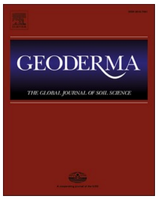
Geoderma 443 (2024) 116846

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma



Energy stored in soil organic matter is influenced by litter quality and the degree of transformation – A combustion calorimetry study

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ARTICLE INFO

Handling Editor: C. Rumpel

Keywords:
Energy content
Combustion enthalpy
Bioenergetics
Soil depth gradients
Tree species effect
Bomb calorimetry

ABSTRACT

The turnover and stabilization of organic matter (OM) in soils depend on mass and energy fluxes. Understanding the energy content of soil organic matter (SOM) is therefore of crucial importance, but this has hardly been studied so far, especially in mineral soils. In this study, combustion calorimetry (bomb calorimetry) was applied to determine the energy content (combustion enthalpy, $\Delta_C H$) of various materials: litter inputs, forest floor layers (OL, OF, OH), and bulk soil and particulate organic matter (POM) from topsoils (0–5 cm). Samples were taken from 35-year-old monocultural stands of Douglas fir (*Pseudotsuga menziesii*), black pine (*Pinus nigra*), European beech (*Fagus sylvatica*), and red oak (*Quercus rubra*) grown under highly similar soil, landscape and boundary conditions. This allowed to investigate the influence of the degree of transformation and litter quality on the $\Delta_C H$ of SOM. Tree species fuel the soil C cycle with high-energy litter (38.9 ± 1.1 kJ g^{-1}C) and fine root biomass (35.9 ± 1.1 kJ g^{-1}C). As plant material is transformed to SOM, $\Delta_C H$ decreases in the order: OL (36.8 ± 1.6 kJ g^{-1}C) $\geq$ OF (35.9 ± 3.7 kJ g^{-1}C) $>$ OH (30.6 ± 7.0 kJ g^{-1}C) $>$ 0–5 cm bulk soil (22.9 ± 8.2 kJ g^{-1}C). It indicates that the energy content of OM decreases with transformation and stabilization, as microorganisms extract energy from organic compounds for growth and maintenance, resulting in lower-energy bulk SOM. The POM fraction has 1.6-fold higher $\Delta_C H$ compared to the bulk SOM. Tree species significantly affect $\Delta_C H$ of SOM in the mineral soil with the lowest values under beech (12.7 ± 3.4 kJ g^{-1}C). The energy contents corresponded to stoichiometric and isotopic parameters as proxies for the degree of transformation. In conclusion, litter quality, in terms of elemental composition and energy content, defines the pathway and degree of the energy-driven microbially mediated transformation and stabilization of SOM.

1. Introduction

Soil organic matter (SOM) is of central importance for the global carbon (C) cycle because soils represent one of the biggest C reservoirs on Earth (Friedlingstein et al., 2022). The significance of SOM for a healthy, sustainable soil functioning and its connection to ecosystem services that are indispensable for human welfare is indisputable (Lehmann et al., 2020). SOM represents a continuum of progressively decomposed organic compounds mainly provided by primary producers, being metabolized by adapted dynamic microbial communities and subsequently being stabilized (Lehmann and Kleber, 2015). Thus, the microbial metabolism exerts strong control over organic matter (OM) cycling and storage in soil (Liang et al., 2017).

The partitioning of OM utilization between catabolic and anabolic pathways was mostly studied by mass balances and ratios related to microbial biomass such as the microbial carbon use efficiency (CUE) (Geyer et al., 2019), but both the driving forces and the complex mechanisms behind the high variability of microbial CUE in soil systems are not yet fully understood (Adingo et al., 2021). Beside C and nutrients, all living chemoheterotrophic organisms need energy to accommodate their requirements for growth and maintenance metabolism (del Giorgio and Cole, 1998; Kästner et al., 2021). For instance, it was shown in chemostat experiments, that the energy content of a substrate determines whether and how much of it will be catabolized or allocated to microbial structural compounds (Linton and Stephenson, 1978). However, to our knowledge, no comparable studies have been conducted

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https://doi.org/10.1016/j.geoderma.2024.116846
Received 25 October 2023; Received in revised form 4 February 2024; Accepted 29 February 2024

with soils. In fact, knowledge of the biological energy demand and turnover, as well as energy supply in soil, is central to gaining a complete picture of OM transformation and stabilization in soils. This research is therefore increasingly coming into focus (Calabrese et al., 2021; Chakrawal et al., 2022; Derrien et al., 2023).

An integral part of new bioenergetic concepts and models are the energy contents of both material from primary producers and organic material already present in the soil. However, its determination particularly in mineral soils with typically low OM content is challenging (Barros et al., 2007). Indirect approaches such as the nominal oxidation status of carbon (NOSC) (LaRowe and van Cappellen, 2011), the degree of reduction (Chakrawal et al., 2020), or proximate analysis (Channiwala and Parikh, 2002), can be used to estimate the energy content of OM mainly based on its elemental composition. For SOM, with its extremely heterogeneous and variable structure (Kleber et al., 2007; Kleber et al., 2021), this alone represents a major challenge. Alternatively, the energy content of OM as a whole can be quantified by combustion calorimetry.

Combustion calorimetry or constant volume calorimetry, also known as bomb calorimetry, is considered the standard method to determine energy contents of OM (Malucelli et al., 2020; Minas da Piedade, 1999), where the chemical energy of the sample is converted to heat during a rapid oxidation in a closed vessel filled with pressurized pure O_2. The energy of all compounds in the sample, including volatiles, is captured. The standard method (ISO, 1928:2020) is of limited use for mineral soils with low organic C content because incomplete or absence of combustion may occur. In these cases, an additional fuel (often benzoic acid) is required for ignition (Sarge et al., 2014) but it may still not achieve a complete combustion of the sample (Minas da Piedade, 1999). Thus, the application of combustion calorimetry is mostly limited to studies of plant biomass (Currie, 2003; van Cleve, 1971; Villanueva et al., 2011), litter layers (Reiners and Reiners, 1970), extracted dissolved OM (Dufour et al., 2022), and other organic substrates (Harvey et al., 2016) and is rarely used to study mineral soil samples (Bölscher et al., 2017). To our knowledge, there is no systematic study of SOM, including mineral soils, using combustion calorimetry to investigate energy contents along a transformation gradient.

This research was conducted at a post-mining site that was recultivated with a uniform soil material, where previous accumulation of plant or coal material are negligible (Lorenz and Thiele-Bruhn, 2019). We studied soil samples from monocultural stands of Douglas fir (*Pseudotsuga menziesii*), black pine (*Pinus nigra*), European beech (*Fagus sylvatica*) and red oak (*Quercus rubra*) that were grown for 35 years under identical soil, geomorphological and management conditions similar to a common garden experiment. Therefore, differences in SOM properties resulted from in-situ processes determined by tree species and mediated by the microbial community (Lopez-Capel et al., 2005; Lorenz et al., 2020; Lorenz et al., 2021; Lorenz and Thiele-Bruhn, 2019). In more detail, we applied combustion calorimetry to determine energy contents (measured as combustion enthalpy) of litter inputs (foliar litterfall, fine root biomass), organic forest floor horizons (OL – litter, OF – fragmented, OH – humified) as well as the mineral topsoil horizon (0–5 cm), representing different stages of the continuum from plant litter to SOM. The objective was to investigate (i) the effect of the transformation degree of litter and (ii) the influence of tree species (i.e. litter quality in terms of elemental composition of litterfall and root tissues) on the energy contents of SOM. Additionally, the bulk soil material (0–5 cm) was fractionated into particulate organic matter (POM) and mineral-associated organic matter (MaOM) and analyzed by combustion calorimetry to compare the energy content of the less transformed POM with the bulk soil material. Previously published data on C:H:N:S:O stoichiometry as well as the natural abundance of ^{13}C and ^{15}N of litterfall, roots, forest floor horizons and the bulk soil material from the mineral topsoil (Lorenz et al., 2020; Lorenz and Thiele-Bruhn, 2019) was used to assess the relationship of energy contents with biogeochemical properties. Changes in the elemental and isotopic composition from plant litter to OM along soil depth gradients served as proxies for SOM transformation (Lorenz et al., 2020; Soldatova et al., 2024). With this study design, we addressed the following hypotheses:

- H1: The energy content (combustion enthalpy) of OM will decline in the continuum from plant litter to SOM because microorganisms exploit energy to meet their requirements.
- H2: Tree species influence the combustion enthalpy of the litter input due to differences in the composition and thus the energy content of the biomass, especially between coniferous and deciduous tree species.
- H3: The combustion enthalpy of SOM in mineral topsoil differs between tree species due to different degrees of transformation and/or stabilization.
- H4: Particulate OM represents an energy-rich fraction of SOM in an intermediate stage of transformation from litter to OM stabilized in the soil.

2. Materials and methods

2.1. Study site

The study was conducted at the afforested spoil heap ‘Sophienhöhe’, which is located in the northwest of the lignite open-cast mine ‘Hambach’ near Jülich, Germany (N 50° 56.11’, E 6° 26.56’). There, boundary conditions regarding soil, climate, topography and management were highly similar, equivalent to a common garden experiment (Lorenz and Thiele-Bruhn, 2019). The studied monocultural stands of Douglas fir (*Pseudotsuga menziesii*), black pine (*Pinus nigra*), European beech (*Fagus sylvatica*) and red oak (*Quercus rubra*) were afforested in 1982 on the western exposed slopes of the spoil heap that were recultivated with the same parent material consisting of 20–25 % loess loam and 75–80 % sandy gravel material. The soil was characterized by a loamy sand texture, > 40 % of coarse fragments and strongly acidic pH (4.3 ± 0.7) (Lorenz and Thiele-Bruhn, 2019). The soil did not contain measurable amounts of lignite-bearing carbon or inorganic carbon from carbonates. Differences in soil properties (Table A1) and SOM formation were primarily caused by the influence of tree species. In the 35 years after afforestation, Dystric Skeletic Regosols (IUSS Working Group WRB, 2022) had developed under all tree species with sequences of the diagnostic organic horizons OL (litter), OF (fragmented) and OH (humified) that were classified as Moder (Zanella et al., 2018b). Dependent on the thickness of the OH horizon, Dysmoder (OH $\geq$ 1 cm) was the dominant humus form that coexisted in some patchy sections with Eumoder (OH < 1 cm) under Douglas fir, beech and oak, while Dysmoder had consistently developed under pine (Lorenz and Thiele-Bruhn, 2019).

2.2. Sampling scheme and sample preparation

Each species stand is subdivided into six to ten plots, 1780 ± 660 m^2 in size, by skid trails established in slope line. For each of the four species stands, five plots were selected for sampling of the litterfall, roots, forest floor horizons and mineral soil (Fig. A1). Details on the sampling scheme and sample preparation are given by Lorenz et al. (2020). Briefly, in each of the five plots per tree species stand, a litter trap was established to collect the litterfall samples monthly over one year. The foliar fraction of the 12 monthly samples of each litter trap were pooled into one composite sample and dried at 60 °C (Ukonmaanaho et al., 2016). Five litterfall samples per tree species resulted in a total number of 20 litterfall samples. In addition, an extensive root sampling campaign in the same five plots with five replicate samples taken from (i) the entire organic forest floor (OL + OF + OH) and (ii) the 0–5 cm soil horizon (Fig. A1) yielded 200 root samples (<5mm; four tree species, five plots, five replicate samples, 2 depths) (Lorenz et al., 2020). A representative subset of 20 fine root samples (five per tree species) was selected for this study and dried at 105 °C.

Forest floor samples were taken with a steel frame (20 cm × 20 cm) and carefully separated into the organic litter, fragmented, and humified horizon, OL, OF and OH, respectively, according to Zanella et al., (2018a). At the same spots, bulk soil samples were taken in 0–5 cm depth from excavated 50 cm × 50 cm × 50 cm pits. To ensure representativeness, forest floor and soil samples were taken from four positions within each plot (Fig. A1) and samples from similar depths were subsequently pooled. In total 60 forest floor samples (20 from each horizon, OL, OF and OH) and 20 mineral soil samples were collected (five per depth in each tree species stand). Forest floor samples were dried at 60 °C and visible roots were carefully removed. Mineral soil samples were passed through a 2 mm sieve, roots were removed and the mineral soil was dried at 60 °C.

The bulk soil material from 0 to 5 cm was fractionated using a density-based approach according to Golchin et al. (1997) that was simplified in order to separate POM with a density < 2.0 g cm^{-3} from MaOM with a density > 2.0 g cm^{-3}. Briefly, 30 ml of a sodium polytungstate solution ($Na_6(H_2W_{12}O_{40})$, TC-Tungsten Compounds) was added to 10 g of air-dried soil and shaken overhead for 16 h at 15 rpm. Samples were then vortexed and centrifuged for 60 min at 2800 g. The supernatant was vacuum filtrated through a 0.45 µm membrane filter (Sartorius, Göttingen, Germany). The filter residue represents the POM fraction and includes free and occluded POM. It was rinsed into a glass petri dish, dried at 45 °C, and weighed. In order to obtain sufficient POM from all 20 samples (five per tree species), the procedure was performed three times for each sample and the POM material obtained from these triplicates was finally pooled.

All samples (20 litterfall, 20 roots, 20 OL, 20 OF and 20 OH from the forest floor, 20 bulk mineral soil from 0 to 5 cm and 20 POM from 0 to 5 cm) were ground and homogenized using a ball mill (Retsch MM400, Retsch GmbH, Haan, Germany) for further analysis.

2.3. Combustion calorimetry

A subset of all homogenized samples was dried at 105 °C and stored in a desiccator until combustion calorimetric measurements were performed. The energy content was determined as combustion enthalpy ($\Delta_C H$) according to DIN 51900-1:2000-04 and DIN 51900-3:2005-01 (comparable with ISO 1928:2020) using an adiabatic combustion calorimeter (IKA C 4000 A, IKA-Analysentechnik, Heitersheim, Germany). Benzoic acid ($\geq$99.5 %; Carl Roth GmbH; $\Delta_C H = 26.45$ kJ g^{-1}) was used to determine the heat capacity C_v of the combustion calorimeter and to calibrate the system. In this measurement, the chemical energy of the sample is converted to heat during an abrupt oxidation in a closed, O_2 saturated pressure vessel. Under isochoric measurement conditions, the heat of combustion corresponds to the internal energy change and considering the expansion work the combustion enthalpy is obtained. Due to the complexity of SOM, the stoichiometry of the material and the calculation of the expansion work can only be a rough estimate. However, as demonstrated by Korth et al. (2017), the difference between the combustion enthalpy as total amount of energy and the internal energy is small ($<$0.2 %). Therefore, the measured heat of combustion can be interpreted as combustion enthalpy. The terms energy content and combustion enthalpy were interchangeably used in this study. It must be noted, that positive values are reported for the combustion enthalpy of exothermic reactions in this study, while, when comparing exothermic and endothermic reactions, according to thermodynamic convention, the release of heat (exothermic) is indicated by negative values.

For organic materials (litterfall, roots, OL, OF, OH and the POM fraction) 1 g of sample were weighed into combustion bags ($\Delta_C H =$ 46.298 kJ g^{-1}) and placed in a quartz crucible inside the bomb. A volume of 5 ml of distilled water (25 °C) was added to the bomb to collect gases containing N and S from the combustion reaction (details below). A tungsten wire was fixed between two electrodes inside the bomb to close a circuit and a cotton thread was attached to the wire. The sealed bomb was pressurized with pure oxygen (purity 99.998 mol%, ALPHAGAZ) to 30 bar. An electrical impulse was used to initiate the reaction by igniting the cotton thread that subsequently ignites the sample (Sarge et al., 2014). After the combustion reaction, the residual material was weighed to account for the ash content of the analyzed sample. The temperature increase due to the combustion in the adiabatic calorimeter can be used to calculate the energy content as combustion enthalpy $\Delta_C H$ using the following equation:

$$Energy\ content = \Delta_C H = \frac{C_v \bullet \Delta T - Q_{ext}}{m} \tag{1}$$

where C_V [J K^{-1}] is the heat capacity of the specific calorimeter at constant volume, ΔT [K] the temperature difference before and after the combustion, Q_{ext} [J] the energy contained in external energy sources and m [g] the mass of the sample. External energy sources are the combustion enthalpies of the cotton thread (50 J) and the ignition wire (30 J) as well as the solution enthalpy of the formed nitrogen oxides (Q_N) and of the sulfur oxides (Q_S). To quantify Q_N and Q_S, the aqueous solution residing inside the bomb was analyzed for the concentration of nitrate and sulfate using a modular Ion Chromatograph (Metrohm, Herisau, Switzerland) with a Shodex IC SI-90 4E column (4.0 mm × 250 mm). The energy releases by formation of nitric and sulfuric acid were calculated as follows:

$$Q_N = 0.97 \bullet m_{NO_3} \tag{2}$$

$$Q_S = 3.14 \bullet m_{SO_4} \tag{3}$$

where m_{NO_3} and m_{SO_4} represent the absolute mass [mg] of formed nitrate and sulfate derived from ion chromatography. Analysis of the C content in the combustion residues confirmed that OM had been completely burned in all samples. Therefore, the OM content of each sample could be calculated based on the mass loss during combustion, and the C content was calculated taking into account the previously determined stoichiometry. In this study, the combustion enthalpy is reported either as kJ g^{-1} of sample or normalized to the organic C content of the sample kJ g^{-1}C. Normalization was done to better characterize the energetic signature of OM, particularly in the mineral soil samples.

To achieve complete combustion of OM in the mineral soil samples, benzoic acid was added in a 1:1 (w/w) ratio as an auxiliary combustion source. Accordingly, the energy contained in the benzoic acid was taken into account when calculating the combustion enthalpy of the external energy sources (Eq. (1)). In case the combustion failed or was incomplete (recognizable by black soot in the sample residue), the respective measurement was discarded and repeated. All measurements were done in triplicate.

2.4. Elemental stoichiometry and isotopic composition

Data on the elemental stoichiometry of SOM (focusing on the main elements, C:H:N:S:O) as well as the natural abundance of ^{13}C and ^{15}N of litterfall, roots, forest floor horizons and the bulk soil material from the 0–5 cm (Table A1 and A2) was taken from previous studies (Lorenz et al., 2020; Lorenz and Thiele-Bruhn, 2019). In this study, the elemental stoichiometry and isotopic composition of the POM fraction were characterized in a similar way. Total contents of C, N, S, and H were determined using the simultaneous CHNS combustion analyzer Vario EL cube (Elementar Analysensysteme GmbH, Langenselbold, Germany), while for the determination of the O content an elemental analyzer EA3000 (HEKAtech GmbH, Wegberg, Germany) was used. The natural abundance of the stable isotopes ^{13}C and ^{15}N was determined by an elemental analyzer (Flash EA 1112; Thermo Fisher Scientific, Bremen, Germany) coupled to an isotope ratio mass spectrometer (IRMS, DeltaV Advantage; Thermo Fisher Scientific). Isotope composition was reported in delta notation (δ^{13}C ‰ and δ^{15}N ‰) relative to Vienna Pee-Dee Belemnite (VPDB) for C and relative to atmospheric N_2 for N.

2.5. Statistical analyses

The following statistical analyses were conducted to test the significance of differences in combustion enthalpies between the litter inputs (litterfall vs. roots), the SOM fractions (bulk soil vs. POM) as well as the effect of tree species on the combustion enthalpies of the different materials. First, boxplots and one-way analysis of variance (ANOVA) were performed to examine the data structure. The residuals of ANOVA were tested for normality and homoscedasticity using residual plots as well as the Shapiro-Wilk and Levene's test. Comparing litterfall against roots and bulk soil against POM was done using two-sample t-tests and Wilcoxon signed rank test, depending on fulfilment of the test conditions. The tree species effect on the combustion enthalpies of the different materials was analyzed as follows: normal distributed and homoscedastic data were tested for significant differences between tree species by one-way ANOVA followed by the Tukey's honest significant difference (HSD) post hoc test. The Welch-ANOVA followed by a pairwise t-test with Bonferroni-Holm correction was used for normal distributed but heteroscedastic data. In case data was not normal distributed but homoscedastic the Kruskal-Wallis test was applied followed by the Dunn test.

The effects of soil depth and tree species as well as their interaction within the soil profiles was tested using a two-way ANOVA. Because the residuals of the two-way ANOVA were not normal distributed and heteroscedastic, and attempts to obtain normal distributed and homoscedastic data through data transformation (log, square root, box-cox) failed, the non-parametric Scheirer-Ray-Hare test (Mangiafico, 2016) was applied. According to this test, the interaction of tree species and soil depth was not significant ($P = 0.965$). Therefore, the influence of soil depth and tree species on the energy contents in the soil depth gradients were tested separately by using one-way ANOVA or its alternative non-parametric tests as described above.

Spearman's rank order correlation and regression analyses was performed in order to evaluate the relationship of energy contents with the elemental stoichiometry as well as the isotopic signatures of SOM. All data analyses were conducted with the R 4.3.1 statistical software (R Core Team, 2023).

3. Results

3.1. Combustion enthalpy of litter inputs

On average of all four tree species, the combustion enthalpy of the foliar fraction of litterfall (38.9 ± 1.1 kJ g^{-1}C) was significantly higher compared to the values of the fine root biomass (35.9 ± 1.1 kJ g^{-1}C, Fig. 1). This observation was also made for each of the tree species. The largest differences between combustion enthalpies of litterfall and roots were found in the coniferous species of Douglas fir and pine. Furthermore, the needles of these tree species (Douglas fir: 39.6 ± 0.7 kJ g^{-1}C, pine: 40.0 ± 0.5 kJ g^{-1}C) had significantly higher energy contents than the leaves of beech (37.8 ± 0.6 kJ g^{-1}C) and oak (38.4 ± 0.3 kJ g^{-1}C, Fig. 1). In contrast, the combustion enthalpy of the fine root biomass was not significantly different between tree species, but there was only a tendency of higher energy contents in fine roots of deciduous species (beech: 36.1 ± 0.6 kJ g^{-1}C; oak: 36.5 ± 1.5 kJ g^{-1}C) compared to the conifers (Douglas fir: 35.9 ± 0.5 kJ g^{-1}C, pine: 35.2 ± 1.1 kJ g^{-1}C, Table A2).

3.2. Combustion enthalpy in soil depth gradients

The combustion enthalpy decreased significantly with increasing soil depth from the organic forest floor horizons OL (36.8 ± 1.6 kJ g^{-1}C), OF (35.9 ± 3.7 kJ g^{-1}C) and OH (30.6 ± 7.0 kJ g^{-1}C) to the mineral soil horizon in 0–5 cm (22.9 ± 8.2 kJ g^{-1}C, Fig. 2). This general trend was observed at all sites of the investigated tree species stands. The energy decrease from OL to 0–5 cm ($\Delta_C H_{OL} - \Delta_C H_{0\text{-}5\ cm}$) ranged from 6.5 kJ g^{-1}C (Douglas fir) up to 24.0 kJ g^{-1}C (beech).

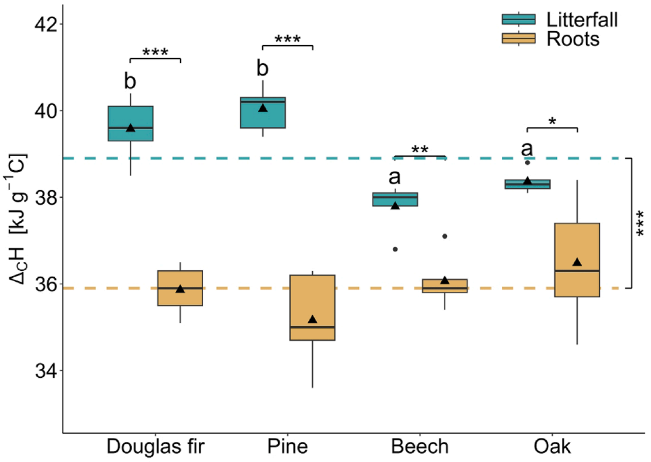


Fig. 1. Boxplots of combustion enthalpies $\Delta_C H$ [kJ g^{-1}C] of foliar litterfall and fine roots. Black triangles represent mean values. Dashed horizontal lines represent the mean of all samples of litterfall (teal) and roots (ocher). Significant differences between litterfall and root are marked with: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). Differences between tree species are marked by different letters.

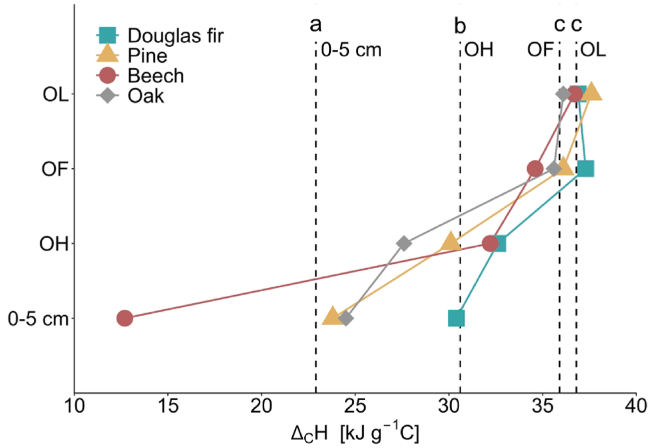


Fig. 2. Depth gradients of combustion enthalpies $\Delta_C H$ [kJ g^{-1}C] from the upper organic forest floor horizon OL down to the bulk SOM in mineral soil at 0–5 cm depth. Dashed vertical lines represent the mean of all samples of OL, OF, OH and 0–5 cm. Differences between the overall mean values of the horizons are marked by different letters. For detailed information about statistical differences, see Table 1.

In the upper two organic forest floor horizons, OL and OF, there was a tendency of higher combustion enthalpies under Douglas fir and pine compared to beech and oak but the effect of tree species was not significant (Table 1). However, the differences between tree species became larger with increasing soil depth and were significant in the mineral soil horizon in 0–5 cm (Table A1). Significantly lowest combustion enthalpies in 0–5 cm were determined under beech with 12.7 ± 4.3 kJ g^{-1}C, and the highest under Douglas fir with 30.4 ± 3.2 kJ g^{-1}C.

3.3. Combustion enthalpies of the bulk soil and the POM fraction

The POM fraction isolated from the 0–5 cm mineral soil samples of all tree stands had a significantly higher combustion enthalpy (33.7 ± 3.6 kJ g^{-1}C) compared to the total of SOM in bulk soil material (22.9 ± 8.2 kJ g^{-1}C). The differences between POM and bulk soil SOM were even significant under beech and oak (Fig. 3; Table A3). The effect of

Table 1
Combustion enthalpies $\Delta_C H$ [kJ g^{-1}C] in soil depth gradients reported as mean ± SD. *P* values refer to the significance level of differences between the soil horizons separated by tree species or in all samples. Differences in combustion enthalpies between soil horizons are marked by different lowercase letters. Significant differences between tree species are marked by capital letters in parentheses ($P < 0.001$).

	All samples	Douglas fir	Pine	Beech	Oak
OL	36.8 ± 1.6c	36.9 ± 2.7b	37.6 ± 0.9b	36.7 ± 1.1b	36.1 ± 1.2b
OF	35.9 ± 3.7c	37.3 ± 2.6b	36.1 ± 1.7b	34.6 ± 4.2b	35.6 ± 4.0b
OH	30.6 ± 7.0b	32.6 ± 4.3ab	30.1 ± 2.5a	32.2 ± 7.5b	27.7 ± 11.5ab
0–5 cm	22.9 ± 8.2a	30.4 ± 3.2a(B)	23.8 ± 7.0a(B)	12.7 ± 4.3a(A)	24.5 ± 6.2a(B)
P value	**<0.001**	**0.009**	**0.001**	**<0.001**	**0.03**

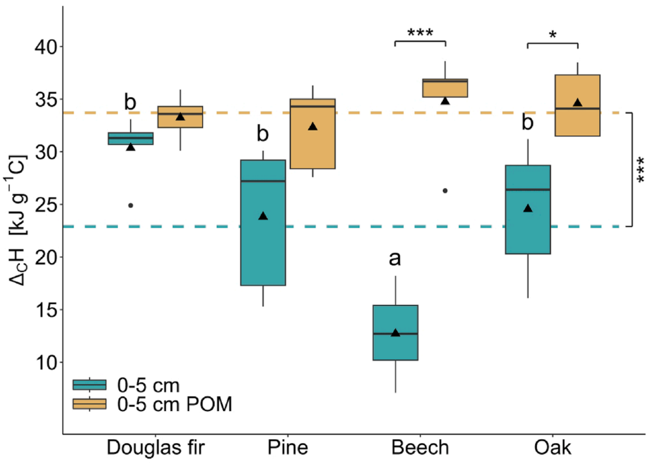
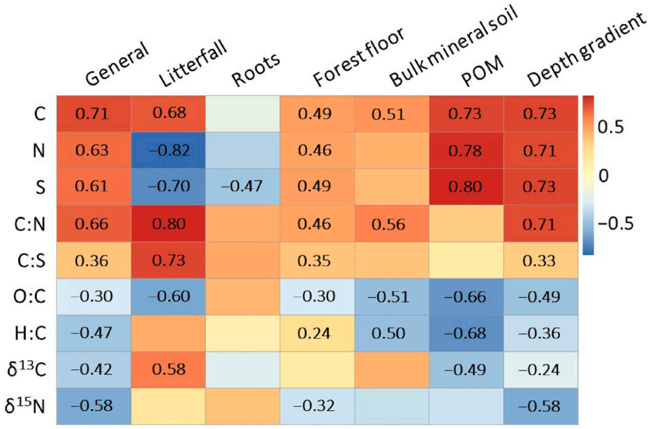


Fig. 3. Boxplots of combustion enthalpies $\Delta_C H$ [kJ g^{-1}C] of bulk SOM and POM in 0–5 cm mineral soil samples. Black triangles represent mean values. Dashed horizontal lines represent the overall mean of bulk SOM (teal) and POM (ocher). Significant differences between bulk SOM and POM are marked with: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). Differences between tree species are marked by different letters.

tree species on the combustion enthalpies of POM was less pronounced than for bulk soil material. Because the energy content of MaOM was often too small for calorimetric quantification, it was roughly estimated using weighted balances. The maximum energy content of MaOM was around 22 kJ g^{-1}C, but in most samples it was < 10 kJ g^{-1}C.

3.4. Relationship of combustion enthalpies to the elemental and isotopic composition

Spearman's rank correlation analysis of the samples covering the whole continuum from plant litter to SOM (incl. litterfall, roots, OL, OF, OH, bulk mineral soil and the POM fraction) revealed that the combustion enthalpy (kJ g^{-1}C), is positively correlated with the C, N, and S content as well as the stoichiometric ratios C:N and C:S, and negatively correlated with the O:C and H:C ratios as well as the natural abundance of δ^{13}C and δ^{15}N (Fig. 4). The relationship between the C content and the energy content of the samples was determined by regression analysis. Fig. 5 shows that the relationship between the C content and the combustion enthalpy followed a logarithmic curve along the transformation gradient: litterfall > roots = OL > OF ≥ POM (0–5 cm) ≥ OH > 0–5 cm (bulk soil). The more transformed the plant material was the less C and less energy per unit C remained in the organic material. When relating the energy content to the mass of the sample (i.e. kJ g^{-1} of plant material or soil), the relationship with the C content follows a linear function (Fig. A2).



	General	Litterfall	Roots	Forest floor	Bulk mineral soil	POM	Depth gradient
C	0.71	0.68		0.49	0.51	0.73	0.73
N	0.63	−0.82		0.46		0.78	0.71
S	0.61	−0.70	−0.47	0.49		0.80	0.73
C:N	0.66	0.80		0.46	0.56		0.71
C:S	0.36	0.73		0.35			0.33
O:C	−0.30	−0.60		−0.30	−0.51	−0.66	−0.49
H:C	−0.47			0.24	0.50	−0.68	−0.36
δ^{13}C	−0.42	0.58				−0.49	−0.24
δ^{15}N	−0.58			−0.32			−0.58

Fig. 4. Heatmap of the Spearman's rank order correlation analysis depicting correlations between elemental and isotopic composition and the combustion enthalpy $\Delta_C H$ in general (whole data set; number of observations = 140) and for the different subsets: litterfall (number of observations = 20), roots (number of observations = 20), forest floor (OL, OF, OH; number of observations = 60), bulk mineral soil (0–5 cm; number of observations = 20), POM (0–5 cm; number of observations = 20) and depth gradient (OL, OF, OH, 0–5 cm; number of observations = 80). In case of significant correlation ($P < 0.05$), the correlation coefficients are shown.

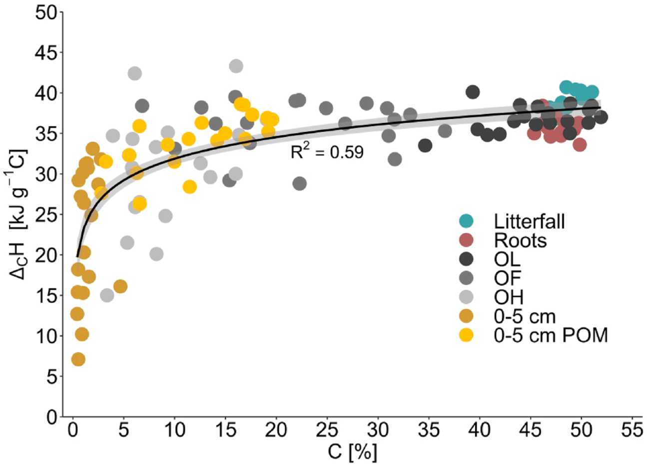


Fig. 5. Relationship between combustion enthalpy $\Delta_C H$ [kJ g^{-1}C] and C content [%] in the investigated continuum from plant litter to SOM. The regression was best described by a logarithmic curve ($\Delta_C H = 3.837 \log (C) + 23.034$; with $R^2 = 0.59$ and $P < 0.001$).

However, when conducting Spearman's rank correlation analysis for the subset of litter inputs, some differences to the outlined general pattern were found, for instance a negative correlation of N and S with the combustion enthalpy (Fig. 4). The correlation trends of the combustion enthalpy with elemental and isotopic composition within the sample subsets of the soil depth gradient (OL, OF, OH, 0–5 cm) were similar to the general trends (Fig. 4). However, the correlation between the isotopic ratios and the combustion enthalpy differed between the analyzed subsets. For instance, δ^{15}N correlates positively with the combustion enthalpy in litterfall and root material while a negative correlation was found in the other subsets. Furthermore, the C:N ratio was positively correlated with the energy content, irrespective of the data subset analyzed.

4. Discussion

The design of our study enabled us to investigate the influence of litter quality, represented by different tree species, and degree of transformation, represented by different soil horizons, on SOM properties (Lorenz et al., 2021) with regard to its energy content determined as combustion enthalpy. To our knowledge, this is the first time that combustion calorimetry, although it is widely recognized as the standard method for the determination of energy contents (Minas da Piedade, 1999), was systematically applied in soil research to study the turnover of organic matter in soil profiles including the mineral topsoil horizon. In our study, combustion calorimetry was successfully used to analyze samples from organic and mineral soil horizons with OC contents ranging from 0.56 to 50.39 %.

4.1. Energy content evolution controlled by OM transformation

The comparative evaluation of the combustion enthalpy of litter inputs and SOM in different soil horizons down to the mineral soil gives insights into the evolution of energy contents along the continuum of progressively decomposing primary plant compounds. The combustion enthalpy of plant biomass is influenced by many factors such as plant species or genotype, edaphic conditions, irradiance, seasonality of storage compound production and its chemical composition (Golley, 1961; Hnilička et al., 2020). The higher combustion enthalpy of the foliar litterfall fraction compared to fine roots (Fig. 1) is in accordance with other observations (Ovington, 1961). Aboveground plant organs contain higher proportions of high-energy substances such as fats (39.6 kJ g^{-1} OM) and proteins (23.6 kJ g^{-1} OM), while roots contain a high percentage of non-structural carbohydrates (<17.6 kJ g^{-1} OM) and less proteins (Lamprecht, 1999; Lieth, 1975; Yan et al., 2018).

On the way from plant material to SOM, the combustion enthalpy decreased in the following order: litterfall $\geq$ OL $\geq$ OF > OH > 0–5 cm bulk soil. The energy content of OL is close to but slightly lower compared to that of the litterfall. Ovington and Heitkamp (1960) reported similar energy declines in their studies of OM transformation gradients (covering leaves, OL, and OF + OH material) in organic layers of soils under different tree species in Great Britain using combustion calorimetry. In addition, studies using differential scanning calorimetry combined with thermogravimetry (DSC-TG) in experiments with bare fallow (Barré et al., 2016), soil depth gradients (Stone and Plante, 2015), and incubation studies (Plante et al., 2011) revealed an energy loss during OM degradation. The energy discharge with progressing transformation can be assigned to the metabolic activity of the decomposer community. Depending on their requirements, heterotrophic soil organisms use the available primary plant material either as energy source for catabolism or as carbon and nutrient source to synthesize new biomass (anabolism). Since anabolic reactions mainly consume (Gibbs) energy they depend on the (Gibbs) energy gained by catabolic reactions in the microbial cell (LaRowe and Amend, 2016). During these processes, the elemental and isotopic composition of the utilized organic matter in soil changes with an increase of relative nutrient concentrations (Berg, 2000) indicated by the narrowing of C:N and C:S ratios (see depth gradients in Tab. A2). This is accompanied by an enrichment of ^{13}C and ^{15}N in soil depth gradients due to an increasing relative contribution of microbial biomass and its residues (Lorenz et al., 2021; Nel et al., 2018). Therefore, the combustion enthalpy of OM in the continuum from primary plant biomass to microbially transformed SOM compounds (Fig. 5) is positively correlated to parameters of the C:N:S stoichiometry and negatively to $\delta^{13}C$ and $\delta^{15}N$ (Fig. 4). It should be noted that the enthalpy of combustion of OM as an admixture in a largely mineral matrix is influenced by the latter. Endothermic reactions of the minerals in the bomb calorimeter as well as the sorption energies of the organic molecules associated in supramolecular structures and even more the binding energies of organic-mineral associations reduce the measured values (Ahmad and Martsinovich, 2022; Kaiser and Zech, 1997). However, this reflects the total energy status of OM in mineral soil, unlike, for example, combustion calorimetry of OM artificially extracted and purified from soil.

Barré et al. (2016) interpreted from long-term bare fallow experiments that the decrease of the energy content is an indication of the residual accumulation of persistent SOM because microorganisms preferentially use high-energy OM and leave behind material with low energy contents. The decrease of the energy content applies also for the here observed depth gradients with the lowest combustion enthalpy in bulk soil in 0–5 cm (Table 1). In comparison to the bulk soil, the POM fraction in the mineral soil horizon is characterized by higher energy compounds (Fig. 3), overlapping in its combustion enthalpy partly with OF samples, but especially with OH samples (Fig. 5). This indicates that there is still high-energy material in the soil left for microbial transformation, which originates largely from OH material (and less from OF) transferred into the topsoil by bioturbation and/or other transport processes. Williams et al. (2018) demonstrated that the energy content in POM fractions is higher than in MaOM, which underpins our measurements of the energy contents of bulk soil SOM and POM fraction (Table A3) and calculated estimates for the MaOM fraction. Basically, the POM fraction is characterized by a retarded degradation due to its biochemical properties, resulting from preceding transformation that leaves behind less degradable components (Angst et al., 2016; Kögel-Knabner, 2017), and/or its spatial inaccessibility (Cotrufo et al., 2015; Witzgall et al., 2021). From an energetic perspective, the mainly plant-derived POM fraction is in an intermediate degradation state and it has been suggested that it remains in soil because the energetic investment (e.g. production of enzymes) by microorganisms is high for its depolymerization in relation to the energy gained from its mineralization under the current growth conditions in the soil system (Fontaine et al., 2007; Henneron et al., 2022). In short, biodegradation would be energetically less favorable (Dufour et al., 2022) and therefore retarded. Consequently, the POM fraction remains in the soil even though it contains a lot of energy. Combustion calorimetry is suitable for characterizing the total energy content of OM with its interactions with the mineral matrix of the soil. However, it does not necessarily provide information on the amount of energy that can be utilized by microorganisms.

In contrast to our results, other theoretical concepts stated that the energy content of SOM should be higher compared to that of litter due to its aliphatic structure and relative enrichment with microbial N-rich residues (Gunina and Kuzyakov, 2022). This was supported by empirical observations in litterbag studies (Bocock, 1964), decomposition studies of pine needles (Rovira et al., 2008) or litter in forest floors of oak forests (Chavez-Vergara et al., 2014) as well as soil depth gradients (Barros et al., 2020) pointing to an ongoing discussion. It appears that the determination of energy contents depends on the nature of the sample analyzed in terms of its OC concentration versus the contribution of minerals (Peltre et al., 2013) and the calorimetric method used (e.g. combustion calorimetry vs. DSC-TG). We need more robust empirical thermodynamic data on microbial mediated OM turnover and OM stabilization mechanisms, particularly in mineral soils under various edaphic conditions, to clarify the apparent contradictions in the literature.

4.2. Energy contents influenced by litter quality

This study, with its common garden experimental study design with similar systems and boundary conditions, allows for relating differences in combustion enthalpy to tree species identity (Lorenz and Thiele-Bruhn, 2019). Tree species fuel the C cycle in soil systems with the input of above- and belowground biomass (Janzen, 2015) characterized by different combustion enthalpies. The needles of Douglas fir and pine had significantly higher combustion enthalpies compared to the leaves of beech and oak (Fig. 1), which is in accordance with other studies (Gorham and Sanger, 1967; Ovington and Heitkamp, 1960). This can be related to the elemental composition and attributed to biochemical properties of the foliar litterfall that differed significantly between tree species (Table A2, for detailed discussion see Lorenz et al., 2020). For instance, needles are sclerophyllous, rich in C and high-energy lignin (26.3 kJ g^{-1} OM, Lieth, 1975) as well as characterized by high C:N ratios and low N concentrations (Augusto et al., 2015). This results in a positive correlation between the combustion enthalpy and the C:N ratio as well as a negative correlation with the N content of the litterfall (Fig. 4). Furthermore, contents of plant secondary metabolites such as tannins and terpenes are higher in needles compared to leaves (Kanerva et al., 2008; Smolander et al., 2012) and terpenes are known to have one of the highest energy contents (46.9 kJ g^{-1} OM) among biomolecules (Lieth, 1975). As pointed out by Gravalos et al. (2016), the C content represents the main energy source in plant materials, which confirms the positive relationship between C and combustion enthalpy in the litterfall (Fig. 4). In contrast to the litterfall, the combustion enthalpy in fine root biomass of the four tree species was not significantly different, although there was a tendency for higher values in the fine roots of deciduous species compared to conifers (Fig. 1). In line with this, Currie (2003) determined higher energy content in oak roots compared to pine. This may be due to higher proportions of high-energy compounds in deciduous tree roots. For instance, lignin contents in fine roots decrease in the order oak > beech > Douglas fir (Hobbie et al., 2010), which corresponds to the order of decreasing energy contents in this study (Table A2, Fig. 1).

The combustion enthalpies of the forest floor horizons OL, OF and OH were not (significantly) affected by tree species identity but the differences in combustion enthalpies between tree species increased with increasing soil depth and were significant in the mineral soil horizon (Fig. 2). Although the effect of tree species was not significant in the forest floor horizons, it is obvious that there is an imprint of litterfall on the upper forest floor horizons. This is because slightly higher combustion enthalpies in OL and OF of Douglas fir and pine (Table 1) corresponded to higher combustion enthalpies of their litterfall (Fig. 1).

The different combustion enthalpies of SOM in the mineral soil can be interpreted as result of the degree of transformation. The lowest combustion enthalpy and thus strongest OM transformation was detected in SOM under beech (12.7 ± 3.4 kJ g^{-1}C). In general, combustion enthalpy in SOM correlated positively with the C concentration and C:N ratio and negatively with the N concentration of the litterfall (Table A5). Previous findings confirm that SOM turnover under beech is enhanced and related to the elemental composition and nutrient supply by the litter input (Lorenz et al., 2020). This is further supported by the stoichiometric ratios of SOM in the mineral soil, with the lowest bulk soil C:N ratio of 8.5 ± 1.1 found under beech (Table A1). This C:N ratio is in the range of microbial biomass C:N of 4.5 to 12.5 (Mooshammer et al., 2014), which may be due to a significant contribution of microbial derived compounds (Kästner et al., 2021; Lorenz et al., 2021). Thus, the highest OM turnover under beech coincides with the highest energy loss of 24.0 kJ g^{-1}C from plant biomass to SOM in the bulk mineral soil. This, together with the significantly lowest C concentration of 0.56 ± 0.19 % in 0–5 cm under beech, suggests that most of the C from litter inputs was used as an energy source and respired by microorganisms.

Although the C concentration remaining in mineral soil under beech was the lowest compared to the other tree species, it was more stabilized by organo-mineral associations, as indicated by the significantly highest MaOM fraction (Table A4). It appears that the energy content of the bulk soil indicates whether the SOM is more stabilized in organo-mineral associations or remains in the soil as POM. The difference between the combustion enthalpy in POM and bulk soil was related to the proportion of MaOM. Highest differences ($\Delta_C H_{POM} - \Delta_C H_{bulk\ soil} = 22.0$ kJ g^{-1}C) were associated with the highest share of MaOM under beech (97.1 wt%, Table A4), while the opposite trend with lowest differences (2.8 kJ g^{-1}C) and the lowest share of MaOM (84.8 wt%) was observed for soils under Douglas fir. Vice versa, it is presumed that the stabilization mechanisms via organo-mineral interactions contribute to the energy contents of bulk soil SOM. In dependence on the chemistry of the organic ligand and the mineral, different types of bonds with varying binding energy will be created (Newcomb et al., 2017). The stability, and thus the persistence, of organo-mineral associations is related to the energy input required (i) to remobilize OM from these bonds (Bernard et al., 2022; Kleber et al., 2015), and (ii) to subsequently utilize the liberated OM. It has been shown that the combustion of stabilized SOM generates less energy than unprotected SOM (Hemingway et al., 2019; Henneron et al., 2022). Consequently, the different energy contents of OM depths profiles in soils under different tree species result from the degree of the microbially mediated transformation of plant litter inputs and their potential for stabilization with the soil mineral matrix.

5. Conclusion

This study showed that combustion calorimetry can be successfully applied to determine energy contents of OM in soil samples, including mineral soil, covering a wide range of C contents (0.56 – 50.39 %). The obtained empirical data on the energy content of SOM provide new insights into SOM status, stabilization and utilization by microorganisms. It should be noted, however, that combustion calorimetry may have its limitations when investigating samples with low SOM content (OC < 0.5 %), such as subsoil samples.

Our study provided fundamental insights into the energy discharge as OM turnover progresses and in dependence on litter quality. Thus, valuable information was obtained for the recent controversial debate on whether OM transformation leads to energy dissipation or residual accrual of energy-rich compounds in soils. According to the hypotheses, the amount of energy stored per C unit decreased in the continuum from plant litter inputs to SOM indicating that as OM turnover progressed, microorganisms exploit energy from organic compounds for growth and maintenance, leaving behind SOM consisting of plant and microbial residues with lower energy content. The overall energy content of bulk SOM in mineral soil horizons is the sum of the POM fraction, consisting of relatively energy-rich and incompletely processed plant-derived compounds, and the MaOM fraction, which is energy-depleted due to further transformation and/or stabilization. Apparently, the microbial degradation of POM or parts thereof in topsoil is delayed. Consequently, POM in soil represents an intermediate degradation product and energy reservoir that is particularly utilized when environmental conditions and/or microbial community composition and activity change.

The energy content of OM differs greatly depending on the litter-producing tree species. Remarkably, these differences are not relevant for litter layers (OL) but become more pronounced as microbial transformation progresses. As a result, the combustion enthalpy of SOM in mineral topsoil differs between tree species due to different pathways and degrees of transformation and/or stabilization in the mineral soil matrix. It is concluded that litter quality, in terms of its stoichiometry, molecular composition and thus its usable energy content, forms the basis for the pathway and extent of the energy-driven microbial transformation and stabilization of SOM.

CRediT authorship contribution statement

Marcel Lorenz: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Thomas Maskow:** Methodology, Supervision, Validation, Writing – review & editing. **Sören Thiele-Bruhn:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the colleagues of the Soil Science Department of Trier University, especially E. Sieberger, P. Ziegler, M. Hill, M. Heinrich, and B. Backes for assistance and support during laboratory and fieldwork. Many thanks to M. Kästner and A. Miltner for fruitful discussion. We acknowledge financial support from the Trier University and the German Research Foundation (DFG-SPP 2322 with the projects: Coordination Project, TH 678/25-1, DriverPool TH 678/26-1, MA 3746/9-1, and TherMic MA 3746/8-1). We also like to thank the three anonymous reviewers who contributed greatly to improving the readability of the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2024.116846.

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