

REFeree #1

Response: We sincerely thank the referee for the careful and constructive review of our manuscript. We appreciate the recognition of the study's relevance.

Main comment

Referee: "Main comment"

I agree with the authors on their main issues surrounding LOI which has led to LOI being perceived as a low precision method for estimating SOC. They rightly point out lack of consensus on the optimal temperature and ignition duration for measuring SOM, how to account for any mass loss from clay minerals and the cost and time involved to measure both LOI-SOM and DC-SOC to create customized equations as confounding factors. This is explained and structured well within the manuscript. However, further clarification on the analytical procedures used to generate the TC, IC and SOC data in this study would be of benefit to the readers to convince us that comparison of the three LOI methods selected by the authors, which have different heating durations and temperatures, would enable the aim of the research to be met.

The conclusion that an optimized LOI protocol (LOI at 400 °C for 16 h) offered the best compromise among accuracy, repeatability, and scalability when compared with dry combustion analyses and can provide scientifically defensible soil organic carbon estimates at lower cost is a welcome contribution to the vast literature in this area but only if the analytical measurements that underpin this statistical analysis and hence conclusion are robust."

Response: We agree and thank the referee for identifying the need to make the scope of the comparison more explicit. The experiment aimed to compare three existing LOI protocols as complete analytical workflows, rather than to independently quantify the effects of ignition temperature and ignition duration. The protocols represent published procedures that differ substantially in their temperature-duration combinations: 360 °C for 2 hours (Schulte and Hopkins, 1996), 400 °C for 16 hours (USDA, 2022), and 550 °C for 3 hours (Hoogsteen et al., 2015).

Because both temperature and duration vary among these protocols, the present design is not factorial and cannot statistically separate their independent effects. To meet that mechanistic objective, a factorial experiment would be required that varies temperature at a common duration and duration at a common temperature. Our practical question was instead how results from these established protocols, when implemented as operational methods, can be reliably transferred to SOC measured by dry combustion. We have bounded the recommendation accordingly and no longer describe 400 °C for 16 hours as a universal optimum.

CHANGES IN THE MANUSCRIPT:

Abstract

Among the three established LOI protocols evaluated in this study, LOI400 provided the best overall balance of predictive accuracy, repeatability, and operational scalability.

Section 2.2.2 – Loss on ignition

Three established LOI protocols were evaluated as complete analytical workflows: 360 °C for two hours (Schulte and Hopkins, 1996), 400 °C for sixteen hours (USDA, 2022), and 550 °C for three hours (Hoogsteen et al., 2015). Because ignition temperature and duration differ across these protocols, the experimental design was not factorial and cannot separate their independent effects. Instead, the objective was to compare the performance of these established protocols as operational methods for predicting DC-SOC.

Conclusions

Among the three established LOI protocols evaluated in this study, LOI400 provided the best overall balance between predictive accuracy, repeatability, analytical cost, and scalability.

Specific comments

2.4. Experimental design, Lines 344 – 347. Comparing the intrinsic measurement errors of the different methods.

Referee: “Operator-related variability and many sample characteristics were considered by the authors that would affect analytical variability but how do the authors account for the variability caused by heterogeneity of the samples when comparing samples using widely different sample preparation techniques? Is it appropriate to expect agreement or compare accuracy, precision and variability of results obtained by a dry combustion method which uses a finely processed (<0.25 mm), 30 mg sample size to a coarser (<2 mm), 8 g sample size used in the LOI method? The difference in sample heterogeneity between fine and coarse particles and mg to g sample weights would be large (depending on soil type).”

Response: We thank the referee for raising this important point. We agree that particle size and analytical subsample mass can influence measurement variability and that the original manuscript did not distinguish this clearly enough.

Both workflows began from the same processed soil material. Samples were oven-dried at 65 °C for 24-48 hours, ground, sieved to <2 mm, and homogenized by

passing the material through a Havert RT splitter three times. The LOI aliquot (8 g) was taken from this homogenized <2 mm material. For DC, a subsample of the same homogenized material was ground further to <0.25 mm before approximately 30 mg was weighed for elemental analysis. Thus, neither method began with an unprocessed heterogeneous field sample.

Nevertheless, the differences in final particle sizes and analytical masses are real. Finer grinding can reduce particle-scale heterogeneity for the very small DC aliquot, whereas the much larger LOI mass can reduce subsampling error by integrating more material. The present experiment cannot independently separate the effects of particle size and analytical mass. We therefore agree that our original wording of “intrinsic measurement errors” was too strong. The reported CVs are better interpreted as repeatability of the complete analytical workflows, including their respective sample preparation and analytical subsampling steps.

CHANGES IN THE MANUSCRIPT:

Section 2.4 Experimental design / repeatability

To compare the repeatability of the analytical workflows, we calculated the coefficient of variation (CV), defined as the ratio of the standard deviation to the mean of replicate measurements. These CVs represent the combined variability associated with analytical subsampling, sample preparation, measurement, and, for the LOI analyses, operator effects. They should therefore not be interpreted as instrument-specific analytical error, which would be only one factor contributing to overall uncertainty in results.

Specific comment:

Referee: “Would the accuracy, precision or variability of the LOI protocols improve and agree better with the DC results if you used finely processed (<0.25mm) soil for the LOI measurement i.e. standardised one of these parameters?”

Response: This is an important question. Finer grinding could plausibly reduce particle-scale heterogeneity and improve LOI repeatability, particularly where organic matter is unevenly distributed among aggregates or particle-size fractions. However, the present experiment was not designed to quantify that effect, so our data cannot establish whether using <0.25 mm material would significantly improve LOI-DC agreement.

We have therefore added this as a limitation and future research need rather than speculating about the magnitude of an untested effect. We think testing operational workflows for a range of commonly used protocols is a useful start for more systematic evaluation of the LOI method.

CHANGES IN THE MANUSCRIPT:

Section 4. Future research

Because particle size differences between LOI and DC could reduce variability associated with particle-scale heterogeneity, future research would benefit from testing the effect of grinding LOI material to <0.25 mm.

Specific comment:

Referee: "What would happen if you heated soil samples at 360°C for 16h, 400°C for 16h and 550 °C for 16 h standardizing the heating duration? What about standardizing the temperature at 400 °C then comparing results obtained from samples heated for 2 h, 3 h and 16 h duration?"

Response: We agree that these comparisons would provide valuable mechanistic information. A factorial experimental design in which temperature and ignition duration are varied independently would be the appropriate approach for determining their separate effects and identifying an optimal temperature-duration combination.

However, our dataset cannot confidently predict the results of those untested combinations, so we do not extrapolate beyond the evidence provided by the experiment. The temperature-duration combinations were intentionally retained as specified by the three published protocols because the study was designed as a comparison of operational protocols rather than a factorial optimization experiment.

CHANGES IN THE MANUSCRIPT:

Materials and Methods section 2.2.2.

Because ignition temperature and duration differ across these protocols, the experimental design was not factorial and does not allow their independent effects to be separated. Instead, the objective was to compare the performance of these established protocols as operational methods for predicting DC-SOC.

Specific comment:

Referee: "Would your recommendation for the LOI400 protocol still stand if the analytical variability on the LOI measurements had been thoroughly investigated? Any comment?"

Response: Within the scope of the present study, LOI400 remains the best-supported recommendation among the three protocols evaluated, but we agree that the wording should be explicitly bounded (see changes in manuscript mentioned earlier). Nonetheless, for the comparisons made here, LOI400 had the

lowest observed CV among the LOI protocols tested (5.3%, compared with 7.6% for LOI360 and 29.6% for LOI550), which indicates stronger repeatability. Although LOI360 and LOI400 showed similar predictive performance, the transfer-equation intercept being near zero for LOI400 indicates that the need to compensate for incomplete combustion or mass loss from minerals is minimized. Additional analytical variables would be useful to test, but the results suggest that avoiding complicating factors from either incomplete combustion or mineral mass loss helps reduce measurement variability.

Specific comment:

Referee: "Introduction, Paragraph 3, Lines 53 – 54 Despite its enduring widespread use as a low-cost assessment of soil fertility and health, LOI is perceived in the scientific community as insufficient for change detection.

References required here to support this statement. Perceived as insufficient for change detection by whom? I suggest this all-encompassing statement should be qualified or removed. LOI as a measure of soil organic matter content or indeed SOC can be sufficient for many researchers' needs in the scientific community, it depends on the research context and soil type in question."

Response: We agree. The original statement aligns with the many conversations we have had with scientists working on detecting soil carbon change, which is naturally anecdotal. The original wording was too broad and implied a present-day consensus that we cannot substantiate. We have therefore removed the statement that LOI is generally "perceived in the scientific community" as insufficient for change detection. We also clarified that LOI itself can be suitable for measuring SOM and detecting changes in SOM when the same analytical procedure is applied consistently, including in routine and commercial soil testing.

In addition, we found a paper by Goldin (1987) documenting longstanding concerns about non-organic mass loss during ignition while also showing that LOI can be useful in non-calcareous soils when many samples must be processed. We used that reference to frame the analytical concern more precisely and to emphasize that suitability is context-dependent.

CHANGES IN THE MANUSCRIPT:

Introduction

LOI has long been used in soil and ecological studies because of its simplicity, rapid analytical throughput, and capacity to process large numbers of samples (Goldin, 1987). It also remains relevant to routine soil testing, as recommended procedures for the North Central United States include a 360 °C for 2 hours LOI method within guidance intended for public, private, and industrial soil-testing

laboratories (Combs and Nathan, 1998; Nathan and Gelderman, 2012). These procedures recommend local regression calibration of LOI against reference SOM values rather than unrestricted transfer of a single relationship. Notably, however, the reference procedures retain the conventional assumption that SOM contains 58% C, illustrating how an assumed OM:C proportion can remain embedded in analytical workflows even when LOI results are calibrated by regression rather than converted directly using that proportion. Concerns regarding LOI-derived estimates of SOM or SOC are therefore longstanding because mass loss during ignition may include not only organic matter but also water associated with clay minerals, carbonate-derived CO₂, and other mineral-related losses (Goldin, 1987; Pribyl, 2010). Consequently, the suitability of LOI-derived SOC estimates depends on the analytical protocol, soil properties, magnitude of change, monitoring objective, and the validity of the method-transfer equation used.

Reference added:

Goldin, A.: Reassessing the use of loss-on-ignition for estimating organic matter content in noncalcareous soils, *Communications in Soil Science and Plant Analysis*, 18, 1111-1116. <https://doi.org/10.1080/00103628709367886>, 1987.

Combs, S. M. and Nathan, M. V.: Soil organic matter, in: *Recommended Chemical Soil Test Procedures for the North Central Region*, edited by: Nathan, M. V. and Gelderman, R., North Central Region Research Publication No. 221 (Revised), Missouri Agricultural Experiment Station SB 1001, Columbia, Missouri, 12.1-12.6, 1998.

Nathan, M. V. and Gelderman, R.: Introduction, in: *Recommended Chemical Soil Test Procedures for the North Central Region*, North Central Regional Research Publication No. 221 (Revised), Missouri Agricultural Experiment Station SB 1001, Columbia, Missouri, iii-iv, 2012.

Specific comment:

Referee: "Introduction, Paragraph 4, Lines 67 – 69 This paper proposes using method transfer equations derived from linear regression, offering a more flexible alternative to fixed conversion factors, which are limited in their ability to account for non-SOM mass losses during LOI.and"

Introduction, Paragraph 6, Lines 84 – 86

"This approach offers a simple and inexpensive alternative because it estimates SOM from the mass lost after heating soil in a muffle furnace, and it has been widely used to estimate SOC using a standard conversion such as the van Bemmelen factor (Schulte and Hopkins, 1996)"

“I question whether many people who use LOI as a measure of SOM actually still use the van Bemmelen conversion factor to calculate SOC directly? I believe most current researchers use their own ground truthing with some form of dry combustion analysis combined with stable isotope analysis ($\delta^{13}\text{C}$) or Thermogravimetric analysis (TGA) to understand the mineral/inorganic component of their samples then use their own empirical equations, site-specific conversion factor and/or linear regression-based models. The references included to support this statement in this section of the manuscript are very few and quite old. Any further comment on how widespread the use of a LOI measurement with a standard conversion to estimate SOC is? Are there any more recent examples?”

Response: We agree that the original wording overstated our knowledge of how widely the van Bemmelen conversion factor is used in current practice. We did not identify a formal survey quantifying how often current researchers or commercial laboratories use each approach.

However, LOI remains part of routine soil-testing practice. Recommended procedures for the North Central Region, which were developed for public, private, and commercial soil-testing laboratories, include LOI for SOM determination and retain the conventional assumption that SOM contains 58% C (Combs and Nathan, 1998; Nathan and Gelderman, 2012).

Recent studies also continue to apply or evaluate the van Bemmelen factor. For example, Matteau et al. (2021) converted LOI-derived SOM to SOC using the van Bemmelen factor, and Heikkinen et al. (2021) explicitly evaluated the commonly used factor of 1.724 for converting between SOM and SOC.

Thus, we think the historical and ongoing use of conversion factors, with the implied assumption that the SOM-to-SOC ratio can stand in for conversion between methods, remains relevant to our study's motivation. Nonetheless, we agree that describing their use simply as “widely used” is unnecessarily broad. We have revised the introduction to distinguish historical use from contemporary practice and added more recent references as well as regional soil-testing guidance relevant to commercial laboratory practice.

CHANGES IN THE MANUSCRIPT:

Introduction

The van Bemmelen factor has historically been one of the most widely recognized approaches for interconverting SOM and SOC, based on the assumption that SOM contains approximately 58% carbon. This assumption also remains embedded in recommended soil-testing procedures, such as those for the North Central United States, which include LOI for SOM determination and are intended for public, private, and commercial soil-testing laboratories (Combs and Nathan, 1998;

Nathan and Gelderman, 2012). Although some contemporary applications use region-specific calibration or regression relationships with direct carbon measurements, simple conversion factors still appear in soil studies and analytical interpretations (e.g., Matteau 2021, Heikkinen 2021). Their continued relevance is important because a proportional conversion cannot account for method-specific factors, such as incomplete combustion and non-SOM mass losses during LOI.

Reference added:

Combs, S. M. and Nathan, M. V.: Soil organic matter, in: Recommended Chemical Soil Test Procedures for the North Central Region, edited by: Nathan, M. V. and Gelderman, R., North Central Region Research Publication No. 221 (Revised), Missouri Agricultural Experiment Station SB 1001, Columbia, Missouri, 12.1-12.6, 1998.

Nathan, M. V. and Gelderman, R.: Introduction, in: Recommended Chemical Soil Test Procedures for the North Central Region, North Central Regional Research Publication No. 221 (Revised), Missouri Agricultural Experiment Station SB 1001, Columbia, Missouri, iii-iv, 2012.

Matteau, J. P., Célécourt, P., Létourneau, G., Gumiere, T., Walter, C., and Gumiere, S. J.: Association between irrigation thresholds and promotion of soil organic carbon decomposition in sandy soil, Sci. Rep., 11, 6733, <https://doi.org/10.1038/s41598-021-86106-4>, 2021.

Heikkinen, J., Keskinen, R., Regina, K., Honkanen, H., and Nuutinen, V.: Estimation of carbon stocks in boreal cropland soils – methodological considerations, Eur. J. Soil Sci., 72, 934-945, <https://doi.org/10.1111/ejss.13033>, 2021.

Materials and Methods

Referee: “Some further detail on the laboratory analyses is required to allow the reader to determine whether the analysis has been carried out robustly.

2.2. Lab analysis , 2.2.1. Dry combustion Lines 210 – 215 and 221 – 222. *More details of the sample preparation steps prior to DC would be helpful to give an understanding of sample homogenization and additional details on the DC instrument and methods of analysis for TC and IC such as:*

- *What weight/size of sub-sample was taken from the original sample to prepare the ground <0.25 mm fraction*
- *How long was the <0.25 mm fraction dried at 105 °C to ensure an accurate oven dry weight*

- *Be more specific about the model of FlashSmart EA used as there are many different configurations of the Thermo instrument*
- *Provide detail of the acidification step for removing IC from the samples and if any steps were taken to ensure no organic carbon was lost through acid leaching*

Were any official methods used i.e. ISO or UNE methods as referencing a method would save adding a lot of additional detail to this text?"

Response: Thank you for pointing out these additional areas where we should be more explicit about the details for the protocols used. The common sample-preparation step is now explicitly connected to the DC section: samples were first dried at 65 °C for 24-48 hours, ground, sieved to <2 mm, and homogenized through a Havert RT splitter three times. A subsample of that homogenized fraction was then ground to <0.25 mm for DC.

For the instrument description, the configuration can be specified as “FlashSmart Elemental Analyzer, NC Soil configuration” (Thermo Fisher Scientific, Waltham, MA, USA), which is a distinct manufacturer configuration for soil C/N analysis that uses thermal-conductivity detection.

We also now clarify an important point in the referee’s acidification question. The aliquot combusted for TC was not acid-pretreated to remove carbonate. IC was measured independently on a separate aliquot by pressure calcimetry. The calcimetry reaction was performed in a sealed vessel after addition of 2 mL of 6 M HCl containing 3% FeCl₂, and SOC was subsequently calculated as TC – IC. Therefore, we did not apply an acid leaching step to the TC aliquot that could remove organic C before combustion.

The analyses followed published soil analytical procedures and laboratory QA/QC methods cited in the manuscript (including Schepers et al., 1989, for combustion and Sherrod et al., 2002, for pressure calcimetry) rather than an ISO or UNE method.

CHANGES IN THE MANUSCRIPT:

Section 2.2.1 – Dry combustion

After the <2 mm preparation and homogenization, 20 mL of soil was ground further to <0.25 mm. The <0.25 mm fraction was dried at 105 °C overnight before approximately 30 mg was weighed for analysis. Samples were flash combusted in an oxidative environment using a FlashSmart Elemental Analyzer (NC Soil configuration; Thermo Fisher Scientific, Waltham, MA, USA), and CO₂ was quantified using a thermal conductivity detector. Total C and inorganic C were determined independently. Inorganic C was measured on a separate aliquot using

a pressure calcimeter to quantify the pressure increase from CO₂ evolved after adding 2 mL of 6 M HCl containing 3% FeCl₂ in a sealed reaction vessel (Sherrod et al., 2002). DC-SOC was calculated as TC – IC; the aliquot used for TC combustion was not acid-pretreated or leached.

Referee: “2.2.2. Loss on ignition Line 233. Sixty-two Fisher brand FB-965-E crucibles were filled with 8 g of < 2-mm oven-dry soil per run.

- It would be useful to include the dimension or capacity (metric volume) of the porcelain crucibles used to the method.
- Why was 8g sample used? This is not a usual weight to use in my experience. Any comment?”

Response: We agree that these implementation details should be explicit. The manufacturer's catalog for Fisherbrand FB-965-E high-form porcelain crucibles specifies a nominal capacity of 30 mL and dimensions of 43 mm top outer diameter x 37 mm height. We have added those specifications.

The 8 g analytical mass was a laboratory-specific adaptation to the crucible dimensions and the workflow used in this experiment. We do not present 8 g as the USDA-NRCS prescribed mass. Instead, the revised text distinguishes our implementation from the USDA-NRCS procedure on which the 400 °C for 16 hours temperature-duration treatment was based. The 8 g mass still provided a relatively large homogenized analytical subsample and allowed mass loss to be resolved with a balance readable to 0.01 g.

CHANGES IN THE MANUSCRIPT:

Section 2.2.2 – Loss on ignition

Sixty-two Fisherbrand FB-965-E high-form porcelain crucibles (30 mL capacity; 43 mm top outer diameter x 37 mm height) were filled with 8 g of homogenized <2 mm soil per run. The 8 g analytical mass was a laboratory-specific adaptation to the crucible dimensions and analytical workflow. The relative measurement error for mass loss between analyzing 8 and 15 g of soil would not be detectable with a measurement precision of 0.01 g.

Referee: “Was a repeat of the oven dry at 105°C overnight step carried out to ensure no further weight loss due to retained moisture or continued drying at 105°C until no further weight loss is noted? This is common practice.”

Response: Samples were dried at 105 °C overnight, cooled in a desiccator, and weighed before ignition. We did not perform a separate dry-cool-weigh cycle to experimentally verify constant mass for each sample. We have made this explicit so the manuscript does not imply that constant mass was independently verified.

The analytical samples had already undergone prior drying and homogenization before the final 105 °C overnight step. Nevertheless, because we did not perform repeated drying cycles, we describe the exact procedure used rather than claiming experimental verification of constant mass.

CHANGES IN THE MANUSCRIPT:

Section 2.2.2 – Loss on ignition

Before furnace ignition, samples were oven-dried at 105 °C overnight, cooled in a desiccator, and weighed to 0.01 g. Repeated dry-cool-weigh cycles to independently verify constant mass were not performed.

Referee: “Line 239. Was there a specific reason why LOI was presented in g kg⁻¹ rather than the usual % value?”

Response: We thank the referee for pointing out this typo. Equation 1 was modified accordingly.

CHANGES IN THE MANUSCRIPT:

Equation 1

$$\text{LOI (\%)} = [(\text{ODSM} - \text{ISM}) / \text{ODSM}] * 100$$

Minor comments

Referee: “Results and Discussion 3.3. Repeatability and evaluation of measurement error, Line 609 multi-sensor SOC models that use different indices (e.g., NDVI, SAVI, EVI)

It would be helpful to readers unfamiliar with these metrics to include abbreviations in full i.e. normalised difference vegetation index and so on.”

Response: We agree and have expanded each abbreviation at first use.

CHANGES IN THE MANUSCRIPT:

Results and Discussion

Multi-sensor SOC models that use different spectral vegetation indices, such as the normalized difference vegetation index (NDVI), soil-adjusted vegetation index (SAVI), and enhanced vegetation index (EVI), together with topographic attributes and other environmental covariates.

Technical correction

Referee: “References, Line 790 Stanley, P., et al

Check – publication title split unnecessarily over two lines”

Response: We thank the referee for identifying this formatting issue. We corrected the Stanley et al. reference so the article title appears continuously and consistently with the other references.

CHANGES IN THE MANUSCRIPT:

References

Stanley, P., Spertus, J., Chiartas, J., Stark, P. B., and Bowles, T.: Valid inferences about soil carbon in heterogeneous landscapes, *Geoderma*, 430, 116323, <https://doi.org/10.1016/j.geoderma.2022.116323>, 2023.