Quantifying organic carbon in particulate and mineral-associated fractions of calcareous soils – A method comparison

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ABSTRACT

Accurate quantification of soil organic carbon (OC) pools is essential to study the dynamics of OC in soils. Therefore, organic matter is often separated into physical fractions with distinct turnover times, e.g. particulate (POM) and mineral-associated organic matter (MAOM), after which their OC content is measured. Calcareous soils are under-represented in such fractionation studies, because the required analytical differentiation between OC and inorganic carbon (IC) is not straightforward and implies more processing time, sample mass and equipment. Here, we review the performance of four methods to quantify OC in POM and MAOM fractions of soils containing 3–16 g IC kg^{-1} and 10–25 g OC kg^{-1} (n = 16). We assessed the similarity and consistency of the data obtained with the different methods. Furthermore, we checked how their ability to distinguish OC from IC was influenced by the fractionation and in particular by the dispersion with sodium hexametaphosphate (SHMP). The four methods were: 1) Elemental carbon analysis after removal of IC by acid fumigation (OC$_{fum}$); 2) Elemental carbon analysis after removal of IC with aqueous acid (OC$_{aq}$); 3) Elemental carbon as determined by Rock-Eval 6 thermal analysis (OC$_{RE6}$); and 4) Elemental carbon analysis after removal of OC by loss on ignition and subsequent quantification of OC by difference between total carbon and IC (OC$_{LOI}$). We found that the OC$_{fum}$, OC$_{aq}$, and OC$_{LOI}$ methods produced similar OC contents for bulk soils, whereas OC$_{RE6}$ results were slightly but significantly lower. Total recovered OC$_{RE6}$ contents of the summed POM and MAOM fractions were similar to the bulk soil OC$_{RE6}$ contents. In contrast, the recovered OC$_{fum}$, OC$_{aq}$ and OC$_{LOI}$ contents were slightly higher than respective bulk soil OC contents (108–112%), especially in soils with high IC/OC ratios. We show that this was not caused by operational artefacts or chemical changes that occurred during OC fractionation, but rather likely indicated error propagation. We conclude that all 4 methods can reliably quantify OC in POM and MAOM fractions in calcareous soils and that the optimal choice depends on the required accuracy of the results and the available sample mass, lab equipment and processing time. Based on our findings and practical considerations, we provide guidance for the selection of the most optimal OC quantification method to study OC dynamics in calcareous soils.

1. Introduction

Soil organic carbon (OC) is of fundamental importance in global carbon cycling and related ecosystem functioning (Shukla et al., 2022). To understand the response of soil OC to climate change and land use, OC is often quantified in soil organic matter (SOM) fractions with contrasting turnover times (Lavallee et al., 2020; Ramírez et al., 2021). The separation of SOM into particulate organic matter (POM) and mineral-associated organic matter (MAOM) is a relatively easy and frequently used method to isolate two fractions with contrasting properties with regard to their persistence, functioning and formation (Lavallee et al., 2020): POM mainly consists of relatively large SOM

Table 1

Overview of the methods used to analyse Organic Carbon (OC) in bulk soils and particulate (POM) and mineral-associated (MAOM) fractions, OC_{Sch} was analysed in bulk soil only due to high required sample mass. IC = Inorganic Carbon; TC_{LECO} = Total Carbon as measured on a LECO Truspec CHN Analyser; micro CN Analyser = micro Flash 2000 Organic Elemental CN Analyser; LECO CN Analyser = LECO Truspec CHN Analyser. For a full explanation of the OC methods: See the Supplementary Materials and Methods.

Method abbreviation	Method summary	Reference
OC_{aq}	IC was removed by adding 80 μL of 10% aqueous hydrochloric acid ($HCl_{(aq)}$) to 15–20 mg soil subsamples, the remaining OC in the soil was measured on a micro CN Analyser.	(Nieuwenhuize et al., 1994)
OC_{fum}	IC was removed via acid fumigation. First, 33 μL of 1% HCl was added to 15–20 mg soil subsamples in a 96-wells plate. The wells plate was subsequently placed in a desiccator, together with a beaker containing 100 mL of 37% fuming HCl and left overnight. The remaining OC in the soil was measured on a micro CN Analyser	(Harris et al., 2001; Walthert et al., 2010)
OC_{LOI}	OC was determined by difference between TC_{LECO} content and IC content after removing OC by loss on ignition (LOI): 0.4 g soil was combusted for 3 h at 550 °C in a muffle furnace and the IC content of the ash was measured on a LECO CN Analyser. IC content was corrected for the weight loss during LOI before subtraction from TC_{LECO}, as measured in a subsample prior to LOI.	(Mayer et al., 2019)
OC_{RE6}	CH–, CO– and CO_2-effluents were measured during ramped pyrolysis and oxidation of 60 mg soil in a Rock-Eval 6 Analyser. OC content was derived by adding the integrated effluent signals assigned to organic carbon from all thermograms.	(Disnar et al., 2003)
OC_{Sch}	OC was estimated by difference between TC_{LECO} content and IC content measured by adding 7 mL of 4 M HCl solution to 10 g of soil in a Scheibler apparatus and volumetric quantification of the CO_2 produced after 2 h. Gas volume was converted to IC_{Sch} content via a calibration curve determined with pure calcite.	(Horváth et al., 2005) and ISO 10693 (1995)

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Nieuwenhuize, J., Maas, Y. E. M., and Middelburg, J. J., 1994, Rapid analysis of organic carbon and nitrogen in particulate materials: Marine Chemistry, v. 45, no. 3, p. 217–224.

Walthert, L., Graf, U., Kammer, A., Luster, J., Pezzotta, D., Zimmermann, S., and Hagedorn, F., 2010, Determination of organic and inorganic carbon, δ13C, and nitrogen in soils containing carbonates after acid fumigation with HCl: Journal of Plant Nutrition and Soil Science, v. 173, no. 2, p. 207–216.

compounds with no or little association with the soil mineral matrix, whereas MAOM mainly consists of small SOM compounds that are associated with soil minerals in organo-mineral complexes and fine aggregates. Separating POM from MAOM based on their size has, in spite of this operational distinction, proven to provide fractions with distinct turnover times (Poeplau et al., 2018). This fractionation was therefore recommended as a time- and cost-effective method for broad-scale studies (Lavallee et al., 2020) and has since then been adopted in multiple studies (e.g. (Duan et al., 2023; Li et al., 2023; Xiao et al., 2022)). However, the presence of inorganic carbon (IC) complicates the quantification of OC in fractions of calcareous soils. Usually, IC is removed prior to elemental CN analysis, or OC is estimated by difference between total carbon (TC) and IC content (Harris et al., 2001; Mayer et al., 2019; Vuong et al., 2013). These extra analytical steps require additional sample mass and processing time, limiting feasibility of broad-scale fractionation studies. Calcareous soils are therefore under-represented in OC fractionation studies, although these soils cover more than 30% of the earth's land surface (Marschner, 2011).

Different OC quantification methods exist that differentiate between OC and IC (Mayer et al., 2019; Xu et al., 2021), but not all methods are suitable for fractionation studies, for example due to limitations in available sample mass for the separate fractions. Moreover, the accuracy of OC quantifications in calcareous POM and MAOM fractions has, to the best of our knowledge, not yet been evaluated (Baldock et al., 2013; Poeplau et al., 2018; Sanderman et al., 2013). Obtaining 100% OC recovery after fractionations can be a challenge (Poeplau et al., 2013; Rumpel et al., 2004) and the risk of deviating recoveries is further increased in calcareous soils by the extra analytical steps to differentiate between IC and OC. OC losses during fractionation and incomplete IC removal may cause deviating recoveries, but also the dispersion of the soil samples with sodium hexametaphosphate (SHMP), as part of the fractionation procedure: Firstly, SHMP remains present in the MAOM fraction and exerts a buffering effect on pH (de Morais et al., 2020) which may influence IC removal of acid-based methods used to differentiate between IC and OC. Secondly, SHMP may change the nature of OC and IC, since it desorbs OC from soil minerals by the phosphate surplus (Hinsinger, 2001) and dissolves and adsorbs to IC (de Morais et al., 2020; Hatch and Rice, 1939; Thomson, 1936).

Here, we present the performance characteristics of four different methods to quantify OC in calcareous POM and MAOM fractions. We selected methods that are suitable for low sample masses and high sample numbers and that do not require a conversion factor to quantify OC. The similarity and consistency of the OC results obtained by these methods were assessed for bulk soils and fractions. Additionally, we explored the influence of the fractionation procedure, especially the dispersion step using SHMP, on the distinction between OC and IC. Finally, we provide guidance on the selection of the optimal OC method based on the required accuracy of the results and the available sample mass, lab equipment and processing time.

The four methods to quantify OC in POM and MAOM fractions are: 1) Elemental carbon analysis after removal of IC by acid fumigation (OC_{fum}); 2) Elemental carbon analysis after removal of IC with aqueous acid (OC_{aq}); 3) Elemental carbon as determined by Rock-Eval 6 thermal analysis (OC_{RE6}); and 4) Elemental carbon analysis after removal of OC by loss on ignition and subsequent quantification of OC by difference between TC and IC (OC_{LOI}) (see Table 1 and Supplementary Information for more information on the OC methods). For bulk soils, we also estimated OC by difference between TC and IC measured by volumetric quantification in a calcimeter based on the Scheibler method (OC_{sch}), to include a more standard indirect OC method than OC_{LOI} for a broader method comparison in bulk soils (the OC_{Sch} method requires too much sample mass to analyse the separate fractions).

2. Materials and methods

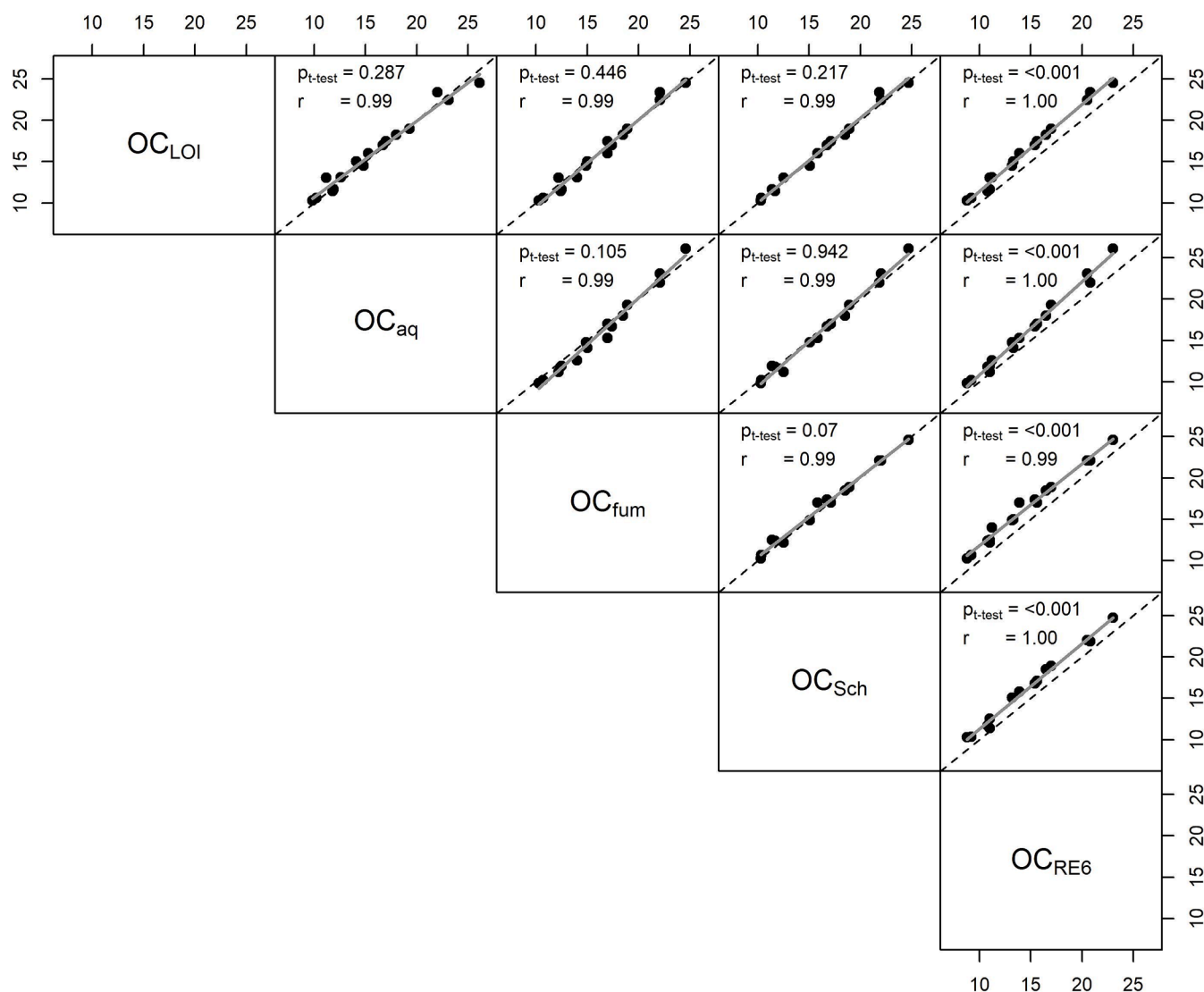We used sixteen different soil samples from calcareous fluvisols in

Fig. 1. Soil organic carbon (OC) contents in calcareous bulk soils (n = 16) in g kg^{-1}, as measured by different OC methods: OC$_{LOI}$, OC$_{aq}$, OC$_{fum}$, OC$_{Sch}$, OC$_{RE6}$. See Table 1 and the Supplementary Materials and Methods for a further explanation of the methods. Gray solid line: visualization of linear correlation ($y = ax + b$) to assess consistency among OC methods. Black dashed line: The 1:1 line. p$_{t-test}$: p-value of two-tailed paired *t*-test with a Bonferroni-corrected significance level α = 0.005. r: correlation coefficient obtained from linear correlation ($y = ax + b$). Note that the intercept is not shown.

the Netherlands with a clay content between 10 and 31%, and that contain 3–16 g IC kg^{-1} and 10–25 g OC kg^{-1} (Table S1). These soils are classified as Mn25a and Mn35a according to Dutch soil classification (De Bakker and Schelling, 1989), have been reclaimed from the sea and frequently contain shell fragments. A subsample of 10 g air-dried <2 mm soil was fractionated into POM and MAOM (Poeplau et al., 2018; Baldock et al., 2013; Sanderman et al., 2013). In short, soils were dispersed in 40 mL of 5 g l^{-1} sodium hexametaphosphate (SHMP) and wet-sieved over 50 μm in an automated wet sieving system (FRITSCH) to separate POM from MAOM. The fractions were dried at 105 °C. Bulk soils and fractions were ground to powder before determining OC content with the different OC methods. We similarly dispersed bulk soils in SHMP, skipped the wet fractionation step, and analysed OC in the 105 °C dried and ground bulk soils, to investigate whether SHMP dispersion affects OC quantification. To further evaluate the OC methods, we applied all methods on two calcareous reference soils of the global proficiency program WEPAL (ISE-989: calcareous river clay; and ISE-879: calcareous marine clay). More details on the physical size fractionation, carbon detection methods and data processing are described in the Supplementary Information.

Since there is no golden standard, all OC methods were compared against all other methods with linear correlations ($y = ax + b$, using the correlation coefficient as measure for consistency) and two-tailed paired t-tests to detect differences between the OC methods with pairing per sample (n = 16). A Bonferroni correction (α / number of tests) was used to correct for the large number of t-tests. Recovered OC and TC contents were calculated as the weighted sum of the dry weights and respective carbon contents of the two fractions. Recovered OC and TC contents were compared with bulk soil OC and TC contents via two-tailed paired t-tests with pairing per sample. OC and TC recoveries were calculated by dividing the recovered OC and TC contents by the carbon contents of the respective bulk soils.

3. Results and discussion

Comparison of OC methods. Generally, all OC methods (OC$_{fum}$, OC$_{aq}$, OC$_{LOI}$, OC$_{RE6}$, OC$_{Sch}$) led to accurate results, since all but one OC contents of the WEPAL samples (n = 69) were within the 95% confidence interval of the consensus value (see Supplementary Information). Similar OC contents were produced for all OC methods for both bulk

soils and POM fractions ($p_{t\text{-test}} > \alpha$; range r: 0.99–1.00), except for the OC_{RE6} method which produced OC contents that were significantly ($p_{t\text{-test}} < 0.001$) and consistently (range r: 0.99–1.00) lower than the other methods (Fig. 1 and Fig. S1). The results of the different OC methods were less consistent for the MAOM fractions (range r: 0.86–0.98) and less in agreement (lower $p_{t\text{-test}}$-values). The OC_{RE6} contents were again slightly but significantly lower than OC contents obtained with all other methods ($r \geq 0.95$, Fig. S2), in line with findings in literature showing that Rock-Eval 6 underestimates OC by $\pm 10\%$ (Disnar et al., 2003; Saenger et al., 2013). This underestimation of OC coincides with an overestimation of IC, since TC as measured by elemental carbon Analyser (LECO) and Rock-Eval are equivalent (Table S1). This underestimation of OC is likely due to the fact that during pyrolysis, part of the OC evolving as CO_2 and CO at high temperature is erroneously attributed to IC. As (Disnar et al., 2003) suggest, this systematic misattribution can probably be solved by using a correction factor.

Carbon recovery. OC recovered based on the sum of the fractions (Fig. S3) was good when using the OC_{RE6} method ($103 \pm 7\%$,), but the other OC methods led to recoveries that were significantly higher than 100% (OC_{fum}: 108 ± 8 %; OC_{aq}: 112 ± 15 %; OC_{LOI}: $107 \pm 10\%$). OC contamination during fractionation, spilling, subsampling errors and other systematic operational errors did not explain these overestimated OC recoveries, because the sample mass recovery was $98 \pm 1\%$ and the TC recoveries as measured by Rock-Eval and elemental carbon Analyser were $99 \pm 3\%$ and $100 \pm 4\%$ respectively (Fig. S4). We also excluded possible effects of operational errors on OC_{LOI} recovery due to varying LOI conditions (e.g. duration and ignition temperature (Hoogsteen et al., 2015)) between bulk soils and fractions, since these samples were measured simultaneously. We subsequently explored the potential effect of dispersion with SHMP on the OC_{fum}, OC_{aq} and OC_{LOI} recoveries. OC recoveries of bulk soils that were dispersed did not differ significantly from the respective OC contents before dispersion, with recoveries of 97–101% (Fig. S5), indicating that the OC_{fum}, OC_{aq} and OC_{LOI} methods are not affected by chemical changes induced by SHMP. However, the concentration and buffering capacity of SHMP can be higher in MAOM fractions than in bulk soils after fractionation, since the same amount of SHMP ends up in a smaller soil mass (50–80% of bulk soils ended up in MAOM fraction). Because a higher buffering capacity could theoretically reduce acid dissolution of IC, and thus cause an overestimation of OC, we measured OC_{aq} content in MAOM fractions after increasing the acid to soil ratio by factors of 2, 2.5 and 3 (Supplementary Information). Adding more acid did not lower the OC_{aq} contents (Table S2), indicating that proton-binding to SHMP did not limit the dissolution of IC. We thus conclude that the OC_{fum}, OC_{aq} and OC_{LOI} methods were not affected by dispersion with SHMP. We conclude that the elevated OC recoveries most likely derived from error propagation caused by the larger number of processing steps for these methods compared to the OC_{RE6} method, and that this error propagation becomes increasingly apparent at higher IC/OC ratio's (Fig. S6).

Choosing the optimal OC method. We conclude that all four OC methods can be used to accurately measure OC content in calcareous bulk soils and POM and MAOM fractions. Nevertheless, one method may serve specific needs better than others. In practice, choosing the optimal OC method depends on the desired accuracy of the results and the available sample mass, laboratory equipment and processing time. We provide guidance on selecting the most optimal method based on these practical considerations and the results of our study in the Supplementary Information (Fig. S7 and Table S3), to facilitate including currently under-represented calcareous soils in global assessments of soil organic carbon dynamics.

Declaration of Competing Interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116558.

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