

Repeatability and Spatiotemporal Variability of Emerging Soil Health Indicators Relative to Routine Soil Nutrient Tests

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Ideal indicators suitable for routine soil health evaluation would be rapid, cost-effective, sensitive to management, and exhibit low analytical variability. Permanganate-oxidizable C (POXC), mineralizable C, and soil protein are rapid and emerging soil health indicators of labile organic matter (OM), but their repeatability and spatiotemporal variability remain largely unknown. We examined the repeatability and spatiotemporal variability of each indicator relative to routine soil nutrient tests. Grid soil samples were collected three times during a corn (*Zea mays* L.) growing season at three sites in Ohio. Analytical variability of indicators ranked from highest to lowest mean coefficients of variation (CVs): mineralizable C (13–23%) > OM via loss-on-ignition (OM-LOI; 11–16%) = POXC (9–19%) > protein (2.6–3.2%) = Mehlich-3 P (1.8–2.6%) = Mehlich-3 K (3.0–3.8%) > pH ($\leq 1\%$). Temporal variability of indicators ranked from highest to lowest mean CVs: mineralizable C (22–37%) > OM-LOI (16–25%) = POXC (9–21%) = Mehlich-3 P (14–33%) = Mehlich-3 K (6–33%) \geq protein (7–13%) > pH (1.7–3.6%). Almost all soil properties exhibited moderate to strong spatial autocorrelations, occurring at range distances ≤ 76 m. Our results collectively suggest: (i) mineralizable C and POXC have analytical variability similar to that of OM-LOI, whereas soil protein similar to Mehlich-3 extractable nutrients; (ii) the soil health indicators exhibit temporal variability to a degree similar to routine soil nutrient tests; and (iii) the soil health indicators display spatial variability to a similar extent as the routine soil nutrient tests and therefore do not require greater soil sampling densities within a field.

Abbreviations: AIC, Akaike's information criterion; BCA, bichinchonic acid assay; CV, coefficient of variation; LOI, loss-on-ignition; OM, organic matter; POXC, permanganate-oxidizable C.

Soil organic matter (OM) is a key determinant and indicator of both soil fertility and soil health (Reeves, 1997; Weil and Magdoff, 2004). Organic matter influences numerous soil functions, including soil structural stability, water holding capacity, biological activity, and nutrient retention and release (Tiessen et al., 1994; Weil and Magdoff, 2004). Most standard soil analyses performed by commercial soil testing facilities include the measurement of soil OM (via weight loss on ignition) as part of routine soil nutrient testing program (NCERA-13, 2015). However, soil OM is known to respond very slowly over time to management changes, and hence may not provide an early indication of potential soil OM accrual or loss (Wander, 2004; Mirsky et al., 2008). Thus, indicators that are more sensitive than total OM are needed to monitor short-term management-induced changes (Doran and Parkin, 1994; Doran and Zeiss, 2000; Doran, 2002; Wienhold et al., 2004; Karlen et al., 1997, 2003, 2008). The soil OM pool includes a continuum of compounds varying in nutrient content and biological availability (Schmidt et al., 2011; Lehmann and Kleber, 2015). The labile OM pool in particular is a good candidate for soil health assessment, as it would be responsive to

Core Ideas

- POXC, mineralizable-C, and protein are emerging soil health indicators.
- POXC and mineralizable-C have analytical variability similar to OM via loss-on-ignition.
- Soil protein has smaller analytical variability similar to Mehlich-3 extractable nutrients.
- Soil health indicators exhibit spatiotemporal variability to a similar extent as routine nutrient tests.
- Soil health indicators do not require greater soil sampling densities within a field.

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management changes (usually within one to three years of implementation) (Wander, 2004; Weil and Magdoff, 2004; Culman et al., 2013).

Permanganate oxidizable C, mineralizable C, and soil protein are rapid and affordable soil tests that focus on the fast-cycling, labile OM pool. A measure of the biologically-available soil C pool, POXC is related to key soil quality indicators including microbial biomass and a processed pool of C (Weil et al., 2003; Mirsky et al., 2008; Culman et al., 2012b). Permanganate oxidizable C is also sensitive to management practices (Stine and Weil, 2002; Weil et al., 2003; Culman et al., 2012b; Lucas and Weil, 2012; Culman et al., 2013). Mineralizable C based on short-term aerobic incubation (1 to 3 d) is a general indicator of biological activity (Wang et al., 2003), and is sensitive to management practices (Culman et al., 2013; Ladoni et al., 2015). A comprehensive study showed that POXC and mineralizable C were often correlated, but differentially influenced by management practices (Hurisso et al., 2016). In that study, POXC was related more to practices that promote soil OM building (e.g., no-till and compost addition) than mineralizable C, which was associated more with practices that lead to soil OM mineralization (e.g., tillage and leguminous cover cropping). Protein-N represents by far the largest fraction of organic-N containing compounds in soil OM, with estimates ranging from 30 to 45% of total soil N (Gillespie et al. (2011) and references therein). Soluble proteins have also been found to be the principal source of mineralizable N (Németh et al., 1988; Matsumoto et al., 2000), indicating that soil proteins can serve as a reservoir of N that is released through mineralization processes (Jan et al., 2009; Nannipieri and Paul, 2009). Some studies have reported that soil protein is also responsive to management practices, such as tillage and crop rotational diversity (Moebius et al., 2007; Moebius-Clune et al., 2008; Roper et al., 2017; Hurisso et al., 2018).

The three measurements described above are also part of indicators included in commercially-available soil health assessments, such as the Cornell Assessment of Soil Health Framework (Moebius-Clune et al., 2016). In addition, POXC, mineralizable C and soil protein have been identified as potential soil health indicators by recently established initiatives, such as the Soil Health Institute (<https://soilhealthinstitute.org/>); however, they have yet to be adopted widely by commercial soil testing facilities that handle a high volume of samples. These laboratories might be more receptive to incorporating these rapid and inexpensive soil tests into their current portfolios of services if the measurements were easily repeatable (i.e., low analytical variability). Unfortunately, there is no published information on the repeatability of POXC and soil protein, and scant information on mineralizable C repeatability, for which the studies by Sherrod et al. (2012) and Wade et al. (2018) are the only reports to our knowledge.

Another key factor to improve the accuracy of soil test results is understanding the spatial and temporal variability associated with soil properties. Total soil C and N are generally stable in time and less variable in space and only affected by long-term changes in soil management (Ruffo et al., 2005). Since POXC, mineralizable

C and soil protein are measures of the labile OM pool, they are more likely to display greater spatiotemporal variability than total soil C and N. Geostatistics are commonly used to assess spatial heterogeneity of soil properties using model parameters derived from semivariograms, including range distances to describe the extent of spatial autocorrelation (Ettema and Wardle, 2002; Kral et al., 2012). For example, Robertson et al. (1993) found soil organic C, soil pH, and soil test P to be spatially autocorrelated over range distances of 7 to 26 m in an annually-mown and never cropped field. In an adjacent field that was plowed and cropped annually for decades, they determined range distances of 23 to 64 m for the same soil parameters. Their findings demonstrate that the distribution of soil properties within a field can be spatially structured over distances of a few meters to hundreds of meters. But published information on spatial variability of POXC, mineralizable C, and soil protein does not exist. Therefore, a better understanding of spatiotemporal variability for these relatively new soil health indicators is needed to help guide farmers and farm advisors on the appropriate sampling densities required to account for inherent field spatial variability.

The objectives of this study were: (i) to compare the repeatability of POXC, mineralizable C and soil protein measurements with those of routine soil nutrient tests within a single analytical lab, and (ii) to assess in-field spatial and temporal variability of each indicator relative to routine soil nutrient tests. For the purpose of this study, routine soil nutrient tests are defined as those recommended by Land Grant Universities (NCERA-13, 2015) and most commonly reported in the U.S. North Central Region (specifically, soil OM via loss of weight on ignition, soil pH, and Mehlich-3 extractable P and K).

MATERIALS AND METHODS

Study Area, Soil Sampling, and Processing

Soils used in this study were collected from agricultural cropland fields located in Knox, Mahoning and Wood Counties in Ohio (Table 1). The fields were sampled three times over the course of corn growing season in 2016. The first sampling event took place in June and corresponded with soil sampling for pre-sidedress nitrate test in corn, which is typically at V4-V8 growth stage in Ohio (Tremblay et al., 2012), hereafter referred to as *V4 sampling* for the sake of greater simplicity. The second sampling event occurred at the end of July when corn was at silking or R1 growth stage (hereafter called *R1 sampling*). The third and final set of samples were collected in October when corn was at physiological maturity or R6 growth stage (hereafter called *R6 sampling*). For further details on corn growth and development stages in Ohio, see Thomison et al. (2017). Soils were sampled at different time points during growing season to gain insights into short-term temporal dynamics in measures of the labile OM pool, which is a nutrient pool for both plants and the soil food web, relative to routine soil nutrient tests.

At each field site, soil samples were collected in a grid pattern (46 m by 61 m) to facilitate geostatistical analysis, using semivariograms (Kral et al., 2012) to assess spatial autocorrelation in the

Table 1. Description of Ohio study sites.

County (location)	Soil series (taxonomic class)	Management practice	Soil texture		
			Sand	Silt	Clay
			g kg soil ⁻¹		
Knox 40°17' N 82°35' W	Condit silt loam (Fine, illitic, mesic Typic Epiaqualfs) and Pewamo silty clay loam (Fine, mixed, active, mesic Typic Argiaqualfs) with 0 to 2% slopes	Corn (<i>Zea mays</i> L.), soybean [<i>Glycine max</i> (L.) Merr.] and wheat (<i>Triticum aestivum</i> L.) rotation involving tillage before corn planting	260 ± 25†	443 ± 27	297 ± 23
Mahoning 40°56' N 80°40' W	Bogart loam (Fine-loamy, mixed, active, mesic Aquic Hapludalfs) and Jimtown loam (Fine-loamy, mixed, superactive, mesic Aerlic Endoaqualfs) with 2 to 6% slopes	Corn-soybean rotation involving chisel plow tillage in the spring before corn planting and broiler chicken (<i>Gallus gallus domesticus</i>) litter application (4.5 Mg ha ⁻¹ each spring for over 5 yr) until 2014 when the practice was replaced with mineral P and K fertilizers	423 ± 77	350 ± 74	227 ± 15
Wood 41°28' N 83°33' W	Hoytville clay loam (Fine, illitic, mesic Mollic Epiaqualfs) with 0 to 1% slopes	Corn-soybean-wheat rotation involving tillage in the spring before corn planting and in the fall after corn harvest	471 ± 40	158 ± 52	370 ± 14

† Values are means with one standard deviation.

soil health indicators relative to routine soil nutrient tests. The first sampling area (anchor point) was established 10 m (~12 rows of corn) from the edge of the field and the remaining nineteen sampling points were oriented to this anchor point, every 15.2 m apart (Fig. 1). At each center grid point, 12 individual soil cores were randomly collected within a radius of 3.0 to 4.5 m (four to six rows of corn). The soil cores were collected using a probe (2.5 cm i.d.) to a depth of 0- to 20-cm from the middle of the row. All 12 cores from each sampling point in the grid were combined to form a composite soil sample for that grid point. Even though our sampling density and number of cores per sample was slightly higher than what is typically practiced in the agriculture industry in Ohio (5 to 8 cores per composited soil sample; ~0.5 to 5 acres per soil sample), we make the assumption in this study that spatial variability in these fields can be scaled-up to larger areas.

Once in the laboratory, all soil samples were sieved to pass an 8-mm screen, air-dried, and ground to pass through a 2-mm sieve

with a Dynacrush flail grinder, as commonly practiced in most commercial soil testing facilities in the North Central Region (NCERA-13, 2015). All soil samples were analyzed in a single analytical replicate except those from the R6 sampling, which were analyzed in triplicate to evaluate analytical variability. In the case of soil samples analyzed in triplicate, all three replicate subsamples from each composite soil were analyzed at the same time by a single technician within the same analytical laboratory.

Permanganate Oxidizable C

Analysis of POXC was based on the method described by Weil et al. (2003), with minor modification as described in Culman et al. (2012a). A 20-mL volume of 0.02 mol L⁻¹ KMnO₄ solution was added to a 50-mL centrifuge tube containing 2.5 g of soil and shaken for 2 min on a horizontal shaker. The soil was allowed to settle for 10 min, after which 0.5 mL of the supernatant was transferred into a second 50-mL centrifuge tube containing 49.5 mL of deionized water. Sample absorbance values were read at 550 nm with a 96-well spectrophotometric plate reader and calibrated using standard concentration curves of KMnO₄ solutions.

Mineralizable C

Mineralizable C (i.e., C respired upon rewetting of dried soils) was measured during 1 d of aerobic incubation (Franzluebbers et al., 2000; Franzluebbers, 2016). Permutations of this method exist among researchers, including incubation time (1 to 3 d), sieve size (2–6 mm), and amount of water added. We employed a methodology amenable to a high-throughput framework. A 10-g soil sample was weighed into a 50-mL polypropylene centrifuge tube. 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 after drying soil overnight in an oven at 105°C. The tubes were capped tightly with lids containing rubber septum and incubated at a room temperature of 23°C for 24 h. The headspace air was mixed by pumping a syringe three times before taking a 1-mL air sample to determine the concentration of CO₂

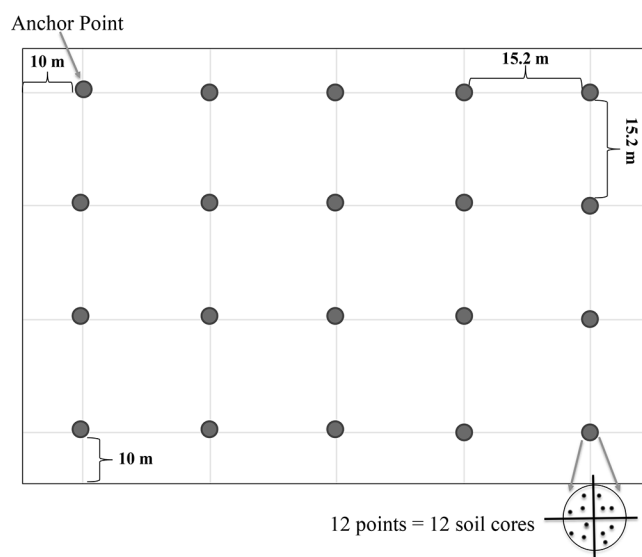


Fig. 1. Soil sampling design for repeatability and spatiotemporal variability assessment of soil properties at three locations in Ohio. The sampling protocol followed a grid pattern which consisted of 20 points spaced at 15.2 m by 15.2 m. A composite soil sample of 12 cores was taken at each sampling point in the grid.

by injecting the air sample into an LI-820 infrared gas analyzer (LI-COR, Biosciences, Lincoln, NE). Mineralizable C was calculated as the difference between a sample and a blank control, using the headspace volume and the ideal gas law (Zibilske, 1994).

Soil Protein

Extractable soil protein was determined using a neutral sodium citrate (pH 7) buffer (Hurisso et al., 2018). A 24-mL volume of 0.02 mol L⁻¹ sodium citrate buffer solution was added to 3.0 g of soil contained in a 50-mL glass centrifuge tube. The soil-extractant mixture was shaken for 5 min (180 strokes min⁻¹) and subjected to a high temperature and pressure in an autoclave (121°C, 30 min). The extracts 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 protein was measured using the colorimetric bicinchoninic-acid (BCA) assay (Thermo Scientific, Pierce, Rockford, IL) with a 96-well spectrophotometric plate reader at 562 nm. Sample absorbance readings were calibrated using a standard curve of 0 to 2000 µg mL⁻¹ bovine serum albumin. Autoclaved-citrate extractable soil protein content of each sample was calculated by multiplying protein concentration in soil extracts by the volume of extractant used, and dividing the results by the weight of soil used.

Routine Soil Nutrient Tests

All routine soil analyses followed the procedures outlined and recommended by Land Grant Universities in the North Central Region (NCERA-13, 2015). Soil OM was determined by loss of weight on ignition (OM-LOI) in a high temperature oven at 360°C for 2 h (Combs and Nathan, 1998). Soil water pH was measured with a glass electrode in a 1:1 soil/water (w/v) mixture (Peters et al., 2012). Extractable soil P and K were determined using the Mehlich-3 extractant (Mehlich, 1984) and analyzed with an inductively coupled plasma spectrometer as described by Frank et al. (1998) and Warncke and Brown (1998). In addition, particle size was determined by the standard hydrometer method according to Gee and Bauder (1986).

Statistical Analyses

To estimate the relative magnitude of analytical and temporal variability associated with each soil test, coefficient of variation (CV) was calculated as standard deviation normalized by the mean, separately for each grid point ($n = 20$). For analytical variability, the CV calculations were based on measurements performed on three replicate subsamples of the same composite soil at each grid point from R6 sampling. The CV calculations for temporal variability were based on measurements performed on a single replicate sample at each grid point from V4, R1, and R6 sampling times. The CV value was then used as a response variable in analysis of variance model where soil tests and grid points were treated as fixed and random effects, respectively. Data was checked for parametric statistical assumptions and analyzed by using the PROC MIXED procedure in SAS (SAS ver. 9.4, SAS Institute Inc., Cary, NC). Differences between means were sepa-

rated using the PDIF option of the LSMEANS statement with Tukey's HSD adjustment at $p < 0.05$ unless reported otherwise. All graphs were made with the function *ggplot()* from the *ggplot2* package (RStudio Team, 2016).

The presence of spatial autocorrelations was first assessed by calculating and plotting sample semivariograms of the dataset by using custom R codes (RStudio Team, 2016). The semivariance calculation was according to the formula:

$$\gamma(h) = \frac{1}{2N_h} \sum_{i=1}^{N_h} [Z(s_i) - Z(s_i + h)]^2$$

where N_h is the number of observation pairs separated by a distance of h , $Z(s_i)$ is the value of the variable of interest at location s_i , and $Z(s_i + h)$ is the value of that same variable of interest at a location at distance h from s_i . Then theoretical semivariogram models that account for spatial correlations, including exponential, Gaussian, power and spherical were fitted to the data, separately for each site and sampling time. The Akaike Information Criterion (AIC) was used to compare model performances. Spatial autocorrelation was considered to be present when AIC of at least one of the semivariogram models that accounted for spatial correlations was lower than AIC from the null model that assumed no spatial autocorrelation (Burnham et al., 2011; Symonds and Moussalli, 2011). Range distances derived from the best-fit model were used to describe the extent of spatial dependence. The proportion of model sample variance explained by structural variance [$C/(C_0 + C)$] was calculated as a normalized measure of spatial dependence, using nugget (C_0) and the sill variance ($C_0 + C$) from best-fit models. The values of [$C/(C_0 + C)$] range from 0 to 1, where [$C/(C_0 + C)$] < 0.25, 0.25 < [$C/(C_0 + C)$] < 0.75, and [$C/(C_0 + C)$] > 0.75 respectively indicate weak, moderate, and strong spatial dependence (Robertson et al., 1993; Cambardella et al., 1994). For the purpose of spatial and temporal variability assessment, we used only one analytical replicate from the R6 soils that were analyzed in triplicate by randomly selecting one replicate using the function *sample_n()* from the *dplyr* package.

Pearson's correlation coefficients were also calculated using the function *rcorr()* from the *Hmisc* package to assess the relationships among the different soil tests across sites and sampling times. Significant correlations were identified at $p < 0.05$ or $p < 0.10$.

RESULTS AND DISCUSSION

Descriptive Statistics

Across sites, measured soil POXC ranged from 93 to 991 mg kg⁻¹, mineralizable C ranged from 7 to 95 mg kg⁻¹ and soil protein ranged from 3.6 to 9.6 mg g⁻¹ (Table 2). These values are within the ranges of POXC, mineralizable C and soil protein values found in the literature (Culman et al., 2012b; Hurisso et al., 2016; Fine et al., 2017). Wood County generally had larger values of organic matter (POXC, mineralizable C, soil protein, OM-LOI), likely reflecting the higher percentage of clay relative to the other sites (Tables 1 and 2).

Table 2. Descriptive statistics for the various soil tests by site and time of sampling ($n = 20$ for each mean value).

Statistic	Knox			Mahoning			Wood		
	V4	R1	R6	V4	R1	R6	V4	R1	R6
POXC†									
mg kg soil ⁻¹									
Mean	456	491	422	493	459	395	638	668	724
Std. Dev.	86	88	91	68	127	167	72	61	78
Minimum	319	319	290	406	318	94	437	511	589
Maximum	657	656	612	662	887	991	736	769	908
Mineralizable C									
mg kg soil ⁻¹									
Mean	50	52	39	41	33	22	44	46	40
Std. Dev.	15	16	14	18	19	7	11	8	7
Minimum	21	33	11	19	12	7	22	24	28
Maximum	76	91	61	95	90	41	60	58	55
Protein									
mg kg soil ⁻¹									
Mean	5.6	5.1	5.0	6.7	6.2	5.3	6.5	6.5	7.0
Std. Dev.	0.6	0.5	0.7	0.9	1.0	0.7	0.7	0.5	0.4
Minimum	4.5	4.4	3.6	5.2	5.1	4.0	5.7	5.9	6.2
Maximum	6.7	6.0	6.0	9.0	9.6	7.0	7.6	7.6	7.7
OM-LOI‡									
g kg soil ⁻¹									
Mean	25	25	22	23	23	15	29	29	33
Std. Dev.	6	5	7	3	4	3	3	4	9
Minimum	16	18	10	18	18	9	25	21	3
Maximum	40	35	33	29	37	22	34	37	54
pH									
Mean	5.6	5.6	5.9	5.7	5.6	5.7	6.3	6.2	6.3
Std. Dev.	0.4	0.3	0.4	0.3	0.5	0.4	0.1	0.2	0.3
Minimum	5.0	5.1	5.3	5.2	5.1	5.1	6.0	5.9	5.8
Maximum	6.5	6.1	6.4	6.2	6.8	6.5	6.5	6.7	7.1
Mehlich-3 P									
mg kg soil ⁻¹									
Mean	112	125	105	79	83	44	61	66	78
Std. Dev.	36	44	31	43	58	28	8	13	15
Minimum	63	64	49	44	40	17	49	44	52
Maximum	203	231	184	200	275	150	78	89	105
Mehlich-3 K									
mg kg soil ⁻¹									
Mean	187	170	157	351	339	189	208	204	224
Std. Dev.	34	35	36	69	123	43	9	13	11
Minimum	124	96	85	214	199	123	189	183	202
Maximum	259	237	218	454	727	280	225	230	243

† POXC, permanganate-oxidizable carbon.

‡ OM-LOI, soil organic matter determined via loss-on-ignition.

Soil health indicators were generally positively related to routine soil measurements, but the strength of the relationship varied considerably (Table 3). In particular, OM-LOI was correlated with POXC, mineralizable C and soil protein. The range of r^2 values of the correlations between OM-LOI and POXC, mineralizable C, and soil protein were 0.20 to 0.54, 0.19 to 0.36, and 0.24 to 0.61 respectively, depending on site and sampling time (data not shown). Consistent with results presented here, Fine et al. (2017) also reported significant relationships of OM-LOI with POXC ($r^2 = 0.52$), mineralizable C ($r^2 = 0.61$), and soil protein ($r^2 = 0.45$) for samples collected from a wide range of soils and cropping systems in the Mid-Atlantic, Midwest, and Northeast regions.

Analytical Variability

Soil testing laboratories require soil tests with relatively small analytical variability so that meaningful data can be generated consistently from samples submitted for analysis. Thus, a primary question of interest when adopting new methodology is to what extent are emerging soil health indicators repeatable? This question focuses on the precision of these relatively new indicators that are ideal for routine soil health evaluation, using CV values from a group of laboratory replicates of the same composited soil sample to assess analytical variability. Across all sites, POXC, mineralizable C and soil protein mean CV values ranged from 9 to 19, 13 to 23 and 2.6 to 3.2%, respectively. Trends in the analytical variability of each indicator were largely consistent across sites, with organic matter measurements CV values following the pattern: mineraliz-

Table 3. Pearson correlation coefficients (*r*) and *p*-values for soil health and soil nutrient tests calculated across all sites and sampling times (*n* = 180).

	POXC†	Mineralizable C	Protein	OM-LOI‡	pH	Mehlich-3 P	Mehlich-3 K
POXC	1.000	***	***	***	***	NS§	*
Mineralizable C	0.464	1.000	***	***	***	***	NS
Protein	0.703	0.365	1.000	***	***	NS	***
OM-LOI	0.627	0.451	0.511	1.000	***	¶	¶
pH	0.695	0.455	0.460	0.491	1.000	¶	NS
Mehlich-3 P	0.005	0.407	0.093	0.132	-0.135	1.000	*
Mehlich-3 K	0.167	0.123	0.573	0.139	0.054	0.155	1.000

* Significant at *p* < 0.05; *** Significant at *p* < 0.001.
† POXC, permanganate-oxidizable carbon.
‡ OM-LOI, soil organic matter determined via loss-on-ignition.
§ NS, not statistically significant.
¶ Significant at *p* < 0.10.

able C > OM-LOI = POXC > soil protein (Fig. 2). Soil pH had the lowest analytical variability, likely given its logarithmic scale, and Mehlich-3 P and K were intermediate in variability.

These results demonstrate that analytical variability associated with POXC and mineralizable C are on a similar scale to OM-LOI, but soil protein has at least fourfold smaller variability compared with the other organic matter measurements (POXC,

mineralizable C, and OM-LOI). The relatively large analytical variability associated with POXC and mineralizable C will need to be considered in routine soil testing. Since high analytical variability associated with a soil test can obscure differences among imposed management practices, measurements of OM-LOI, POXC and mineralizable C may need to be run with analytical replication. In particular, mineralizable C is known to be inher-

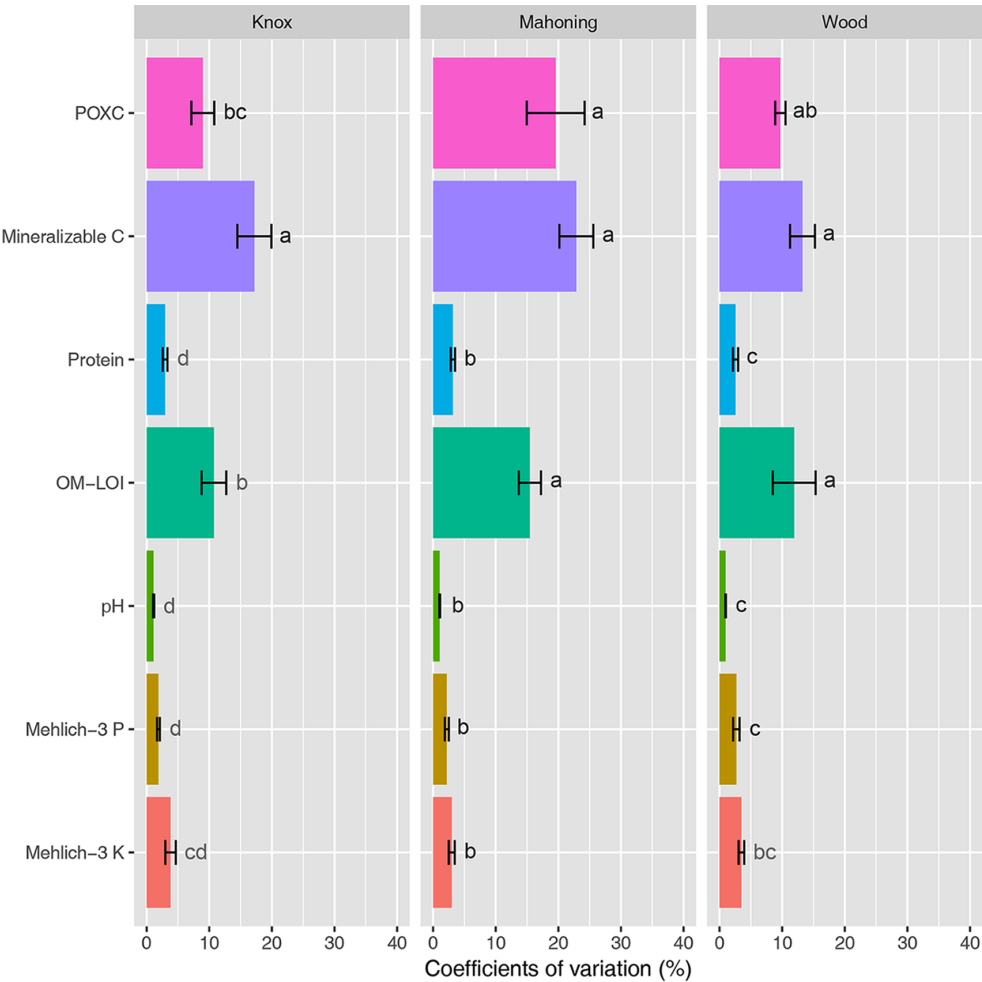


Fig. 2. Analytical variability, expressed as coefficients of variation (CV), associated with emerging soil health indicators and routine soil nutrient tests. Each bar represents the mean (*n* = 20) of CV values calculated based on measurements performed on a group of three replicate subsamples from the same composite soil from the R6 sampling. Within a site, mean CV values followed by different lowercase letters were significantly different at *p* < 0.05. Error bars represent the standard error of the mean. POXC, permanganate-oxidizable carbon; LOI, loss-on-ignition.

ently a variable measurement (Ahn et al., 2009; Zagal et al., 2009; Morrow et al., 2016; Wade et al., 2018). Consistent with our findings, Wade et al. (2018) also found a considerable amount of analytical variability in mineralizable C within a laboratory, which was highly dependent on the type of soils they studied. Although our results indicated similarity between POXC and OM-LOI in terms of analytical variability, it is worthwhile to mention that the former has an advantage of being more responsive to changes in soil management than OM evaluated on a total soil C basis (Weil et al., 2003; Culman et al., 2012b, 2013; Huriisso et al., 2016; Morrow et al., 2016).

Temporal Variability

Across all three sites, temporal trends were also evident, but were different depending on the site and indicator. At the Knox and Mahoning sites, soil health and soil nutrient measures typically were highest at the V4 or R1 sampling and declined at the R6 sampling (Table 2). Whereas at the Wood site, most soil health and soil nutrient indicators were greatest at the R6 sampling. The soil at the

Wood site has more clay than soils at Knox and Mahoning sites (Table 1), which may have contributed to these differential effects. The temporal patterns observed in POXC and mineralizable C at the Knox site were similar to those reported by Culman et al. (2013), peaking at R1 sampling; however, the values peaked at V4 sampling at the Mahoning site and R6 sampling at the Wood site. Such inconsistent patterns in the mean values measured for each soil health indicator and soil nutrient test (Table 2) could be due to variations in uptake and losses of nutrients and addition of labile OM via rhizodeposition and root turnover during the growing season.

To further determine the extent to which the soil health indicators vary temporally relative to routine soil nutrient tests, we computed CV values using measurements performed on soils from each sampling time points. Patterns in the magnitude of temporal variability of each indicator were less consistent across sites, but mean CV values for organic matter measurements followed the order: mineralizable C (22 to 37%) ≥ OM-LOI (16 to 25%) ≥ POXC (9 to 21%) = soil protein (7 to 13%; Fig. 3). Soil pH had consistently lower temporal variability than the soil health indicators considered in this study except soil protein. Whereas Mehlich-3 P and K were either statistically similar or higher in temporal variability than POXC and soil protein, with their respective mean CV values ranging from 14 to 33% and 6 to 33% (Fig. 3). Overall, data presented here demonstrate the tendency for both the soil health indicators and routine soil nutrient tests to change considerably over the course of a growing season. Thus, these results underscore the need to collect soil samples at the same time of the year, as is usually recommended due to known variability in routine soil test measurements.

Spatial Variability

Another key question we addressed was to what extent do these emerging soil health indicators vary spatially relative to routine soil nutrient tests. Exploring this question could help provide very useful information for designing appropriate soil sampling strategies needed to address in-field spatial variability (e.g., whether soil cores should be taken every 10 m or every 1000 m across a field).

Almost all soil properties examined here exhibited moderate to strong spatial dependence, with the proportion of model sample variance explained by structural variance, that is, $C/(C_0 + C)$, ranging from 0.26 to 0.98 depending on site and sampling time (Table 4). At the Wood site, most soil tests (in particular soil nutrient tests) did not exhibit spatial autocorrelation. The discussion regarding spatial autocorrelations and range distances is therefore focused on the Knox and Mahoning sites unless specified otherwise. Spatial range distances differed between sites and among sampling times for most indicators, but not consistently. At the Knox and Mahoning sites, POXC was spatially autocorrelated over a distance ranging from 43 to 76 m (Table 4). For mineralizable C, the range distances were between 23 and 76 m, whereas range distances of 31 to 76 m were determined for soil protein across sites and sampling times (Table 4).

Similar inconsistent spatial autocorrelation trends were found for most routine soil nutrient tests as for the soil health indicators. The sole exception was soil test P, which was consistently autocorrelated to a spatial distance of 76 m independent of site

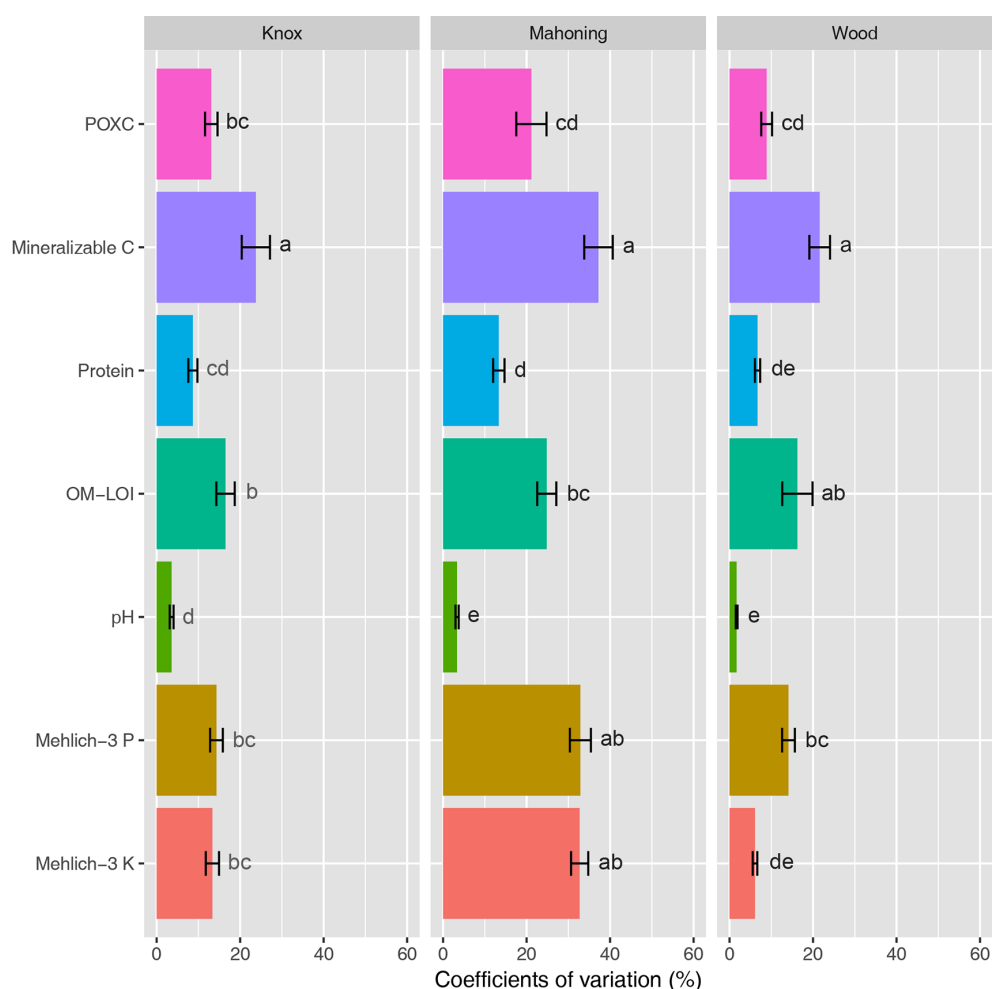


Fig. 3. Temporal variability, expressed as coefficients of variations (CV), associated with emerging soil health indicators and routine soil nutrient tests. Each bar represents the mean ($n = 20$) of CV values calculated based on measurements performed on a single replicate sample at each grid point from V4, R1 and R6 samplings. Within a site, mean CV values followed by different lowercase letters were significantly different at $p < 0.05$. Error bars represent the standard error of the mean. POXC, permanganate oxidizable carbon; LOI, loss-on-ignition.

and sampling time (Table 4). Other researchers have also reported spatial autocorrelations for soil P over a distance ranging from 64 to 78 m in corn-based cropping systems (Robertson et al., 1993; Cambardella et al., 1994; Cambardella and Karlen, 1999). The OM-LOI range distance was between 10 and 76 m across sites and sampling times (Table 4), which is also consistent with values found in the literature. Maresma and Ketterings (2017) determined spatial range distance of 38 to 79 m for OM-LOI in corn fields, depending on site and sampling season, and Baxter et al. (2003) reported OM-LOI range distance of 49 m for a field under winter barley (*Hordeum vulgare* L.) production for 10 yr. For total soil C (determined via dry combustion), Cambardella et al. (1994) reported spatial autocorrelation over a distance of 104 m, which is bigger than the spatial ranges for OM-LOI in our study and those of others (Baxter et al., 2003; Maresma and Ketterings, 2017). Such differences in spatial ranges could be

due to differences in field management history (Robertson et al., 1993; Cambardella and Karlen, 1999).

These results collectively demonstrate that the distribution of the soil health indicators can be spatially autocorrelated over distances of few meters to 76 m depending on site and sampling time. The fact that spatial autocorrelations occurred at range distances ≤ 76 m for both the soil health indicators and routine soil nutrient tests (Table 3) suggests that the three soil health indicators investigated here exhibit in-field spatial variability to a similar extent as the routine soil nutrient tests. Therefore, the sampling densities typically used for routine soil nutrient analysis (a composite soil sample of 5 to 8 cores with each composite soil sample representing ~ 0.5 to 5 acres), with cores taken approximately 76 m apart, should also be adequate for the measurement of POXC, mineralizable C and soil protein.

Table 4. Spatial dependence and spatial range from best-fit semivariograms for emerging soil health indicators and routine soil nutrient tests by site and time of sampling.[†]

Site	Model [‡]	V4		Model	R1		Model	R6	
		Spatial dependence [§]	Range		Spatial dependence	Range		Spatial dependence	Range
			m			m			m
POXC									
Knox	Sph	0.81	76	Sph	0.87	76	Exp	0.68	47
Mahoning	Sph	0.87	43	Gau	0.91	76	Sph	0.98	76
Wood	Exp	0.80	42	— [¶]	—	—	Sph	0.90	76
Mineralizable C									
Knox	Exp	0.78	76	Exp	0.68	76	Sph	1.00	23
Mahoning	Gau	0.89	76	Gau	0.89	76	—	—	—
Wood	Exp	0.95	76	—	—	—	Exp	0.26	17
Protein									
Knox	Sph	0.90	31	Exp	0.59	76	Sph	1.02	32
Mahoning	Sph	0.44	44	Exp	0.98	76	Exp	0.46	76
Wood	—	—	—	—	—	—	Exp	0.67	76
OM-LOI									
Knox	Sph	1.32	22	Exp	0.69	10	Sph	1.13	76
Mahoning	Gau	0.52	10	Sph	0.68	76	Sph	0.76	26
Wood	—	—	—	—	—	—	—	—	—
pH									
Knox	Gau	0.98	76	Sph	1.09	76	Sph	1.72	24
Mahoning	Gau	0.94	36	Gau	0.92	76	Gau	0.94	28
Wood	—	—	—	—	—	—	—	—	—
Mehlich-3 P									
Knox	Gau	0.94	76	Gau	0.92	43	Gau	0.84	76
Mahoning	Exp	0.87	76	Exp	0.97	76	Gau	0.92	76
Wood	—	—	—	—	—	—	Gau	0.91	32
Mehlich-3 K									
Knox	Sph	1.00	36	Sph	1.00	28	Sph	1.00	54
Mahoning	Sph	1.00	23	Sph	1.00	76	Sph	0.67	76
Wood	—	—	—	Exp	1.00	59	Sph	0.87	31

[†] POXC, permanganate-oxidizable carbon; OM-LOI, soil organic matter determined via loss-on-ignition.

[‡] Model semivariograms: Exp = Exponential; Gau = Gaussian; Sph = Spherical.

[§] Spatial dependence = $[C/(C_0+C)]$, where (C_0) and $(C_0 + C)$ respectively represent the nugget and the sill variance. The higher the value the greater the strength of spatial correlation over the range of the separation distance.

[¶] Negative sign (—) indicates lack of spatial dependence (see the statistical analysis section for more details as to how the presence vs. absence of spatial dependence was determined).

CONCLUSION

This study examined repeatability and spatiotemporal variability of emerging soil health indicators relative to routine soil nutrient tests that are commonly used in the US North Central Region. Among the soil health indicators, the analytical variability of POXC and mineralizable C was relatively large and on a similar scale to that of OM-LOI. In contrast, soil protein was found to be analytically the least variable and similar to Mehlich-3 extractable nutrients, suggesting that measurements of soil protein should be easily repeatable in a soil testing laboratory. Our results also suggested that the soil health indicators considered in this study can vary temporally during a growing season to a similar degree as routine soil nutrient tests; as a consequence, soil samples for measuring POXC, mineralizable C and soil protein should likely be taken at the same time of the year, as is typically recommended in routine soil nutrient testing. Data presented here also showed that both the soil health indicators and routine soil nutrient tests exhibited spatial autocorrelations over similar range distances of ≤ 76 m, suggesting that measurements of POXC, mineralizable C and soil protein should not require greater soil sampling densities within a field than what is typically used for routine soil nutrient testing.

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