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Absolute values and precision of emerging soil health indicators as affected by soil sieve size

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ABSTRACT

Permanganate-oxidizable carbon (POXC), mineralizable carbon (C), and protein are rapid and inexpensive soil health indicators, which focus on the fast-cycling labile soil organic matter. We tested the effect of soil processing (sieve size) on measured values and analytical variability (i.e., precision) of each indicator. Soil samples were hand-sieved to < 8-mm, < 2-mm, or ground to pass through a 2-mm sieve. Mean values of POXC, protein and mineralizable C were higher in < 2-mm than in either ground or < 8-mm soils. Soils sieved to < 8-mm were significantly more likely than either < 2-mm or ground soils to result in higher analytical variability of POXC and protein, but sieve size did not affect the analytical variability of mineralizable C. These results collectively demonstrate that the use of larger size fractions like 8-mm sieved soils for measuring POXC and protein not only leads to lower absolute values, but also increases analytical variability and therefore minimize their precision.

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Introduction

Soil health – also commonly referred to as soil quality – has emerged as a concept that emphasizes the need for a holistic approach to adequately assess the biological, physical, and chemical functional status of soils, with an overall goal of guiding producers' management decisions towards achieving increased productivity, resilience, and other agroecosystem services (Doran 2002, 2008; Karlen, Ditzler, and Andrews 2003; Karlen et al. 1997; Wienhold, Andrews, and Karlen 2004). Soil health indicators are especially useful when they are (i) sensitive to changes in management practices, (ii) reflect important soil ecosystem functions, (iii) rapid and inexpensive, and (iv) suitable for the high-throughput framework of commercial soil testing laboratories (Doran and Parkin 1994; Doran and Zeiss 2000).

Permanganate-oxidizable C (POXC), mineralizable carbon (C), and soil protein – which focus on the soil's active organic matter (OM) that fuels the soil food web and nutrient availability – all satisfy the above criteria of an ideal soil health indicator. However, they have yet to be adopted widely, with only a few commercial soil testing labs currently offering these tests (e.g., the Cornell Assessment of Soil Health Framework; Moebius-Clune et al. 2016). Measurement of POXC involves reaction of readily oxidizable soil C fraction with dilute potassium permanganate (KMnO_4) solution, resulting in reduction in color intensity that is measured colorimetrically (Culman, Freeman, and Snapp 2012a; Weil et al. 2003). The soil C fraction measured in such a way has been shown to be sensitive to management practices (e.g., Culman et al. 2012b; Lucas and Weil 2012; Morrow et al. 2016; Stine and Weil 2002). Short-term mineralizable C (i.e., carbon dioxide [CO_2] burst or respiration upon rewetting of dried soils; Franzluebbers et al. 2000; Haney and Haney 2010; Haney et al. 2001) is a measure of general biological activity – usually measured during 1- to 3-day aerobic incubation – and also sensitive to management practices (Culman et al. 2013; Ladoni, Basir, and Kravchenko

2015). A study by Hurisso et al. (2016) found relative enrichment of POXC in soils managed with organic inputs from compost additions and conservation tillage practices compared to mineralizable C, which was related more with conventional tillage and crop rotations involving leguminous cover crops. Soil protein represents the largest pool of organically-bound nitrogen (N) in soil OM that is readily mineralizable by microbial activity, potentially becoming available for plant uptake (Hurisso et al. 2018; Jan et al. 2009; Nannipieri and Paul 2009; Roberts and Jones 2008). Soil protein analysis involves extraction with weak and neutral sodium citrate buffer at high temperature and pressure, followed by quantification with bicinchoninic-acid (BCA) assay (Hurisso et al. 2018).

Grinding soil to pass through a 2-mm sieve is standard and recommended practice in most commercial soil testing laboratories (e.g., NCERA-13 2015). Grinding is a rapid way to homogenize a composited soil sample and mixes the soil to maximize the precision of measurements (NCERA-13 2015). However, the process of grinding also disrupts aggregate formation and exposes physically protected nutrient pools (Grandy and Robertson 2007). This is especially relevant with soil health indicators that target biologically-active measures of N and C. But the effects of soil grinding on final results and analytical variability associated with the relatively new soil health indicators remain largely unknown.

The objective of this study was to determine how soil processing (i.e., sieve size) impacts measured concentrations and precision of POXC, mineralizable C, and soil protein within a soil testing laboratory. In this paper, soil sieve size refers to soils that are hand-sieved to pass through 8-mm, 2-mm, or ground to pass through a 2-mm sieve.

Material and methods

Study site description

The three locations selected for this study represent three major crop production regions in Ohio, USA. The Knox County site in central Ohio (40° 17' N lat., 82° 35' W long.) consisted of Condit silt loam (fine, illitic, mesic Typic Epiaqualfs) and Pewamo silty clay loam soils (fine, mixed, active, mesic Typic Argiaquolls), with 0 to 2% slopes. This field was managed under corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.]-wheat (*Triticum aestivum* L.) rotation with reduced tillage before corn planting. The Mahoning County site in northeast Ohio (40° 56' N lat., 80° 40' W long.) was on Bogart loam (fine-loamy, mixed, active, mesic Aquic Hapludalfs) and Jimtown loam (Fine-loamy, mixed, superactive, mesic Aeric Endoaqualfs) soils, with 2 to 6% slopes. The field at the Mahoning County was managed under corn-soybean rotation, with chisel-plow tillage in the spring before corn planting. This field received broiler chicken (*Gallus gallus domesticus*) litter application ($\sim 4.5 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ each spring for over five years) until 2014, when the practice was stopped and replaced with mineral phosphorous (P) and potassium (K) fertilizers. The Wood County site in northwest Ohio (41° 28' N lat., 83° 33' W long.) was on Hoytville clay loam soil (fine, illitic, mesic Mollic Epiaqualfs) with 0 to 1% slopes. This field was managed under corn-soybean-wheat rotation, involving tillage in the spring prior to corn planting followed by deep disk chisel tillage in the fall after corn harvest.

Soil sampling and processing

In 2016, soil samples were collected in a grid pattern, which consisted of 20 points spaced at 15.2 m by 15.2 m and established within a 0.4-ha corn field at three sites mentioned above. The anchor point (i.e., first sampling area) was established 10 m (~ 12 rows of corn) from the edge of the field and the remaining sampling points were oriented to this anchor point, every 15.2 m apart. At each center grid point, 12 soil cores of 2.5 cm diameter \times 20 cm depth were randomly sampled within a radius of 3–4.5 m (4–6 rows of corn) from the middle of the row and were combined to form a composite sample for each grid point. Study site characteristics and soil sampling protocol are more fully described in Hurisso, Culman, and Zhao (2018).

Once in the laboratory, each composited soil sample was separated into three size classes: i) hand-sieved through an 8-mm sieve to thoroughly mix the cores and air-dried, hereafter referred to as *< 8-mm for greater simplicity*, ii) a subsample of the 8-mm sieved fraction was hand-sieved through a 2-mm sieve, hereafter referred to as *< 2-mm*, and iii) a subsample of the 8-mm sieved fraction was ground with a flail grinder and passed through a 2-mm sieve, as commonly practiced in commercial soil testing labs (NCERA-13 2015), hereafter referred to as *ground*. For each size class, the soil health tests outlined below were run on a group of three analytical replicates of the same composited soil sample. All three replicate subsamples from each composited soil were analyzed at the same time by a single technician within the same analytical laboratory.

Soil analysis

Permanganate-oxidizable carbon

Analysis of POXC was based on the method described by Weil et al. (2003), with slight modification as described in Culman, Freeman, and Snapp (2012a). Briefly, three replicate subsamples of the same composited, air-dried soil (2.5-g) from each size class were weighed into 50-mL centrifuge tubes. Twenty-milliliters of 0.02 mol L⁻¹ KMnO₄ was added to each tube and the mixture was shaken for exactly 2 min (240 strokes min⁻¹) and allowed to settle for exactly 10 min. Following settling, 0.5-mL of the supernatant was transferred into another 50-mL centrifuge tube and mixed with 49.5-mL of deionized water. Sample absorbance values were read with a spectrophotometer at 550 nm and used to calculate POXC concentration.

Mineralizable carbon

Measurement of mineralizable C was based on a 1-day aerobic incubation (e.g., Franzluebbers 2016; Franzluebbers et al. 2000). There are permutations of mineralizable C measurement methods used by researchers, including incubation time and amount of water added. Here, we used a method that is amenable to a high-throughput framework necessary for adoption by commercial soil testing laboratories. Three replicate subsamples of the same composited, air-dried soil (10-g) from each size class were weighed into three polypropylene 50-mL centrifuge tubes. To each tube, deionized water was added to adjust soil water content to 50% water-holding capacity, which was determined based on the difference in weight between a saturated soil that was allowed to drain for an hour and the weight of an oven-dried soil at 105°C for ~ 24 h. The tubes were capped tightly with lids containing rubber septum and incubated at ~ 23°C for 24 h. The headspace air was mixed by pumping a syringe three times before taking a 1-mL air sample and injecting it into an LI-820 infrared gas analyzer (LI-COR, Biosciences, Lincoln, NE) to estimate the concentration of CO₂ released during the 24 h incubation. Mineralizable C was calculated as the difference between CO₂ concentration in the sample and ambient CO₂ concentration in the blank control, using the headspace volume and the ideal gas law (Zibilske 1994).

Soil protein

Soil protein measurement was conducted following the procedure described by Hurisso et al. (2018). Three replicate subsamples of the same composited, air-dried soil (3-g) from each size class were weighed into three 50-mL glass centrifuge tubes. Twenty-four milliliters of 0.02 mol L⁻¹ sodium citrate buffer solution (pH 7) was added to each tube. The soil-extractant mixture was shaken for 5 min (180 strokes min⁻¹) and then subjected to a high temperature and pressure in autoclave (121° C, 15 psi) for 30 min. Following autoclaving, the tubes were allowed to cool to room temperature, shaken for 3 min (180 strokes min⁻¹), and then clarified by centrifugation (10,000 × g, 3 min). The quantity of extracted proteins was measured using the colorimetric BCA assay (Smith et al. 1985). Ten microliters of the clarified extract and 200 µL of the BCA reagent solution (Thermo Scientific, PierceTM, Rockford, IL) were pipetted into a 96-well plate and incubated at 60°C for 60 min. Sample absorbance values were read using a spectrophotometric plate reader at 562 nm and calibrated using

a standard curve of 0–2000 $\mu\text{g mL}^{-1}$ bovine serum albumin. Soil protein values were calculated by multiplying protein concentration in soil extracts by the volume of extractant used, and dividing the results by the weight of soil used.

Statistical analysis

Analysis of variance (ANOVA) was performed using the PROC MIXED procedure of the Statistical Analysis System (SAS ver. 9.4, SAS Institute Inc 2015) for testing the effect of sieve size on measured values and precision of each indicator. To determine the effect of sieve size on analytical variability (i.e., precision), coefficients of variation (CVs) were calculated separately for each grid point as standard deviation divided by the mean of three analytical replicate measurements from the same composited soil sample. The CV values were then used as response variables in a linear model with sieve size as predictor variable. In the model, sieve size and grid points were treated as fixed and random effects, respectively. When ANOVA indicated significance ($P \leq 0.05$), differences between means were separated using the PDIFF option of the LSMEANS statement with the Tukey’s HSD adjustment at the P level of 0.05. All graphing was performed using the *ggplot()* function from the *ggplot2* package (Wickham 2009) in R (R Development Core Team 2016).

Results and discussion

Effects of sieve size on absolute measured values

Across all three study locations, POXC concentrations in 8-mm, 2-mm, and ground soils ranged from 233 to 684, 357 to 911, and 252 to 854 mg kg^{-1} soil, respectively (Table 1). These values are within the ranges of POXC values reported by Culman et al. (2012b) and Hurisso et al. (2016), who measured POXC values of 69 to 1468 mg kg^{-1} soil and 24 to 1469 mg kg^{-1} soil, respectively, for samples collected from a wide range of soils and cropping systems across the United States. There were significant differences in POXC values among soil size fractions, with the 2-mm soils have greater POXC values than both the 8-mm and ground soils across all three sites (Figure 1). There were no significant differences in mean POXC values between ground and 8-mm soils except at the Wood site, where means of POXC were higher in ground soil than 8-mm soil.

Table 1. Descriptive statistics of three emerging soil health test values as affected by soil sieve size at three locations in Ohio, USA ($n = 60$ for each mean value). †.

Statistic	Knox			Mahoning			Wood		
	8-mm	2-mm	Ground	8-mm	2-mm	Ground	8-mm	2-mm	Ground
POXC (mg kg^{-1} soil)									
Mean	407	495	411	342	450	373	560	787	737
SD	76	90	89	54	64	70	49	59	64
Minimum	323	357	279	233	359	252	442	683	596
Maximum	569	709	621	446	643	512	684	911	854
Mineralizable C (mg kg^{-1} soil)									
Mean	33	42	38	21	18	22	39	46	40
SD	11	15	11	9	5	9	3	5	5
Minimum	11	11	16	8	9	8	33	35	31
Maximum	50	71	56	35	29	43	45	55	50
Protein (mg g^{-1} soil)									
Mean	5.1	6	5	4.8	7.3	5.2	6.6	7.7	7
SD	1.1	0.7	0.7	1	2.9	0.7	0.5	0.5	0.4
Minimum	3.5	4.4	3.6	3.5	2.4	4	5.7	6.9	6.2
Maximum	7.9	7.2	6.2	7.1	14.1	6.8	7.4	8.7	7.7

† POXC = permanganate oxidizable carbon; SD = standard deviation.

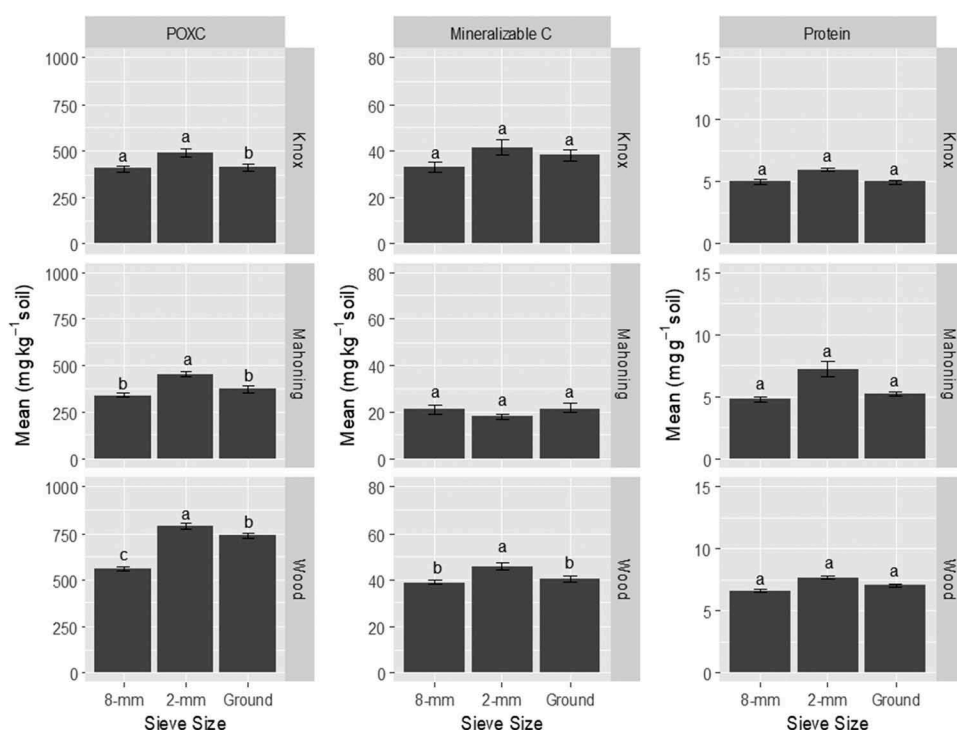


Figure 1. Mean values of permanganate oxidizable C (POXC), mineralizable C and soil protein as affected by soil sieve size at three locations in Ohio, USA. For each indicator, subsamples from the same composited soil were analyzed in triplicate separately for each grid point before being averaged over all of the 20 grid points. In the statistical model, soil sieve size and grid points were treated as fixed and random effects, respectively. Within the same site, mean values followed by different lowercase letters were significantly different at the $p \leq 0.05$ level. Error bars represent the standard error of the mean ($n = 60$).

Mineralizable C values ranged from 8 to 50, 9 to 71, and 8 to 56 mg kg⁻¹ soil in 8-mm, 2-mm, and ground soils, respectively (Table 1). However, means of mineralizable C were statistically not different among grind size classes except at the Wood site, where the 2-mm soils had 1.2 times higher mineralizable C than that of ground and 8-mm soils (Figure 1). Previous studies have shown that soil disturbance induced by sieving through smaller sieve openings leads to greater C mineralization, generally supporting the results found at the Wood site (Figure 1). Franzluebbers (1999), for example, found a 10–60% greater C mineralization (3-day incubation) in soils sieved with ≤ 2 -mm sieve openings than those sieved with ≥ 4.7 -mm sieve openings. Wade et al. (2018) also measured greater mineralizable C (1-day incubation) in 2-mm sieved soils than 8-mm sieved soils. Such an observation is usually attributed to the release of aggregate-protected potentially labile source of organic matter following disruption of macroaggregates (Franzluebbers 1999; Grandy and Robertson 2007).

Soil protein values ranged from 3.5 to 7.9, 2.4 to 14, and 3.6 to 7.7 mg g⁻¹ soil in 8-mm, 2-mm, and ground soils, respectively (Table 1). These soil protein values agree with those of Fine, van Es, and Schindelbeck (2017), who reported soil protein values for a large number of samples ($n = 2451$) from a wide range of soils in the US Mid-Atlantic, Midwest, and Northeast regions. In that study, median soil protein values for coarse-, medium-, and fine-textured soils were 9.0 (range: 1.3–31.1), 11.7 (range: 0–242.2), and 14.5 (range: 2.2–60) mg g⁻¹, respectively. Trends in soil protein values were similar to the patterns observed in POXC, with 2-mm sieved soils having greater protein values than either ground or 8-mm sieved soils across all three sites (Figure 1). There were no significant differences in soil protein values between ground and 8-mm sieved soils except at the Wood site.

Effects of soil grinding versus sieving on precision

To examine how *analytical precision* of the three emerging soil health indicators would be affected by soil grinding versus sieving, we used coefficient of variation (CV) from a group of three analytical replicates of the same composited soil sample. For all indicators but mineralizable C, analytical variability differed significantly between ground and sieved soil samples. Permanganate-oxidizable C had the highest CV values in 8-mm soil (20 to 35%), followed in decreasing order by those in ground soil (9 to 9.7%) and 2-mm soil (8.4 to 9.0%; [Figure 2](#)). At Mahoning, CV value of POXC measured in ground soil was almost twice as large as that of 2-mm soil (19% vs. 10%, respectively), but differences in CV values of POXC between ground and 2-mm soils were not significant at Knox and Wood sites. For protein, CV values followed the order: 8-mm soil (5 to 11% CV) > 2-mm soil (2.4 to 2.6% CV) ≥ ground soil (2.6 to 3.2% CV). At Mahoning, CV value of protein measured in 2-mm soil was almost 6-times higher than that of ground soil (17.9% vs. 3.2%, respectively), but did not differ significantly between these two size fractions at Knox and Wood sites. Inherent soil variability and repeated application of broiler chicken litter (4.5 Mg ha⁻¹ yr⁻¹) at the Mahoning site might explain the reason for the inconsistent patterns observed in POXC and protein CV values between ground and 2-mm sieved soils compared with those observed at the other two study sites. These results collectively demonstrate that both POXC and protein were analytically more variable in 8-mm size fractions than in either 2-mm sieved soil or ground soil.

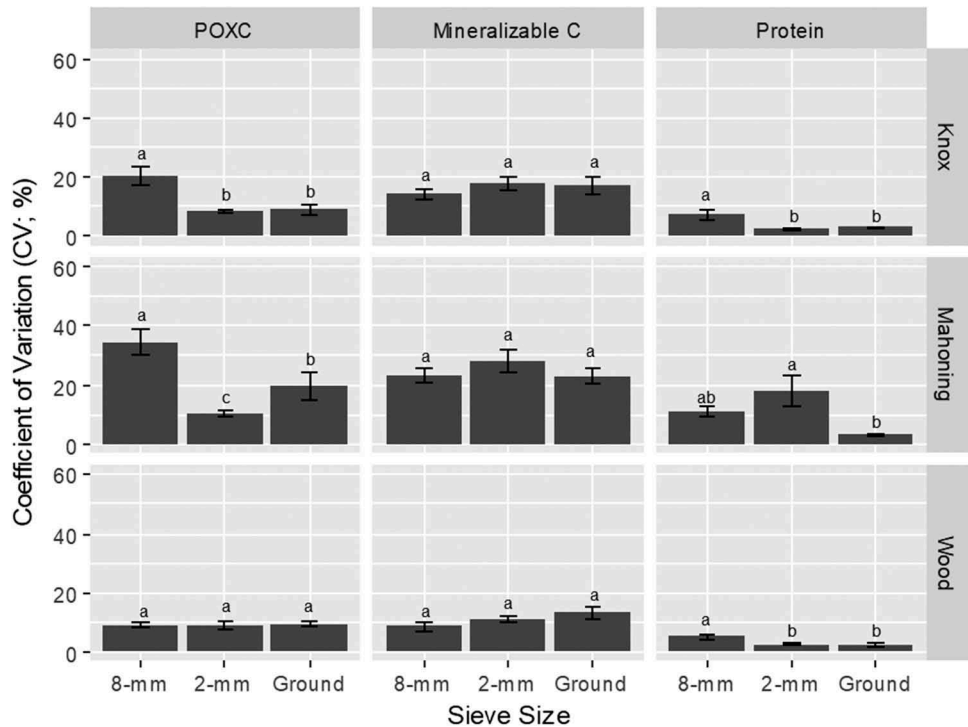


Figure 2. The effect of soil sieve size on analytical precision (as determined by coefficient of variation, %CV) of emerging soil health indicators: permanganate oxidizable C (POXC), mineralizable C, and soil protein at three locations in Ohio, USA. For each indicator, subsamples from the same composited soil were analyzed in triplicate to calculate CV values separately for each grid point prior to averaging over all of the 20 grid points. In the statistical model, CV values were used as response variables with soil sieve size as predictor variables. Within the same site, mean CV values followed by different lowercase letters were significantly different at the $p \leq 0.05$ level. Error bars represent standard of the mean ($n = 60$).

Conclusions

Overall, results presented here demonstrate that the use of larger size soil fractions such as an 8-mm sieved soil for measuring POXC and soil protein is more likely to result in lower absolute values, higher analytical variability and thus minimize their repeatability. Surprisingly, 2-mm sieved soil had the greatest POXC and protein values across all sites, and greatest mineralizable C value at 1 site. Our findings that analytical variability of mineralizable C did not differ significantly between ground and sieved soils (Figure 2) also agree with the observations of Wade et al. (2018), who concluded that the method of soil processing (i.e., ground versus sieved) has little to no influence on the repeatability of mineralizable C measurement for a given site or soil type.

Greater adoption of soil health testing will require that commercial soil testing labs incorporate additional methodologies into their current portfolios of analysis offered. Understanding the trade-offs and implications of soil processing on analysis results and repeatability is a key step to more wide-spread adoption of these emerging soil health indicators. The data presented here show that class size does impact both final values and repeatability of analysis. For commercial soil testing labs that handle a high volume of samples, hand-sieving soils is often not practical, as the time commitment is too great. Grinding is the preferred approach and these results justify grinding soils to reduce analytical error and maximize repeatability. Future work should seek to better understand the mechanism(s) that cause a reduction in total values in POXC and protein values when samples are ground versus sieved to 2-mm.

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