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Linking soil organic matter thermal stability with contents of clay, bound water, organic carbon and nitrogen

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## **Abstract**

Thermogravimetry is a technique measuring mass change during programmed heating. In soil analysis, it is used for determination of content of volatile fractions, thermally labile and stable fractions of soil organic matter and minerals. One method of data analysis uses the determination of mass losses in 10°C temperature areas. In the past, their mutual correlation revealed several larger temperature areas of mass losses, which appeared to be universal for all types of soils equilibrated at the same relative humidity. However, it is unclear if mass losses in these temperature areas are connected with biogeochemical functions or processes in soil. In this work, using data from more than 300 soils of different types, geographical origin, and land uses we demonstrate their linear correlation with content of organic C, total N and clay content and biological activity or their combinations. In particular, the results showed that mass losses between 200-300°C, which is related to thermally labile fraction, correlates best with total organic C and less with total organic N. From 300-450°C represents a more stabilized soil organic matter pool and can be best described by either by C or N contents. Mass loss 450-550°C correlates strongly with clay content, which suggests a connection to organo-clay complexes. The low temperature interval of 30-200°C, which corresponded to weakly and strongly bound water showed a strong connection with clay content, but a weaker connection to microbial activity. The developed equations were corrected and verified using additional soil sample sets. The correlations along with their universal applicability lead to conclusion about the possible connection of mass losses in these temperature areas to, still unknown, biogeochemical soil functions. The obtained equations may represent a new approach of rapid and universally applicable

“mathematical” fractionation requiring only contents of soil organic carbon, total nitrogen, clay and water in soil equilibrated at 76% relative humidity.

**Key words:** soil organic matter, stability, fractionation, modeling, thermogravimetry

## 1. Introduction

“Soil is a complex system composed of a set of interconnected physical, chemical and biological factors that function as a whole” ([Minasny et al., 2008](#)). Primarily, soil heterogeneity and complexity is caused by soil forming factors being climate, topography, geological parent material, biota, time ([Jenny, 1994](#)) and anthropogenic influences. The interplay between forming factors gives rise to a high variability of soil forming processes and results in formation of different soil types covering a wide range of properties and composition. The variation and interactions between soil forming factors are believed to be the reason for the absence of a generic model useful for the prediction of properties in a wide range of soils ([Schmidt et al., 2011](#)). However, many soils share similar features in their composition (e.g. C/N content, amino acids composition, phenotypic and genotypic characteristics of soil microbial communities ([Fierer et al., 2009](#))) and these may be used for the development of a universally applicable model for the prediction of soil properties irrespective their origin and composition ([Siewert and Kucerik, 2015](#)).

Soil organic matter (SOM) is a key parameter that determines quality, productivity and ecological functioning of soils. SOM contains a large portion of the global terrestrial carbon pool, and for this reason soils are currently being discussed for their capacity to offset increasing levels of atmospheric CO<sub>2</sub> ([Schmidt et al., 2011](#)). This is reflected in

the emphasis in developing methods for rapid assessment of SOM turnover rates, sizes and composition of different C pools and also to better understand SOM protection mechanisms ([Pallasser et al., 2013](#)).

Methods of thermal analysis are gradually gaining interest in soils science. Generally, they belong to the family of techniques that use heat to measure properties of soil exposed to a temperature program. One of the most frequently used techniques is calorimetry (both isothermal and non-isothermal) which is based on measurement of temperature that gives enthalpy upon instrument calibration. In soil science calorimetry techniques have been used to evaluate the quality of organic matter ([Barros et al., 2011b](#); [Plante et al., 2005](#)), insight into carbon cycling ([Herrmann et al., 2014](#)), soil microbiological activity ([Barros et al., 2011a](#); [Barros et al., 2015](#); [Barros et al., 2007](#); [Barros et al., 2010](#)), stability of water molecule bridges ([Ondruch et al., 2017b](#); [Schaumann and LeBoeuf, 2005](#)), properties of soil aliphatic crystallites ([Ondruch et al., 2017a](#)), role of cations in soil organic matter stability ([Diehl et al., 2014](#)) or phase transitions in soil organic matter ([LeBoeuf and Zhang, 2009](#); [Young and LeBoeuf, 2000](#)).

Another widely used thermoanalytical technique is thermogravimetry (TG), which is based on the determination of mass change of soil at either non-isothermal or isothermal conditions. For soil analysis, mostly linear heating programs are used ([Fernández et al., 2011](#)). As a result, TG analyzes thermal or thermo-oxidative stability of soils, which is determined mainly by binding energies, accessibility of SOM components, their mutual interactions and other factors ([Plante et al., 2009](#)). TG provides both quantitative (mass loss) and qualitative (temperature) information. Alone,

or in a combination with other detectors, TG has been used to determine the ratio between thermally labile and stable soil organic matter ([Peikert et al., 2015](#); [Tamimi et al., 2017](#)), stability of soil organic matter components ([Manning et al., 2005](#)), the shift in stability of soil organic matter supramolecular complexes induced by small organic molecules and cations ([Buurman et al., 2002](#)), the content ([De la Rosa et al., 2008](#); [Lopez-Capel et al., 2006](#)) and presence ([Siewert and Kucerik, 2015](#)) of pyrogenic carbon, stability of organic matter fractions such as humic acids ([Rotaru et al., 2008](#)) or for distinguishing of organic and inorganic carbon ([Kristl et al., 2016](#)).

Traditional TG data evaluation employs a temperature or time derivative TG curve (DTG). The local minima in the DTG curve are interpreted as a change in degradation mechanisms reflecting the change in composition of degraded material. To illustrate it, Figure 1 reports the TG curves (black line) of soil investigated in this work and DTG (bold grey line) and an alternative way of data analysis discussed further on.

To avoid soil-dependent differences, an alternative approach of TG data evaluation representing the determination of mass losses in predefined temperature intervals is sometimes used e.g. ([Kristl et al., 2016](#)). An alternative way of this approach ([Siewert, 2001](#)) suggests the use of mass losses in 10°C temperature intervals (here referred as TML) of air dried soils equilibrated at constant relative humidity (RH). This approach is based on the view that progressive mass loss determined by using TG is a separation of soil parts according to their stability, and separation of mass losses into 10°C intervals represent a good way to minimize the data number while keeping reproducibility of mass loss determination. The constant RH is used to determine the mass losses under thermodynamically comparable conditions, i.e. soils are in

equilibrium with their environment under the same moisture conditions. Up to now, this approach was tested only for soils equilibrated at 76% RH and lead to the discovery of connections between TML and soil properties such as soil organic carbon (SOC), total nitrogen content (TN), clay content ([Siewert, 2001](#); [Siewert, 2004](#)), and microbiological activity indicators reflected in amount of CO<sub>2</sub> released by soil microorganisms ([Kucerik et al., 2013](#); [Kucerik and Siewert, 2014](#); [Siewert et al., 2012](#)). The most important TML, which will be further discussed in this work and their characteristics and methods of determination together with coefficients of determination are reported in Table 1. Furthermore, the combination of TMLs has been demonstrated to correlate with total SOM content represented by mass losses from 110 to 550 °C (referred here as LTML<sub>110-550</sub>). The correlation enabled the development of equations for SOM determination using soil organic C (or total N) and clay for a wide range of soil types from various climatic zones and soil management ([Kucerik et al., 2016](#)). The deviation from the equations seems to be useful to distinguish fresh, undecomposed organic material from more highly decomposed soil organic matter ([Kucerik et al., 2016](#)). Next, correlating all TMLs with each other (autocorrelation method) in the temperature interval 30-950°C revealed several temperature areas in which TMLs significantly correlated. For better understanding, we report an exemplary raster graph showing the coefficients of determination as grey shades (Figure 2). It is hypothesized that these temperature areas indicate the presence of molecules or soil parts, which are mutually interconnected either directly or as a result of biotic and/or abiotic soil forming processes ([Siewert and Kucerik, 2015](#)). The temperature areas are marked as A-F in Figure 2 and are also summarized in Table 2. Briefly, the areas A (30-100°C) and B

(100-200°C) represent loosely and strongly bound water, respectively, C (200-300°C), D (300-450°C) , E (450-550°C) are temperature areas representing fractions of soil organic matter (and possibly pyrogenic carbon) of increasing thermal stability and G (above 550°C) represents soil inorganic matter. F is the combination of the C, D and E temperature areas. It is important to note that the mass loss in F area is also caused by elimination of water bound in clay minerals, which has implications to understanding to soil forming processes (Kucerik et al., 2016).

The comparison of the traditional DTG-based approach with TML-based approach reveals that the minima in DTG are soil dependent, therefore, this approach is applicable to distinguish differences in soil composition ([Peikert et al., 2015](#)). On the contrary, the temperature areas A-F obtained from TML-based approach are the same for different soil types, which indicates generic similarities in composition of soils and their stability fractions ([Siewert and Kucerik, 2015](#)). This is in accordance to [Fierer et al. \(2009\)](#), who argued that many soils share a common set of ecological mechanisms that govern biogeochemical processes. However, the connection of the fractions A-F to biogeochemical cycles has not been clarified yet, in particular C, D, and E which correspond to decomposition of SOM. We assume that if there is a connection, then they represent functional soil fractions and not only mathematical “artefacts”.

Therefore, the aim of this work is to answer the question about the meaning and potential functioning of the fractions, especially C, D and E. We assume that their potential function would be connected to content and dynamics of soil properties such as content of organic C, N, clay and microbiological stability. As aforementioned, the soil parameters can be obtained from TML reported in Table 1, thus we will perform the

correlation analysis between TML (Table 1) and temperature areas (Table 2). The second aim is to validate the obtained relationships by predicting the temperature areas in an independent soil sample set.

## **2. Experimental**

### ***2.1 Overall strategy and soil samples***

Figure 3 summarizes the overall strategy of this paper. Briefly, we analyzed 301 soils from contrasting climatic and geological areas in South and North America, Antarctica, Siberia, Africa and Western Europe. The sample set included soils with the least possible influence of human activity as well as soils from agricultural use and long term agricultural experiments. The content of SOC ranged between 0.2 to 27% and clay content from 10 to 65%. Further descriptions of the soils and their properties can be found in [Kucerik et al., 2013](#); [Kucerik et al., 2016](#); [Kucerik and Siewert, 2014](#); [Siewert et al., 2012](#); [Siewert and Kucerik, 2015](#). Most soils were collected from 0-30 cm (which in natural soils usually contains more than one genetic soil horizon), gently air dried, and sieved through a 2 mm sieve in order to avoid changes in composition. Prior to thermogravimetric analysis, the samples were equilibrated at 76% relative humidity at 20°C in order to ensure comparable conditions during analysis. As aforementioned, the equilibration is important to assure that the soils are in equilibrium with its environment and that all soils are measured under the same thermodynamically comparable conditions (similarly as defined for example by IUPAC or NIST). Incomparable conditions, i.e. variable partial pressure and temperature, would result in incomparability of experimental data. The temperature of 20°C and relative humidity 76% were selected

as easily achievable in most laboratories. Higher temperatures can cause irreversible changes in soil structure (Diehl et al., 2014). The 76% RH can be reached using oversaturated solution of common NaCl, it prevents soils from compaction, which could occur at low RH and simultaneously from microbiological activity, if the RH is too high. Second reason is that water is a part of soil and thus it should be involved in the mass analysis. Due to its volatility, its content varies depending also on soil properties, thus only comparable external conditions assure the comparability of experimental data. The reasoning for using these conditions is also discussed in earlier works ([Kucerik et al., 2013](#); [Kucerik et al., 2016](#); [Kucerik and Siewert, 2014](#); [Siewert, 2001](#); [Siewert, 2004](#); [Siewert and Kucerik, 2015](#)).

## **2.2 Determination of soil properties**

Soil properties were determined via standard methods. This included determination of organic carbon and nitrogen contents using dry combustion via element-analyzer (Carlos Erba), with samples ground to 0.2mm and dried at 105°C before analysis. The carbonate content was measured by elemental analysis before and after treatment of soils with HCl. Texture was analyzed by using standard pipette-analysis with gravimetric determination ([Siewert, 2001](#)).

## **2.3 Thermogravimetry**

The moisture-equilibrated soils were analyzed by thermogravimetry (TG) in duplicate and results were averaged for use in further calculations. 0.5-1 g of soil was placed in alumina crucibles and analyzed under a constant air stream enriched with water to 76% relative humidity at 25°C with a flow rate 200 mL min<sup>-1</sup>. A heating rate of 5°C min<sup>-1</sup> was

applied from room temperature (around 25°C) to 950°C. Due to relatively high sample mass, the results did not differ significantly, maximally up to several %. Figure 1 presents an average record of all the results of thermal mass losses and first derivative (DTG) of mass loss of the investigated samples including the variance of mass losses.

## **2.4 Data Analysis**

The recorded data were used to calculate mean thermal mass losses (TML) in 10 °C temperature intervals for every sample in the temperature range from 30 to 950°C. This resulted in 93 TMLs for each soil sample. The TML of each sample was then divided by the total sample mass to obtain mutually comparable values in mg per g of a sample.

For simplified data presentation the following abbreviations are used. TML<sub>40</sub> refers to thermal mass loss between 30 and 40°C, TML<sub>50</sub> refers to thermal mass loss between 40 and 50°C, etc. thus, the suffix indicates the upper limit of thermal mass losses (TML) in 10°C temperature increase steps.

For presentation of temperature areas C, D, E and their combinations we used abbreviation LTML, (larger thermal mass loss). The index indicates lower and upper temperature limit (e.g., LTML<sub>100-200</sub> corresponds to temperature area between 100 and 200°C). The values are presented in mg g<sup>-1</sup> sample weight.

In this work, for correlation we used LTMLs A to F in Table 2, while selected TMLs are reported in Table 1. The search for interrelations between TML and LTML was carried out in Microsoft Excel with linear and non-linear (power and exponential) models. For this purpose, LINEST function based on the least squares method was used to calculate a dependency between LTML and one or more TMLs. In addition, to complete

the information about relationships between TML and LTML, the search was extended to different temperature areas than reported in Table 2 (LTML).

The calculations were carried out using following equations.

1. linear one parametric fitting, i.e. we correlated one TML with a LTML (SOM fraction) according to equation (a)

$$\text{LTML} = a \times \text{TML}_1 + b \quad (\text{a})$$

2. linear fitting with two parameters according to the equation (b)

$$\text{LTML} = c \times \text{TML}_1 + d \times \text{TML}_2 + e \quad (\text{b})$$

3. linear combination of three parameters according to the equation (c)

$$\text{LTML} = f \times \text{TML}_1 + g \times \text{TML}_2 + h \times \text{TML}_3 + i \quad (\text{c})$$

Where  $a, b, c, d, e, f, g, h, i$  are fitted parameters.

As a measure of goodness of fit we used coefficients of determinations ( $R^2$ ) as a primary approach. This part is reported as step 1 in Figure 3.

## **2.5 Correction and verification**

The obtained equations based on correlation between TML and LTML (TG equations) were converted into relationships between LTML and soil properties. More specifically, the TMLs were replaced by the respective equations reported in Table 1, which connect TML with soil properties such as soil respiration and biogenic elements and clay contents. This transformation gave relationships referred to as the “EA equations” (i.e. elemental analysis equations). The obtained EA equations were improved using an additional set of soil samples (Step 3 in Figure 3). Briefly, the measured and calculated values were compared and parameters of linear regression were used for their

improvement. The sample set for correction consisted of soils from major climatic regions of Eastern and Central Europe (Russia and Germany) and represented a variety of parent materials under natural vegetation, agriculture and forestry. The properties of these soils are described in detail in ref. ([Siewert, 2001](#)) where 54 soils were investigated. Due to limited availability of samples, selected soils numbered in ref. ([Siewert, 2001](#)) as 19, 25 and 48 were not used here. The 51 soils used for verification in the current work included both natural and agricultural soils sampled mostly in the  $A_p$  or  $A_h$  with underlying horizons (i.e. in 0-5, 5-30) up to 30 cm depth and, in a few cases, samples from 30-60 cm depth. The soil types included mainly podzols, luvisols, chernozems and Kastanozems. The SOC of these soils varied between 0.2 - 5.8 % of air dried soil mass, the TN between 0.04 and 0.45% and the clay content between 9 and 55%.

For the validation of the EA equations (step 4 in Figure 3), a third soil sample set was used. The sample set consisted of 32 silty loam type from long-term agricultural experiment V505 at Bad Lauchstädt (Saxony-Anhalt, Germany) exposed to different agricultural practices, sampled in November 2014 and October 2015 from 0-30 cm horizon. In particular, SOC varied between 1.59 and 2.71, TN 0.13-0.24 and clay between 23-27%. The long-term agricultural experiments are conducted by Helmholtz Centre for Environmental Research - UFZ Bad Lauchstädt ([Franko and Merbach, 2017](#)).

### **3. Results**

#### *3. 1 Correlation between TML and LTML*

The results of one-parametric correlations (Equation a) between LTML and their combinations (Table 2) with selected TML (Table 1) are reported in Table 3.

The results show various degrees of correlation between LTML and TML, the highest  $R^2$  in each column are given in bold. It can be seen that the highest  $R^2$  were observed for TML<sub>330</sub> and LTML<sub>300-450</sub>. The last two rows in Table 3 were added to improve the information about other possibilities for prediction of SOM fractions.

Higher  $R^2$  values in Table 4 compared to Table 3 reflect the importance of the inclusion of a second TML for the correct determination of LTML or SOM fractions. In most cases the increase of  $R^2$  was significant when including a second parameter.

The introduction of a third TML did not change the  $R^2$  significantly (data not shown). The TML with the highest influence on LTML did not change either. One exception was observed for TML<sub>200-300</sub>, which was significantly influenced by TML<sub>540</sub>, TML<sub>250</sub> and TML<sub>330</sub>, whereas in the two parametric equations a similar  $R^2$  was calculated for interrelations of LTML<sub>200-300</sub> to TML<sub>250</sub> and TML<sub>320</sub> (Table 4).

No significant increase in  $R^2$  was found when non-linear (power and exponential) models were applied to equations with one, two and three parameters.

Table 5 summarizes the equations of the most significant relationships between LTML and TML. Furthermore, it shows also their transformation into relationships between LTML and soil properties determined with standard methods. Due to low  $R^2$  (though still statistically significant) the TML corresponding to microbiological activity was not used further.

### *3.2 Corrections of developed models*

The models developed in this work models require correction due to uncertainties in determination of SOC, TN and clay content (last column in Table 1), uncertainties in the mutual relationship between TMLs and LTMs (Table 3 and 4), uncertainty in TG measurements and limited accuracy of soil property determination with standard methods used to develop the models in Table 5 (second column), were corrected with an additional soil sample set. Briefly, the equations in Table 5 were used to model LTML and compared with measured data. Figure 4a and b report examples of this pre-validation for predictions based on Equations 1 and 2.

In Figure 4 a linear trend ( $p < 0.0001$  and  $R^2 = 0.89$ ) was observed between calculated and measured LTML. Similar trends were observed also for predictions using other equations (e.g. Figure 4b). In most cases the slope between calculated and predicated values differed from ideal situation of slope = 1 and a Y-intercept = 0. Therefore, for correction, the results of linear regression between calculated and measured values were used. Table 6 summarizes the obtained regression equations and models, which were corrected based on these equations. The coefficients of determination in the column reflect the uncertainty in practical applications of these models. It is worth reminding, that these equations are valid for soils equilibrated at 76% RH and thus, for practical use, water content in soil under these conditions must be determined separately.

### *3.3 Validation of models*

The models were validated using a third independent soil sample set. Figures 5a and b show examples of validation of the corrected equations 6 and 11. Both Figures show an agreement between modeled and measured SOM fractions.

#### **4. Discussion**

The results showed the close correlation between the LTML and soil properties, which suggests the functionality of LTML in terms of biogeochemical soil processes. The validation confirmed that they are useful for the prediction of fractions of different stability using a combination of several soil properties. Also, the equations extend the understanding of modelled LTML.

Wang et al. ([Wang et al., 2011](#)) showed that  $LTML_{30-200}$  (A and B temperature area) is connected mainly with elimination of loosely and chemically bound water. Water content in air dried soils is regulated both by SOM and clay minerals. The obtained results imply the dominant influence of clay on this temperature interval (Table 3), which is enhanced by combination with SOC or TN (Table 4). Interestingly, this interval can also be connected with microbiological activity, which reflects the connection between soil microorganisms and water sorption capacity.

The next temperature interval  $LTML_{200-300}$  (temperature area C) is connected with degradation of thermally labile organic molecules. Recent works showed that  $LTML_{200-300}$  was the most significantly affected during both short (9 days ) and longer (89 days) laboratory incubation days at 20°C and pF=1.8 ([Kucerik et al., 2013](#); [Siewert et al., 2012](#)). Therefore, it is likely that the OM degraded in this temperature area is represented partially or mostly by microbiologically labile SOM components or organic

components in various states of decomposition such as fresh plant residues and microbial tissues, exudates, particulate organic matter, microbial biomass C, soluble C, potentially biologically degradable C, extractable with various reagents ([Haynes, 2005](#)).

The one-parametric correlation indicates a connection between LTML<sub>200-300</sub> and SOC or TN, but also to total SOM content. This is rather surprising because the amount of fresh, not yet transformed organic residues varies across the soil samples and depends on the supply of fresh inputs prior to sampling. This influences the total SOM content that depends on the balance between organic matter input, formation of SOM and its mineralization ([Cotrufo et al., 2015](#)). Likely for this reason, the short-term mineralizable SOC (1 day) correlates well with longer-term (28 days) SOC mineralization rates ([Haney et al., 2008](#)) and with the more stabilized pool of active SOC ([Hurisso et al., 2016](#)). It still remains unclear if LTML<sub>200-300</sub> corresponds to the whole or only to a part of labile SOM fraction. The results based on the DTG approach e.g. ([Gregorich et al., 2015](#); [Katsumi et al., 2016](#); [Ma et al., 2016](#); [Peikert et al., 2015](#)) indicate that this interval might be wider, i.e. the upper temperature can reach 400°C or more. We hypothesize that this difference can be caused by different experimental conditions used, in particular, in the current work, the reaction atmosphere was enriched by water vapor, which can accelerate the thermal degradation processes.

More importantly, some authors question the connection between mass loss at low temperatures and labile SOM fraction ([Schiedung et al., 2016](#)) and some results even indicate that there is no relationship between thermo-oxidative and biological stabilities whatsoever ([Helfrich et al., 2010](#); [Kuz'yakov et al., 2006](#); [Schiedung et al., 2016](#)). On the contrary, other authors have demonstrated that this connection exists, e.g. ([Barre et al.,](#)

[2016](#); [Lopez-Capel et al., 2005](#); [Peltre et al., 2013](#)). This inconsistency makes this issue still open for further research.

The LTML<sub>300-450</sub> (temperature area D) represents the middle temperature area and thus reflects degradation of a more stabilized SOM fraction. The LTML<sub>300-450</sub> showed significantly lower changes during microbial degradation of organic matter in incubation experiments in comparison with LTML<sub>200-300</sub> (refs [Kucerik et al., 2013](#); [Siewert et al., 2012](#)). The highest correlation was observed for the combination of SOC and TN in a two parameter fitting. Importantly, one parametric fitting implied no connection with clay content (Table 3). [Bimueller et al. \(2014\)](#) demonstrated that in soil, carbon and nitrogen mineralization are closely coupled processes during the decay of plant residues, but in mineral associated fractions these processes are decoupled. The inclusion of SOC and TN in the equation for predicting LTML<sub>300-450</sub> confirms that the fraction consists of fresh, but stabilized material, with no direct influence of clay. For this reason, we hypothesize that the fraction LTML<sub>300-450</sub> reflects the degradation of molecules stabilized by various mechanisms such as inherently thermally stable biomolecules (e.g. lignin-derived aromatic carbons, melanoidins, tannins or aliphatic compounds ([Kleber et al., 2011](#))), molecules stabilized by mutual intermolecular interactions (e.g. humic substances ([Piccolo, 2002](#); [Rotaru et al., 2008](#)), or molecules physically protected in soil aggregates.

The LTML<sub>300-450</sub> showed the highest  $R^2$  in all calculations with a maximum  $R^2$  for interrelations with SOC and TN (TML<sub>330</sub> and TML<sub>410</sub> respectively). The autocorrelation analyses (Figure 2) also gave additional hints on possible subfractionation at TML<sub>320</sub> and TML<sub>380</sub>. The large number of TML, which can be used for modelling LTML<sub>300-450</sub> is a

specific feature of this temperature area. In addition to results in Table 3 and 4, the correlations revealed also influences of  $TML_{40}$  and  $TML_{230}$  which, however, have unknown physical meanings.

The  $LTML_{450-550}$  (temperature area E) was separated from  $LTML_{300-550}$  due to the influence of clay on dynamics of thermal mass losses in  $LTML_{450-550}$  ([Siewert, 2004](#)). The highest  $R^2 = 0.64$  for a one parametric fitting for correlation with clay content confirms this connection. However,  $R^2$  was lower comparing to previous study ([Siewert, 2004](#)), which could be caused either by higher content of pyrogenic carbon, ([Kucerik et al., 2016](#); [Siewert and Kucerik, 2015](#)) to a small extent also to silt-sized-stabilized SOM in analyzed soils and clay mineralogy type or Al/Fe oxides content. The stabilization of organic matter by adsorption on clay minerals belongs to the most prominent protection mechanisms ([Lehmann and Kleber, 2015](#)), the resulting organo-mineral structures are more persistent and their combustion occurs at higher temperature than the labile ones ([Barre et al., 2016](#)). Therefore, the significant influence of clay seems to support our assumption that  $LTML_{450-550}$  can be linked to degradation of organo-mineral structures.

This connection is also confirmed by the connection to TN, because the abundant N-containing molecules in soils are proteins and peptides (40 to 60%, ([Khalili et al., 2011](#)), which have a high affinity to soil minerals, in particular to fine clay fractions ([Kleber and Johnson, 2010](#); [Kleber et al., 2007](#); [Knicker, 2011](#); [Kögel-Knabner, 2006](#); [Miltner et al., 2009](#); [Pronk et al., 2013](#)) and positively correlate with water-stability of soil aggregates and long term C and N storage (e.g. ([Gillespie et al., 2011](#)) and references therein).

Variation of the TML leads to a maximum  $R^2 = 0.95$  for one parametric equations for the correlation of  $LTML_{450-550}$  with  $TML_{500}$  (Table 3). A significant increase of  $R^2$  to 0.96 for

interrelations between LTML<sub>450-550</sub> and clay can also be observed in combination with TN as a second parameter. This confirms the fraction LTML<sub>450-550</sub> consists mostly of organo-clay complexes.

The LTML<sub>110-550</sub> that is used frequently for determination of total SOM content was discussed in detail previously ([Kucerik et al., 2016](#)). In the current work, the relationship for its determination was slightly modified to improve its applicability. Unlike the LTML<sub>200-550</sub>, the LTML<sub>110-550</sub> includes also volatiles, partial degradation products and water bound by clay minerals. Interestingly, the TML<sub>200-550</sub> can be described only by TN with significantly higher  $R^2$  than TML<sub>110-550</sub>.

## 5. Conclusions

The stability of SOM fractions can be, as defined in this work, described using the content of biogenic elements such as organic carbon and nitrogen, content of reactive surfaces (e.g. clays) and amount and properties of transport media (water). Interestingly, the equations show a constant ratio of the first two parameters, while the only variable parameter is water content. For this reason, water, or more specifically the mechanism of water binding in equilibrated soil, is key for understanding soil forming processes, carbon sequestration and related biological processes.

The observed connection of LTML to soil properties is a good precondition to use the LTML for soil fractions based on either TG or EA data. However it requires further validation, in particular their connection to results of classical biological, chemical and physical fraction methods such as for example size and density fractionation. This would connect their meaning also to mechanisms of SOM protection. After this step, the method could be useful for rapid prediction of SOM fractions and their dynamics.

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*Table 1: TML with known interrelations to soil properties including regression equations and coefficients of determination. The subscript refers to upper temperature of the 10°C thermal mass loss, i.e. TML<sub>100</sub> corresponds to TML between 90 and 100°C in soil equilibrated at 76% RH, heating rate 5°Cmin<sup>-1</sup>, dynamic air atmosphere enriched by water vapors.*

Thermal mass loss (TML)	Soil parameters	Determination method	Equation	R <sup>2</sup>
TML <sub>100</sub> *	Biological activity	CO <sub>2</sub> -respiration	Respiration = -21.4 + 12.3 × TML <sub>100</sub>	0.68
TML <sub>120</sub>	Clay content	pipette method	clay = 4 × TML <sub>120</sub> + 9.8	0.76
TML <sub>300</sub> *	Biological activity	CO <sub>2</sub> -respiration	Respiration = 4.33 × (TML <sub>300</sub> ) <sup>1.18</sup>	0.74
TML <sub>330</sub>	Soil organic carbon content (SOC)	elemental analysis	OC = 1.18 × TML <sub>330</sub> - 0.05	0.91
TML <sub>350</sub>	Soil organic carbon content (SOC)	elemental analysis	OC = 1.48 × TML <sub>350</sub> - 0.08	0.98
TML <sub>330</sub>	Soil organic nitrogen content (TN)	elemental analysis	N = 0.1118 × TML <sub>330</sub> - 0.023	0.95
TML <sub>410</sub>	Soil organic nitrogen content (TN)	elemental analysis	N = 0.1813 × TML <sub>410</sub> - 0.038	0.96
TML <sub>520</sub>	Clay content	pipette method	clay = 27 × TML <sub>520</sub> + 1.0	0.87

\* 133 soil samples for calibration and 83 for validation from [32]; the rest from [29] where 52 samples was used for calibration and 394 for validation.

*Table 2. Summary of main temperature areas and their meanings.*

	Mass loss in temperature area	Abbreviation in text	Dominant soil components or SOM-fractions
A	30 -100	LTML <sub>30-100</sub>	Mainly loosely (physically) bound water (mainly on SOM)
B	100 – 200	LTML <sub>100-200</sub>	Mainly strongly (chemically) bound water (on surfaces, clays and organo-clay complexes), organic volatiles
C	200 – 300	LTML <sub>200-300</sub>	Degradation of labile SOM
D	300 – 450	LTML <sub>300-450</sub>	Degradation of intermediate stability SOM pool
E	450 – 550	LTML <sub>450-550</sub>	Degradation of stable SOM content and larger part of pyrogenic carbon
F	110 – 550	LTML <sub>110-550</sub>	Corresponds roughly to the total SOM content (see text)
G	> 550	Not mentioned	Decay of mineral carbon (carbonates)

LTML: large thermal mass losses or thermal mass losses in dedicated temperature areas

Table 3. Coefficients of determinations ( $n=301$ ) between selected thermal mass losses in 10 °C temperature increase steps (TML) and temperature areas (LTML) using one-parametric Equation (a) and meanings of TML and LTML

			Temperature area (LTML)			
			A + B	C	D	E
			30 – 200	200-300	300-450	450-550
			Moisture+volatile s	Labile OC	Stabilized OC	Organo- clay OC
TML	meaning					
100	Microbial activity		0.75	0.47	0.46	0.63
120	Clay content		<b>0.96</b>	0.21	0.16	0.59
300	Microbial activity		0.12	0.83	0.93	0.36
330	Organic carbon		0.15	<b>0.91</b>	<b>0.98</b>	0.32
350	Organic carbon		0.15	0.89	0.97	0.28
410	TN		0.21	0.86	0.96	0.36
530	Clay content		0.48	0.01	0.01	<b>0.64</b>
110-550	SOM		0.56	0.86	0.82	0.61
Highest found coefficients of determination with free variation of TML						
	max $r^2$ :		0.96	0.92	0.98	0.95
	with TML:		120	370	340	500
<b>Bold</b>	<b>values:</b>		maximum	$R^2$	in	the
MLI: mass losses on ignition with modified lower temperature limit						
						column

Tab. 4: Coefficients of determinations ( $n=301$ ) between mass losses in LTML representing the content of different SOM fractions and TML using equation (b) with two parameters (TML1 and TML2).

			Temperature area			
		A + B	C	D	E	MLI
		30 – 200	200-300	300-450	450-550	110-550
TML <sub>1</sub>	TML <sub>2</sub>	Moisture+volatiles	Labile OC	Stabilized OC	Organo-clay OC	SOM content
120	100	0.96	0.60	0.65	0.65	0.74
330	100	0.80	0.90	0.97	0.61	0.90
350	100	0.80	0.91	0.98	0.61	0.90
410	100	0.78	0.88	0.97	0.61	0.91
530	100	0.83	0.59	0.59	0.88	0.74
330	120	<b>0.96</b>	0.91	0.97	0.68	0.95
350	120	0.96	<b>0.92</b>	0.98	0.67	<b>0.96</b>
410	120	0.96	0.88	0.97	0.67	0.95
530	120	0.96	0.35	0.29	0.81	0.60
350	330	0.15	0.91	0.98	0.35	0.78
410	330	0.22	0.91	<b>0.998</b>	0.35	0.82
530	330	0.60	0.90	0.97	0.95	0.88
410	350	0.23	0.91	0.99	0.35	0.78
530	350	0.62	0.91	0.98	0.95	0.82

530	410	0.64	0.8 8	0.97	<b>0.96</b>	0.91
Highest coefficients of determination with two TML						
90	150	0.996				
120	200	0.98				
130	330	0.97				
250	320		0.9 6			
330	410			0.998		
210	500				0.97	

**Bold values:** maximum R<sup>2</sup> in column

*Table 5: Regression equations of selected relationships between LTML and TML (in mg per g sample) (“TG relationship”) and their transformation into primary soil parameters equations (in g/kg soil (“EA relationship”) using equations reported in Table 1*

	TG relationship between LTML and TML	EA relationship between LTML and soil parameters
1	$LTML_{200-300} = 10.3 \times TML_{330} - 0.18$	$LTML_{200-300} = 8.70 \times SOC - 1.42$
2	$LTML_{200-300} = 19.7 \times TML_{410} - 1.12$	$LTML_{200-300} = 109 \times TN - 5.09$
3	$LTML_{300-550} = 14.7 \times TML_{350} + 9.62 \times TML_{530} + 1.54$	$LTML_{300-550} = 10 \times SOC + 0.36 \times \text{clay} + 0.44$
4	$LTML_{300-550} = 21.2 \times TML_{410} + 7.4 \times TML_{530} + 1.11$	$LTML_{300-550} = 117 \times TN + 0.27 \times \text{clay} + 5.3$
5	$LTML_{300-450} = 13.2 \times TML_{350} + 3.31$	$LTML_{300-450} = 8.98 \times SOC + 2.65$
6	$LTML_{300-450} = 12.5 \times TML_{330} + 1.38 (TN)^*$	$LTML_{300-450} = 112 \times TN - 1.19$
7	$LTML_{450-550} = 2.8 \times TML_{410} + 7.6 \times TML_{530} + 0.76$	$LTML_{450-550} = 15.4 \times TN + 0.28 \times \text{clay} + 1.6$
8	$LTML_{450-550} = 1.9 \times TML_{350} + 7.8 \times TML_{530} + 0.96$	$LTML_{450-550} = 1.3 \times SOC + 0.29 \text{ clay} + 0.57$
9	$LTML_{110-550} = 10 \times TML_{120} + 25 \times TML_{330} - 2 (SOC)^*$	$LTML_{110-550} = 21 \times SOC + 2.5 \times \text{clay} - 4.4$
10	$LTML_{110-550} = 9.8 \times TML_{120} + 33.5 \times TML_{410} - 5.1$	$LTML_{110-550} = 200 \times TN + 2.2 \times \text{clay} - 14.6$
11	$LTML_{200-550} = 24.7 \times TML_{330} + 9.8 \times TML_{530} - 3.41 (SOC)^*$	$LTML_{200-550} = 20.8 \times SOC + 0.36 \times \text{clay} - 2.1$
12	$LTML_{200-550} = 24.7 \times TML_{330} + 9.8 \times TML_{530} - 3.41 (TN)^*$	$LTML_{200-550} = 223 \times TN + 0.36 \times \text{clay} - 8.87$

13	$LTML_{200-550} = 16.0 \times TML_{330} + 14.6 \times TML_{410} + 6.32 (TN)^*$	$LTML_{200-550} = 223.5 \times TN + 6.1$
14	$LTML_{200-550} = 14.6 \times TML_{330} + 16.0 \times TML_{410} + 6.32 (SOC)^*$	$LTML_{200-550} = 12.3 \times SOC + 88.3 \times TN + 10.3$
15	$LTML_{30-550} = 12.4 \times TML_{120} + 24 \times TML_{330} - 1.15 (TN)^*$	$LTML_{30-550} = 215 \times TN + 3.1 \times \text{clay} - 36.5$
16	$LTML_{30-550} = 12.7 \times TML_{120} + 25.1 \times TML_{350} + 1.35$	$LTML_{30-550} = 17.1 \times SOC + 3.2 \times \text{clay} - 31$

\*  $TML_{330}$  can be used for both SOC and TN determination. The information in parenthesis refers to SOC or TN as it was used. Equations 9 and 10 are adopted from Kucerik et al. (2016)

*Table 6: Summary of relationships between calculated and measured values of LTMLs used for corrections of models from Table 5 and the coefficient of determination ( $R^2$ ) from verification*

Eq.	LTML	Correlation between calculated (Y) and measured (X) values using equations from tab. 5	Corrected models from tab. 5	Coefficient of determination ( $R^2$ )
1	LTML <sub>200-300</sub>	$Y = 3.5 + 0.73 \times X$	$Y = 6.4 \times \text{SOC} + 2.5$	0.90
2	LTML <sub>200-300</sub>	$Y = 4.3 + 0.73 \times X$	$Y = 80 \times \text{TN} + 1.4$	0.69
3	LTML <sub>300-550</sub>	$Y = 0.85 + 1.03 \times X$	$Y = 8.5 \times \text{SOC} + 0.31 \times \text{clay} + 1.4$	0.98
4	LTML <sub>300-550</sub>	$Y = -4.7 + 1.2 \times X$	$Y = 140 \times \text{TN} + 0.29 \times \text{clay} + 1.7$	0.95
5	LTML <sub>300-450</sub>	$Y = 1.1 + 1.01 \times X$	$Y = 9.1 \times \text{SOC} + 3.8$	0.97
6	LTML <sub>300-450</sub>	$Y = 3.8 + 1.14 \times X$	$Y = 128 \times \text{TN} + 2.4$	0.94
7	LTML <sub>450-550</sub>	$Y = -1.3 + 1.09 \times X$	$Y = 16.8 \times \text{TN} + 0.31 \times \text{clay} + 0.4$	0.89
8	LTML <sub>450-550</sub>	$Y = -0.6 + 1.07 \times X$	$Y = 1.4 \times \text{SOC} + 0.31 \text{ clay}$	0.90
9	LTML <sub>110-550</sub>	$Y = -6.8 + 0.72 \times X$	$Y = 15.1 \times \text{SOC} + 1.8 \times \text{clay} - 10$	0.97
10	LTML <sub>110-550</sub>	$Y = 9.7 + 0.81 \times X$	$Y = 162 \times \text{TN} + 1.8 \times \text{clay} - 2.1$	0.89
11	LTML <sub>200-550</sub>	$Y = 7.1 + 0.76 \times X$	$Y = 15.8 \times \text{SOC} + 0.3 \times \text{clay} + 5.8$	0.99
12	LTML <sub>200-550</sub>	$Y = 13.4 + 0.93 \times X$	$Y = 207 \times \text{TN} + 0.34 \times \text{clay} + 5.1$	0.89
13	LTML <sub>200-550</sub>	$Y = 4.2 + 1.02 \times X$	$Y = 228 \times \text{TN} + 10.4$	0.92
14	LTML <sub>200-550</sub>	$Y = 0.9 + 0.91 \times X$	$Y = 11.2 \times \text{SOC} + 80.4 \times \text{TN} + 10.3$	0.97
15	LTML <sub>30-550</sub>	$Y = 30 + 0.73 \times X$	$Y = 157 \times \text{TN} + 2.3 \times \text{clay} + 3.3$	0.88
16	LTML <sub>30-550</sub>	$Y = 20 + 0.77 \times X$	$Y = 13.2 \times \text{SOC} + 2.5 \times \text{clay} - 3.9$	0.93

## Captions to Figures

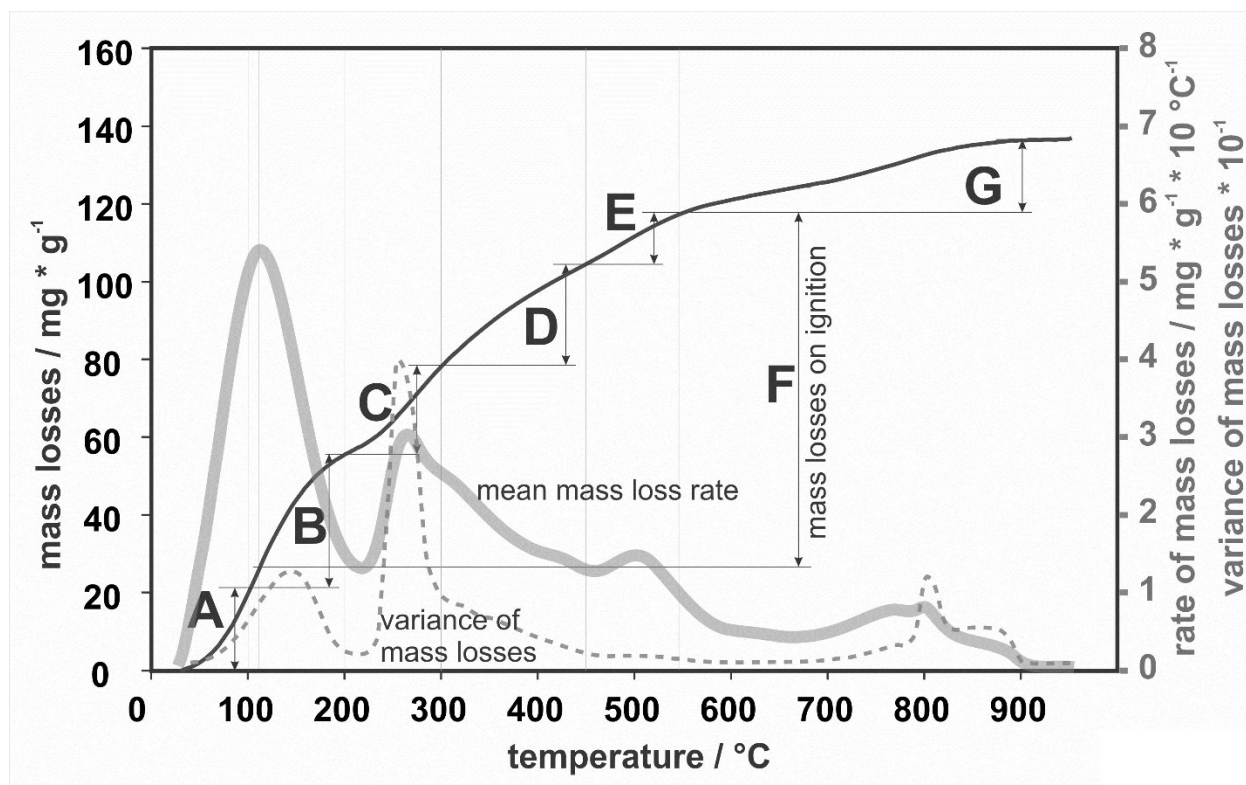


Figure 1. An averaged TG and derivative TG (DTG) profile of soils investigated in this work and alternative way of fractionation (A-G)

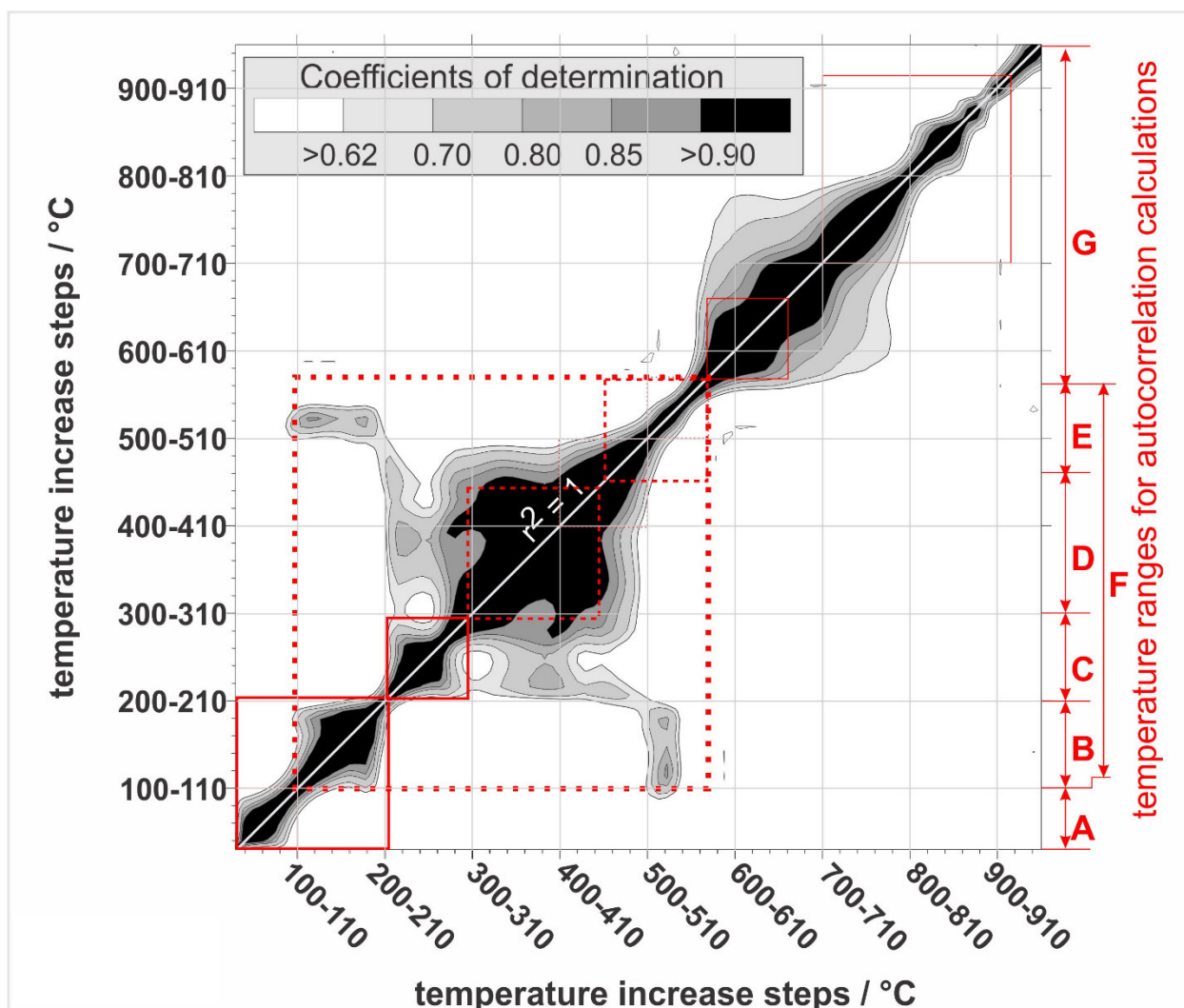


Figure 2 Raster plot summarizing the coefficients of determination for autocorrelations between thermal mass losses in 10 °C temperature increase steps (TML). The dark areas with significant  $R^2$  define the temperature limits for temperature areas reported in Table 2. The areas A (30-100°C) and B (100-200°C) represent loosely and strongly bound water, respectively, C (200-300°C), D (300-450°C), E (450-550°C) are temperature areas representing fractions of soil organic matter (and possibly pyrogenic carbon) of increasing thermal stability and G (above 550°C) represents soil inorganic matter. F is the combination of the C, D and E temperature areas.

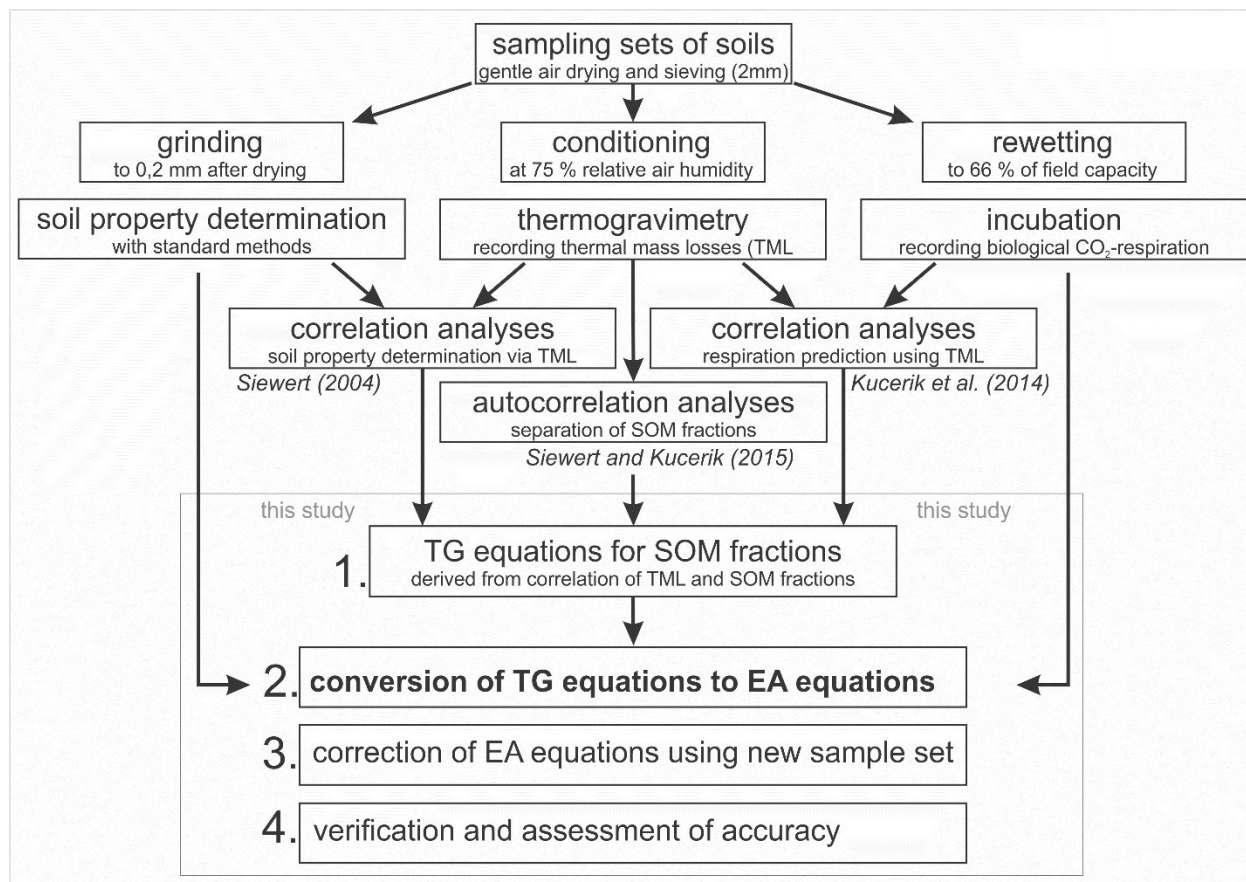


Figure 3: Overview of the applied methodical approach and the content of this study. The term “TG equations” stands for the correlations based on TML and MLTA, their recalculation using soil properties gives term “EA equation”.

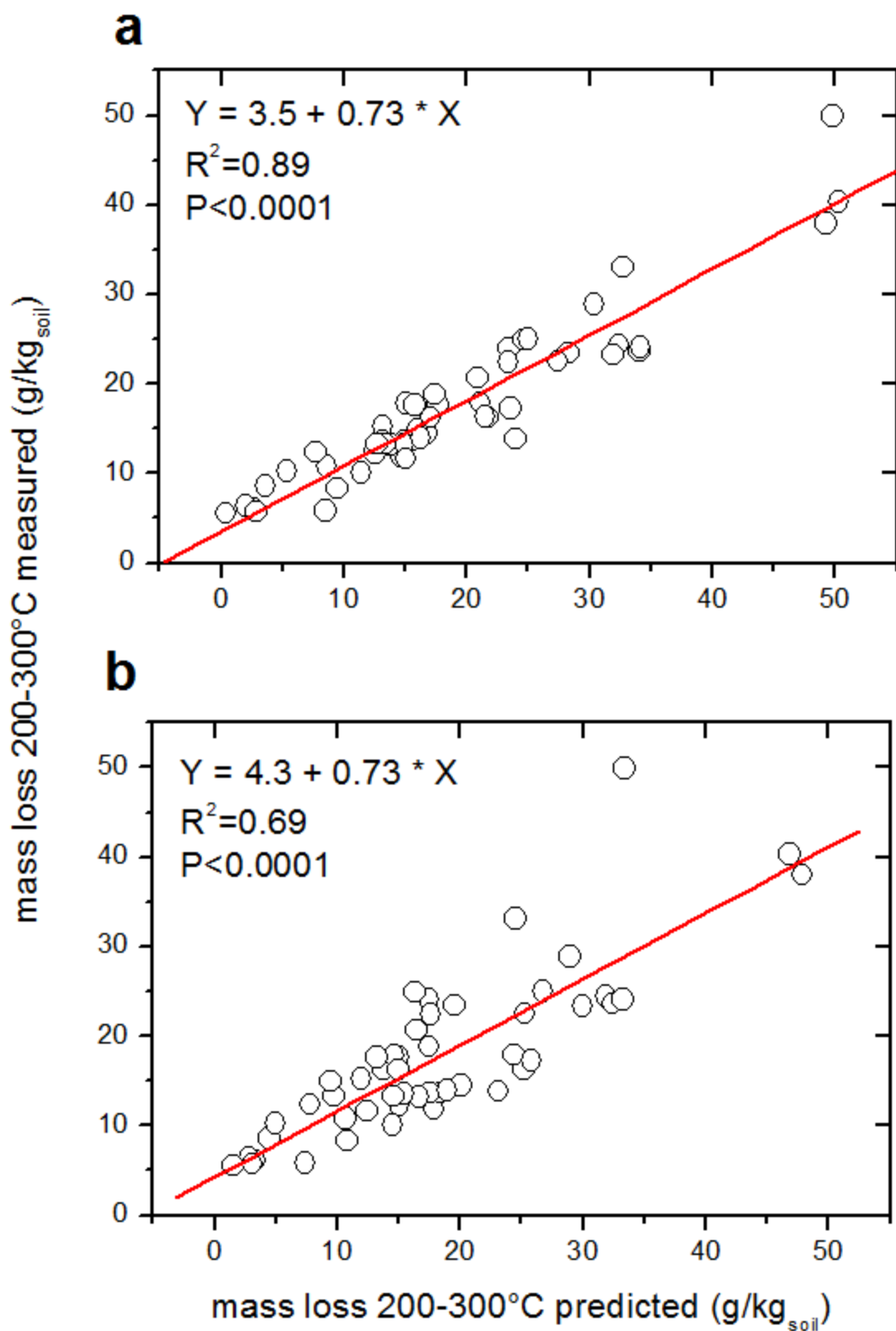


Figure 4. Comparison of measured mass loss between 200-300°C and calculated (predicted) values obtained using Equation 1 (a) and Equation 2 (b) from Table 5. The regression parameters were further used for correction of the EA equations.

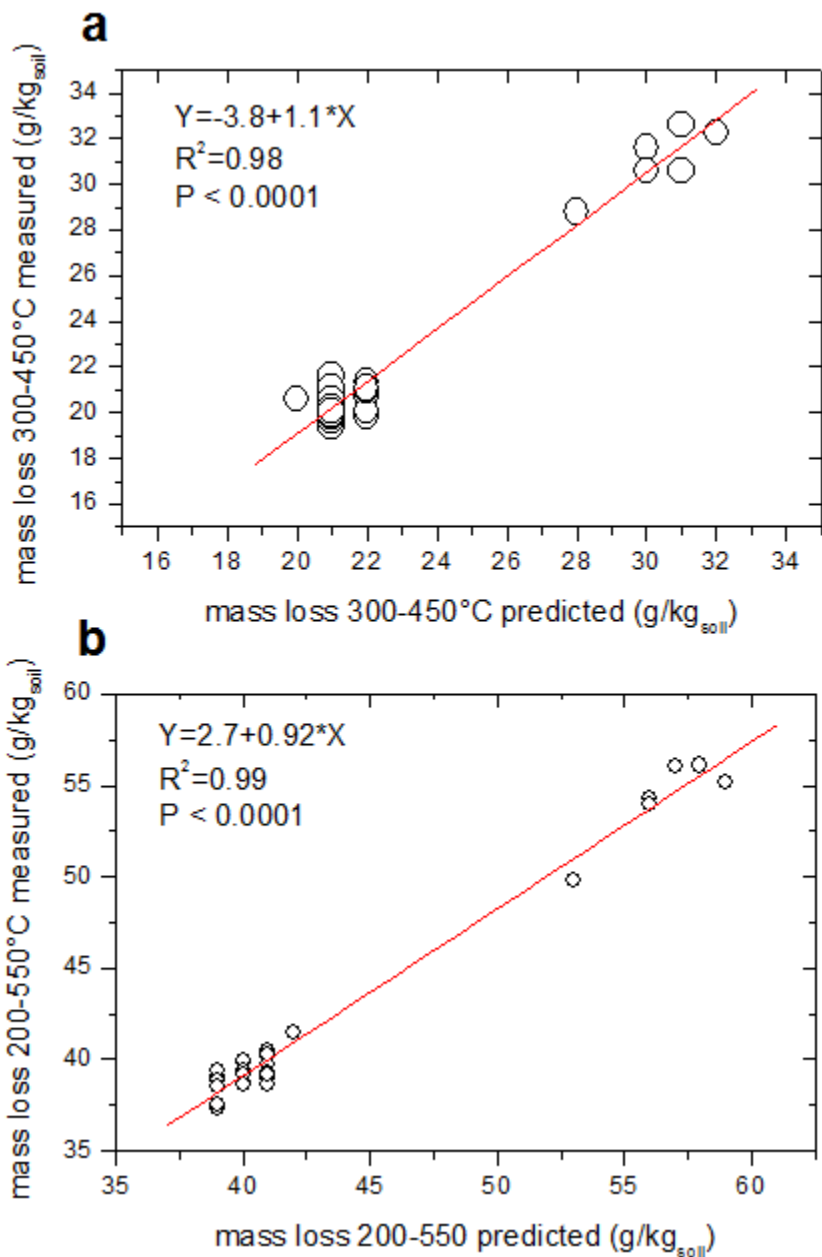


Figure 5. Comparison of predicted and measured mass losses between 300-450°C using equation 6 (a) and 200-550°C using equation 11 (b) from Table 6.

