

Estimating soil organic carbon through loss on ignition: effects of ignition conditions and structural water loss

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Summary

Loss on ignition (LOI) is one of the most widely used methods for measuring organic matter content in soils but does not have a universal standard protocol. A large number of factors may influence its accuracy, such as furnace type, sample mass, duration and temperature of ignition and clay content of samples. We conducted a series of experiments to quantify these effects, which enabled us to derive (i) guidelines for ignition conditions (sample mass, duration and temperature), (ii) temperature-specific soil organic matter (SOM) to soil organic carbon (SOC) conversion factors and (iii) clay content-dependent correction factors for structural water loss (SWL). Bulk samples of a sandy soil (4% clay) and a silt loam soil (25% clay) were used to evaluate the effects of ignition conditions. Samples with a range of clay contents (0–50%) were used to quantify conversion and correction factors. Two furnaces, one without and one with pre-heated air, did not show significant differences in terms of within-batch LOI variability. In both furnaces less combustion occurred close to the door, which necessitated tray turning at half-time as this reduced the standard deviation per batch significantly. Variation in mass loss declined exponentially with sample mass (range, 0.15–20 g). The LOI increased with duration at lower temperatures ($\leq 550^{\circ}\text{C}$) for the sandy soil. At greater temperatures (600 and 650°C), no effect of duration was found. For the silt loam soil, LOI values increased with duration for each temperature, which was attributed to SWL. The SOM to SOC conversion factor decreased strongly with temperature at an ignition duration of 3 hours from 0.70 (350°C) to 0.57 (500°C) and stabilized around 0.55 between 550 and 650°C , indicating that at temperatures $\geq 550^{\circ}\text{C}$ all SOM had been removed. The clay correction factor for SWL increased from 0.01 to 0.09 as the temperature of ignition increased from 350 to 650°C . To minimize within-batch LOI variation we recommend a standard ignition duration of 3 hours, tray turning at half-time, a sample mass ≥ 20 g and temperatures equal to or greater than 550°C . To avoid over-estimates of SOM through structural water loss, the presented SWL correction procedure should always be applied.

Introduction

Precise quantification of soil organic matter (SOM) stocks is necessary to study organic matter dynamics over a period of time. Worldwide, the two most commonly used methods for quantifying SOM are loss-on-ignition (LOI) and wet-oxidation (WO). These methods remove organic matter either by heat application alone (LOI) or by heating combined with chemical destruction (WO) using a reagent such as hydrogen peroxide, sodium hypochlorite or disodium peroxodisulphate. After removal, the gravimetric mass loss is measured to estimate the organic matter content of the soil. A major limitation of chemical destruction during WO is the risk

of incomplete oxidation, which implies that a correction factor is needed. This correction factor varies from soil to soil and depends largely on the reaction conditions, sample size and type of reagent used (see Mikutta *et al.*, 2005). Despite the fact that a large number of oxidation protocols exist, none reaches a removal efficiency that is constant across soil types or a removal efficiency of 100% (Mikutta *et al.*, 2005). Hence, a soil-specific multiplication factor is needed for each sample to correct for incomplete oxidation.

The type of reagent used does not influence the accuracy of the LOI method, as only physical destruction takes place. Therefore, this method might be more precise for estimating SOM. However, the LOI method has no standard protocol and involves three potentially confounding factors: incomplete combustion of SOM at low temperatures (Ball, 1964), the removal of structural water

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from clay minerals (Sun *et al.*, 2009) and the decomposition of soil carbonates at large temperatures (Kasozzi *et al.*, 2009). Estimates of SOM are inaccurate when soil samples containing clay are combusted and no correction factors are applied. Inaccuracies arise because of the removal of H₂O-groups from sesquioxides and clay elements, and dissipation of H₂O from decomposable carbonates and hydrated salts (Ball, 1964; Schulte & Hopkins, 1996; De Vos *et al.*, 2005). Dehydration of clay minerals involves the dissipation of unbound water, interlayer water and finally the loss of hydroxyl (or lattice OH) water held by clay minerals (Grim, 1953). The latter process is also known as dehydroxylation.

As the observed mass loss from a soil sample involves combusted SOM and any loss of water that was present in it, several authors (including De Vos *et al.*, 2005) have proposed clay correction factors to account for water losses. Houba *et al.* (1997) estimated a clay correction factor for LOI empirically for a large number of Dutch soils to be 7% of the fraction smaller than 2 µm (sieve-pipette method) for water bound to clay minerals at an ignition period of 3 hours at 550°C. As dehydration and dehydroxylation are both temperature and time dependent (Grim, 1953), the clay correction factor proposed by Houba *et al.* (1997) can be only applied under the same ignition conditions.

The third confounding factor that affects the accuracy of SOM estimations is the presence of carbonates (such as CaCO₃, CaMg(CO₃)₂), which decompose at ignition temperatures above 600°C (decarbonation; Kasozzi *et al.*, 2009).

There is little consistency in the literature as to which combination of ignition temperature and duration should be employed to determine SOM contents accurately. A wide variation exists in terms of both ignition temperature (300–850°C: Ball, 1964; Abella & Zimmer, 2007) and ignition duration (2–28 hours: Ranney, 1969; Konen *et al.*, 2002). Ball (1964) observed that an ignition temperature of 375°C for 16 hours gives more accurate SOM estimations than ignition at 850°C for 0.5 hour, because at greater temperatures more structural water loss occurs. There is, however, a large risk of incomplete combustion at 375°C.

Furthermore, there appears to be considerable variation in sample mass (0.2–20 g) and the type of ignition furnace employed (Lowther *et al.*, 1990; Grewal *et al.*, 1991). Wang *et al.* (2011) recommended a sample mass less than 5 g and preferably between 2 and 4 g. We question whether such a small amount of soil will yield an accurate and precise estimate of the SOM content, because it is widely reported that variance decreases with increasing sample mass (Geelhoed & Glass, 2004). Moreover, large within-batch variation can be expected for furnaces that do not use pre-heated air, which are commonly used for SOM analysis. To obtain a more equal heat distribution within the muffle, furnaces with pre-heated air are recommended (Fisher Scientific; M. Evers, personal communication). We analysed to what extent variation between pseudo-replicates can be attributed to furnace type and to a non-homogeneous distribution of SOM in the sample. We also investigated if we could observe a near-door effect: heat losses close to the door will lead to reduced combustion of SOM in the crucibles there. This was expected from previous experiences (Chemical

Biological Soil Laboratory, Wageningen, the Netherlands: A. van Leeuwen, personal communication) and might be overcome by turning the tray with crucibles through 180° halfway through the combustion. As the LOI method is widely used to analyse organic matter in soils, there is a clear need to refine the procedures and to develop a worldwide standard. The objectives of this study were to (i) give an overview of analytical procedures that are used worldwide, including furnace type, sample mass, and ignition temperature and duration, (ii) compare mass loss patterns of both a sandy and a silt loam soil for two different furnace types that are commonly used for LOI analysis, (iii) determine the effects of sample mass on variation in mass loss measurements for both soils, (iv) investigate if the door effect is reduced by tray turning at half time, (v) determine the effects of ignition duration and temperature on mean mass loss and (vi) quantify structural water loss from clay elements and conversion factors to estimate SOC from SOM for soils with a wide range of clay contents ignited at different temperatures.

Materials and methods

Literature review

A literature review was conducted by using the Scopus abstract database. In addition, the Wageningen University Library (www.library.wur.nl) and Google Scholar were consulted. We made an inventory of reported types of furnaces, sample masses and ignition conditions (temperature and duration) used for LOI determination. Publications that referred to other sources for specifications regarding using the LOI method were excluded.

Soil samples

Soil sample set 1. We collected bulk samples from a podzol (sand) profile and a fluvisol (silt loam) profile. The sites were located in Wageningen (Bornse weilanden, sand; de Hoeveslagen, silt loam; Buringh, 1951). These fields were chosen because there was a large contrast in soil texture. The clay contents (volume fraction ≤ 2 µm) were 4% (sandy soil) and 25% (silt loam soil) as derived from measured optical laser diffractometry values (LD; see below).

Soil sample set 2. Bulk soil samples were collected in arable and grassland fields in different parts of the Netherlands with known particle size distribution to obtain a wide range of clay contents. The carbonate content was not known in advance. In total, 24 fields were sampled with a clay content (mass fraction ≤ 2 µm) ranging from 1 to 50%. The clay content was measured with the sieve-pipette method (SP). Because the LD method and SP method measure different soil particle diameters resulting in different textural fractions, LD values had to be converted to SP values (Vandecasteele & De Vos, 2001; see File S1).

Clay types varied among samples; 12 samples contained marine clay and 12 samples contained fluvial clay. In Dutch marine clay soils, the clay minerals present are mainly illite and smectite (about 30–50%). Kaolinite and chlorite represent about 10–15% of the clay fraction. In fluvial clay, vermiculite also occurs and the smectite

Table 1 Overview of samples and ignition conditions per experiment. The Dutch standard procedure (NEN-5754, 1992) is also presented

Experiment	Soil samples	Furnace type ^a	Tray turning	Sample mass(es) / g	Temperature(s) / °C	Duration / hours	Total batches	No. of pseudo-replicates per batch	Statistical design	Statistical analysis
1: LOI	S1	CWF, AAF	No	5	550	3	4 CWF	32	2 × 2, 2 replicates	ANOVA
2: LOI	S1	AAF ^b	No	0.15; 0.30; 1; 2; 5; 10; 20	550	3	4 AAF	21	Latin square	ANOVA
3: LOI	S1, S2 (3 s)	AAF	Yes	20	550	3	16	21	4 × 2, 2 replicates	ANOVA
4: LOI	S1	AAF	Yes	20	350; 400; 450; 500; 550; 600; 650	3; 6; 9; 12; 24	35	7	Block design	Linear regression
5a: LOI	S2 (17 s)	AAF	Yes	20	350; 400; 450; 500; 550; 600; 650	3	21	3 ^c	Block design	Non-linear regression
5b: WCO (Equations (1) and (2))	S2 (17 s)	NA	NA	NA	NA	NA	3	NA	–	Non-linear regression
5c: Reproducibility WCO	S1, S2 (3 s)	NA	NA	NA	NA	NA	NA	6	–	Paired t-test
SD SOC _{WCO} vs. SD SOC _{LOI}	S2 (17 s)	AAF						3		
NEN-5754	NA	Not specified	No	5–10 g	550	3	NA	NA	NA	NA

^aCWF = carbolite wire furnace, without pre-heated air; AAF = analytical ashing furnace, with pre-heated air.

^bBased on the results of the comparison of both furnaces, the AAF furnace was used for the remaining part of the study.

^cEvery batch contained three pseudo-replicates of quartz sand, which were placed in the middle row of the furnace (row 4; total no. of rows is 7).

NA = not applicable.

fraction in younger deposits (post-Roman) is much smaller than in older ones (Breeuwsma, 1987). Furthermore, the free iron oxide content of the smectite-illite minerals in the clay fraction < 0.04 µm of marine clay soils is 1–3% greater than the clay fraction in fluvial clay soils. Quartz sand was used in this study as a control without clay.

Sampling procedure

The bulk samples of soil sample set 1 were obtained from a grassland field experiment on a sandy and a silt loam soil. The 0–30 and 30–60-cm soil layers were sampled with an auger using stratified sampling. Each field was divided into 48 plots and within each plot four random samples were taken from each of the two layers.

Soil sample set 2 was obtained from the 0–30-cm soil layer of 20 farmer's fields. A total of 40 soil samples were taken from each field in a zigzag pattern. A composite sample per field was obtained by mixing the 40 samples thoroughly in a bucket. This was stored in a plastic bag and brought to the laboratory. Soil samples were oven-dried for a period of 24 hours at a temperature of 105°C. As previous studies have not shown a systematic effect of grinding on reproducibility of analytical results (Houba *et al.*, 1993), the soil samples in this study were only crushed and sieved (< 1.8 mm). However, Schumacher *et al.* (1990) reported that random sampling after sieving and grinding is the most time efficient method to obtain a homogeneous soil sample for LOI measurements. After

sieving, the soil fines (< 1.8 mm) were stored in plastic bags at room temperature. Subsamples were taken by spooning (after Carver, 1981), which we defined as the random insertion of a spoon into the sample and lifting the spoon vertically.

Analytical methods, data processing and analysis

Four experiments (1–4) were conducted to derive guidelines for furnace type and ignition conditions (sample mass, duration and temperature). Experiment 5 was set up to derive temperature-specific SOM to SOC conversion factors and clay-content-dependent correction factors for structural water loss (SWL). The experiments and corresponding objectives are presented below. The analytical methods that were used are described in the text under each experiment and summarized in Table 1. The Dutch standard procedure (NEN-5754, 1992) is also listed in the table and was taken as a starting point.

Experiment 1: Effect of furnace type on variation in LOI values (Objective 2). The soils from sample set 1 were used and two Carbolite furnace types (Carbolite, Hope Valley, UK) were compared: the carbolite wire furnace (CWF; no pre-heated air) and the analytical ashing furnace (AAF; pre-heated air). A completely randomized design was chosen with oven type and soil type as factors. This resulted in four treatments: CWF-sand, AAF-sand, CWF-silt loam and AAF-silt loam. The experiment was executed in duplicate (eight batches in total). Each batch consisted of 32

crucibles (CWF: eight rows \times four columns) or 21 crucibles (AAF: seven rows \times three columns). To test for differences in spread of the LOI values between oven types, we calculated standard deviations as follows: (i) within row (60 values); (ii) within column (28 values); and (iii) within batch (eight values). Analyses of variance were done with soil and oven as fixed factors with interactions and batch as a random factor for cases (i) and (ii). Assumptions of normality were assessed. We also analysed the 212 observations as coming from a split-plot design with oven, soil and their interaction as fixed factors (for the main plot = batch), batch as a random factor, and row and column within oven as fixed factors (for the subplot = crucible). To get appropriate tests for row and column effects within oven, row \times batch and column \times batch were included as well. The ignition conditions were: 550°C, 3 hours and a dry soil sample mass of 5 g.

Experiment 2: Effect of sample mass on reproducibility of LOI values (Objective 3). Soil samples from sample set 1 were used. Seven different masses were chosen to assess the effect of sample mass on reproducibility of LOI values. A Latin square design was used (seven batches with seven different masses per batch) such that each sample mass occurred in triplicate in each row (see File S1, Figure S2 for the layout). The chosen masses increased more or less exponentially and ranged from 0.15 to 20 g (see Table 1 for details). The experiment was conducted with the AAF furnace. Analysis of variance was conducted (LSD or Dunnett's T3 in the case of unequal variances, $P < 0.05$). In line with the Latin square design, batch and row number were included as block factors. The standard deviation of the LOI values of each row was taken as a response variable. Homogeneity of variances was assessed.

Experiment 3: Effect of tray turning on the door effect (Objective 4). In line with the results of experiment 1 (significantly reduced LOI values close to the furnace door for the silt loam sample of sample set 1) we tested whether tray turning halfway through reduced the LOI standard deviation in a batch. If turned, the tray was taken out after 1.5 hours, turned 180° and put back in the furnace. We used four bulk soil samples: the silt loam sample of set 1 and three samples of set 2. Samples of set 2 were selected on the basis of the clay content, because the door effect was only observed for the loamy soil sample of set 1 in Experiment 1. The clay percentages of the selected soil samples of set 2 were 20, 31 and 40%. Sample masses per crucible were 20 g. Every bulk sample was analysed four times (four batches each containing 21 crucibles). Two out of four batches were turned. This resulted in a total of 16 batches (see Table 1 for an overview). The ignition conditions were 550°C and 3 hours and the AAF furnace was used. Analysis of variance was conducted with turning and clay percentage as factors and LOI standard deviation per batch as dependent variable. For the turning/non-turning contrast, we used a one-sided test as we expected the LOI standard deviation to decline with turning.

Experiment 4: Effect of temperature and duration (Objective 5). To assess the effect of temperature and duration on ignition

losses, soil samples from sample set 1 were used. The aim was to minimize duration by determining at which temperature LOI values became stable over time. Seven different temperatures that were often reported for LOI analysis were chosen (see File S1, Table S2). The temperatures ranged from 350 to 650°C. Temperatures greater than 650°C could not be used because the crucibles melted. Five ignition times were chosen and varied from 3 to 24 hours. This resulted in 35 different temperature-time combinations. Each batch consisted of 14 crucibles (seven pseudo-replicates per soil type), which were placed in two columns (one column sand, one column loam). Regression analysis was used to test the effect of duration for each temperature. For all batches, the tray was turned halfway through and the sample mass was 20 g. The AAF furnace was used.

Experiment 5: Effect of temperature on water loss from clay (Objective 6). Before conducting Experiment 5, all bulk samples (set 1 and set 2) were tested for carbonate content with the methods described in File S1. Samples from carbonatic soils (eight in total: the silt loam from set 1 and seven samples from set 2) were excluded from the experiment. The remaining 17 samples consisted of eight samples containing fluvial clay and nine samples containing marine clay. The range in clay contents did not change and still extended from 0 to 50%. Four replicates of quartz sand were used as control without clay. The ignition duration was 3 hours and temperatures ranged from 350 to 650°C. Six samples were placed in one batch (three pseudo-replicates per sample). The middle row (row 4) was filled with three pseudo-replicates of quartz sand (see File S1, Figure S2 for the positioning of the samples in the furnace). The average LOI value was determined for each temperature. The following model was fitted to quantify structural water loss from clay elements:

$$\text{SOC} = a_T \times (\text{LOI}_T - b_T \times C), \quad (1)$$

where SOC (%) is the soil organic carbon content obtained by wet chemical oxidation (WCO, see below), LOI is mass loss of SOM (%), C is the soil clay content (%), a_T is the carbon content of SOM (kg kg^{-1}), b_T is the clay correction factor for structural water loss (kg kg^{-1}) and the suffix T refers to the ignition temperature. The parameters a_T and b_T were fitted with nonlinear regression using SPSS (19th edition). The minimum value of a_T and the maximum value of b_T were estimated as a function of ignition temperature. For a_T , an exponential equation was fitted:

$$a_T = \alpha + \beta \gamma^T. \quad (2)$$

For b_T we used a logistic equation following general dehydration curves of clay minerals as presented by Grim (1953):

$$b_T = \delta + \frac{\epsilon}{1 + e^{-\kappa(T-\lambda)}}. \quad (3)$$

Genstat (15th edition SP1) was used to fit Equations (2) and (3) to the data. Soil OC values were determined after WCO by sulphochromic acid as described by Houba *et al.* (1997). This

Table 2 Effect of furnace type (CWF, without pre-heated air, and AAF, with pre-heated air) on average loss-on-ignition (LOI) values per row for a sandy and loamy bulk soil sample

Row no.	Sand		Loam		Row no.	Column no.		
	CWF	AAF	CWF	AAF		1	2	3
	SE = 0.019	SE = 0.023	SE = 0.027	SE = 0.034		19	20	21
1	2.96	2.95	4.15	4.00	7	16	17	18
2	2.98	2.97	4.22	4.06	6	13	14	15
3	2.99	2.99	4.31	4.11	5	10	11	12
4	3.02	2.91	4.29	4.11	4	7	8	9
5	2.98	2.93	4.37	4.12	3	4	5	6
6	3.01	2.97	4.36	4.15	2	1	2	3
7	2.95	2.93	4.29	4.06	1			
8	2.97	NA	4.26	NA				

Ignition conditions were 550°C and 3 hours. A sample mass of 5 g was used. A row runs from the left to the right side of the furnace (see the image next to the table for crucible positions within the AAF furnace). Standard errors (SE) for the means per row are based on the pooled within-row standard deviations ($n = 8$ for CWF, $n = 7$ for AAF). NA = not applicable

method results in complete oxidation and therefore no correction factor is needed (Walinga *et al.*, 1992). We used glucose ($C_6H_{12}O_6$) as a standard substance and conducted the spectrophotometric measurements at a wavelength of 585 nm. Seven stock solutions with a C-content that increased from 0 to 20 mg per 5 ml were used to derive a calibration curve. We adjusted the calculation procedure because the prescribed equation includes a double correction factor for the blank. We also suspected chemical deviations from Beer's law for the association of chromate in acid solutions (File S1, Box S3). All samples from sample set 2 were analysed in triplicate. These replicates were fitted separately with Equation (1) and the average a_T and b_T values together with their standard deviations were calculated. Furthermore, we compared standard deviations of measurements obtained by the WCO procedure and the LOI method. The same LOI dataset was used as for determining the clay correction factor (17 non-carbonatic soil samples ignited at 550°C for 3 hours). The standard deviation (SD) of each sample was calculated from three pseudo-replicates. The data were analysed using a paired t -test.

Results

Literature review (Objective 1)

The literature search resulted in 40 publications from the period 1960–2011 in which the LOI method was used for assessing organic matter in sediments, compost and soils (File S1, Table S2). Many studies were characterized by a lack of information about details of the applied LOI method, and the reported analytical conditions were very diverse. In 65% of the studies, the type of furnace was not reported. In the remaining 35% of the studies, eight different makes of furnaces were reported. Sample mass was reported in only 62% of the studies and varied between 0.2 and 20 g (Christensen & Malmros, 1982; Wright *et al.*, 2008) with an average of 5.5 g. Heating time varied from 30 minutes to 32 hours and ignition temperature ranged from 350 to 1000°C. No significant

correlations were found between year of publication and sample mass, ignition duration and temperature.

Effect of furnace type on LOI (Objective 2, Experiment 1)

The average mass loss of the sandy sample in the CWF furnace was 2.98% (SD = 0.06) for the two batches (Table 2). We found nearly the same values for the AAF furnace: 2.95% (SD = 0.06). For the silt loam soil we found large absolute differences between furnaces: the average LOI value was 4.29% for the CWF (SD = 0.09) and 4.09% (SD = 0.09) for the AAF (see File S1, Table S3 for the output of the analysis of variance). Large LOI ranges within batch were observed for both furnaces. The maximum difference was 0.22% for the sandy soil and 0.42% for the silt loam soil.

No consistent gradient in LOI values with distance from the furnace door was found for the sandy soil, whereas for the silt loam soil sample ignition losses were smallest close to the door in both furnaces (Table 2). This door effect seemed less pronounced for the AAF than for the CWF furnace: differences in average LOI values between the middle row and the front row were smaller for the AAF as compared with the CWF furnace.

The effect of furnace type on variation was assessed for both soil types in three analyses (standard deviation per row, column and batch). All three tests showed no significant differences in spread of LOI values between furnaces, so pre-heated air did not improve analytical precision (see File S1, Table S4 for the output of the analysis of variance). Because the door effect was less pronounced for the AAF furnace, we decided to conduct the subsequent experiments only with the AAF model.

Effect of sample weight on LOI (Objective 3, Experiment 2)

The effects of the factors batch number and row number were not significant for the sandy or the silt loam bulk soil. The variation in standard deviations was not equal across masses and decreased with increasing sample mass. The natural logarithm was taken to

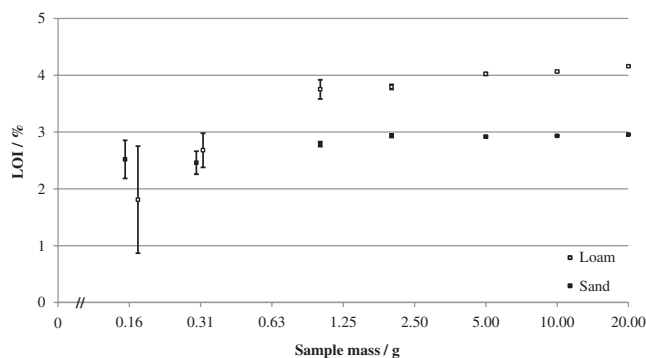


Figure 1 Effect of sample mass on average loss-on-ignition (LOI) values for a sandy and loamy bulk soil sample. Ignition conditions were 550°C for 3 hours. The AAF furnace was used. All sample weights were analysed in septuplicate. Standard errors of the mean (SEM) are presented. Sample masses are presented on a log scale. To avoid visual overlap in SEM values, for the loamy soil sample 0.02 g was added to the sample masses of 0.15 and 0.30 g.

stabilize variation of the response variable (sample mass). The natural logarithm was also taken of each mass and plotted against the natural logarithm of standard deviation (File S1, Figure S3). For the sandy soil, a significantly smaller standard deviation ($P = 0.047$) was observed for sample masses of 20 g than for masses of 5 g (standard protocol; NEN-5754). For the silt loam soil, we also found that standard deviations decreased with increasing sample mass. Standard deviations of sample masses of 20 g were slightly smaller than those for masses of 5 g, but this was not significant ($P = 0.116$). Furthermore, we observed for both soil samples that mean LOI values increased with sample mass (Figure 1).

Effect of tray turning on LOI (Objective 4, Experiment 3)

In Experiment 3, we established the effect of turning the tray on the possible decrease in standard deviation per batch. For the sandy soil of sample set 1, no significant effect of tray turning was found. Standard deviations per batch (SD_b) were 0.04 without turning and 0.03 with turning. For the silt loam soil (set 1), tray turning had a significant effect on the SD_b : a decline from 0.10 (without turning) to 0.06 (with turning) was observed. The effect of tray turning for three bulk samples of set 2 is presented in Figure 2. Analysis of variance showed that the effects of clay percentage ($P = 0.366$) and the interaction of clay percentage and turning ($P = 0.890$) were not significant. Because the explained variation of the model (R^2 adjusted = 0.04) was very small, an ANOVA was conducted with only turning as treatment factor and clay percentage as block factor. This improved the explained variation (R^2 adjusted = 0.16). The effect of turning was significant (one-sided $P = 0.03$).

Combined effect of ignition temperature and duration on LOI (Objective 5, Experiment 4)

The results of the sandy and silt loam bulk sample are treated separately below.

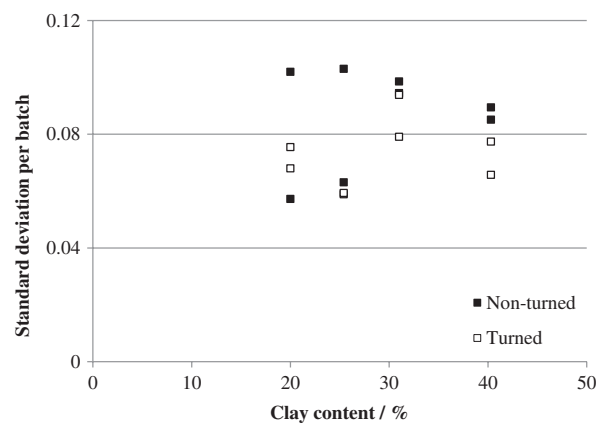


Figure 2 The effect of turning the tray after 1.5 hours on standard deviations per batch for different soil types. Four soils were used with clay contents that ranged from 20 to 40%. Ignition conditions were 550°C for 3 hours. The sample mass was 20 g and the AAF furnace was used.

Sandy soil (sample set 1). In Experiment 4, mass loss increased significantly with ignition duration (3–24 hours) at temperatures between 350 and 550°C (Figure 3a). At 600 and 650°C, no clear effects of ignition duration on LOI values were observed.

Silt loam soil (sample set 1). Over the whole range of temperatures, average LOI values increased with ignition time (Figure 3b). Mass loss was least at a temperature of 350°C for 3 hours ($LOI = 2.22\%$, $SD = 0.02$) and nearly tripled at 650°C for 24 hours ($LOI = 6.60\%$, $SD = 0.13$). This indicates that structural water loss from clay elements is a process that takes place at greater temperatures (> 550°C) and continued at increasing duration of ignition.

Effect of temperature on structural water-loss from clay minerals (Objective 6, Experiment 5)

Values of coefficients a_T and b_T at increasing ignition temperatures are presented in Figure 4 (Experiment 5). The value of coefficient a_T , the fraction of soil organic matter that is carbon, declined with increasing temperature up to 500°C, and stabilized at greater temperatures. The minimum value of a_T was 0.55 kg kg⁻¹. The value of b_T increased in a sigmoidal fashion with a maximum value for b_T of 0.09 kg kg⁻¹.

Reproducibility of measured soil organic carbon by using wet chemical oxidation

Standard deviations of SOC as determined by both LOI and WCO are presented in Figure 5. To compare SDs of both methods, LOI values were converted to SOC by multiplication with a factor 0.55 (horizontal asymptote in Figure 4a). The SD_{WCO} value was significantly larger than that of SD_{LOI} ($P = 0.0002$).

Discussion

The observed effects of furnace type, sample mass, tray turning, ignition temperature and duration on LOI measurements (mean

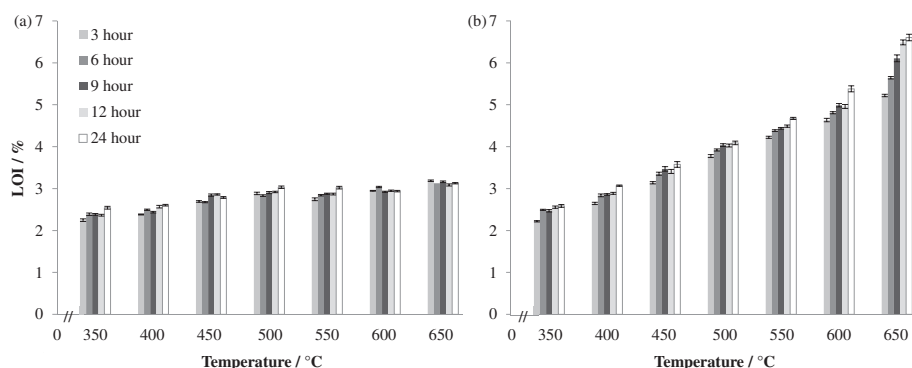


Figure 3 Effect of ignition temperature and duration on loss-on-ignition values of a sandy (4% clay; a) and silt loam (25% clay; b) soil sample. The sample mass was 20 g and the AAF furnace was used.

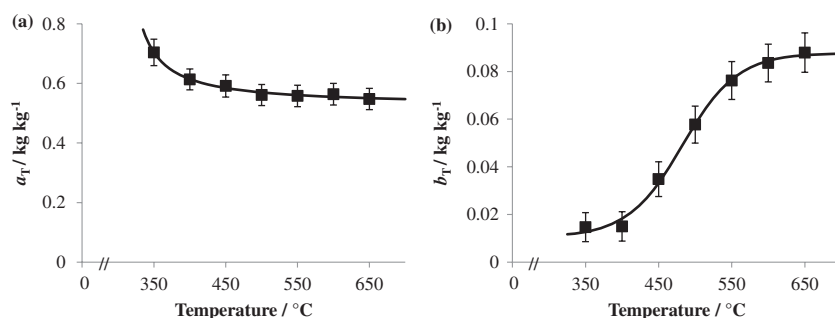


Figure 4 Relationship between furnace temperature and SOM to SOC conversion factor a_T (a) and clay correction factor b_T (b). Values of curvature parameters are presented in File S1, Table S5. The duration of ignition was 3 hours. The sample mass was 20 g (SOM; loss-on-ignition). The AAF furnace was used and the tray was turned halfway through the combustion.

and standard deviation) call for standardization of procedures and materials to arrive at accurate and comparable estimates of organic carbon content of soils. We found that furnace type did not affect variation in LOI values and that within-batch variation can be reduced by turning the tray halfway through the ignition, in all probability counteracting heat losses close to the door (Table 2 and Figure 2). Furthermore, we showed that the precision of LOI measurements increased with sample masses up to 20 g (Figure 1 and File S1, Figure S3). At temperatures greater than 550°C, no effect of heating time on LOI values was observed for the sandy bulk soil sample, while for the silt loam soil ignition losses continued to increase with time. We showed that the conversion factor from SOM (estimated through LOI) to SOC decreased at an ignition duration of 3 hours from 0.70 at 350°C to 0.55 kg kg⁻¹ at 650°C (Figure 4a). Finally, we showed that the multiple linear regression approach was successful in deriving temperature-dependent clay-correction factors (Figure 4b).

Our review of the literature regarding the LOI method employed by different authors (File S1, Table S2) showed a wide diversity of procedures and/or incomplete methodological information. We observed an LOI gradient in both of the furnaces used for the silt loam soil, with ignition values being least close to the furnace door. This was more pronounced for the CWF furnace than for the AAF furnace (larger differences in average LOI values between the front row and the centre row; Table 2).

Although all samples were well mixed in our experiments, we found that standard deviations declined with increasing sample mass in a logarithmic fashion for sample masses up to 20 g. This is in line with the literature about a large range of materials including ores and living tissues, all demonstrating that the variance

of replicate measurements decreases with increasing sample mass (Ingamells, 1974; Kratochvil, 1981; Geelhoed & Glass, 2004).

The WCO procedure uses sample masses between 300 and 500 mg and therefore we suspected that the WCO procedure suffers from large inaccuracies. One could consider refining the WCO method by increasing the sample mass. It is, however, impossible to increase sample masses to much larger values because of the molarity of the chromate solution and incomplete oxidation. Increasing the molarity of the solution is only possible to about 1.5 times the current concentration (a further increase in molarity results in incomplete dissolution of K₂Cr₂O₇). The SD values of WCO were significantly larger than the SD values of LOI (Figure 5). Therefore, we conclude that the LOI method (using a sample mass of 20 g) is able to generate results with a much larger degree of precision than the commonly used wet chemical oxidation procedure for determining SOC.

Furthermore, we observed that mean LOI values increased with sample mass. This cannot be attributed to the effect of sample position as each mass occurred once in each row (following the Latin square design). Therefore, this is most likely the result of differences in thermal conductivity between soil and air: relatively more SOM particles in small sample masses (<1 g) were not surrounded by soil but by air, as in larger samples (see File S1, Figure S4 for an image). It is known that the thermal conductivity of air is about 10 times smaller than the conductivity of quartz sand. This is probably why smaller LOI values were found for masses in the order of 1 g than for masses of 5 g and larger.

To minimize the effect of heat losses close to the furnace door on within-batch variation of LOI, the effect of tray-turning halfway through was assessed. Because standard deviations per batch were

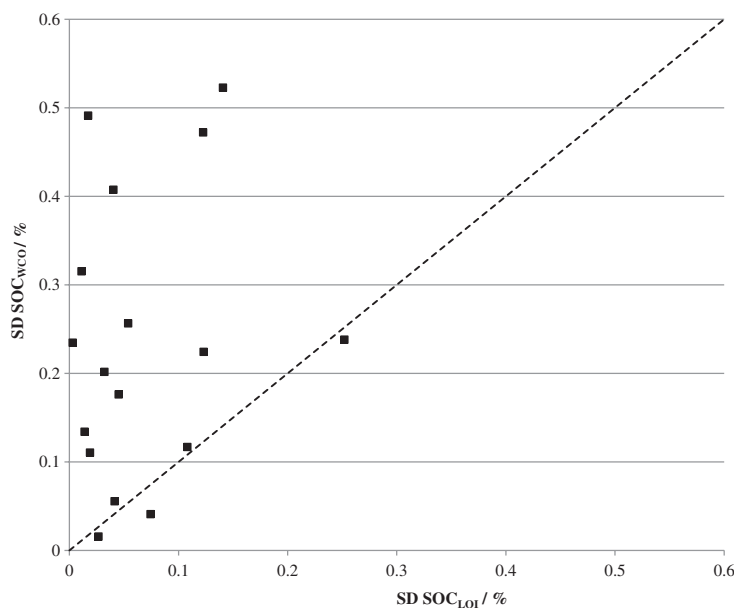


Figure 5 Relationship between standard deviation (SD) of the loss-on-ignition method (LOI) and the wet-chemical oxidation method (WCO). LOI values were converted to SOC with a factor of 0.55. A 1:1 line is plotted. Seventeen different soil samples were used with a clay content that ranged from 1 to 50%. All samples were analysed in triplicate. The LOI was conducted in the AAF furnace at 550°C for 3 hours, a sample mass of 20 g was used and the tray was turned halfway through the ignition period. The WCO method requires that a sample mass of 0.3 g is used.

significantly smaller for turned batches than for non-turned batches we recommend that the tray should always be turned.

For the sandy sample, heating time increased ignition losses at temperatures $\leq 550^\circ\text{C}$ (Figure 3a). At 600 and 650°C, no effect of heating time was observed (thus all SOM was removed). For the silt loam soil, ignition losses increased with duration for all temperatures, which is in accordance with Grim (1953). As structural water loss is a time- and temperature-dependent process, correction factors had to be calculated. We did this for temperatures ranging from 350 to 650°C and a duration of 3 hours (no consistent effect of heating time was observed for the sandy soil at temperatures $\geq 600^\circ\text{C}$). The value of coefficient a_T , the conversion factor of SOM to SOC, declined strongly with increasing temperatures up to 500°C, and stabilized between 550°C ($a_T = 0.56$) and 650°C ($a_T = 0.55$). This shows that at temperatures $\geq 550^\circ\text{C}$ all organic matter is removed, which is in line with Schnitzer *et al.* (1959) and Turner & Schnitzer (1962), who have shown with thermobalances that further mass losses of SOM in mineral soils and pure organic matter samples, respectively, do not occur at temperatures $\geq 550^\circ\text{C}$.

Structural water loss from clay increased in a sigmoidal fashion (Figure 4b). This is in accordance with dehydration curves and differential thermal curves, as demonstrated by Grim (1953) and Pansu & Gautheyrou (2006). At 550°C, we obtained a value of about 0.075, which is slightly larger than the empirical value of 0.07 given by Houba *et al.* (1997). Although factors that are used for correction of structural water loss are not universal, Grim (1953) demonstrated that for nearly all clay types, SWL increases in a sigmoidal fashion with temperature, except for allophane and sepiolite. This indicates that an LOI method that circumvents the need for clay correction as suggested by Ball (1964) is needed. Davies (1974) showed that a temperature between 375 and 450°C does not lead to loss of hydroxyl water. This is, however, not in

accordance with our results (structural water loss at 400°C) and with those of a large number of authors who showed that dehydration occurs at temperatures in excess of 300°C (Nutting, 1943; Pansu & Gautheyrou, 2006). Moreover, smaller ignition temperatures result in incomplete oxidation of SOM. Therefore, we recommend that a clay correction factor for analysing SOM by the LOI method should be used.

Conclusions

The results of our experiments to test the effects of ignition conditions (furnace type, sample mass, temperature and duration) on loss-on-ignition measurements using samples of sandy to clay soils from a temperate climate led us to conclude the following:

- No differences in precision between furnaces were found: the furnace without pre-heated air attained the same degree of precision as the furnace with pre-heated air so both furnace types can be used for the LOI method.
- Turning the tray at half-time reduces the standard deviation and overcomes the effects on LOI heat losses close to the furnace door.
- A sample mass of at least 20 g should be used to minimize variation in LOI measurements.
- To attain complete oxidation an ignition temperature of at least 550°C is needed.
- A clay correction factor should always be used, to avoid over-estimating the SOM content by correcting for structural water loss.

Further research should investigate the introduction of correction factors for CaCO_3 and free iron as those components contribute to an additional mass loss at temperatures $\geq 550^\circ\text{C}$.

Supporting Information

The following supporting information is available in the online version of this article:

File S1. Estimating soil organic carbon through loss on ignition: effects of ignition conditions and structural water loss.

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