

Carbon stored in sand and stable aggregates (SA)

The SA fraction was most sensitive to increased soil temperature. Soil warming not only led to a decrease in carbon concentration, but also to a large mass loss of the SA fraction. Similar sensitivity of the SA fraction to changes in environmental conditions has been observed by

Guidi *et al.* (2014) and Poeplau and Don (2013), who found a strong decrease after conversion from grassland to forest. The results of our study clearly indicate destabilisation and break-up of aggregates along the warming gradient, since the mass of the fine SC fraction showed the opposing trend. The negative effect of soil warming on aggregate stability has been reported previously (Lavee *et al.*, 1996, Rillig *et al.*, 2001), but many different mechanisms may be involved. It is not even clear whether the depletion of SOC leads to a decrease in aggregation (Lavee *et al.*, 1996) or whether the break-up of aggregates due to warming leads to destabilisation of SOC. The latter could have biological reasons, such as changes in soil fauna (mostly earthworms), fungal hyphae, the rooting system and the abundance of certain proteins or polysaccharides or complex biofilms fostering soil aggregation (Qurashi & Sabri, 2012, Rillig *et al.*, 2001, Tisdall & Oades, 1979, Traore *et al.*, 2000). Between +16.6°C and +40°C (grass-free) we found a very pronounced decrease in SA fraction mass, which might indicate the importance of an intact rooting system and root-associated fungal hyphae for aggregate stability (Tisdall & Oades, 1979). The more persistent aggregate-binding agents usually found in Andosols, such as allophanes and ferrihydrites, have been found to primarily stabilise microaggregates (<20 µm) (Lehtinen *et al.*, 2015), which were part of the SC fraction in the present study. Thus, they did probably not influence the stability of larger aggregates. The change in $\delta^{13}\text{C}$ in the SA fraction supports the theory of SOC decomposition due to aggregate break-up. While the SOC stored in the SA fraction consisted of younger and less decomposed SOC than the SOC in the bulk soil (lower $\delta^{13}\text{C}$ value), it was enriched in ^{13}C compared with the bulk soil after soil warming of more than 9.9°C in the upper and 16.6°C in the lower layer. Only the most stable aggregates and presumably organic coatings on sand grains remained undecomposed at higher temperatures. The additional SOC that was sequestered at 0.6°C warming was almost

entirely found in the SA fraction, which further underlines the sensitivity of this fraction to environmental change, but also its importance for SOC accrual (Deneff *et al.*, 2001).

Carbon stored in the silt and clay fraction

The SC fraction is operationally divided into a very recalcitrant, passive SOC pool (rSOC) and a slow-cycling SOC pool associated with silt and clay minerals (SC-rSOC). The rSOC fraction is isolated by a chemical fractionation step which mimics very aggressive decomposition. It is meant to correspond functionally to an inert pool that does not change under any environmental change excluding wild fires (Zimmermann *et al.*, 2007). However, the claim that the molecular structure of organic matter, rather than environmental conditions, controls its long-term persistence in the soil has been disproven (Dungait *et al.*, 2012, Schmidt *et al.*, 2011). Indeed, while rSOC was the most recalcitrant fraction in the present study, as revealed by the lowest $\delta^{13}\text{C}$ value, it responded similarly to soil warming as the SC-rSOC fraction (67 and 78% SOC loss in top- and subsoils of the +40°C warmed soil, respectively). Irrespective of soil temperature, rSOC accounted for 25% of the total SC pool. This is in line with findings by Leifeld and Lützw (2013) that the activation energy of chemical and microbial (enzyme-catalysed) oxidation are not correlated, which indicates that they are determined by different sample properties. It is also in line with findings by Poeplau and Don (2013) that the rSOC fraction responded equally to land use change to the SC-rSOC fraction. It can therefore be concluded that these two fractions differ in their chemical composition, recalcitrance and radiocarbon age (Helfrich *et al.*, 2007), but not, or only slightly, in their response to significant environmental changes. Poeplau and Don (2013) reported that these two fractions were about 30% less responsive to land use change than the bulk SOC, which is of the same order of magnitude as the relative enrichment of the two SC

fractions in the present warming experiment. Chemical oxidation might thus not be appropriate to isolate a SOC fraction that is biologically stable under environmental change.

Change in SOC fraction distribution after extreme soil warming and its implications

Soil warming led to a shift in SOC fraction distribution. In both layers, the soil was relatively enriched in those fractions that are considered to be more stable. However, this was mostly due to a shift in soil mass of the fractions after aggregate breakdown. Altogether, the temperature sensitivity of SOC in the five fractions investigated was not as specific and distinct as expected, as revealed by the very similar ^{13}C signal and thus relative ^{13}C enrichment within all fractions, or at least those attached to the mineral soil (SA, SC, rSOC) and the bulk soil. This confirms findings by (Conen *et al.*, 2006) that young and old SOC fractions were equally mineralised after warming. Likewise, Shaver *et al.* (2006) reported no significant changes in SOC distribution in different fractions after incubating a tundra soil at 7°C and 15°C. However, in the present study we found a response, especially in the lower layer, which was more decoupled from fresh C supply than the upper layer, that was perfectly in line with the response by fractions to land use change reported by Poeplau and Don (2013). They found that SOC stored in the rSOC, SC-rSOC and DOC fractions was less sensitive to environmental change than SOC stored in POM and SA. This relative enrichment of more stable fractions is also in line with findings in other studies (Cheng *et al.*, 2011, Xu *et al.*, 2013). Yet, in agreement with Conen *et al.* (2006) and Shaver *et al.* (2006), we found that this was not primarily caused by differences in SOC turnover due to chemical recalcitrance, but by physical destabilisation of aggregates and thus a decline and growth of fractions as such, not only of the SOC stored within them. The SOC of silt or clay particle size that was previously stored in aggregates was found in the SC fraction after aggregate break-up. The

successive growth of the SC fraction with increasing temperature was a very strong predictor for the total proportion of SOC stored in that fraction, as illustrated in Figure 6. Thus, carbon attached to silt and clay was not relatively enriched due to higher stability or lower temperature sensitivity as the bulk soil, but due to the fact that the mass of this fraction increased. This relationship illustrates the difficulty of matching modelled and measured SOC pools to predict SOC dynamics after any environmental change by characterising a soil in steady state conditions or at one specific point in time.

Soil organic carbon fractions are not static reservoirs through which carbon molecules pass at a certain pace, even if they may be distinguishable by aggregate size or stability, particle size or density. On the contrary, they are dynamic fragments of a complex continuum that responds to environmental change in several ways. This study showed repeatedly that the most sensitive and most diagnostic fraction (the SA fraction) is not at all homogeneous with regard to the nature and age of the SOC stored within. It contains both the youngest and oldest SOC, which becomes subject to decomposition after the stabilisation mechanism is deactivated. This is in line with the claims i) that SOC stability is governed by its accessibility rather than by its recalcitrance (Dungait *et al.*, 2012) and ii) that changes in environmental conditions can trigger changes in accessibility, and thus decomposition (Schmidt *et al.*, 2011). Understanding the response of SOC to temperature increase should thus involve a more mechanistic approach that targets the main mechanisms for SOC depletion besides catalysed during microbial metabolism. Our findings are therefore well in line with the concept raised by Conant *et al.* (2011), who suggested to focus on the temperature response of individual SOC stabilization processes rather than to measure temperature sensitivity of young and old carbon pools alone. In this study, we have not measured aggregate dynamics directly, although the strong mass loss in the SA fraction indicated aggregate breakdown with

warming. In future studies investigating climate change effects on SOC, aggregate dynamics should play a key role.

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List of Tables

Table 1: Mixed effect model p-values for the significance of the difference between unwarmed and warmed soil in terms of: Soil organic carbon (SOC) concentration [g kg^{-1} fraction] (shown as relative change in Figure 1a and 1b), SOC mass [g kg^{-1} bulk soil] (shown in Figure 2a and 2b), and $\delta^{13}\text{C}$ (shown in Figure 5) in the topsoil (0-10 cm) and subsoil (20-30 cm) layers. ns = not significant; POM = particulate organic matter, DOC = dissolved organic carbon, SA = SOC in sand and aggregates, SOC-rSOC = SOC attached to silt and clay, rSOC = resistant SOC.

		Topsoil (0-10 cm)						Subsoil (20-30 cm)					
		Soil temperature increase at 10 cm [°C]						Soil temperature increase at 10 cm [°C]					
Fixed effect	Fraction	0.6	1.9	3.9	9.9	16.6	40	0.6	1.9	3.9	9.9	16.6	40
SOC conc.	Bulk SOC	ns	ns	0.02	<0.01	<0.01	<0.01	0.02	ns	ns	ns	<0.01	<0.01
	POM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	DOC	ns	ns	0.03	0.01	<0.01	<0.01	ns	ns	ns	ns	0.03	0.07
	SA	ns	ns	0.03	<0.01	<0.01	<0.01	0.03	ns	ns	ns	0.2	<0.01
	SC-rSOC	ns	ns	ns	0.02	<0.01	<0.01	ns	ns	0.04	0.02	<0.01	<0.01
	rSOC	ns	ns	0.03	ns	<0.01	<0.01	ns	ns	0.04	0.03	<0.01	<0.01
SOC mass	Bulk SOC	ns	ns	0.02	<0.01	<0.01	<0.01	0.02	ns	ns	ns	<0.01	<0.01
	POM	ns	ns	ns	ns	0.066	0.01	<0.01	ns	ns	ns	0.04	0.03
	DOC	ns	ns	0.03	ns	<0.01	<0.01	ns	ns	ns	ns	0.03	0.07
	SA	ns	ns	0.02	<0.01	<0.01	<0.01	0.02	ns	ns	ns	ns	ns
	SC-rSOC	ns	0.05	ns	ns	ns	0.03	ns	ns	ns	ns	0.02	0.01
	rSOC	ns	0.01	ns	ns	ns	0.03	ns	ns	ns	ns	0.02	<0.01
$\delta^{13}\text{C}$	Bulk SOC	ns	ns	ns	ns	0.09	<0.01	ns	ns	ns	ns	<0.01	<0.01

POM	ns	ns	ns	ns	0.07	ns	ns	ns	ns	ns	ns	ns
DOC	ns	ns	ns	0.04	0.03	ns	ns	ns	ns	0.088	ns	0.09
SA	ns	ns	ns	ns	0.04	<0.01	0.05	ns	ns	ns	<0.01	<0.01
SC	ns	ns	ns	ns	<0.01	<0.01	0.08	ns	ns	ns	0.04	0.01
rSOC	ns	ns	ns	ns	<0.01	<0.01	<0.01	ns	ns	ns	<0.01	0.02

Figure captions

Figure 1: Schematic overview of the applied fractionation procedure.

Figure 2: Relative change in carbon concentration [g kg^{-1}] in each fraction (DOC = dissolved organic carbon, POM = particulate organic matter, SA = SOC in sand and aggregates, SC-rSOC = SOC attached to silt and clay, rSOC = resistant SOC) and in bulk soil as a function of soil warming for the (a) topsoil (0-10 cm) and (b) subsoil (20-30 cm). Relative fraction mass of the three solid fractions silt and clay (SC), sand and aggregates (SA) and particulate organic matter (POM) for (c) the 0-10 cm and (d) 20-30 cm soil layer. Error bars indicate standard errors.

Figure 3: Cumulative total soil organic carbon (SOC) in each fraction at all soil temperatures investigated for the (a) topsoil (0-10 cm) and (b) subsoil (20-30 cm); and distribution of total SOC in different fractions at all soil temperatures investigated for the (c) topsoil (0-10 cm) and (d) subsoil (20-30 cm). (rSOC = resistant SOC, SC-rSOC = SOC attached to silt and clay, SA = SOC in sand and aggregates, POM = POM = particulate organic matter, DOC = dissolved organic carbon). Error bars indicate standard errors.

Figure 4: Correlation between total soil organic carbon (SOC) in the silt and clay (SC) fraction and resistant SOC (rSOC) in the SC fraction, with equation of the fit and the correlation coefficient (R^2).

Figure 5: $\delta^{13}\text{C}$ values in each fraction and in bulk soil as a function of soil temperature in the (a) topsoil (0-10 cm) and (b) subsoil (20-30 cm). The dotted line expresses the uncertainty in the DOC observations due to incompleteness of the dataset. POM = particulate organic matter, DOC = dissolved organic carbon, SA = SOC in sand and aggregates, SC = silt and clay, rSOC = resistant SOC. Error bars indicate standard errors.

Figure 6: Proportion of total soil organic carbon (SOC) in the silt and clay (SC) fraction [%] as a function of relative mass of the SC fraction [%], with correlation coefficients of the fits (R^2).











