

Structural Stability Conditions Soil Carbon Gains from Compost Management and Rotational Diversity

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Understanding processes that underlie soil carbon gains and enhance structural stability is key to sustainable production of intensively managed row crops. Long term consequences of crop diversity and interactions with compost management have rarely been tested with economically feasible, moderate rates of organic amendments. We investigated soil aggregation and carbon pools over twenty years on a field crop experiment that allowed quantification of the impact of rotational diversity within two integrated management regimes, one based on compost (3 Mg ha⁻¹) and the other inorganic fertilizer, both at about 100 kg N ha⁻¹ annually. The Living Field Laboratory (LFL) experimental study where the study was conducted is located at the W.K. Kellogg Biological Station in southwest Michigan. The rotational diversity treatments investigated included continuous corn (*Zea mays* L.) (CC), corn–soybean [*Glycine max* (L.) Merr.] rotation (CS), corn–soybean–wheat (*Triticum aestivum* L.) rotation (CSW), and corn–soybean–wheat rotation with a cover crop (CSWco). Soil C status in 1993 was 2584 g m⁻² and by 2013 increased in integrated fertilizer and compost plots to 3026 and 3672 g kg⁻¹, respectively. Compost management enhanced soil labile C, organic C and the proportion of large macroaggregates (>2000 µm size fraction). Rotational diversity did not influence total soil C but was positively associated with soil aggregate stability and aggregate carbon pool size. The study findings were consistent with integrated organic and inorganic nutrient management as interacting positively with crop diversity, to support gains in soil structural stability and C accrual.

Abbreviations: CC, continuous corn; CS, corn–soybean rotation; CSW, corn–soybean–wheat rotation; CSWco, corn–soybean–wheat rotation with a cover crop; DAP, days after planting; IC, integrated compost; IF, integrated fertilizer; LFL, living field laboratory; MWD, mean weight diameter; POXC, permanganate oxidizable carbon; PSNT, pre-side dress nitrate test; SOC, soil organic carbon.

Cropping system productivity and sustainability depend on soil organic matter dynamics, including the turnover of labile carbon and nitrogen and the development of stabilized pools (Wander, 2004). Soil organic carbon (SOC) plays an important role in soil productivity and cropping system resilience, as well as being a key sink in the terrestrial carbon cycle (1500 Pg) (Kong et al., 2005). Understanding which management practices influence SOC accrual and sequestration of C in field crop systems thus plays a crucial role in agricultural sustainability, mitigating against negative environmental impacts.

Agricultural nutrient management practices that incorporate organic nutrient sources, integrated with judicious use of inorganic fertilizer inputs, have shown promise as practical and farmer adoptable options. Integrated nutrient management holds the promise of environmental protection through reduction of nutrient losses and energy use, compared to conventional systems that rely on large doses of agro-chemical inputs (Pearson, 2007). Crop diversification has also

Core Ideas

- Soil structure and carbon pools were quantified in a 20-year field crop trial.
- Compost management influenced soil aggregate size, labile carbon, and total carbon.
- An increased rotational diversity from one to five crops enhanced soil aggregate stability.
- Use of moderate compost increased soil carbon by 20% relative to fertilizer.

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widely been regarded as a sustainability principle with positive agroecosystems benefits at multiple scales, and some of the benefits include enhanced net primary productivity, nutrient retention and resilience (Jackson et al., 2007; Snapp et al., 2010). Nevertheless, there are still major unknowns on underlying processes responsible for nutrient retention and agroecosystems resilience. For example, soil aggregation, has often been linked to agroecosystems productivity as an indicator of soil health, yet mechanisms and controls of its formation are poorly understood. Consequently, identifying controls and mechanisms of aggregate formation is key to understanding the longevity of C pools under integrated nutrient management systems and across crop diversity gradients. Studies have demonstrated that SOC accrual is directly linked to the return of fresh organic material to the soil (Rasmussen et al., 1980), disturbance regime (Grandy and Robertson, 2006), and mixtures of residue quality may support microbial diversity and processes that enhance SOC through stabilization of C (Kallenbach et al., 2015; Liang and Balser, 2010). Thus the inclusion of cover crops and the addition of manure may increase SOC levels in soils that are not C saturated. However, the efficiency of C gain is known to vary depending on dominant and active decomposers in the system that in turn may strongly be influenced by biochemical quality of plant residues (Bending et al., 2002). Duration of living cover and root system inputs are related factors by which crop diversification in rotational sequences may influence soil structure and carbon status (Grandy and Robertson, 2007). Soil aggregate dynamics appear to play an important role in SOC accrual, C sequestration and cycling (Tisdall and Oades, 1982). Soil aggregate formation and stabilization in turn influence a wide range of biological and chemical processes that regulate SOC (Tiemann and Grandy, 2015). Consequently, aggregate stability is among the key soil quality indicators that are important for informing management choices. Aggregate size distribution controls soil pore space size and connectivity, which in turn influence soil microbial activity and SOC mineralization (Ananyeva et al., 2013; Tiemann and Grandy, 2015). Under crop diversity gradients, variation in rooting depths, differences in amounts and quality of root exudates produced by different crops and changes in root biomass are expected to have profound effects on aggregate formation and soil carbon stabilization (Ball et al., 2005). Over time, aggregates can be composed of SOC coming from different time periods, with recently added organic material located at the outer perimeter of aggregates (Kavdir and Smucker, 2005). It is crucial to understand SOC dynamics in integrated nutrient management systems as well as the role that rotational diversity in C accrual and storage in row crop systems. However, studies are often constrained by the long time required to discern appreciable changes in SOC, and to understand how aggregates may interact with SOC and management practices.

In this study, we examined two management systems that follow recommended practices, namely integrated fertilizer (IF) (where judicious fertilizer use is tailored to take into account biological N fixation and management history), and integrated

compost (IC). The period it took for the trial as well as its unique design allowed the evaluation of IF and IC and interactions with rotational crop diversity on SOC and soil structural stability. We investigated the role of rotational diversity through a gradient from simple to increasingly complex crop sequences: continuous monoculture corn (*Zea mays* L.) (CC), corn–soybean [*Glycine max* (L.) Merr.] rotation (CS), corn–soybean–wheat (*Triticum aestivum* L.) rotation (CSW), and corn–soybean–wheat with winter cover crops (CSWco). The LFL experiment reported on here is the first study we know to experimentally manipulate nutrient management regimes along a crop biodiversity gradient for twenty years for row crop production system (Snapp et al., 2010). Our study presented a novel opportunity to examine the effects of rotational diversity and nutrient management (fertilizer or compost based), to assess soil C pools and soil aggregation dynamics on the same soil series under farmer-relevant management options.

This provides a unique opportunity to quantify how management and diversity, singly and in combination, influenced fast responding measures of labile pools (Weil et al., 2003), in relationship to slow response variables such as soil structure, and total organic C (Paul et al., 2013). There are reports on positive correlations between permanganate oxidizable C (POXC) and other soil biologically mediated C fractions (Culman et al., 2013), yet there remains a lack of understanding regarding the relationship between POXC and soil structural stability. We hypothesized that gains in POXC would be closely associated with water stable aggregation, and that POXC would be a sensitive indicator of decadal changes in soil organic matter.

The objectives of this field study were to: (i) quantify the long-term response of soil C and soil structural stability to rotational diversity in integrated compost and integrated fertilizer management systems, (ii) compare the response of the measures between the two nutrient management regimes, and (iii) explore the relationship between POXC and soil structural stability.

MATERIALS AND METHODS

Site Description and Experimental Design

This study was conducted in the Living Field Laboratory (LFL) established in 1993 at the W.K. Kellogg Biological Station for Long Term Ecological Research located in Kalamazoo County, MI (42°24' N, 85°24' W, elevation 288 m). The area receives approximately 90 cm of precipitation annually, about half as snow. The site is located on a mixture of Kalamazoo and Oshtemo sandy loam soils (both Typic Hapludalfs) developed from glacial outwash. The Ap horizon of both soils reaches a depth of 20 to 30 cm and an average bulk density of 1.3 Mg m⁻³ to a depth of 20 cm (Robertson et al., 1997).

The LFL was designed to investigate the effects of biodiversity (cover crops and rotational diversity) and the addition of composted dairy manure in four management systems. The focus in this study is on four levels of diversity, and two of the management systems: integrated fertilizer (IF) and integrated compost (IC), as designated by Dr. Harwood at the start of the LFL, and described

in earlier studies (Sanchez et al., 2004) The term “integrated” in this case refers to following recommended management practices that reduce toxicity of herbicide application (in-row banding of herbicide, and use of less toxic chemical formulations) and stringent accounting of N inputs using pre-side dress nitrate test (PSNT) and N analysis of composted dairy manure to adjust inorganic N fertilizer doses by taking into account other N sources. Synthetic fertilizer N was applied in the form of liquid fertilizer (20 kg N ha^{-1}) at planting, and side dressed as ammonium nitrate ($70\text{--}130 \text{ kg N ha}^{-1}$) in corn. Wheat was fertilized with urea at 80 kg N ha^{-1} in the IF systems. On the other hand, composted dairy manure was the primary source of N in the IC systems. The IF systems received P fertilizer in form of triple superphosphate at a rate of 50 kg ha^{-1} of P_2O_5 and K fertilizer in the form of K chloride at a rate of 84 kg ha^{-1} just before planting (late April and early May each year). Over the duration of this experiment, compost applied had a C to N ratio ranging from 11:1 to 13:1 and provided sufficient levels of P as indicated by soil testing (44.1 to 53.4 mg kg^{-1}). The management goal was for N rate applied to IF and IC systems to be equivalent, with $\sim 80 \text{ kg N ha}^{-1}$ applied annually to wheat, and 130 kg N ha^{-1} applied to corn in the form of fertilizer, or compost and reduced rates of fertilizer. No N was applied to soybean (Sanchez et al., 2004).

The experimental design was a split plot, randomized complete block with four blocks (Sanchez et al., 2004) with individual plot size of 15 m by 4.5 m. The main plots within blocks were IF and IC, and split plots were rotational diversity plots comprising of continuous corn, corn–soybean rotation, corn–soybean–wheat rotation, and the most diverse system of corn–soybean–wheat rotation with a cover crop. Every year, each phase of the rotations was present enabling sampling for all crop diversity and nutrient management treatment combinations. Cover crops at the beginning of the experiment included red clover (*Trifolium pretense* L.) frost-seeded into winter wheat in March and crimson clover (*Trifolium incarnatum* L.) inter-seeded in corn plots in the initial decade of the experiment. Later in 2006, crimson clover was replaced with seeding of a cereal rye (*Secale cereale* L.) cover crop after corn harvest to ensure reliable establishment of cover in all cover crop systems.

Crop Management

Winter cover crop split plots were maintained on the same locations throughout this long-term experiment. Late March of each year red clover was frost-seeded into wheat at a rate of $20 \text{ kg seed ha}^{-1}$. Cereal rye was planted at a rate of $125 \text{ kg seed ha}^{-1}$ following corn harvest within 2 wk of 1 November each fall. On or around 20 April every year, all plots received glyphosate [*N*-(phosphonomethyl) glycine] at the rate of 0.5 kg ha^{-1} a.i. on the cover crop and winter fallow split plots. This was done to minimize weed biomass accumulation in the systems annually. Pre-emergence corn herbicide mixture of mesotrione {2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione} at 0.2 kg ha^{-1} a.i., *S*-metolachlor {2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]

acetamide} at 1.9 kg ha^{-1} a.i., and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) at 0.7 kg ha^{-1} a.i. were applied on all corn plots in late May. Corn insecticide {chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] was applied on continuous corn plots at the rate of 1.3 kg ha^{-1} a.i. at planting. All corn plots were chisel plowed to a depth of 0 to 25 cm and seed bed preparation was performed with a soil finisher or field cultivator. A row cultivator was used on all corn plots. To eliminate the effect of weed competition on plant N availability, yield rows were hand-weeded following row cultivation each year.

Based on fertilizer recommendation for corn in the region, the IF system received P fertilizer in the form of triple superphosphate (0–45–0) at a rate of 50 kg ha^{-1} of P_2O_5 and K fertilizer in the form of potassium chloride (0–0–63) at a rate 84 kg ha^{-1} of K_2O , whereas the IC system had sufficient levels of P and K and did not receive fertilizer (Vitosh et al., 1995). Pioneer corn hybrid 36W66 (103-d corn) was planted in rotated and continuous corn plots at a population of $81,500 \text{ plants ha}^{-1}$. At 32 d after planting (DAP) plots were hand-thinned to a stand of $69,160 \text{ plant ha}^{-1}$.

Soil C, POXC, and pH

Soils cores were taken in November 2013 at five random positions per plot using a 1.9-cm diameter soil probe to three depths (0–5, 5–20, and 20–25 cm). The depths included the entire zone of influence associated with plant roots and cover crop residue incorporation following a sampling depth scheme of Six et al. (2000) with an addition of 20- to 25-cm depth following earlier surveys at the same site (Snapp et al., 2010). We used these depth increments in consistency with earlier study at the main site (Six et al., 2000). The scheme enabled us to study the zones where greater differentiation in soil aggregation attributable to agronomic treatments was expected. Soils from the same depth were composited, sieved with a 6-mm mesh sieve while still field moist, air dried for 3 d and then stored at 4°C until analysis for SOC and POXC. POXC analysis was based on Weil et al. (2003). Briefly, 2.5 g of air-dried soil were weighed into polypropylene 50-mL screw-top centrifuge tubes, 18 mL of deionized water and 2 mL of $0.2 \text{ mol L}^{-1} \text{ KMnO}_4$ stock solution were added and tubes were shaken for exactly 2 min at 240 oscillations per minute on an oscillating shaker. After allowing to settle exactly 10 min, 0.5 mL of the supernatant were transferred into a second 50-mL centrifuge tube and mixed with 49.5 mL of deionized water. An aliquot (200 μL) of each sample was loaded into a 96-well plate containing a set of replicated internal standards, including a blank of deionized water, four standard stock solutions (0.00005 , 0.0001 , 0.00015 , and $0.0002 \text{ mol L}^{-1} \text{ KMnO}_4$), a soil standard and a solution standard (laboratory reference samples). Sample absorbance was read at 550 nm with a SpectraMax M5 microplate reader using SoftMax Pro software (Version 5.4.1, Molecular devices, Sunnyvale, CA).

Total SOC was determined on samples that were pulverized to fine powder with a shatterbox mill 8515 (SPEX) and analyzed by dry combustion in a CHNS analyzer (Costech ECS 4010,

Costech Analytical Technologies, Valencia, CA). Aggregate associated carbon concentration was also determined by dry combustion method using subsamples of whole soil and soil aggregates obtained for each size fraction.

Soil pH was measured in field moist soil weighed to 15 g into replicate extraction cups, 30 mL deionized water was added to each cup, tightly capped and shaken for a few seconds. The cap was removed to allow the solution to equilibrate with the atmosphere for at least 30 min following which a pH meter standardized at pH 7 and 4 was placed into the slurry and a measurement taken to the nearest 0.01.

Water Stable Aggregate Assay

In November 2013, a second sampling was conducted at five locations per plot for water stable aggregate determination. First, residues and litter were brushed aside prior to sampling so that soil C and N values and aggregate associated C reflected the mineral component only using the procedure by Grandy and Robertson (2007). Then PVC cores (height 30 cm, diameter 7.6 cm) were hammered into moist ground, with care so as to minimize disturbance of soil aggregates, then cores were pulled with a vertical force. Soils were obtained at three depths of 0 to 5, 5 to 20, and 20 to 25 cm; these intervals included the uppermost layer (0–5 cm) where changes in soil structural stability due to organic matter accumulation and decomposition would be captured, an intermediate layer (5–20 cm) where disturbance on aggregate stability due to tillage would mostly occur, and lastly a depth profile (20–25 cm) that would have minimal influence from surface organic matter accumulation and minimal tillage influence. Simultaneous to aggregate sampling, three samples per plot were taken for bulk density analysis by driving an 8-cm diameter corer into soil at three depths (0–5, 5–20, and 20–25 cm). Oven-dried samples were weighed to calculate mass per unit volume.

For water stable aggregate analysis, the sampled soils were refrigerated at 4°C at the field laboratory prior to being processed within 72 h of sampling by passing samples through an 8-mm sieve and gently breaking soil clods along natural fracture planes, before being air dried. Water stable aggregates were then determined on a triplicate of 100 g air-dried composite soil subsamples for each plot by wet sieving in water at 23°C through a series of sieves with 2000-, 250-, and 53- μm openings following the method of Grandy and Robertson (2007). A subsample weighing 100 g was fractionated by wet sieving as follows: soil was spread evenly onto a 2000- μm sieve and slaked for 5 min with distilled water. The soil was then sieved for 2 min by oscillating the sieves 50 times up and down with a stroke length of 3 cm.

Large macroaggregates retained on the 2000- μm sieve mesh were backwashed into pre-weighed pans for drying. Large (>2000 μm) floating litter was removed, while soil passing through the 2000- μm sieve was transferred to a 250- μm sieve and the process was repeated to obtain the small macroaggregate fraction (250–2000 μm). The sieving process was repeated

once more using a 53- μm sieve to separate microaggregates (53–250 μm) from the silt and clay fraction (<53 μm). All pans and soil solutions were placed in an oven at 60°C until dry. Sand content (all particles >53 μm) was determined on all aggregate size fractions by collecting a 5-g aggregate subsample from the collected aggregates and dispersing the subsample in 0.5% sodium hexametaphosphate for 24 h on a rotary shaker at 150 rpm. Following this step, the suspension was decanted into a 53- μm sieve, and sand trapped on the sieve was backwashed into pre-weighed pans, dried for 24 h and weighed after cooling.

Computation of Stability Index

Mean weight diameter was computed as the summation of the average aggregate size remaining on each sieve, multiplied by the percent of total sample represented by the respective aggregate class as outlined by Kemper and Rosenau (1986).

The mean weight diameter (MWD) of aggregates is computed as

$$\text{MWD} = \sum_{i=1}^n x_i w_i$$

where x_i is the mean diameter (mm) of the aggregate class, and w_i is the proportion of each aggregate class i to the weight of the soil sample (Kemper and Rosenau, 1986).

Statistical Analyses

Analysis of variance was performed on soil data with PROC MIXED procedure in SAS v 9.4 (SAS Institute, Cary, NC) to conduct a two-way analysis of variance (ANOVA) to determine the effects of nutrient management regime, rotational diversity and their interaction using split plot design. Treatment effects on aggregate proportions, aggregate stability, SOC, POX-C were determined using ANOVA with cropping system as the fixed effect and block as a random factor. Sampling depths were analyzed separately. Significant effects were further investigated with a test of least significant differences at $p = 0.05$ for main effects and interactions. Normality of residuals was tested using the Shapiro-Wilk test and data were square-root-transformed before statistical analysis to meet the requirements of normality; means were compared with an adjusted Tukey's pairwise means comparison procedure in PROC MIXED. A regression analysis of POXC against the soil aggregate stability index (i.e., MWD) was also performed to explore the relationship between labile soil carbon and soil structural stability using PROC REG procedure.

RESULTS

Nutrient Management and Diversity on Soil C

After two decades of experimentation in the Living Field Laboratory, total soil organic carbon between the two nutrient management systems was significantly different ($p < 0.05$). By 2013, differences in SOC were most pronounced at upper depths: At depth 0 to 5 cm, the mean SOC for IC was 15.0 g kg^{-1} compared with 11.1 g kg^{-1} for IF (Fig. 1). On the other hand, at depth 5 to 20 cm, the mean SOC value for the IC system was

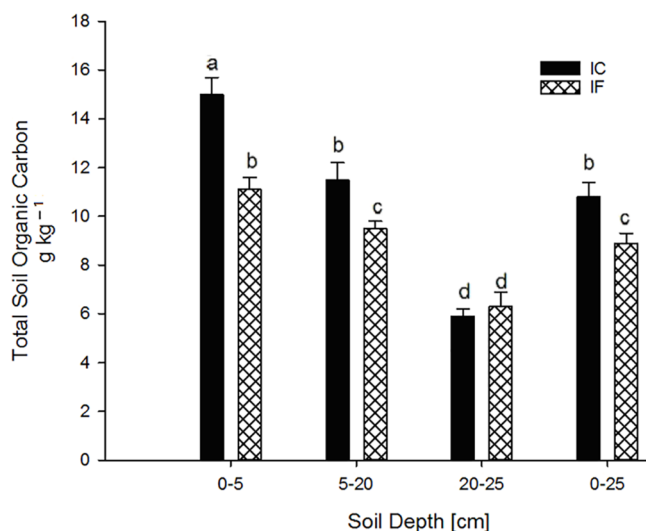


Fig. 1. Distribution of total soil organic carbon (SOC) along the depth profile in integrated compost (IC) and integrated fertilizer (IF) management systems of the Living Field Laboratory at W.K. Kellogg Biological Station for Long Term Ecological Research, Michigan, in 2013. Error bars represent standard errors of the difference (SED) between means. Means with the same letter are not significantly different ($p < 0.05$).

also higher ($p < 0.05$) averaging 11.5 g kg^{-1} as compared with 9.5 g kg^{-1} for IF. At the deeper depth, no differences were observed between IC and IF (5.9 and 6.3 g kg^{-1} respectively; Fig. 1). On an area basis the topsoil had 2.5 kg C m^{-2} at the start of the experiment (Snapp et al., 2010). Two decades later, in this study, IF soil had 3.0 kg C m^{-2} and IC soil had 3.7 kg C m^{-2} for gains of 20 and 48%, respectively.

The labile C pool as measured by POXC was affected by nutrient management ($p < 0.05$), but not by rotational diversity ($p = 0.1932$; Table 1). The magnitude of difference among POXC values varied by depth and was greatest in the upper soil layer (0–5 cm) where the mean POXC value for IC system was 557 mg C kg^{-1} compared with 423 mg C kg^{-1} in the IF system (Fig. 2). At the intermediate depth (5–20 cm), POXC

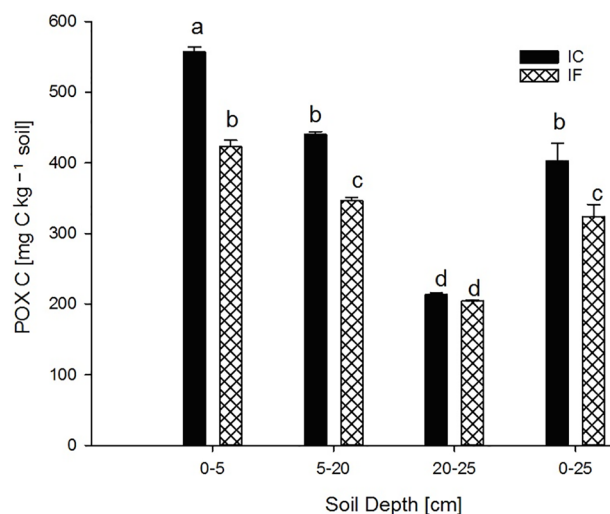


Fig. 2. Distribution of permanganate oxidizable carbon (POXC) along the depth profile in integrated compost (IC) and integrated fertilizer (IF) management systems of the Living Field Laboratory at W.K. Kellogg Biological Station for Long Term Ecological Research, Michigan, in 2013. Error bars represent standard errors of the difference (SED) between means. Means with the same letter are not significantly different ($p < 0.05$).

registered a mean value of 440 mg C kg^{-1} in the IC system and 346 mg C kg^{-1} in the IF system, accordingly. However, at lower depth (20–25 cm), the mean POXC values between IC and IF systems were not different (213 and 204 mg C kg^{-1} , respectively; Fig. 2). Overall, POXC and aggregate stability were positively correlated ($r^2 = 0.58$, $p < 0.05$; Fig. 3).

Aggregate associated C ranged from 8.1 to 12.6 g kg^{-1} (sand-free aggregates) in the large macroaggregate (2000–8000 μm) class (Fig. 4). Aggregate associated C was strongly influenced by crop rotational diversity, where the highest total aggregate C values were observed in the polyculture system (CSWco), among large macroaggregates (Fig. 4). On the other hand, lowest values of aggregate associated C were observed in monoculture systems.

Table 1. Effects of nutrient management and rotational diversity on soil pH, bulk density, soil organic C (SOC), and permanganate oxidizable C (POXC) at 0- to 25-cm depth in November 2013 in the Living Field Laboratory trial at the W.K. Kellogg Biological Station, Hickory Corners, MI, USA. Values are means with standard errors in parentheses.

Nutrient management	Rotational diversity†	pH	Bulk density Mg m ⁻³	SOC g kg ⁻¹	POXC mg kg ⁻¹
Integrated compost	Monoculture (CC)	6.67 (0.10)	1.38 (0.05)	9.0 (1.8)	375 (56)
	Biculture (CS)	7.09 (0.09)	1.40 (0.05)	10.1 (1.3)	341 (48)
	Triculture (CSW)	7.00 (0.08)	1.36 (0.06)	10.2 (1.4)	391 (46)
	Polyculture (CSWco)	6.89 (0.07)	1.34 (0.04)	12.0 (1.3)	438 (55)
Integrated fertilizer	Monoculture (CC)	7.63 (0.06)	1.38 (0.03)	6.4 (1.1)	271 (37)
	Biculture (CS)	7.74 (0.03)	1.40 (0.05)	7.5 (1.4)	347 (27)
	Triculture (CSW)	7.41 (0.15)	1.37 (0.04)	9.3 (0.9)	323 (31)
	Polyculture (CSWco)	7.77 (0.04)	1.36 (0.04)	8.6 (1.2)	348 (35)
ANOVA‡					
Management (M)		0.003	NS	0.04	0.043
Diversity (D)		NS	NS	NS	0.193
M × D		NS	NS	NS	NS

† Crop diversity treatments: CC, continuous corn; CS, corn and soybean rotation; CSW, corn, soybean, and wheat rotation; CSWco, corn, soybean, and wheat rotation with a cover crop.

‡ ANOVA p -value significance was set at $\alpha = 0.05$; NS, no significance.

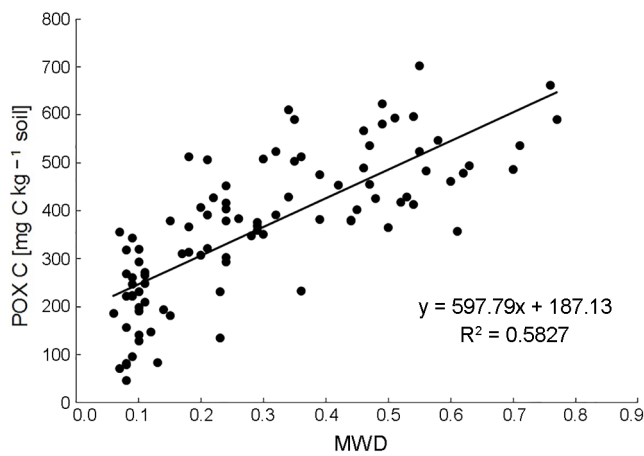


Fig. 3. Trends in permanganate oxidizable carbon (POXC) across the aggregate stability index (mean weight diameter [MWD]) in integrated nutrient management systems of the Living Field Laboratory at W.K. Kellogg Biological Station for Long Term Ecological Research, Michigan, in 2013.

This pattern of aggregate-C accumulation with rotational complexity was similar across nutrient management systems.

Water Stable Aggregates

Two decades after the trial was initiated, both nutrient management and crop diversity were found to have affected large macroaggregates and microaggregates (Table 2). At a depth of 0 to 5 cm, the polyculture system (CSWco) under IC management registered the highest MWD (0.74 mm). Comparatively, the mean MWD value for polyculture under IF management was 0.60 mm. Of all treatments for the depth 0 to 5 cm, the least MWD was observed in CC under IF nutrient management (0.40 mm; Table 2).

Considering the entire topsoil (0–25 cm), a similar pattern was observed: MWD results indicated improvements in soil structural stability for IC compared to IF nutrient management, and for rotational diverse systems compared to monocul-

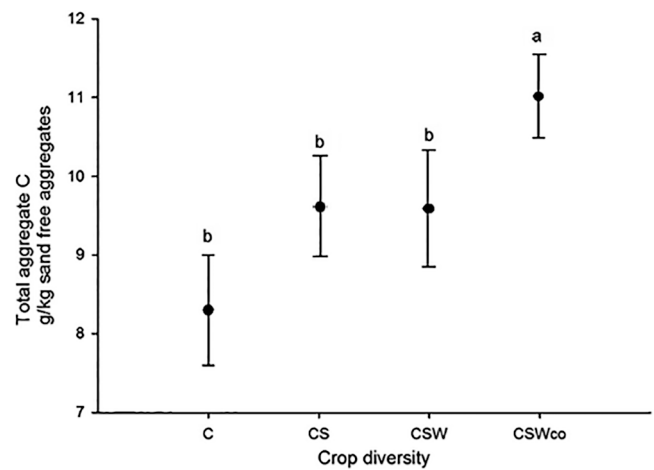


Fig. 4. Crop diversity effects on aggregate associated total soil organic carbon concentration in large macroaggregates size class (8000–2000 μm) from the Living Field Laboratory at the W.K. Kellogg Biological Station for Long Term Ecological Research, Michigan. C, continuous corn; CS, corn-soybean rotation; CSW, corn-soybean-wheat rotation; CSWco, corn-soybean-wheat rotation with a cover crop. Error bars represent standard errors of the difference (SED) between means ($p < 0.05$).

ture (Table 3). The MWD for IC across cropping systems was 0.32 mm, the MWD for IF was 0.28 mm, and aggregation was in almost all cases least under monoculture (CC) and highest under CSWco. Nutrient management and diversity both influenced large macroaggregates; consider the CSWco treatment where the large macroaggregates ($18.3 \text{ g } 100 \text{ g soil}^{-1}$) represented a 22% increase compared to the same system in IF. A similar pattern was observed in microaggregates. However, IC had a large effect on small macroaggregates relative to IF, and moderate crop diversity effects were observed for this size class (Table 3).

Corn Yield

We observed no effect on corn yield in 2013 for either type of diversity (rotational or cover crop) treatment; however, there

Table 2. Effects of nutrient management and rotational diversity on water-stable aggregate size fraction distribution and mean weight diameter (MWD) at 0- to 5-cm depth in the Living Field Laboratory trial at the W.K. Kellogg Biological Station, Hickory Corners, MI, USA. Values are means with standard errors in parentheses.

Nutrient management	Rotational diversity†	Water-stable aggregate size fraction			MWD
		>2000 μm	2000–250 μm	250–53 μm	
		g 100 g soil ^{−1}			mm
Integrated compost	Monoculture (CC)	19.2 (0.4)	24.7 (1.8)	22.4 (1.4)	0.48 (0.01)
	Biculture (CS)	21.1 (0.8)	21.8 (1.8)	20.8 (1.2)	0.52 (0.02)
	Triculture (CSW)	24.4 (1.0)	23.3 (1.1)	22.8 (0.8)	0.58 (0.02)
	Polyculture (CSWco)	32.4 (0.9)	30.1 (0.4)	29.3 (1.3)	0.74 (0.01)
Integrated fertilizer	Monoculture (CC)	16.2 (1.2)	31.0 (1.3)	30.1 (1.5)	0.40 (0.03)
	Biculture (CS)	18.2 (0.7)	30.8 (1.3)	29.4 (1.8)	0.43 (0.02)
	Triculture (CSW)	21.7 (0.6)	28.8 (0.6)	26.1 (1.4)	0.51 (0.01)
	Polyculture (CSWco)	25.4 (0.7)	28.3 (0.7)	28.9 (0.4)	0.60 (0.01)
ANOVA‡					
Management (M)		0.014	0.013	0.014	0.0100
Diversity (D)		<0.0001	NS	0.012	<0.0001
M × D		0.023	0.003	0.01	NS

† Crop diversity treatments: CC, continuous corn; CS, corn and soybean rotation; CSW, corn, soybean, and wheat rotation; CSWco, corn, soybean, and wheat rotation with a cover crop.

‡ ANOVA p -value significance was set at $\alpha = 0.05$; NS, no significance.

was a trend for compost management to be associated with higher corn yield (10,800 kg ha⁻¹, standard deviation 750 kg ha⁻¹), compared to IF management corn yield (9600 kg ha⁻¹, standard deviation 720 kg ha⁻¹). Further, there may well be economic benefits with this system, considering that compost corn was produced at half the rate of N fertilizer compared to IF corn.

DISCUSSION

Soil Carbon Pools

Over this 20-yr study, compost-based management was associated with topsoil gains in SOC, by almost 50% relative to the initial level of 2.5 kg m⁻². This is a substantial gain, taking into account the moderate compost doses used here, 3 Mg ha⁻¹. Change in SOC is difficult to detect because of the slow pace of the processes involved in its formation and accrual, and field experimentation often involves amendments applied at economically-unfeasible rates, to facilitate discovery in SOC response. Heterogeneity of background SOC and analytical variability compound the challenges (Kong et al., 2005) in determination of SOC changes. Increase in soil C has been shown previously at this site, some 8 yr later, which was also associated with compost management (Sanchez et al., 2004). We did not observe rotational diversity effects on soil C, findings that corroborate those of Snapp et al. (2010) who compared compost, organic and fertilizer management 15 yr after the start of the experiment and found compost management to be key to soil C accrual.

We investigated aggregation, as an important regulator of soil organic matter (Grandy and Robertson, 2006). Several studies have shown that the addition of composted organic material to the soil results in an increase in aggregation, especially at high doses (Valarini et al., 2009; Medina et al., 2004; Caravaca et al., 2006; Annabi et al., 2007; Wortmann and Shapiro, 2008). At this LFL site we observed that soil aggregate stability was enhanced in IC relative to IF, and specifically the proportion of large macroaggregates (>2000 µm) were high in compost

amended plots. Compost effects on aggregation and soil C were most notable at the upper depth (Table 2). Similarly, other field studies have found a pattern of macroaggregate and C accrual that is consistent with macroaggregates as providing maximum protection for soil C conservation (Bouajila and Gallali, 2008; Grandy and Robertson, 2007).

Our findings provide further evidence of a positive relationship between labile soil C accrual (i.e., POXC) and increases in soil structural stability (Fig. 3). This concurs with observations that labile C appears to promote structural stability and this in turn protects C, for a virtuous cycle (Denef et al., 2001).

Changes in SOC attributable to management are often expensive and difficult to measure, hence other metrics that can help explain soil aggregate stability in the context of soil management are attractive options for farmers and extension advisors (Culman et al., 2013).

A recent study was consistent with POXC as an assay that could quantify labile C in a relatively rapid and inexpensive manner across a wide range of soil types, ecosystems, and geographic areas, and found a strong positive relationship between POXC and SOC, as well as microbial biomass C (Culman et al., 2012). Furthermore, POXC demonstrated high sensitivity to changes in management; however, there is a knowledge gap concerning POXC and other soil health properties such as soil structural stability. In our study POXC values were influenced by IC management, and positively associated with soil aggregation ($r^2 = 0.58$, $p < 0.05$; Fig. 3). To our knowledge this is the first study to explore such a relationship.

Water Stable Aggregates

We found evidence that soil structure was influenced by both management and rotational diversity at the LFL site. Changes in soil aggregation were most apparent in the top soil depth (0–5 cm; Table 2) but were also observed for the plow layer (0–25 cm; Table 3). These findings demonstrate that spatial scales (vertical

Table 3. Effects of nutrient management and rotational diversity on water-stable aggregate size fraction distribution and mean weight diameter (MWD) at 0- to 25-cm depth in the Living Field Laboratory trial at the W.K. Kellogg Biological Station, Hickory Corners, MI, USA. Values are means with standard errors in parentheses.

Nutrient management	Rotational diversity†	Water-stable aggregate size fraction			MWD
		>2000 µm	2000–250 µm	250–53 µm	
		g 100 g soil ⁻¹			mm
Integrated compost	Monoculture (CC)	11.0 (1.9)	13.9 (3.7)	11.6 (4.0)	0.26 (0.05)
	Biculture (CS)	12.4 (2.2)	13.7 (3.7)	10.9 (4.0)	0.29 (0.05)
	Triculture (CSW)	13.6 (2.5)	12.4 (3.5)	10.5 (3.4)	0.31 (0.06)
	Polyculture (CSWco)	18.3 (3.2)	13.8 (3.2)	12.1 (3.6)	0.41 (0.07)
Integrated fertilizer	Monoculture (CC)	9.3 (1.6)	11.2 (3.0)	8.5 (3.0)	0.22 (0.04)
	Biculture (CS)	11.2 (1.8)	10.6 (2.5)	8.3 (2.7)	0.26 (0.04)
	Triculture (CSW)	12.6 (2.2)	11.1 (2.7)	9.0 (3.0)	0.29 (0.05)
	Polyculture (CSWco)	15.0 (2.5)	13.6 (3.6)	11.3 (3.9)	0.34 (0.06)
ANOVA‡					
Management (M)		0.012	0.015	0.009	0.015
Diversity (D)		<0.0001	0.009	0.001	<0.0001
M × D		0.035	0.042	NS	NS

† Crop diversity treatments: CC, continuous corn; CS, corn and soybean rotation; CSW, corn, soybean, and wheat rotation; CSWco, corn, soybean, and wheat rotation with a cover crop.

‡ ANOVA p -value significance was set at $\alpha = 0.05$; NS, no significance.

distribution) are important in terms of distribution of aggregates and their associated properties.

Carbon inputs through compost additions, in the IC treatment are expected to stimulate microbial polysaccharides and other compounds that stabilize aggregates (Roberson et al., 1991; Angers and Mehuys, 1989); leading to the high aggregate stability that we observed in IC plots. Further, polyculture systems in our study exerted similar effects to compost with respect to the proportion of whole soil found in the small macroaggregate (250–2000 μm) and microaggregate (53–250 μm). Biochemical properties of the root and residues inputs in the rotational diversity treatments, as well as quantities of those residues, are likely factors that control the pace of aggregate formation as well as decomposition dynamics (Kavdir and Smucker, 2005; Liang and Balser, 2010). The association between rotational diversity and soil structural stability has profound implications for ameliorating soil health in degraded cropping systems. Enhanced C inputs from biomass cannot alone explain soil aggregation patterns we observed along the diversity gradient, as monoculture corn produces copious amounts of biomass (estimated at 5.7–8.4 Mg ha^{-1}) compared with soybean and wheat (3.7 and 3.9 Mg ha^{-1} , respectively). The quality of residues involved, the role of cover crops (vegetative cover that is persistent over the winter as found in the cover crop diversified systems) and root system inputs may all play significant roles (Grandy and Robertson, 2007; Angers and Caron, 1998; Kavdir and Smucker, 2005).

Soil aggregation can be increased by diversifying farming systems with various cover crops, as shown by Roberson et al. (1991) in orchard soils of California where introduction of cover was associated with rapid gains in the stability of soil macroaggregates as indicated by slaking resistance of cover crop versus fallow (clean cultivated or herbicide) treatments. Similarly, Hermawan and Bomke (1997) found greater aggregate structure (i.e., larger MWD) following growth of winter cover crops on lowland soils in British Colombia. However, in a study adjacent to this LFL site in Michigan, 8 yr of cereal rye cover crop did not increase aggregation relative to bare winter management, in a rotation sequence that included 2 yr of corn followed by soybean (Snapp and Surapur, 2018). This underscores the slow processes involved and long time periods required to observe effects of winter cover on soil properties. It is also apparent through this LFL study, and others, that biodiversity seems to help augment soil structural stability, and introduction of a rye cover crop alone as in the Snapp and Surapur (2018) study, this may not be sufficient to support gains in structural stability.

Taken together, the patterns of aggregate formation and C accrual in our study, and the nearby LTER site (Tiemann and Grandy, 2015), show that diversity of C inputs is important in conditioning soil carbon gains as well as soil aggregate stability. Equally important, integrated nutrient management practices can markedly alter C enrichment in aggregate size classes consequently impacting the long-term C status in row crop production.

CONCLUSION

Over two decades of experimentation, our study demonstrated that integrated compost outperformed integrated fertilizer in terms of soil aggregation, total and labile SOC accrual. Rotational diversity was associated with gains in microaggregates, compared to corn monoculture, and acted as a substitute for compost amendment relative to soil structural gains. Compost associated gains in structural stability were noted at this LFL site after 8 yr, whereas rotational diversity effects on aggregation and soil C accrual have only become observable after twenty years. Our findings highlight the complex interplay between management factors in aggregate formation, stabilization and SOC pools over multiple decades.

A decadal or longer time frame needs to be taken into account by policymakers, as highlighted by the loss of soil health properties associated with simplified field crop sequences as shown here, which is only became apparent through long-term field experimentation.

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