Geoderma 366 (2020) 114235

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Assessing the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric: An interlab comparison across soils

Jordon Wade[a,*], Gabriel Maltais-Landry[b], Dawn E. Lucas[b], Giulia Bongiorno[c,d], Timothy M. Bowles[e], Francisco J. Calderón[f], Steve W. Culman[g], Rachel Daughtridge[h], Jessica G. Ernakovich[i], Steven J. Fonte[j], Dinh Giang[j], Bethany L. Herman[g], Lindsey Guan[e], Julie D. Jastrow[l], Bryan H.H. Loh[i], Courtland Kelly[j], Meredith E. Mann[g], Roser Matamala[l], Elizabeth A. Miernicki[a], Brandon Peterson[f], Mirjam M. Pulleman[c,m], Kate M. Scow[k], Sieglinde S. Snapp[n,o], Vanessa Thomas[n], Xinyi Tu[n], Daoyuan Wang[k], Nicolas A. Jelinski[p], Garrett C. Liles[q], Felipe H. Barrios-Masias[r], Devin A. Rippner[k], Maria L. Silveira[b,s], Andrew J. Margenot[a,*]

[a] Department of Crop Sciences, University of Illinois at Urbana-Champaign, United States
[b] Soil and Water Sciences Department, University of Florida, United States
[c] Soil Biology Group, Wageningen University, the Netherlands
[d] Department of Soil Science, Research Institute of Organic Agriculture (FiBL), Germany
[e] Department of Environmental Science, Policy, & Management, University of California, Berkeley, United States
[f] Central Great Plains Research Station, USDA-ARS, United States
[g] School of Environment & Natural Resources, The Ohio State University, United States
[h] Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, United States
[i] Department of Natural Resources and the Environment, University of New Hampshire, United States
[j] Department of Soil and Crop Sciences, Colorado State University, United States
[k] Department of Land, Air and Water Resources, University of California Davis, United States
[l] Environmental Science Division, Argonne National Laboratory, United States
[m] International Center for Tropical Agriculture (CIAT), Colombia
[n] Department of Plant, Soil and Microbial Sciences, Michigan State University, United States
[o] Center for Global Change and Earth Observations, Michigan State University, United States
[p] Department of Soil, Water and Climate, University of Minnesota, United States
[q] College of Agriculture, California State University Chico, United States
[r] Department of Agriculture, Veterinary and Rangeland Sciences, University of Nevada, Reno, United States
[s] Range Cattle Research and Education Center, University of Florida, United States

ARTICLE INFO

Handling Editor: Ingrid Kögel-Knabner

ABSTRACT

Soil organic matter is central to the soil health framework. Therefore, reliable indicators of changes in soil organic matter are essential to inform land management decisions. Permanganate oxidizable carbon (POXC), an emerging soil health indicator, has shown promise for being sensitive to soil management. However, strict standardization is required for widespread implementation in research and commercial contexts. Here, we used 36 soils—three from each of the 12 USDA soil orders—to determine the effects of sieve size and soil mass of analysis on POXC results. Using replicated measurements across 12 labs in the US and the EU (n = 7951 samples), we quantified the relative importance of 1) variation between labs, 2) variation within labs, 3) effect soil mass, and 4) effect of soil sieve size on the repeatability of POXC. We found a wide range of overall variability in POXC values across labs (0.03 to 171.8%; mean = 13.4%), and much of this variability was attributable to within-lab variation (median = 6.5%) independently of soil mass or sieve size. Greater soil mass (2.5 g) decreased absolute POXC values by a mean of 177 mg kg^{-1} soil and decreased analytical variability by 6.5%. For soils with organic carbon (SOC) > 10%, greater soil mass (2.5 g) resulted in more frequent POXC values above the limit of detection whereas the lower soil mass (0.75 g) resulted in POXC values below the limit of detection for SOC contents < 5%. A finer sieve size increased absolute values of POXC by 124 mg kg^{-1} while decreasing the analytical variability by 1.8%. In general, soils with greater SOC contents had lower analytical

* Corresponding authors.
E-mail addresses: jordonwade@gmail.com (J. Wade), margenot@illinois.edu (A.J. Margenot).

https://doi.org/10.1016/j.geoderma.2020.114235
Received 18 December 2019; Received in revised form 25 January 2020; Accepted 27 January 2020

variability. These results point to potential standardizations of the POXC protocol that can decrease the variability of the metric. We recommend that the POXC protocol be standardized to use 2.5 g for soils < 10% SOC. Sieve size was a relatively small contributor to analytical variability and therefore we recommend that this decision be tailored to the study purpose. Tradeoffs associated with these standardizations can be mitigated, ultimately providing guidance on how to standardize POXC for routine analysis.

1. Introduction

Soil organic matter is a vital component of ecosystem functioning (Schmidt et al., 2011), as well as crop production (Oldfield et al., 2019; Sanderman et al., 2017). For decades, the characterization of organic matter in soils has been predominantly described through chemical extractions or fractionations (Lehmann and Kleber, 2015). Increasingly, this paradigm is being left behind in favor of a model that integrates chemical composition, physical accessibility, and biological activity to describe organic matter dynamics (Blankinship et al., 2018; Dungait et al., 2012; Lehmann et al., 2008).

The soil health framework emphasizes the degree to which dynamic properties of a soil can be optimized for multifunctionality. While there are intrinsic properties of each soil, the chemical, physical, and biological components of soil organic matter form the core of soil health (Lal, 2016). Soil health seeks to unify these previously disparate components of soil into a cohesive framework for implementation in agroecosystems (Kibblewhite et al., 2008). To aid in the implementation of this framework, a novel set of metrics are being developed with usability by land managers as one of the central goals (Doran and Zeiss, 2000). As these metrics undergo development, vetting, and calibration, an emerging soil health indicator is the fraction of carbon (C) that is oxidized by potassium permanganate ($KMnO_4$), which we will refer to here as permanganate oxidizable C (POXC) (Culman et al., 2012; Moebius-Clune et al., 2017). While POXC is a chemically-defined fraction of soil C, it is often thought or proposed to be reflective of a biologically active pool (Moebius-Clune et al., 2017; NRCS, 2019) and has been shown to be related to microbial community composition (Ramírez et al., 2019). Recent work has also shown that POXC is positively related to aggregate stability (Fine et al., 2017; Wade et al., 2019) and inversely related to dispersible clays (Jensen et al., 2019), suggesting a potential physical component of this measurement as well. This interrelatedness of POXC to multiple components of soil health—as well its sensitivity to changes in management, potential for high throughput, and relatively low equipment costs—have made it an attractive metric for soil health assessments (Bongiorno et al., 2019). One unique aspect of the soil health framework is the focus on indicator usability and interpretability. Soil health indicators must provide information that is both reliable and actionable for land managers in their decision making process.

Table 1
Classification and characteristics of soils used in multilab comparison of permanganate oxidizable C (POXC). Soils are A horizons for mineral soils and O horizons for Histosols and Gelisols. Soil series information, including USDA classification and land use is available for each soil in Table S1.

Soil ID	USDA Order	SOC (%)	C:N	pH	Clay (g kg^{-1})	Sand (g kg^{-1})	CEC[a] (m_{eq} 100 g^{-1})	Location
1	Oxisol	1.7	12.7	5.0	716	79	9.1	Kisumu, Kenya
2	Oxisol	0.6	9.7	4.8	480	243	12.5	CA, USA
3	Oxisol	1.4	11.7	5.6	694	74	11.6	Vihiga, Kenya
4	Vertisol	1.8	14.7	7.7	446	81	32.0	CA, USA
5	Vertisol	1.6	11.2	6.1	392	269	25.3	CA, USA
6	Vertisol	1.0	12.4	7.0	540	135	31.1	CA, USA
7	Histosol	8.3	14.8	7.7	236	502	31.2	CA, USA
8	Histosol	37.7	25.3	5.3	125	500	2.0	MN, USA
9	Histosol	29.7	16.0	7.7	102	414	55.8	FL, USA
10	Inceptisol	3.3	10.8	4.8	176	395	8.9	RI, USA
11	Inceptisol	1.2	15.5	4.5	281	311	7.0	CA, USA
12	Inceptisol	1.5	11.4	6.6	317	156	21.4	CA, USA
13	Mollisol	3.7	14.6	6.0	275	66	26.9	IL, USA
14	Mollisol	1.1	10.4	8.1	284	330	15.3	MO, USA
15	Mollisol	3.1	12.4	6.1	256	105	14.7	CA, USA
16	Alfisol	1.1	10.1	8.0	228	292	17.4	IL, USA
17	Alfisol	0.9	8.4	6.5	300	200	19.1	CA, USA
18	Alfisol	2.4	10.2	5.7	75	850	26.6	IL, USA
19	Ultisol	0.8	nd[b]	6.1	236	503	3.0	FL, USA
20	Ultisol	4.1	21.7	5.8	128	668	8.0	CA, USA
21	Ultisol	7.0	22.0	5.4	225	474	20.4	CA, USA
22	Entisol	8.9	68.5	8.1	203	418	92.3	FL, USA
23	Entisol	0.9	8.9	7.3	25	925	21.1	CA, USA
24	Entisol	1.5	13.9	6.4	107	680	11.2	CA, USA
25	Andisol	8.0	19.6	6.0	103	819	9.4	CA, USA
26	Andisol	7.4	19.6	5.8	77	743	11.7	CA, USA
27	Andisol	4.4	21.1	6.3	0	918	10.5	CA, USA
28	Spodosol	6.2	13.2	4.7	0	950	10.0	FL, USA
29	Spodosol	2.3	25.0	4.4	25	900	3.7	FL, USA
30	Spodosol	2.7	11.9	5.7	0	364	24.8	FL, USA
31	Aridisol	1.3	9.9	7.8	0	671	24.9	CA, USA
32	Aridisol	0.7	12.2	7.1	0	874	18.9	NV, USA
33	Aridisol	0.2	nd	8.1	716	79	7.4	NV, USA
34	Gelisol	17.5	19.9	6.9	480	243	25.0	AK, USA
35	Gelisol	20.8	15.8	5.3	694	74	22.5	AK, USA
36	Gelisol	32.8	29.8	5.2	446	81	31.7	AK, USA

[a] CEC = cation exchange capacity.
[b] nd = non-detectable total soil N (< 0.05%).

For decades, permanganate oxidation has been used to describe the portion of soil organic matter that is thought to turnover quickly and have a relatively short residence time (Blair et al., 1995; Matsuda and Schnitzer, 1972; Willard et al., 1956). Oxidation by relatively dilute solutions (< 0.5 mol L^{-1}) of permanganate (MnO_4^-) has been used to describe both C (Lefroy et al., 1993; Loginow et al., 1987) and N dynamics (Bundy and Bremner, 1973; Carski and Sparks, 1987). The concentration of MnO_4^- used in these evaluations have varied over an order of magnitude, ranging from 20 mmol L^{-1} to 333 mmol L^{-1} (Loginow et al., 1987; Weil et al., 2003). Higher concentrations with longer shaking times have been found to produce inconsistent results (Tirol-Padre and Ladha, 2004) and are less sensitive to changes in management (Lucas and Weil, 2012; Weil et al., 2003), prompting the use of lower concentrations and shorter reaction times. While there is a broad consensus to use 20 mL of 0.02 mol L^{-1} MnO_4^-, slight variations in shaking time still exist, with both 12 (Culman et al., 2012; Hurisso et al., 2016; Weil et al., 2003) and 10 min (Bongiorno et al., 2019; Moebius-Clune et al., 2017; NRCS, 2019) of total reaction time being utilized. However, even these slight variations are indicative of a broader convergence from previous times of up to 24 h (Tirol-Padre and Ladha, 2004).

Although there has been convergence on the concentration of the solution and reaction time, other potential methodological considerations have been less studied. Of particular interest for standardization are the mass of soil reacted and the sieve size through which that soil has been passed. These methodological decisions have the potential to influence both absolute values of POXC (i.e. sensitivity), as well as the analytical variability (i.e. repeatability). Balancing these considerations is essential to ensure reliable quantification across edaphic contexts. More broadly, these methodological decisions have implications for the utility of POXC to inform land management decisions. Here, we will examine how soil mass and sieve size influence absolute POXC values and the analytical variability. In order to differentiate between treatment effects and lab or operator effects, soils were sent to twelve labs in the United States and Europe. The objectives of this study were to determine 1) changes in absolute POXC values associated with soil mass and sieve size decisions, 2) the range of potential within-lab variability of POXC, and 3) the relative contributions of soil mass and sieve size to analytical variability of POXC. To evaluate the robustness of these findings, we examined these sources of variability using three soils from each of the twelve soil orders of the USDA classification system (n = 36 soils total).

We hypothesized that a decrease in soil mass from 2.5 g to 0.75 g would increase absolute POXC values due to the greater ratio of oxidant (MnO_4^-) to substrate (soil organic C [SOC]). However, we hypothesized that this lower soil mass would result in greater variability from sample to sample (i.e. between analytical reps), increasing the analytical variability. We also hypothesized that decreasing sieve size from < 2.0 mm to < 0.5 mm would expose physically occluded organic matter to oxidation, increasing absolute values of POXC and would produce more consistent values. Finally, we hypothesized that within-lab variability would be the largest contributor to the variability of the metric, i.e. that internal lab practices would outweigh the variability associated with methodological considerations.

2. Materials and methods

2.1. Soil sampling and characterization

Three soils for each of the twelve USDA orders were obtained from a combination of archived collections and field sampling. Surface A horizons were sampled for mineral soils and O horizons were obtained for Histosols and Gelisols. Soils were air-dried and sieved to < 2 mm prior to characterization for chemical and physical properties. Soil pH was determined in water (1:2 m/v) after equilibrating for 30 min (Thomas, 1996). Soil texture was determined by the hydrometer method, using overnight shaking (16 h) in sodium hexametaphosphate to disperse mineral particles (Bouyoucos, 1962). Total organic C was determined by dry combustion chromatography (Nelson and Sommers, 1996) and soil organic C (SOC) was estimated for soils after gravimetric determination of potential carbonates using dilute HCl (Harris et al., 2001).

Soils analyzed in the current study included three soils from each of the twelve USDA soil orders and encompassed a wide range of soil physicochemical properties (Table 1). Soil organic carbon contents ranged from 0.21% to 37.7% by mass (median = 2.3%, mean = 6.4%). Clay contents ranged from 0.0 g kg $soil^{-1}$ to 716.5 g kg^{-1} soil (median = 226.6 g kg^{-1} soil, mean = 244.3 g kg^{-1} soil) and sand contents ranged from 66.5 g kg^{-1} soil to 949.8 g kg^{-1} soil (median = 404.7 g kg^{-1} soil, mean = 439.1 g kg^{-1} soil). Further soil physicochemical properties are summarized in Table 1. Soil series and USDA taxonomic classification information are summarized in Table S1.

2.2. Sample processing and distribution

In order to minimize artifacts due to soil processing, we air-dried, hand sieved the soil by gently pressing soil through < 0.5 mm or < 2.0 mm sieves, and then homogenized the sample before sending to twelve different laboratories in the US and Europe. Sieve sizes of < 0.5 mm and < 2.0 mm were based on the two most common sieve sizes used for high-grade chemical analyses (e.g. synchrotron, mass spectroscopy) and in commercial soil test labs, respectively. Then, each participating laboratory performed the $KMnO_4$ oxidation on five analytical replicates using a mass of 0.75 g (± 0.02 g) or 2.50 g (± 0.05 g). The mass of 0.75 g was empirically derived *a priori* as the mass for which low SOC soils produced detectable levels of POXC and high SOC soils were within the maximum limit of quantification (e.g. 4800 mg POXC kg^{-1} for 0.75 g soil). All oxidations were performed by the same operator within each laboratory.

2.3. Permanganate oxidation and reading by colorimetry

We performed each oxidation using the methods of Lucas and Weil (2012), specifically the protocol outlined by Culman et al. (2012) (https://lter.kbs.msu.edu/protocols/133). In brief, soils were weighed into 50 mL polypropylene tubes prior to the oxidation step. We initiated the oxidation reaction by adding 18 mL of deionized water and 2 mL of 0.2 mol L^{-1} $KMnO_4$ (final reaction concentration = 0.02 mol L^{-1} MnO_4^-) to each tube containing pre-weighed soil, shaking for exactly 2 min on a reciprocal shaker and allowing to settle for exactly 10 min. After settling for exactly 10 min, we terminated the reaction by transferring 0.5 mL of supernatant into a fresh 50 mL tube with 49.5 mL of deionized water, which we then inverted to mix thoroughly, resulting in a homogenized 1:100 dilution. Since oxidation occurs over time and total C oxidization is time sensitive, consistency in the timing of termination between replicates and across batches is essential to reproducible measurements. To minimize variability in reaction termination time, the five analytical replicates were run in sequence. The 1:100 dilution was then transferred to either microcuvettes or a 96-well plate reader and analyzed by UV–Vis spectrophotometry to quantify MnO_4^- remaining in solution by absorbance at 550 nm. After the reaction has been terminated and the 1:100 dilution completed, the MnO_4^- in solution should be consistent and the subsequent quantification step is less time sensitive. No difference in analytical variability or absolute values were found between readings from microcuvettes and readings from 96-well plate readers ($F_{1,10}$ = 1.7, p = 0.225, data not shown). Adjusted absorbance was then calculated by subtracting the mean of three blanks from the raw absorbance for each sample.

We then used the adjusted absorbance to calculate the total POXC using the following equation:

$$\text{POXC (mg\;\;soil)} = \frac{[[0.02\;\text{mol\;L}^{-1} - (a + (b \times \text{Abs}_{adj}))] \times (9000\;\text{mg\;C\;mol}^{-1}) \times (0.02\;\text{L\;solution})]}{\text{Mass(kg)}} \quad (1)$$

where 0.02 mol L^{-1} is the initial concentration of the oxidation solution, 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 mol^{-1} is the assumed mass of C oxidized by 1 mol of Mn^{7+} oxidizing to Mn^{4+} (Weil et al., 2003), 0.02 L is the volume of solution in the oxidation step, and *Mass* is the mass of soil (in kg) reacted in the tube. To maximize consistency between labs, we calculated a and b for each batch and then used the resulting equations to simultaneously calculate POXC values from adjusted absorbance values (Abs_{adj}). To maximize consistency across labs, we used the same $KMnO_4$ concentrations to construct all standard curves: 0.020 mol L^{-1}, 0.015 mol L^{-1}, 0.010 mol L^{-1}, and 0.005 mol L^{-1}. Using the theoretical maximum of MnO_4^- reduced per unit of soil mass (9000 mg C mol^{-1} MnO_4^-), we considered values between 0 and 1440 mg kg^{-1} soil valid for the 2.5 g soil treatment and 0–4800 mg kg^{-1} soil valid for the 0.75 g soil treatment. Values outside of this range were excluded from consideration in measurements of analytical variability, but were used to calculate detection rates. All values are expressed on an air-dried weight basis.

2.4. Statistical analyses

All statistical analyses were run in RStudio version 1.2.5001 (RStudio Team, 2019). Absolute values were determined by averaging all values for each combination of soil, mass, and sieve size. Averaging for each group was performed using the *group_by()* and *summarise()* command in the *dplyr* package (Wickham et al., 2019).

Given that the sieving was performed in one location, we considered these pre-oxidation treatments—mass and sieve size—fixed effects in our statistical model. Thus, we assume that any variation due to sieve size and mass occur independently of any lab-specific variation, i.e. these effects are not nested within each laboratory. Similarly, the variability attributed to each soil was considered fixed and not nested within each laboratory. Thus, our initial linear model to assess sources of variation was:

$$Y = \text{Lab} + \text{Soil} \times \text{Mass}_{\text{treatment}} \times \text{Sieve Size}_{\text{treatment}} + \varepsilon \quad (2)$$

where Y is the coefficient of variation (CV; expressed as a %) of the five analytical replicates. We then eliminated interaction terms that did not significantly contribute to analytical variability ($p > 0.10$) to develop our reduced model. Sieve size was retained due to its interaction with soil (Table 2).

To determine whether each soil should be assessed individually or grouped by soil order, we compared a reduced model where *Soil* referred to each of the 36 unique soils with a reduced model where *Soil*

Table 2
Summary of POXC values for each soil and treatment. Values are mean, median, and median absolute difference (all in mg POXC kg^{-1} soil). Analytical variability (% coefficient of variation) for each soil is also summarized. Averages (mean and median) are n = 50 for 0.75 g and n = 60 for 2.5 g. CV values are averaged from each of the 12 labs.

Soil ID	2.5 g				0.75 g			
	< 0.5 mm		< 2.0 mm		< 0.5 mm		< 2.0 mm	
	Average	CV (%)	Average	CV (%)	Average	CV (%)	Average	CV (%)
1	342, 324, 41	8.7	274, 259, 57	11.0	373, 323, 211	23.7	317, 251, 198	21.1
2	287, 278, 52	9.3	253, 230, 41	11.9	361, 271, 102	25.0	250, 202, 141	37.3
3	274, 267, 42	10.0	247, 237, 41	12.1	390, 329, 150	27.7	321, 315, 123	22.9
4	497, 516, 55	5.8	410, 414, 57	5.1	612, 616, 151	15.9	502, 509, 106	16.8
5	557, 573, 32	4.6	476, 511, 48	4.5	643, 655, 156	11.2	611, 593, 163	13.9
6	345, 357, 37	8.5	278, 282, 41	10.3	464, 390, 172	27.6	315, 275, 120	26.3
7	1155, 1166, 224	3.8	917, 929, 166	7.4	1891, 1965, 414	4.6	1435, 1412, 322	8.6
8	–	–	–	–	4450, 4687, 124	4.6	3660, 3657, 564	6.4
9	–	–	–	–	4319, 4414, 180	1.7	4040, 4127, 222	2.1
10	427, 385, 60	7.2	347, 317, 72	8.7	514, 457, 207	14.5	525, 392, 162	14.0
11	268, 275, 57	14.4	252, 239, 76	12.7	380, 271, 196	37.9	437, 293, 198	41.4
12	554, 556, 73	4.3	497, 496, 87	6.7	600, 562, 159	12.7	548, 477, 192	15.3
13	625, 627, 45	5.1	576, 582, 40	7.8	774, 750, 163	11.1	731, 749, 111	14.9
14	241, 202, 32	11.3	195, 159, 34	10.5	270, 231, 96	30.8	241, 227, 102	26.6
15	852, 847, 70	4.3	710, 674, 136	6.7	1214, 953, 195	11.7	1104, 814, 130	11.5
16	245, 231, 46	8.5	190, 182, 38	12.0	288, 214, 115	27.9	343, 235, 72	26.5
17	229, 219, 24	11.6	234, 218, 40	12.9	283, 180, 136	47.1	299, 234, 131	23.1
18	638, 630, 57	4.2	553, 549, 77	7.1	905, 768, 160	14.1	813, 721, 124	10.4
19	167, 153, 35	17.4	174, 165, 36	16.9	244, 212, 117	44.7	212, 204, 120	21.0
20	907, 931, 110	2.2	640, 645, 116	7.1	1230, 1199, 289	7.0	865, 856, 227	13.7
21	1171, 1210, 90	2.2	946, 959, 131	4.0	1650, 1648, 295	5.3	1243, 1218, 241	11.6
22	374, 387, 43	5.9	365, 357, 41	9.9	409, 404, 130	18.9	475, 433, 131	26.4
23	447, 493, 63	6.6	389, 410, 37	8.2	531, 547, 123	14.7	519, 496, 141	28.2
24	618, 617, 122	6.9	387, 386, 57	11.9	792, 739, 209	14.6	492, 482, 136	24.9
25	984, 1011, 365	8.3	967, 946, 210	8.0	1590, 1499, 638	12.6	1495, 1404, 324	11.7
26	831, 852, 306	9.7	705, 691, 156	9.6	1231, 1247, 484	16.4	1009, 1073, 245	16.9
27	420, 439, 188	14.7	378, 395, 93	12.8	612, 560, 141	20.4	534, 473, 167	21.8
28	1039, 1033, 234	6.8	1075, 1034, 152	6.7	1719, 1765, 470	9.2	1664, 1641, 291	8.8
29	514, 505, 61	8.7	501, 489, 43	5.7	714, 640, 123	17.9	690, 598, 171	27.5
30	780, 807, 72	4.4	829, 839, 100	5.9	997, 988, 159	10.9	1084, 1048, 201	12.9
31	333, 348, 47	5.1	313, 333, 52	6.2	380, 324, 85	15.8	343, 328, 143	19.4
32	302, 229, 47	9.4	215, 144, 51	20.9	600, 274, 66	25.8	537, 195, 90	26.7
33	102, 90, 24	30.5	149, 70, 18	24.5	115, 111, 53	27.3	96, 90, 56	38.3
34	–	–	–	–	4486, 4657, 141	1.0	4291, 4416, 153	2.0
35	–	–	–	–	2953, 2519, 1236	12.4	2429, 2410, 747	10.5
36	–	–	–	–	4318, 4521, 298	3.1	4094, 4145, 397	3.7

referred to the USDA Soil Order. We compared these reduced models using the Akaike Information Criteria (AIC) (Akaike, 1974) and the likelihood ratio test (Perneger, 2001), both of which indicated that characterizing each soil uniquely was a more parsimonious model fit. Analytical variability by soil order can be found in Table S2 and Fig. S1.

Our final reduced model was estimated using the *lm()* command. The resulting linear regression was log transformed to meet linear regression assumptions. Statistical significance of each variable was determined with the *Anova()* command in the *car* package (Fox and Weisberg, 2019). Normality of residuals was ascertained using the *shapiro.test()* command to conduct a Shapiro-Wilk test of normality. Additional verification of normality of residuals, homogeneity of variance, and the leverage of individual observations was performed visually using the *plot()* command. We quantified relative contributions of each variable (i.e. lab, soil, or soil mass/sieve size treatment) to overall analytical variability using the estimated marginal means for each variable. Estimated marginal means (also referred to as least-square means) for each factor or set of factors in the final reduced model were obtained using the *emmean()* command in the *emmeans* package (Lenth, 2019). Therefore, each estimate of analytical variability—whether by soil, lab, soil × sieve size, or otherwise—is an estimated marginal mean, which accounts for other sources of variability in our model (Eq. (2)). In order to properly determine effect sizes of each treatment and the relative precision associated with that treatment, we used estimation statistics, rather than significance testing (Ho et al., 2019a). Unlike significance testing, which uses *p*-values to measure against the null hypothesis of no difference, estimation statistics uses a bootstrapped estimate of effect size and the precision associated with that effect size. This circumvents overreliance on $p < 0.05$, which can often lead to a lack of reproducibility of effects (Halsey et al., 2015), particularly when using categorical variables, e.g. soil mass and sieve size. Estimation statistics were performed using the *dabest()* command in *dabestr* package (Ho et al., 2019b). Due to the highly skewed distributions of mass and sieve size effects, robustness to outliers was ensured by using 5000 bias-corrected and accelerated bootstrap resamplings (Efron, 1987) and using the median, rather than the mean value. Estimated effect sizes and the associated 95% confidence intervals are quantified and included in each plot.

3. Results and discussion

3.1. Soil characterization and POXC values

Detectable POXC values—defined as 0 to 1440 mg kg^{-1} soil for the 2.5 g soil treatment and 0 to 4800 mg kg^{-1} soil for the 0.75 g soil treatment—spanned nearly the entire detection range for both masses. For the 2.5 g mass, POXC values ranged from 4 to 1406 mg POXC kg^{-1} soil (median = 427 mg POXC kg^{-1} soil, mean = 504 mg POXC kg^{-1} soil). For the 0.75 g mass, POXC values ranged from 0.8 mg to 4790 mg POXC kg^{-1} soil (median = 651 mg POXC kg^{-1} soil, mean = 1161 mg POXC kg^{-1} soil), indicating a 34% increase in the median measured POXC value by decreasing the mass of soil analyzed. Average POXC values and analytical variability for each combination of soil and treatment are listed in Table 3. The median absolute deviation—which is similar to standard deviation, but is more robust for our skewed data—ranged from 18 mg POXC kg^{-1} soil to 1236 mg POXC kg^{-1} soil (Table 2). The wide range of absolute differences in POXC values underscores how much the POXC values can vary for the same soil. The 95% confidence interval for the median absolute deviation was 126–178 mg POXC kg^{-1} soil (mean = 152 mg POXC kg^{-1} soil, data not shown) indicating that absolute deviations of ~150 mg POXC kg^{-1} soil are likely to occur. Since greater deviations tend to happen for soils with higher average POXC values, there is a need to relativize error, such as using the coefficient of variation or a percent change. Given the range of soil properties and detected POXC values, these values are likely representative of a broad set of edaphic conditions.

3.2. Methodological effects on absolute values of POXC

One of the most common processing treatments for soils is sieving or grinding of the soils prior to analysis. The resulting sieve size classes are especially salient in the measurement of soil C fractions, due to the physical occlusion of C within soil structures (von Lützow et al., 2008). Accordingly, we hypothesized that the smaller sieve size (< 0.5 vs < 2.0 mm) would increase POXC values, a hypothesis that was largely confirmed (Fig. 1a). On average, the < 0.5 mm sieve size increased POXC values by 124 mg POXC kg^{-1} soil (median = 56 mg POXC kg^{-1} soil). This is a further extension of the results of Hurisso et al. (2018b), who found that decreasing sieve size from < 8 mm to < 2 mm increased POXC values an average of 141 mg POXC kg^{-1} soil across three soils. However, our results show that the size of this effect can vary considerably, from −188 mg POXC kg^{-1} soil (i.e. a decrease in POXC) to 876 mg POXC kg^{-1} soil. On a relative basis, this is an average of 4.0% greater POXC values at the < 0.5 mm sieve size than at the < 2.0 mm (range = −272.6% to 37.6%). It is expected that smaller sieve size would increase POXC values and that this would vary, but not that POXC values would decrease (6 of 36 soils). While there was no apparent physicochemical characteristic(s) underlying these six soils (soils 11, 17, 22, 29, 30, and 33) that could explain this unexpected result, five of the six decreases were for soils with < 100 mg POXC kg^{-1} soil or < 20% relative decrease. Therefore, while < 0.5 mm sieve size has an inconsistent direction on POXC values, the resulting analyses are likely to be substantively similar. This finding also suggests that it may not be possible to standardize sieve size in a “one size fits all” approach.

In addition to decisions regarding sieve size, the mass of soil required for analysis is vital to consider in the standardization of POXC because it can change the amount of SOC present in the sample for oxidation by a fixed amount of MnO_4^-. Our hypothesis that a decrease in soil mass from 2.5 g to 0.75 g would increase POXC values was mostly confirmed. The 0.75 g mass had POXC values that were 177 mg POXC kg^{-1} soil greater than the 2.5 g mass (median = 111 mg POXC kg^{-1} soil), an average increase of 32.4% (range = −5.0% to 114.6%). This trend was consistent for 35 of the 36 soils, with the exception of soil 31 for which POXC slightly decreased by 16 mg POXC kg^{-1} soil. While this ratio of soil (and soil SOC) to 0.02 mol L^{-1} $KMnO_4$ has not been specifically evaluated for POXC, the effect of soil-to-solution ratios has been shown to influence absolute values for a variety of soil chemical extractions, including extractable organic carbon (Kaiser et al., 2001), inorganic N (Li et al., 2012), phosphate (Fuhrman et al., 2005), heavy metals (Yin et al., 2002), and even pH (Hendershot et al., 1993).

Standardization of a soil mass-to-solution ratio for POXC may provide consistency among studies and should be more thoroughly investigated. Previous work using $KMnO_4$ to measure labile C standardized the analysis on the basis of total SOC, rather than on a soil mass basis (Blair et al., 1995). However, this practice has gone largely unadopted due to the increased labor associated with its use. Although standardizing on a mass basis is scientifically rigorous, it may be less amenable to the high-throughput context of commercial soil test labs, which often employ a volumetric scoop for rapid soil preparation (Hoskins and Ross, 2011; Miller et al., 2013; Mylavarapu and Miller, 2014; Peck, 2015). This further modification of POXC for high throughput analysis (and more generally any soil test) may be a

Table 3
Soil IDs included in each processing treatment and the proportion of assays that resulted in MnO_4^- concentration within the range of detection.

Sieve Size	Mass	Soil IDs excluded	*n*	Detection limit (mg POXC kg^{-1} soil)	% Detectable
< 0.5 mm	0.75 g	none	2141	4800	96.0
	2.50 g	8, 9, 34–36	1835	1440	99.5
< 2.0 mm	0.75 g	none	2142	4800	95.7
	2.50 g	8, 9, 34–36	1833	1440	99.1

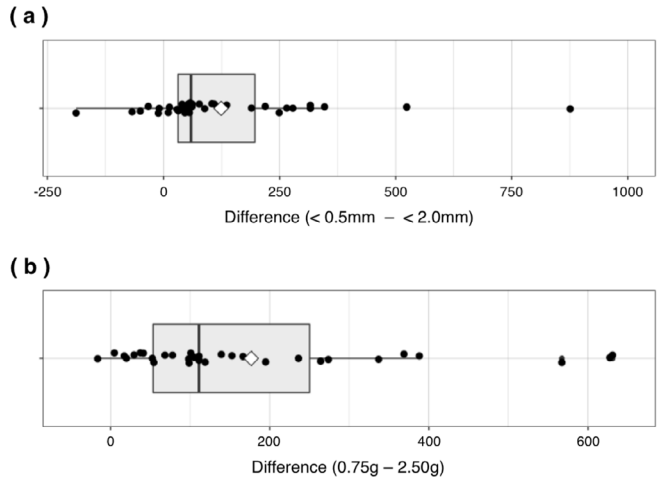

Fig. 1. Absolute differences in POXC values between (a) sieve sizes and (b) mass. Note the differences in scale. Values are estimated marginal means for each soil using Eq. (2) and are in mg POXC kg^{-1} soil.

significant source of variation, necessitating lab proficiency testing for appropriate quality assurance/quality control (QA/QC). While QA/QC might establish tolerance limits, it would not address the potential for an unequal distribution of bias across SOC contents.

3.3. Methodological considerations and detection limits

The finite amount of MnO_4^- in the oxidizing solution (0.4 mmol) results in upper limits of quantification for the measurement of POXC. These upper limits change based on the soil mass, which is reflected in the exclusion of high SOC soils (> 10% SOC) from consideration at 2.5 g (Table 4). For the soils included at each soil mass, nearly all of the samples were within the limits of detection (> 95%). However, the proportion of values falling within the detection limits was not consistent across SOC contents (Fig. 2). In the 2.5 g soil mass, which were exclusively using soils < 10% SOC, detection rates fell off at SOC contents < 1.0% due to non-detectable levels of POXC in samples from both < 0.5 mm and < 2.0 mm sieve sizes (Fig. 2c and d). However, detection rates were generally higher for 2.5 g than for 0.75 g (Table 3). This difference is largely attributable to the lower absolute values of POXC measured using 2.5 g relative to 0.75 g soil (Fig. 1b). The lower POXC values at 2.5 g results in a greater proportion of POXC values within detection limits, increasing the overall detection rate. In the 0.75 g soil mass, we found decreased detection at both high SOC contents (> 10%) and at low SOC contents (< 5%) in the < 0.5 mm sieve size. This combination resulted in values that were simultaneously above and below detection limits. Samples analyzed with 0.75 g mass at < 2.0 mm sieve size maintained detection rates at higher SOC contents (> 10%). For 0.75 g mass, soils with lower SOC contents (< 5.0%) and < 2.0 mm sieve size had a sharper decrease in detection than for < 0.5 mm, presumably due to increased consumption of MnO_4^- (i.e. higher POXC values) associated with the < 0.5 mm sieve size (Fig. 1a). Thus, while 0.75 g may allow for soils with a broader range of SOC contents to be measured for POXC, soils with greater SOC content will require an increased number of replications to ensure detectable values. Similarly, 2.5 g soil masses may provide consistent detection at SOC contents < 10%, but greater replication will be required at very low SOC values (< 1%). These results collectively show that across SOC contents, replication is needed to ensure a sufficient number of detectable values.

3.4. Overall analytical variability

The coefficient of variation of the five analytical replicates for each unique set of soil, sieve size, and soil mass ranged from 0.04 to 171.8% (median = 7.85, mean = 13.41; data not shown). The distribution of the total analytical variability was highly positively skewed, i.e. had several extreme high values. The 95% confidence interval for the overall distribution—after transformation to meet normality assumptions and backtransformation into natural values—was 7.2 to 8.0% with a mean overall variability of 7.6% (data not shown). These values of POXC analytical variability largely agree with the values obtained by Hurisso et al. (2018a) and are comparable to the analytical variability of Mehlich-3 extractable P and K also evaluated therein. However, a clearer understanding of the contributing sources of this variability will help improve the reliability and robustness of this metric across contexts.

3.5. Inter-lab analytical variability

We found salient inter-lab effects on the variability of POXC measurements (Table 4, $p < 0.00001$), although soil mass also influenced analytical variability (Table 4, $p < 0.00001$). Additionally, each soil had differing levels of analytical variability (Table 4, $p < 0.00001$), an effect which varied by sieve size (Table 4, $p = 0.009$). Although F-values can often be used as measures of overall effect, the transformations needed to meet assumptions of normality prevent a direct comparison of F-values to be made. Therefore, we will examine the overall contribution (in backtransformed values) in the following sections to quantify the relative contributions of each of these sources to the overall observed analytical variability.

3.6. Intra-lab analytical variability

A significant source of variation of any soil measurement is attributable to lab-specific variability. Here, we found that analytical variability ranged from 2.9 to 15.8% within a given lab (Fig. 2; median = 6.5, mean = 7.7). Most labs (25 to 75% quartiles) had intra-lab analytical variability ranging from 5.1 to 10.7%. There were no systematic differences in reliability between labs that used a 96-well plate reader and labs that used microcuvettes ($F_{1,10} = 1.7$, $p = 0.225$, data not shown). Internal practices such as $KMnO_4$ reagent storage conditions, consistency of reaction times, error in the dilution step, or pipetting errors prior to dilution may have contributed to this variability, though these are beyond the scope of the current study. However, the suitability of POXC for high-throughput commercial testing labs (Bongiorno et al., 2019) and the potential for substantial inter-laboratory variability (Table 4; $p < 0.00001$) suggest that POXC would benefit from lab proficiency testing.

3.7. Contribution of soil mass to analytical variability

Alterations of the mass of soil used in POXC analysis can produce differences in absolute values (Fig. 1b), but the implications of soil mass for analytical variability are understudied. We hypothesized that a greater soil mass (2.5 g) would allow for a greater and thus more

Table 4
Final reduced model assessing relative sources of analytical variability (%CV) for all detectable POXC values. All experimental factors were initially included in the model and non-significant effects ($p \geq 0.10$) were eliminated from the model one at a time. Final model was log transformed to meet assumptions of normality.

Experimental Factor	*df*	F-value	*p*-value
Lab	11	62.1	< 0.00001
Mass	1	438.7	< 0.00001
Sieve size	1	47.0	< 0.00001
Soil ID	35	35.5	< 0.00001
Soil ID × Sieve size	35	1.7	0.009

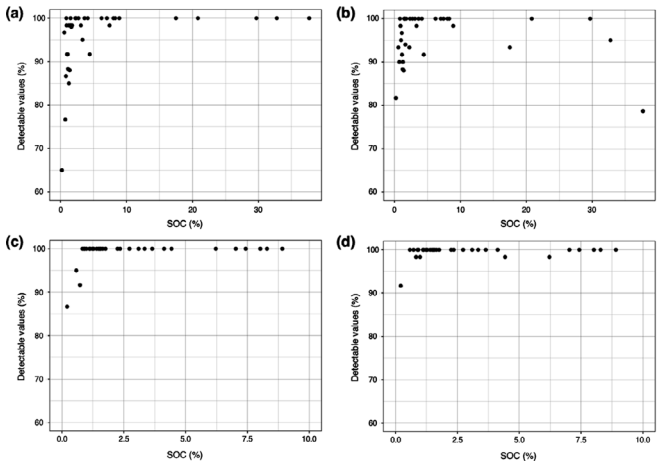

Fig. 2. Detections rates (%) as a function of SOC for (a) < 2.0 mm and 0.75 g, (b) < 0.5 mm and 0.75 g, (c) < 2.0 mm and 2.5 g, and (d) < 0.5 mm and 2.5 g. Dashed lines indicate average detection across all soils for that set of sieve size and soil mass.

consistent mass of SOC in a given sample, resulting in lower analytical variability than for a lower soil mass (0.75 g). Confirming this hypothesis, analytical variability was 6.5% greater for POXC values measured with 0.75 g relative to the 2.5 g (Fig. 3). Median analytical variability more than doubled from 5.1% at 2.5 g to 11.6% at 0.75 g. This increase in median CV value is equivalent to the median lab-specific variability (Fig. 4a), underscoring the importance of this standardization for routine, repeatable POXC analysis.

3.8. Contribution of sieve size to analytical variability

The standardization of soil sieve size prior to analysis is a common consideration for soil analytical methods. As soil aggregates are broken and the physically protected soil C is exposed, this organic matter becomes more susceptible to oxidation, regardless of chemical composition (Dungait et al., 2012). Therefore, we hypothesized that a finer sieve size (i.e. < 0.5 mm) would produce a more consistent measurement of POXC than a larger sieve size (i.e. < 2.0 mm), yielding lower analytical variability. While this hypothesis was largely confirmed (Fig. 4b), the magnitude of this difference was smaller than expected. A 1.8% decrease in median CV from 8.4% in < 2 mm sieve size to 6.6% in < 0.5 mm represents a modest improvement in analytical variability. This difference is much lower than the ~ 10% decreases that Hurisso et al. (2018b) reported for soils sieved to < 8.0 mm (CV ≈ 20%) to < 2.0 mm (CV ≈ 10%). Thus, both the absolute variability and the relative change in that variability was lower in our study than in Hurisso et al. (2018b). However, the variability at < 2.0 mm was comparable across both studies (CV ≈ 10%). Our results and those of Hurisso et al. (2018b) collectively demonstrate higher analytical variability of POXC values in coarser sieve size treatments. However, sieving to smaller sizes seems to produce diminishing returns in terms of analytical variability. Additionally, manual sieving used in this study, as is commonly practiced by research labs, produced similar analytical variability as samples mechanically flail ground to the same size (< 2.0 mm), as is commonly employed in commercial test lab settings (Hurisso et al., 2018b).

While sieve size exerted a strong main effect on analytical variability (Table 4, Fig. 4b), the relationship was not straightforward, as indicated by the soil × sieve size interaction (Table 3, $p = 0.009$). Upon further examination of the interaction term, we found differences in both direction and magnitude of this effect (Fig. 5). The magnitude of the sieve size effect on analytical variability—the absolute value of the difference between < 0.5 mm and < 2.0 mm sieve sizes—ranged from 0.04% in soil 28 to 10.6% in soil 32 (mean Δ = 1.64%). The magnitude of this difference was inversely related to SOC content ($F_{1, 70} = 4.4$, $p = 0.039$, data not shown). Thus, soils with a lower SOC content expressed larger differences in analytical variability between sieve sizes. In most soils (31 out of 36), this entailed lower analytical variability at < 0.5 mm sieve size than at < 2.0 mm, as hypothesized. However, several soils exhibited increased variability at the < 0.5 mm sieve size, and this did not appear to be explained by physicochemical properties. Of the soils with this inverse relationship, three of the five (soils 11, 27, and 28) had negligible (< 1%) changes in variability, whereas soils 17 and 19 had larger changes in variability (3.6% and 5.9%, respectively). Nevertheless, the trend of substantially lower or effectively unchanged analytical variability using the < 0.5 mm sieve size was consistent across soils.

3.9. Soil-specific sources of variability

One of the more formidable hurdles to widespread implementation of POXC is the differing degree of soil-specific analytical variability (Table 4; $p < 0.00001$). Using the standard soil physicochemical properties examined here (Table 1), we found that SOC content had the strongest effect on soil-specific variability. We found similar, negative logarithmic relationships between SOC and soil analytical variability for both < 0.5 mm and < 2.0 mm sieve sizes (Fig. 6a and b). Thus, lower SOC contents had greater overall analytical variability than higher SOC contents, independent of sieve size. At lower SOC contents, the amount of SOC per sample is likely more susceptible to slight variations between replicates, ultimately increasing analytical variability.

3.10. Conversion between masses and sieve sizes

Differences in methodology—varying the soil mass and sieve size—resulted in changes in absolute POXC values by both sieve size and mass (Fig. 1a and b, respectively). The differences in absolute values attributable to these methodological differences prevent direct comparison of POXC values across treatments. To facilitate comparisons, we developed equations to convert among POXC values derived using different masses and sieve sizes (Table 5). For these soils, conversions between soil mass and sieve size treatments were generally accurate, with R^2 values > 0.90. The root mean square error (RMSE)—a measurement of the expected error of the estimate in mg POXC kg^{-1} soil—ranged from 55 to 100 mg POXC kg^{-1} soil for soils < 10% SOC. Conversions within a given sieve size were more accurate than conversions within a mass (R^2 values and lower RMSE). Of the two sieve

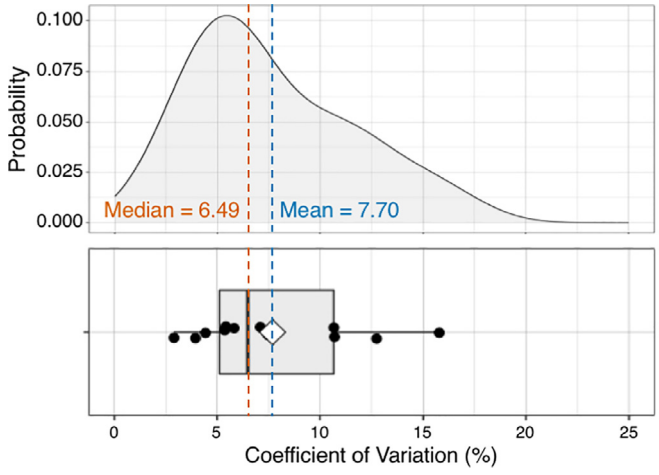

Fig. 3. Range of analytical variability that is attributable to within-lab sources. Top represents the continuous probability function, while the bottom indicates the specific variation for each of the twelve labs. Values are estimated marginal means from Eq. (2) for each lab.

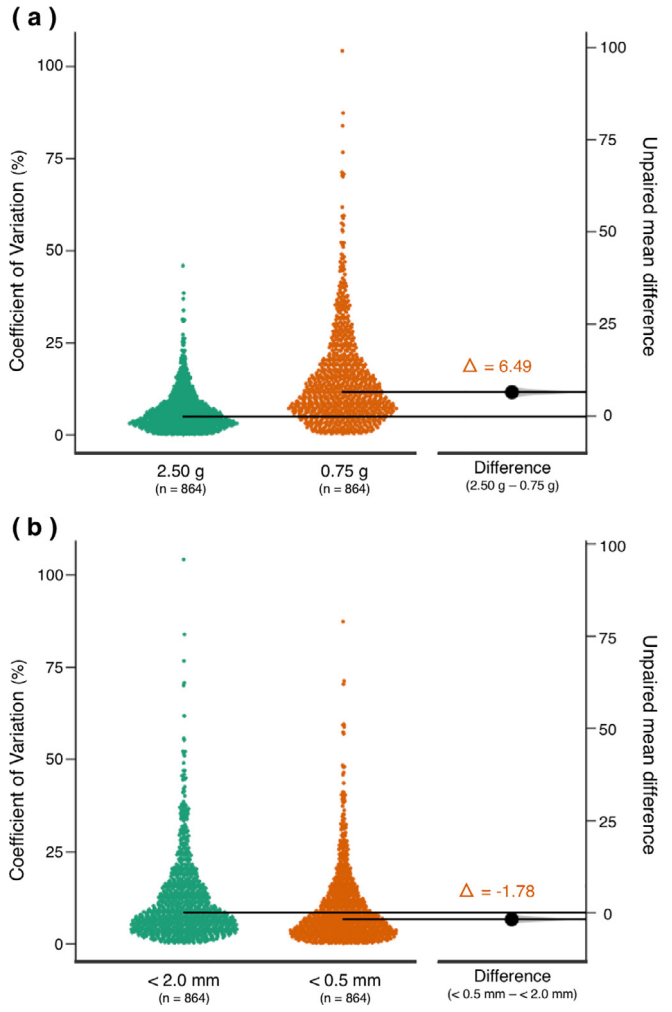

Fig. 4. Changes in analytical variability of permanganate oxidizable C (POXC) values due to change in (a) soil mass and (b) sieve size. Values are back-transformed estimated marginal means for each unique combination of soil, soil mass, sieve size, and lab. The 95% confidence intervals for the difference between treatments is based on bias-corrected accelerated bootstrap resampling with 5000 resamplings.

sizes, conversions were most accurate in the < 0.5 mm sieve size (R^2 = 0.968, RMSE = 53 mg POXC kg^{-1} soil). Therefore, we found that approximations can be made across these soil mass and sieve size treatments.

4. Future work

To date, the most prominent adaptations of the POXC measurement in soils have altered the concentration of MnO_4^- (Weil et al., 2003) and the mass of soil (Culman et al., 2012) but still accepting the underlying chemistry assumptions. Future work should address two central assumptions to further refine the measurement. First, assumptions about the oxidation-reduction processes should be examined. As Gruver (2015) noted, the assumed relationship of 1 mol Mn^{7+} oxidizing 9000 mg of C (Eq. (1)) assumes that $C^0 \rightarrow C^{4+}$ and $Mn^{7+} \rightarrow Mn^{4+}$, a stoichiometric relationship of 0.75 mol C to 1 mol Mn. At circumneutral pH, Mn^{7+} reduces to Mn^{4+}, but at acidic pH, a nearly complete reduction of Mn^{7+} to Mn^{2+} would be expected (Ladbury and Cullis, 1958). The redox state of SOC can vary considerably by texture (Keiluweit et al., 2018, 2017) and can reflect the composition of C inputs (Spokas, 2010). Because the stoichiometry of carbon oxidation and permanganate reduction is a necessary assumption in the calculation of

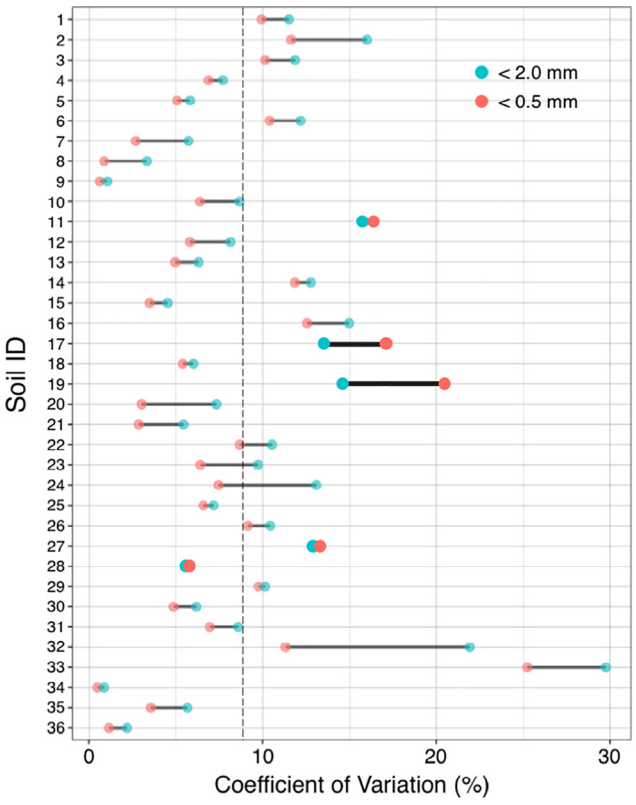

Fig. 5. Interactive effects of sieve size and soil on analytical variability on permanganate oxidizable C (POXC) values. Bolded soils indicate that CV of < 0.5 mm was greater than < 2.0 mm. Dashed line indicates mean CV for all soils (CV = 8.86). All values are backtransformed estimated marginal means using Eq. (2).

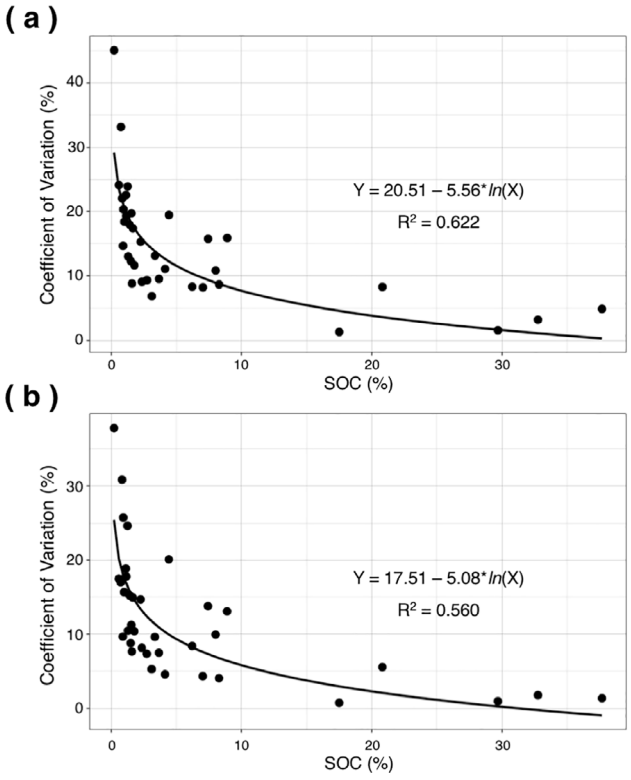

Fig. 6. Analytical variability of permanganate oxidizable C (POXC) for each soil by SOC content at (a) sieve size < 2.0 mm and (b) sieve size < 0.5 mm (mass = 0.75 g for both).

Table 5
Equations to convert between processing treatments for soils with SOC below 10%. All calculations are using mg POXC kg^{-1} soil. Bootstrapped 95% confidence intervals for each equation can be found in Table S3.

Conversion	Equation	RMSE[a]	R^2
< 2.0 mm: 0.75 g → 2.5 g	$POXC_{2.5g} = 0.633*POXC_{0.75g} + 56.40$	55.4	0.954
< 0.5 mm: 0.75 g → 2.5 g	$POXC_{2.5g} = 0.608*POXC_{0.75g} + 85.95$	59.4	0.968
0.75 g: < 0.5 mm → < 2.0 mm[b]	$POXC_{<2mm} = 0.821*POXC_{<0.5mm} + 43.76$	100.0	0.938
2.5 g: < 0.5 mm → < 2.0 mm	$POXC_{<2mm} = 0.820*POXC_{<0.5mm} + 27.72$	67.7	0.932

[a] Root mean squared error, in mg POXC kg^{-1} soil.
[b] Conversion including soils with SOC above ~10%: $POXC_{<2mm} = 0.902*POXC_{<0.5mm} - 13.42$, RMSE = 135.1, $R^2 = 0.986$.

POXC, this value should be empirically determined for soil conditions (e.g., texture, SOC quality) that can impact the conversion of a measure of MnO_4^- reduction to a SOC concentration. It is possible that "the use of a constant stoichiometric relationship when calculating [POXC] may be more a matter of convenience than accuracy" (Gruver, 2015). Secondly, quantification of POXC on a SOC, not soil mass basis (i.e., constant ratio of MnO_4^-:SOC) as in Lefroy et al. (1993) and Blair et al. (1995) could represent a substantial improvement in the repeatability of the metric by accounting for nonlinearities (Gruver, 2015). Establishment of a consistent ratio of reducing agent (soil C) to oxidizing agent (MnO_4^-) may be necessary to ensure the reliability of a measurement based on a reduction-oxidation reaction.

Although methodological considerations have been central in many discussions, the question remains: "What fraction of soil C is the permanganate oxidizing?" Several evaluations of this question have been largely inconclusive (Margenot et al., 2017; Romero et al., 2018). Better understanding the nature of POXC is critical to its use as an indicator of soil health given the mechanistic assumptions of this operationally defined C fraction implicit in its description as "active C" or "microbial food" (NRCS, 2019).

5. Recommendations

The refinement of soil methods is essential to providing reliable tools for land management decisions. However, land managers often prefer to use multiple indicators to inform their decisions, particularly within the realm of soil health (Andrews et al., 2002). In pursuance of this goal, soil health indicators have often been proposed as heuristic in place of more accurate yet labor-intensive measurements. For the current proposition and application of POXC as an operational metric sensitive to management, sensitivity and/or precision must be weighed carefully against usability and ease of implementation. While we did not exhaustively test all potential sample processing treatments, we have focused on two methodological variations that have (in our experience) proved especially problematic. Accordingly, we developed the following recommendations with two goals of (1) minimizing analytical variability while (2) maximizing the utility of the POXC metric across soil contexts. Given the range of soil characteristics in our current dataset, we believe these recommendations are applicable across nearly all soil contexts.

- In-house quality control practices: Our data demonstrate that one of the least-generalizable, yet most significant sources of variation is within-lab variability (Fig. 3). However, our data suggests that low variability (< 5%) is easily attainable. In lieu of external lab proficiency testing for POXC, individual labs are recommended to develop in-house quality control practices, such as internal reference soils or technician performance testing, to minimize this significant source of variability.
- Soil mass of 2.5 g: A soil mass of 2.5 g resulted in lower analytical variability (Fig. 4a), but was not suitable for soils with SOC contents > 10% by mass due to full consumption of MnO_4^- (i.e., no quantification possible). This threshold roughly corresponds to the ~12%SOC threshold that distinguishes mineral from organic soils in USDA Soil Taxonomy (Soil Survey Staff, 2014). Therefore, for studies comparing both mineral and organic soils, we recommend a lower mass (0.75 g). Standardization of soil mass within the same study or monitoring program is recommended because mass can markedly affect POXC values, and this magnitude of change is greater than any other source of variability assessed here.
- Sieve size of < 2.0 mm: A finer sieve size decreased analytical variability (Fig. 4b) for the majority of soils (Fig. 5) by 1.8%. However, the additional sieving to < 0.5 mm requires more labor and/or time. Given the negligible decrease in variability at < 0.5 mm sieve size, we believe this additional labor time is an opportunity cost for other in-house quality control metrics (see recommendation 1) that contribute a larger amount of variability.
- Replication: Our data shows that POXC, like many other soil metrics, has a substantial degree of analytical variability. Therefore, analytical replication is necessary, although the reasoning for increased replication varies. Soils with lower SOC contents generally have higher variability (Fig. 6a and b), necessitating additional replicates to ascertain that sample POXC values are an accurate approximation of the population mean. At SOC contents > 10% or < 5%, greater replication is needed to ensure that the calculated POXC values are within detection limits (Fig. 3), but POXC is most often measured using one to three replicates (NRCS, 2019). The issue of underpowered or uncertain hypothesis testing is ubiquitous in soil science analyses (Ladoni et al., 2015; Welsch et al., 2019). Calculated replication numbers can be found in Table S4. While we do not have values for other standard soil measurements for the current dataset, Hurisso et al. (2018a) found that POXC had comparable analytical variability to organic matter via loss-on-ignition, a common component of agronomic soil tests.

These recommendations balance a tradeoff between the sensitivity and the reliability of the metric. Specifically, the lower absolute POXC values at 2.5 g (Fig. 1b) reduces the overall sensitivity of the metric, relative to values based on 0.75 g. The decision to use a greater soil mass also decreases the range of SOC contents at which the measurement is viable, potentially complicating analyses. For example, all three Gelisols and two of the three Histosols fully consumed the 0.4 mmol of MnO_4^- when 2.5 g soil was employed, preventing measurement of POXC. We believe that these considerations are outweighed by the substantial decrease in analytical variability (Fig. 4a, $\Delta = 6.5\%$) and consistency with previously published literature (Culman et al., 2012).

6. Conclusions

As with any emerging soil health metric, POXC must be thoroughly evaluated prior to widespread adoption. Thus, standardization of methods and a clearer understanding of relative sources of variability are essential steps along the path towards implementation in commercial soil test labs. Across a wide range of edaphic properties, we found that research labs (n = 12, US and EU) differed in their within-lab variability, which ranged from 3 to 16%. A finer sieve size (< 0.5 mm) increased the absolute values of POXC (mean = 124 mg POXC kg^{-1} soil; median = 56 mg POXC kg^{-1} soil) and decreased the

analytical variability 1.8%, relative to < 2.0 mm sieve size. Using a greater soil mass (2.5 g) decreased the absolute POXC values (mean = 177 mg POXC kg^{-1} soil; median = 111 mg POXC kg^{-1} soil) and the analytical variability (Δ = 6.5%). However, at the greater soil mass the full consumption of MnO_4^- (i.e., 'bleaching') for soils with SOC > 10% exceeded the limit of quantification and meant that POXC could not be measured. Conversely, at the lower soil mass (0.75 g), some soils with SOC < 5% could be below the detection limit. Although variability in POXC measurements was in part soil-specific, it was generally inverse to SOC content. Therefore, we recommend that routine POXC analysis of < 10% SOC soils (most mineral soils) be conducted using multiple analytical replicates and a soil mass of 2.5 g. For analyses that include high organic matter soils (> 10% SOC), we recommend decreasing soil mass to 0.75 g for more appropriate comparisons across soils. While the < 0.5 mm sieve size decreased analytical variability relative to < 2.0 mm (Δ = −1.8%), the increase in labor associated with the finer sieve size suggests that this additional effort is likely not merited. Given the wide range of edaphic contexts in the current study, we believe that these recommendations are robust across soil and climatic contexts.

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 are grateful for the contributions of Cheryl Mackowiak, Jehangir H. Bhadha, Ashley Smyth, and Yuncong Li in helping source several soils for this study. Additionally, we are grateful to Willeke van Tintelen for technical assistance in the laboratory. Rachel C. Daughtridge's work was supported in part by the U.S. Department of Energy, Office of Science, Office of Workforce Development for Teachers and Scientists (WDTS) under the Science Undergraduate Laboratory Internships Program (SULI).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114235.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19, 716–723. https://doi.org/10.1109/TAC.1974.1100705.
Andrews, S.S., Karlen, D.L., Mitchell, J.P., 2002. A comparison of soil quality indexing methods for vegetable production systems in Northern California. Agric., Ecosyst. Environ. 90, 25–45.
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.
Blankinship, J.C., Berhe, A.A., Crow, S.E., Druhan, J.L., Heckman, K.A., Keiluweit, M., Lawrence, C.R., Marín-Spiotta, E., Plante, A.F., Rasmussen, C., 2018. Improving understanding of soil organic matter dynamics by triangulating theories, measurements, and models. Biogeochemistry 140, 1–13.
Bongiorno, G., Bünemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., de Goede, R., 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.
Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils. Agron. J. 54, 464–465.
Bundy, L.G., Bremner, J.M., 1973. Determination of ammonium n and nitrate n in acid permanganate solution used to absorb ammonia, nitric oxide, and nitrogen dioxide evolved from soils. Commun. Soil Sci. Plant Anal. 4, 179–184.
Carski, T.H., Sparks, D.L., 1987. Differentiation of soil nitrogen fractions using a kinetic approach 1. Soil Sci. Soc. Am. J. 51, 314–317.
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., Mirksy, S.B., Spargo, J.T., Wander, M.M., 2012. 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.
Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. Appl. Soil Ecol. 15, 3–11.
Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Glob. Change Biol. 18, 1781–1796. https://doi.org/10.1111/j.1365-2486.2012.02665.x.
Efron, B., 1987. Better bootstrap confidence intervals. J. Am. Stat. Assoc. 82, 171–185.
Fine, A.K., van Es, H.M., Schindelbeck, R.R., 2017. Statistics, scoring functions, and regional analysis of a comprehensive soil health database. Soil Sci. Soc. Am. J. 81, 589–601.
Fox, J., Weisberg, S., 2019. An {R} Companion to Applied Regression. Sage, Thousand Oaks, CA.
Fuhrman, J.K., Zhang, H., Schroder, J.L., Davis, R.L., Payton, M.E., 2005. Water-soluble phosphorus as affected by soil to extractant ratios, extraction times, and electrolyte. Commun. Soil Sci. Plant Anal. 36, 925–935.
Gruver, J., 2015. Evaluating the sensitivity and linearity of a permanganate-oxidizable carbon method. Commun. Soil Sci. Plant Anal. 46, 490–510.
Halsey, L.G., Curran-Everett, D., Vowler, S.L., Drummond, G.B., 2015. The fickle P value generates irreproducible results. Nat. Methods 12, 179.
Harris, D., Horwáth, W.R., van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or CARBON-13 isotopic analysis. Soil Sci. Soc. Am. J. 65, 1853. https://doi.org/10.2136/sssaj2001.1853.
Hendershot, W.H., Lalande, H., Duquette, M., 1993. Soil reaction and exchangeable acidity. Soil sampling and methods of analysis 2.
Ho, J., Tumkaya, T., Aryal, S., Choi, H., Claridge-Chang, A., 2019a. Moving beyond P values: data analysis with estimation graphics. Nat. Methods 1.
Ho, J., Tumkaya, T., Aryal, S., Choi, H., Claridge-Chang, A., 2019b. Moving beyond P values: everyday data analysis with estimation plots. BioRxiv 377978.
Hoskins, B., Ross, D., 2011. Soil Sample Preparation and Extraction. The Northeast Coordinating Committee for Soil Testing.
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., 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.
Hurisso, T.T., Culman, S.W., Zhao, K., 2018a. 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.
Hurisso, T.T., Culman, S.W., Zone, P., Sharma, S., 2018b. Absolute values and precision of emerging soil health indicators as affected by soil sieve size. Commun. Soil Sci. Plant Anal. 1–9.
Jensen, J.L., Schjønning, P., Watts, C.W., Christensen, B.T., Peltre, C., Munkholm, L.J., 2019. Relating soil C and organic matter fractions to soil structural stability. Geoderma 337, 834–843.
Kaiser, K., Kaupenjohann, M., Zech, W., 2001. Sorption of dissolved organic carbon in soils: effects of soil sample storage, soil-to-solution ratio, and temperature. Geoderma 99, 317–328.
Keiluweit, M., Gee, K., Denney, A., Fendorf, S., 2018. Anoxic microsites in upland soils dominantly controlled by clay content. Soil Biol. Biochem. 118, 42–50.
Keiluweit, M., Wanzek, T., Kleber, M., Nico, P., Fendorf, S., 2017. Anaerobic microsites have an unaccounted role in soil carbon stabilization. Nat. Commun. 8, 1771.
Kibblewhite, M., Ritz, K., Swift, M., 2008. Soil health in agricultural systems. Philos. Trans. R. Soc. London B: Biol. Sci. 363, 685–701.
Ladbury, J.W., Cullis, C.F., 1958. Kinetics and mechanism of oxidation by permanganate. Chem. Rev. 58, 403–438.
Ladoni, M., Basir, A., Kravchenko, A., 2015. Which soil carbon fraction is the best for assessing management differences? a statistical power perspective. Soil Sci. Soc. Am. J. 79, 848–857.
Lal, R., 2016. Soil health and carbon management. Food Energy Secur. 5, 212–222.
Lefroy, R.D., 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, 399–402.
Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60.
Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirick, S., Jacobsen, C., 2008. Spatial complexity of soil organic matter forms at nanometre scales. Nat. Geosci. 1, 238.
Lenth, R., 2019. emmeans: Estimated Marginal Means, aka Least-Squares Means.
Li, K., Zhao, Y., Yuan, X., Zhao, H., Wang, Z., Li, S., Malhi, S.S., 2012. Comparison of factors affecting soil nitrate nitrogen and ammonium nitrogen extraction. Commun. Soil Sci. Plant Anal. 43, 571–588.
Loginow, W., Wisniewski, W., Gonet, S.S., Ciescinska, B., 1987. Fractionation of organic carbon based on susceptibility to oxidation. Polish Journal of Soil Science (Poland).
Lucas, S.T., Weil, R.R., 2012. Can a labile carbon test be used to predict crop responses to improve soil organic matter management? Agron. J. 104, 1160–1170. https://doi.org/10.2134/agronj2011.0415.
Margenot, A.J., Calderón, F.J., Magrini, K.A., Evans, R.J., 2017. Application of DRIFTS, 13C NMR, and py-MBMS to characterize the effects of soil science oxidation assays on soil organic matter composition in a Mollic Xerofluvent. Appl. Spectrosc. 71, 1506–1518.
Matsuda, K., Schnitzer, M., 1972. The permanganate oxidation of humic acids extracted from acid soils. Soil Sci. 114, 185–193.
Miller, R.O., Gavlak, R., Horneck, D., 2013. Soil, Plant, and Water Reference Methods for the Western Region, 4th ed. Western Coordinating Committe on Nutrient Management.
Moebius-Clune, B.N., Moebius-Clune, D.J., Gugino, B.K., Idowu, O.J., Schindelbeck, R.R., Ristow, A.J., van Es, H.M., Thies, J.E., Shayler, H.A., McBride, M.B., Kurtz, K.S.M., Wolfe, D.W., Abawi, G.S., 2017. Comprehensive Assessment of Soil Health – The

Cornell Framework Manual, 3.1. ed. Cornell University, Geneva, NY.
Mylavarapu, R., Miller, R., 2014. Soil Preparation, Measurement, and Storage. In: Soil Test Methods From the Southeastern United States. Southern Extension and Research Activity Information Exchange Group.
Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter, in: Methods of Soil Analysis Part 3—Chemical Methods, SSSA Book Series. pp. 961–1010.
NRCS, 2019. Recommended Soil Health Indicators and Associated Laboratory Procedures (Technical Note No. 450– 03). US Department of Agriculture 3.
Oldfield, E.E., Bradford, M.A., Wood, S.A., 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. Soil 5, 15–32.
Peck, T.R., 2015. Standard Soil Scoop, in: Recommended Chemical Soil Test Procedures for the North Central Region. North Central Regional Research Publication.
Perneger, T.V., 2001. Sifting the evidence: likelihood ratios are alternatives to P values. BMJ Br. Med. J. 322, 1184.
Ramírez, P.B., Fuentes-Alburquenque, S., Díez, B., Vargas, I., Bonilla, C.A., 2019. Soil microbial community responses to labile organic carbon fractions in relation to soil type and land use along a climate gradient. Soil Biol. Biochem., 107692.
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.
RStudio Team, 2019. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA.
Sanderman, J., Creamer, C., Baisden, W.T., Farrell, M., Fallon, S., 2017. Greater soil carbon stocks and faster turnover rates with increasing agricultural productivity. Soil 3, 1–16.
Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49.
Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, D.C.
Spokas, K.A., 2010. Review of the stability of biochar in soils: predictability of O: C molar ratios. Carbon Manage. 1, 289–303.
Thomas, G., 1996. Soil pH and soil acidity. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America, Madison, WI, pp. 475–490.
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. https://doi.org/10.2136/sssaj2004.9690.
von Lützow, M., Kögel-Knabner, I., Ludwig, B., Matzner, E., Flessa, H., Ekschmitt, K., Guggenberger, G., Marschner, B., Kalbitz, K., 2008. Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. Z. Pflanzenernähr. Bodenk. 171, 111–124. https://doi.org/10.1002/jpln.200700047.
Wade, J., Culman, S.W., Sharma, S., Mann, M., Demyan, M.S., Mercer, K.L., Basta, N.T., 2019. How does phosphorus restriction impact soil health parameters in midwestern corn-soybean systems? Agron. J.
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. https://doi.org/10.1079/AJAA200228.
Welsch, J., Songling, C., Buckley, H.L., Lehto, N.J., Jones, E.E., Case, B.S., 2019. How many samples? soil variability affects confidence in the use of common agroecosystem soil indicators. Ecol. Ind. 102, 401–409.
Wickham, H., François, R., Henry, L., Müller, K., 2019. dplyr: A Grammar of Data Manipulation.
Willard, H.H., Furman, N.H., Bricker, C.E., 1956. Oxidations with standard potassium permanganate solutions. Elements of quantitative analysis, theory and practice. D. Van Nostrand Co., Princeton, NJ 217.
Yin, Y., Impellitteri, C.A., You, S.-J., Allen, H.E., 2002. The importance of organic matter distribution and extract soil: solution ratio on the desorption of heavy metals from soils. Sci. Total Environ. 287, 107–119.