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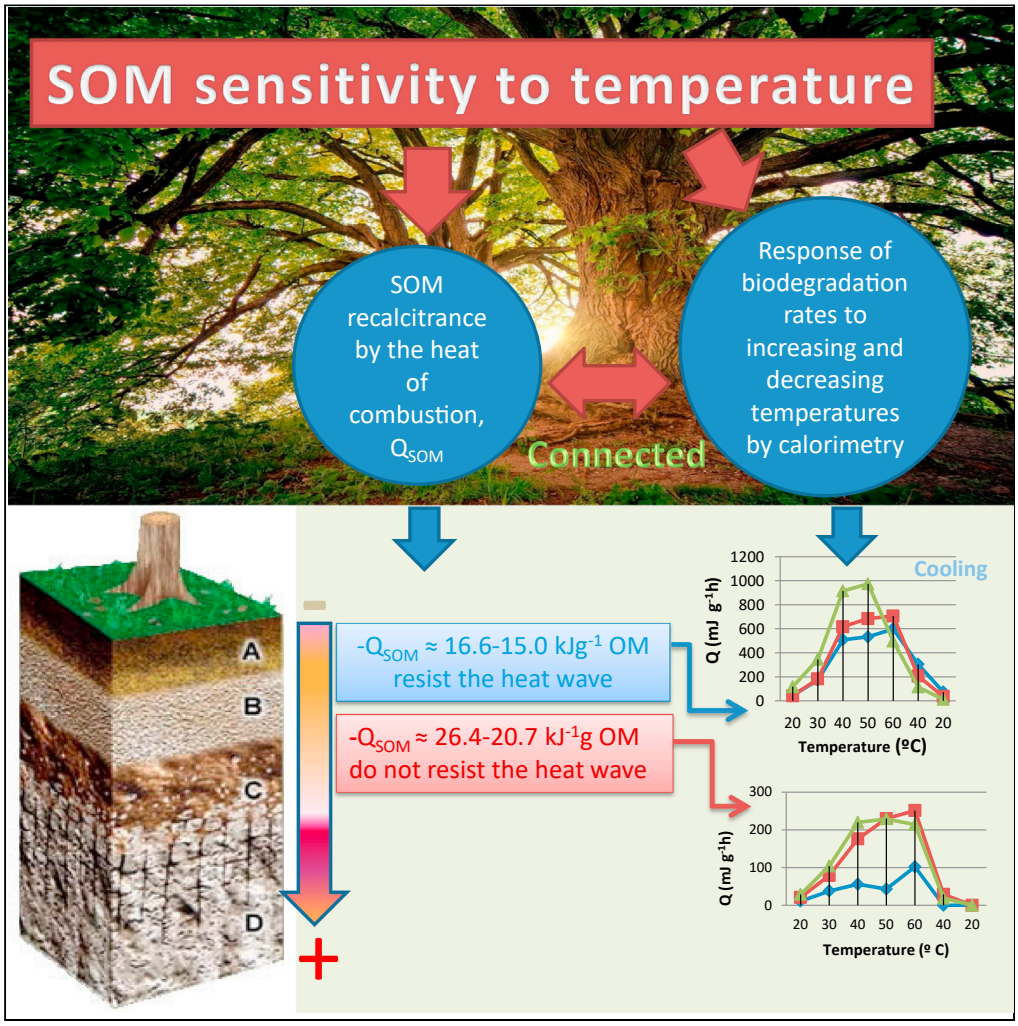

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Highlights

SOM from different soil horizons is subjected to an extreme calorimetric heat wave

Soil samples are from Atlantic oak forests

LF soil horizon resists the heat wave

Mineral soil samples do not resist the heat wave

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The effect of extreme temperatures on soil organic matter decomposition from Atlantic oak forest ecosystems

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SUMMARY

This work designs a heatwave with a calorimeter to analyze the response of soils from oak forest ecosystems to increasing temperature from 20°C to 60°C and to cooling from 60°C to 20°C. Calorimetry measures the heat rate of the soil organic matter decomposition and the response to increasing and decreasing temperatures directly. It was applied to soil samples representing different soil horizons with organic matter at different degree of decomposition given by their heat of combustion, calculated by differential scanning calorimetry. Results showed temperature-dependent decomposition rates from 20°C to 40°C or 50°C typical for enzymatic activity. From 40°C to 60°C, changes in the rates are less predictable. Data analysis during cooling showed that all samples suffered losses of their enzymatic capacity and that only those with the heat of combustion values close to that of carbohydrates resisted the heat wave.

INTRODUCTION

The study of the effect of temperature on soil encompasses different research fields because temperature controls many soil processes, such as dissolved organic carbon export, rates of soil mineralization and soil organic matter bio decomposition, nutrient assimilation by soil microorganisms and plants, weathering of base cations, soil structure, and forest productivity (Strömgren and Linder, 2002; Jungqvist et al., 2014), all of them with direct effects on soil fertility, and therefore, on food production. The effect of temperature on soil bio decomposition impacts on global warming too. The rate at which soil organic matter, SOM, is decomposed is temperature dependent and a source of greenhouse gases to the atmosphere (Brinkman and Sombroek, 1996). The high multidisciplinary nature of this subject makes this research complex and limits the development of more knowledge about it.

Bio decomposition takes place by different enzymes involved in microbial metabolic reactions, as well as by exoenzymes, which are temperature dependent, responding by accelerating bio decomposition rates as temperature increases, but also slowing down owing to thermal deactivation of enzymes. There is a wide number of high-impact works explaining that effect at the range of temperatures at which enzymes work (Davidson and Janssens, 2006). Those rates are measured by different methods at field and/or at lab conditions (Steinweg et al., 2012) at the temperatures at which enzymes are active, commonly from 4°C to 40°C. At these experimental conditions, the most widely used models to study the temperature-rate relation are the Q_{10} temperature coefficient, a measure of the effect of a 10°C rise in temperature on any chemical reaction that is usually applied to soils too (Meyer et al., 2018), and the Arrhenius equation connecting the increase in temperature with the activation energy of any reaction (Alster et al., 2016). Both involve exponential correlation between rates and temperatures and both have pros and cons. Q_{10} is highly variable (Chen et al., 2020) which hinders interpretation of data giving controversial results. The Arrhenius equation is not as extended as Q_{10} because of some discrepancies to settle the kind of correlation between the rate and temperature, being linear or exponential depending on soils and depending on the SOM availability to microbial decomposers due to SOM physical protection (Davidson and Janssens, 2006; Alster et al., 2016).

Another important goal is to link the effect of temperature to the SOM chemical composition (Tang et al., 2017; Meyer et al., 2018) to predict the impact of global warming on different soil ecosystems. This is challenging because of the high molecular complexity of SOM. One of the most extended criteria is the

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classification of SOM in labile and recalcitrant (Kleber, 2010). Nevertheless, the qualitative nature of the terms recalcitrant and labile SOM, the existing limitations to quantify them, and to connect them to the existing metabolic indicators (Davidson and Janssens, 2006) make that the relation between the SOM nature and the response to temperature is not well understood yet. For this reason, a part of this research field focuses on searching for additional alternatives to improve understanding and knowledge about this subject (Von Lützow and Kögel-Knabner, 2009;Alster et al., 2016; Hansen et al., 2018).

Calorimetry is one of the options. Calorimetry measures bio decomposition rates by the heat rate dissipated by the reaction. Some works are showing up how some calorimeter models can contribute to this field (Suurkuusk et al., 2017). These models have the singularity of monitoring the response of the heat rate from SOM bio degradation to increasing and decreasing temperatures continuously and in real time. The method has been proved with soil samples by increasing the temperature within the range at which enzymatic activity and temperature correlates. Results showed that it can be applied to calculate the Q_{10} and the activation energy by the Arrhenius equation (Barros et al., 2015, 2016). The advantage of this methodological alternative is to go beyond those limits. It is possible to simulate a heatwave, monitoring the soil reaction to harsh temperatures, and track how soil recovers when temperature declines. In a previous paper (Hansen et al., 2018), the method was tested with various mineral soils with differing SOM properties, and the SOM sensitivity to temperature was analyzed under new arguments, such as the maximum temperatures at which SOM decomposition is active and by contrasting which soils do or do not resist the heatwave. This recent application brings the opportunity to apply the heatwave to different soil ecosystems.

This paper intends to study the sensitivity to temperature of labile and recalcitrant SOM by designing a calorimetric heatwave and by the direct monitoring of the SOM bio decomposition in different soil layers from oak forests. It is assumed that the organic layer from the soil surface (the LF layer made by oak organic substrates at a slight degree of decomposition) would represent the labile material, while the mineral soil under it would represent more transformed and recalcitrant SOM. In some of the samples used for this study, there is an intermediate humic layer in between the soil surface and the mineral soil. Their SOM properties were studied by elemental and thermal analysis. SOM thermal properties derived from the thermal analysis are studied to provide a scalar to the degree of recalcitrance of SOM. These SOM properties are related to the heat rates from SOM decomposition recorded at differing temperatures by calorimetry, heating from 20°C to 60°C first, and cooling after from 60°C to 20°C. The extreme temperature values are selected in order to monitor the response of soil beyond the range at which temperatures and rates are correlated and linked to their enzymatic activity. The soil response to cooling and the contrast of samples which resisted the heatwave or not are introduced as additional criteria to settle SOM sensitivity to temperature and compared to the Q_{10} values determined for these samples.

RESULTS

SOM elemental and thermal properties

All samples were characterized by elemental and thermal analysis. Samples DC, G, and K, were collected in the southwest of Ireland. The SOM evolution with depth could be tracked in three different soil horizons: LF, H, and M. LF samples come from the soil surface and represent organic material partially decomposed. The H layers are humified organic matter at a higher degree of decomposition. Mineral soil samples (M) are characterized by organic matter at a higher degree of decomposition interacting with the soil mineral matrix. Samples ROG, BW, and NF were collected in the southeast of UK. They have only two horizons: LF and M. Table 1 shows the C, N, and SOM percentages of the samples, the C/N ratio, and the heat of combustion of SOM, Q_{SOM}. SOM percentages and Q_{SOM} were determined by simultaneous TG-DSC. Raw data from TG-DSC are in Data S1.The different SOM thermal fractions were calculated by TG and can be observed in Figure 1 (Data S1).All samples present two thermal fractions called Exo 1 and Exo2. The Exo1 is the percentage of labile material and the Exo2, the recalcitrant one given by TG (Plante et al., 2009; Fernández et al., 2011). Char is the material derived from the mineral soil fractions presented in the TG-DSC vials after SOM combustion. LF samples from the UK have similar Exo1 and Exo2 percentages and a higher percentage of mineral char than LF samples from Ireland, which have a higher Exo1 than Exo 2 percentages, suggesting higher degree of decomposition in LF samples from the UK. Samples from Ireland show the Exo1 percentage decreases and the Exo2 and char percentages increases with soil depth. The same evolution with depth is observed in the UK samples. SOM evolution with depth also involves depletion of the C content (Table 1). Q_{SOM} was directly determined from the DSC plots (Plante et al., 2009; Barros et al., 2020). The

Table 1. Elemental properties and heat of combustion of samples

Samples	C_{tot} (%)	N (%)	SOM (%)	C/N	$-Q_{SOM}$ (kJ g^{-1}SOM)
ROG LF	38.53	1.55	75	24.85	15.4 ± 0.7
BW LF	45.82	2.46	83	18.62	15.1 ± 0.8
NF LF	44.09	2.04	82	21.61	15.1 ± 0.8
DC LF	50.18	1.44	95	30.72	15.0 ± 0.5
G LF	51.30	1.67	97	30.72	15.9 ± 0.4
K LF	50.73	1.72	95	29.49	16.4 ± 0.5
DCH	41.00	1.74	82	23.56	16.6 ± 0.5
GH	34.00	1.70	73	20.00	18.7 ± 0.6
KH	40.00	2.00	83	20.00	19.3 ± 0.5
ROG M	5.00	0.26	11	19.23	21.9 ± 1.1
BWM	10.86	0.62	23	17.52	20.7 ± 1.0
NFM	12.33	0.49	18	25.16	22.2 ± 1.1
DCM	5.60	0.28	8	20.00	26.4 ± 0.7
G M	4.70	0.22	9	21.36	25.0 ± 0.7
KM	10.12	0.51	21	19.84	21.3 ± 0.6

Carbon (C_{tot}), Nitrogen (N) and SOM percentages, as well as C/N ratio and heat of combustion ($-Q_{SOM}$) of samples used in this study. Samples LF are from the soil surface, samples H correspond to the soil layer between LF and mineral soil, and M samples are mineral soils. Soils DC, G, and K were collected in Ireland. Samples ROG, BW, and NF are from the UK.

SOM combustion in the DSC is an exothermic reaction involving negative values of Q_{SOM}. It is observed as a clear evolution of Q_{SOM} absolute values to increase with soil depth. SOM on the soil surface (LF layer) has $-Q_{SOM}$ values similar to those reported for carbohydrates like glucose (15.65 kJ g^{-1}) (Gary et al., 1995). H samples from Ireland have higher $-Q_{SOM}$ values than the LF ones, indicating a higher degree of reduction of SOM in these samples. H values are close to those reported for lignocellulosic material (16.13–18.15 kJ g^{-1}) and cellulose (17.28–16.61 kJ g^{-1}) (Wróblewska et al., 2009; Blokhin et al., 2011). All mineral samples have the most negative Q_{SOM}, with values at a similar degree of reduction as reported for lignin (21.45–23.50 kJ g^{-1}) and some peats (25–26 kJ g^{-1}) (Gary et al., 1995; Voitkevich et al., 2012; Hansen et al., 2018). Evolution of all these elemental and thermal properties evidence SOM composition varies with soil depth. Changes in the thermodynamic properties of soils can be attached to modifications in their chemical nature (LaRowe and Van Capellen, 2011).

Response to temperature

Figure 2 shows the evolution of the average of the heat rates (n = 2) to heating from 20°C to 60°C and to cooling from 60°C to 40°C and 20°C determined by calorimetry. Raw data from calorimetric measurements are in Data S2. All of them have a similar response to increasing temperature from 20°C to 40°C, evidencing the typical correlation existing between temperature and metabolic rates measured in this case as a heat rate (Bradford et al., 2008; Barros et al., 2015). Results in Figure 2 show that relationship seems to be linear or exponential depending on the samples. Differences among samples in response to temperature appear at temperatures higher than 40°C, where most of them abandon the correlation with temperature presenting an unpredictable and variable evolution. LF samples from Ireland (Figure 2A) show a trend to increase the heat rate from 20°C to 50°C in samples GLF and KLF, both showing a similar profile also when cooling. Sample DCLF shows a heat rate tending to decrease at temperatures higher than 40°C with a stable heat rate at 50°C and 60°C. LF samples from the UK (Figure 2D) have a different profile after 40°C. ROGLF and BWLF reach the maximum heat rate at 40°C and keep that maximum more or less stable from 50°C to 60°C. Sample NFLF reaches the maximum heat rate at 40°C, stays up 50°C to decay from 50°C to 60°C. All LF samples decrease the heat rate during cooling from 60°C to 20°C. The heat rate at 40°C during cooling is in every case lower than that at 40°C during heating. All LF samples resisted the heatwave and show heat activity at 20°C after the extreme temperatures.

Samples from the H horizon (DCH, GH, and KH) show the SOM transition from the soil surface to the mineral soil and all the three have similar heat rates (Figure 2B). All of them reach a maximum heat rate at 50°C

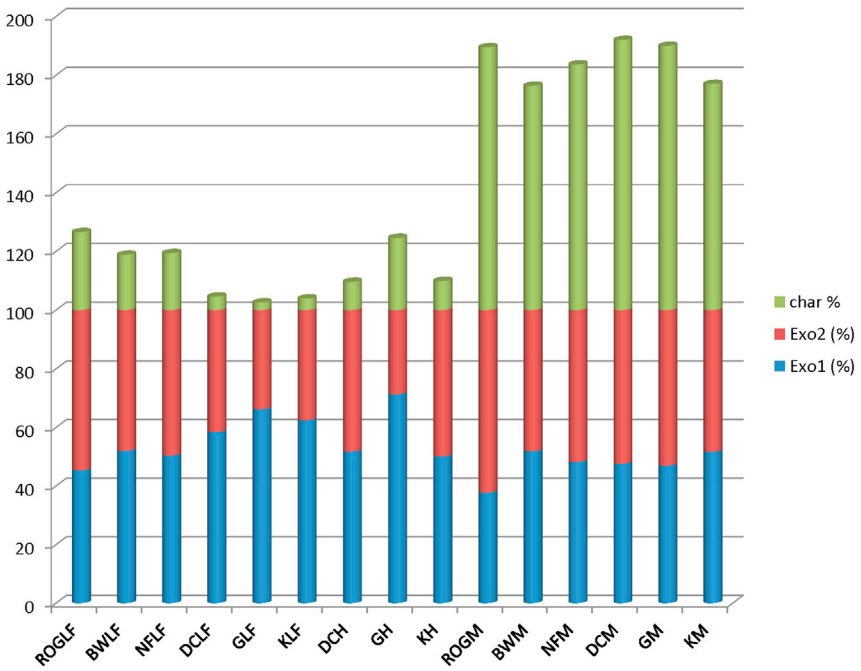

Figure 1. SOM thermal fractions and mineral char

Evolution of the different SOM thermal fractions, Exo1 (percentage of labile SOM), Exo2 (percentage of recalcitrant SOM), and char (percentage of the mineral residue after combustion) in the samples used in this study. Samples ROG, BW, and LF come from three different plots in the UK. Samples DC, G, and K were collected in three different sites in Ireland. LF, H, and M represent the different soil layers following a depth gradient. The percentage of mineral char is normalized to the sample size. Exo 1 and Exo 2 fractions are normalized to the soil organic matter combusted on dry weight basis.

followed by an acute decay at 60°C. Only sample DCH presents a heat rate at 20°C after the heatwave, similar to that before heating. The other two samples did not resist the heatwave.

All mineral samples from Ireland (Figure 2C) have a similar profile, analogous to that observed for the sample NFM from the UK (Figure 2E), while samples ROGM and BWM from the UK show a different pattern. During heating, ROGM and BWM maintain the same profile as that observed in their respective LF samples. The rest of the M samples increase heat rate with temperature from 20°C to 40°C, show an acute depletion at 50°C, a new increase at 60°C to decrease again at 40°C and 20°C during the cooling period. None of the mineral samples resisted the heatwave showing no activity at 20°C after cooling.

Differences in the heat rates among samples at different temperatures were analyzed by one-way ANOVA. The statistical experimental design can be observed in Figure S1. Data fulfilled the necessary normality (Shapiro Wilk) and homoscedasticity (Brown-Forsythe Test) linked to parametric statistics. LF samples from the UK (ROGLF, BWLF, and NFLF) (3 levels, n = 42 per level of significance) showed no significant differences in the response to temperature at $p < 0.05$.

Comparison of data from the Irish LF samples (DCLF, GLF, and KLF) gave no significant differences among sites (3 levels, n = 14; $p < 0.05$). When ANOVA is applied to compare heat rates of LF samples from the UK and Ireland (6 levels, n = 14) differences among sites were not significant either. LF samples present a homogeneous response to extreme temperatures despite being from different locations. Results are shown in Figure 3A.

The t test showed that the heat rates values reached after the heat wave at 20°C were not significantly different than the initial ones, but rates at 40°C when cooling were significantly lower than rates at 40°C when heating in all cases (n = 24; $p< 0.05$).

One-way ANOVA among the mineral samples from the UK (3 levels, n = 42, $p< 0.05$) indicated significant differences. Tukey's test revealed ROGM as the sample responding differently than the other ones

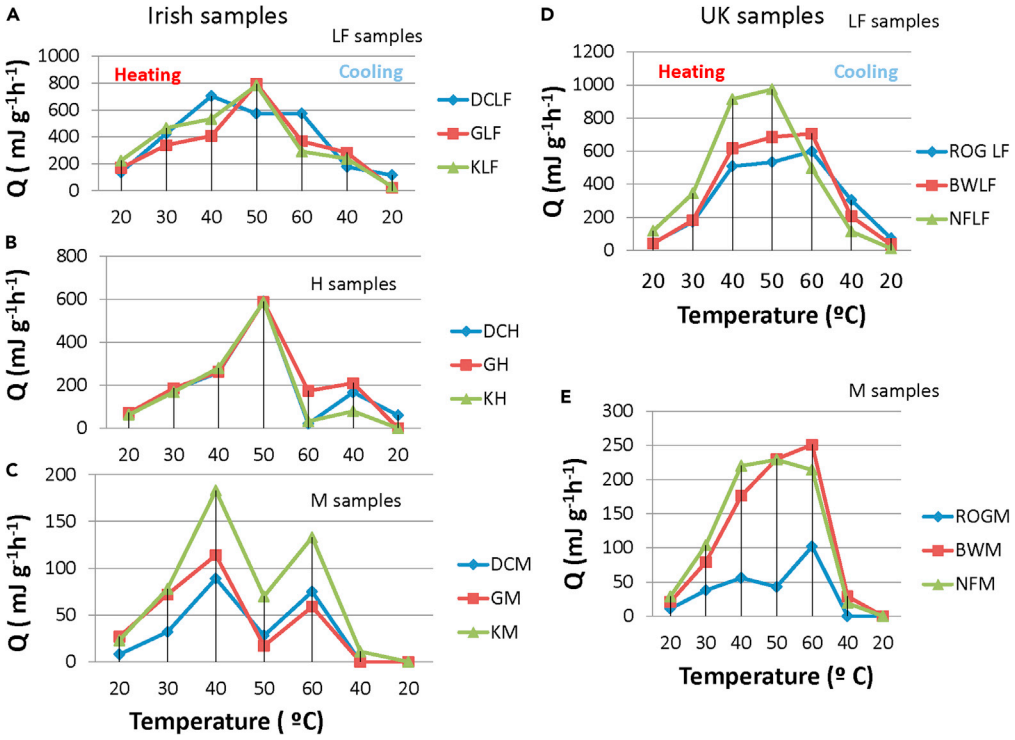

Figure 2. Evolution of the heat rates with temperature

Averaged heat rates for soil duplicates, Q, given in millijoulesper gram of sample and hour, mJ g^{-1} h^{-1}, versus increasing and decreasing temperatures for all samples in this study. The left side of the plots represents the evolution of heat rates with increasing temperature from 20°C to 60°C. It is symbolized in red as "Heating". The right side of the plots represents the evolution of the heat rates with decreasing temperatures. It is symbolized in blue as "cooling". Each plot shows the profile of the response of the heat rate to the temperatures in samples collected in Ireland (DC, G, and K samples) grouped on the left side of the figure (plots a, b, and c) and in samples from the UK (ROG, BW, and NF) grouped on the right side of the figure (c, e). Symbols LF, H and M, represent the different soil layers analyzed.

(Figure 3B).Comparison of M samples from Ireland (3 levels, n = 42) yielded no significant differences, but the contrast among M samples from the UK and Ireland (6 levels, n = 84; $p < 0.05$) revealed differences between Ireland and the UK. Tukey's test indicated that the response to temperature in BWM and NFM from the UK were significantly different than DCM from Ireland (Figure 3B).The response to the temperature of all LF horizons was significantly different than that of mineral samples in all cases (2 levels, n = 168) (Figure 3C). Therefore, the observed differences in the elemental and thermal properties of soils cause different behaviors to temperature. These differences show up as the degree of SOM transformation increases because the original substrate (LF samples) shows a similar response.

The common aspect among soils is that all LF samples resisted the heatwave while all mineral samples did not. Q_{SOM} values of samples increased with soil depth. This increment is accompanied by a depletion of the Exo1 fraction of SOM (the labile fraction) and by an increment of Exo2 (recalcitrant fraction) and mineral char. Q_{SOM} values are close to those reported for carbohydrates in all LF samples. Q_{SOM} values are more reduced than carbohydrates in all M samples with values ranging from about 26 to 21 kJ g^{-1} SOM (Table 1) indicating a similar degree of reduction as substrates like some aminoacids, lignin, biochar, and peats (Gary et al., 1995; Malucelli et al., 2020; Leifeld et al., 2020). H samples from Ireland have Q_{SOM} values higher than LF ones, ranging from 16.6 to 19.3 kJ g^{-1} SOM. All LF samples resisted the heatwave. Only one H sample resisted the heatwave, DCH, with a Q_{SOM} value of 16.6 kJ g^{-1} SOM, the closest to values in LF samples. Samples GH and KH, with Q_{SOM} values of 18.7 and 19.3 kJ g^{-1} SOM did not resist the heatwave. All M samples with SOM more reduced than carbohydrates also did not resist the heatwave. The resilience to temperature seems to decrease as the degree of reduction of SOM increases. This possible relation was checked by Pearson's correlation. Results yielded significant negative linear correlations among the Q_{SOM} values and the heat rates of the samples at different temperatures (n = 15, $p < 0.05$)

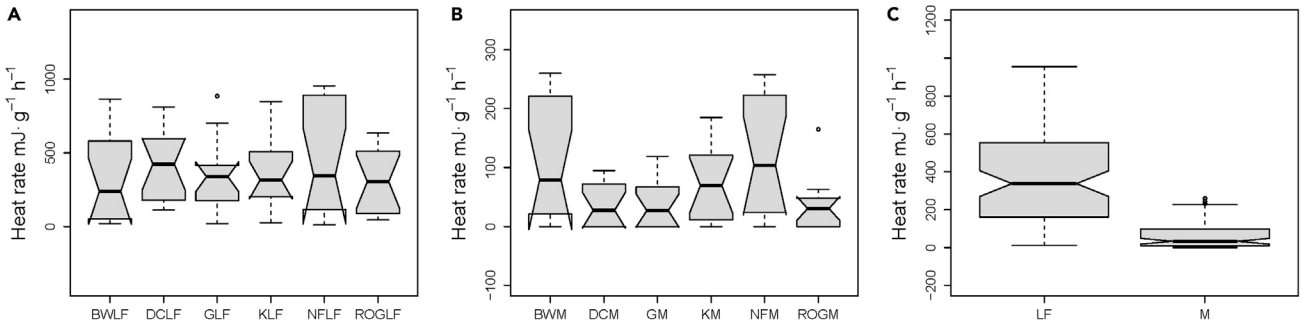

Figure 3. Boxplot notches overlapping for medians of the heat rates

A and B show boxplots of the samples from the different sampling locations. Figure 3A represents the range, medians, and quantiles, of heat rates in mJ g^{-1} h^{-1} for the different temperatures in LF samples and Figure 3B, the ones for the M samples (six levels representing the different locations, n = 84, $p < 0.05$). Figure 3C shows the boxplots of heat rates of all LF samples with the M ones (two levels, n = 168, $p < 0.05$).

(Table 2). Therefore, heat rates decline with an increasing degree of reduction of the samples. The depletion of the heat rates with increasing degree of reduction of samples (increased Q_{SOM} in absolute terms, avoiding the negative sign) could be interpreted as higher recalcitrance of SOM. Comparison among samples based on their resistance to the heat wave (heat rates values at 20°C after the heat wave) suggests that the recalcitrant SOM would be more sensitive to temperature than the labile SOM, that is samples with Q_{SOM} values higher than 16.6 kJ g^{-1} SOM.

Q_{10} values determined for the samples are shown in Table 3. They were highly variable among samples with no correlation with any of the other indicators used in this paper. The only observed trend is the decrease of Q_{10} values with increasing temperature along with the temperature range at which heat rates and temperature correlates (20°C–40°C). That means the sensitivity to temperature decreases as temperature increases.

Cluster analysis of samples based on their Q_{SOM} values, Q_{10} values, and heat rates when heating from 20°C to 40°C and after the heatwave at 40°C and 20°C is shown in Figure 4. Results indicate that temperature sensitivity discriminates between the three different soil horizons. Two main clusters are separating LF from mineral and H samples. Sensitivity in mineral samples does not show up clusters based on the geographical location.

All samples present lower heat rates at 40°C after the heatwave than their heat rate values at that temperature when heating. It can be interpreted as the loss of enzymatic activity (LEA) after extreme temperatures. Based on this assumption, the percentage of LEA by the samples at 40°C and 20°C after the heat wave was determined by the following equation:

$$(LEA) = (Q_h\text{-}Q_c)\ 100/Q_h \qquad \text{(Equation 1)}$$

Where Q_h is the heat rate at a certain temperature during heating and Q_c is the heat rate at the same temperature during cooling. Results give LEA as a percentage. They are listed in Table 4. Q_{SOM} shows a weak but significant positive Pearson's correlation (n = 15; r= 0.63; $p< 0.05$) with LEA at 40°C and 20°C after heating, indicating that samples at a higher degree of reduction are the ones losing more enzymatic capacity under an extreme heatwave.

This fact connects to the role of the soil thermodynamic properties with the sensitivity to temperature based on the following equation for irreversible processes:

$$\Delta G = \Delta H\text{-}T\Delta S \qquad \text{(Equation 2)}$$

Where ΔG is the Gibbs Energy change of the reaction, ΔH the enthalpy change, T is temperature, and ΔS the entropy change. The thermodynamic characterization from the Q_{SOM} values for some of these samples reported in a previous paper (Barros et al., 2020) showed that the calculated entropy change, ΔS, was positive and contributes little to the Gibbs energy. Therefore, soil reactions involving SOM combustion and

Table 2. Pearson's correlation among the Q_{SOM} and heat rates, Q, at different temperatures (n = 15)

		Q20	Q30	Q40	Q50	Q60	Q40c	Q20c
Q_{SOM} kJ g^{-1} SOM	r	−0.66	−0.79	−0.83	−0.88	−0.71	−0.82	−0.65
	p	<0.01	<0.001	<0.001	<0.001	<0.01	<0.001	<0.01

Q_{SOM} is given in kJ g^{-1}SOM. Q is in mJ g^{-1} h^{-1}. Data Q40c and Q20c are the heat rates at 40°C and 20ºC along the cooling period of the experiment. Samples Q20 to Q60 symbolize the heat rate along the heating phase of the experiment. r is the correlation coefficient. Q_{SOM} values influence the heat rates of the samples at the temperatures of the experiments.

SOM biodegradation would be mainly enthalpic processes. Q_{SOM} values approach to the ΔH of SOM, which is negative for the SOM combustion in the DSC. It allows determining ΔG through several models connecting the enthalpies and Gibbs energy to the degree of reduction of SOM (Roels, 1983; Sandler and Orbey, 1991). Q_{SOM} would yield the degree of reduction of SOM by the following equation (Sandler and Orbey, 1991):

$$Q_{SOM} \approx \Delta_c H_{SOM} = \gamma_C \, (-109 \text{ kJ Cmol}^{-1}) \quad \text{(Equation 3)}$$

Where $\Delta_c H_{SOM}$ is the enthalpy of SOM combustion, γ_C represents the degree of reduction of Carbon, C, in SOM, and −109 kJ $Cmol^{-1}$ is the oxycaloric quotient for the aerobic reaction (Sandler and Orbey, 1991; Gary et al., 1995; Barros et al., 2020; Barros, 2021). γ_C yields the Gibbs energy change for the SOM combustion in the DSC by the following relation (Sandler and Orbey, 1991):

$$\Delta_c G = \gamma_C \, (-110.23 \text{ kJ Cmol}^{-1}) \quad \text{(Equation 4)}$$

Where $\Delta_c G$ is the Gibbs energy change for the combustion of organic substrates. Considering results in previous works, SOM at a higher degree of reduction would involve more negative ΔG values (Barros et al., 2020; Barros, 2021)that would be interpreted as increased potential for spontaneous SOM combustion as temperature increases. Here, what we find is that SOM more reduced than carbohydrates in H and M samples, and therefore, with more negative Gibbs energy values and higher potential to combust spontaneously, are the ones that did not resist the heatwave. The organic layer on the soil surface would be more resistant to extreme temperatures than mineral soils under it in this case.

DISCUSSION

Most of the existing studies about the effect of temperature on SOM decomposition focus on the range of temperatures that correlates to bio decomposition rates, by calculating the Q_{10} or by applying the Arrhenius equation (Davidson and Janssens, 2006; Von Lützow and Kögel-Knabner, 2009). Calorimetry can be applied in the same way (Barros et al. 2015, 2016) and can be used to determine Q_{10} values as shown here. In this particular case, Q_{10} values are indicating the temperature sensitivity when heating from 20°C to 40°C, revealing how sensitive are soils to increase their metabolic rates with the increase of temperature in 10°C. Q_{10} results also show how that sensitivity decreases as temperature increases, something well known about this metabolic indicator (Kruse and Adams, 2008).

Figure 2 displays how the heat rate increases proportionally to the increase of temperature from 20°C to 40°C in most of the cases and even up to 50°C in some of the samples. This proportionality is generally linked to the enzymatic activity (Ma et al., 2017; Hobbs et al., 2013) and used by most of the literature about this subject, as the main criteria to understand the effect of temperature on soil. Nevertheless, that correlation does not explain the role of SOM nature on soil sensitivity to temperature and it cannot be applied as the sole criteria for a clear description of the impact of temperature on soils (Davidson and Janssens, 2006; Von Lützow and Kögel-Knabner, 2009; Carey et al., 2016). The controversy relies on settling whether SOM recalcitrance or SOM lability is more or less sensitive to increasing temperature (Tang et al., 2017; Klimek et al., 2016). This paper sheds some light in that sense by changing the experimental design going beyond the temperatures at which enzymes work with technologies like calorimetry. Bringing soils to extreme temperatures as 60°C, the sensitivity can be tested under a simple concept: which soils resist it and which ones do not in the short term. Then, connecting that response to soil properties linked to their lability and/or recalcitrance enables us to discern which ones define better the temperature resistance. Thermal analysis constitutes an option because it yields the heat of combustion of SOM, Q_{SOM}, which is directly related to

Table 3. Q_{10} values of samples

Q_{10}samples	20°C–30°C	30°C–40°C
ROGLF	4.20 ± 0.62	2.95 ± 0.26
BWLF	4.35 ± 0.50	3.34 ± 0.54
NFLF	2.92 ± 0.13	2.65 ± 0.04
DCLF	2.45 ± 0.02	1.66 ± 0.29
GLF	2.09 ± 0.13	1.21 ± 0.01
KLF	2.00 ± 0.18	1.14 ± 0.13
ROGM	3.45 ± 0.27	1.47 ± 0.02
BWM	3.76 ± 0.61	2.22 ± 0.44
NFM	3.58 ± 0.57	2.11 ± 0.05
DCM	4.00 ± 0.62	2.82 ± 0.16
GM	2.66 ± 0.23	1.58 ± 0.04
KM	3.39 ± 0.18	2.34 ± 0.06

Q_{10} averaged for the LF and M samples from 20°C to 30ºC and from 30°C to 40ºC during the heating period of the measurement (n = 2 ± SD).

the degree of reduction of organic substrates by well-known principles (Roels, 1983; Sandler and Orbey, 1991). Results here show significant evidence that the degree of reduction of SOM given by Q_{SOM} is connected to the heat rate, a measure of the rate at which SOM is decomposed. As the degree of reduction of SOM increased, the heat rate decreased. Samples at a degree of reduction given by a Q_{SOM} value lower than −18.7 kJ g^{-1} OM (more negative) did not resist the heat wave. Therefore, these results support theories postulating that recalcitrant organic matter is more sensitive to temperature than the labile one (Klimek et al., 2016; Bol et al., 2003) evidencing that the degree of reduction of SOM could be linked to SOM recalcitrance. As a consequence, the soil surface with partially degraded SOM from oak leaves resists better the extreme temperatures than the mineral soil under it, and therefore, would constitute an important temperature tampon for protecting the soil. Deciduous forests could play a relevant role in protecting our soils under our global warming environment for that reason.

The design of this work allows tracking of what happens after 40°C. The trends in Figure 2 show that temperature correlation is lost about 40°C–50°C, suggesting those are the temperatures at which enzymes start to denaturalize. The temperature at which the correlation is lost would depend on the nature of the enzymes and their thermal stability (Hansen et al., 2018; Collins et al., 2005). Figure 2 also shows that the soil response after those temperatures is more unpredictable and variable. Some hypothesis for that behavior is given in a previous paper (Hansen et al., 2018). On the one hand, there are enzymes in soils that can be thermally stable up to 60°C (Collins et al., 2005; Li et al., 2009), on the other hand, there are also thermophile microorganisms, and there are abiotic reactions like direct oxidation of lignin that can be stimulated by increasing temperatures too (Hansen et al., 2018). Probably, the different behavior observed at 50°C and 60°C here and in the previous paper (Hansen et al., 2018) is a compendium of all those features. Then, the observed rates at 40°C and 20°C along the cooling experimental phase reflect the remaining activity after the heatwave. Their direct comparison to the values determined during heating provides a quantitative measure of what is called here LEA, a value for the impact of the heatwave on soil samples. The degree of reduction of SOM could be one of the factors affecting LEA, given by a weak but significant ($r = 0.63$; $p<0.05$) positive correlation suggesting that recalcitrant SOM could present higher losses of their enzymatic capacity than labile SOM as temperature increases to extreme values.

Q_{SOM} is one of the doors to the thermodynamic characterization of SOM and will allow us to check the temperature sensitivity from a thermodynamic perspective in the future. The application of Equation 2 as presented here, suggests that temperature sensitivity would increase as Gibbs energy of SOM turns more negative. That is, the degree of reduction of SOM would inform us about their potential to decompose spontaneously with increasing temperature. Deeper research in this regard would allow us to predict soil survival under increasing environmental temperatures, as well as to discern soil ecosystems more or less sensitive to global warming. This paper helps to show up that thermal analysis and calorimetry are tools

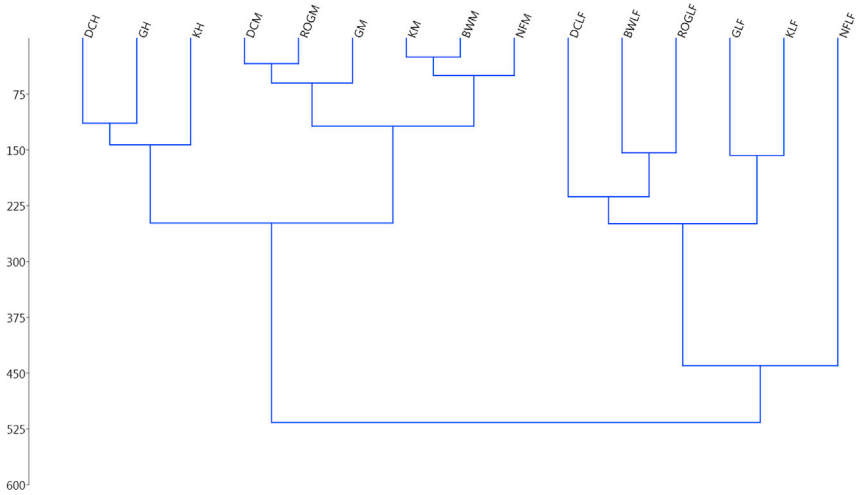

Figure 4. Cluster dendrogram of samples based on their Q_{SOM}, Q_{10}, and heat rates at 40°C and 20°C when heating and cooling

which can enrich knowledge about the temperature sensitivity of soil ecosystems by a different perspective than that just focused on the small ranges of temperatures at which enzymes are active.

Limitations of the study

The paper is introducing a new alternative to study the impact of temperature on soils. For this reason, it has not been applied to a high number of soil samples yet. It is reporting initial results about the potential of the methods and indicators proposed in this work.

The measurement is done with soil samples enclosed in stainless steel calorimetric ampoules, and the calorimetric recording takes about 10 days. Therefore, it is important to avoid the effect of the lack of oxygen or CO_2 excess on the heat rates during the measurement. In the experimental protocol proposed here, ampoules are opened twice during the heating and cooling phases to equilibrate O_2/CO_2 inside the calorimetric ampoule. It is important to consider that fact when designing the experimental phase because it can affect results.

Table 4. LEA percentages

Samples	LEA 40°C (%)	LEA 20°C (%)
ROGLF	40	0
ROGM	100	100
BWLF	67	0
BWM	84	100
NFLF	87	90
NFM	91	100
DCLF	75	34
DCH	36	15
DCM	100	100
GLF	30	88
GH	20	100
GM	100	100
KLF	56	87
KH	72	100
KM	100	100

Percentages for the loss of enzymatic activity (LEA) of samples during the calorimetric measurement.

The study is done in the short term. It just measures the immediate response of soil to increasing and decreasing temperature. From our perspective, the next question would be if samples that did not resist the heatwave can recover the enzymatic activity linked to microbial action again after a certain time. Those results could influence some of the conclusions in this work.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103527.

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AUTHOR CONTRIBUTIONS

All authors in this paper have equally contributed to this work.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Soil samples, DC, G, and K (LF, H, and M soil horizons)	Ken Byrne. University of Limerick. Ireland	https://www.ul.ie/scieng/dr-ken-byrne
Soil samples ROG, BW, NF (LF and M soil horizons)	Elena Vanguelova. Alice Holt Forest Research Station. UK	https://www.forestresearch.gov.uk/staff/dr-elena-vanguelova/
Deposited data		
Raw Tabulated data TG-DSC measurements	Data S1	https://doi.org/10.17632/xc8hs2wc65.1
Raw Tabulated data microcalorimeter TAM III	Data S2	https://doi.org/10.17632/xc8hs2wc65.1
Statistical experimental design	Figure S1	This paper
Software and algorithms		
Origin Pro Lab	OriginLab	https://www.originlab.com/
Past 3	PAST	https://past.en.lo4d.com/windows
R Core Team (2021)	R Foundation for Statistical Computing	https://www.r-project.org/
Other		
Elemental Analyzer LECO TruSpec CHNS	Instrumental Analysis Unit. RIAIDT. CACTUS Lugo. University of Santiago de Compostela. Spain	https://www.usc.gal/gl/investigacion/riaidt/analise/modules/equipamento/macromostra/equipamento_0001.html
TG-DSC1 Mettler Toledo	Instrumental Analysis Unit. RIAIDT. CACTUS Lugo. University of Santiago de Compostela. Spain	https://www.usc.gal/gl/investigacion/riaidt/analise/modules/equipamento/dsc/equipamento_0004.html
Microcalorimeter TAM III. TAM Instruments (Waters)	Instrumental Analysis Unit. RIAIDT. CACTUS Lugo. University of Santiago de Compostela. Spain	https://www.usc.gal/gl/investigacion/riaidt/analise/modules/equipamento/dsc/equipamento_0003.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for materials and methods should be directed to and will be fulfilled by the lead contact, Dr. Nieves Barros (nieves.barros@usc.es).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Tabulated raw data from the calorimetric and TG-DSC measurements are deposited as supplemental information.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

Soil samples

Soil samples were collected at six semi-natural oak woodland sites in south west Ireland and at the Alice Holt research station in the UK. The dominant soil types are Podzol and Cambisol. Samples were all

collected about August/September 2016. Sampling criteria was common for every site and it is specified in detail in a previous paper (Barros et al., 2020). Three soil layers were collected at each site: the loose litter (L) and fermented (F) layer (LF samples) from the soil surface; the humic layer, H, in samples from Ireland (DC, G and K samples); and samples from the top 5 cm of the mineral layer (M samples). At each study site, between three and five sampling plots were identified. Within each stand, samples from those sampling plots were mixed together and prepared for laboratory analysis.

Samples for elemental and thermal analysis were air dried at laboratory temperature. LF and H samples were ground and the mineral samples (M samples) sieved through 2 mm.

Samples for calorimetric measurements were kept at the humidity they had after sampling, and mineral samples were sieved at 2 mm too. Then, about 100 g of each sample from different locations and soil layers were kept in separated polyethylene bags and frozen at −21°C until the calorimetric measurements. All samples were treated under the same experimental conditions. Therefore, comparison among samples is possible (Rubin et al., 2013).

Elemental and thermal analysis

The soil Carbon, C, and Nitrogen, N, content was determined by combustion with a LECO elemental analyzer (TrusSpec CHNS).

Thermal properties were obtained by simultaneous thermogravimetry (TG) and differential scanning calorimetry (DSC)(TGA-DSC1 Mettler Toledo). About 7 mg for LF samples, 10 mg for H samples and 20 mg for M samples were placed in 100 mL open aluminum pans under a dry air flow of 50 mL min^{-1} for a temperature ramp from 50 to 1000°C at a heating rate of 10°C min^{-1} (Fernández et al., 2011).

The quantity of soil organic matter (SOM) is directly obtained from the TG traces as the total mass loss between 180°C and 600°C normalized to the mass of the sample. DTG curves (first derivative of TG traces) yields the different SOM fractions based on the maximum temperature of the combustion peaks in the DTG curves. All samples have a first combustion peak with a maxima ranging from 307°C and 340°C giving an Exo1 SOM fraction considered as labile SOM (Fernández et al., 2011; Plante et al., 2009), and a second peak at a maxima from 404°C to 434°C called Exo 2 and considered as recalcitrant SOM. After the temperature scan there are remains of the combustion constituted by mineral parts of the samples called mineral char that is also reported here. The percentage for the Exo 1 and Exo 2 fractions taking part of SOM composition is obtained directly from the TG traces and normalized to the SOM content. The mineral char is not considered here as a part of SOM, therefore it is quantified by normalizing to the sample size combusted.

DSC gives the profile of the energy released by SOM along with the temperature ramp as a DSC plot. The integration of the plot yields the heat of combustion of the samples, Q_{SOM}, in kilo joules per gram of SOM, kJ g^{-1} SOM, a measurement of the energy content of SOM connected to the degree of reduction of organic substrates (Barros et al., 2020).

Raw data from the TG-DSC analysis are provided as supplemental material, Data S1. Soil thermal properties are usually highly reproducible. Therefore only one measurement per soil sample was run. The error is assumed to be 5% and given as the uncertainty for Q_{SOM} values in Table 1.

Calorimetric measurements

Calorimetry measures the heat rate of SOM microbial decomposition.

For the calorimetric measurements 10 g of each of the samples were defreeze at the initial temperature of the experiments (20°C). After this initial stabilization, samples were brought to 60% of their water holding capacity (WHC) and stabilized again after rewetting for about 8–10 days, depending on the samples, inside polyethylene bags with a water container. The entire stabilization process is previously recorded with the calorimeter in order to check when samples are stable after this treatment. About 400–500 mg (LF samples) and 700-800 mg (H and M samples) of stabilized samples are introduced into a 4 mL stainless steel calorimetric ampoule, sealed, and taken into a calorimeter (TAM III, TA Instruments) with six channels. Therefore, six samples can be run at the same time.

Heat rates (φ = dQ/dt) are directly registered by the calorimeter in microwatts per gram of sample. For the measurement it was designed a step-scan of isothermal temperatures to measure the response of soils to increasing and decreasing temperature by the heat rates. Increasing temperatures at 20, 30, 40, 50 and 60°C tested the response of the heat rates to the heating to extreme temperatures. Decreasing temperatures from 60°C to 40 and 20°C tested the ability of the samples to recover from the heatwave. The duration of each of the isothermal phases was about 15 h, extending the duration to 48 h at 20°C after the heatwave. The increase of temperature in between each isothermal period was at 0.042°C/min and took about 4 h. Cooling took about 4 h at 0.083°C/min. The whole measurement takes about one week. To ensure the Oxygen consumption and CO_2 released inside the calorimetric ampoules did not affect the heat rates, ampoules were taken out of the calorimeter and opened twice along the measurement: during the scan from 40 to 50°C, and from 40 to 20°C. Samples were re-inserted in the calorimeter 30 min before the next isothermal period started, after incubation during the temperature scan period (4 h) inside an oven at the temperature of the next calorimetric measure. Identical calorimetric scan was previously done with empty calorimetric ampoules as a blank to avoid the effect of the heating in the stainless steel at each temperature by the following equation:

$$(dQ/dt)_{metabolism} = (dQ/dt)_{soil} - (dQ/dt)_{empty\ ampoule} \quad \text{(Equation 5)}$$

The calorimeter continuously records the heat rate in microwatts in a result file that can be plotted versus time (φ-t plots). The raw tabulated date is provided as supplemental material Data S2. The quantitative heat rate for each temperature is determined by integration of each of the isothermal phases in the φ-t plots. Integration was performed for the same period for all samples, 10 h, to give the heat rate, Q, in millijoules per gram of soil and hour (mJ g^{-1} h^{-1}). The TAM III software performs the total integration of the entire experiment as a result file. The integration for 10 h of each isothermal phase was designed by Origin Pro Lab software.

The heat rates, Q, for each temperature was used to determine the Q_{10} for 20–30°C and for 30–40°C by the following equation:

$$Q_{10} = (R_2/R_1)e^{(10/T2-T1)} \quad \text{(Equation 6)}$$

Where R_1 is Q at temperature T_1, and R_2 is Q at temperature T_2 ($T_2 > T_1$)

QUANTIFICATION AND STATISTICAL ANALYSIS

One TG-DSC measurement was done per soil sample (n = 15). The error, about 5%, is reported as the uncertainty for Q_{SOM} values in Table 1. Calorimetric measurements were done with soil duplicates. Heat rate data, Q, are given as the average of duplicates of each soil layer and sampling site for each temperature (n = 200). The experimental design for the statistical analysis is summarized as supplemental material in Figure S1. The significance of differences among the same soil layers from different locations and for the different layers was studied by one way ANOVA after checking normality (Shapiro-Wilk test) and homoscedasticity (Brown-Forsythe Test). These comparisons were done with Origin Pro Lab software. The test was designed considering the heat rate at different temperatures from each soil location as levels of the studied factor (six locations, six levels; n = 84, $p < 0.05$). Significant differences were analyzed by Tukeys'test using Origin Pro Lab software too. Results are shown in Figure 3, where differences among levels of the factor (sampling locations, six levels, n =84) are represented as overlaps of boxplots notches as described by Chambers et al. (Chambers et al., 1983) using R (R CoreTeam,2021). Comparison of heat rates at 40 and 20°C after the heat stress at 60°C with the initial ones during heating was done by a paired sample ttest for LF and M samples (six locations, n = 24) with Origin Pro Lab software. Pearson's correlation was applied to study the relation between the thermal soil properties and the response of samples to temperature with Origin Pro Lab software too. The correlation obtained among Q_{SOM} values and the heat rates averaged for each of the different temperatures during the calorimetric measurements is shown in Table 2 (n = 15, where n represents the total number of samples). Pearson's correlation was also applied to study the effect of Q_{SOM} on LEA correlating Q_{SOM} data with LEA obtained for 40 and 20°C (n = 15). The hierarchical relation among soil layers, sites, soil thermal properties and heat rates was done by cluster analysis using Past 3 software and it is shown in Figure 4.

Additional resource

Plot analysis from TG-DSC and calorimetric measurements are performed with Origin Pro Lab software.

Q_{10} calculation by Equation 6 is done with Q_{10} calculator by Physiology Web. "*Description*: https://www.physiologyweb.com/calculators/q10_calculator.html".