

Winter wheat cover crop increased subsoil organic carbon in a long-term cotton cropping system in Tennessee

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ABSTRACT

Long-term cover-cropping and no-tillage practices can facilitate soil organic carbon (SOC) accumulation in agroecosystems for soil health and climate mitigation benefits. However, the contribution of these conservation management practices to SOC gain from the subsoil layers is not extensively studied. To understand this knowledge gap, it is essential to determine the distribution of total SOC and SOC fractions in response to management practices across the soil profile. Therefore, this study was conducted by leveraging a 40-year replicated field experiment in a continuous cotton (*Gossypium hirsutum*) system. The management treatments examined included three cover crop treatments: 1) hairy vetch, HV (*Vicia villosa*); 2) winter wheat, WW (*Triticum aestivum* L.), and 3) no cover crop, NC; and two tillage treatments: 1) chisel-tillage, CT (10 cm deep) and 2) no-tillage, NT. Soil samples were collected from four depths (0–5, 5–10, 10–30, and 30–60 cm) and analyzed for total SOC and C fractions such as microbial biomass (MBC), particulate organic matter (POM-C), and mineral-associated organic matter (MAOM-C). Results showed that the profile-scale (0–60 cm) SOC stock was greater for WW (33.7 Mg ha⁻¹) compared to HV (29.1 Mg ha⁻¹) and NC (24.4 Mg ha⁻¹), while the tillage effect was non-significant (28 Mg ha⁻¹ for NT and 29.3 Mg ha⁻¹ for CT). The NT increased SOC in the top 5-cm layer, which was reflected in the concentrations of POM-C and MBC. Below the tillage depth (10–30 cm), SOC accrual was greater under CT (10.4 Mg ha⁻¹) than NT (8.9 Mg ha⁻¹), despite similar profile-scale SOC stocks. Further analysis revealed that total SOC in topsoil and subsoil were driven by POM and MAOM-related parameters, respectively. We also observed a limited accumulation of C in POM fraction (0.2–6.1 g C kg⁻¹ soil in tilled topsoil and 0.01–0.71 g C kg⁻¹ soil in subsoil) with a weak relationship with total SOC concentration (topsoil slope = 0.29, subsoil slope = 0.13). Contrastingly, accumulation of C in MAOM fraction was greater (2.7–12.7 g C kg⁻¹ soil in topsoil and 0.6–5.4 g C kg⁻¹ soil in subsoil), and it was strongly related to total SOC concentration than POM-C, especially in the subsoil (topsoil slope = 0.43, subsoil slope = 0.71). Growing deep-rooted winter wheat cover crop increased SOC in the subsoil, indicating the need for climate, soil, and ecosystem-specific management practices for profile-scale net SOC accumulation and redistribution in agroecosystems.

1. Introduction

The loss of soil organic carbon (SOC) in agroecosystems is primarily attributed to intensive productivity-oriented management activities. Land-use conversion from natural to agricultural systems resulted in a 40–90 Pg decline in global SOC (Lal, 2004; Smith, 2008; Don et al., 2011). This decline in SOC has increased awareness of the importance of global C budgets and has accelerated SOC storage evaluations under long-term agricultural management practices (Kirschbaum, 2000; Olson, 2013). Implementing long-term cover cropping and no-tillage practices, two most popular conservation management practices

across the United States (US), has been shown to improve SOC accumulation across different agro-ecoregions of the US (West and Post, 2002; Blanco-Canqui and Lal, 2008; Jagadamma and Lal, 2010; Chalise et al., 2019; Singh et al., 2020). No-tillage reduces residue decomposition compared to conventional tillage due to no or minimal soil disturbance, which helps in soil aggregation and protects SOC from microbial decomposition (Six et al., 2000; West and Post, 2002; Franzluebbers, 2010). However, several studies showed that profile-scale SOC stock was similar in no-tilled and tilled soils, indicating that increased SOC in topsoil under no-till is compensated by increased SOC in subsoil under tilled soil (Luo et al., 2010; Olson and Al-Kaisi, 2015; Jagadamma et al.,

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2019). Integration of cover crops during the non-cash crop growing season has also increased SOC in agroecosystems by providing additional aboveground and belowground C inputs to soil (Poeplau and Don, 2015; Jian et al., 2020). In general, grass cover crops (e.g., winter wheat, triticale, oats) produce more biomass compared to legumes (e.g., hairy vetch, clovers, Austrian winter pea) and brassicas (e.g., radish, turnip, canola) (Cherney et al., 1991; Sassenrath and Farney, 2019). A significant C contribution from cover crops comes from root-biomass, which contributes more C to the subsoil (Rasse et al., 2005). For example, Amsili and Kaye (2021) reported a significant increase in root C input by a triticale (grass type cover) compared to crimson clover (legume) and canola (brassica) cover crops. Previous research has demonstrated that the effect of conservation practices on SOC storage depends not only on the type and duration of the practices but also on the chemical composition and turnover rates of SOC.

The SOC comprises heterogeneous C compounds with a broad molecular-size range, oxidation and reduction potential, and stability (Lehmann and Kleber, 2015). Agroecosystem management practices impart a strong control on the chemical composition and stability of SOC (Jagadamma and Lal, 2010). However, determining the changes in total SOC with management practices is challenging because SOC accumulation in detectable quantity is a slow process. Instead of total SOC, active SOC fractions with shorter turnover time and distinct chemistry, such as particulate organic matter C (POM-C) and microbial biomass C (MBC), can serve as early indicators for management-induced changes (Cambardella and Elliott, 1994; Poeplau et al., 2018; Six et al., 1998). On the contrary, the mineral-associated organic matter C (MAOM-C) fraction represents the SOC fraction with a longer-turnover time and is composed of dissolved organic carbon (DOC) and microbial by-products adsorbed and/or co-precipitated to the surface of soil minerals (von Lützow et al., 2007; Poeplau et al., 2018). Given the continued interest in SOC stabilization and destabilization in agricultural soils, it is critical to understand the profile distribution of functionally diverse C fractions under contrasting management practices.

Most previous studies on changes in SOC distribution in response to agroecosystem management practices have ignored the effect of soil depth (Yost and Hartemink, 2020) despite the estimate that more than 50% of the global SOC stock resides below 30 cm soil depth (Jobbágy and Jackson, 2000). In addition, the subsoil micro-environment possesses several characteristics that indicate its potential for longer-term SOC storage compared to that in the topsoil (Fontaine et al., 2007; Rumpel and Kögel-Knabner, 2011). These characteristics include, 1) greater soil volume that comprises C-depleted mineral fraction, 2) an increased proportion of slow-cycling root C, and 3) a less favorable micro-climate for SOC mineralization due to the reduced substrate availability and microbial activity (Fierer et al., 2003; Lorenz and Lal, 2005; Stewart et al., 2007; Rumpel and Kögel-Knabner, 2011). These potential SOC sink characteristics of subsoil raised concerns about our current understanding of the impact of agricultural management practices on subsoil SOC dynamics and their consequent implication on the global carbon cycle. Previous studies have quantified SOC in subsoils of varying depths in response to agricultural practices (Babujia et al., 2010; Christopher et al., 2009; Luo et al., 2010; Poirier et al., 2009; Sainju et al., 2005). However, results so far have been inconsistent, meaning that profile-scale SOC concentration and distribution were either increased (Babujia et al., 2010), decreased (Christopher et al., 2009), or unchanged (Poirier et al., 2009; Luo et al., 2010; Jagadamma et al., 2019) under long-term management practices. Due to the paucity of studies focused on subsoil SOC and the lack of consistency among the existing results, the magnitude, and direction of SOC change in the subsoil in response to management changes are still unclear.

Although our understanding of the SOC response to agroecosystem management practices has improved in recent years, global C cycle model performances can be improved by adequate representation of depth-wise data on total SOC and functional pools (Jenkinson and Coleman, 2008; Rumpel and Kögel-Knabner, 2011). Given the

differences in SOC sources, quality, and stabilization mechanisms between topsoil and subsoil, a more site-specific understanding of the role of agroecosystem management practices on profile-scale SOC accumulation and distribution is warranted (Freibauer et al., 2004; Lorenz and Lal, 2005; Angst et al., 2018). Therefore, in this study, our objective was to determine how contrasting long-term tillage and cover cropping practices affect the profile-scale total SOC stock as well as SOC distribution in different functional C fractions. We hypothesized that, (i) the positive effect of no-tillage on SOC accumulation will be limited to the topsoil, (ii) cover crops with extensive root systems will increase profile-scale SOC stock, and (iii) different C functional fractions control SOC accumulation in topsoil and subsoil.

2. Materials and methods

2.1. Experimental site and treatments

This study was conducted at the University of Tennessee's West Tennessee Research and Education Center in Jackson, Tennessee, US (35° 37' N, 88°50' W), leveraging a long-term agricultural field experiment that has been maintained since 1981. The mean annual temperature and precipitation of the study site are 15.6 °C and 1300 mm, respectively. The soil is a well-drained Lexington silt loam (fine-silty, mixed, thermic Ultic Hapludalfs) developed over a marine deposit. The experiment is laid out as a randomized complete block design (RCBD) with a split-split plot arrangement of treatments: N rates assigned to main plots, cover crops to sub plots, and tillage types to sub-sub plots. There are four rates of N fertilization: 0, 34, 67, 101 kg N ha⁻¹ yr⁻¹; four treatments of cover crops: crimson clover (*Trifolium incarnatum* L.) (CC), hairy vetch (*Vicia villosa*) (HV), winter wheat (*Triticum aestivum* L.) (WW), and no-cover control (NC); and two types of tillage: no-tillage (NT) and chisel-tillage (CT), with cotton (*Gossypium hirsutum*) as the primary cash crop. Each treatment has four replications with each experimental unit measured 12 m × 8 m, which included eight rows of cotton. A detailed description of the experimental design and all management treatments is presented in Nouri et al. (2021). For the current study, following treatments were considered. These treatments are two tillage (NT and CT) and three cover crops (NC, HV, and WW) that receive 67 kg N ha⁻¹ yr⁻¹ as urea. The cotton lint yield did not increase beyond 67 kg N ha⁻¹ yr⁻¹ (Nouri et al., 2021), so we only considered this economically optimum N rate treatment in this study. The CT plots were chisel-tilled once a year to a depth of 10 cm and then leveled with a harrow. Cover crops were sown in October–November and chemically terminated in April–May using Gramoxone SL 2.0 (N, N'-dimethyl-4, 4'-bipyridinium dichloride) at a rate of 3.51 L ha⁻¹ before cotton planting at a rate of 86,500 ha⁻¹. In addition to N, other fertilizers were applied yearly based on soil test recommendations (Phosphorus as triple superphosphate at 101 kg P₂O₅ ha⁻¹ and potassium as potash muriate at 134 kg K₂O ha⁻¹). Cotton is mechanically harvested, usually between October and November.

2.2. Soil sampling, processing, and analyses

2.2.1. Soil sampling

Soil cores (8 cm diameter and 60 cm long) were collected using a hydraulic probe in June 2020. Four cores were taken from the inter-row spaces of each plot and transported to the laboratory in a cooler with ice packs. In the laboratory, each core was split into four depth increments: 0–5, 5–10, 10–30, and 30–60 cm. Two core segments per plot were used for root biomass determination, and the other two cores were used to analyze soil properties.

2.2.2. Bulk soil analysis

Two depth-separated core segments per plot were weighed to record the fresh weight of soil before they were processed for other analyses. A subsample (10 g) from field-moist soil samples was collected and oven-

dried at 105 °C for 48 h to measure gravimetric moisture content. Wet soil weight, gravimetric moisture content, and soil volume were used to determine the soil bulk density (BD). The composited field-moist soil was sieved through a 4 mm sieve and a subsample was stored under 4 °C until MBC analysis. The remaining soil was air-dried and sieved through a 2 mm sieve to analyze basic properties (Supplementary Table 1), SOC, and functional C fractions. The air-dried and 2 mm sieved samples were further pulverized using a vial rotator (SampleTek model 200) and used for the determination of total SOC concentration (SOC_{con}) using an Elementar vario MAX cube CN analyzer (Elementar, Hanau, Germany). Depth-wise SOC stock was calculated using the layer SOC_{con} , layer thickness, and respective BD data (Lal et al., 1998). Since the BD values were mostly similar across tillage practices (Supplementary Table 2), we opted for depth-wise SOC stock calculation rather than the equivalent soil mass (ESM) approach, which is a preferred method when different land management treatments significantly alter the soil mass over a predetermined soil depth (Ellert and Bettany, 1995; Meurer et al., 2018).

Soil MBC was determined by following the modified chloroform fumigation and extraction procedure (Vance et al., 1987). Fresh 4 mm sieved 15 g soils were fumigated for 48 h in the dark. After fumigation, soils were extracted with 45 ml 0.5 M K_2SO_4 solution, followed by shaking at 200 rpm for 1 h, centrifuging for 2 min at 2500 rpm, and filtering the supernatant using Whatman no. 42 filter paper. On the day of incubation, 15 g of the unfumigated soil samples were extracted using the same protocol. The fumigated and unfumigated filtrates were analyzed for C concentration using Elementar vario TOC cube CN analyzer in liquid mode (Elementar, Hanau, Germany). Microbial biomass C was determined as the difference in measured C concentration between fumigated and unfumigated samples and divided by the extraction efficiency coefficient of 0.45 (Beck et al., 1997).

2.2.3. Soil physical fractionation

We used a combination of size and density-based fractionation methods to separate bulk soil into POM and MAOM fractions following the modified Par+Den1 fractionation method (Poeplau et al., 2018). We conducted a preliminary fractionation test to determine the general proportion of SOC associated with occluded POM (POM within the aggregates), free POM (POM outside the aggregates), and sand fraction (density greater than 1.8 g cm^{-3} and size greater than $53 \mu\text{m}$). We found that the SOC associated with occluded POM and sand fraction was negligible in contrasting treatments and soil depths ($< 2\%$ of the total SOC). Therefore, we omitted the steps to isolate the occluded POM and C associated with sand fraction from the subsequent data analysis procedures. Air-dried and 2-mm sieved 15 g soil was added to 60 ml of 0.5% sodium hexametaphosphate solution to disperse the soil particles. The soil solution was shaken for 18 h in a reciprocal shaker at 200 rpm and then passed through a $53 \mu\text{m}$ sieve. The sand and POM fractions were retained on the $53 \mu\text{m}$ sieve, while the silt and clay sized MAOM fraction was passed through the sieve and collected in a beaker. To separate the POM from the sand fraction, the combined sand and POM fraction were treated to density fractionation using a 1.8 g cm^{-3} sodium polytungstate (SPT) solution. The resulting mixture was centrifuged for 1 h at 1069 g, and the floated POM was aspirated. The recovered MAOM and POM fractions were washed three times with Milli-Q water to remove any residual SPT solution and dried at 60 °C. Before measuring organic C concentration, the recovered POM and MAOM fractions were weighed, and the proportions of their weight (%) were described as fPOM and fMAOM. The dried fractions were further pulverized using a vial rotator (SampleTek model 200), and organic C concentrations in the recovered POM (POMCp) and MAOM (MAOMCp) fractions (g C kg^{-1} POM or MAOM) were measured using the Elementar vario MAX cube CN analyzer (Elementar, Hanau, Germany). The C associated with POM and MAOM fractions on a unit soil weight basis (POM-C and MAOM-C in g C kg^{-1} soil) was calculated by using Eqs. (1) and (2).

$$\text{POM-C} = \text{fPOM} \times \text{POMCp} \quad (1)$$

$$\text{MAOM-C} = \text{fMAOM} \times \text{MAOMCp} \quad (2)$$

2.3. Soil organic C stratification ratios

The stratification ratios (SR) were calculated for total SOC, POM-C, and MAOM-C as the ratio of C concentrations at 0–5 cm soil depth (topmost soil layer) vs. at 10–30 cm soil depth (layer immediately below the tillage depth) (Eq. 3).

$$\text{SR}_X = C_X(0-5 \text{ cm}) / C_X(10-30 \text{ cm}) \quad (3)$$

where X is the SOC fraction (total SOC, POM-C and MAOM-C) and C is their respective concentrations.

2.4. Root biomass determination

Root biomass was determined according to Amsili and Kaye (2021). The core segments were gently broken by hand, and the material from two cores per plot for each depth increment was composited. These soil samples were soaked overnight in 1% Liquinox solution to assist soil dispersion and root separation. The next day, the soil solution was passed through a 0.5 mm sieve. Washing roots through a 0.5 mm sieve can capture up to 95% of the root biomass (Böhm, 1979; Amato and Pardo, 1994). The remnants collected on the sieve were suspended in water to clean roots from associated soil and other debris. The collected roots were oven-dried at 50 °C for 48 h and then weighed to calculate the root biomass.

2.5. Statistical analysis

Analysis of variance was performed using the linear mixed-effect model in SAS v9.4 statistical package (SAS Institute, Inc., Cary, NC). The least-square means were separated using Fisher's least significant difference (LSD) test at $P \leq 0.05$. Tillage, cover crops, and their interactions were considered as fixed effects and replication as random effects. Statistical analyses were conducted separately for each soil depth. We performed principal component analysis (PCA) by following the method described in Andrews et al. (2002) to identify a minimum set of SOC fractional properties that drives changes in total SOC concentration (SOC_{con}) in the tillage zone (combining data from 0 to 5 and 5–10 cm soil depth layers; representing topsoil) and soil layers beyond the tillage depth (combining 10–30 and 30–60 cm soil depth layers; representing subsoil). We retained the variables that showed significant differences at $P \leq 0.05$ in at least one of the PCA treatments. Principal components (PCs) are the linear combinations of the variables that account for the maximum variance within the dataset. The PCs with eigenvalues (amount of variance explained by each factor) higher than 1 were selected to determine profile-scale SOC_{con} distribution drivers (Brejda et al., 2000). Individual variables received a weight (factor loading) within each PC according to its contribution to the PC. We selected the variables with the highest weight from each PC to build the minimum dataset of soil parameters. Finally, to check how well the minimum dataset determines the profile-scale SOC_{con} distribution, we performed a multiple regression analysis using R statistical software (R Core Team, 2021), using the minimum dataset parameters as independent variables and SOC_{con} as a dependent variable at $P \leq 0.05$ (Andrews et al., 2002; Jagadamma et al., 2008).

3. Results

3.1. Total SOC stock and concentration

After 40 years of cover cropping, the whole profile (0–60 cm) SOC stock was greater in WW (33.7 Mg ha^{-1}) compared to HV (29.1 Mg

ha^{-1}) and winter fallow (24.4 Mg ha^{-1}) (Fig. 1a). Integration of winter cover crops, particularly WW, resulted in more SOC accumulation in the subsoil layers. Combining 10–30 cm and 30–60 cm depths, WW resulted in 23% and 34.5% more SOC stock than HV and NC, respectively. Between the two tillage treatments, statistically similar whole profile SOC stock was found across the cover crop treatments (28.8 Mg ha^{-1} for NT and 29.4 Mg ha^{-1} for CT) (Fig. 1b). Although NT resulted in greater SOC stock in the 0–5 cm depth (7.42 Mg ha^{-1}) compared to CT (5.6 Mg ha^{-1}), the trend was reversed below the tillage depth of 10 cm and resulted in greater SOC stock under CT in the 10–30 cm depth compared to NT (10.4 vs. 8.88 Mg ha^{-1} , $P < 0.05$). The total SOC concentration (SOC_{con}) also varied almost similarly to SOC stock in response to treatments and soil depths (Table 1 and Supplementary Table 2). The interaction of tillage and cover crops was not significant for SOC stock and concentrations in most depths except for SOC stock at subsoil depth intervals of 10–30 cm and 30–60 cm (Supplementary Table 2).

3.2. SOC fractions

Microbial biomass C sharply decreased from topsoil to subsoil layers (Fig. 2). At the 0–5 cm soil layer, CT under winter fallow showed the least (245 mg kg^{-1}), and NT under HV cover showed the highest (430 mg kg^{-1}) MBC concentration (Fig. 2 and Supplementary Table 2). At this depth, NT resulted in 28% more MBC than CT across the cover crop treatments. At 5–10 cm, NT and CT showed similar MBC when winter cover crops (HV and WW) were grown. However, CT had 25% lower MBC in winter fallow plots than NT. Immediately below the tillage depth (10–30 cm), CT showed 17% higher MBC than NT across the cover crop treatments, with WW showing the highest increase (Fig. 2). All tillage and cover crop combination treatments had similar MBC concentrations at 30–60 cm depth intervals (Fig. 2 and Supplementary Table 2).

In the top 0–5 cm layer, NT had two times higher POM-C concentration than CT (3.86 and 1.11 g kg^{-1} for NT and CT, respectively) (Table 1 and Supplementary Table 2). The trend remained the same at the 5–10 cm layer with a decrease in magnitude (0.80 vs. 0.44 g kg^{-1} for NT and CT, respectively). No significant change in POM-C concentration between tillage practices was observed in subsoil depth intervals (Table 1). A significant increase in POM-C was observed with the integration of cover crops at 0–5 cm and 10–30 cm soil layers. The highest

increase in POM-C was observed in topsoil 0–5 cm layer with HV cover (3.49 g kg^{-1}), followed by WW (2.58 g kg^{-1}) and NC (1.40 g kg^{-1}) across the tillage practices ($P < 0.05$, Table 1). At 10–30 cm layer, the highest POM-C was observed under WW (0.43 g kg^{-1}) followed by HV (0.34 g kg^{-1}) and NC (0.17 g kg^{-1}) ($P < 0.05$, Table 1). Between the two tillage treatments, no significant difference in MAOM-C concentration was found in 0–5 cm and 5–10 cm soil layers (Table 1). However, the MAOM-C was higher under CT than NT in the subsoil layers: 37% increase in 10–30 cm and 71% increase in 30–60 cm (Table 1). Across the cover crop treatments, MAOM-C was similar for all the treatments in all soil layers except at 5–10 cm depth. At this depth, the highest increase in MAOM-C was observed under WW (7.23 g kg^{-1}), followed by HV (4.98 g kg^{-1}) and NC (5.04 g kg^{-1}). For both POM-C and MAOM-C fractions, the interaction effect of tillage and cover crops was mostly non-significant except for the MAOM-C fraction at 5–10 cm (Supplementary Table 2).

3.3. Carbon stratification ratios

Stratification ratios were calculated between the topmost surface soil layer (0–5 cm) and the soil layer immediately beneath the plow layer (10–30 cm) for total SOC (SR_{SOC}), POM-C ($\text{SR}_{\text{POM-C}}$), and MAOM-C ($\text{SR}_{\text{MAOM-C}}$). Results showed that tillage treatments significantly influenced SR_{SOC} , and $\text{SR}_{\text{POM-C}}$ (Supplementary Table 2). The mean values under NT and CT were 3.52 and 2.42, respectively, for SR_{SOC} and 17.09 and 4.02, respectively, for $\text{SR}_{\text{POM-C}}$ (Fig. 3a and 3c). Mean values of $\text{SR}_{\text{MAOM-C}}$ were not different between NT and CT practices (Fig. 3b). Effects of cover crops and tillage $\times$ cover crop interaction on SR were not significantly different for total SOC and the fractions (Fig. 3b, Supplementary Table 2).

3.4. Drivers of total SOC concentration change in topsoil and subsoil

We used PCA to determine a minimum set of SOC fractional properties responsible for variations in profile-scale total SOC concentration (SOC_{con}). We found that 10 out of 16 C-related parameters measured differed by at least one treatment (tillage or cover crop) or soil depth. These properties are MBC, POM and MAOM fractional abundance (fPOM , fMAOM), CN ratio of POM and MAOM fractions (POMCN and MAOMCN), C concentration in POM and MAOM fractions (POMCp ,

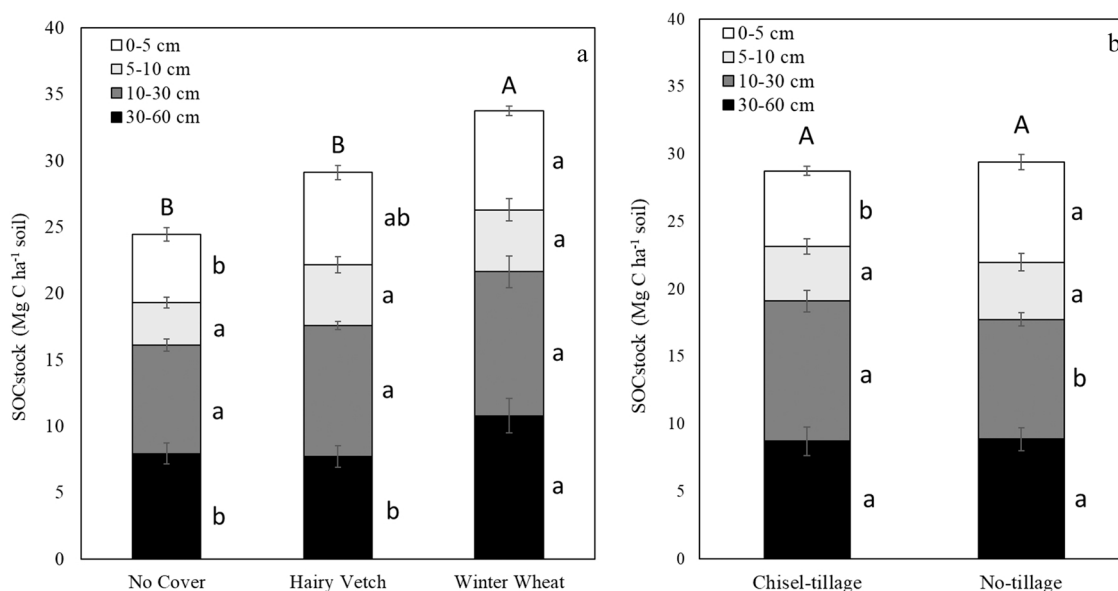


Fig. 1. Depth-wise soil organic carbon (SOC) stock under long-term cover cropping (a) and tillage practices (b). The upper-case letters indicate significant differences in whole profile (0–60 cm) SOC stock among treatments and the lower-case letters indicate significant differences in SOC stock among treatments for each soil depth ($P \leq 0.05$).

Table 1
Tillage and cover crop effects on total soil organic carbon, particulate organic carbon, and mineral-associated organic carbon for each soil depth.

	Soil depth (cm)	Tillage		Cover crops		
		CT	NT	NC	HV	WW
SOC _{con} (g C kg ⁻¹ soil)	0–5	9.78Ab	12.98Aa	9.68Ab	12.17Aa	12.29Aa
	5–10	6.44Ba	6.20Ba	5.16Ba	6.32Ba	7.49Ba
	10–30	4.13Ca	3.59Ca	3.61Ca	3.63Ca	4.34Ca
	30–60	2.37Da	2.36Da	2.29Dab	1.96Db	2.85Da
POM-C (g C kg ⁻¹ soil)	0–5	1.12Ab	3.86Aa	1.40Ab	3.49Aa	2.58Aab
	5–10	0.44Bb	0.80Ba	0.46Ba	0.70Ba	0.70Ba
	10–30	0.34Ba	0.29Ba	0.17Bb	0.34Bab	0.43Ba
	30–60	0.12Ca	0.06Ca	0.05Ca	0.04Ca	0.18Ca
MAOM-C (g C kg ⁻¹ soil)	0–5	6.54Aa	6.70Aa	4.88Aa	7.27Aa	7.71Aa
	5–10	5.37Aa	6.12Aa	5.04Aab	4.98Ab	7.23Aa
	10–30	4.09Ba	2.98Bb	3.11Ba	3.78a	3.71Ba
	30–60	2.79Ba	1.63Bb	1.75Ba	2.39Ba	2.48Ba

Means followed by the upper-case letters indicate significant differences among soil depths at $P \leq 0.05$. Means followed by lowercase letters indicate significant differences among treatments for each soil depth ($P \leq 0.05$). CT, Chisel-tillage; NT, No-tillage; NC, no cover; HV, hairy vetch cover crop; WW, winter wheat cover crop. SOC_{con}, total SOC concentration; POM-C: C associated with POM fraction; MAOM-C, C associated with MAOM fraction.

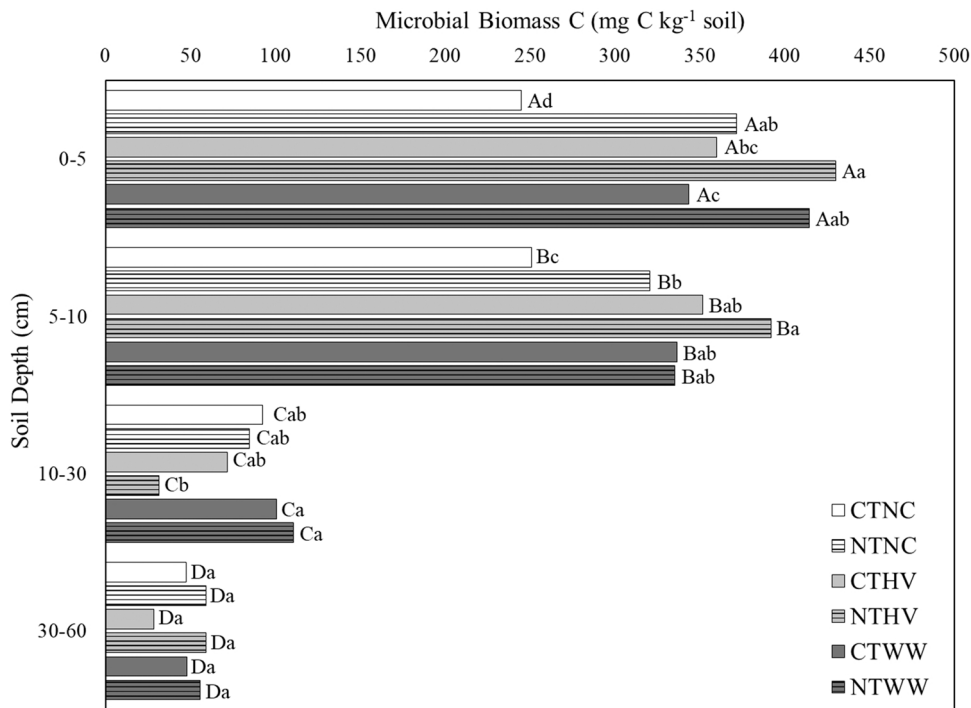


Fig. 2. Depth-wise distribution of microbial biomass carbon (MBC) under long-term tillage and cover cropping practices. The upper-case letters indicate significant differences in MBC among soil depth and the lowercase letters indicate significant differences in MBC among treatments for each soil depth ($P \leq 0.05$). CT, Chisel-tillage; NT, No-tillage; NC, No Cover; HV, Hairy Vetch; WW, Winter Wheat.

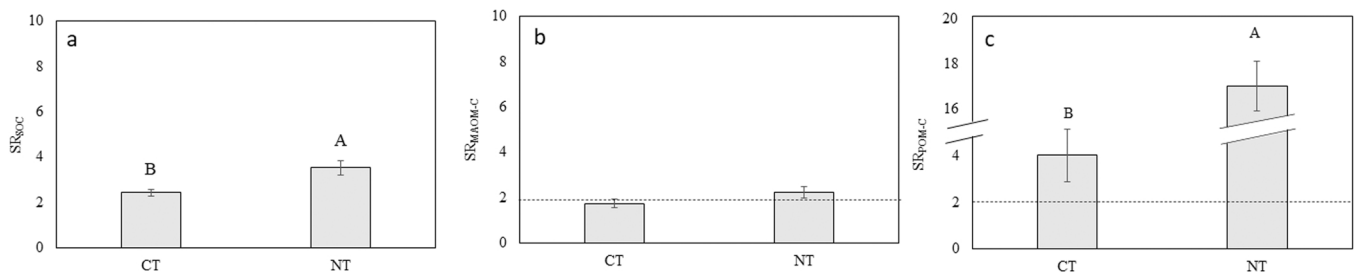


Fig. 3. Carbon stratification ratio (SR) for total SOC (SR_{SOC}) (a), MAOM-C (SR_{MAOM-C}) (b), and POM-C (SR_{POM-C}) (c) as affected by long-term tillage practices. CT, Chisel-tillage; NT, No-tillage. The upper-case letters indicate significant differences between the two tillage treatments ($P \leq 0.05$). No letters denote non-significance ($P > 0.05$).

Table 2

Principal component analysis for the determination of total SOC concentration drivers.

PCA output	Topsoil (0–10 cm)			Subsoil (10–60 cm)			PC4
	PC1	PC2	PC3	PC1	PC2	PC3	
Eigenvalues	3.85	1.93	1.34	3.97	1.72	1.21	1.00
Proportion of Variance	0.39	0.18	0.12	0.40	0.17	0.12	0.10
Cumulative Variance	0.39	0.57	0.69	0.40	0.57	0.69	0.79
Eigenvectors							
MBC ^a	0.75	0.21	-0.03	0.58	0.15	0.49	0.28
fPOM	0.78	0.34^b	-0.36	0.74	-0.32	0.46	-0.29
POMCp	0.82	-0.05	0.13	0.39	0.41	-0.40	-0.39
POMCN	0.71	-0.06	0.30	0.48	0.71	0.09	-0.14
POM-C	0.88	0.23	-0.28	0.79	-0.18	0.39	-0.34
fMAOM	-0.16	0.21	0.83	-0.51	-0.27	-0.03	-0.64
MAOMCp	0.51	-0.82	0.12	0.80	-0.30	-0.45	0.01
MAOMCN	-0.15	-0.48	-0.19	0.70	-0.09	-0.14	0.35
MAOM-C	0.50	-0.82	0.15	0.78	-0.32	-0.46	-0.02
Troot	0.32	0.32	0.52	0.28	0.78	-0.07	-0.10

^a MBC, microbial biomass carbon; fPOM, fractional proportion of particulate organic matter (POM); POMCp, C concentration in the recovered POM-fraction; POMCN, CN ratio of POM; POM-C, C associated with POM fraction in unit soil weight basis; fMAOM, fractional proportion of mineral associated organic matter (MAOM); MAOMCp, C concentration in the recovered MAOM-fraction; MAOMCN, CN ratio of MAOM; MAOM-C, C associated with MAOM fraction in unit soil weight basis; Troot, total root biomass.

^b Factor loadings in bold indicate that the indicators are highly weighted and included in the multiple regression analysis.

MAOMCp), total C associated with POM and MAOM fractions per unit soil weight basis (POM-C, MAOM-C), and total root biomass (Troot) (Table 2 and Supplementary Table 3). On these 10 SOC related parameters, PCA was performed separately for the tillage zone (topsoil representative: 0–10 cm) and the depth below the tillage zone (subsoil representative: 10–60 cm) to determine if the parameters associated with SOC functional fractions drive SOC_{con}. We selected three PCs for 0–10 cm and four PCs for 10–60 cm soil layers accounting for 69% and 79% of the cumulative sample variance, respectively (Table 2). Indicators associated with the greatest absolute eigenvector values were selected from each PC to determine SOC_{con} drivers at the respective soil depth layers. These indicators were POM-C in PC1, fPOM in PC2, and fMAOM in PC3 for the topsoil and MAOMCp in PC1, Troot in PC2, MBC in PC3, and MAOMCN in PC4 for the subsoil (Table 2). The multiple regression analysis was conducted to identify the drivers of SOC_{con} from the PCA selected data set variables for both topsoil and subsoil (Table 2). The analysis revealed that fPOM and POM-C fractions drive topsoil SOC_{con} ($R^2 = 0.43$, $P < 0.001$), and MBC and MAOMCp fractions drive subsoil SOC_{con} ($R^2 = 0.35$, $P < 0.001$), while the other selected parameters were not included in the model as they were not statistically significant in explaining SOC_{con} (Table 3).

Table 3

Regression equations to determine total SOC concentration drivers for topsoil and subsoil.

Soil depth	Equation	Adjusted R ²	P value
Topsoil (0–10 cm)	$SOC_{con} = 5.83 + 0.8 * POM-C + 2 * fPOM$	0.43	< 0.001
Subsoil (10–60 cm)	$SOC_{con} = 1.2 + 3.16 * MAOMCp + 0.01 * MBC$	0.35	< 0.001

SOC_{con}, total SOC concentration; POM-C, C concentration in particulate organic matter (POM) in unit soil weight basis; fPOM, fractional proportion of POM; MAOMCp, C concentration in the recovered mineral-associated organic matter (MAOM) fraction; MBC, microbial biomass carbon.

3.5. Carbon accumulation capacity in POM and MAOM fractions

Total SOC_{con} across the treatments and soil depths ranged from 3.2 to 14.5 g kg⁻¹ in the tillage depth (topsoil representative: 0–10 cm) and 1.28–5.75 g kg⁻¹ in the soil layers below the tillage depth (subsoil representative: 10–60 cm). The POM-C and MAOM-C concentrations ranged from 0.23 to 6.1 g C kg⁻¹ soil and 2.1–12.7 g C kg⁻¹ soil, respectively for topsoil and 0.01–0.71 g C kg⁻¹ soil and 0.58–5.43 g C kg⁻¹ soil, respectively for subsoil (Fig. 4). Regression analysis revealed that MAOM-C had a relatively stronger linear relationship with SOC_{con} (Fig. 4c and 4d) than POM-C (Fig. 4a and 4b) in the subsoil (Slope_{MAOM-C} = 0.71 vs. Slope_{POM-C} = 0.13), compared to topsoil (Slope_{MAOM-C} = 0.43 vs. Slope_{POM-C} = 0.29).

4. Discussion

4.1. Profile SOC storage under conservation management practices

This study evaluated the impacts of long-term conservation management practices to identify cropping systems that can increase SOC across the soil profile compared to the conventional practices. We found that 40 years of no-tillage adoption improved topsoil SOC stock but had similar SOC stock to that of shallow-tilled systems when 60 cm soil profile SOC stock was compared (Fig. 1b), consistent with the results from previous global meta-analyses and review studies (West and Post, 2002; Luo et al., 2010; Meurer et al., 2018). This seems to be particularly important in hot and humid climatic conditions that favor SOC decomposition over accumulation (Franzluuebbers, 2010; Dimassi et al., 2014; Jagadamma et al., 2019) as opposed to cold and drier climates where significant SOC accumulation occurs under no-till relative to different frequencies and intensities of tillage (Jagadamma and Lal, 2010; VandenBygaart et al., 2010; VandenBygaart, 2016). Thus, changes in soil micro-climate appear to have strong control on the magnitude and direction of no-till impacts on SOC changes.

In this study, average soil bulk density values were similar for NT (1.22 Mg m⁻³) and CT (1.27 Mg m⁻³) in the 0–5 cm soil layer after 40 years of management (Supplementary Fig. 1). Thus, tillage induced changes in topsoil SOC stock were largely due to the changes in total SOC and fractional SOC pools. However, the NT systems showed a noticeably higher BD (1.32 Mg m⁻³) than the CT system (1.14 Mg m⁻³) in the soil layer immediately below the tillage depth (10–30 cm) (Supplementary Fig. 1). Higher BD in NT subsoil layers may restrict organic C migration from the topsoil to subsoil compared to CT system (Bronick and Lal, 2005; Garcia-Franco et al., 2015), which was also evident from the higher stratification of SOC fractions under NT compared to CT (Fig. 3). Furthermore, the subsoil compaction under NT may limit the oxygen availability, thereby limiting the microbial activity (Balesdent et al., 2000), which is supported by the sharp reduction in MBC beyond the 0–10 cm tillage depth (Fig. 2). The increased SOC stock in the surface soil layers of NT treatment was compensated by a 13% increase in SOC stock by the shallow-tilled CT treatment in 10–30 cm depth (Fig. 1b), which can be primarily attributed to the incorporation of crop residues by tillage and residue C distribution beyond the top 10 cm tillage layer.

Despite the positive effect of long-term NT on SOC accumulation being restricted to the topsoil, profile SOC accumulation was increased when a grass-type winter cover crop (WW) was integrated into the system (Fig. 1a and 1b). Integrating winter cover crops introduces greater soil cover and increases the quantity of the above and below-ground residue input (Poeplau and Don, 2015; Jian et al., 2020). We found a 9–35% increase in profile-scale SOC stock from the combined effect of a winter cover crop and the main crop (cotton) relative to the main crop without the cover crop (NC) (Fig. 1a).

The effect of cover crops on SOC changes depends on cover crop species type. Some studies demonstrated that growing cover crops with higher quality residues (e.g., lower CN ratio) such as legumes increases

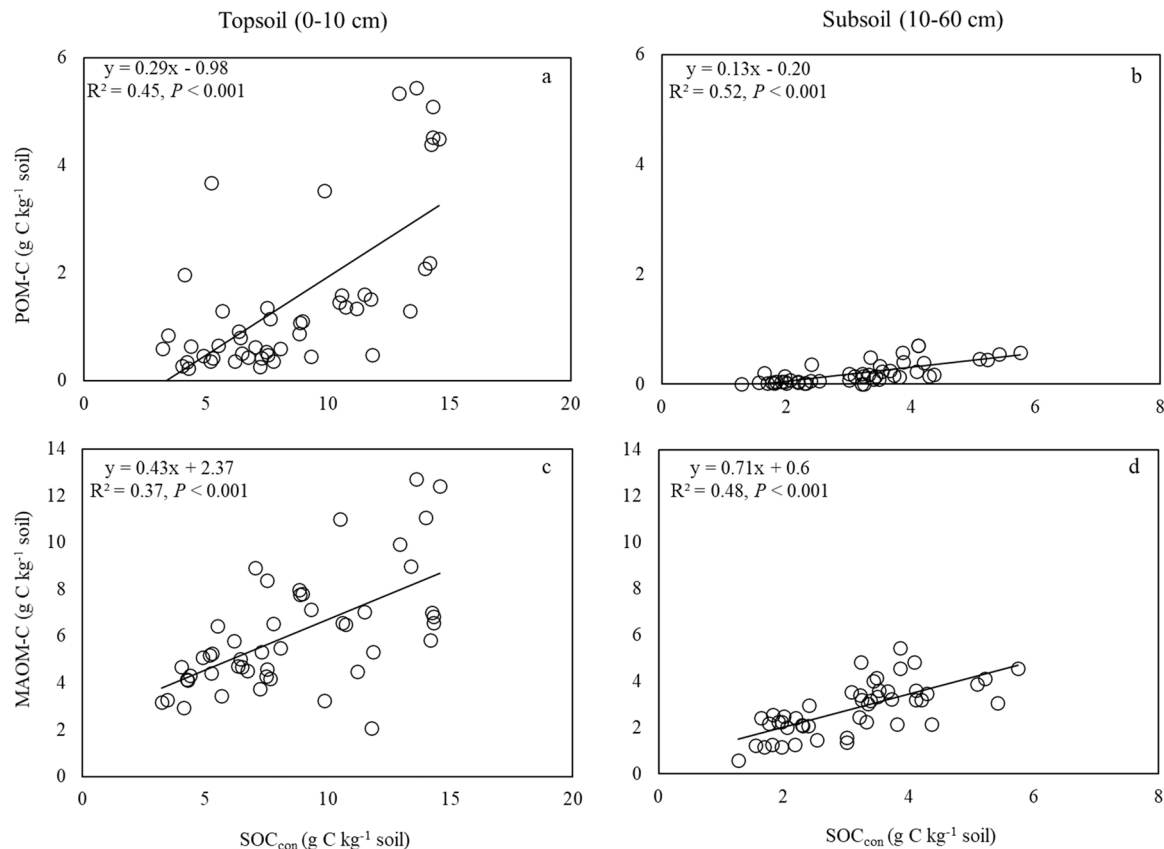


Fig. 4. Relationship of total SOC concentration (SOC_{con}) with POM-C (a,b) and MAOM-C (c,d) for all the treatments. (a,c) represent soil samples from tillage zone (topsoil representative: 0–10 cm); and (b,d) represent soil samples beyond the tillage depth (subsoil representative: 10–60 cm).

SOC due to their more efficient utilization by the soil microbes, resulting in greater production of microbial biomass and by-products and subsequent stabilization as soil organic matter (e.g., Boddey et al., 2010; Kallenbach et al., 2015). Consistent with these studies, we found a 12.7% increase in SOC_{con} , 28% increase in MBC, and ~200% increase in POM-C concentrations under HV compared to winter fallow in 0–5 cm soil layer (Fig. 2 and Table 1). However, this favorable effect of HV cover crop on SOC accumulation observed in the surface soil layer was not reflected in the profile SOC stock as WW integrated systems showed greater SOC_{con} and SOC stock in the subsoil relative to HV and winter fallow (Fig. 1a).

Profile SOC stock is typically influenced by many factors including above- and below-ground biomass input, intrinsic soil properties, physical disturbance, movement of organic matter through the soil profile, and microbial activity. The emerging literature on soil organic matter stabilization emphasizes that a part of DOC from residue decomposition is rapidly incorporated into microbial biomass and its by-products, thereby directly contributing to the MAOM-C formation through stabilization onto the mineral surfaces (Cotrufo et al., 2013). This finding points to a potentially significant role the movement of DOC through soil profile play in subsoil SOC accumulation. Also, the observed increase in subsoil SOC stock under WW treatment compared to HV and NC treatments (Fig. 1a) can be attributed to deeper root distribution of the wheat crop. Although root biomass was statistically similar among cover crops in the surface soil layers, WW had 39% and 76% more root biomass than HV and NC treatments, respectively, in the 30–60 cm depth (Supplementary Table 2 and 3). Previous studies have reported that grass cover crops contribute to more SOC accumulation by increasing total C input from extensive root biomass and higher root CN ratio (Lorenz and Lal, 2005; Sainju et al., 2005; McDaniel et al., 2014; Amsili and Kaye, 2021). The tight coupling of roots-microbes-minerals

in the subsoil favors greater stabilization of C accumulated in the subsoil than in the surface soil (Hicks Pries et al., 2017; Sokol and Bradford, 2019).

4.2. SOC functional fractions driving profile-scale SOC distribution

We identified the specific C fractions driving the topsoil and subsoil SOC_{con} under the long-term management practices using the univariate, multivariate, and regression analyses. The results showed that different C functional fractions dictate change in SOC_{con} in surface soil and subsoil (Table 3). Generally, POM is more sensitive to management-mediated changes in the soil than total SOC (Cambardella and Elliott, 1992; Plaza-Bonilla et al., 2014). In accordance with this, our results showed significant improvement of POM and related parameters in topsoil layers by conservation management practices (Fig. 3c, Table 1). We also found POM related parameters (POM-C and fPOM) as the most robust predictors of topsoil SOC_{con} across the management treatments (Table 3). In the subsoil, the change in SOC_{con} was best explained by MBC and MAOM-C concentrations (MAOM-Cp) (Table 3). Plant residues or POM are progressively decomposed and converted to microbial by-products, and such microbial by-products are subsequently stabilized on the mineral surface to form MAOM fraction (Cotrufo et al., 2013). Previous studies have reported that 10–20% of the added aboveground litter is retained in the MAOM-C fraction (Haddix et al., 2020). With environmental changes (e.g., weathering activity via high rainfall) and management interventions (e.g., tillage activity), decomposition of crop residues and aggregate protected POM in the soil surface enhances, leading to increased leaching of DOC to the subsoil. This increased presence of DOC in the subsoil could increase microbial uptake of C to increase MBC and subsequent sorption on the soil mineral fraction (Sanderman and Amundson, 2008; Osanai et al., 2020).

4.3. Implications of C functional pools for SOC storage

We found that POM-C and MAOM-C exhibited different strengths of relationship with SOC_{con} in topsoil and subsoil depth intervals. A maximum POM-C concentration of ~6 g C kg⁻¹ soil was noted when SOC_{con} was 12–14 g C kg⁻¹ soil, indicating limited C storage in POM, and beyond this SOC_{con}, additional C storage was realized through MAOM accrual (Fig. 4). Although soil C saturation follows a hierarchical pattern from POM to MAOM pools (Gulde et al., 2008), we observed limited POM accumulation in our study, supported by the weak relationship between POM-C and SOC_{con} (topsoil slope = 0.29, subsoil slope = 0.13) (Fig. 4a and 4b). This low POM retention is likely due to our hot and humid climate, conducive to SOC mineralization over accumulation in agroecosystems (Franzluebbers, 2010; Jagadamma et al., 2019). This trend is also found in other ecological regions. For example, a review study including data from 67 agricultural sites concluded that, on average, POM-C contributed 22% of the total SOC (Gregorich et al., 2006). Another study based on European grasslands and forests concluded that although the C accrual potential of POM fraction is unlimited, the majority of the C is stored in the MAOM fraction with a saturation limit of 50 g SOC kg⁻¹ soil, indicating inherent high SOC accrual capacity as MAOM-C (Cotrufo et al., 2019). Unlike our study, these past studies have mostly ignored the effect of soil depth on the dynamics of SOC physical fractions in response to agricultural management practices.

Contrary to our POM-C results, accumulation of MAOM-C was unconstrained in our study, with MAOM-C dominating both in the surface soil (tillage zone) and subsoil (soil layers beyond tillage zone) SOC_{con} (Table 1). Consequently, we found a relatively stronger relationship between MAOM-C with SOC_{con} (Topsoil slope = 0.43, Subsoil slope = 0.71) (Fig. 4c and 4d). Increased accumulation of MAOM-C, especially in the subsoil, was likely a legacy effect of long-term conservation management practices, which increases C inputs in the subsoil through increased root biomass (Supplementary Table 2 and 3), and potential vertical movement of DOC through soil profile (Presley et al., 2004; Osanai et al., 2020). Across all treatments and depths, POM-C and MAOM-C contributed 11% and 85%, respectively, of total SOC in our study (Table 1). Our results reiterate that effective SOC accumulation strategies in agroecosystems require consideration of climate, soil, and ecosystem-specific management practices. For example, implementing no-tillage alone may not be sufficient to increase whole-profile SOC stock compared to tilled systems; instead, it may redistribute the SOC near the soil surface. However, if more than one conservation practices are adopted in tandem, such as growing winter cover crops along with NT adoption, particularly in regions with hot and humid climatic conditions and/or low native SOC content, it can not only improve surface soil SOC accumulation but can also distribute more C to the subsoil.

5. Conclusion

Our results demonstrated that adopting long-term conservation management practices such as NT and cover cropping differently influenced the topsoil and subsoil SOC stocks. NT increased SOC accumulation in the surface soil. While NT increased SOC accumulation in the surface soil, profile SOC accumulation was more impacted by cover crops irrespective of tillage. In particular, the addition of a grass cover crop increased SOC stock in the subsoil. While the MAOM fraction generally revealed more C storage capacity than the POM fraction, we found that POM and MAOM-related parameters drove total SOC differently in topsoil and subsoil. Although POM and MAOM fractions responded positively to conservation management practices, POM-C had greater control over topsoil SOC storage, whereas the MAOM fraction had more control over subsoil SOC storage. Our results indicate the need to develop soil and environment-specific cropping systems by integrating complementary conservation management practices to increase profile-scale SOC storage. The results also stress the need for more data

on subsoil SOC dynamics in response to depth sensitive SOC management strategies to help develop policies to mitigate greenhouse gas emissions and foster healthy soils.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2022.105521.

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