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Trade-offs between soil organic carbon surface gains and subsurface losses following 60 years of contrasting tillage and crop rotations

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Abstract

Conservation tillage and diversified crop rotations are known to enhance soil organic carbon (SOC) storage in surface soils (0–20 cm), while their long-term effects on subsurface SOC storage remain poorly understood. This study examined the 60+ year impacts of tillage and rotation on surface and subsurface SOC storage and aggregation at the Triplett–Van Doren Experiment in Ohio, at two locations with contrasting soil types (Wooster silt loam and Hoytville clay loam). Soil cores were sampled in 2024 from the full factorial randomized complete block design including three tillage intensities (moldboard plow [MP], chisel plow [CP], and no-tillage [NT]) and three crop rotations (continuous corn [*Zea mays* L.], corn–soybean [*Glycine max* (L.) Merr.], and corn–forage–forage), segmented into five depths (0–10–20–30–50–100 cm), and analyzed for SOC, particulate and mineral-associated organic C (POC and MAOC) stocks, and mean weight diameter (MWD). Tillage and depth had greater influence on SOC stocks than rotation. In the 0- to 10-cm depth, NT stored more SOC and MAOC than MP and CP at both locations, more POC at Hoytville, and had greater MWD at Wooster. At the 30- to 50-cm depth, MP stored more SOC and MAOC than NT and CP at both locations. At the 50- to 100-cm depth at Hoytville, MP and CP stored more POC than NT. Thus, tillage disturbed SOC in the surface soil and distributed SOC into subsurface soil depths. The depth-dependent nature of SOC storage after six decades underscores the importance of including subsurface soils in SOC assessments.

Plain Language Summary

Soil carbon storage and soil structure can improve in surface soils when farmers reduce soil disturbance and grow more diverse crops. However, more long-term

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Abbreviations: CC, continuous corn; CFF, corn–forage–forage; CP, chisel plow; CS, corn–soybean; MAOC, mineral-associated organic carbon; MP, moldboard plow; MWD, mean weight diameter; NT, no-tillage; POC, particulate organic carbon; SOC, soil organic carbon; SOM, soil organic matter.

research is needed to understand what happens in deeper soil layers. This study examined soil carbon and structure after over 60 years of farming practices at two sites in Ohio with different soil types using soil cores collected from 0 to 1 m in soil depth. Management comparisons were across three tillage levels (heavy plowing, moderate plowing, and no-till) and three crop systems (continuous corn, corn–soybean rotation, and corn with forage crops). No-till and heavy plowing practices corresponded to highest carbon in surface and subsurface soils, respectively. Overall, results show that measuring deeper soil responses to tillage is important for quantifying soil carbon storage.

1 | INTRODUCTION

Eastern regions of the Corn Belt in the US Midwest have lost approximately half of their initial soil organic carbon (SOC) levels since the mid-1800s due to intensive agricultural practices (Grace & Robertson, 2021). This estimate is for the top 0–30 cm of soil profiles, with even greater losses when considering whole soil profiles from 0 to 1 m (Sanderman et al., 2017; Sanford et al., 2012). This SOC loss has extreme consequences for soil health and agroecosystem productivity in a changing climate when resilience and resistance is key to sustainability (Y. Zhang et al., 2020). Regenerative agricultural practices like conservation tillage and crop rotational diversity can help rebuild SOC and promote long-term soil health in row crop systems (Lehmann et al., 2020; Nunes et al., 2020; Veum et al., 2022).

SOC and aggregation are standard soil health indicators (Bagnall et al., 2023; Natural Resource Conservation Service, 2025) that are generally higher under reduced tillage and greater crop rotational diversity (Gonzalez-Maldonado et al., 2025; Jin et al., 2021; Liptzin et al., 2022; Martin et al., 2022; Rieke et al., 2022; Zuber et al., 2015). Conservation tillage can mitigate SOC loss by reducing the physical disruption of soil organic matter (SOM) and aggregates relative to conventional tillage (Blanco-Canqui & Ruis, 2018; Blanco-Canqui et al., 2011; Six et al., 2000). Evidence of increased SOC storage under conservation tillage is strongest in surface soils, with mixed results for subsurface soils and soil profiles (Angers & Eriksen-Hamel, 2008; Blanco-Canqui et al., 2021; Córdova et al., 2025; Haddaway et al., 2017; Luo et al., 2010; Ogle et al., 2019). In fact, intensive plowing practices can increase subsurface and whole-profile SOC storage relative to less intensive tillage practices (Blanco-Canqui & Lal, 2008; Nicoloso & Rice, 2021; Olson et al., 2005). While tillage disrupts surface SOM and aggregates, it incorporates aboveground crop residues and belowground surface-level roots into subsurface soils, where there is increased potential for stabilization of plant-derived C in mineral associations and as microbial necromass (Angst et al., 2021; Yan et al., 2025). Though the redistribution of SOC into subsurface soil depths is not necessarily beneficial to surface soil health, with respect to supplying nutrients to crops and supporting other ecosystem services, the stability of subsurface SOC is integral in supporting agroecosystem productivity by improving water and nutrient supplies and increasing resilience to disturbance and climate change (Hicks Pries et al., 2023; Sierra et al., 2024). Therefore, assessing SOC stocks across surface, subsurface, and whole profile soils is essential for comparing storage potential across tillage intensities, and assessing other management practices in place, such as crop rotational diversity.

Relative to continuous monoculture rotations, crop rotational diversity can increase surface SOC storage, and often has compounding effects with tillage intensity on SOM stratification in subsurface soils and soil profiles (Deiss et al., 2021; Gautam et al., 2025; Nicoloso & Rice, 2021). Crop diversity influences the quantity and quality of plant inputs, with implications for soil health (Chahal et al., 2021; Martin & Sprunger, 2022). Multispecies crop rotations increase the diversity and chemical complexity of aboveground and belowground organic matter inputs relative to monoculture systems (Deiss et al., 2021; Sprunger et al., 2020; Tiemann et al., 2015). In particular, perennial systems provide continuous surface cover, persistent root presence, and enhanced aggregate formation, all factors that promote long-term soil health and SOC stabilization (Córdova et al., 2025; Martin & Sprunger, 2022; Peixoto et al., 2022; Sprunger et al., 2017, 2020). Several studies have shown interactive effects of tillage and crop rotation on surface soil health, soil aggregation, and SOC storage (Gonzalez-Maldonado et al., 2025; Jin et al., 2021; Martin et al., 2022; Zuber et al., 2015), with fewer studies examining subsurface dynamics (Gál et al., 2007; Gautam et al., 2025; Weidhuner et al., 2021).

Conclusions about surface and subsurface SOC dynamics are best informed when considering multiple fractions that range in source, stability, and function (Angst et al., 2023). Total SOC can be physically fractionated into particulate organic C (POC) and mineral-associated organic C (MAOC), which are distinct in chemistry, turnover, and ecological function, and are sensitive to tillage and crop management (Aziz et al., 2014; Cotrufo & Lavallee, 2022; Jilling et al., 2021; King et al., 2024; Prairie et al., 2023; Villarino et al., 2021). Plant residues are the primary component of POC, especially those that have been fragmented and partially decomposed (Lavallee et al., 2020; Leuthold et al., 2024). In comparison, MAOC is smaller and protected, and derived from more soluble compounds such as root exudates, rhizodeposits, and microbial necromass (Cotrufo & Lavallee, 2022; Lavallee et al., 2020; Sokol & Bradford, 2019; Villarino et al., 2021). The two fractions are also distinct in their distribution across soil profiles. While POC is more concentrated in surface soils than in subsurface soils, driving faster SOC turnover, it can also be root- and microbe-derived and contribute to SOC stabilization, even in subsurface soils (Franzluebbers, 2024; Fulton-Smith et al., 2024; Si et al., 2024; van der Pol et al., 2022; Witzgall et al., 2021; Zhou et al., 2024). Meanwhile, total SOC storage is dominated by MAOC, with increased abundance at depth (Fulton-Smith et al., 2024; Zhou et al., 2024). Yet, the impact of tillage and crop rotation on POC-MAOC dynamics in subsurface and whole-profile soils is underexplored relative to surface soils, especially in relation to total SOC storage (Gautam et al., 2025), and in field conditions with contrasting inherent soil properties such as soil type (Even & Cotrufo, 2024).

In this study, we address the knowledge gap of the impact of tillage and rotation management on surface, subsurface, and whole-profile SOC storage and aggregation at the Triplett–Van Doren experiment, with two locations that differ in soil type. Our objective was to evaluate the long-term (60+ years) effects of tillage and crop rotation practices on total SOC, MAOC, and POC storage and aggregation in US Midwest corn (*Zea mays* L.) production systems. Our overall hypothesis is that management will change SOC stratification in the profile by modifying SOM inputs, which regulates SOC movement, stability in aggregates, and interactions with minerals as follows: (1) in surface soils (0–20 cm), reduced tillage and extended rotations with perennial forages will increase SOC by preserving aggregates that physically protect POC and enhance microbial and root contributions to MAOC; (2) in subsurface soils (20–100 cm), SOC will be higher in systems with more intensive tillage and C-rich root systems (corn and perennials), through the mixing of POC into depths with slower microbial processing and depolymerization, and the increased availability of reactive mineral surface area for MAOC formation; (3) in soil profiles (0–100 cm), SOC storage will be comparable among systems, due to redistribution of SOC in the profile that balances out stratification described in (1) (surface gains under regenerative practices) and (2) (subsurface gains under tillage and C-rich root systems).

2 | MATERIALS AND METHODS

2.1 | Field experiment description

The Triplett–Van Doren No-Tillage and Crop Rotation Experiment is located in Wooster, OH (40˚45′ N, 81˚54′ W) and in Hoytville, OH (41˚13′ N 83˚45′ W) (Dick et al., 2013). The Wooster location was established in 1962 and the Hoytville location was established the following year. The locations have contrasting soil types: a Wooster silt loam (well-drained, fine-loamy, mixed, mesic Typic Fragiudalfs) and a Hoytville clay loam (poorly drained, fine, illitic, mesic Mollic Ochraqualfs) (Soil Survey Staff, 2010). The experiment features a randomized complete block design with three replicates of a two-way full factorial arrangement including three tillage intensity levels and three crop rotations.

The levels of tillage are moldboard plow (MP), chisel plow (CP), and no-tillage (NT). The MP treatment consists of inversion of the surface 0- to 20-cm soil depth with an MP that buries all crop residues. The CP treatment was tilled with a paraplow until 1983, and a CP since then; at least 30% of previous crop residues are retained on the soil surface. Corn and soybean (*Glycine max* (L.) Merr.) residues are spread evenly using a chaff spreader mounted on the rear of the combine to distribute residue evenly throughout the harvested widths. In MP and CP treatment plots, secondary tillage is implemented with a field disc (up to two passes) and then completed with a soil finisher to get a smooth seedbed. Primary and secondary tillage operations occur in the fall at Hoytville and spring at Wooster. The NT treatment consisted of retaining all crop residue on the soil surface with minimal to no disturbance.

The crop rotations include a monoculture continuous corn (CC) treatment, a 2-year corn–soybean (CS) treatment, and a 3-year corn–forage–forage (CFF) treatment. Forage crops include oats (*Avena sativa* L.) as a nurse crop that is seeded with a grass-legume mix of species such as orchard grass (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* (Schreb.) Darbysh), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and clover (*Trifolium repens* L.). Tillage is performed annually on all tillage treatment plots, with the exception of the CFF rotation. In the CFF system, tillage occurs after corn and the first forage phase (Forage 1), but not after the second forage phase (Forage 2), as it represents a continuation of Forage 1.

Agronomic management practices follow Tristate Guidelines and are described in detail by Dick et al. (2013). In general, lime was applied as needed, and corn systems were fertilized with nitrogen. Corn is harvested as grain in all rotations. The plot size is 22.3 m × 4.3 m at Wooster and 30.5 m × 6.4 m at Hoytville. For the forage treatments, only forage biomass is harvested, and not smaller cereal grain, and at Wooster the species composition changed in the early

2000s. Forage biomass is harvested three times per year; Forage 1 is not always able to be harvested three times due to poor growth. Additional agronomic details about the experiment can be found in Dick et al. (2013).

2.2 | Soil sampling and processing

Soil samples were collected in spring 2024 before planting. All of the treatments were going into the same corn growing year. Sampling occurred at Wooster on April 15–23 and at Hoytville on April 30–May 5. Wooster was sampled before all tillage operations on May 15–30, 2024. Hoytville was sampled after primary and secondary tillage the previous fall on November 16 and 28, 2023. Three replicate samples per plot were collected with a handheld hammer probe (0- to 30-cm depth × 5 cm diameter) and segmented into three depth increments of 0–10, 10–20, and 20–30 cm. Immediately adjacent to the handheld hammer probe sampling locations, three separate soil cores (0- to 100-cm depth × 5 cm diameter) were collected with a hydraulic probe and segmented into depth increments of 0–30, 30–50, and 50–100 cm. All cores were collected to 100 cm. This study uses the 0–10, 10–20, and 20–30 cm samples from the handheld hammer probe, and the 30–50 and 50–100 cm samples from the hydraulic probe; this approach minimizes compaction and increases precision of depth segmentation in the surface 0–30 cm (Sharma et al., 2020). Soil samples ($n = 810$) were sieved to 8 mm and dried at 40˚C until a constant weight (Even et al., 2025). Bulk density was determined using the intact core method and corrected for coarse fragment content (>8 mm). Gravimetric soil moisture was measured as the mass of oven-dried soil per volume of field moist soil. The three replicates were pooled by depth for aggregate and SOC analyses ($n = 270$). The 8 mm soil was used for aggregates, and a subsample was ground to <2 mm for SOC analyses.

2.3 | Lab analyses

2.3.1 | Total SOC

Total soil C (including organic and inorganic C) was measured by Spectrum Analytic Inc (Washington Court House) using a LECO CN828 elemental analyzer and corrected for inorganic C content to estimate SOC content. Inorganic C was estimated using diffuse reflectance infrared Fourier transform spectroscopy (mid-DRIFTS). Spectra were obtained with an X,Y Autosampler (Pike Technologies Inc.) coupled with a Nicolet iS50 spectrometer (Thermo Fisher Scientific Inc.) using finely ground (roller grinder, 16 h) and dry (40˚C for >72 h) soils, potassium bromide as background, 8 cm^{-1} resolution, 24 co-added scans, 4000–700 cm^{-1} range, and four spectral replicates (Deiss et al., 2020). Neural network regression models were trained using legacy data (733 samples) from the National Cooperative Soil Survey with inorganic C determined by the pressure calcimeter method (Dreimanis, 1962). Model performance for the independent validation set (25% of dataset) achieved RMSE = 0.137%, RPD = 14.4, and $R^2 = 0.995$, indicating high predictive accuracy. Total SOC stocks (Mg ha^{-1}) were calculated by multiplying SOC concentrations (g kg^{-1}), bulk densities corrected for coarse fragments (g cm^{-3}), and soil depth increment lengths (cm). Whole-profile SOC stocks (0–100 cm) were calculated as the sum of SOC in all individual depth increments for each replicate block.

2.3.2 | Particulate and mineral-associated organic carbon

Physical SOM fractionation followed the density fractionation protocol described in Leuthold et al. (2023) that was adapted from Golchin et al. (1994). First, 20 mL of sodium polytungstate (SPT), adjusted to a density of 1.85 g cm^{-3}, was added to 6.00 g soil in a 50 mL Falcon tube, along with 12 glass beads. Samples were shaken on a reciprocal shaker for 18 h, inverted, and then rinsed down with SPT to the 40 mL line. Tubes were centrifuged (3600 rpm at 20˚C) for 20 min. The supernatant was aspirated over a 20-µm nylon filter with a vacuum filtration set up, and triple rinsed with deionized water. The particulate organic matter (POM) fraction was rinsed off the filter into pre-weighed and labeled POM pans. The remaining sample in the tube was rinsed down with deionized water, and centrifuged for 20 min, and the supernatant was discarded; this rinse/centrifuge/discard step was repeated twice, followed by final centrifugation for 25 min. Samples were washed into pre-weighed and labeled mineral-associated organic matter (MAOM) pans. The pans were oven dried (60˚C) to a constant weight. Total sample mass recovery was above 95% for all samples.

The POM and MAOM fractions were individually analyzed for total C by rapid combustion using a Vario UNICUBE elemental analyzer (Elementar). The average C recovery in POC and MAOC fractions across all samples was 61% of total SOC, with greater average recovery at Hoytville compared to Wooster in each depth increment (75% vs. 64% in 0–10 cm, 73% vs. 62% in 10–20 cm, 67% vs. 56% in 20–30 cm, 61% vs. 49% in 30–50 cm, and 72% vs. 34% in 50–100 cm). The non-recovered SOC could be attributable to analytical mismatches between elemental analyzers or the physically disrupting nature of the fractionation protocol, especially for subsurface soil depths at Wooster with higher silt content and poorer aggregation than at Hoytville. Previous studies employing density fractionation indicate non-recovered C could be from either fraction during separation (Crow et al., 2007; Helbling et al., 2021; Leuthold et al., 2023, 2024; Plaza et al., 2019). Stocks were calculated as described for total SOC.

2.3.3 | Soil aggregates

Soil aggregate stability was tested using a mechanical wet-sieving machine as described in Yoder (1936) with sieve sizes of 2000, 250, and 53 µm. First, 50.00 g of 8 mm sieved soil was oscillated in tap water at 30 cycles per minute for 10 min. After oscillation, soil aggregates on each sieve were washed into small trays and dried at 40°C, weighed separately by aggregate-size class (i.e., 2000–8000, 250–2000, 53–250, <53 µm). All fractions were separated per class, dried, and weighed except for the <53 um fraction that was calculated as the difference of the initial weight minus total weight of the other fractions. The portion of sample in each of these classes were then multiplied by the mean within that class size (5000, 1125, 151.5, and 26.5 µm) to determine mean weight diameter (MWD) in µm.

2.4 | Statistical analyses

Data analysis was conducted in R version 4.2.2 (R Core Team, 2022) and figures were produced with the "ggplot2" package (Wickham, 2016). Linear mixed effects models were used to evaluate the effects of tillage and crop rotation on SOC stocks and MWD at different depths at each location, using a linear mixed effect model with tillage, rotation, soil depth, and their interactions as fixed effects and replicate block as a random effect ("lmer" function, "lme4" package; Bates et al., 2015). For these "individual depth" models, log transformations were performed on total SOC stocks at both locations, MAOC stocks at Hoytville, and MWD at Wooster, and POC stocks were square root-transformed for both locations. After transformations, each model met assumptions of normality and constant variance. Total SOC, MAOC and POC concentrations; bulk density; and percent sand, silt, and clay were run through the same models without requiring transformations to meet assumptions (See Table S1 for analysis of variance results). Whole-profile stocks (0–100 cm) at each location were analyzed in linear mixed effect models with tillage, rotation, and their interaction as fixed effects and replicate block as a random effect. For all models, the significance levels of individual and interaction effects were tested with the "Anova" function ("car" package; Fox & Weisberg, 2019). In the "individual depth" models, the three-way interaction of tillage, rotation, and depth was used to evaluate depth-specific stock and MWD differences across nine tillage-rotation systems. Compact letter displays were produced with the "cld" function ("multcomp" package; Hothorn et al., 2008) using group means estimated with the "emmeans" function ("emmeans" package; Lenth, 2023). The *p*-values were adjusted using Tukey's honest significant difference test.

3 | RESULTS

3.1 | Total SOC stocks

Tillage and soil depth interacted to affect total SOC stocks at both locations ($p < 0.001$; Table 1), as well as SOC concentrations and bulk density ($p < 0.05$; Table S1). Depth-specific differences in SOC stocks were observed despite no significant whole-profile (0–100 cm) effects (Tables 1 and 2). Stocks were highest in the 50- to 100-cm depth, which was driven by the greater bulk density and soil volume of the 50- to 100-cm depth, given that concentrations were highest in the 0- to 10-cm depth (Tables S2 and S3). Averaged across rotations in the 0- to 10-cm depth, SOC was significantly higher under NT than CP and MP at Hoytville (+11 and +17 Mg ha^{-1}) and under NT and CP than MP at Wooster (both +5 Mg ha^{-1}; $p < 0.05$; Figure 1). Although the three-way interactions of tillage, rotation, and depth on SOC stocks (Table 1) and SOC concentrations (Table S1) were not significant, SOC (0–10 cm) was highest in NT–CFF and lowest in CP–CS, for both locations ($p > 0.05$; Figure 1; Table S3). Total SOC stock responses to management shifted in subsurface depths (>20 cm; Figure 1). In the 20- to 30-cm depth at Wooster, SOC stocks were significantly greater under MP and CP than NT (+5 Mg ha^{-1}; $p < 0.05$; Figure 1). In the 30- to 50-cm depth, MP significantly exceeded NT and CP in SOC storage (+9 and +13 Mg ha^{-1} at Hoytville; +5 and +7 Mg ha^{-1} at Wooster; $p < 0.05$; Figure 1).

3.2 | Mineral-associated organic carbon stocks

Tillage and soil depth interactions on MAOC stocks and concentrations were significant at both locations ($p < 0.05$; Table 1; Tables S1 and S4), while tillage did not impact whole-profile MAOC stocks to a significant extent (Tables 1 and 2). In the 0- to 10-cm depth, MAOC stocks were significantly higher under NT than CP and MP at Hoytville (+7 and +10 Mg ha^{-1}) and under NT and CP than MP at Wooster (+3 and +5 Mg ha^{-1}; $p < 0.05$; Figure 2). In the 20- to 30-cm depth, MAOC stocks were significantly higher under both MP and CP than NT at both locations (all +4 Mg ha^{-1}; $p < 0.05$; Figure 2). In the 30- to 50-cm depth, MP significantly exceeded CP and NT in MAOC storage at Hoytville (both +8 Mg ha^{-1}) and Wooster (both +4 Mg ha^{-1}; $p < 0.05$; Figure 2).

TABLE 1 Summary of results from mixed effect linear models, including *F*-statistics and *p*-values, for stocks and mean weight diameter across all soil depths (“individual”) and for whole-profile stocks (“profile”).

Property	Model	Factor	Hoytville	Wooster
SOC (Mg ha^{-1})	Individual	Depth (D)	173***	593***
		Tillage (T)	1	7***
		Rotation (R)	1	2
		D × T	12***	11***
		D × R	1	2
		T × R	1	2
		D × T × R	1	1
	Profile	T	1	0
		R	1	1
		T × R	2	1
MAOC (Mg ha^{-1})	Individual	D	101***	33***
		T	3	1
		R	3	2
		D × T	8***	7***
		D × R	1	1
		T × R	2	1
		D × T × R	1	0
	Profile	T	1	0
		R	2	1
		T × R	2	0
POC (Mg ha^{-1})	Individual	D	19***	22***
		T	3	1
		R	1	4*
		D × T	6***	0
		D × R	1	2
		T × R	2	1
		D × T × R	1	0
	Profile	T	1	2
		R	0	2
		T × R	1	0
MWD (μm)	Individual	D	11***	208***
		T	8***	6***
		R	5*	74***
		D × T	1	12***
		D × R	2	6***
		T × R	1	3*
		D × T × R	1	1

Note: No asterisk indicates nonsignificance. Abbreviations: MAOC, mineral-associated organic carbon; MWD, mean weight diameter; POC, particulate organic carbon; SOC, soil organic carbon.

* and *** represent significance at the 0.05 and 0.001 probability level.

TABLE 2 Soil profile (0–100 cm) carbon stocks (Mg ha^{-1}) under different tillage intensities and crop rotations at Hoytville and Wooster.

Location	Tillage	Rotation	Total SOC (Mg ha^{-1})	MAOC (Mg ha^{-1})	POC (Mg ha^{-1})
Hoytville	Moldboard plow	CC	221 ± 5	123 ± 4	30 ± 1
		CS	210 ± 11	123 ± 7	30 ± 4
		CFF	200 ± 5	111 ± 2	31 ± 2
	Chisel plow	CC	201 ± 9	104 ± 6	28 ± 4
		CS	198 ± 12	110 ± 4	23 ± 2
		CFF	209 ± 13	119 ± 12	30 ± 4
	No-till	CC	200 ± 8	106 ± 4	22 ± 5
		CS	200 ± 8	117 ± 6	30 ± 1
		CFF	219 ± 3	130 ± 7	26 ± 4
Wooster	Moldboard plow	CC	177 ± 9	67 ± 10	18 ± 1
		CS	166 ± 8	62 ± 7	16 ± 2
		CFF	170 ± 13	66 ± 10	16 ± 1
	Chisel plow	CC	164 ± 3	62 ± 7	21 ± 3
		CS	172 ± 3	61 ± 3	22 ± 7
		CFF	176 ± 6	69 ± 5	16 ± 1
	No-till	CC	171 ± 4	62 ± 3	23 ± 2
		CS	162 ± 3	61 ± 5	23 ± 4
		CFF	167 ± 1	63 ± 7	18 ± 5

Note: Mean and standard error values are among tillage and crop rotation systems at each location ($n = 3$).

Abbreviations: CC, continuous corn; CFF, corn–forage–forage; CS, corn–soybean; MAOC, mineral-associated organic carbon; POC, particulate organic carbon; SOC, soil organic carbon.

3.3 | Particulate organic carbon stocks

For POC stocks and concentrations, a tillage and soil depth interaction occurred at Hoytville ($p < 0.001$) whereas a main rotation effect occurred at Wooster in POC stocks ($p < 0.05$; Table 1; Tables S1 and S5). Averaged across rotations at Hoytville, POC stocks in the 0- to 10-cm depth were significantly higher under NT than CP and MP, but in the 30- to 50-cm depth higher under MP than CP and NT, and in the 50- to 100-cm depth higher under MP and CP compared to NT (all +2–3 Mg ha^{-1}; $p < 0.05$; Figure 3). Averaged across tillage and depth at Wooster, POC stocks were higher in CC than CFF ($p < 0.05$; Table 1), with most pronounced in the 50- to 100-cm depth (Figure 3). Whole-profile POC stocks did not differ among treatments at either location to a significant extent ($p > 0.05$; Tables 1 and 2).

3.4 | Soil aggregation

At Wooster, soil depth significantly interacted with tillage and separately with rotation to impact MWD of soil aggregates, and these interactions were strengthened by variability in MWD at depth, while at Hoytville, depth, tillage, and rotation each had significant effects on MWD, with less of a depth effect (Table 1). In the 0- to 10-cm depth at Wooster, MWD was significantly larger for NT than CP, and both compared to MP ($p < 0.05$; Figure 4). At Wooster in the 0- to 10-cm, 10- to 20-cm, and 20- to 30-cm depths across tillage, MWD was significantly larger for CFF than CS and CC ($p < 0.05$; Figure 5). The absence of MWD patterns at Hoytville (Figure 4) could be attributed to the consistent proportion of aggregates larger than 2000 μm outweighing the abundance of smaller aggregate fractions (Figure 5), which could be related to the abundance of clay content over silt and sand content (Tables S6–S8).

4 | DISCUSSION

4.1 | Tillage intensity impacted surface SOC storage and aggregation more than crop rotation

Assessing the long-term (60+ years) effects of conservation practices on SOC storage and aggregates in surface soils (i.e., 0–10–20 cm soil depths), we hypothesized that reduced tillage and extended rotations with perennial forages would increase SOC by preserving aggregates that physically protect POC and enhance microbial and root contributions to MAOC. This hypothesis was verified in the 0- to 10-cm depth, where relative to systems that were tilled, no-till systems had higher SOC and MAOC at both locations and higher POC at Hoytville with clay soils. Also, at Wooster in the 0- to 10-cm depth,

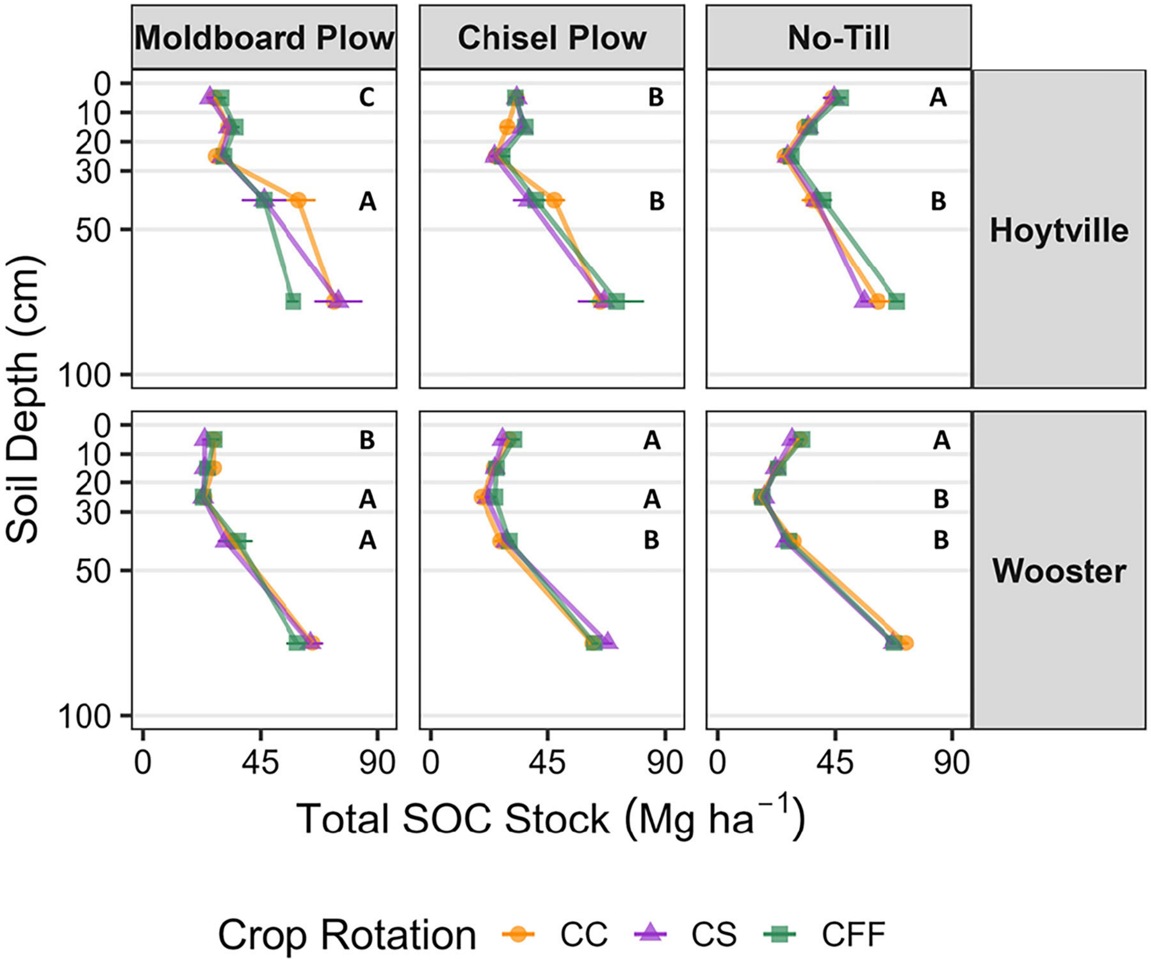

FIGURE 1 Total soil organic carbon stocks (Mg ha^{-1}) in different soil depths under different tillage intensities and crop rotations at Hoytville and Wooster. Points are colored and shaped by crop rotation, and faceted by tillage intensity and location. Points and error bars represent mean and standard error values among tillage and crop rotation systems in individual depths at each location ($n = 3$). Uppercase black letters indicate significant differences between tillage treatments averaged across crop rotations ($n = 9$). The absence of letters indicates nonsignificant differences. CC, continuous corn; CFF, corn-forage-forage; CS, corn-soybean; SOC, soil organic carbon.

soil aggregates were larger in no-till systems and under CFF rotations. Greater SOC and larger soil aggregates under conservation practices in surface soils indicates enhanced soil health (Bagnall et al., 2023; Liptzin et al., 2022; Natural Resource Conservation Service, 2025; Rieke et al., 2022). Reducing disturbance in agricultural systems, such as through reduced tillage intensity enhances SOC storage by lowering disruption of aggregates that physically protect and slow oxidation of plant-derived C, including structural and solubilized plant inputs that, respectively, contribute to POC and MAOC (Even & Cotrufo, 2024; Li et al., 2023; Luo et al., 2010; Prairie et al., 2023). While soil health benefits of reduced tillage intensity on SOC storage in the 0- to 10-cm depth did not extend into the 10- to 20-cm depth, differences in aggregation at Wooster extended into the 10- to 20-cm depth. The depth-specific management responses of soil health indices in the 0- to 10-cm and 10- to 20-cm depths highlight the interpretive value in segmenting surface soil samples into incremental depths over examining a combined 0- to 20-cm depth sample for soil health assessments.

The response of surface (0–10 cm) POC and MAOC storage to reduced tillage varied by location and tillage intensity; compared to moldboard, no-till increased MAOC at both locations and POC for clay soils of Hoytville, whereas CP increased MAOC for silt soils at Wooster only. Thus, compared to no-till, CP was an intermediate reduced tillage management strategy for increased SOC storage in corn production systems, especially as MAOC in silt loam soils, which aligned with previous studies (Blanco-Canqui et al., 2021; Gautam et al., 2025; Haddaway et al., 2017). Furthermore, surface SOC storage, especially as POC, was more sensitive to tillage at Hoytville than Wooster, which aligns with greater differences in surface (0–20 cm) SOC concentrations between tillage intensities at Hoytville (Gonzalez-Maldonado et al., 2025). Taken together, tillage mediated differences in surface SOC storage (0–10 cm) were primarily governed by

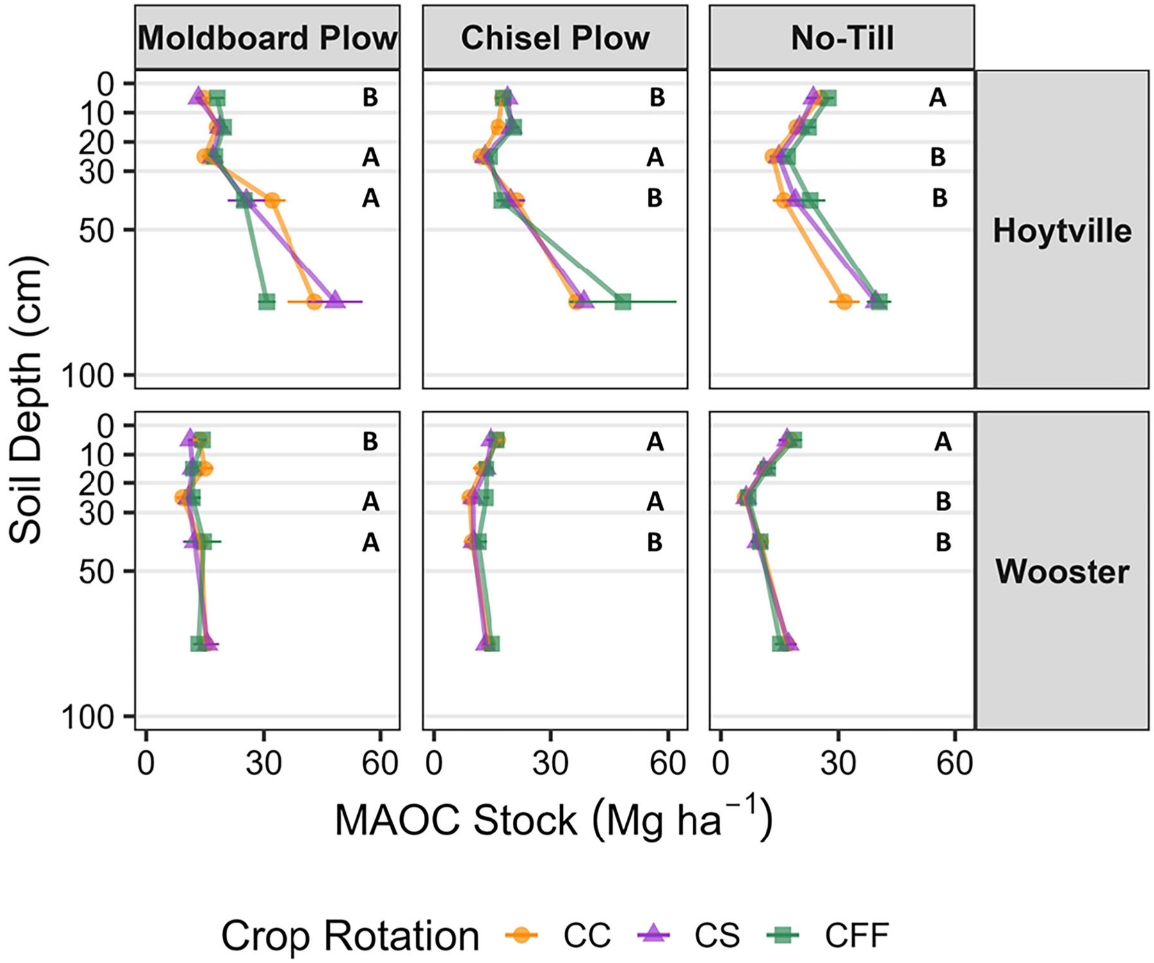

FIGURE 2 Mineral-associated organic carbon stocks (Mg ha^{-1}) in different soil depths under different tillage intensities and crop rotations at Hoytville and Wooster. Points are colored and shaped by crop rotation and faceted by tillage intensity and location. Points and error bars represent mean and standard error values among tillage and crop rotation systems in individual depths at each location ($n = 3$). Uppercase black letters indicate significant differences between tillage treatments averaged across crop rotations ($n = 9$). The absence of letters indicates nonsignificant differences. CC, continuous corn; CFF, corn-forage-forage; CS, corn-soybean; MAOC, mineral-associated organic carbon.

MAOC at Wooster and both POC and MAOC at Hoytville. The increased sensitivity of POC to tillage at Hoytville could reflect higher capacities of clay particles to contribute to aggregate formation and occlude plant-derived C compared to silty soils at Wooster (Six et al., 2002). Our findings highlight fraction-specific responses and suggest that both fast- and slow-cycling fraction contributions to SOM formation respond differently to management depending on soil inherent properties. While MAOC generally cycles slower than POC (Cotrufo & Lavallee, 2022), recent evidence shows that there are many different forms of MAOC, including more reactive forms that provide bioavailable N to microbes for transformation into plant-available N to support crop growth (Jilling et al., 2025; Leuthold et al., 2025). Thus, the differing sensitivities of MAOC and POC fractions in surface soils to reduced tillage intensity have implications for SOM dynamics and nutrient cycling in agroecosystems.

Increased crop rotational diversity had inconsistent effects on SOC storage and soil aggregation in surface soils. Diversifying corn production systems with perennial forage crops improved surface soil aggregation at Wooster. Surface soil aggregates were larger for CFF rotations than CC rotations, signaling likely benefits of above- and belowground plant C inputs sourced from perennial crops as they contribute to the formation of stable aggregates and enhancement of soil health (de Camargo Santos et al., 2025; Deiss et al., 2021; Gonzalez-Maldonado et al., 2025; Martin et al., 2022; K. Zhang et al., 2021). Despite crop rotation effects on soil aggregation at Wooster, SOC storage did not differ across rotations at either location, even after more than six decades of consistent management. Thus, there is considerable interpretive benefit in including additional indices, such as aggregate stability, when assessing soil health under conservation practices on a site-specific basis.

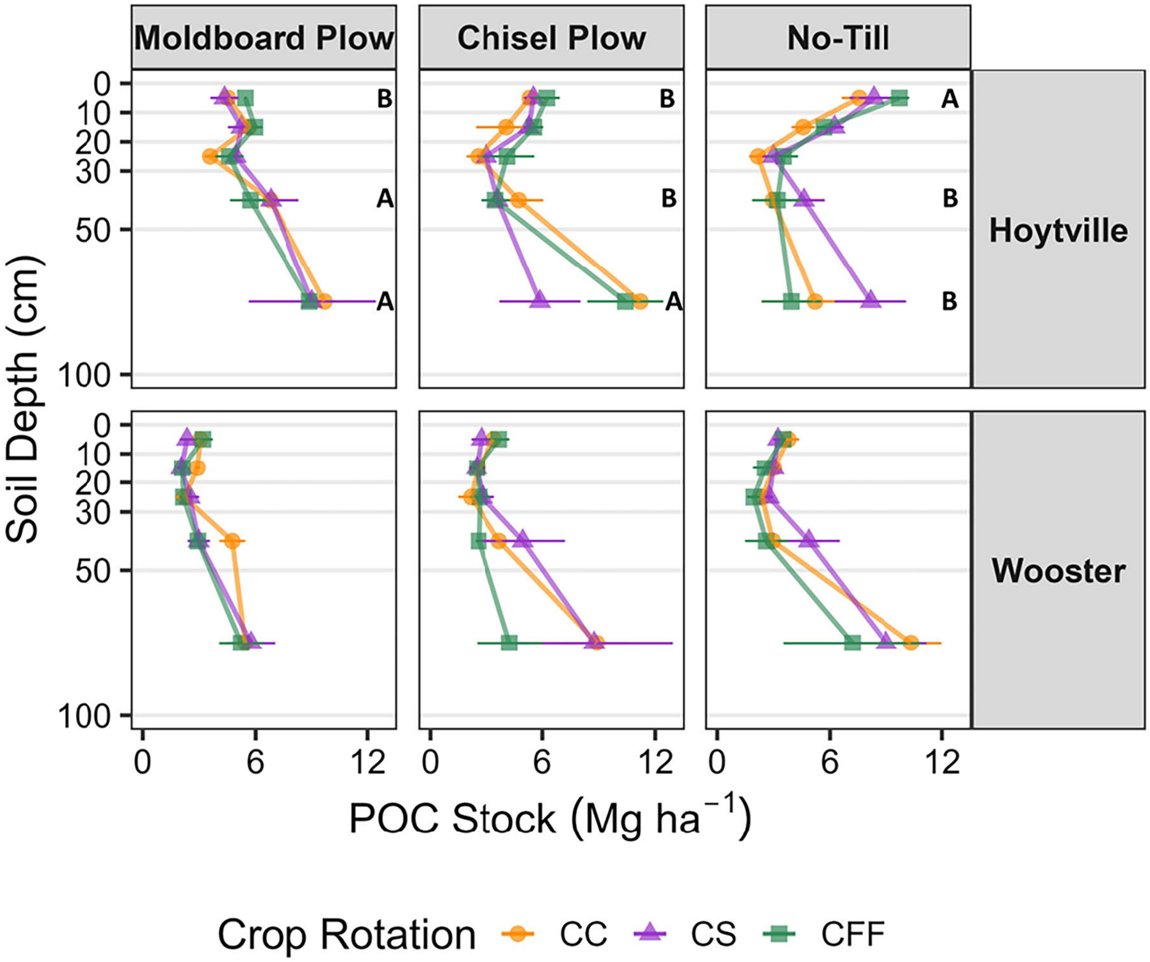

FIGURE 3 Particulate organic carbon stocks (Mg ha^{-1}) in different soil depths under different tillage intensities and crop rotations at Hoytville and Wooster. Points are colored and shaped by crop rotation, and faceted by tillage intensity and location. Points and error bars represent mean and standard error values among tillage and crop rotation systems in individual depths at each location ($n = 3$). Uppercase black letters indicate significant differences between tillage treatments averaged across crop rotations ($n = 9$). The absence of letters indicates nonsignificant differences. CC, continuous corn; CFF, corn-forage-forage; CS, corn-soybean; POC, particulate organic carbon.

4.2 | Tillage intensity impacted subsurface SOC storage more than crop rotation

Examining subsurface soil depths, our hypothesis that SOC storage would be higher in systems with more intensive tillage, by mixing of POC into depths with slower microbial processing and depolymerization, and increased availability of reactive mineral surface area for MAOC formation was supported. Relative to no-till, tillage with an MP increased subsurface SOC, especially as MAOC in the 20- to 30-cm and 30- to 50-cm depths at both locations, and as POC storage in the 50- to 100-cm depth at Hoytville with higher clay content. Intensive tillage practices like MP mechanically distribute SOC deeper into the soil profile, where it is generally processed more slowly due to reduced microbial activity and has increased propensity to form stable mineral associations, as we observed by measuring MAOC, due to greater surface area availability to bind with organic molecules, which often results in increased subsurface SOC storage relative to conservation tillage practices (Christopher et al., 2009; Gál et al., 2007; Haddaway et al., 2017; Hicks Pries et al., 2023; Luo et al., 2010; Ogle et al., 2019; Schrumpf et al., 2013). The greater potential for POC storage under moldboard and CP treatments in the 50- to 100-cm depth in the Hoytville clay loam over the Wooster silt loam could be attributed to locational differences in soil type and aggregation. The higher clay content at Hoytville in subsurface depths could have provided more reactive mineral surfaces to protect C-rich plant inputs (Kaiser & Guggenberger, 2008; Sposito, 2008), sourced from both the mixing of aboveground plant residue and surface SOM and plant-derived C from deep roots. In addition, subsurface occlusion of SOM in aggregates was more evident at Hoytville, which exhibited much greater aggregate stability and a consistent presence of aggregates larger than 2 mm. These aggregation patterns at Hoytville did not vary substantially with depth, in stark

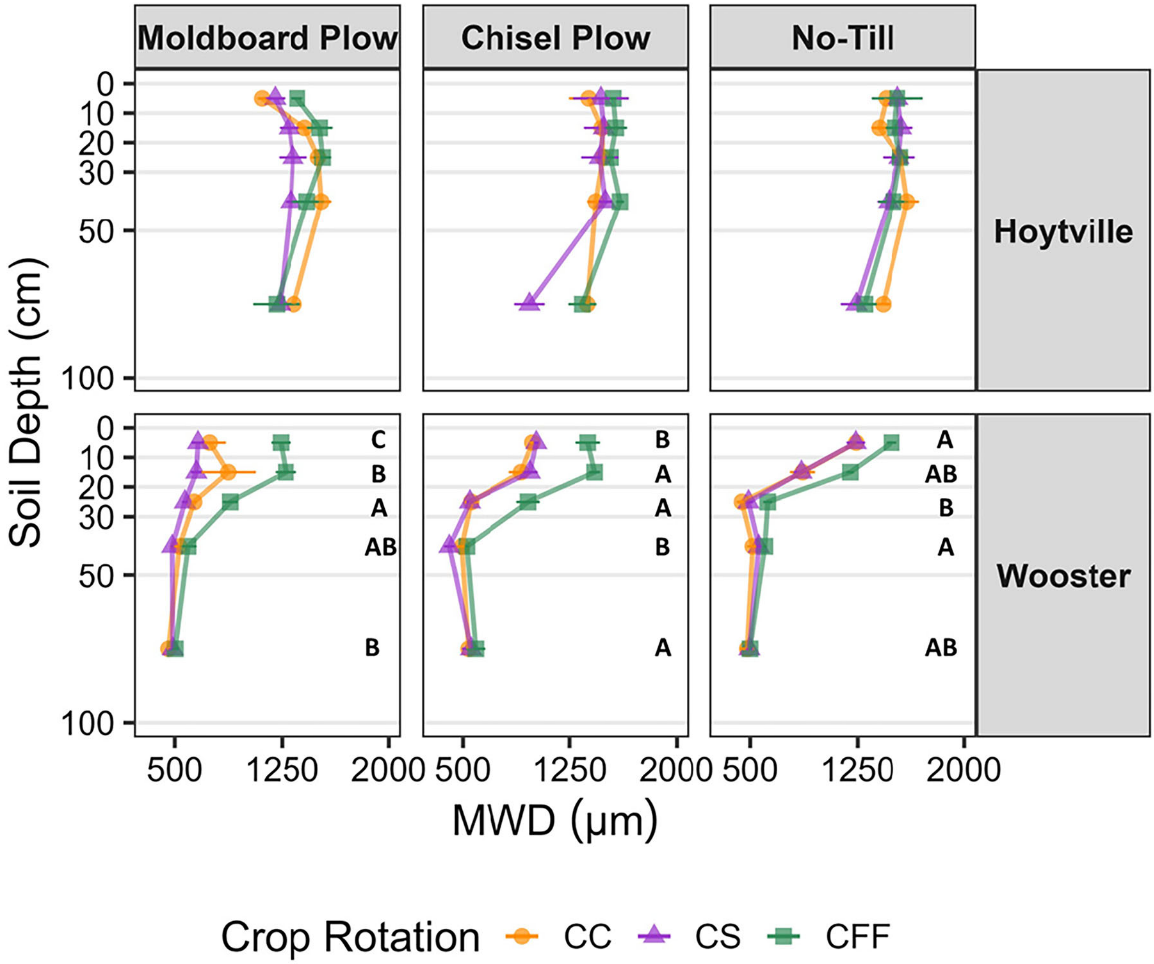

FIGURE 4 Soil aggregate mean weight diameter (MWD, μm) in different soil depths under different tillage intensities and crop rotations at Hoytville and Wooster. Points are colored and shaped by crop rotation, and faceted by tillage intensity and location. Points and error bars represent mean and standard error values among tillage and crop rotation systems in individual depths at each location ($n = 3$). Uppercase black letters indicate significant differences between tillage treatments averaged across crop rotations ($n = 9$). The absence of letters indicates nonsignificant differences. CC, continuous corn; CFF, corn-forage-forage; CS, corn-soybean; MWD, mean weight diameter.

contrast to the decline in MWD at Wooster with increasing depth.

Our study is novel in demonstrating that relative increases in subsurface SOC storage in tilled annual cropping systems can extend well below the plow layer, and persist at depths two to four times deeper after more than six decades of contrasting tillage management. We illustrate management effects on subsurface depth (20–50 cm) SOC being mirrored in MAOC, as the dominant form of SOC with increasing abundance at depth (Fulton-Smith et al., 2024; Zhou et al., 2024). We also provide evidence of subsurface depth (50–100 cm) POC increases in tilled corn production systems in clay loam soils (Hoytville), supplementing other studies that have reported deep POC accumulation under perennial systems that are not tilled (Peixoto et al., 2022; van der Pol et al., 2022). Furthermore, the experimental duration of 60+ years of this study in locations characterized by different soil types demonstrates the influence of site-specific inherent factors on SOC dynamics and supplements the limited literature of POC-MAOC relationships and aggregation in surface and subsurface soils in multi-decade field experiments (Blanco-Canqui et al., 2021; Even & Francesca Cotrufo, 2024; Gál et al., 2007; Gautam et al., 2025; Weidhuner et al., 2021).

4.3 | Soil carbon storage stratification balanced out in soil profiles

Whole-profile total SOC, MAOC, and POC stocks (0–100 cm) did not differ significantly among tillage, rotation, or their interactions. This finding supports the hypothesis that stratified SOC distributions, with respect to surface SOC enrichment under regenerative practices and greater subsurface SOC accumulation under tillage, can offset one another, resulting in comparable SOC stocks when integrated across the entire soil profile. The contrasting effects of reduced

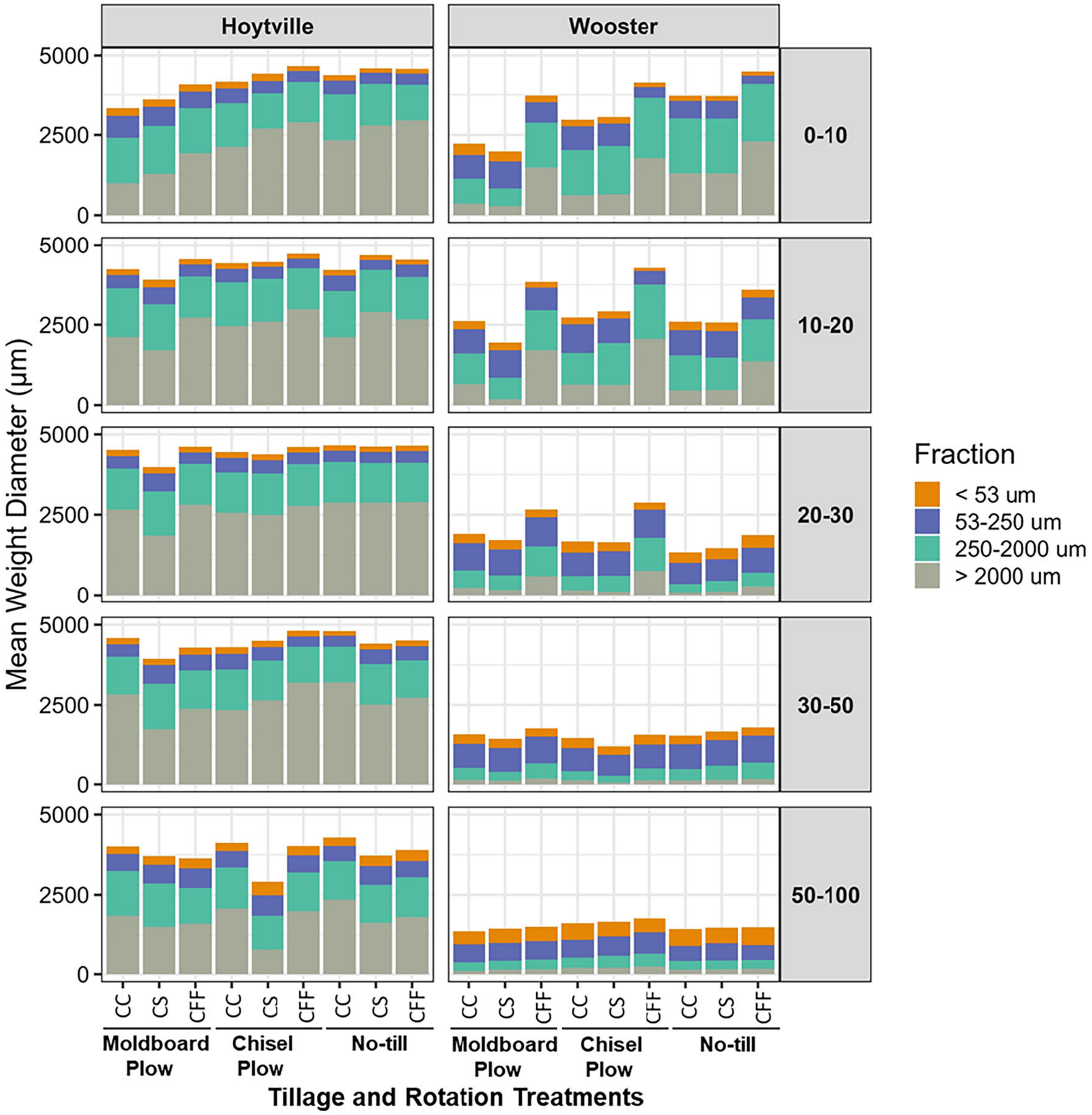

FIGURE 5 Mean weight diameter (MWD) and soil aggregate fractions (relative proportion as the height of each inside bar indicating the proportion of aggregates in each class) in different soil depths (cm) under different tillage intensities and crop rotations at Hoytville and Wooster. Bars are stacked by each tillage intensity and crop rotation combination, colored by relative proportion in each aggregate fraction, and faceted by location and depth. CC, continuous corn; CFF, corn-forage-forage; CS, corn-soybean; MWD, mean-weight diameter.

tillage intensity on surface and subsurface SOC storage are not novel, with many studies reporting similar relationships with higher stores under reduced tillage in surface soil depths and lower or comparable levels in subsurface soil depths (Christopher et al., 2009; Gál et al., 2007; Haddaway et al., 2017; Luo et al., 2010; Ogle et al., 2019). Most of these studies report no management differences in whole-profile C stocks from the dilution of stratifications. More recently. Blanco-Canqui et al. (2021) challenged these other studies and reported that SOC storage was higher under NT practices in soil profiles (0–100 cm), but not surface soils. As 70% of global SOC is stored in depths below 20 cm, where SOC generally cycles more slowly than in surface depths, subsurface SOC stores are essential in maintaining agroecosystem productivity, improving water and nutrient supply, and bolstering resilience to environmental disturbances and climate change, signaling the need for quantification of subsurface SOC storage (Lal, 2020; Sierra et al., 2024).

Based on the distinct surface and subsurface soil depth patterns in SOC storage, we recommend future soil sample collections for SOC stock surveys extend up to 1 m into the soil profile, with clear delineation of multiple surface depth increments and segmenting subsurface depths into multiple increments (i.e., 0–10–20–30–50–100 cm or 0–10–25–50–100 cm). This recommendation aligns with other studies that emphasize the importance of whole-profile soil sampling to accurately inform carbon crediting markets and climate models (Blanco-Canqui et al., 2021; Hicks Pries et al., 2023; Sierra et al., 2024). We further acknowledge that it is impractical for producers to sample the whole soil profile when assessing soil health via SOC measurements. However, if a producer is part of a C market program, we strongly suggest (1) sampling to at least 50 cm when implementing different tillage practices in order to more accurately quantify SOC stocks, and (2) segmenting samples into depths that best capture surface (0–10 cm) and subsurface (30–50 cm) effects to best capture trade-offs in SOC storage, as whole-profile stocks did not differ by treatment. The most appropriate depth segmentation and even deeper sampling requirements can be determined by the depth of a disturbance, such as tillage, management practice, such as irrigation or drainage, and other factors that may be important on a site-specific basis, such as crop rooting depth. We further recommend the use of soil health indicators, such aggregate stability, and measurement of different SOC fractions, such as POC and MAOC, to inform management decisions (Cotrufo et al., 2019). In other words, SOC storage should not be the sole indicator of soil health status and increased SOC should not be the sole goal of conservation agriculture (Rakkar et al., 2025). Few studies have examined how subsurface SOC storage relates to aggregation using soils collected from long-term field experiments (Gál et al., 2007; Gautam et al., 2025; Weidhuner et al., 2021). In our study, evaluating soil aggregation and POC-MAOC dynamics in subsurface soil depths provided additional insight into how site-specific inherent factors influence SOC stabilization, beyond what could be inferred from surface SOC alone. These findings reinforce the importance of including deeper soil layers in SOC stock assessments and of measuring multiple SOC fractions that differ in turnover and function when evaluating management effects on SOC dynamics, soil health, and agroecosystem function (Angst et al., 2023; Olson & Al-Kaisi, 2015; Raffeld et al., 2024; Sierra et al., 2024).

4.4 | Managing agroecosystem trade-offs: Implications for soil carbon dynamics

Differences in surface SOC storage and aggregation hold implications for agroecosystem function. Greater SOC and larger soil aggregates under conservation practices in surface soils indicates enhanced soil health (Bagnall et al., 2023; Liptzin et al., 2022; Rieke et al., 2022), resulting from reduced tillage and greater crop diversity (Gonzalez-Maldonado et al., 2025; Jin et al., 2021; Martin et al., 2022; Zuber et al., 2015). Gonzalez-Maldonado et al. (2025) found that most diverse rotation (CFF) at the Triplett–Van Doren Experiment increased topsoil (0–20 cm) concentrations of SOC and soil health indices of permanganate oxidizable C, mineralizable C, and autoclaved-citrate extractable protein at both locations. No-till increased these SOC and nitrogen-related soil health indicators in the Hoytville clay loam, but not in the Wooster silt loam (Gonzalez-Maldonado et al., 2025). Altogether, these findings indicate that improvements in soil health are closely associated with greater SOC storage under reduced tillage and diversified cropping systems, especially in surface soils (0–20 cm), which are most important from an agronomic perspective. Beyond soil health benefits, increased SOC levels in more diverse no-till systems also can translate into improved agroecosystem performance, such as greater grain yield and resiliency to weather extremes (de Camargo Santos et al., 2025). Thus, implementing regenerative practices that target both tillage and crop management practices provides a practical pathway to sustain productivity while enhancing agroecosystem resilience.

Our study adds to the aforementioned literature on agroecosystem productivity and surface soil health at the Triplett–Van Doren Experiment by revealing that reducing tillage intensity has trade-offs on surface and subsurface SOC storage. Our study is novel in demonstrating that, compared with no-till, moldboard plowing results in relative increases in subsurface SOC and MAOC storage extending to twice the depth of the plow layer (20–50 cm vs. 0–20 cm), and in subsurface POC storage extending up to five times the depth (30–100 cm). While very few studies have documented such differences, it is critical to acknowledge that the increased SOC storage under intensive tillage practices is not necessarily a benefit. The redistribution of SOC to subsurface depths at the expense of surface SOC disrupts the natural stratification pattern of SOC in soil profiles. This has consequences for ecosystem service provision that most depend on surface soils, such as the supply of nutrients, water, and oxygen to crops that support agroecosystem productivity. The natural abundance of SOC in surface soils also regulates ecosystem responses to global change, land use, and short- and long-term disturbance (Hicks Pries et al., 2023).

4.5 | Limitations

A limitation of this study is that we do not consider changes in SOC across time, but rather we examine SOC at a single timepoint post-establishment. Higher relative levels of SOC under a given management practice do not necessarily reflect SOC gains, but may instead indicate slower rates of SOC loss, as demonstrated by Sanford et al. (2012). However, even without baseline SOC or bulk density data in our study, SOC stock comparisons after 60 years of consistent management are valuable for informing relative treatment differences on a site-specific basis. Previous studies at the Triplett–Van Doren Experiment have reported historical bulk density and SOC concentrations across the soil profile (Dick et al., 1986) and surface (0–30 cm) SOC changes comparing tillage intensities and crop rotations (Dick, 1983; Rakkar et al., 2025) and in the top 0–50 cm of the soil profile for select systems (Mestelan et al., 2021). Our study provides additional insight into SOC dynamics by extending soil sampling to 100 cm and including measurements of SOC fractions that differ in turnover and function after more than six decades of contrasting tillage and rotation management.

5 | CONCLUSIONS

The effects of regenerative tillage and crop management strategies on SOC storage and aggregation were not consistent across surface and subsurface soil depths. While reduced tillage increased surface layer SOC storage, particularly within the MAOC pool, these relative gains were offset by decreased SOC storage in subsurface layers, notably between 30–50 cm. Differences in total SOC and MAOC were confined to the upper 0–50 cm of the soil profile, with no detectable variation at 50–100 cm. In contrast, POC stocks exhibited greater variability in the 30- to 50-cm and 50- to 100-cm depths than in the upper 0–30 cm. These contrasting depth responses resulted in an overall balance of SOC storage across the 0–100 cm profile. Even though whole-profile (0–100 cm) SOC stocks were similar among management systems, other critical dimensions of agroecosystem performance, such as crop productivity and resilience to adverse weather extremes (de Camargo Santos et al., 2025) and soil aggregate stability, were more pronounced in systems with reduced tillage, and greater crop diversity with perennial components. These improvements, which were not evident under more conventional management, underscore the need to consider a broader suite of soil and ecosystem functions when making management decisions to increase SOC storage (Anand et al., 2025; Ogle et al., 2019). Such attributes are fundamental for conserving soil and sustaining key ecosystem services, including crop yield, soil health, and water quality. Ultimately, our results show the importance and value of quantifying whole profile C stocks, at select depth intervals of interest that are consistent across time and space, and including multiple SOC sources that range in stability and function to understand the impact of tillage and rotation management on SOC storage capacities in agroecosystems.

AUTHOR CONTRIBUTIONS

Katherine Naasko: Formal analysis; investigation; methodology; visualization; writing—original draft; writing—review and editing. **Aline de Camargo Santos**: Investigation; writing—review and editing. **Christine D. Sprunger**: Resources; supervision; writing—review and editing. **Leonardo Deiss**: Conceptualization; funding acquisition; investigation; project administration; resources; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

Anand, S. K., Singh, R., Mohanty, B., Rajan, N., & Calabrese, S. (2025). Classifying the potential for soil organic carbon gain under regenerative agriculture. *Environmental Research Letters*, *20*(4), 044045. https://doi.org/10.1088/1748-9326/adc292

Angers, D. A., & Eriksen-Hamel, N. S. (2008). Full-inversion tillage and organic carbon distribution in soil profiles: A meta-analysis. *Soil Science Society of America Journal*, *72*(5), 1370–1374. https://doi.org/10.2136/sssaj2007.0342

Angst, G., Mueller, K. E., Castellano, M. J., Vogel, C., Wiesmeier, M., & Mueller, C. W. (2023). Unlocking complex soil systems as carbon sinks: Multi-pool management as the key. *Nature Communications*, *14*(1), 2967. https://doi.org/10.1038/s41467-023-38700-5

Angst, G., Mueller, K. E., Nierop, K. G., & Simpson, M. J. (2021). Plant-or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology and Biochemistry*, *156*, 108189. https://doi.org/10.1016/j.soilbio.2021.108189

Aziz, I., Mahmood, T., & Islam, K. (2014). Impact of long-term tillage and crop rotation on concentration of soil particulate organic matter associated carbon and nitrogen. *Pakistan Journal of Agricultural Research*, *51*(4), 827–834.

Bagnall, D. K., Rieke, E. L., Morgan, C. L., Liptzin, D. L., Cappellazzi, S. B., & Honeycutt, C. W. (2023). A minimum suite of soil health indicators for North American agriculture. *Soil Security*, *10*, 100084. https://doi.org/10.1016/j.soisec.2023.100084

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Blanco-Canqui, H., & Lal, R. (2008). No-tillage and soil-profile carbon sequestration: An on-farm assessment. *Soil Science Society of America Journal*, *72*(3), 693–701. https://doi.org/10.2136/sssaj2007.0233

Blanco-Canqui, H., & Ruis, S. J. (2018). No-tillage and soil physical environment. *Geoderma*, *326*, 164–200. https://doi.org/10.1016/j.geoderma.2018.03.011

Blanco-Canqui, H., Schlegel, A. J., & Heer, W. F. (2011). Soil-profile distribution of carbon and associated properties in no-till along a precipitation gradient in the central Great Plains. *Agriculture, Ecosystems & Environment*, *144*(1), 107–116. https://doi.org/10.1016/j.agee.2011.07.004

Blanco-Canqui, H., Shapiro, C., Jasa, P., & Iqbal, J. (2021). No-till and carbon stocks: Is deep soil sampling necessary? Insights from long-term experiments. *Soil and Tillage Research*, *206*, 104840. https://doi.org/10.1016/j.still.2020.104840

Chahal, I., Hooker, D. C., Deen, B., Janovicek, K., & Van Eerd, L. L. (2021). Long-term effects of crop rotation, tillage, and fertilizer nitrogen on soil health indicators and crop productivity in a temperate climate. *Soil and Tillage Research*, *213*, 105121. https://doi.org/10.1016/j.still.2021.105121

Christopher, S. F., Lal, R., & Mishra, U. (2009). Regional study of no-till effects on carbon sequestration in the Midwestern United States. *Soil Science Society of America Journal*, *73*(1), 207–216. https://doi.org/10.2136/sssaj2007.0336

Córdova, S. C., Kravchenko, A. N., Miesel, J. R., & Robertson, G. P. (2025). Soil carbon change in intensive agriculture after 25 years of conservation management. *Geoderma*, *453*, 117133. https://doi.org/10.1016/j.geoderma.2024.117133

Cotrufo, M. F., & Lavallee, J. M. (2022). Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration. *Advances in Agronomy*, *172*, 1–66. https://doi.org/10.1016/bs.agron.2021.11.002

Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience*, *12*(12), 989–994. https://doi.org/10.1038/s41561-019-0484-6

Crow, S. E., Swanston, C. W., Lajtha, K., Brooks, R., & Keirstead, H. (2007). Density fractionation of forest soils: Methodological questions and interpretation of incubation results and turnover time in an ecosystem context. *Biogeochemistry*, *85*, 69–90. https://doi.org/10.1007/s10533-007-9100-8

de Camargo Santos, A., Culman, S. W., & Deiss, L. (2025). Sixty years of crop diversification with perennials improves yields more than no-tillage in Ohio grain cropping systems. *Field Crops Research*, *331*, 109993. https://doi.org/10.1016/j.fcr.2025.109993

Deiss, L., Margenot, A. J., Culman, S. W., & Demyan, M. S. (2020). Optimizing acquisition parameters in diffuse reflectance infrared Fourier transform spectroscopy of soils. *Soil Science Society of America Journal*, *84*, 930–948. https://doi.org/10.1002/saj2.20028

Deiss, L., Sall, A., Demyan, M. S., & Culman, S. W. (2021). Does crop rotation affect soil organic matter stratification in tillage systems?. *Soil and Tillage Research*, *209*, 104932. https://doi.org/10.1016/j.still.2021.104932

Dick, W. A. (1983). Organic carbon, nitrogen, and phosphorus concentrations and pH in soil profiles as affected by tillage intensity. *Soil Science Society of America Journal*, *47*, 102–107. https://doi.org/10.2136/sssaj1983.03615995004700010021x

Dick, W. A., Triplett, G. B., & Van Doren, D. M. (2013). *Triplett-Van Doren long-term tillage and crop rotation data*. Ohio Agricultural Research and Development Center. http://hdl.handle.net/1811/55716

Dick, W. A., Van Doren D. M., Jr., Triplett, G. B., & Henry, J. E. (1986). *Influence of long-term tillage and rotation combinations on crop yields and selected soil parameters. Results obtained for a Mollic Ochraqualf soil* (Research Bulletin 1180). The Ohio State University Ohio Agricultural Research and Development Center.

Dreimanis, A. (1962). Quantitative gasometric determination of calcite and dolomite by using Chittick apparatus. *Journal of Sedimentary Research*, *32*, 520–529. https://doi.org/10.1306/74D70D08-2B21-11D7-8648000102C1865D

Even, R. J., & Francesca Cotrufo, M. (2024). The ability of soils to aggregate, more than the state of aggregation, promotes protected soil organic matter formation. *Geoderma*, *442*, 116760. https://doi.org/10.1016/j.geoderma.2023.116760

Even, R. J., Machmuller, M. B., Lavallee, J. M., Zelikova, T. J., & Cotrufo, M. F. (2025). Large errors in common soil carbon measurements due to sample processing. *Soilless*, *11*, 17–34. https://doi.org/10.5194/soil-11-17-2025

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage.

Franzluebbers, A. J. (2024). Soil depth distribution of particulate organic carbon and nitrogen in the southeastern United States. *Agricultural & Environmental Letters*, *9*(2), e70005. https://doi.org/10.1002/ael2.70005

Fulton-Smith, S., Even, R., & Cotrufo, M. F. (2024). Depth impacts on the aggregate-mediated mechanisms of root carbon stabilization in soil: Trade-off between MAOM and POM pathways. *Geoderma*, *452*, 117078. https://doi.org/10.1016/j.geoderma.2024.117078

Gál, A., Vyn, T. J., Michéli, E., Kladivko, E. J., & Mcfee, W. W. (2007). Soil carbon and nitrogen accumulation with long-term no-till versus moldboard plowing over-estimated with tilled-zone sampling depths. *Soil and Tillage Research*, *96*, 42–51. https://doi.org/10.1016/j.still.2007.02.007

Gautam, A., Anuo, C. O., Kladivko, E., Vyn, T. J., & Armstrong, S. (2025). Does 46 years of conservation tillage and crop rotations change soil carbon and nitrogen distribution and storage?. *Soil and Tillage Research*, *254*, 106702. https://doi.org/10.1016/j.still.2025.106702

Golchin, A., Oades, J., Skjemstad, J., & Clarke, P. (1994). Study of free and occluded particulate organic matter in soils by solid state 13C Cp/MAS NMR spectroscopy and scanning electron microscopy. *Australian Journal of Soil Research*, *32*, 285–309. https://doi.org/10.1071/SR9940285

Gonzalez-Maldonado, N., Deiss, L., Ali, F., & Culman, S. W. (2025). In-season temporal variability of soil carbon and nitrogen pools after half a century of a tillage and crop rotation gradient. *Soil and Tillage Research*, *252*, 106566. https://doi.org/10.1016/j.still.2025.106566

Grace, P., & Robertson, G. P. (2021). Soil carbon sequestration potential and the identification of hotspots in the eastern Corn Belt of the United States. *Soil Science Society of America Journal*, *85*(5), 1410–1424. https://doi.org/10.1002/saj2.20273

Haddaway, N. R., Hedlund, K., Jackson, L. E., Kätterer, T., Lugato, E., Thomsen, I. K., Jørgensen, H. B., & Isberg, P.-E. (2017). How does tillage intensity affect soil organic carbon? A systematic review. *Environmental Evidence*, *6*, 30. https://doi.org/10.1186/s13750-017-0108-9

Helbling, E., Pierson, D., & Lajtha, K. (2021). Sources of soil carbon loss during soil density fractionation: Laboratory loss or seasonally variable soluble pools?. *Geoderma*, *382*, 114776. https://doi.org/10.1016/j.geoderma.2020.114776

Hicks Pries, C. E., Ryals, R., Zhu, B., Min, K., Cooper, A., Goldsmith, S., Pett-Ridge, J., Torn, M., & Berhe, A. A. (2023). The deep soil organic carbon response to global change. *Annual Review of Ecology, Evolution, and Systematics*, *54*(1), 375–401. https://doi.org/10.1146/annurev-ecolsys-102320-085332

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Jilling, A., Grandy, A. S., Daly, A. B., Hestrin, R., Possinger, A., Abramoff, R., Annis, M., Cates, A., Dynarski, K., Georgiou, K., Heckman, K., Keiluweit, M., Lang, A., Phillips, R., Rocci, K., Shabtai, I., Sokol, N., & Whalen, E. D. (2025). Evidence for the existence and ecological relevance of fast-cycling mineral-associated organic matter. *Communications Earth & Environment*, *6*, 690. https://doi.org/10.1038/s43247-025-02681-8

Jilling, A., Keiluweit, M., Gutknecht, J. L., & Grandy, A. S. (2021). Priming mechanisms providing plants and microbes access to mineral-associated organic matter. *Soil Biology and Biochemistry*, *158*, 108265. https://doi.org/10.1016/j.soilbio.2021.108265

Jin, V. L., Wienhold, B. J., Mikha, M. M., & Schmer, M. R. (2021). Cropping system partially offsets tillage-related degradation of soil organic carbon and aggregate properties in a 30-yr rainfed agroecosystem. *Soil and Tillage Research*, *209*, 104968. https://doi.org/10.1016/j.still.2021.104968

Kaiser, K., & Guggenberger, G. (2008). Mineral surfaces and soil organic matter. *European Journal of Soil Science*, *54*(2), 219–236. https://doi.org/10.1046/j.1365-2389.2003.00544.x

King, A. E., Amsili, J. P., Córdova, S. C., Culman, S., Fonte, S. J., Kotcon, J., Masters, M. D., McVay, K., Olk, D. C., Prairie, A. M., Schipanski, M., Schneider, S. K., Stewart, C. E., & Cotrufo, M. F. (2024). Constraints on mineral-associated and particulate organic carbon response to regenerative management: Carbon inputs and saturation deficit. *Soil and Tillage Research*, *238*, 106008. https://doi.org/10.1016/j.still.2024.106008

Lal, R. (2020). Managing soils for negative feedback to climate change and positive impact on food and nutritional security. *Soil Science and Plant Nutrition*, *66*, 1–9. https://doi.org/10.1080/00380768.2020.1718548

Lavallee, J. M., Soong, J. L., & Cotrufo, M. F. (2020). Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology*, *26*(1), 261–273. https://doi.org/10.1111/gcb.14859

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., & Rillig, M. (2020). The concept and future prospects of soil health. *Nature Reviews Earth & Environment*, *1*, 544–553. https://doi.org/10.1038/s43017-020-0080-8

Lenth, R. (2023). *emmeans: Estimated marginal means, aka least-squares means*. https://doi.org/10.32614/CRAN.package.emmeans

Leuthold, S. J., Haddix, M. L., Lavallee, J., & Cotrufo, M. F. (2023). Physical fractionation techniques. In M. Goss & M. Oliver (Eds.), *Encyclopedia of soils in the environment* (2nd ed., pp. 68–80). Elsevier. https://doi.org/10.1016/B978-0-12-822974-3.00067-7

Leuthold, S. J., Lavallee, J. M., Haddix, M. L., & Cotrufo, M. F. (2024). Contrasting properties of soil organic matter fractions isolated by different physical separation methodologies. *Geoderma*, *445*, 116870. https://doi.org/10.1016/j.geoderma.2024.116870

Leuthold, S. J., Lavallee, J. M., Haddix, M. L., Schipanski, M., Poffenbarger, H. J., Castellano, M. J., & Cotrufo, M. F. (2025). Quantifying the contribution of MAOM to mineral nitrogen pools under various soil organic matter conditions. *Biology and Fertility of Soils*, *61*(8), 1391–1404. https://doi.org/10.1007/s00374-025-01945-x

Li, P., Ying, D., Li, J., Deng, J., Li, C., Tian, S., Zhao, G., Wu, C., Jiao, J., Jiang, M., & Hu, F. (2023). Global-scale no-tillage impacts on soil aggregates and associated carbon and nitrogen concentrations in croplands: A meta-analysis. *Science of the Total Environment*, *881*, 163570. https://doi.org/10.1016/j.scitotenv.2023.163570

Liptzin, D., Norris, C. E., Cappellazzi, S. B., Bean, G. M., Cope, M., Greub, K. L. H., Rieke, E. L., Tracy, P. W., Aberle, E., Ashworth, A., Bañuelos Tavarez, O., Bary, A. I., Baumhardt, R. L., Borbón Gracia, A., Brainard, D. C., Brennan, J. R., Briones Reyes, D., Bruhjell, D., Carlyle, C. N., … Honeycutt, C. W. (2022). An evaluation of carbon indicators of soil health in long-term agricultural experiments. *Soil Biology and Biochemistry*, *172*, 108708. https://doi.org/10.1016/j.soilbio.2022.108708

Luo, Z., Wang, E., & Sun, O. J. (2010). Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. *Agriculture, Ecosystems & Environment*, *139*(1–2), 224–231. https://doi.org/10.1016/j.agee.2010.08.006

Martin, T., Culman, S., & Sprunger, C. D. (2022). Quality or quantity? Determining the impact of fine root traits on soil health in row crop agriculture. *Journal of Soil Science and Plant Nutrition*, *22*(2), 2322–2333. https://doi.org/10.1007/s42729-022-00811-1

Martin, T., & Sprunger, C. D. (2022). Sensitive measures of soil health reveal carbon stability across a management intensity and plant biodiversity gradient. *Frontiers in Soil Science*, *2*, 917885. https://doi.org/10.3389/fsoil.2022.917885

Mestelan, S., Smeck, N., Sprunger, C., Dyck, A., & Dick, W. (2021). Four decades of continuously applied tillage or no-tillage on soil properties and soil morphology. *Agrosystems, Geosciences & Environment*, *4*, e20195. https://doi.org/10.1002/agg2.20195

Natural Resource Conservation Service. (2025). *Technical note no. 470-16*. USDA NRCS Soil Health Division. https://directives.nrcs.usda.gov/sites/default/files2/1748890495/TN%20470-16%20Soil%20Health%20Testing%20to%20Support%20Conservation%20Planning.pdf

Nicoloso, R. S., & Rice, C. W. (2021). Intensification of no-till agricultural systems: An opportunity for carbon sequestration. *Soil Science Society of America Journal*, *85*(5), 1395–1409. https://doi.org/10.1002/saj2.20260

Nunes, M., Karlen, D. L., Veum, K. S., Moorman, T. B., & Cambardella, C. A. (2020). Biological soil health indicators respond to tillage intensity: A US meta-analysis. *Geoderma*, *369*, 114335. https://doi.org/10.1016/j.geoderma.2020.114335

Ogle, S. M., Alsaker, C., Baldock, J., Bernoux, M., Breidt, F. J., McConkey, B., Regina, K., & Vazquez-Amabile, G. G. (2019). Climate and soil characteristics determine where no-till management can store carbon in soils and mitigate greenhouse gas emissions. *Scientific Reports*, *9*(1), 11665. https://doi.org/10.1038/s41598-019-47861-7

Olson, K. R., & Al-Kaisi, M. M. (2015). The importance of soil sampling depth for accurate account of soil organic carbon sequestration, storage, retention and loss. *Catena*, *125*, 33–37. https://doi.org/10.1016/j.catena.2014.10.004

Olson, K. R., Lang, J. M., & Ebelhar, S. A. (2005). Soil organic carbon changes after 12 years of no-tillage and tillage of Grantsburg soils in southern Illinois. *Soil and Tillage Research*, *81*(2), 217–225. https://doi.org/10.1016/j.still.2004.09.009

Peixoto, L., Olesen, J. E., Elsgaard, L., Enggrob, K. L., Banfield, C. C., Dippold, M. A., Nicolaisen, M. H., Bak, F., Zang, H., Dresbøll, D. B., Thorup-Kristensen, K., & Rasmussen, J. (2022). Deep-rooted perennial crops differ in capacity to stabilize C inputs in deep soil layers.

Scientific Reports, *12*(1), 5952. https://doi.org/10.1038/s41598-022-09737-1

Plaza, C., Giannetta, B., Benavente, I., Vischetti, C., & Zaccone, C. (2019). Density-based fractionation of soil organic matter: Effects of heavy liquid and heavy fraction washing. *Scientific Reports*, *9*(1), 10146. https://doi.org/10.1038/s41598-019-46577-y

Prairie, A. M., King, A. E., & Cotrufo, M. F. (2023). Restoring particulate and mineral-associated organic carbon through regenerative agriculture. *Proceedings of the National Academy of Sciences*, *120*(21), e2217481120. https://doi.org/10.1073/pnas.2217481120

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Raffeld, A. M., Bradford, M. A., Jackson, R. D., Rath, D., Sanford, G. R., Tautges, N., & Oldfield, E. E. (2024). The importance of accounting method and sampling depth to estimate changes in soil carbon stocks. *Carbon Balance and Management*, *19*(1), 2. https://doi.org/10.1186/s13021-024-00249-1

Rakkar, M., Deiss, L., & Dick, W. A. (2025). Soil carbon storage or sustainable conservation agriculture practices—Which should be our goal?. *Journal of Environmental Quality*, *54*, 1586–1597. https://doi.org/10.1002/jeq2.70073

Rieke, E. L., Bagnall, D. K., Morgan, C. L. S., Flynn, K. D., Howe, J. A., Greub, K. L. H., Mac Bean, G., Cappellazzi, S. B., Cope, M., Liptzin, D., Norris, C. E., Tracy, P. W., Aberle, E., Ashworth, A., Bañuelos Tavarez, O., Bary, A. I., Baumhardt, R. L., Borbón Gracia, A., Brainard, D. C., … Honeycutt, C. W. (2022). Evaluation of aggregate stability methods for soil health. *Geoderma*, *428*, 116156. https://doi.org/10.1016/j.geoderma.2022.116156

Sanderman, J., Hengl, T., & Fiske, G. J. (2017). Soil carbon debt of 12,000 years of human land use. *Proceedings of the National Academy of Sciences*, *114*(36), 9575–9580. https://doi.org/10.1073/pnas.1706103114

Sanford, G. R., Posner, J. L., Jackson, R. D., Kucharik, C. J., Hedtcke, J. L., & Lin, T. L. (2012). Soil carbon lost from Mollisols of the North Central USA with 20 years of agricultural best management practices. *Agriculture, Ecosystems & Environment*, *162*, 68–76. https://doi.org/10.1016/j.agee.2012.08.011

Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kögel-Knabner, I., & Schulze, E. D. (2013). Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. *Biogeosciences*, *10*(3), 1675–1691. https://doi.org/10.5194/bg-10-1675-2013

Sharma, S., Wilson, T., Ochsner, T., & Warren, J. G. (2020). Sampling probes affect bulk density and soil organic carbon measurements. *Agricultural & Environmental Letters*, *5*(1), e20005. https://doi.org/10.1002/ael2.20005

Si, Q., Chen, K., Wei, B., Zhang, Y., Sun, X., & Liang, J. (2024). Dissolved carbon flow to particulate organic carbon enhances soil carbon sequestration. *Soilless*, *10*(2), 441–450. https://doi.org/10.5194/soil-10-441-2024

Sierra, C. A., Ahrens, B., Bolinder, M. A., Braakhekke, M. C., von Fromm, S., Kätterer, T., Luo, Z., Parvin, N., & Wang, G. (2024). Carbon sequestration in the subsoil and the time required to stabilize carbon for climate change mitigation. *Global Change Biology*, *30*, e17153. https://doi.org/10.1111/gcb.17153

Six, J., Conant, R. T., Paul, E. A., & Paustian, K. (2002). Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil*, *241*, 155–176. https://doi.org/10.1023/A:1016125726789

Six, J., Paustian, K., Elliott, E. T., & Combrink, C. (2000). Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Science Society of America Journal*, *64*(2), 681–689. https://doi.org/10.2136/sssaj2000.642681x

Soil Survey Staff. (2010). *Keys to soil taxonomy* (11th ed.). USDA-NRCS.

Sokol, N. W., & Bradford, M. A. (2019). Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. *Nature Geoscience*, *12*, 46–53. https://doi.org/10.1038/s41561-018-0258-6

Sposito, G. (2008). *The chemistry of soils* (2nd ed.). Oxford University Press.

Sprunger, C. D., Martin, T., & Mann, M. (2020). Systems with greater perenniality and crop diversity enhance soil biological health. *Agricultural & Environmental Letters*, *5*(1), e20030. https://doi.org/10.1002/ael2.20030

Sprunger, C. D., Oates, L. G., Jackson, R., & Robertson, G. (2017). Plant community composition influences fine root production and biomass allocation in perennial bioenergy cropping systems of the upper Midwest, USA. *Biomass and Bioenergy*, *105*, 248–258. https://doi.org/10.1016/j.biombioe.2017.07.007

Tiemann, L. K., Grandy, A. S., Atkinson, E. E., Marin-Spiotta, E., & McDaniel, M. D. (2015). Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecology Letters*, *18*(8), 761–771. https://doi.org/10.1111/ele.12453

van der Pol, L. K., Nester, B., Schlautman, B., Crews, T. E., & Cotrufo, M. F. (2022). Perennial grain Kernza® fields have higher particulate organic carbon at depth than annual grain fields. *Canadian Journal of Soil Science*, *102*(4), 1005–1009. https://doi.org/10.1139/cjss-2022-0026

Veum, K. S., Zuber, S. M., Ransom, C., Myers, R. L., Kitchen, N. R., & Anderson, S. H. (2022). Reduced tillage and rotational diversity improve soil health in Missouri. *Agronomy Journal*, *114*(5), 3027–3039. https://doi.org/10.1002/agj2.21156

Villarino, S. H., Pinto, P., Jackson, R. B., & Piñeiro, G. (2021). Plant rhizodeposition: A key factor for soil organic matter formation in stable fractions. *Science Advances*, *7*(16), 1–13. https://doi.org/10.1126/sciadv.abd3176

Weidhuner, A., Hanauer, A., Krausz, R., Crittenden, S. J., Gage, K., & Sadeghpour, A. (2021). Tillage impacts on soil aggregation and aggregate-associated carbon and nitrogen after 49 years. *Soil and Tillage Research*, *208*, 104878. https://doi.org/10.1016/j.still.2020.104878

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer.

Witzgall, K., Vidal, A., Schubert, D. I., Höschen, C., Schweizer, S. A., Buegger, F., Pouteau, V., Chenu, C., & Mueller, C. W. (2021). Particulate organic matter as a functional soil component for persistent soil organic carbon. *Nature Communications*, *12*, 4115. https://doi.org/10.1038/s41467-021-24192-8

Yan, Y., Shi, J., Fan, Z., Peng, Y., & Wang, X. (2025). Changes in long-term land use alter deep soil microbial necromass and organic carbon stabilization. *Journal of Environmental Management*, *384*, 125589. https://doi.org/10.1016/j.jenvman.2025.125589

Yoder, R. E. (1936). A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. *Agronomy Journal*, *28*, 337–351. https://doi.org/10.2134/agronj1936.00021962002800050001x

Zhang, K., Maltais-Landry, G., & Liao, H. L. (2021). How soil biota regulate C cycling and soil C pools in diversified crop rotations. *Soil Biology and Biochemistry*, *156*, 108219. https://doi.org/10.1016/j.soilbio.2021.108219

Zhang, Y., Marx, E., Williams, S., Gurung, R., Ogle, S., Horton, R., Bader, D., & Paustian, K. (2020). Adaptation in U.S. Corn Belt increases resistance to soil carbon loss with climate change. *Scientific Reports*, *10*(1), 13799. https://doi.org/10.1038/s41598-020-70819-z

Zhou, Z., Ren, C., Wang, C., Delgado-Baquerizo, M., Luo, Y., Luo, Z., Du, Z., Zhu, B., Yang, Y., Jiao, S., Zhao, F., Cai, A., Yang, G., & Wei, G. (2024). Global turnover of soil mineral-associated and particulate organic carbon. *Nature Communications*, *15*(1), 5329. https://doi.org/10.1038/s41467-024-49743-7

Zuber, S. M., Behnke, G. D., Nafziger, E. D., & Villamil, M. B. (2015). Crop rotation and tillage effects on soil physical and chemical properties in Illinois. *Agronomy Journal*, *107*(3), 971–978. https://doi.org/10.2134/agronj14.0465

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