

Measuring Rotation and Manure Effects in an Iowa Farm Soil Health Assessment

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ABSTRACT

Data from on-farm sites with known management histories are needed to quantify soil biological, chemical, and physical properties influencing carbon stocks and soil health. Surface (0–15 cm) and deep core (0–122 cm) soil samples were collected from fields under two rotations in Boone County, IA. The first was a 5-yr corn [*Zea mays* (L.)], soybean [*Glycine max* (L.) Merr.], corn, oat [*Avena sativa* (L.)], and alfalfa [*Medicago sativa* (L.)] rotation to which 0, 18, or 36 Mg ha⁻¹ of a manure/biosolids mixture was applied prior to planting corn. The second was an 8-yr rotation with 6 yr of mixed grass and legume pasture followed by corn and an oat crop within which the pasture mixture was reestablished. Soil samples were collected evenly across the predominant soil map units (SMUs). Bulk density (BD), soil organic C (SOC), water-stable aggregates (WSA), microbial biomass carbon (MBC), pH, Mehlich-3 and diethylenetriaminepentaacetic acid (DTPA) extractable nutrients, electrical conductivity (EC), and nitrogen (total-, NH₄⁻, and NO₃⁻-N) were measured. Surface SOC data were consistent with Soil Survey values for the various SMUs. Crop rotation effects were more noticeable than manure/biosolid application rate effects. Data from this study were combined with previously published SOC data in Iowa. Results suggest extended rotation systems or those with cover crops may increase SOC 8 ± 4 g kg⁻¹ compared to corn–soybean rotations (33 vs. 25 g kg⁻¹). This study provides on-farm reference values for soil health assessment tools and draws attention to the importance of inherent soil properties for these assessments.

Core Ideas

- Farms with known management histories contribute to soil health research.
- Rotation-based systems may increase soil organic carbon when combined with appropriate tillage.
- Inherent soil properties must be considered in soil health assessments.
- Rotation effects were more noticeable than manure effects on soil health groupings.

TILLAGE, CROP rotation, nutrient applications, and other land management practices influence soil health by modifying near-surface (0–15 cm) dynamic properties such as soil organic carbon (SOC), bulk density, and aggregate stability. The rate and magnitude by which those soil properties change are variable and site-specific, but generally bound by inherent soil characteristics. Since the 2000s, efforts to develop soil health assessment have been focused on the time required for changes to occur (i.e., days, years, decades) and the spatial scales at which those changes are detectable (i.e., row, field, watershed). Along with these temporal and spatial areas of emphasis, there is an interest in whether specific soil indicators change independently or with others.

Soil treatment effects tend to be more pronounced as time increases, particularly if the contrasting treatments cover a wide disturbance continuum (Karlen et al., 2017). A series of 4-yr studies with various crop rotation and tillage practices across the United States and Canada found frequent within-year temporal fluctuations in soil properties and processes that must be recognized when comparing management effects (Krupinsky et al., 2006; Liebig et al., 2006; Mikha et al., 2006; Pikul et al., 2006). Comparing single practice effects, such as presence or absence of tillage, demonstrated varied responses across sites. For example, infiltration was more responsive to tillage than crop rotation (Pikul et al., 2006), while soil chemical properties showed a tillage effect at only half of the study sites (Mikha et al., 2006). The amount of soil disturbance or fallow had no consistent effects on soil biological properties when the results were grouped by location (Liebig et al., 2006). Another comparison of no-till systems with 10 crops over a 4-yr period showed few significant soil property changes, except for a particulate organic matter (POM) difference based on the amount of residue returned each year (Krupinsky et al., 2006).

In contrast to studies that might evaluate the effect of a management change immediately or a few years after, agronomic evaluations of treatments that have been in place for numerous years or decades have successfully documented soil changes, particularly for samples collected at shallow sampling depths, such as 0 to 5 cm (Karlen et al., 1991, 2006, 2013; Veum et al., 2014). For example, following 26 yr of continuous corn,

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Abbreviations: BD, bulk density; DTPA, diethylenetriamine-pentaacetic acid; EC, electrical conductivity; MBC, microbial biomass carbon; POM, particulate organic matter; SMUs, soil map units; SOC, soil organic C; WSA, water-stable aggregates.

soil under moldboard plowing had lower organic carbon (20 vs. 30 g kg⁻¹), fewer water stable aggregates (30% vs. 48%), and decreased microbial biomass carbon (179 vs. 504 µg g⁻¹) when compared to a no-till treatment (Karlen et al., 2013). Also, these studies document how inherent soil properties affect soil health indicators. For example, across 25 soil properties following 32 yr of corn on silt loam soils, eight were affected by stover harvest and 15 were affected by tillage (Moebius-Clune et al., 2008). Inherent soil properties affected rotation and treatment effects on the well-studied Morrow Plots in Illinois, as recent soil electrical conductivity mapping demonstrated little influence from the site's experimental design (Nafziger and Dunker, 2011). As demonstrated in these studies that look at the effects of soil management changes, soil health indicators are a product of inherent and dynamic soil properties.

Along with time, the spatial scale at which evaluations are conducted affects the magnitude of detectable change. Different sampling schemes and data interpretation criteria are needed as scale increases from intra-row to watershed assessment (e.g., Collado and Karlen, 1992; Grigera et al., 2007; Tomer et al., 2006). Field variability versus management-induced soil property changes can be difficult to detect (Beehler et al., 2017; Cambardella et al., 1994; Hammac et al., 2016; Necpálová et al., 2014). Tillage practices, including earthworm activity, and associated equipment traffic patterns can increase spatial heterogeneity (Grigera et al., 2007; Kaspar and Parkin, 2011; Kaspar et al., 2001; Williams et al., 2016, 2017). Therefore, to ensure SOC and soil health assessments are accurate and meaningful, many samples, sites, and sampling strategies are needed to account for spatial and temporal variability (Idowu et al., 2003; Necpálová et al., 2014; Zhang et al., 2011).

Collecting soil physical, chemical, and biological indicator data from multiple sites and types of experiments is important because changes in one soil property may or may not cause changes in other soil properties. For example, 9 yr of continuous animal manure application increased SOC, POM-C, MBC, and soil P, but had no effect on aggregate stability (Delate et al., 2013). In another study, Jin et al. (2015) found that the fraction of water stable aggregates did not increase in plots receiving biosolids for 20 yr, but changes were noted at another site after receiving biosolids for only 8 yr. Both sites showed a nonlinear increase in total C stocks as manure application rates increased (Jin et al., 2015). One possible reason for this apparent discrepancy is that development of stable soil aggregates is influenced not only by total carbon content, but also by root derived C and decomposition (Gale et al., 2000). Furthermore, crop selection and rotation are important factors affecting soil health indicators. For example, root distribution and wheel traffic patterns can vary across fields and affect where roots grow and the level of soil compaction they may have to overcome to acquire water and nutrient resources (Kaspar and Parkin, 2011; Kaspar et al., 1991, 2001).

From a modeling perspective, the time needed for soil properties to change, scale at which the changes occur, and inherent properties that influence those changes must be accounted for within each soil type. Findings from existing studies can be expanded to other soils with similar texture, bulk density, and soil organic matter content (Merante et al., 2017; Wösten et al., 2001). Collecting site specific data from numerous soil series, associations, and textural classes will help refine our predictive capabilities (Liebig et al., 2006; Manns et al., 2014; Mikha et al., 2006; Necpálová et al.,

Table 1. National, county, and farm-scale distribution of sampled soil series.†

Soil series	Nationwide	Boone County, IA	
		Study farm	
		ha	
Clarion	925,142 (27%)‡	35,670 (33%)	27 (30%)
Canisteo	817,257 (23%)	29,731 (28%)	26 (29%)
Nicollet	595,299 (17%)	17,291 (16%)	17 (19%)
Webster	617,316 (18%)	11,612 (11%)	14 (15%)
Okoboji	298,135 (9%)	6,891 (6%)	2 (2%)
Harps	202,517 (6%)	5,599 (5%)	4 (4%)
Total hectares	3,455,666	106,795	91
Total area§	10,448,662	144,152	91
Percent of area	33%	74%	100%

† Data collected from <http://apps.cei.psu.edu/soiltool/semtool.html>.

‡ Percentages in parentheses indicate the soil series area divided by the total hectares from the six soil series in each column.

§ For the nationwide data, total area is the land hectares for all counties across the United States reporting some area in the six soil series, excluding water; for Boone County, total area is the land hectares in the county; for Study farm, total area is the size of the sampled area in hectares.

2014). These site specific data can include capturing the farmer's or land manager's knowledge regarding how various management practices are influencing soil properties on his/her farm (Gruver and Weil, 2007; Liebig and Doran, 1999; Romig et al., 1995). This combination of changing management practices and a site's inherent landscape soil properties can make it difficult to predict how soil properties will respond (Karlen et al., 2006).

The primary objective for this study was to quantify and compare several soil health indicators, including soil organic carbon stocks, under different management practices that had been followed for over 20 yr on a well-characterized farm in central Iowa. This study complements previously published profile N (Karlen and Colvin, 1992), soil aggregation (Collado and Karlen, 1992), weed bank (Buhler et al., 2001), economic and labor evaluations (Karlen et al., 1995), soil morphology and crop yield (Steinwand et al., 1996), as well as soil test evaluations (Karlen et al., 2002) conducted on the same farm. This current study provides a novel contribution to these previous studies by measuring a broader range of soil health indicators.

MATERIALS AND METHODS

The experiment collected soil samples from four field-scale treatments on a previously studied farm near Boone, IA (National Research Council, 1989). The treatments were: (i) a 5-yr crop rotation consisting of corn–soybean–corn–oat–alfalfa that received 0 Mg ha⁻¹ (dry weight) of a manure biosolids mixture applied prior to planting corn; (ii) the same rotation but with 18 Mg ha⁻¹ (dry weight) of the manure/biosolids mixture applied prior to planting corn; (iii) the same rotation but with 36 Mg ha⁻¹ (dry weight) of the manure/biosolids mixture applied prior to planting corn; and (iv) an 8-yr rotation of corn, oats, and 6 yr of pasture, to which no manure/biosolids mixture was applied.

The tillage practices for each rotation began with moldboard plowing following the hay or pasture crops (Karlen et al., 2002). The first application of manure/biosolids occurred prior to moldboard plowing in the autumn. In spring the fields were disked twice, corn was planted and a rotary hoe was used for early-season weed control. A Buffalo ridge-till cultivator was used to build ridges that were ~20 cm high. The ridges remained intact until the

Table 2. A comparison of measured and soil survey projected SOC concentrations within the surface 15 cm.

Map unit	Description	Slope %	Horizon	Depth cm	Soil survey SOC values			Study farm
					Minimum	Representative	Maximum	Avg†
90	Okoboji mucky silt loam	0 to 1	H1	0 to 23	70	78	87	46
95	Harps loam	0 to 2	Ap	0 to 20	23	41	58	47
107	Webster silty clay loam	0 to 2	Ap	0 to 20	23	41	58	37
507	Canisteo silty clay loam	0 to 2	Ap	0 to 20	23	41	58	37
55	Nicollet loam	1 to 3	Ap	0 to 20	23	35	52	31
138B	Clarion loam	2 to 5	Ap	0 to 23	12	20	35	24
138C	Clarion loam	5 to 9	Ap	0 to 20	12	21	35	22
138C2	Clarion loam, moderately eroded	5 to 9	Ap	0 to 15	6	15	20	20

† Calculated by multiplying total soil mass × TOC measurement value for each 0 to 15-cm depth segment and then averaging for each soil map unit.

following spring when they were truncated during soybean planting. After two rotary hoe passes, the ridges were rebuilt to a height of ~20 cm with the cultivator. In late August, oat was broadcast as the soybean crop began to senesce. Soybean was harvested and oat was allowed to grow until it was killed by low temperatures. The manure/biosolids material was applied in spring prior to truncating the ridges to incorporate the material and plant the second corn crop. Once again, after two rotary hoe passes, the ridges were rebuilt to a height of ~20 cm with the cultivator. In spring, after the second corn crop, the ridges were disked and an oat–legume mixture was planted (Karlen et al., 2002). In general, crop residues remained on the soil surface and were redistributed during the crop harvest and ridge rebuilding processes. At planting, the top 5 to 10 cm of each ridge was removed and distributed to the inter-row area. The planting, rotary hoe, cultivation, and ridge-building operations collectively provided weed control for the row-crops (National Research Council, 1989). Weeds were not considered a problem in the oat crop since they were cut and baled with the straw (Karlen et al., 2002).

The 5-yr crop rotation (corn–soybean–corn–oat–alfalfa) had been followed for 37 yr prior to sampling in 2006 (National Research Council, 1989). The manure/biosolids mixture consisted of solids from the local municipal waste treatment plant plus animal manure from the farm. It had been applied twice every 5 yr, prior to planting corn, since the mid-1980s. Urea was applied to corn and oat crops at planting (34 kg N ha⁻¹), and 34 kg K₂O ha⁻¹ was applied to corn and soybean (National Research Council, 1989). The 8-yr rotation is not directly mentioned in the National Research Council (1989) report, and although none of the remaining field and sampling records specify an exact start date it is implied that this rotation had been in place for at least 16 yr prior to collecting soil samples for this study.

The four field-scale treatments were distributed across several soil series. Soil types were identified using field maps and Soil Survey data (Soil Survey Staff, 2017; Table 1). Three soil series noted on the most recent Soil Survey were not shown on the maps generated at the time of sampling. They included: Okoboji silty clay loam 0–1% slopes (0.53 ha), Spillville loam 2–5% slopes (0.81 ha), and Clarion loam 9–14% slopes (0.61 ha). The soil series from which samples were collected (Table 2) represented 97.9% of the mapped hectares.

All six soil series (Clarion, Canisteo, Nicollet, Webster, Okoboji, and Harps) are common within the Des Moines Lobe in central Iowa and south-central Minnesota. Overall, in counties where these soils are found they account for 33% of the land area, but

in Boone County, IA, they comprise 74% of the landscape. The study site had a similar distribution of soils and was therefore considered representative of soils from this region (Table 1). Across the landscape, Okoboji and Harps are located in pothole areas, Webster at level slopes, Canisteo at an intermediate slope position, and Clarion and Nicollet at higher elevations (Andrews and Dideriksen, 1981).

Soil samples were collected from each of the four treatments. Based on Soil Survey data, the soil series at each sampling site was recorded and used to group the data by soil organic carbon content into low, medium, and high categories to provide sufficient sample counts for Chi-squared analysis. The distribution of core and hand sampling locations for each treatment and SOC values were compared with the assumption that the samples were collected from the mapped soil series noted in 2006. There were 53 locations across the farm selected for sampling. At each location, core and hand samples were collected to evaluate surface and profile soil properties. Previous research at this site had evaluated these two aspects of soil health separately and this project provides both data concurrently. For example, Karlen and Colvin (1992) evaluated profile soil properties and Karlen et al. (2002) evaluated surface (0–20 cm) soil properties.

For this current study, 53 soil cores were collected in 2006 to a depth of 122 cm. At each sampling site, a 4.45-cm diameter soil core was taken with a Giddings probe and divided into segments representing 0 to 15, 15 to 30, 30 to 61, 61 to 91, and 91 to 122 cm. Within a 6.1 m radius of each site, 12 surface samples (0–5 and 5–15 cm) were collected with a 3.18-cm diameter hand probe and composited by depth.

Soil physical, chemical, and biological properties were measured on the collected air-dried soil samples. The moisture content of all samples was determined by drying a subsample at 104°C and used to estimate soil bulk density by dividing the dry weight for each sampling depth segment by its volume.

Water stable aggregates were measured using a modified Yoder sieving machine, set to 30 strokes per minute for 5 min. A 100-g sample of 8-mm sieved soil was placed on a nest of sieves with 2, 1, 0.5, and 0.25-mm screen sizes. The fraction of soil retained on each screen was dried, weighed, and used to calculate the percent macroaggregation (>250 µm). Any rocks and roots were subtracted from the fraction of soil retained on the screen. Sand content was not used as a correction factor.

Soil sieved to 2 mm was used for the following measurements. Soil pH and electrical conductivity (EC) were measured using 1:1 soil/water solutions. The MBC was measured following

the chloroform fumigation incubation-extraction summarized in Rice et al. (1996). Micronutrients (Cu, Fe, Mn, Zn) were extracted using DTPA (Lindsay and Norvell, 1978). Other plant nutrients (Ca, P, K, Mg) were extracted with Mehlich-3 (Mehlich, 1984). A 2 M KCl using a 1:5 soil/solution ratio was used to extract NH_4^- , and NO_3^- -N (Mulvaney, 1996) and the solution was analyzed using flow injection analysis on a Lachat QC 800 (Loveland, CO).

Total organic C and N were analyzed on 2-mm sieved and pulverized samples using dry combustion on a Thermo Finnigan Flash 1112 Elemental Analysis (EA) at 900°C. As Soil Survey maps provide soil organic matter (SOM), all SOM values were converted to SOC by multiplying SOM values by 0.58. The term SOC is used throughout this paper. Soil organic C refers to non-carbonate C measured in the soil samples by dry combustion (i.e., TOC). While the conversion factor of 0.58 is used commonly, researchers over several decades have noted ranges in the factor occur based on soil type and mineralogy (Kasozi et al., 2009).

The following paragraphs describe the statistical analysis used on the dataset and presented in this paper. The collected soil data are available for interested readers in the supplementary information. The data were summarized and statistically analyzed using Microsoft Excel (v. 2010) and R (R Core Team, 2016). Statistical tests included two sample *t*-tests, ANOVA, Kruskal–Wallis, Chi-squared, and linear discriminant analysis. Data distribution assumptions were evaluated using quantile–quantile plots, histograms, and the Shapiro–Wilk test. Homogeneity of variances was tested by comparing the ratio of the largest and smallest group variances in an F_{\max} test with the degrees of freedom for the numerator and denominator calculated as the number of data points in each respective group minus one. If one or more test assumptions were violated, a Kruskal–Wallis test was used to compare group means if possible, and those results were also reported with the ANOVA *p*-value.

Profile C, NH_4^- , NO_3^- -N, and P quantities were calculated by multiplying sample mass by the measured concentrations for each depth segment and summing for the entire sampling depth. Other Mehlich-3 and DTPA extractable nutrients were reported by mass at 0 to 5- and 5 to 15-cm depths. For surface samples (0–15 cm), the percent SOC was calculated using a weighted concentration average for the 0 to 5- and 5 to 15-cm samples. That value was then multiplied by the mass associated with the 0 to 15 cm core sample to estimate C stocks for that depth segment.

A linear discriminant analysis evaluated if the surface (0–15 cm) physical, chemical, and biological measurements grouped by treatment. Discriminant function analysis was selected because this type of analysis allowed us to evaluate if the four treatments were separating into different groups based on the measured soil properties. The soil measurements used in the discriminant analysis were the values reported for both the 0 to 5- and 5 to 15-cm samples, including bulk density, pH, electrical conductivity, microbial biomass C, percent organic C, aggregate stability, and soil test P. Both sample depths were included in the discriminant analysis because we were interested to see if the treatment groupings were potentially consistent across sample depths. When presenting the data, the average values for these measurements across the two depths are shown.

The supplemental data file contains information for all these measurements separated into the two sample depths (0–5 and 5–15 cm) to allow for additional data analysis.

Linear discriminant analysis (*lda*) was conducted in R using the MASS package (Venables and Ripley, 2002) and *lda* command following the methods summarized by Holland (2017), Maindonald and Braun (2007), and Manly (2005). Since a dataset with no missing values was needed for this analysis, three missing data points were removed. Therefore, the adjusted sample sizes for discriminant analysis was 24, 24, 33, and 22 for the 5-yr rotation with 0, 18, and 36 Mg ha^{-1} manure/biosolids applied and the 8-yr rotation, respectively. For example, 24 samples for the 0 Mg ha^{-1} manure/biosolids treatment occurred because there were 12 sample locations (Table 3) and two sample depths collected at that location (0–5 and 5–15 cm). Prior probabilities were kept as the default to these sample distribution proportions. The predicted group assignments were calculated with and without cross validation to compare the stability of these group predictions. Leave-one-out cross validation was used. An individual observation was left out, all other observations were used to fit the model, and then the likelihood for this observation to fit into each of the four treatments was calculated (Maindonald and Braun, 2007). The percent accurate assignments for both methods are presented and was calculated as the number of samples in a given rotation and manure management treatment assigned correctly divided by the total number of samples from this treatment.

In addition to the discriminant analysis, individual ANOVAs or Kruskal–Wallis tests for each soil property, without splitting by depth, were conducted and their *p*-values were reported. Our primary objective was to conduct a linear discriminant analysis using all indicator variables to evaluate systematic soil groupings, but we conducted these additional tests to show if there were any potential trends occurring by treatment within a given property. All *p*-values are reported to allow readers to use their own judgment with respect to the *p*-values denoting statistical and practical importance.

Four soil carbon analyses were conducted using the data. First, the sampling statistical power for the study was calculated to consider how effective the sampling strategy might detect SOC differences among the four treatments. This analysis was motivated by Necpálová et al., (2014) and used statistical power tables provided by Zar (2010) and Rotton and Schönemann (1978). The second analysis considered how the manure/biosolid compost would decompose over time. The manure/biosolid mixture was assumed to be 100% SOM that would consist of 58% SOC. The average decomposition rate per year was assumed to be 20% following Magdoff and van Es (2010). The third analysis compared the average SOC values from the collected soil samples and the expected SOC content using Soil Survey values. The Soil Survey values were computed by taking the average SOC content of a given soil series and weighting this value by the area covered by that soil series. For example, if soil series A averaged 3 g kg^{-1} SOC and covered 20% of a given area and soil series B averaged 5 g kg^{-1} SOC and covered 80% of a given area, the weighted average SOC would be 4.6 g kg^{-1} ($3 \times 0.2 + 5 \times 0.8$). The average SOC value for each soil series was used as the expected SOC content (Table 2) and the area that each soil series covered (Table 3).

Table 3. Distribution of core and hand sampling locations by treatment and soil type.

Sample distribution	138B†	138C	138C2	55	107	507	90	95	Total Locations
5-yr rotation, 0 Mg ha ⁻¹	1	0	1	4	1	4	0	1	12
5-yr rotation, 18 Mg ha ⁻¹	2	0	1	3	1	3	1	1	12
5-yr rotation, 36 Mg ha ⁻¹	3	1	2	3	4	3	0	1	17
8-yr rotation, 0 Mg ha ⁻¹	2	2	0	3	3	1	0	1	12
Sum	8	3	4	13	9	11	1	4	53
Total ha‡	12.8	8.0	6.3	17.0	14.3	26.5	1.5	4.1	
% all samples	15	6	8	25	17	21	2	8	
% ha	14	9	7	19	16	29	2	5	
Chi-squared analysis	Sample counts (expected value, Chi-squared value)§								
Organic carbon (g kg ⁻¹) groups	20–24			31–37			46		
5-yr rotation, 0 Mg ha ⁻¹	2 (3.59, 0.704)			9 (7.66, 0.235)			1 (0.75, 0.082)		
5-yr rotation, 18 Mg ha ⁻¹	3 (3.59, 0.097)			7 (7.66, 0.057)			2 (0.75, 2.075)		
5-yr rotation, 36 Mg ha ⁻¹	6 (5.09, 0.164)			10 (10.85, 0.066)			1 (1.06, 0.004)		
8-yr rotation, 0 Mg ha ⁻¹	4 (3.59, 0.047)			7 (7.66, 0.057)			1 (0.75, 0.082)		

† Full soil map unit names are provided in Table 2.

‡ Total hectares for each soil map unit was estimated by selecting areas of interest from the soil survey corresponding to general farm map. (Expected value, chi-squared value)

§ Chi-squared values (Total $\chi^2 = 3.671$, $df = 6$, $p(\chi^2 \geq \text{Total } \chi^2) = 0.72$). Soil series 138B/C/C2 comprised 29.92% of the farm hectares, series 107/55/507 comprised 63.82%, and series 90/95 comprised 6.26%. Expected counts equal the row sums multiplied by these fractions, such as $0.2992 \times 12 = 3.59$.

In the fourth analysis, the SOC values from this study were compared to previous studies conducted on similar soils in central Iowa. These previous studies were separated into two groups. The first group consisted of studies that evaluated soils under some kind of extended crop rotation and were labeled as “long-term rotations.” These studies included extended crop rotations, including data collected in this study from the 5- and 8-yr rotations, as well as studies that included cover crops in a corn–soybean rotation. The second group consisted of studies that evaluated soils under business as usual production practices, such as continuous corn or corn–soybean with no cover crops used. This second group was referred to as “short-term rotations.” The average SOC concentrations for these two groups were compared using a two-sample *t* test.

RESULTS AND DISCUSSION

Distribution of Sampling Sites

Soil series were evenly distributed among the four treatments. The distribution of sampling locations was similar when evaluated by SOC content groupings and treatment ($\chi^2 = 3.6707$, $df = 6$, $p = 0.72$) (Table 3). This indicates the experimental design avoided potential confounding due to uneven soil series distribution across the treatments. A potential example of this would have been if all the high organic matter soils were grouped together in one treatment. Instead, soil series that would be expected, based on the estimates available from Soil Survey maps, to have relative low (20–24 g SOC kg⁻¹), medium (31–37 g SOC kg⁻¹), and high (46 g SOC kg⁻¹) were sampled from across the four treatments. The soil sampling procedure followed a proportional sampling strategy that balanced soil series' distributions across the treatments.

Surface Soil Organic Carbon

Average SOC content ranged from 29 to 34 g SOC kg⁻¹ across the four treatments (Table 4). Collectively, surface (0–15 cm) SOC analyses suggested that the manure/biosolids application had minimal effect on SOC. Results from a one-way ANOVA indicated there was not an increase in SOC due

to manure/biosolids application with an average content of 33 ± 0.14 g SOC kg⁻¹ (3.3%) for 0 to 15-cm samples (ANOVA $p = 0.66$, Kruskal–Wallis $p = 0.59$). A two-way ANOVA (Type III) by sampling depth and treatment also showed no significant SOC differences. Soil samples from 0 to 5 or 5 to 15 cm were not affected by treatment ($p = 0.58$), depth ($p = 0.39$), or the interaction of the two factors ($p = 0.73$).

As sample sizes for each treatment were unequal (Table 3), additional analyses were conducted to determine if those conclusions would change if only 12 of the 17 samples from the 36 Mg ha⁻¹ biosolids/manure treatment were evaluated. Given 17 options for 12 choices, a total of 6188 unique combinations of surface SOC values were generated. Of all possible combinations, the highest possible average was 38 g SOC kg⁻¹ instead of the 33 g SOC kg⁻¹ calculated using all 17 samples. Using a one-way ANOVA ($n = 12$) to analyze the four treatments with this higher SOC average substituted for this treatment indicated no significant treatment effect ($p = 0.19$).

Surface Sample Linear Discriminant Analysis

Linear discriminant analysis results suggested crop rotation had a greater effect on soil properties than applying manure/biosolids. Soil samples from the 8-yr rotation with 6 yr of pasture grouped together more consistently than any of the manure/biosolid application rate treatments within the 5-yr rotation. The first linear discriminant (LD1) accounted for 92.85% of the between-class variance (Table 4). The combination of the group means and coefficients for the first linear discriminant indicated the extended pasture treatment tended to have lower MBC, pH, EC, organic C, aggregate stability, and Mehlich-3 P. The coefficients for the LD1 indicated a contrast between bulk density, pH, SOC, aggregate stability, and soil test P compared to MBC and EC.

When these coefficients were applied to the sample values, the contrast clustered the manure/biosolids treatments together and separated the 8-yr rotation samples. A plot of LD1 and LD2 indicated samples from the 8-yr rotation were grouping separately from the other three treatments, and this effect was most

Table 4. Discriminant analysis of physical, chemical, and biological properties with calculated means (0 to 5, 5 to 15 cm).

Treatment	BD	Microbial biomass C	pH	EC	Organic C	Aggregate stability†	Mehlich-3 P	Predicted assignment accuracy	
	g cm ⁻³	mg kg ⁻¹		μS cm ⁻¹	g kg ⁻¹	%	mg kg ⁻¹	All samples	Leave-one-out
5-yr rotation, 0 Mg ha ⁻¹	1.5	932	7.5	401	34	38	131	46%	38%
5-yr rotation, 18 Mg ha ⁻¹	1.5	1053	7.6	468	33	34	177	50%	29%
5-yr rotation, 36 Mg ha ⁻¹	1.5	1113	7.5	451	33	44	194	67%	64%
8-yr rotation, 0 Mg ha ⁻¹	1.6	650	7.0	345	29	32	52	86%	86%
LDI coefficient‡	-2.45	0.0005	-3.75	0.00015	-0.4	-0.03	-0.02		
p-value§	0.17	0.08	<0.001	0.16	0.25	0.005	<0.001		
Comparison soils¶									
Continuous Corn	1.1	294	6.6	105	25	42	33#		
Corn/Soybean	1.1	310	6.3	103	24	36	42#		

† Percent of aggregates >250 μm in diameter.

§ p-values for ANOVA (bulk density) and Kruskal–Wallis (all other variables) results.

‡ LDI accounted for 92.85% of the between-class variance.

¶ Soil data from Clarion, Canisteo, and Webster soils in central IA in 26 yr of continuous corn or 31 yr in corn–soybean rotation (Karlen et al., 2013). In the presented table, continuous corn and corn/soybean values are averaged across both depths and 5 different soil treatments.

Karlen et al. (2013) values are converted from Bray-1 P to Mehlich-3 values using regression equation from Mallarino and Blackmer (1992).

noticeable for the LDI axis (Fig. S1). However, the discriminant analysis was not separating the manure/biosolid treatments as effectively as shown by the percent accuracy for group assignment with and without cross validation (Table 4). Across all 103 samples, total correct treatment assignments was 62% (64 correct) and was 54% in leave-one-out-cross validation. However, the accuracy varied by treatment group. In both assignment calculations, the percent accuracy for the 8-yr rotation remained consistent at 86%. This finding suggests the samples collected from the 8-yr rotation were more similar to one another because the leave-one-out cross validation did not reduce the predictive accuracy as occurred in the manure/biosolid treatments.

The additional ANOVAs or Kruskal–Wallis tests for each soil property are presented in Table 4 with respective *p*-values. Table 4 also includes information from Karlen et al. (2013) for soils collected following 26 yr continuous corn and 31 yr of corn–soybean systems from similar soil series in Boone County, IA. Aggregate stability measurements from both sites were similar, but SOC, Mehlich-3 extractable P, EC, pH, and MBC were all higher in samples from the long term rotation sites than in samples from the continuous corn or corn–soybean plots. These results suggest that for similar soils in Boone County, IA, diversified crop rotations may modify some soil properties, but not all, more than either continuous corn or corn–soybean systems.

Depth Analysis of Bulk Density, Total Carbon, Nitrogen, Phosphorus, and Other Nutrients

Data from the 2006 sampling, aggregated across all sample depths (0–122 cm), indicated bulk density, NO₃-N, and total carbon were similar for all treatments when analyzed using separate Kruskal–Wallis analyses (Table 5). Karlen and Colvin (1992) found that the 5-yr rotation compared to an adjacent corn–soybean rotation (Baker field) indicated the risk for groundwater pollution due to NO₃-N was the same for both operations. Differences in profile NH₄-N between corn–soybean and 5-yr rotation management practices were previously explained by the addition of anhydrous NH₃ in the corn–soybean rotation (Karlen and Colvin, 1992).

Soil test P results suggested a rotation effect when comparing the 5-yr rotation to the 8-yr rotation data, but did not show

a manure/biosolid rate effect (Kruskal–Wallis *p* = 0.02). Profile NH₄-N was higher in the 8-yr rotation compared to the 5-yr rotation (Kruskal–Wallis *p* = 0.02). However, due to the greater concentration of NO₃-N relative to NH₄-N, the four treatments would not be different based on total N. The higher NH₄-N in the 8-yr rotation compared to the 5-yr rotation could be due to greater N fixation occurring within the pasture phase of the 8-yr rotation. Furthermore, the various manure/biosolids application rates had no consistent effect on either Mehlich-3 extractable elements (Ca, K, and Mg) or DTPA extractable elements (Cu, Fe, Mn, and Zn) (Table 6).

Soil Carbon Results from Four Perspectives

The soil carbon results showed no consistent differences among the four treatments. To gain a better understanding of how the four treatments were potentially changing SOC stocks, the data were evaluated from four perspectives which included: (i) sampling statistical power, (ii) manure/biosolid decomposition, (iii) soil series carbon values, and (iv) soil carbon values from previously published studies.

Farmers implementing a new soil or crop management practice at the field-scale within the Des Moines Lobe could easily cross different soils with SOC levels ranging from 15 to 78 g kg⁻¹ as noted from Soil Survey data (Table 2) and a survey of published values for central Iowa soils (Table S1). Within the fields sampled for this study, measured SOC concentrations ranged from 34 to 81 g kg⁻¹ (Table 2). A brief statistical power calculation provides an example as to how this field variability can affect measuring soil organic carbon at the field-scale. Based on this variability, the number of samples and analytical cost required to detect a treatment response could exceed the resources allocated for soil analysis in most farm budgets. For example, using the ANOVA residual mean squares value for this study (0.9937) to detect a 1% (10 g kg⁻¹) SOC difference among four groups with 12 samples per group, the minimum detectable difference would be $\Phi = 1.2286$. Available power analysis tables suggest the statistical power would be approximately 0.45 at an $\alpha = 0.05$ (Zar, 2010) and approximately 0.60 at an $\alpha = 0.10$ (Rotton and Schönemann, 1978). Detecting an effect at either $\alpha = 0.05$ or 0.10 could lead to an unreasonably high potential for Type II error.

Table 5. Average soil mass and quantities of C, N, and P of all soil profile samples collected to a depth of 1.2 m within the four management treatments.†

Treatment‡	Soil mass kg	Carbon g	NH ₄ -N		NO ₃ -N		P
			mg		mg		
5-yr rotation, 0 Mg ha ⁻¹	1.998	28.6	0.6b	13.8	78.1a		
5-yr rotation, 18 Mg ha ⁻¹	2.039	25.3	0.8b	29.0	70.5a		
5-yr rotation, 36 Mg ha ⁻¹	2.032	23.0	1.0b	26.5	79.6a		
8-yr rotation, 0 Mg ha ⁻¹	2.003	20.2	2.4a	17.5	33.0b		
p-value	0.78	0.36	0.02§	0.38	0.02¶		

† All mass values reported as the total soil collected to a depth of 1.2 m using a 4.4 cm diameter probe for a total sampling volume of 1892 cm³.

‡ Treatment refers to the years in crop rotation and the manure/biosolids amount applied prior to planting corn.

§ Letters assigned across treatments following an additional Kruskal-Wallis analysis for treatments 1, 2, and 3 with a *p*-value of 0.17.

¶ Letters assigned across treatments following an additional Kruskal-Wallis analysis for treatments 1, 2, and 3 with a *p*-value of 0.83.

Table 6. Soil mass and average mass of Mehlich-3 or DTPA extractable elements.

Treatment	Soil mass kg	M3			DTPA			
		Ca	K	Mg	Cu	Fe	Mn	Zn
0–5 cm		mg‡						
5-yr rotation, 0 Mg ha ⁻¹ †	0.614	4112	76	272	3	17	13	4
5-yr rotation, 18 Mg ha ⁻¹	0.586	3836	109	251	4	16	12	5
5-yr rotation, 36 Mg ha ⁻¹	0.640	3999	108	285	5	21	12	5
8-yr rotation, 0 Mg ha ⁻¹	0.627	2674	187	311	2	31	19	3
5–15 cm		mg‡						
5-yr rotation, 0 Mg ha ⁻¹	1.144	8044	109	470	7	35	22	8
5-yr rotation, 18 Mg ha ⁻¹	1.146	7306	147	470	8	39	21	9
5-yr rotation, 36 Mg ha ⁻¹	1.163	7094	167	519	9	42	21	10
8-yr rotation, 0 Mg ha ⁻¹	1.216	5084	161	538	5	62	32	5

† Treatment refers to the years in crop rotation and the manure/biosolids amount applied prior to planting corn.

‡ The total mass of the extractable elements. The ppm can be calculated by dividing the mg by the soil mass column

However, these results are not intended to suggest that all field research is fraught with this unsolvable problem. Farmers and researchers know that different landscape positions can respond differently to management practices (Beehler et al., 2017; Cambardella et al., 1994). Zonal soil and crop management can be used to improve soil resource utilization by providing zone-specific information that might be lost if aggregated across an entire field (Jaynes et al., 2010; Mzuku et al., 2005; Sawchik and Mallarino, 2006). Research must continue into zone-specific soil health management as well.

The second SOC analysis based on manure/biosolids application and decomposition rates suggested none of the treatments applied sufficient material to increase SOC over decades. For example, when averaged across all four treatments surface (0–15 cm) soil bulk density was approximately 1.53 Mg m⁻³ (Table 4). Using this average bulk density value, the 0- to 15-cm depth segment across the farm contained approximately 2295 Mg of soil ha⁻¹. Similarly, when averaged across all four treatments surface (0–15 cm), SOC content (Table 4) was approximately 32.3 g kg⁻¹ for a total of 74 Mg ha⁻¹ (0–15 cm depth). The maximum manure/biosolids application rate (36 Mg ha⁻¹) would increase the surface soil (0–15 cm) SOC pool by about 21 Mg ha⁻¹ prior to each corn crop in the corn–soybean–corn–oat–alfalfa rotation.

Considering 20% of the manure/biosolids would remain after 1 yr (Magdoff and van Es, 2010), SOC in the 0–15 cm was unlikely to increase at the conclusion of the 5-yr rotation due to the manure/biosolids application alone. The maximum application rate applied in the first year would decompose to approximately 0.8 Mg SOC ha⁻¹ before the second corn crop was grown. Following the next manure/biosolids application, all added manure/biosolids would decompose to 0.9 Mg SOC ha⁻¹ at the

conclusion of the 5 yr. This increase would theoretically raise the 32.3 g SOC kg⁻¹ average to 32.6 g ha⁻¹ over the 0- to 15-cm depth segment. This small potential increase in SOC would be difficult to detect given the variability in SOC across the sampled fields.

The third approach for evaluating SOC changes in response to the 5- and 8-yr rotations compared the measured data with Soil Survey values (Soil Survey Staff, 2017). For this study, the average SOC content in the 0- to 15-cm layer was 32 g kg⁻¹. The expected SOC content across all fields computed using Soil Survey values (Table 2) and weighting the values by the area each soil series covered (Table 3) was 34 g kg⁻¹. One possible reason for the 2 g kg⁻¹ difference was that measured SOC values for Okobojo soil were lower than the minimum Soil Survey values (Table 2). Since Okobojo soils tend to be wetter and have higher SOC than other area soils, management practices such as periodic tillage could have reduced the expected values. Except for Okobojo sites, SOC data agreed with Soil Survey estimates for all four treatments. This suggests the 5- and 8-yr rotations were maintaining the Soil Survey predicted SOC levels.

The fourth approach compared this study's SOC values to previously published data from near-by continuous corn or corn–soybean rotations on similar soil types (Table S2, Fig. 1). When measured SOC values were separated by fields with either (i) crop rotations that included multiple years and/or cover crop treatments or (ii) continuous corn or corn–soybean crops (Fig. 1), SOC values were 8 ± 4 g kg⁻¹ higher (two-sample *t* test, *p* < 0.001) from the extended rotations or cover crop treatments. These extended rotations, noted as “long-term” on Fig. 1, averaged 33 mg SOC kg⁻¹. The continuous corn or corn–soybean data, noted as “short-term” on Fig. 1, averaged 25 mg SOC kg⁻¹. This comparison focused on C concentration in the surface soil and did not account

for bulk density differences that may have influenced total profile carbon values.

Challenges for Merging Field and Laboratory Soil Carbon Assessments

The four analyses outlined above suggest several findings and challenges for producers interested in maintaining and improving soil carbon. The treatments used on this farm were maintaining SOC levels when compared to Soil Survey values. Using either the 5- or 8-yr rotation could be increasing soil organic carbon relative to soils that do not have as long of a crop rotation, but more paired studies are needed to explore this comparison fully. The routine, but not annual, application of the manure/biosolids mixture was not sufficient to increase soil organic carbon over time. In studies where manure application occurs annually, soil organic carbon stocks may increase (Delate et al., 2013; Jin et al., 2015).

However, organic carbon in agricultural soils can become carbon saturated due to soil texture and climate limitations (Hassink, 1997; Hassink and Whitmore, 1997). If mineral surfaces are filled, increases in soil organic carbon would occur through increased particulate organic matter content and increase aggregate protection of these particulate carbon sources (Cambardella and Elliott, 1992; Magdoff and van Es, 2010). If organic materials are added to soils routinely and at elevated application rates, an entirely new soil horizon can develop such as occurred with pluggen soils in Europe in which a new soil horizon between 30 and 130 cm thick developed (Blume and Leinweber, 2004).

When farmers are considering the effects of their management practices on soil carbon, they must consider how soil inherent and dynamic properties are working together to retain this soil carbon. Multiple biogeochemical mechanisms are responsible for the cycling of soil C and N. Management systems should seek to balance SOC stabilization and mineralization to ensure that soil C stocks are maintained and nutrients are able to become available for plants (Hurisso et al., 2016). While maintaining adequate surface residues have soil and water protection benefits that cannot be ignored, farmers must also consider how root derived C will help build SOC over time (Gale and Cambardella, 2000). Types of carbon substrate, such as plant sugars compared to lignin, follow different decomposition trajectories. These trajectories are in turn influenced by moisture content as greater moisture content can increase decomposition rates. Surface residues can dry more quickly than residues within the soil, and this low moisture content can increase the length of time surface residues remain on the soil (Schomberg et al., 1994). Maximum relative microbial activity tends to occur when neither water nor air are limiting at approximately 60% water-filled pore space (Linn and Doran, 1984). Biogeochemical factors, such as texture, temperature, moisture, substrate, and microbial populations, interact with a given farmer's management activities to determine SOC concentrations in the field.

Farmers know SOC is important. Farmers value SOC data, which is provided to them when measured as SOM on common soil tests or evaluated in field using their own observations. Producers commonly mention SOM as a key soil health indicator (Gruver and Weil, 2007; Romig et al., 1995). A 2013 survey of Iowa farmers indicated 76% of farmers viewed SOM as a very important characteristic to use when judging soil health. When these farmers considered the current SOM content of their soils,

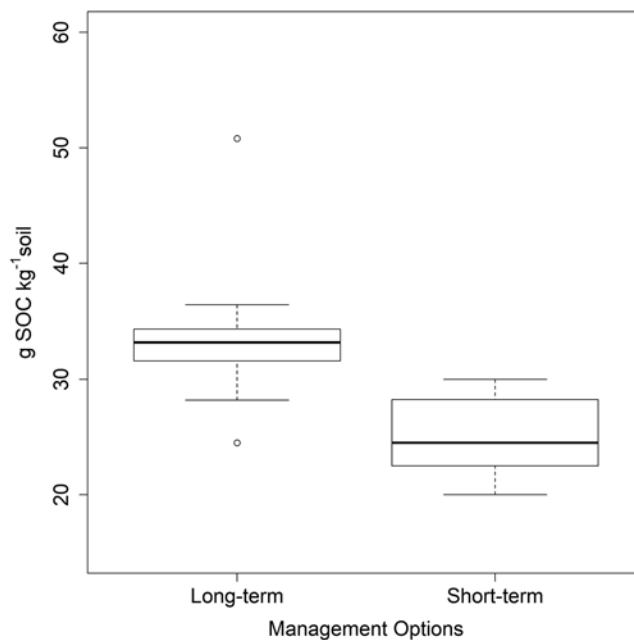


Fig. 1. Graphical presentation of soil carbon response data from 26 crop management treatments (Table S2) for average SOC (g C kg^{-1} soil) under long ($n = 14$) and short-term ($n = 12$) rotations on central Iowa soils.

respondents noted their amounts as poor (2%), fair (17%), good (41%), very good (26%), and excellent (5%) (Arbuckle, 2013).

Liebig and Doran (1999) provided farmers with SOM percent ranges and qualitative terms, such as “good” and “problem” soils. They compared farmers’ assessments of soil properties with laboratory assessments. When evaluating the SOM content of a self-defined “good soil,” farmers were able to accurately match laboratory analyzed results 67% of the time and were near-accurate 12%. However, when considering a self-defined “problem-soil,” farmers had an accurate perception 42% of the time and a near-accurate perception 58% of the time. Farmers were more likely to have an accurate perception of “good” soils than “problem” soils. This accuracy was based on comparing farmers’ ability to correctly place the SOM content into one of 5 classes that matched the laboratory results (i.e. less than 2%; between 2.1 and 4%; between 4.1% and 6%, etc.). If farmers were one class away from the laboratory results, these were considered near-accurate (Liebig and Doran, 1999).

If a farmer wants to increase the SOC content of a field and move a soil from a “problem soil” to a “good soil” and asks a researcher about the best methods to achieve this goal, what kind of answer might they receive? Similar to farmers, researchers know SOC is important, but measuring minimum detectable differences to a degree of agreed on statistical accuracy could result in an increased number of samples beyond the ability, interest, or finances of an individual farmer (Necpálová et al., 2014). Soil organic carbon is spatially (Beehler et al., 2017; Cambardella et al., 1994) and temporally variable (Mikha et al., 2006). Variations in laboratory procedures, such as between air-drying and oven-drying soil samples can cause variations in reported values (Hoskins, 2002). Using remote sensing to quantify soils can scan large areas more quickly, but does introduce variability in measured values as well (Mulder et al., 2011). Sampling at different times of the year, using different sample depths, and having samples analyzed by

different laboratories or methods could result in finding some kind of SOC difference when one is not occurring.

An illustration of these challenges is provided by recent studies from Moore et al. (2014) and Basche et al. (2016) that measured SOC in corn–soybean systems that included a cereal rye (*Secale cereale* L.) cover crop. On a field that followed a corn–soybean rotation with a winter rye cover crop for 13 yr, Basche et al. (2016) detected increases of 10–11% in field capacity water content and 21–22% in plant available water. However, the researchers noted their sampling approach was not sufficient to detect potential changes in SOC and pointed to the recent study by Moore et al. (2014) as an example where a greater sampling intensity detected an SOC increase from cover crops (Basche et al., 2016). Moore et al. (2014) studied rye cover crops in a corn silage–soybean rotation after 9 yr and detected an increase from 29 to 33 g SOC kg⁻¹ ($p < 0.05$) between a field that had cover crops and one that did not have any cover crops in the rotation. Moore et al. (2014) reported SOM, and these values were converted to SOC for this discussion.

However, these SOC increases stay within an individual category (i.e., 4.1% to 6% SOM) that Liebig and Doran (1999) provided to farmers in their questionnaire. These category ranges could always be modified for future studies, but the findings of Liebig and Doran (1999) are still relevant. Adequate management for “good” or “problem” soils can certainly involve more than just managing SOC, such as addressing other physical (compaction) or chemical (inadequate nutrients) resource concerns. These findings suggest developing robust soil health baselines through soil sampling should provide useful reference information for farmers against which soil management effects can be evaluated. But the key question remains, how will farmers measure changes of this magnitude reliably, such as an increase in SOC of 10%? Or, if entire rotations were changed, how can farmers detect a potential 25% increase as suggested by the compilation of previous studies (Fig. 1)?

Measuring how soil properties change under different management practices, including extended crop rotations is not a new research question for soil scientists (e.g., Page and Willard, 1947; Lemaire et al., 2015). Much remains to be learned from on-farm research regarding how inherent and dynamic soil properties and processes combine within different management systems (Karlen et al., 2017). Collectively, our research efforts have generated decades of valuable data that can now be aggregated and compared to help provide new soil health insights. This study again documents that extended crop rotations followed for many cycles can enhance soil properties relative to short-term or less diversified crop rotations on similar soil types, but these effects are mediated by inherent soil properties. Therefore, the knowledge gained from on-farm studies such as this can provide science-based data that can be used to improve soil resource management for current and future generations.

SUMMARY AND CONCLUSIONS

Four field-scale treatments were evaluated on a 91 ha farm in Boone County, IA. Three consisted of a 5-yr corn–soybean–corn–oat–alfalfa rotation during which 0, 18, or 36 Mg ha⁻¹ of a manure/biosolids mixture was applied prior to each corn crop. The fourth treatment was an 8-yr rotation consisting of corn (without manure/biosolids), oat, and 6 yr of pasture. Each treatment had

a similar distribution of soils within the various farm fields. Soil organic carbon was similar across the four treatments from 0 to 15 cm. The routine, but infrequent, manure/biosolids addition was not sufficient to cause a detectable increase in SOC using this study’s methods. Profile (0–122 cm) C and NO₃-N concentrations were not different among the four treatments, but NH₄-N and P exhibited rotation effects. The measured SOC content for all four treatments agreed with Soil Survey values, thus indicating the management approaches being used on this farm were maintaining soil carbon resources.

Future directions for research may be guided by this study’s finding that crop rotation on central Iowa soils may improve soil carbon stocks compared to business as usual rotations. However, this finding can be refined by gathering additional data sources and new on-farm data. Changing management systems holistically may have a greater effect on soil resources than simply adding organic amendments to non-rotation production systems. Whether these substantial management changes are feasible requires research into the environmental, economic, and social opportunities available in a given area. As researchers project how soils may change based on altered management practices, they must consider the biogeochemical processes shaping how soils respond to these new farm operations and activities. The data presented in this study contributes to this larger process by presenting data associated with 5- and 8-yr rotations in central Iowa soils. As shown in this study, on-farm collaborations can create productive research outcomes in which researchers and farmers work together to ensure agricultural resilience and sustainability.

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SUPPLEMENTAL MATERIAL

Supplemental material includes a table of the variation in soil C by soil series in central Iowa (Table S1), soil C management effects on central Iowa soils (Table S2), and a scatterplot of the linear discriminant analysis results (Fig. S1). Reference for supplemental table citations are also included.

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