

Calorimetry measures the response of soil organic matter biodegradation to increasing temperature

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Abstract The temperature sensitivity of soil organic matter (SOM) is receiving an increasing interest due to its importance in the global carbon cycle and potential feedbacks to climate change. It constitutes a controversial topic in soil science due to different constraints involving the models employed, together with methodological limitations. It is welcome the introduction of new methods and indicators that can assess the sensitivity to temperature of the SOM macromolecule continuum on a more global basis. Calorimetry can be an attractive alternative if the SOM degradation is studied based on the heat rate under a gradient of temperature. The design of new calorimeters permits to do those measurements in real time through a temperature scan mode. We have applied and designed a preliminary protocol with this new type of calorimeters to calculate the activation energies and Q_{10} values of soil samples with different recalcitrance. The calculation was run on short-term basis and continuously through a temperature gradient from 18 to 35 °C for 1 week. Results showed fast adaptation of microbial decomposition rates to increasing temperature and enough sensitivity of the method to detect changes in the heat rate involving SOM thermal properties. Labile substrates as carbohydrates

showed up as potential rulers explaining E_a and Q_{10} changes which fitted the rule of thumb connecting both.

Keywords Calorimetry · Soil · Q_{10} · E_a · Temperature

Introduction

Most studies focused on the temperature response of soil organic matter (SOM) biodegradation indicate the importance of the impact that soil biodegradation has on climate change. The soil carbon (C) pool is much larger than the atmospheric pool, and thus, small changes in soil C could deeply affect the CO₂ exchange rate between the biosphere and atmosphere [1, 2]. This is widely reported as the main reason for the effort to improve knowledge of SOM sensitivity to temperature. Despite much research, a consensus has not yet emerged on the temperature sensitivity of SOM decomposition [3], thus making it impossible to establish a global model. Most studies have measured CO₂ fluxes over a small range of temperature at laboratory conditions and over quite large intervals of time, e.g., CO₂ released per hour or per day. Field studies make integrated CO₂ measurements over even longer time scales (days–weeks) and usually at only two temperatures [4]. Although these measurements give direct data on the CO₂ flux from soil to the atmosphere, the data do not provide the information necessary to understand the underlying mechanisms and therefore the dependence on SOM quality.

SOM is generally classified as of high or low C quality [5, 6] based on the recalcitrance toward CO₂ production. However, biodegradation of aromatic and aliphatic substrates occurs through biochemical paths that are less CO₂ dissipative than the “high quality,” more labile, carbohydrate substrates. Therefore, the lack of agreement on SOM

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sensitivity to temperature may be a consequence of using inappropriate biodegradation indices or inadequacies in the methodologies employed. This may explain some of the disagreement about the temperature sensitivity of recalcitrant SOM biodegradation [7–9].

Correlations were recently found between CO₂ rates and the chemical nature of the C pools [5] reporting a positive correlation between CO₂ rates and labile substrate indicators such as O-alkyl-C and negative correlations with aromaticity of substrates. This observed reduction of CO₂ rates during decomposition of aromatic substrates or low-quality C pools is probably a consequence of the biochemical mechanism of degradation and not an indication of the actual rate of bioprocessing of SOM by the microbial community. Biodegradation of SOM is controlled by a heterogeneous microbial community associated with and part of the SOM that is capable of degrading both low- and high-quality C pools. The relative activities of different classes of microorganisms depend on the existing environmental conditions and affect the CO₂ rate and temperature dependence.

Biochemical paths of SOM biodegradation may or may not release CO₂ as a product, but all of them have in common dissipation of heat. The heat released by microbial metabolism can be used as a measure of the biodegradation rate and is easily quantified by direct calorimetry. High sensitivity calorimeters permit continuous measurements of heat rate without disturbing the living system at a detection level of nanowatts [10]. Biocalorimetry has been widely applied in microbiology and has also been used to monitor SOM biodegradation [11, 12]. Calorimeters have steadily improved over the years and now allow simulation of temperature changes from 15 to 100 °C while monitoring the microbial response directly. Application of calorimetry to SOM biodegradation can thus provide an innovative way to measure the response of SOM to changing temperature with enhanced sensitivity and accuracy that could complete the information obtained by CO₂ measurements alone. Calorimeters have also recently been adapted to simultaneously measure heat and CO₂ rates from soil biodegradation [13, 14]. Such measurements thus provide direct information on the rate of carbon loss, i.e., as the CO₂ rate, while the heat rate measures the rate of all the biodegradation processes in the SOM continuum, considering the SOM as a whole instead of constituted by different, more or less recalcitrant C pools.

In this work, a calorimetric method is applied to soil samples with more or less recalcitrant SOM based on thermal properties. The SOM response to continuously changing temperature is obtained from the heat rate of microbial decomposition of SOM. The microbiological response is monitored directly and in real time. The main goal is to test the potential of these new calorimeters to

monitor the biodegradation rate changes at different temperatures as well as to check how results fit with models commonly employ to describe SOM sensitivity to temperature as the Q_{10} and Arrhenius equation.

Experimental

Soil samples

Soil samples used for this study were a Cambisol [15] collected at different depths (5–10; 10–20; and 20–30 cm) in Borreiros–Viveiro (43°37′51.94″N 7°37′22.63″).

Elemental and thermal analysis

C content of the Cambisol samples was determined with a LECO Elemental analyzer.

Thermal properties of the samples were determined by thermogravimetry (TG) (TGA-DSC1 Mettler Toledo). For TG analysis, samples were dried, crushed in an agate mortar and placed in 100 µL open aluminum pans under a dry air flow of 50 mL min⁻¹. The temperature ramp was from 50 to 600 °C at 10 °C min⁻¹. DTG curves (first derivative of TG traces) indicate the resistance of SOM to thermal oxidation in air [16, 17]. TG traces determine the quantity of SOM combusted and volatilized and quantify SOM fractions with different resistance to oxidation as defined by the temperatures at the maxima of the different combustion peaks in the DTG curves. The T50-TG of SOM is defined as the temperature at which 50 % of the SOM mass is lost. A higher T50-TG temperature indicates higher thermal stability.

Calorimetric measurements

SOM degradation rates through microbial metabolism were determined by calorimetry in a TAM III (TA Instruments) with six channels. For calorimetric measurements, samples were air-dried after sampling for 3 days at room temperature (21 °C), sieved (0.5 mm), placed in polyethylene bags and stored at 4 °C for 1 month. Immediately before calorimetric measurements, samples were amended with water at 60 % of the water holding capacity (WHC) and stabilized for 4 days inside polyethylene bags at the initial temperature of the calorimetric measurement (18 °C). After this pre-treatment, 0.800 g of soil was sealed into 4 mL stainless steel ampoules and placed in the calorimeter. Duplicates from each depth were used to determine reproducibility. A first calorimetric run was done with six samples, 2 from each depth. Two independent measurements were taken with all samples, and on the

whole, four samples from each depth were studied. By this procedure, reproducibility is clearly stated.

The model of calorimeter used (TAM III) permits measurements of heat rates while scanning temperature continuously or in a stepwise mode. The stepwise mode allows interrupting the scan at selected temperatures by introducing isothermal periods at different temperatures in the scan. The range of temperatures selected was from 18 to 35 °C simulating the environmental temperatures that can be reached in summer in the place where Cambisol was taken in summer too. The temperature was increased from 18 to 35 °C with isothermal periods of 22 h at 18, 21, 25, 30 and 35 °C. The increase in temperature between isothermal periods was designed to take place for 3 h at 0.017, 0.022 and 0.028 °C min⁻¹, respectively. The heat rate was measured and recorded as power–time plots during the whole scan, recording the changes in the heat rate and the adaptation to the new temperatures at each isothermal period.

Because soil samples were enclosed in the calorimetric ampoules for 1 week due to the design of the temperature scan, the influence of the temperature scan design on heat rate values was determined. Because accumulation of CO₂ and/or depletion of O₂ could potentially alter the results, some of the soil samples were taken out of the calorimeter for 3 h during the scan period, opened, covered with a polyethylene lid permeable to O₂ and CO₂ but not to water evaporation and equilibrated at the new isothermal condition in a chamber inside the calorimeter. After 3 h, the ampoules were closed and reintroduced into the calorimeter at the end of the scan period. The heat flow rate versus time curves and heat flow rates values obtained were compared with those determined from samples kept in the calorimeter along the whole measurement.

The isothermal data between scanning periods were analyzed by correcting for blank baselines collected with empty ampoules:

$$(dQ/dt)_{\text{metabolism}} = (dQ/dt)_{\text{measured}} - (dQ/dt)_{\text{baseline}} \quad (1)$$

where $(dQ/dt)_{\text{measured}}$ is the heat flow rate, ϕ_R , in microwatts (μW) of the soil samples and $(dQ/dt)_{\text{baseline}}$ is the heat flow rate of the empty ampoules.

Response of SOM biodegradation to temperature was studied by applying the Arrhenius model, and activation energy (E_a) of samples was determined from the slopes of plots of a form of the Arrhenius equation:

$$\ln(\phi_R) = \ln A - (E_a/R)(1/T) \quad (2)$$

where ϕ_R is the heat flow rate in μW , R the gas constant and $\ln A$ a pre-exponential factor.

Q_{10} was determined by the following equation [18]:

$$Q_{10} = (R_2/R_1)e^{(10/T_2 - T_1)} \quad (3)$$

where R_1 is the ϕ_R value at temperature T_1 , R_2 is the ϕ_R value at temperature T_2 ($T_2 > T_1$).

Statistical analysis

The heat rates are given as the average of four replicates and the standard deviation of the mean. The significance of the variation in the temperature dependence of the heat rates was tested by one-way ANOVA. Comparison of heat rates obtained through different experimental conditions was made by the paired-sample Wilcoxon signed-rank test. Statistical analysis was done using the Origin Pro Lab software.

Results

Thermal properties of samples

Figure 1 shows that all of the Cambisol samples have two different mass loss rates with maxima at 342 ± 3 and 470 ± 4 °C in the sample collected at 0–10 cm, at 308 ± 8 and 455 ± 4 °C in the 10–20 cm sample and at 295 ± 3 and 463 ± 3 °C in the 20–30 cm sample. The lower-temperature peak defines an Exo1 fraction, and the higher-temperature peak in the Cambisol samples defines an Exo2 fraction with recalcitrant substrates. Elemental and thermal properties of these samples are given in Table 1. T50-TG increased with depth. There was a clear depletion of OM and C percentages with depth. Exo1 fraction decreased and the Exo2 significantly increased with depth. This is responsible for a decreasing in the Exo1 to Exo2 ratio with soil depth (Table 1). These properties denoted an increase in SOM recalcitrance as depth increases.

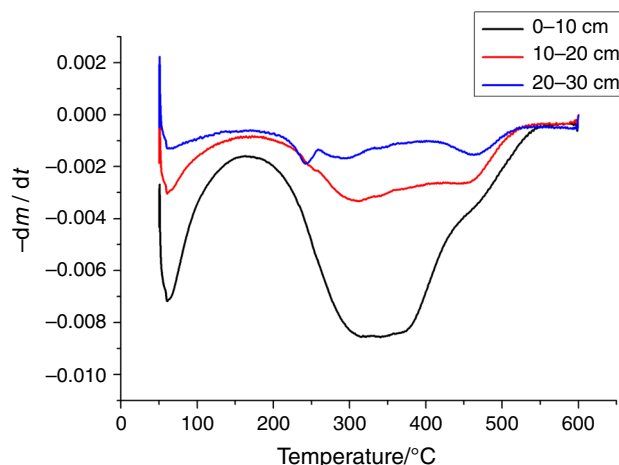


Fig. 1 DTG curves of Cambisol samples collected from different depths

Table 1 Elemental and thermal properties of the Cambisol samples

| Samples/cm | C/% | OM/% | Exo1/% | Exo2/% | T50-TG/°C | Exo1/Exo2 |
|------------|-----------|-----------|--------|--------|-----------|-----------|
| 0–10 | 8.2 ± 0.2 | 22 ± 2 | 86 ± 8 | 14 ± 4 | 344 ± 1 | 6.1 |
| 10–20 | 5.1 ± 0.4 | 11 ± 1 | 68 ± 6 | 32 ± 4 | 354 ± 1 | 2.1 |
| 20–30 | 2.0 ± 0.1 | 4.4 ± 0.2 | 43 ± 2 | 57 ± 3 | 392 ± 2 | 0.8 |

The Exo1 fraction represents the percentage of labile SOM, while the Exo2 fraction is the percentage of the recalcitrant SOM

Response to increasing temperature

Figure 2 shows the comparison between the heat flow rate of samples enclosed in the calorimeter during the entire measurement with samples that were taken outside the calorimeter during the temperature scan to equilibrate O₂/CO₂ inside the ampoule. There is a clear decrease in the heat flow rate as depth increases, as a consequence in the depletion of the C and OM content.

Samples equilibrated outside the calorimeter showed about a 5-h delay to adapt to the new temperature compared with those kept inside the calorimeter but reached the same ϕ_R values as those kept in the calorimeter during the entire measurement. Comparison of ϕ_R data between treatments was made by the paired-sample Wilcoxon signed-rank test yielding not significant difference between the quantitative ϕ_R values. Therefore, at these scan conditions and soil quantities used (0.8 g), samples can be kept inside the calorimeter during the entire measurement time.

Corrected heat flow rate versus time curves by Eq. 1 are shown in Fig. 3. ϕ_R of all Cambisol samples was stable at 18 °C. Above this temperature, ϕ_R values decreased with time during the isothermal measurements with an increasing negative slope as temperature increased. The slope decreased with depth.

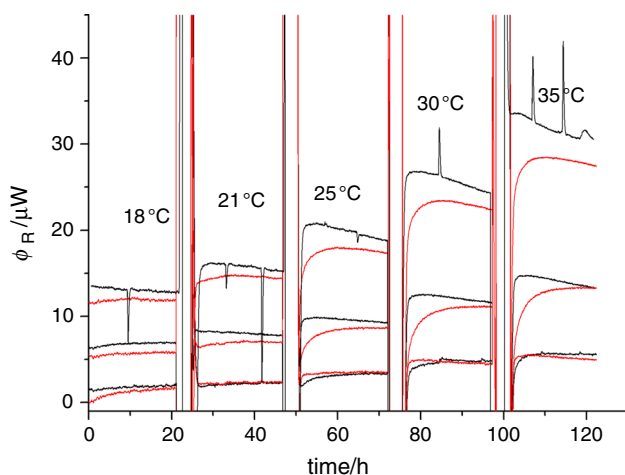


Fig. 2 Power–time curves of Cambisol samples from different depths. *Black lines* are the samples kept inside the calorimeter along the entire measurement. *Red lines* are the samples taken outside the calorimeter to equilibrate O₂/CO₂ inside the ampoules during the scanning of temperature (3 h). (Color figure online)

While samples from the Cambisol deepest soil layers presented stable ϕ_R values during the isothermal phases of the measurement, samples from the Cambisol upper layers had unstable rates. The clear trend of declining ϕ_R values at each temperature made it necessary to introduce criteria to select the ϕ_R values assigned to each temperature. The ϕ_R values selected for Cambisol were the highest (ϕ_{Ri}) and end values (ϕ_{Rf}) reached during each isothermal measurement. Results are given in Table 2.

Comparison of ϕ_{Ri} and ϕ_{Rf} by the paired-sample Wilcoxon signed-rank test showed that the values were not significantly different in the 10–20 and 20–30 cm Cambisol samples, but significantly different in the 0–10 cm Cambisol sample. The range of variation given in Table 2 is based on the average values of two independent measurements involving four replicates, but the paired-sample test was performed with values of each measurement instead of averages. One-way ANOVA tests showed that the observed variance of ϕ_R with temperature was significant in all samples. The maxima heat flow rates reached at each isothermal phase were those for determining the E_a values.

Arrhenius plots, as shown in Fig. 4, yielded significant linear fits for calculation of the activation energy, E_a , given in Table 2. Q_{10} values for heat flow rate are also given in Table 2 for comparison. E_a values do not differ significantly for the Cambisol samples from 0 to 10 and 10 to

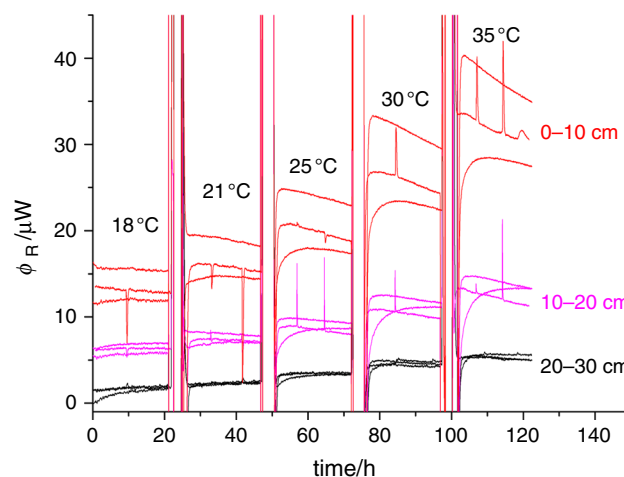


Fig. 3 Power–time curves of three replicates of Cambisol samples at different temperatures

Table 2 Maximum (ϕ_{Ri}) and final (ϕ_{Rf}) heat flow rates of Cambisol determined after correction for blank baselines with empty ampoules

| $T^a/^\circ\text{C}$ | Cam 0–10 cm | | Cam 10–20 cm | | Cam 20–30 cm | |
|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | $\phi_{Ri}/\mu\text{W}$ | $\phi_{Rf}/\mu\text{W}$ | $\phi_{Ri}/\mu\text{W}$ | $\phi_{Rf}/\mu\text{W}$ | $\phi_{Ri}/\mu\text{W}$ | $\phi_{Rf}/\mu\text{W}$ |
| 18 °C | 11 ± 1 | 11 ± 1 | 6 ± 1 | 6 ± 1 | 1.2 ± 0.2 | 1.5 ± 0.2 |
| 21 °C | 13 ± 2 | 13 ± 2 | 8 ± 1 | 7 ± 1 | 1.5 ± 0.1 | 1.7 ± 0.1 |
| 25 °C | 16 ± 2 | 16 ± 2 | 9 ± 1 | 9 ± 1 | 2.3 ± 0.3 | 2.3 ± 0.2 |
| 30 °C | 21 ± 3 | 20 ± 3 | 12 ± 1 | 11 ± 1 | 3.2 ± 0.3 | 3.1 ± 0.2 |
| 35 °C | 26 ± 4 | 25 ± 3 | 14 ± 1 | 13 ± 1 | 3.7 ± 0.2 | 3.5 ± 0.2 |
| Q_{10} | 1.66 ± 0.13 | 1.62 ± 0.13 | 1.65 ± 0.17 | 1.58 ± 0.16 | 1.94 ± 0.11 | 1.65 ± 0.10 |
| $E_a/\text{kJ mol}^{-1}$ | 38 ± 3 | | 38.52 ± 4.00 | | 51.56 ± 3.00 | |

Results are the average of four replicates ($n = 4 \pm \text{SD}$). Uncertainties in E_a are the average computed from the range in the slopes of Arrhenius plots of each individual measurement using ϕ_{Ri} data

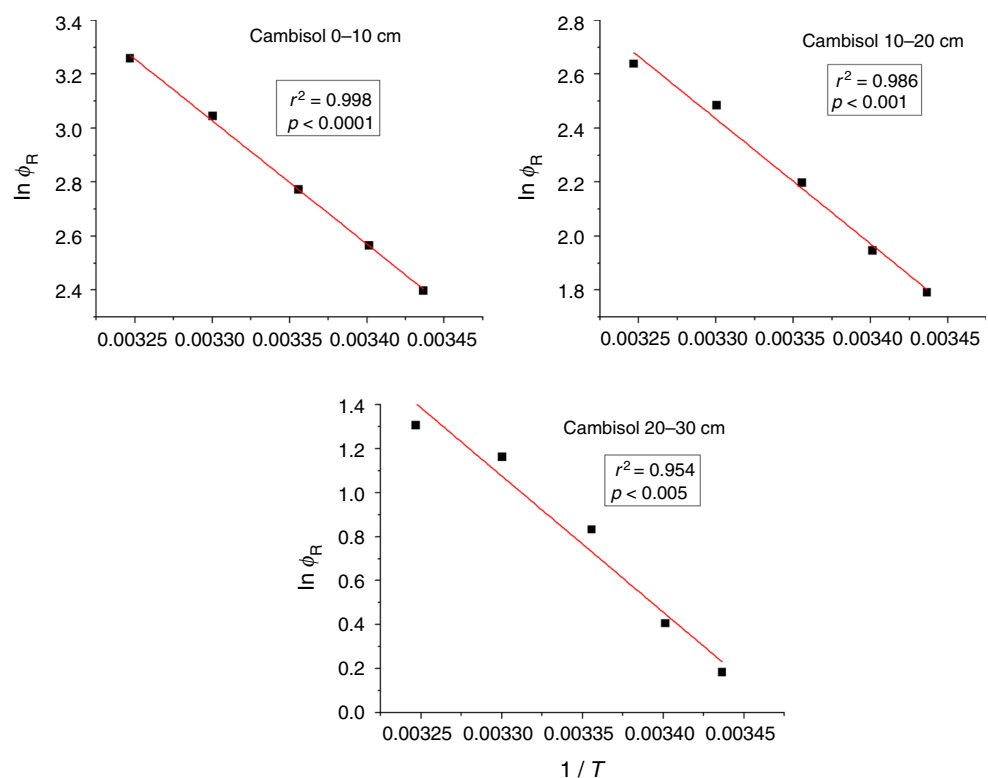
20 cm, but the value from the 20–30 cm was significantly higher than the other ones, suggesting that the slower degradation rates can be associated with a greater activation energy barrier.

Discussion

Establishing the recalcitrance of SOM is difficult because there is yet no general consensus on how to measure biodegradability as an index of recalcitrance [19]. This feature makes difficult to connect the temperature sensitivity of SOM decomposition with the nature of that SOM. Thermal analyses are alternatives for fast assessment of

SOM properties as indicators of biodegradability and stability [20–22]. Temperatures at the maximum rate of SOM thermal decomposition as measured by TG are generally accepted to indicate the recalcitrance [23, 24]. The observed depletion in the Exo1 TG fraction, which is responsible for the decrease in the Exo1/Exo2 ratio and for the increment in the T50-TG, indicates that SOM recalcitrance increased with depth in Cambisol. Ratios of SOM fractions with different thermal stability (Exo1/Exo2) and the T50-TG have been connected with SOM recalcitrance by other authors [25–27] under the assumption that higher thermal stability indicates lower biodegradation rates and higher SOM biological stabilization. Although the reported TG indices can be influenced by the presence of clay minerals [28, 29], metabolic

Fig. 4 Results of the linear fits using the Arrhenius equation. E_a values are determined by the slopes of the straight lines obtained



heat rates determined for these samples indicate the observed evolution of thermal properties with depth is accompanied by decreasing biodegradation rates as suggested in previous papers [14, 22, 26]. The observed changes in the biodegradation rates can be attributed to changes in the SOM chemical composition or to enhancement of SOM physical protection by clays [30, 31] that increases the recalcitrance of SOM as soil depth increases. Therefore, the temperature responses of biodegradation of SOM with different recalcitrance were determined in this study.

Models for the sensitivity of SOM biodegradation to temperature reported in the literature are highly variable and far from describing a general behavior of SOM evolution with temperature changes. The most recent models assume a higher sensitivity of low-quality C, considered as recalcitrant SOM, to increasing temperature [5, 9], but other authors have previously pointed out the limitations of that model [32]. In most of the cases, the temperature response of SOM biodegradation has been described by an Arrhenius model as in this work or by Q_{10} values assigned to different C sources and different soil microbial conditions [6] although there are more models arising [33]. All have used the CO_2 released during the measurement time as a biodegradation rate, and most attempt to isolate different conditions and multiple factors theoretically affecting that response [6, 34]. The relation between rates and temperature found in this work showed that the microbial response to the increasing temperature is very fast and that the observed evolution can only be explained by the SOM progress with depth, specifically to the evolution of the Exo1 fraction attributed to labile material [28]. The depletion of the Exo1 SOM fraction leads E_a values to increase from about 38–51 kJ mol^{-1} , in the range of values reported by other procedures for carbohydrates [32, 35]. In this study, E_a increased with SOM thermal stability measured by thermal analysis. Higher E_a is usually associated with less reactive and more recalcitrant material [3]. Q_{10} values were similar in Cambisol samples from 18 to 35 °C. The obtained values fit well with the rule of thumb that expect Q_{10} values from 1.5 to 2 for E_a ranging from 25 to 50 kJ mol^{-1} assigned to carbohydrates.

The models based on E_a determinations can be debatable, but the main intent of this paper is to show that the response of soil microbial metabolism to temperature can be measured by calorimetry and that such measurements can contribute to better understanding of the processes ruling the mechanism of soil adaptation to environmental temperature, as well as to help to find the best models defining those processes. The described method is sufficiently sensitive to monitor reactions in soils and presents the advantage of changing temperature at various rates, to model daily or annual environmental temperatures at any location, to record responses at extreme temperatures and

to test soil evolution at warming or cooling conditions. The use of heat rate as an indicator of biodegradation rates can be a useful alternative to study soils such as permafrost, wetlands, peatlands, and desert soils where CO_2 is of limited use for quantifying biodegradation rates and the temperature dependence, and also as an indicator to compare with the measurements involving the CO_2 . The goal was to describe a procedure and the technological aspects that are important to take into account in doing such studies that may contribute to the development of this subject.

Conclusions

Calorimetry is a new alternative to apply in the study of the sensitivity of the soil organic matter to temperature.

The biodegradation rates measured as heat flow rate increased with increasing temperature from 18 to 35 °C fitting the Arrhenius model.

The procedure permits calculation of the Q_{10} and E_a based on the heat flow rate. The obtained values followed the rule of thumb connecting both.

Microbial metabolism responds very fast to a change of temperature, reaching maximum heat flow rates after 3–5 h of increasing temperature.

Calorimetry would permit monitoring the response of SOM biodegradation to changing temperature under a wider range of temperatures at heating or cooling conditions that would improve the knowledge about this subject.

Calorimetry detects differences in the response of SOM biodegradation to temperature that could be attached to the SOM nature.

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