



Soil mass and grind size used for sample homogenization strongly affect permanganate-oxidizable carbon (POXC) values, with implications for its use as a national soil health indicator



Mirjam Pulleman^{a,b,*}, Skye Wills^c, Rachel Creamer^a, Richard Dick^d, Rich Ferguson^c, Diane Hooper^c, Candiss Williams^c, Andrew J. Margenot^e

^a Soil Biology Group, Wageningen University, PO Box 47, 6700AA Wageningen, The Netherlands

^b International Center for Tropical Agriculture (CIAT), 763537 Cali, Colombia

^c USDA-NRCS National Soil Survey Center, Lincoln, NE 68508, USA

^d School of Environment and Natural Resources, The Ohio State University, Columbus, OH 43210, USA

^e Department of Crop Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA

ARTICLE INFO

Handling Editor: Ingrid Kögel-Knabner

Keywords:

Labile carbon

Active carbon

Soil organic matter

National monitoring

Method quantitation limits

Analytical variability

ABSTRACT

Permanganate oxidizable carbon (POXC) is increasingly used in soil health assessments as an indicator of labile or active soil organic carbon (SOC). The POXC method owes its popularity to its rapidity and low cost and its responsiveness to soil management. However, the method's sensitivity to variation in methodological parameters may compromise the comparability of POXC values across soils. Here, we measured the effects of soil mass and the method of sample homogenization (grind size) on POXC values for a set of 42 soil samples, representing diverse geographic areas and soil types of the USA. Total SOC contents ranged from 3.0 to 288.4 g kg⁻¹. Ten treatments, combinations of five sample masses (0.25–5.0 g) and two grind sizes (< 2 mm, < 0.18 mm), were evaluated for POXC based on Weil et al. (2003). Results showed that POXC values decreased exponentially with increasing soil mass used, while analytical variability decreased as well. Decreasing grind size from < 2 mm to < 0.18 mm increased POXC values by 49% and decreased analytical variability. Strong correlations between POXC values obtained at two different soil masses ($r = 0.91$ – 0.97) or grind sizes ($r = 0.96$) indicate that general trends in POXC values across soils are maintained irrespective of soil mass or grind size, but the results are not interchangeable since POXC values differ considerably. Therefore methodological parameters, including soil mass and intensity of soil homogenization, should be strictly controlled to ensure comparability. However, standardizing soil mass presents a challenge for its use in national monitoring schemes as none of the soil masses yielded results within the method's quantitation limits for all soils included in our sample set.

A more fundamental problem challenging the comparison and interpretation of POXC values relates to the use of a fixed soil mass for POXC analysis across soils with different SOC contents. Whereas the initial amount of MnO_4^- is fixed, the amount of SOC that takes part in the oxidation reaction is soil specific and not controlled for when using a fixed soil mass. This leads to variation in the $[\text{MnO}_4^-] : \text{SOC}$ mass ratio during the reaction, which affects the size and the biochemical composition of the fraction of total SOC being oxidized. We conclude that the POXC method falls short as an analytical method for measuring a well-defined C pool. Performing POXC analyses based on a fixed SOC mass may improve comparability of POXC values across soils with contrasting SOM contents, but would compromise the main advantages of the POXC method in terms of simplicity, speed and low-cost.

1. Introduction

In recent decades there has been growing demand for indicators of soil health. Soil organic matter (SOM) or soil organic C (SOC) is a key property to measure because it supports important soil and ecosystem

functions such as nutrient storage, carbon sequestration, and provides an energy source for the soil community (Schmidt et al., 2011; Stockmann et al., 2013). However, although total SOC has been widely recognized as a key indicator of soil health or soil quality (Bünemann et al., 2018), it responds too slowly to changes in soil management or

* Corresponding author at: Soil Biology Group, Wageningen University, PO Box 47, 6700AA Wageningen, The Netherlands.

E-mail addresses: mirjam.pulleman@wur.nl, m.pulleman@cgiar.org (M. Pulleman).

<https://doi.org/10.1016/j.geoderma.2020.114742>

Received 16 May 2020; Received in revised form 14 September 2020; Accepted 15 September 2020

0016-7061/ © 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

disturbance to enable timely guidance for sustainable soil management (Lefroy et al., 1993; Marriott and Wander, 2006). Therefore, scientists have attempted to develop early indicators of SOC change and lability, associated with biological activity and potential nutrient mineralization (Blair et al., 1995; Bongiorno et al., 2019) and more recently, SOM stabilization (Cotrufo et al., 2013, 2015; Hurişso et al., 2016).

For over thirty years, potassium permanganate (KMnO_4) oxidation has been used to assess the size of a labile versus total SOC pool. Lefroy et al. (1993) distinguished SOC fractions with incremental degrees of 'lability' based on the amount of total SOC oxidized using MnO_4^- concentrations varying from 0.333 to 0.033 M that were added to soil samples containing a fixed amount of 15 mg SOC. The fraction oxidized by 0.333 M KMnO_4 was then used for the calculation of a Carbon Management Index (Blair et al., 1995). A simplified and less hazardous method for routine quantification of a readily oxidizable SOC fraction was developed by Weil et al. (2003), using a more dilute solution (0.02 M MnO_4^-) and a standard soil mass (5.0 g), instead of SOC mass, and a short oxidation time. Weil et al. (2003) reported that shaking of air-dried soil in a 0.02 M KMnO_4 solution for 2 min produced consistent and management sensitive results, both in the laboratory and in the field using a hand-held colorimeter, and that the addition of a flocculant (CaCl_2) to the permanganate solution could replace the centrifuging step prescribed by Lefroy et al. (1993) and Blair et al. (1995). Further modification by Culman et al. (2012a) and Culman et al. (2012b) employed a lower sample mass (2.5 g). The popularized methods of Weil et al. (2003), Culman et al. (2012a) and Culman et al. (2012b) and variations thereof (Table 1) target the SOC fraction readily oxidized by 20 mL of 0.02 M MnO_4^- and are generally referred to as permanganate-oxidizable carbon (POXC).

Various publications have reported that POXC is sensitive to changes in soil management (Weil et al., 2003; Moebius-Clune et al., 2016; Soil Health Institute, 2017; Bongiorno et al., 2019) and correlates

with total SOC (Culman et al., 2012b; Margenot et al., 2017; Rennert et al., 2017) and with soil biological properties such as mineralizable C, C-mineralizing enzyme activities, and microbial biomass C (Weil et al., 2003; Margenot and Hodson, 2016; Margenot et al., 2017). According to Culman et al. (2012b), POXC demonstrated greater sensitivity than particulate organic C, microbial biomass C or total SOC to variation in soil management and/or environmental conditions and can be used routinely to measure "a relatively processed pool of labile soil C". More recently, Hurişso et al. (2016) suggested that POXC is positively associated with management practices that increase SOC in the long-term. A further attraction for POXC method is its simplicity, low cost and rapidity without the need for expensive equipment, hazardous chemicals, or specialized laboratory technicians.

In the past decade, there has been a rapid increase in the use of the POXC method for studying soil health and/or SOM cycling in temperate (e.g., Hurişso et al., 2016; Bongiorno et al., 2019) and tropical (e.g., de Moraes Sá et al., 2014; Margenot et al., 2017; Thoumazeau et al., 2019) agricultural systems. The growing demand among governments, extension agencies and farmers to monitor soil health at national and regional scales (Bünemann et al., 2018; Moebius-Clune et al., 2016; Soil Health Institute, 2017) has further intensified interest in indicators such as POXC. Recently, POXC was endorsed by USDA NRCS to monitor soil health across the United States as a method to measure a readily available pool of "active C" that supports the microbial population (Stott, 2019). Similarly, in the European Union, research is being conducted on the suitability of POXC as a general soil health indicator (e.g., Van den Elsen et al., 2019; Hanegraaf et al., 2019).

Despite its logistical advantages and purported interpretive value, uncertainties exist concerning the robustness of the POXC method and its comparability across soils due to its sensitivity to multiple methodological parameters (Blair et al., 1995; Gruver, 2015). Moreover, important considerations when validating any method are the accuracy,

Table 1

Summary of sources of variability in permanganate oxidizable carbon (POXC) measurements and how these are being accounted for in widely used standard protocols.

Source of variation (methodological)	Published findings	Relevant studies	Proposed protocols for POXC assay
$[\text{MnO}_4^-]$	$\uparrow [\text{MnO}_4^-]$ \uparrow POXC	Lefroy et al. (1993) Bell et al. (1998) Weil et al. (2003) Gruver (2015)	20 mL of 0.02 M KMnO_4 ^[1,2,3,4]
Soil mass (g) [§]	\uparrow Soil mass \downarrow POXC		5 g ^[1] 2.5 g ^[2,3,4]
Soil preparation	\uparrow Mesh size \downarrow POXC	Weil et al. (2003) Hurişso et al. (2018a) and Hurişso et al. (2018b)	Not specified ^[1] "Soil sample standard prepared with same grinding in the same manner as the unknown samples" ^[2] Sieving over 2 mm mesh ^[3,4]
Degree of soil drying	\uparrow Degree of soil drying \uparrow POXC	Weil et al. (2003)	"All samples being compared should be of equal dryness" ^[1] Air-dried to constant weight ^[2,3,4]
Drying temperature	Drying temperature (air drying, oven drying at 45 °C, 65 °C) did not cause a large shift in POXC	Gash et al. (2020)	"All samples being compared should be of equal dryness" ^[1] Air-dried to constant weight ^[2,3,4]
Shaking duration	\uparrow Shaking duration \uparrow POXC	Weil et al. (2003) Gruver (2015)	2 min ^[1,2,3,4]
Shake intensity	Not available	Not available	120 rpm ^[1,2,3,4]
Settling time (excl. shaking duration)	Not available	Not available	10 min ^[1,2] 8 min ^[3,4]
pH of 0.2 M KMnO_4 stock solution	The pH of the stock MnO_4^- reagent did not affect POXC; Any significant effects of pH on POXC likely result from differences in soil pH	Gruver (2015)	pH 7.2 ^[1,2,3,4]

¹Weil et al. (2003).

²Culman et al. (2012a) and Culman et al. (2012b).

³Schindelbeck et al. (2016).

⁴Stott (2019).

[§]Some protocols recommend to adjust soil mass when absorption levels are very low or final MnO_4^- concentrations are outside certain limits (Soil Survey Staff, 2014a; Schindelbeck et al., 2016).

precision, repeatability and reproducibility of the method, measured as analytical variability (Wade et al., 2018). Important factors that have not been standardized across the widely-used POXC protocols are soil mass used and the grind size for sample homogenization (Table 1). Culman et al. (2012b) reduced the recommended 5.0 g soil mass from Weil et al. (2003) to 2.5 g without any rationale to support this modification. A possible explanation is that using a soil mass of 5.0 g on a high SOC soil would result in complete or near complete MnO_4^- consumption, exceeding the method detection limit. However, combining the recommended 0.40 mmol MnO_4^- with either 5.0 g soil (Weil, et al., 2003) or 2.5 g soil (Culman et al., 2012a, 2012b) may lead to different POXC values when the concentration (20 mM) and volume (20 mL) of MnO_4^- do not combine to provide sufficient excess of permanganate to maintain linearity of the relationship between soil mass and the fraction of MnO_4^- reduced, over the range of SOC levels in the sample set being assessed (Gruver, 2015).

Another factor impacting POXC measurements is the method of sample homogenization. While the majority of routine chemical soil analysis is performed on soil material that is crushed to pass a 2 mm mesh, soil grinding using a Wiley or ball mill is commonly performed to improve method precision. Widely used POXC protocols (e.g., Weil et al., 2003; Culman et al., 2012a, 2012b; Schindelbeck et al., 2016; Stott, 2019) specify assay parameters such as shake duration and intensity, but sample homogenization procedures are not specified in all cases and the soil mass used may vary (Table 1). Published studies reporting POXC measurements employed varying methods of sample homogenization, including sieving over 2 mm versus crushing to < 0.1 mm (Weil et al., 2003), or even a range of mesh sizes from < 0.5 to < 2 mm (Culman et al., 2012b). Hurisso et al. (2018a) reported that decreasing the sieving size from < 4 mm to < 2 mm increased POXC values, indicating that the lack of standardization of the method of sample homogenization likely adds to the multiple sources of variation in measured POXC values.

The objectives of our study were to (i) quantify the impact of the soil mass and grind size used for sample homogenization on POXC results across a diverse and representative range of USA soils; and (ii) evaluate the degree to which soil mass and grind size influence analytical variability of POXC values. We hypothesized that, for a given soil sample, the POXC values obtained increase with decreasing soil mass due to the non-linearity of the relation between soil mass and the fraction of MnO_4^- reduced (Gruver, 2015). With further expect that a decrease in grind size increases POXC values, because more intense grinding will increase the proportion of SOC exposed to the oxidation reaction. The analytical variability is a measure of the precision or repeatability of the method in a given lab and is expected to decrease with increased intensity of sample homogenization (i.e. decreasing grind size) and with greater soil mass. Based on the results obtained, we discuss implications for the robustness of the POXC method and the comparability of POXC values across edaphically diverse soils.

2. Materials and methods

2.1. Soil sample selection

Forty-two samples soil were selected from the Kellogg Soil Survey Laboratory (KSSL) archive using a conditioned Latin hypercube sampling scheme (cLHS software; Roudier et al., 2012). The cLHS protocol uses an objective function to select samples that represent the distribution of all available auxiliary variables. The 42 samples represented 9 of the 12 US soil Orders, including Mollisols (23), Alfisols (5), Ultisols (5), Andisols (2), Entisols (2), Inceptisols (2), Aridisols (1), Histosols (1) and Vertisols (1). The soils were taken from the A horizon except for one sample that came from an O horizon. Horizon designation and taxonomic classification was assigned according to Soil Taxonomy (Schoeneberger et al., 2012; Soil Survey Staff, 2014b). The soil samples varied widely in SOC (3.0–288.4 g kg⁻¹; mean 31 g kg⁻¹), pH

(4.3–8.5; mean 6.2) and clay content (3.6–47.0%; mean 21.5%) ([dataset] Wills et al., 2020). The geographic origin of the selected samples (Fig. S1) and the distribution of SOC concentrations, clay contents and pH values (Fig. S2) are included in the [supplementary materials](#).

Before archiving at room temperature (method 1B1b2a2), the samples had been dried in a low temperature oven at 30–35 °C for 3–7 days (method 1B1b2) and crushed to < 2 mm, using a roller on a flat, metal plate covered with paper to crush the peds (method 1B1b2b). Clayey soils without coarse fragments were crushed using a laboratory jaw crusher (Retsch, Model BB200). Analyses for soil characterization were performed on dry and crushed (< 2 mm) soil and results were reported on an oven-dry basis. Total soil C was determined by dry combustion (method 4H2a1-3a1) and total SOC was calculated by deducting inorganic carbon, if present (method 4E1a1a1a1-2), from total carbon. Soil pH was determined in a 1:1 water solution. Particle-size analysis was determined by sieve and pipette, following pre-treatments for removal of organic matter and soluble salts and chemical dispersion with sodium hexametaphosphate (method 3A1a1a). All methods are described in the KSSL Manual (Soil Survey Staff, 2014a).

2.2. Experimental design and treatments

The experiment was a 2 × 5 factorial with 3 replications of the following treatments: 2 methods of sample homogenization (< 2 mm and < 0.18 mm grind size) and 5 soil sample masses (0.25, 0.5, 1.0, 2.5, or 5.0 g). To prepare < 0.18 mm material, ~15 g of crushed soil (< 2 mm) was finely ground using a planetary ball mill with silicon nitride bowls and balls to pass a 0.18 mm mesh, except for the organic soil, where ~50 g soil was ground in a Wiley mill to pass a 0.18 mm mesh. In the remainder of the text we refer to those two treatments as “grind size”.

2.3. POXC determinations

The POXC method involves reacting a dilute MnO_4^- solution with a soil sample of a given mass for a fixed time. The MnO_4^- solution has a deep violet color that fades as SOC oxidation consumes MnO_4^- . The color of the supernatant after reaction is measured via spectrophotometry and related to the remaining MnO_4^- . A low absorbance value thus signifies high POXC. POXC was determined according to method 6A2a1a1 in the KSSL Manual (Soil Survey Staff, 2014a), based on Weil et al. (2003). Briefly, soil was mixed with 20 mL of 0.020 M KMnO_4 in 50 mL centrifuge tubes. Each sample was thoroughly mixed, allowed to stand for 10 min, and then centrifuged for 10 min at 2000 rpm. A 0.5 mL aliquot of the supernatant was combined with 49.5 mL of deionized water in a 50 mL tube and mixed by inversion. This extract was transferred by pipette to cuvettes and absorbance (550 nm wavelength) was determined on a 4-point calibrated UV-Visible spectrometer (Agilent Technologies, Cary 60 UV-Visible Spectrometer). Each analytical replicate was analysed within the same batch, on the same day, by the same analyst, using the same spectrophotometer. A new standard curve was made daily and applied to all POXC batches that were analysed on that day. POXC was calculated from absorbance (Eq. (1)).

$$\text{POXC (mg kg}^{-1}\text{)} = \frac{(0.020 \text{ mol L}^{-1} - (a + (b \times \text{Abs}_{\text{adj}})) \times (9000 \text{ mg C mol}^{-1}) \times (0.02 \text{ L})}{\text{Soil mass (kg)}} \quad (1)$$

where 0.020 mol L⁻¹ = initial MnO_4^- concentration; *a* is the intercept of the standard curve, *b* is the slope of the standard curve, *Abs_{adj}* is the adjusted absorbance, 9000 = mg C (0.75 mol) assumed to be oxidized by 1 mol MnO_4^- changing from Mn_7^+ to Mn_4^+ (Weil et al., 2003); 0.02 L = volume of KMnO_4^- solution mixed with the soil sample; *Soil mass* = oven-dry mass of soil sample analysed (kg).

2.4. Data processing and analysis

The results obtained for individual sample replicates ([dataset] Wills et al., 2020) were checked for their quantitative validity based on the limits of quantitation for the final MnO_4^- concentrations in the cuvettes. The limits of quantitation are the lowest and highest concentrations of an analyte that can be quantitatively determined with suitable precision and accuracy. Measurements that fall outside of these limits were excluded from further analysis in order to enhance confidence in the results and ensure that our conclusions are supported by quantitatively meaningful data.

The *Practical Quantitation Limit* (PQL) is the minimum analyte concentration (MnO_4^- in case of the POXC method) that can be reliably quantified and pertains to the maximum amount of POXC that can be reliably reported. The PQL was established following the recommendations of the US Environmental Protection Agency (EPA, 2016) for determining the Method Detection Limit (MDL), which for the POXC method at KSSL was 0.004 mM MnO_4^- . To calculate the PQL, the MDL was multiplied by a factor of 3, resulting in a PQL of 0.012 mM. MnO_4^- concentrations below this value were considered quantitatively invalid for this study. No governmental regulation covers the PQL. It comes down to what the laboratory feels comfortable signing their name to, confidently, on a daily basis (EPA, 2006). The KSSL laboratory was comfortable with a factor of 3.

The *Upper Detection Limit* (UDL) is the final MnO_4^- concentration that is statistically distinguishable from a blank sample reading, considering that blank samples show varying final MnO_4^- concentrations due to method imprecision. The UDL thus represents the minimum drop in MnO_4^- concentration to be considered significant, and pertains to the minimum amount of POXC that can be reliably reported. The UDL was estimated as the mean MnO_4^- concentration of blank samples from over 100 validated batches, minus three times the standard deviation of that mean, which yielded an UDL of 0.175 mM. Thus, a final MnO_4^- concentration in the cuvettes of > 0.175 M was considered potentially indistinguishable from a blank sample, and the corresponding POXC results were thus considered quantitatively invalid for this study.

Values of POXC were reported for each sample, based on the average of three analytical replicates for a given sample, grind and mass class. Only samples for which all three replicates yielded results within the quantifiable range (i.e. final MnO_4^- concentrations between the UDL and PQL) were used in further analysis. No further data cleaning or removal of outliers was performed. The analytical variability in POXC values was reported as the coefficient of variation (CV) of three analytical replicates that had POXC results within the quantifiable range. Pearson (r) and Spearman rank (r_s) statistics for linear and non-linear correlations, respectively, were analysed in SPSS v25.

3. Results

3.1. Effect of soil mass and grind size on quantifiable POXC values

POXC values were not validated when measured MnO_4^- concentrations were outside of the method quantitation limits, and hence excluded from further analysis. The proportion of samples that exceeded the UDL (i.e. MnO_4^- concentration not distinguishable from a blank reading) strongly increased with decreasing soil mass and was higher for the < 2 mm than for the < 0.18 mm grind size (Fig. 1). Conversely, the chances of the final MnO_4^- concentration being below the PQL (i.e. below 0.012 mM) increased with increasing soil mass and was higher for the smaller grind size. Overall, 60% of all sample \times treatment combinations had results within the quantifiable range. There was no single treatment combination that provided POXC values for all the soil samples included in this study. Moreover, the combinations of grind size and soil mass that returned POXC results that were within the quantifiable range were clearly associated with SOC content (Fig. 2; Table 2).

For both grind sizes, the highest number of POXC results within the quantifiable range was obtained when using a soil mass of 2.5 g (79 and 88% for the < 0.18 mm and the < 2 mm grind size, respectively; Table 2). When < 2 mm grind size and 2.5 g of soil were used, POXC results could be quantified for soil samples that had SOC contents between 5.0 and 75.8 g kg^{-1} (Fig. 2). When using 5.0 g of soil, POXC could only be quantified for soil samples that had SOC contents up to 25.9 g kg^{-1} . By comparison, when a grind size of < 0.18 mm and a soil mass of 2.5 g was used, POXC could be quantified for samples with SOC contents up to 31.6 g kg^{-1} (79% of soil samples; Table 2). Using 5.0 g of soil, POXC could only be quantified for 38% of the samples and the relation with SOC content was less consistent.

3.2. Soil mass and grind size effects on analytical variability

The coefficient of variation (CV) was calculated from three analytical replicates of sample by treatment combinations. The CV was higher for the < 2 mm grind size (mean 6.0%; median 4.3%) than for the < 0.18 mm grind size (mean 3.8%; median 0.5%; Table 3). The analytical variability also showed a general tendency to decrease with increasing soil mass, from an average of 5.5% (median 0.83%) to 1.2% (median 0.05%) at < 0.18 mm grind size and from an average 8.2% (median 6.8%) to 2.9% (median 0.1%) at < 2 mm grind size. The proportion of samples that had a CV above 10% was relatively high (17.6–41.2%) when using a soil mass of 1.0 g or less, especially at < 2 mm grind size (32.2–41.2% of samples had a CV $> 10\%$; Table 3). Even at a soil mass of 2.5 g and a grind size of < 2 mm, the proportion of samples with CV $> 10\%$ exceeded 20% of the total number of samples for which POXC could be quantified. Overall, the number of samples with a CV $> 10\%$ decreased with increasing soil mass for both grind sizes.

3.3. POXC values as affected by soil mass

POXC values varied widely due to soil mass. Average POXC values across the whole set of soil samples increased with decreasing soil mass, from 425 (± 157) to 3870 (± 2443 mg kg^{-1}) for the < 0.18 mm grind size, and from 430 (± 161) to 3464 (± 1562) mg kg^{-1} for the < 2 mm grind size (Fig. 3a). The non-linearity of the relationship between soil mass and POXC shown in Fig. 3a is extended to the relationship between soil mass and the relative POXC value, or in other words the percentage of the total SOC content of the sample that is oxidized (Fig. 3b). However, the plots shown in Fig. 3 provide a biased picture of soil mass effects on POXC as soil samples high in SOC tend to return results within the method quantitation limits only at low soil masses, whereas for soil samples low in SOC, POXC values within quantitation limits were obtained at high soil masses. This bias is evidenced by the median of the SOC contents of the samples for which POXC could be quantified at a soil mass of 5.0 g (9.7 and 13.6 g kg^{-1}) versus a soil mass of 0.25 g (33.5 and 60.7 g kg^{-1}), for < 0.18 and < 2 mm grind sizes, respectively (Table 2).

Therefore, we proceeded to analyse the effect of soil mass on POXC in an unbiased way, by comparing POXC values obtained at different soil masses, but only including those soil samples for which valid POXC values were obtained for the same set of soil masses (Fig S3; Table S1). There was only one soil sample for which POXC could be quantified using all five soil masses, and only for the < 2 mm grind size. POXC values for this sample ranged from 617 mg kg^{-1} at a soil mass of 5.0 g to 3137 mg kg^{-1} at 0.25 g, a > 5 -fold increase (Fig S3b; Table S1). The relations between soil mass and POXC, limited to those soil samples for which valid POXC values were obtained for the same set of soil masses, confirmed the non-linearity of the inverse relation between soil mass and POXC (Fig. S3). Mean POXC values obtained for the same subset of samples at three soil masses and the < 0.18 mm grind size ($N = 19$) ranged from 1027 (± 198) mg kg^{-1} at a soil mass of 2.5 g to 1546 (± 444) mg kg^{-1} at a soil mass of 0.5 g (Table S1). Similarly, the mean

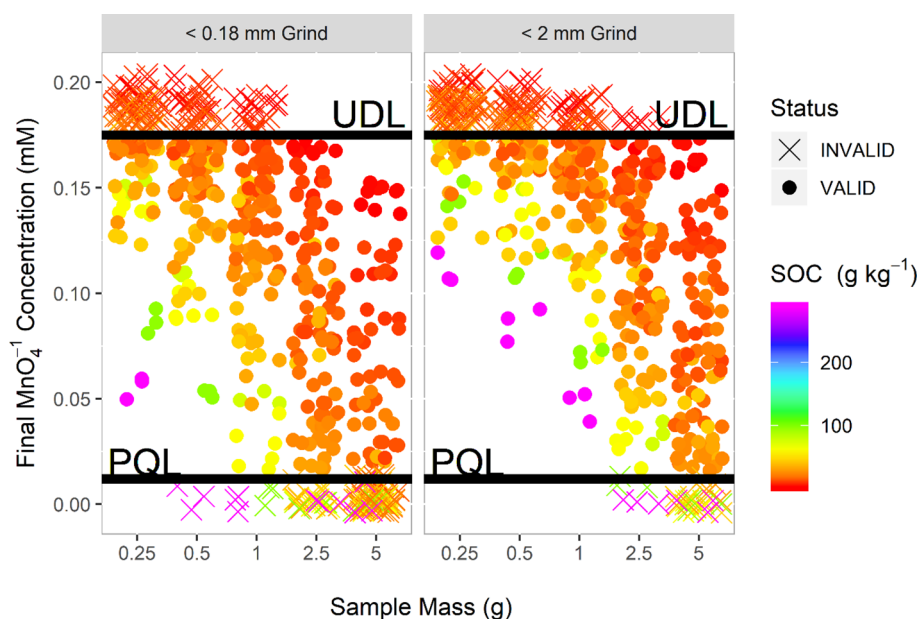


Fig. 1. Final permanganate concentration measured by the spectrophotometer for individual sample replicates (dots) \times grind size and sample mass. Different colors refer to the SOC content of the samples. Results were considered quantitatively invalid for this study when final MnO_4^- concentrations fell between the Upper Detection Limit (UDL) and the Practical Quantitation Limit (PQL) for the method. Note that the final permanganate solution after reaction is diluted 100 times before measurement.

POXC values obtained for the same subset of samples at three soil masses and the < 2 mm grind size ($N = 20$) showed mean POXC values ranging from $523 (\pm 93 \text{ mg kg}^{-1})$ at a soil mass of 5.0 g to $794 (\pm 236) \text{ mg kg}^{-1}$ at 1.0 g, in both cases corresponding to an average increase in POXC values by $\sim 50\%$.

Overall, strong correlations were found between validated POXC values obtained at two different soil masses. Pearson correlation coefficients ranged between 0.91 and 0.97 ($p < 0.001$) and the slope ranged from 1.29–1.52. The lower correlation coefficients represent the coarser grind size and smaller soil masses (Fig. 4).

3.4. POXC values as affected by grind size

To test the hypothesis that POXC values increase with decreasing grind size used for sample homogenization, we used all sample ID \times soil mass combinations for which POXC could be quantified for

both grind sizes ($N = 100$; Table S2). POXC values were consistently greater for the finer grind size (< 0.18 mm) (Fig S4). The increase in POXC values due to grinding to < 0.18 mm was on average $49\% (\pm 25\%)$, when compared to the < 2 mm grinding treatment (Table S2). The correlation between POXC values obtained for the two grind sizes was very strong ($r = 0.96$, $p < 0.001$; Fig. 5a), especially after log transformation to account for the skewed distribution of the two variables ($r = 0.98$, $p < 0.001$; Fig. 5b).

3.5. Relative POXC values as a function of SOC mass.

The marked non-linear decline in POXC values with increasing soil mass shown in Figs. 3 and 4 reflects the non-linear increase in the proportion of MnO_4^- that is consumed during the redox reaction when soil mass increases (Fig. 3b) and from which POXC values are calculated. Fig. 3 shows a strong variation in the fraction of MnO_4^-

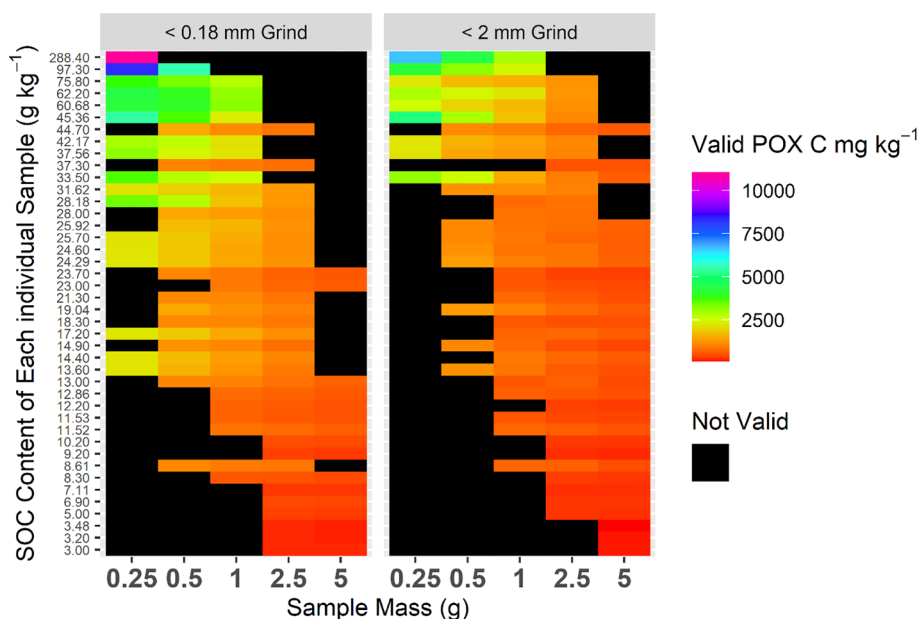


Fig. 2. Effects of soil mass and grind size on POXC values. Samples are sorted by total SOC content. Black cells indicate that at least one of the analytical replicates yielded results outside the quantitation limits of the method for this study.

Table 2

Minimum and maximum POXC values obtained for different soil masses, based on the theoretical limits of the method and on the Upper Detection Limit (UDL) and the Practical Quantitation Limit (PQL) for our study. The percentage of samples ($n = 42$) for which quantitatively valid POXC data were obtained, and the median SOC content, are shown for each soil mass. Two grind sizes (< 0.18 and < 2 mm) were compared.

Soil mass	POXC theoretical limits		POXC practical limits		Valid results		Median of SOC content	
	POXC _(min)	POXC _(max)	POXC _(UDL)	POXC _(PQL)	< 0.18 mm	< 2 mm	< 0.18 mm	< 2 mm
(g)	(mg kg ⁻¹)		(mg kg ⁻¹)		(%)		(g kg ⁻¹)	
0.25	0	14,400	1800	13,536	40	21	33.5	60.7
0.50	0	7200	900	6768	64	40	25.9	33.5
1.00	0	3600	450	3384	76	74	24.0	24.6
2.50	0	1440	180	1354	79	88	14.4	21.3
5.00	0	720	90	677	38	74	9.7	13.6

Table 3

Analytical variability expressed as the coefficient of variation (CV) among three analytical replicates analysed for each sample and treatment combination for which quantitatively valid POXC data were obtained. N is the number of samples or sample by soil mass combinations (out of a total of 42 samples) that returned results within the quantitation limits for all three analytical replicates.

Soil mass	< 0.18 mm				< 2 mm			
	N	CV _{mean}	CV _{median}	CV $> 10\%$	N	CV _{mean}	CV _{median}	CV $> 10\%$
(g)	(%) -				(%)			
0.25	17	5.5	0.83	17.6	9	8.2	6.8	33.3
0.50	27	4.9	0.60	22.2	17	8.6	6.8	41.2
1.00	32	4.5	0.38	18.8	31	6.1	6.8	32.3
2.50	33	2.7	0.12	3.0	37	6.6	5.1	21.6
5.00	16	1.2	0.05	0.0	31	2.9	0.1	6.5
All; mean/median	125	3.8	0.5	12.8	125	6.0	4.3	24.0
All; stdev		6.2				6.6		

consumed (Fig. 3b), and the resulting absolute (Fig. 3a) and relative POXC values (Fig. 3c), depending on sample ID, which is likely related to the large range of SOC values represented by our sample set.

Fig. 6 shows the relationships between the SOC mass (the soil mass multiplied by the sample's SOC content, which represents the amount of SOC that is subjected to the reaction with permanganate) and the fraction of MnO_4^- consumed (Fig. 6a) and between SOC mass and relative POXC values (expressed as the percentage of a sample's total SOC content) (Fig. 6b). Significant ($p < 0.001$) and strong Spearman's rank correlations were found between SOC mass and the fraction of MnO_4^- consumed ($r_s = 0.90$ for both grind sizes; Fig. 6a). The correlation between SOC mass and the relative POXC value was highly significant and negative ($r_s = [-0.78] - [-0.75]$, for the < 2 mm and the < 0.18 mm grind sizes, respectively; Fig. 6b).

3.6. Absolute and relative POXC values across soils, at a fixed soil mass of 2.5 g

To further explore the relationships between sample SOC content and absolute and relative POXC values obtained when using a fixed soil mass, we used the POXC values obtained for the 2.5 g soil mass. This soil mass treatment returned the largest number of validated POXC results for both grind sizes (Table 2). Significant ($p < 0.001$) positive correlations were found between the sample SOC content and the POXC value for samples prepared at < 0.18 ($r_s = 0.80$) and < 2 mm ($r_s = 0.83$) grind size (Fig. 7a). The relative POXC value expressed as a percentage of total SOC content, decreased with increasing SOC content ($r_s = 0.74$ and 0.70 , respectively) (Fig. 7b).

4. Discussion

4.1. Method quantitation limits hinder the applicability of POXC protocols across different soils

Our study showed that the percentage of soils for which POXC values could be reliably quantified depends strongly on soil mass and grind size, and is influenced by the total SOC content of the soil. Importantly, no single soil mass (0.25–5.0 g) provided results within the quantitation limits of the method for all soils included in our study. The soil mass that yielded the highest percentages of quantifiable results for both grind sizes was 2.5 g, which is in line with the protocol of Culman et al. (2012a). Yet, a significant percentage of our soils (11 and 21%, depending on grind size) had POXC values outside the quantitation limits, indicating that a protocol based on a fixed soil mass is not universally applicable across different soils. Based on our sample set, assessing POXC was not feasible for soils that have relatively high (> 76 g C kg⁻¹) or low (< 5 g C kg⁻¹) SOC content, when using a grind size of < 2 mm and a soil mass of 2.5 g. A standard soil mass of 5.0 g (Weil, et al., 2003; Soil Survey Staff, 2014a) had limited applicability to soils with SOC contents > 26 g C kg⁻¹.

The method quantitation limits thus complicate the assessment and comparability of POXC at national levels, and even more so globally, as many soils will yield results outside the quantifiable POXC range for a certain soil mass. A few published studies assessing POXC reported that the experimental conditions of the assay did not account for a surplus of labile C in relatively carbon-rich grassland and garden soils (Calderón et al., 2017; Romero et al., 2018). Calderón et al. (2017) highlighted that increasing the detection limits would require method adjustments, e.g., by increasing the concentration of MnO_4^- and/or decreasing the soil mass used. Interestingly, the POXC protocols for the assessment of active C in version 5 of the KSSL manual (Soil Survey Staff, 2014a) and the protocol of Schindelbeck et al (2016) recommend such adjustments, namely “If samples have < 0.00003 absorbance (A), reweigh smaller

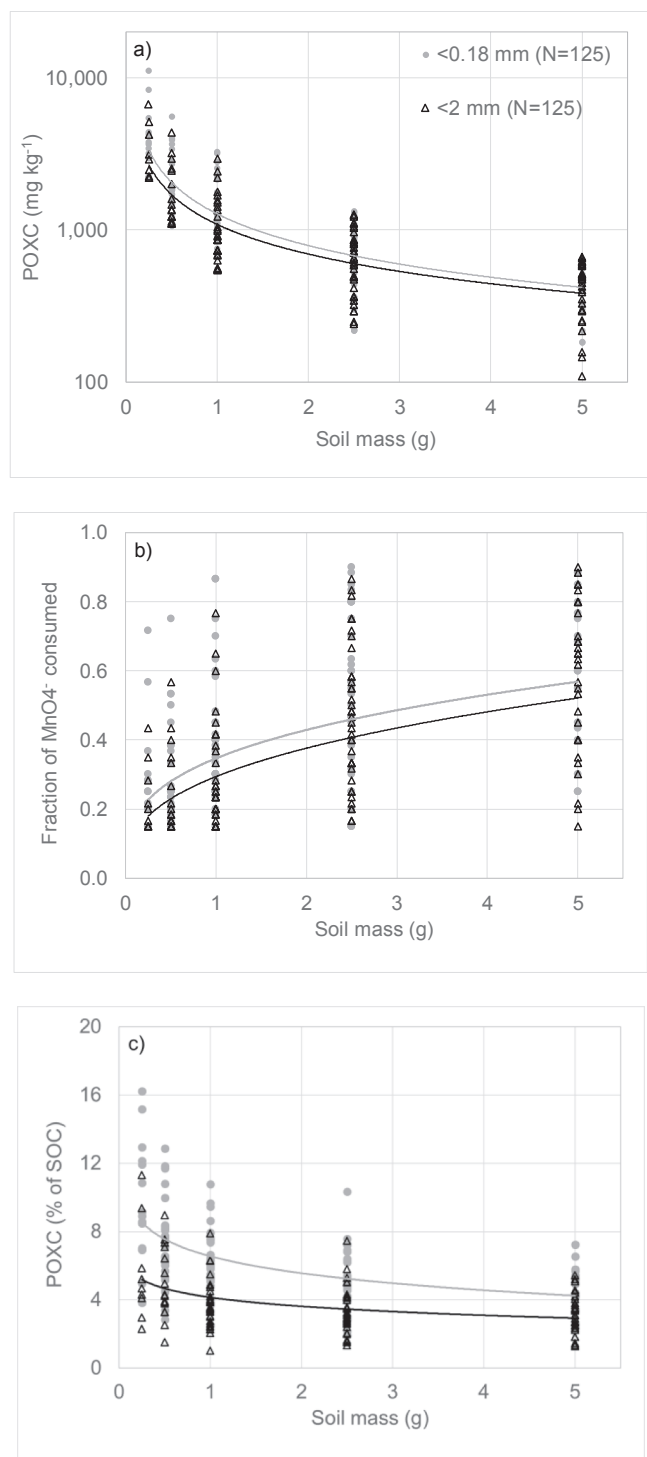


Fig. 3. (a) POXC values obtained; (b) the fraction of MnO_4^- consumed during the oxidation reaction; and (c) relative POXC values, expressed as a percentage of the total SOC content of the sample, for all sample ID \times soil mass combinations for which results were obtained that were within the method quantitation limits for this study, for the <0.18 mm ($N = 125$) and <2 mm ($N = 125$) grind sizes. Note that the POXC values in Fig. 3a are shown on a logarithmic scale.

sample size (e.g., 2.50 g) and re-analyse" (method 6A2a1a1; Soil Survey Staff, 2014a); and "Repeat samples when duplicate sample absorbance readings fall outside the values of the standard curve, adjusting weight of sample used in reaction if necessary" (Schindelbeck et al., 2016). This paper's authors and other researchers (personal communication)

also reported that soil mass is sometimes adjusted for part of the samples within the same study in order to overcome problems with method detection limits as indicated by complete 'bleaching' of the initially purple solution. Based on our literature review, none of the published POXC assessments provide laboratory-specific method quantitation limits, and very few have described how they dealt with results that fall outside of these limits. Reporting method detection and quantitation limits alerts data users of the uncertainties and limitations associated with using these data. We strongly recommend that such information be reported in future studies and refer to EPA (2006) and EPA (2016) for further guidance on the appropriate use and interpretation of method quantitation limits.

4.2. Soil mass affects POXC values

Decreasing or increasing soil mass in order to obtain final MnO_4^- concentrations within method quantitation limits could potentially address issues of non-validated POXC (Soil Survey Staff, 2014a; Schindelbeck et al., 2016). However, our study shows that variation in soil mass causes substantial variation in POXC values, thereby limiting the comparability and interpretability of POXC values. In our study, a clear negative and exponential relationship between soil mass and POXC values was observed. These findings are similar to results reported by Gruver (2015), who compared POXC values for fifteen different masses of a standard soil (0.26 to 4.6 g) based on the Weil et al. (2003) method with and without the addition of acid-washed quartz sand to maintain a constant solid:solution ratio. Gruver (2015) found that the addition of quartz sand did not affect POXC results, which supports our assumption that variation in the ratio between solids and reaction solution should not have affected our results.

Strong Pearson correlations ($0.91 < r < 0.97$; $p < 0.001$) were observed between the POXC values obtained for two different soil masses, where the lowest correlation coefficient ($r = 0.91$) was found for the relation between POXC results at 1.0 and 2.5 g soil mass and a grind size of <2 mm, probably linked to the relatively high CV between analytical replicates at a small soil mass (i.e. 1.0 g) and coarser grind size. While strong correlations between POXC values obtained at different soil masses, a finding that was also reported by Wade et al. (2020), indicate similar trends across the different soils irrespective of the soil mass used, the POXC values obtained at different soil masses cannot be compared directly without conversion. Sample-specific variation in the effect of soil mass for individual soil samples would likely limit the general applicability of such a conversion, as interactions between specific soil characteristics and the amount of SOC available to be reacted determine the outcome of the reaction between soil and MnO_4^- (Blair et al., 1995). We further elaborate on this phenomenon in Section 4.3.

4.3. Implications of the non-linearity of the relationship between soil mass and POXC.

The increase in POXC values with decreasing soil mass, and the non-linearity of this relationship, can be explained by the negative and non-linear relationship between the amount of soil reacting with 20 mL of 0.02 M MnO_4^- (0.40 mmol MnO_4^- in total) and the fraction of MnO_4^- consumed at the end of the assay. In other words: the fraction of total MnO_4^- reduced *per unit mass of soil* decreases non-linearly as soil mass increases. This phenomenon, previously identified by Gruver (2015), and corroborated in the current study for a larger sample set representing diverse US soils, is explained by the kinetics of the oxidation reaction of MnO_4^- with soil. The rate of oxidation is a function of the reaction conditions (MnO_4^- concentration, pH, temperature, among others) and the amount and chemical nature of different MnO_4^- reductants. Reductants include different organic substrates that can be oxidized by MnO_4^- (e.g. lignin or carbohydrate-like species and (poly) phenols; Bose et al., 1999, Romero et al., 2018) and that react at

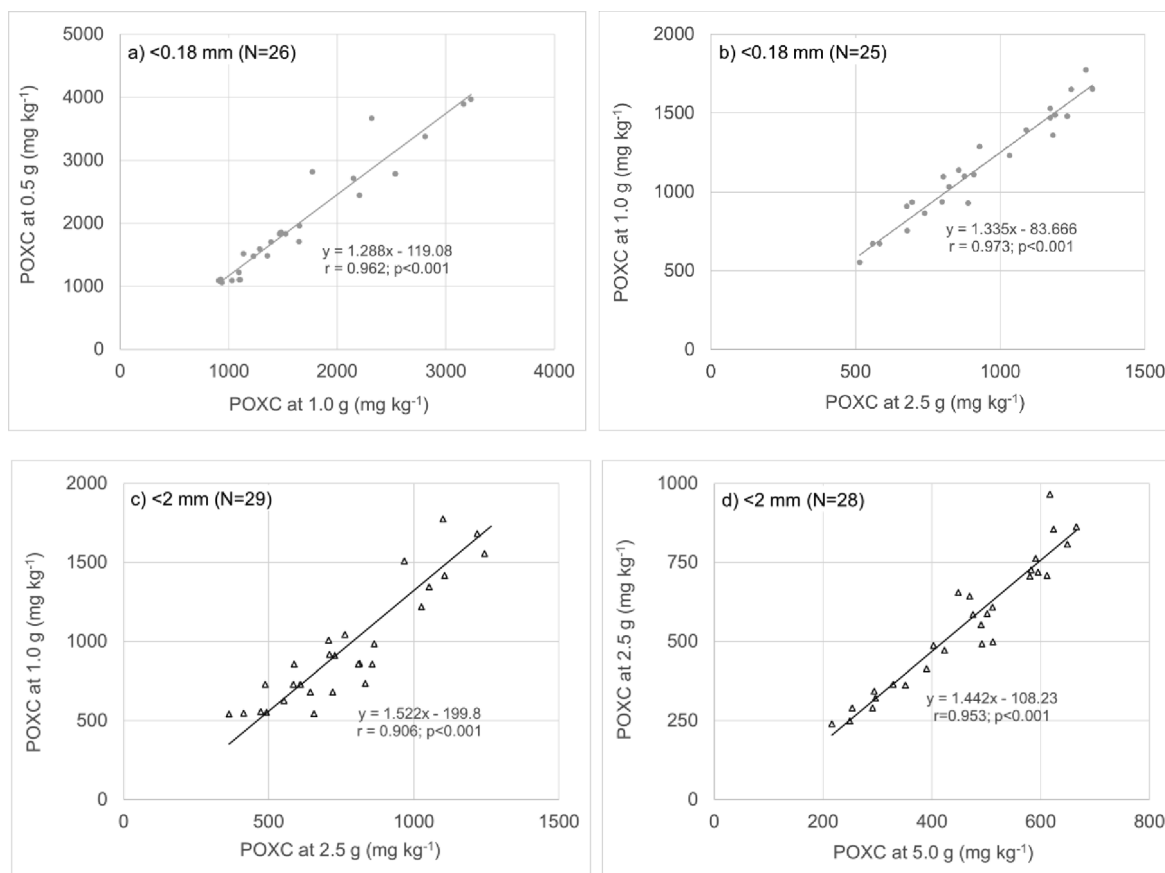


Fig. 4. Pearson correlations between POXC values at smaller soil mass and at larger soil mass for samples for which validated POXC (results within method quantification limits) were obtained for the same two soil masses: a) < 0.18 mm grind size, 0.5 and 1.0 g soil mass (N = 26); b) < 0.18 mm grind size, 1.0 and 2.5 g soil mass (N = 25); c) < 2 mm grind size, 1.0 and 2.5 g soil mass (N = 29); d) < 2 mm grind size, 2.5 and 5.0 g soil mass (N = 28).

different rates proportional to the MnO_4^- concentration. As the concentration of MnO_4^- decreases, reaction rates slow down implying that, as reactions progress, the more rapidly reacting forms of POXC are preferentially oxidized and the remaining MnO_4^- concentration determines the extent to which less quickly reacting forms of POXC will be oxidized. Therefore, a soil with a small amount of oxidizable C will likely result in the oxidation of more recalcitrant C fractions (causing overestimation of “labile C”) than a similar soil with a large amount of oxidizable C. Conversely, a larger soil mass results in a faster decrease of the MnO_4^- concentration during the reaction than a smaller soil mass, therefore the oxidation rates of all forms of oxidizable SOC in the sample are reduced, yielding a lower measured POXC value and an underestimation of “labile C”.

The non-linear relationships between soil mass and POXC shown in Figs. 3a and 4, and between soil mass and the relative POXC value (expressed as a percentage of total SOC content of the sample) shown in Fig. 3c, clearly demonstrate that (i) reactions between soil and MnO_4^- go to varying levels of completion, and (ii) not all potentially permanganate-oxidizable C that can react, does react, on the short time scale of the POXC assay. This indicates that the reaction that takes place during the POXC assay is uncontrolled, leading to different interpretations on the size of the “labile” or “active” carbon pool, depending on the methodological parameters used, including soil mass and grind size.

Furthermore, when we consider that soils with higher SOC contents result in a faster decline in the MnO_4^- concentration during the reaction, a lower oxidation rate of the potentially oxidizable SOC fractions could be expected, leading to an underestimation of the “active” or “labile” C pool. We indeed showed that the correlation between SOC mass (soil mass \times SOC content of the sample) and the percentage of total SOC quantified as POXC was strongly negative (Fig. 6b). This

suggests that the relationship between soil mass and POXC is sample-specific, as the $[\text{MnO}_4^-]$: SOC mass ratio used in the POXC assay affects the size of the fraction of SOC being oxidized, and the biochemical nature of that fraction.

4.4. Grind size used for sample homogenization affects POXC values

As hypothesized, a smaller grind size used for sample homogenization resulted in higher POXC values for a given sample and hence a greater proportion of the total SOC content of that sample being oxidized. On average across soils, the POXC values increased by 49% ($\pm 25\%$) due to the smaller grind size of < 0.18 mm, compared to < 2 mm, irrespective of soil mass. The significant increase in POXC due to grinding to < 0.18 mm is likely due to disintegration of aggregates and particulate organic matter into smaller particles, making potentially oxidizable SOC compounds more available to oxidation and leading to a higher surface area to volume ratio which speeds up chemical and biological reactions (Gruver, 2015; Romero et al., 2018). Soil homogenization by grinding further exposes otherwise protected organic matter and stable C fractions adsorbed to mineral surfaces. Sample-specific effects of grind size likely reflect variation in the characteristics of particulate organic matter and in the level of physical protection among soils. Gruver (2015) found that disruption of soil aggregates (initially sieved over 2 mm) using different shaking treatments in water increased POXC levels by 29–44 %, depending on different levels of intensity of disruption, in a high SOC soil and by 14–18% in a low SOC soil. Nevertheless, the very strong correlation ($r = 0.98$) between POXC values obtained at different grind sizes, a finding that was also reported by Wade et al. (2020), indicates that general trends in POXC values are consistent across grind sizes, but absolute POXC values obtained at

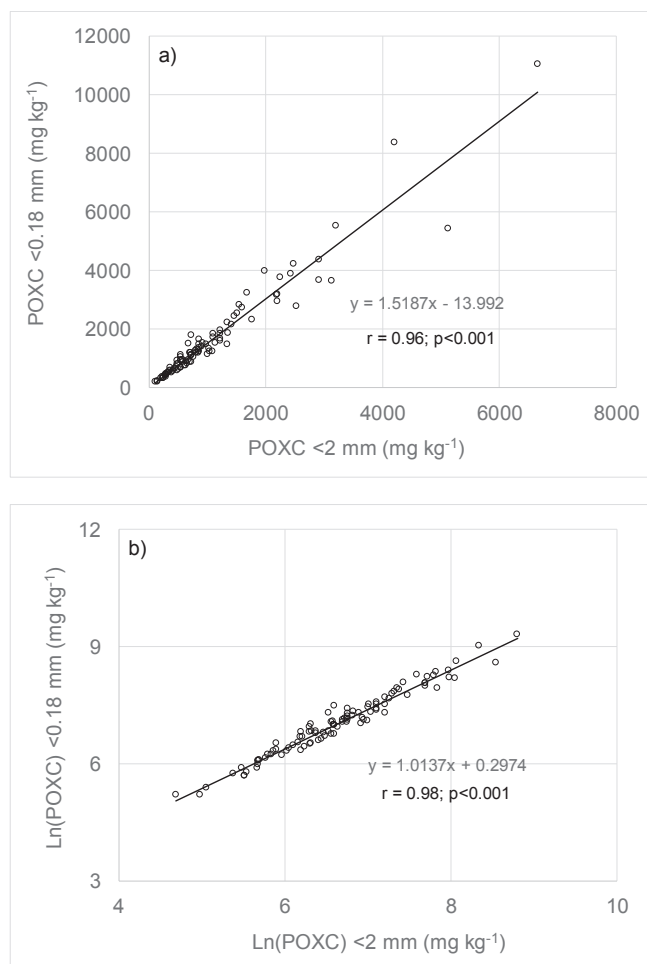


Fig. 5. The correlation between POXC values obtained for the two different grind size treatments (< 0.18 mm and < 2 mm), showing (a) non-transformed and (b) log transformed data. Data shown include all sample ID \times soil mass combinations for which POXC could be quantified (results within method quantitation limits for this study) for both grind sizes ($N = 100$).

different grind sizes are not directly comparable.

Comparing POXC values obtained at different grind sizes could be an appropriate research method to investigate physical protection of C fractions, but when used as a broad soil health indicator, standardization of grind sizes used for soil homogenization is an important prerequisite for POXC values to be comparable across soils. We propose that the gentler soil process of crushing to pass the 2 mm mesh would better reflect the biologically available pool of SOC than grinding to pass a 0.18 mm sieve, while allowing for some level of sample homogenization at reduced sample processing time.

4.5. Analytical variability as affected by soil mass and grind size

Ideally, indicators for routine soil health assessments should have low analytical variability. The variability among three analytical replicates in our study was relatively low with average CVs being 3.8% and 6.0%, for < 0.18 mm for < 2 mm grind sizes respectively. These percentages are lower than Hurisso et al. (2018b) who had CVs of 9–19% for POXC measurements using 2.5 g of soil. Wade et al. (2020) found similar results for analytical variability as in our study, with CVs ranging between 5.1 and 10.7% in the majority of labs participating in an interlaboratory study, depending on soil mass and grind size.

In our study, the treatment combination of 2.5 g soil mass and grinding at < 2 mm resulted in an average CV of 6.4% (median 5.1%). Although finely ground soil samples had lower CVs, this advantage has

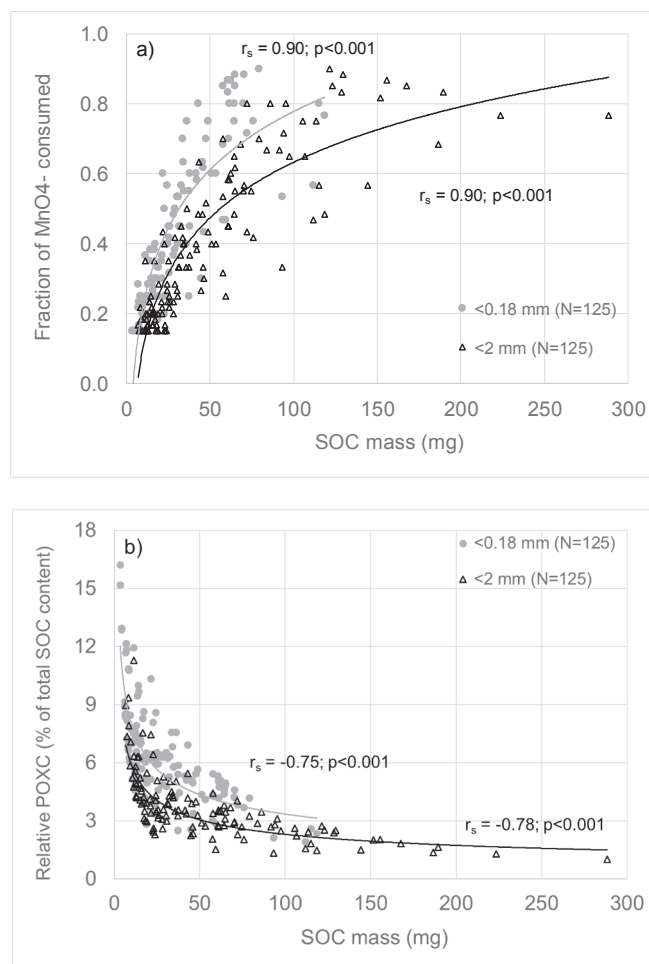


Fig. 6. Non-linear correlation between SOC mass (in mg C; calculated as the soil mass multiplied by the SOC content of a given sample) and (a) the fraction of MnO_4^- consumed and (b) relative POXC values (expressed as a percentage of total SOC content of a sample). These graphs include all validated POXC results for the < 0.18 mm ($N = 125$) and < 2 mm grind sizes ($N = 125$). Correlation coefficients refer to Spearman's rank statistics.

to be weighed against reduced sample processing time, as well as the more intact physical structure for the < 2 mm soil, which likely better reflects “labile” or “active C” under field conditions. Moreover, using soil ground at < 0.18 mm requires the use of smaller soil masses to not exceed the method quantitation limits, which would largely reduce the benefits in terms of decreased analytical variability as can be seen in Table 3.

4.6. Implications for the utility of POXC in national soil health monitoring

Our study showed that determination of POXC is highly sensitive to soil mass and grind size. This implies that the grind size used for soil homogenization should be standardized to enable comparisons of the outcomes across soils and studies. Using the forceful milling of soil to pass a small mesh size (e.g., < 0.18 mm) would not be desirable as it is more labor intensive. Our recommendation for POXC would be to crush and sieve the soil samples to pass a 2 mm mesh which is a widely used soil homogenizing procedure and may better reflect “active SOC” under field conditions.

Standardization of soil mass in POXC protocols, especially when used as a national soil health indicator may be more complicated. This complication derives from our observation that the effect of soil mass on POXC is non-linear and soil-specific. First, there is no single soil mass which consistently produces POXC results within the quantitation limits

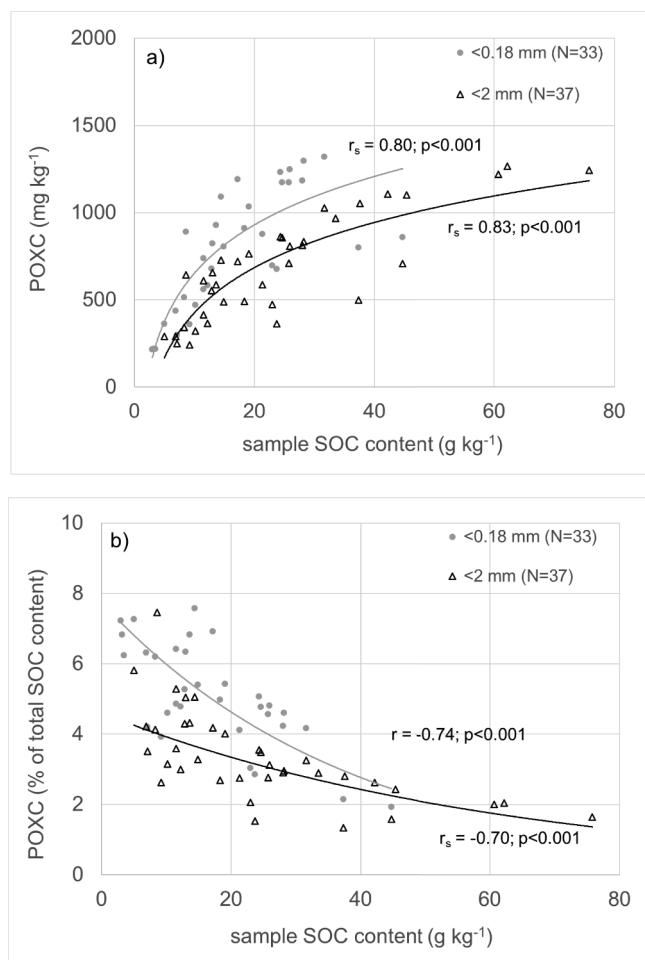


Fig. 7. Relation between sample SOC content (g C kg^{-1} soil) and (a) POXC values; and (b) relative POXC values (expressed as a percentage of total SOC content of a sample). Data shown here were obtained using a fixed soil mass of 2.5 g for samples prepared at < 0.18 ($N = 33$) and < 2 mm ($N = 37$) grind sizes. Correlation coefficients refer to Spearman rank statistics for non-linear correlations.

of the method for a wide range of different soils and adjusting the soil mass for certain samples would make it impossible to compare the results to other samples (Calderón et al., 2017). Second, we showed that the negative relationship that exists between SOC mass and the fraction of total SOC being oxidized by permanganate (Fig. 7b), and subsequently interpreted as the pool of “labile” or “active” carbon, limits the universal interpretability of the POXC method. Calderón et al. (2017), in line with our results (Fig. 7b), concluded that soils high in SOC tend to have proportionally less labile C than soils with lower SOC. What this likely indicates is that in low SOC soils there is rapid oxidation of the smaller amount of labile organic C, followed by greater oxidation of the relatively recalcitrant organic C than would occur in higher SOC soils.

Based on these results, we argue that a fundamental problem of the POXC method is that it is an uncontrolled oxidation reaction that is not a specific “extractant” or measure of a certain C fraction and that it does not consistently measure the same carbon pool across different soils. Therefore, the POXC method as it is currently applied should not be viewed as an approach for measuring a well-defined SOC pool that can be quantitatively compared across a wide range of contrasting soils. Assessment of POXC based on standard soil mass and grind size may allow for comparison of closely associated fields or for monitoring of fields through time where differences in soil properties are relatively modest. However, considering implications for national or regional monitoring schemes, the method should be robust when used in widely

different soil types and vegetation types, and this is where serious problems with quantitation limits and non-linearity can be expected.

The current POXC method should therefore be critically investigated and updated to address variation in POXC due to variation in soil mass and SOC mass. Lefroy et al (1993), Blair et al (1995) and Tirol-Padre and Ladha (2004) performed permanganate oxidation of SOM based on a fixed SOC mass instead of a fixed soil mass. This approach requires that total SOC is measured in advance and would thus compromise the rapidity and cost effectiveness of the POXC method, but it could improve comparability of POXC outcomes across soils varying in total SOC. Further research is needed to systematically evaluate the comparability and sensitivity of POXC values when assessed on a SOC mass basis instead of a soil mass basis, and to elucidate the chemical and functional characteristics of POXC and how these characteristics are affected by the oxidation conditions.

5. Conclusions

Our study confirms the sensitivity of POXC results to two major methodological parameters: soil mass and grind size used for soil homogenization. The following observations have important implications for the suitability and scientific interpretation of the POXC method used for national soil health monitoring:

- (1) None of the soil masses yielded POXC values within quantitation limits of the method for every soil across the diverse set of 42 US soils assessed, thus showing that standardization of soil mass may be impossible in national or regional monitoring programs. A soil mass of 2.5 g and a grind size of < 2 mm provided the largest of quantifiable POXC results across the wide set of soils we studied;
- (2) Strong correlations among POXC values obtained at different soil masses or grind sizes indicate that general trends in POXC values across soils are maintained, independent of soil mass or grind size, but the results are not interchangeable. We suggest that < 2 mm grind size be used in standard monitoring protocols, because minimal grinding and homogenization at < 2 mm is less time consuming and may better reflect “active C” under field conditions.
- (3) The use of a fixed soil mass for POXC analysis across soils with different SOC contents leads to variation in the $[\text{MnO}_4^-]$: SOC mass ratio during the reaction, which affects the size and the biochemical composition of the fraction of total SOC being oxidized. Therefore, we conclude that the POXC assay falls short as an analytical method for measuring a well-defined C pool. Performing POXC analyses based on a fixed SOC mass may enable improved comparability of POXC values across soils with contrasting SOM contents, but would compromise the utility of POXC as a rapid assessment method.

Finally, to enable calibration and interpretation of the results for the detection of land management impacts on soil health across a wide range of soils, further research is required. We recommend evaluation of the comparability and sensitivity of POXC values when assessed on a SOC mass basis rather than a soil mass basis, and investigation of the chemical and functional characteristics of POXC as obtained under different oxidation conditions.

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.

Acknowledgements

We thank Mr. Tom Zimmer, retired Physical Science technician at the Kellogg’s laboratory at the USDA NRCS National Soil Survey Center

(Lincoln, NE), who performed the vast majority of the POXC analyses for this manuscript. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2020.114742>.

References

- Blair, M.J., Moody, P.W., Connolly, B.R.D., Bridge, B.J., 1998. The role of active fractions of soil organic matter in physical and chemical fertility of Ferrosols. *Aust. J. Soil Res.* 36, 809–819.
- Blair, G.J., Lefroy, R.D., Lisle, L., 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. *Aust. J. Agric. Res.* 46, 1459–1466. <https://doi.org/10.1071/AR9951459>.
- Bongiorno, G., Bünnemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., et al., 2019. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. *Ecol. Ind.* 99, 38–50. <https://doi.org/10.1016/j.ecolind.2018.12.008>.
- Bose, S.K., Wilson, K.L., Hausch, D.L., Francis, R.C., 1999. Lignin analysis by permanganate oxidation. II. Lignins in Acidic Organosolv Pulps. *Holzforschung* 53, 603–610.
- Bünnemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R.G., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M.M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality - a critical review. *Soil Biol. Biochem.* 120, 105–125. <https://doi.org/10.1016/j.soilbio.2018.01.030>.
- Calderón, F.J., Culman, S., Six, J., Franzluebbers, A.J., Schipanski, M., Beniston, J., Grandy, S., Kong, A.Y.Y., 2017. Quantification of Soil Permanganate Oxidizable C (POXC) using infrared spectroscopy. *Soil Sci. Soc. Am. J.* 81, 277–288. <https://doi.org/10.2136/sssaj2016.07.0216>.
- Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Glob. Chang. Biol.* 19, 988–995. <https://doi.org/10.1111/gcb.12113>.
- Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat. Geosci.* 8, 776–779. <https://doi.org/10.1038/ngeo2520>.
- Culman, S., Freeman, M., Snapp, S., 2012a. Procedure for the determination of permanganate oxidizable carbon. *KBS POXC Protoc. – Last Revis.* 1, 1–5.
- Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirsky, S.B., Spargo, J.T., Wander, M.M., 2012b. Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. *Soil Sci. Soc. Am. J.* 76, 494–504. <https://doi.org/10.2136/sssaj2011.0286>.
- de Moraes Sá, J.C., Tivet, F., Lal, R., Briedis, C., Hartman, D.C., dos Santos, J.Z., et al., 2014. Long-term tillage systems impacts on soil C dynamics, soil resilience and agronomic productivity of a Brazilian Oxisol. *Soil Tillage Res.* 136, 38–50. <https://doi.org/10.1016/j.still.2013.09.010>.
- EPA, 2016. ENVIRONMENTAL PROTECTION AGENCY. Title 40 - Protection of Environment Chapter I - Subchapter D - WATER PROGRAMS. Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11. < <https://www.govinfo.gov/content/pkg/CFR-2016-title40-vol25/pdf/CFR-2016-title40-vol25-part136-appB.pdf> > .
- EPA, 2006. Region III Quality Assurance MDL Factsheet. “DL- MDL- PQL - What the “L” is going on? What does all this alphabet soup really mean?”. < <https://www.epa.gov/sites/production/files/2015-06/documents/whatthel.pdf> > .
- Gash, C., Mathews, S., Deschene, A., Butcher, K., DeSutter, T., 2020. Permanganate oxidizable carbon for soil health: does drying temperature matter? *Agric. Environ. Lett.* 5. <https://doi.org/10.1002/acl2.20019>.
- Gruver, J., 2015. Evaluating the sensitivity and linearity of a permanganate-oxidizable carbon method. *Commun. Soil Sci. Plant Anal.* 46, 490–510. <https://doi.org/10.1080/00103624.2014.997387>.
- Hanegraaf, M. Van den Elsen, E., De Haan, J. Visser S.M., 2019. Bodemkwaliteitsbeoordeling van landbouwgronden in Nederland – indicatorset en systematiek, versie 1.0 Wageningen Research, Rapport. <https://doi.org/10.18174/498307>.
- Hurisso, T.T., Culman, S.W., Horwath, W.R., Wade, J., Cass, D., Beniston, J.W., Bowles, T.M., Grandy, A.S., Franzluebbers, A.J., Schipanski, M.E., Lucas, S.T., Ugarte, C.M., 2016. Comparison of permanganate-oxidizable carbon and mineralizable carbon for assessment of organic matter stabilization and mineralization. *Soil Sci. Soc. Am. J.* 80, 1352–1364. <https://doi.org/10.2136/sssaj2016.04.0106>.
- Hurisso, T.T., Culman, S.W., Zone, P., Sharma, S., 2018a. Absolute values and precision of emerging soil health indicators as affected by soil sieve size. *Commun. Soil Sci. Plant Anal.* 49, 1934–1942. <https://doi.org/10.1080/00103624.2018.1492597>.
- Hurisso, T.T., Culman, S.W., Zhao, K., 2018b. Repeatability and spatiotemporal variability of emerging soil health indicators relative to routine soil nutrient tests. *Soil Sci. Soc. Am. J.* 82, 939–948. <https://doi.org/10.2136/sssaj2018.03.0098>.
- Lefroy, R.D.B., Blair, G.J., Strong, W.M., 1993. Changes in soil organic matter with cropping as measured by organic carbon fractions and ^{13}C natural isotope abundance. *Plant Soil* 155–156, 399–402. <https://doi.org/10.1007/BF00025067>.
- Margenot, A.J., Hodson, A.K., 2016. Relationships between labile soil organic matter and nematode communities in a California oak woodland. *Nematology* 18, 1231–1245. <https://doi.org/10.1163/15685411-00003027>.
- Margenot, A.J., Pulleman, M.M., Sommer, R., Paul, B.K., Parikh, S.J., Jackson, L.E., et al., 2017. Biochemical proxies indicate differences in soil C cycling induced by long-term tillage and residue management in a tropical agroecosystem. *Plant Soil* 420, 315–329. <https://doi.org/10.1007/s11104-017-3401-z>.
- Marriott, E.E., Wander, M.M., 2006. Total and labile soil organic matter in organic and conventional farming systems. *Soil Sci. Soc. Am. J.* 70, 950–959. <https://doi.org/10.2136/sssaj2005.0241>.
- Moebius-Clune, B.N., Moebius-Clune, D., Gugino, B., Idowu, O.J., Schindelbeck, R.R., Ristow, A.J., van Es, H., Thies, J., Shayler, H., McBride, M., Wolfe, D., Abawi, G., 2016. Comprehensive Assessment of Soil Health - The Cornell Framework Manual. <https://dx.doi.org/10.1080/00461520.2015.1125787>.
- Rennert, T., Ghong, N.P., Rinklebe, J., 2017. Permanganate-oxidizable soil organic matter in floodplain soils. *Catena* 149, 381–384. <https://doi.org/10.1016/j.catena.2016.10.020>.
- Roudier, P., Beaudette, D., Hewitt, A., 2012. A conditioned Latin hypercube sampling algorithm incorporating operational constraints. In: *Digital Soil Assessments and Beyond*, pp. 227–231.
- Romero, C.M., Engel, R.E., D'Andrilli, J., Chen, C., Zabinski, C., Miller, P.R., Wallander, R., 2018. Patterns of change in permanganate oxidizable soil organic matter from semiarid drylands reflected by absorbance spectroscopy and Fourier transform ion cyclotron resonance mass spectrometry. *Org. Geochem.* 120, 19–30. <https://doi.org/10.1016/j.orggeochem.2018.03.005>.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56. <https://doi.org/10.1038/nature10386>.
- Schindelbeck, R.R., Moebius-Clune, B.N., Moebius-Clune, D.J., Kurtz, K.S., van Es, H.M., 2016. Cornell University Comprehensive Assessment of Soil Health Laboratory Standard Operating Procedures 31–38. Cornell University, Geneva, NY.
- Schoeneberger, P.J., Wysocki, D.A., Benhameds, E.C., 2012. Field Book for Describing and Sampling Soils. Government Printing Office.
- Stockmann, U., Adamsa, M., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., Courcelles, V.D.R. De, Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., Odonnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agric. Ecosyst. Environ.* 164, 80–99. <https://doi.org/10.1016/j.agee.2012.10.001>.
- Soil Health Institute, 2017. Enriching Soil, Enhancing Life: An Action Plan for Soil Health. Ed. C. W. Honeycutt. Morrisville. < <https://soilhealthinstitute.org/wp-content/uploads/2017/05/Action-Plan-FINAL-for-flipbook-3.pdf> > .
- Soil Survey Staff, 2014a. Kellogg Soil Survey Laboratory Methods Manual. In: R. Burt and S. S. S. (ed.), editors. Soil Survey Investigations Report No. 42, Version 5.0. USDA NRCS. < https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1253871.pdf > .
- Soil Survey Staff, 2014b. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC.
- Stott, D.E. 2019. Recommended Soil Health Indicators and Associated Laboratory Procedures. Soil Health Technical Note No. 450-03. U.S. Department of Agriculture, Natural Resources Conservation Service < <https://directives.sc.egov.usda.gov/OpenNonWebContent.aspx?content=43754.wba> > .
- Thoumazeau, A., Bessou, C., Renevier, M.S., Trap, J., Marichal, R., Mareschal, L., Decaens, T., Bottinelli, N., Jaillard, B., Chevallier, T., Suvannang, N., Sajjaphan, K., Thaler, P., Gay, F., Brauman, A., 2019. Biofunction*: a new framework to assess the impact of land management on soil quality. Part A: concept and validation of the set of indicators. *Ecol. Ind.* 97, 100–110. <https://doi.org/10.1016/j.ecolind.2018.09.023>.
- Tirol-Padre, A., Ladha, J.K., 2004. Assessing the reliability of permanganate-oxidizable carbon as an index of soil labile carbon. *Soil Sci. Soc. Am. J.* 68, 969–978. <https://doi.org/10.2136/sssaj2004.9690>.
- Van den Elsen, E., Knotters, M., Heinen, M., Römkens, P.F.A.M., Bloem, J., Korthals, G.W., 2019. Noodzakelijke indicatoren voor de beoordeling van de gezondheid van Nederlandse landbouwbodems; De meest relevante fysische, chemische en biologische indicatoren voor het meten van de bodemgezondheid. Rapport 2944, Wageningen Environmental Research. < <http://edepot.wur.nl/475874> > .
- Wade, J., Culman, S.W., Hurisso, T.T., Miller, R.O., Baker, L., Horwath, W.R., 2018. Sources of variability that compromise mineralizable carbon as a soil health indicator. *Soil Sci. Soc. Am. J.* 82, 243–252. <https://doi.org/10.2136/sssaj2017.03.0105>.
- Wade, J., Maitais-Landry, G., Lucas, D.E., et al., 2020. Assessing the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric: an interlab comparison across soils. *Geoderma* 366. <https://doi.org/10.1016/j.geoderma.2020.114235>.
- Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. *Am. J. Altern. Agric.* 18, 3–17.
- Wills, S., Pulleman, M., Ferguson, R. (2020). “USDA POX C samples”, Mendeley Data, v1 <https://doi.org/10.17632/ky83v36t5.1>.