

THESIS

CROPPING SYSTEM, SITE AND TOPOGRAPHIC IMPACTS ON DEEP SOIL CARBON
DYNAMICS IN NO-TILL DRYLAND AGROECOSYSTEMS

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Carolita E. Landers

Department of Soil and Crop Sciences

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Master's Committee:

Advisor: Steven J. Fonte

Co-Advisor: Meagan Schipanski

Joe von Fischer

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ABSTRACT

CROPPING SYSTEM, SITE AND TOPOGRAPHIC IMPACTS ON DEEP SOIL CARBON DYNAMICS IN NO-TILL DRYLAND AGROECOSYSTEMS

Long-term research of no-till management in the US Great Plains has shown that increasing cropping intensity can potentially increase soil organic carbon (SOC) and crop yields compared to traditional winter wheat (*Triticum aestivum L.*)-fallow management systems. However, due to varying climate and topographical factors, SOC accrual rates may change with time and soil depth. We sampled SOC in a long-term experiment 36 years after its establishment. The study is located across three sites in eastern Colorado, and it characterizes a gradient of potential evapotranspiration (PET) with multiple slope positions at each site. Thus, the objectives of this study are to understand 1) the effects of different crop rotations and cropping system intensification on SOC after 36 years in the surface soil in a no-till, dryland system., and 2) how climate and topography influence SOC and SIC dynamics in deeper layers (> 20 cm) and potentially interact with management. The cropping rotations examined were wheat-fallow, wheat-corn (*Zea mays L.*)-fallow, continuous summer cropping and a grass strip to represent the Conservation Reserve Program, all planted across three sites with similar annual precipitation but increasing PET and a slight slope gradient. We found cropping intensity, slope position, soil depth, and site (PET) all independently impacted SOC and SIC concentration (g kg^{-1}) and stocks (Mg ha^{-1}). Aside from the perennial GRASS treatment that consistently had higher SOC to depth, the management effect was seen most pronounced in the surface layers of the soil, but beyond 20 cm, SOC and SIC were influenced more by site and slope. As previously seen, the toe slope

accumulated the most SOC in the surface layers; however, it did not persist in the deeper layers where the summit and side slope positions accumulated more. The findings of this research contribute to addressing the information gap surrounding deep SOC and SIC dynamics and their interactions with climate and management of no-till in dryland agroecosystems. We revealed that while the surface soil SOC responds to intensification, deeper SOC and SIC layers show a more complex interplay between climatic and topographic factors. These insights are crucial for developing sustainable agricultural practices and enhancing carbon sequestration to inform climate change mitigation strategies in the Great Plains.

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INTRODUCTION

Crop production in the Great Plains region of North America has faced numerous challenges since the first European colonizers began farming this region (Peterson et al., 2020). Limited water in the region led to dryland farmers to adopt bare fallows between cropping phases as a means to collect water in the soil profile. However, this practice is inefficient at capturing and retaining precipitation (Peterson et al., 2020). Furthermore, leaving the soil bare for extended period of time, combined with frequent tillage to manage weeds, resulted in soil degradation due to loss of soil organic carbon (SOC) and wind erosion. The development and adoption of no-till farming since the 1980s has helped to address issues of soil degradation and inefficient water use, thus allowing for intensification of cropping rotations (i.e., reducing the frequency of bare summer fallows and adding more crops into annual rotations) and supporting improved resilience in this vulnerable farming region (Peterson et al., 2020). The role of no-till and system intensification have been the subject of considerable research in recent decades due to beneficial impacts on soil water dynamics and SOC (Lehman et al., 2017; Hansen et al., 2012).

Improved SOC with no-till and cropping system intensification is noteworthy, as SOC supports many soil functions and yield stability (Robertson et al., 2018; Osborne et al., 2020; Kane et al., 2021). The total amount of SOC in soil is driven by abiotic, biotic and management factors (Jackson et al., 2017). Abiotic factors such as climate, soil texture, and topography interact with biotic factors, including plant productivity (quantity and litter quality) and the soil biological activity (Castellano et al., 2015; Jackson et al., 2017) to influence soil C inputs and decomposition rates (Burke et al., 1989, 1999). Management factors like tillage, crop choice,

residue retention, and fertilizer application further influence SOC within the confines of a particular abiotic and biotic environment (Lal et al., 2004).

Long-term research in the US Great Plains has been instrumental in advancing our understanding of how agricultural management practices impact SOC and the long-term sustainability of dryland cropping systems, especially in a vulnerable post dust-bowl farming era (Peterson et al., 2012). In this region, potential evapotranspiration exceeds precipitation during much of the year, affecting water capture and retention and the timing of water availability to growing crops. While the inclusion of bare fallows within winter wheat cropping systems can increase water storage and stabilized yields, this practice does not fully take advantage of the available precipitation, thus resulting in low annualized yields and continued soil degradation. Peterson and Westfall (2004) reported that dryland no-till systems with fewer summer fallow periods increased grain water use efficiency by 27% compared to wheat-fallow (WF) systems. Their research and others have informed a change in dryland practices in much of the Great Plains region. For example, Rosenzweig and Schipanski (2019) reported that over nine years, between 2008 to 2016, summer fallow was reduced from 1.8 to 1.3 million hectares (48% to 33%) in dryland cropping systems of the Great Plains region, suggesting important implications for climate change mitigation.

The Great Plains hold significant potential for global carbon storage, but it is also at risk due to past SOC losses and projected future losses due to climate change and land use changes (Follett et al., 2012). Ongoing climate warming and aridification threaten to exacerbate SOC losses in dryland soils due to increased extreme temperature and shifting precipitation patterns (Díaz-Martínez et al., 2024). Maintaining SOC stocks in the Great Plains depends on a delicate balance between plant productivity and decomposition processes, which in turn are all affected

by soils, climate and management (Follett et al., 2015). Given that cropland soils often contain considerably less SOC than grassland soils (Beniston et al., 2014), this suggests that maintaining more continuous vegetation cover in cropping systems could help restore SOC (Follett et al., 2015). Increasing intensity of crop rotations beyond the traditional wheat-fallow system can help achieve this goal, by enhancing carbon inputs and SOC accrual in dryland systems. In agreement with this idea, Robertson et al (2018) simulated SOC changes for different no-till cropping systems and predicted that SOC sequestration rates increased with cropping intensity, with grasslands supporting the highest SOC value.

While SOC dynamics have been extensively studied in the C cycling of terrestrial systems; the soil inorganic carbon (SIC) pool is often ignored, but may be quite important as either a source or sink of atmospheric CO₂ depending on the context and often overlooked in soil carbon research (Raza et al., 2024). In dryland regions, high evapotranspiration paired with moisture deficiency promotes the formation of SIC (Pilli et al., 2023). It is estimated that soil carbonates store about one-third of the total global soil C stock in the top 1 meter of profile, playing an important role in the global C cycle (Lorenz & Lal, 2018). Most SIC accumulation can be attributed to climate and pedogenic formation; however, human decisions can further cause sequestration of SIC indirectly through management effects on soil moisture dynamics and acidification of soils from amending using fertilizers that contain Ca²⁺ and Mg²⁺ (Raza et al., 2021; Lorenz & Lal., 2018). Given the importance of both SOC and SIC in the global carbon cycle, a more holistic approach to understanding carbon sequestration in the Great Plains is needed, especially for deeper soils that have received considerably less attention and likely contain more SIC.

Deep soil horizons account for over 50% of the global soil carbon stock (below ~20 cm) (Gross & Harrison, 2019; Rumpel & Kögel-Knabner, 2011). Deep soil C stocks vary spatially and are typically influenced by climate, topography, and pedologic traits more so than management (Lal, 2014; Rodrigues et al., 2023). Additionally, the radiocarbon age of SOC increases with depth, suggesting SOC is stored longer and is slower to decompose in the deeper layers than in topsoil (Gross & Harrison, 2019; Kirschbaum et al., 2021). Despite its importance, increased stability, and longevity, few studies consider SOC dynamics below 30 cm (Jandl et al., 2014). Thus, to understand how to enhance C storage to its full potential in the U.S. Great Plains, more investigation into deep soil carbon, considering both SOC and SIC, is crucial. Given the rapid growth carbon accounting and marketing, and the need to find viable mitigation strategies in agriculture, it is paramount to investigate deep soil dynamics more thoroughly and expand reliable data on climate-carbon feedbacks for sustainable development.

The Dryland Agroecosystem Project (DAP), established in 1985, was designed to explore how cropping system intensification (under no-till) interacts with climate and topography to influence soil properties, water dynamics, and profitability (Peterson et al., 1993). More specifically, this experiment employed a holistic agroecosystem approach, to understand how the practice of limiting or replacing summer fallow in no-till systems enhances water use efficiency and protects the soil from evaporative losses and erosion, thus increasing productivity and inputs of carbon to the soil (Peterson et al., 1998).

Results from the first 12 years of DAP supported the hypothesis that increased cropping system intensification improves water use efficiency and profitability, while enhancing SOC, at least in the surface (0-5 cm) soil (Peterson et al., 1998; Sherrod et al., 2003, 2005). No-till continuous cropping (CC) systems, though constrained by their C inputs to the soil due to

biomass removal and the limited precipitation environment, show potential in reducing carbon emissions through enhanced water retention, decreased soil erosion, and enhanced SOC sequestration (McGee et al., 1997; Schnarr et al., 2022; Del Grosso et al., 2002). Sherrod et al. (2003) reported a 20% increase in SOC from wheat-fallow to the continuously cropped system in the 0-10 cm soil layer. After 24 years, SOC levels in the CC rotation did not increase significantly relative to WF but maintained similar differences despite 12 years (1998-2009) of frequent drought (Sherrod et al., 2018). Results from the DAP experiment also suggest benefits of cropping system intensification on soil structure, aggregate stability, and biological activity in the surface (0-20 cm) soils (Peterson et al., 1993, 1998; Wood et al., 1990; Sherrod et al., 2003, 2005, 2018; Rosenzweig et al., 2018; Kelly et al., 2020).

Despite the reported benefits on SOC and other soil attributes in the surface (0-20 cm) layers, impacts on deeper soil layers and SIC have largely been ignored, yet may offer valuable insight regarding the overall impact of management and environmental drivers on soil carbon storage. Thus, the objectives of this study were to: 1) understand the effects of cropping system intensification on SOC after 36 years in the surface soil in a no-till, dryland system, and 2) elucidate impacts of climate and topography on SOC and SIC dynamics throughout the soil profile and evaluate potential interactions with management. We leveraged this 36-year-long trial to expand on the extensive body of literature that discusses the effects of intensifying cropping systems, climate, and topography on SOC and SIC accumulation. Additionally, we provide insights into how these factors influence the dynamics of deep C dynamics in no-till dryland agroecosystems.

METHODS AND MATERIALS

Site & Study Design

This research leveraged a long-term, no-till dryland agroecosystem experiment conducted in partnership with local farmers across the Great Plains region of Eastern Colorado. The experiment was established on-farm at three sites (Table 1) in the fall of 1985 to evaluate the effect of cropping system rotations, topography, and climate on crop yield, water dynamics, and soil properties (Peterson et al., 1993). The field sites are located along a potential evapotranspiration (PET) gradient from north to south, with Sterling having the lowest PET and Stratton, intermediate, and Walsh having the highest PET. All three sites are located within a shortgrass steppe biome along a gentle slope and had been managed for at least ten years as a conventionally tilled wheat-fallow (WF) rotation prior to study establishment.

Slope Position and Cropping Systems

Each site contained five cropping system treatments representing different rotations: 1) winter wheat-fallow (WF), 2) winter wheat-corn-fallow (WCF), 3) forage sorghum-hay millet (Fs-HM), 4) winter wheat-forage sorghum-hay millet (W-Fs-HM), and 5) continuous cropping (OPP; where an opportunity crop is designated annually based on weather and market factors) as well as a perennial grass treatment (GRASS), as a reference. To understand the effects of topography, cropping system plots were oriented along the slope (perpendicular to the contour) at each site so that each treatment plot (or strip) contained three slope positions, all less than 5%. All rotations were present in all phases each year, except for OPP, since this treatment had no set rotation (Table S1). Treatment-phase combinations were randomly assigned to 12 strips (6.1 m

wide by 185 to 300 m long, depending on the site) and replicated twice in two blocks at each site, resulting in 24 strips per site. Treatments 1-3 represent a gradient of cropping intensity ranging from one crop every other year (WF) to two crops every 3 years (WCF), and finally, one crop every year (OPP) (Robertson et al., 2018; Sherrod et al., 2005). The lower intensity (WF) had more frequent bare fallow in the summer (rainy) season, while the high-intensity OPP rotation had no summer fallow. The forage-based treatments (Fs-HM and W-Fs-HM) were initiated in 2010 due to the potential flexibility forages can offer producers to produce a viable crop in drier years that isn't vulnerable to total grain crop failure, and to examine the effects of biomass removal on SOC. Before the last 12-years, the forage-based treatments were under consistent management as wheat-corn-millet-fallow systems from 1985-1997 and then wheat-wheat-corn-millet systems from 1998-2009. The perennial grass treatment was established in 1985 by planting an equal mixture of crested wheatgrass, western wheatgrass, side oats grama, little blue-stem, blue grama and buffalograss. The grass treatment serves as a baseline measurement and simulates fields under the USDA Conservation Reserve Program (CRP) treatment. Additional information on each site, their soil texture and slope percentages, and experimental design can be found in Peterson et al. (1993) and Sherrod et al. (2003, 2005).

All crops were seeded using no-till planters. Weeds were managed using herbicide applications as needed throughout the growing season, and fertilizer was applied based on annual available soil tests for N and P and/or previous crop yields (Sherrod et al., 2003).

Soil sampling and analyses

In May (prior to summer crop planting) and July (following wheat harvest) of 2021, we collected two composite cores per slope position within each strip. Samples were collected to a

depth of 80 cm using a tractor-mounted hydraulic Giddings probe with a 1 m long core (~4.2 cm dia.; Giddings Machine Company, Windsor, CO), and each core was divided into six depth intervals of 0-5, 5-10, 10-15, 15-20, 20-40, and 40-80 cm. Soil samples were transported in coolers to Colorado State University and air-dried in the lab. Coarse organic material (plant litter, roots > 2 mm) and rocks were removed by hand, and all soil was sieved to 2 mm and then ground for subsequent analyses. With three sites, two blocks per site, 12 strips per block, three slope positions per strip, and six depths, we collected a total of 1296 samples (Table S1).

Bulk density measurements were taken at each slope position within each replicate strip adjacent to the soil cores (Table. S1). Sharpened metal cylinders (6.7 cm dia. x 5 cm long) were inserted vertically for the 0-5 and 5-10 cm depths. A Madera bulk density probe (3.3 cm diam x 6.8 length; Dickey et al., 1993; Precision Machine Co., Inc.) was used for the 10-20 cm depth. Bulk density for the 20-40 cm and 40-80 cm depths was calculated based on the mass of the cores taken with the Gidding's probe, as these layers are generally more challenging to sample and less prone to compaction in no-till systems. All soil samples were placed in sealed plastic bags for transport to the lab. In the lab, a subsample of each soil was oven-dried at 105 °C for the determination of gravimetric water content and bulk density.

Ground subsamples (~250 mg) of soil from each depth were analyzed for total C and N using a LECO elemental analyzer (LECO Corporation, St. Joseph, Michigan, US). Inorganic C was measured using the modified pressure-calculator method as detailed by Sherrod et al. (2002), which involves reacting 1.0 g of soil with HCl and calculating CaCO₃ based on pressure change with a modified volt meter. SOC was determined at each depth by subtracting inorganic C from total C. SOC stocks (Mg C ha⁻¹) were calculated by multiplying the SOC concentration by the soil bulk density at each corresponding depth and the thickness of the soil

layer being considered. In the case of missing or outlier bulk density measurements, we used values of the adjacent treatment strips with similar management (crops vs. perennial grass).

Statistical Analysis

Analysis of variance (ANOVA) was used to compare sites, slope positions, rotations, and depths, with all these factors and their interactions being treated as fixed effects. To account for the nested structure inherent with treatment rotation strips occurring within blocks, slope positions within strips, and soil depths within cores (positions), block, strip, and slope position were also included within the model as random effects. To ensure that the assumptions of ANOVA were met, we applied log transformations to both SOC concentrations and stocks, and significance was tested at an alpha level of 0.05. When analyzing SIC the model assumptions were not satisfied due to the large number of zeros in the dataset, and the data was square root transformed prior to analysis as the best simple transformation. We also compared shallow vs. deep SOC stocks (i.e., 0-40 vs. 40-80 cm) with just two levels of depth considered in the model, and also SOC stocks in the surface layers with four depths included (0-5, 5-10, 10-15, 15-20 cm). When comparing SOC stocks (i.e., the sum of all depths as one soil profile, 0-80 cm), depth was removed from the model. We conducted an outlier analysis and removed several extreme SOC values, especially from the deep soil layers, where we suspected an incomplete reaction of the CaCO_3 in a few samples. The final dataset excluded SOC values above 3% in soil depths below 40 cm (i.e., twelve samples excluded). All data was analyzed using R software (version 2023.12.1+402).

RESULTS

Soil organic and inorganic carbon concentration

In the surface soil layers (0-20 cm), SOC concentration was influenced by rotation, site, depth, and slope (Table 2). The GRASS treatment had higher SOC concentrations than WF, WCF, and W-Fs-Hm, OPP rotations (57%, 7%, and 8%, 22% greater, respectively, Fig. 1; Table 2). Rotation system did not interact with site or slope, however, we observed a marginally significant interaction with depth in the top 20 cm as the differences between rotation systems decreased with depth ($p=0.051$, Fig. 1, Table 2). Site and slope interacted such that the toe slope had higher SOC in Stratton and Walsh but not in Sterling, where SOC was highest in the side slope position ($p=0.038$, Table 4, Fig. S2). Walsh had less SOC across all slope positions. Slope and depth had a significant interaction where the toe slope presented more SOC in the top 15 cm, while the side slope began to trend higher in the 15-20 cm layer (Fig. S1). Lastly, we found an interaction between site and depth ($p < 0.001$, Fig. S1). At Stratton, SOC was consistently higher at shallower depths (particularly at 5 cm) and gradually decreased with depth, but it still maintained relatively higher SOC levels across all depths compared to other sites. Sterling showed more variation in SOC at the shallowest depths (5 and 10 cm). Walsh, however, had the lowest SOC overall, with smaller differences in SOC between depths, with sensitivity to depth changes compared to Stratton and Sterling.

When considering all soil depths down to 80 cm, site, rotation, and depth influenced SOC concentration (Table 2). The GRASS treatment had higher SOC than all the other treatments, but no differences were observed among the cropping systems (Fig. 2). Slope position did not directly influence SOC. However, there was an interaction between slope and depth (Fig. 3) as well as site and depth (Fig. 4). The decrease in SOC with depth was more pronounced in the toe

slope than at other slope positions. The side and summit slope positions had higher SOC concentrations within the 40-80 cm depth than the toe slope (Fig. 3). Site interacted with soil depth ($p < 0.001$, Fig. 4; Table 2), because Stratton had the highest SOC until Sterling surpassed it in the 40-80 cm depth. Walsh, characterized by the highest PET, exhibited lower SOC concentrations than the other two sites across all depths in the soil profile (Fig. 4). In contrast, Stratton with medium PET (and the highest clay content), had the highest SOC concentrations across the complete profile. Site and slope interacted, such that Stratton consistently showed the highest SOC level, particularly in the summit and side slopes, while Sterling demonstrated more moderate SOC levels. In contrast, Walsh had the lowest SOC concentrations across all slope positions with minimal variation between summit, side, and toe slopes (Table 2, Fig. S2).

The amount of SIC was mainly affected by slope position and soil depth (Table 2). The side slope had the highest concentration of SIC, followed by the summit and toe slope (Fig. 5). The relationship between SIC concentrations and depth were opposite to SOC, with higher amounts in deeper depths and decreasing in surface layers and having least in the GRASS treatment. Slope and depth interacted, with the toe slope consistently having less SIC than the summit and side slope across the entire soil profile. The side slope position generally demonstrated higher SIC until the 40 cm depth, while the summit position tended to have higher values below 40 cm (Fig. 5). The site and slope interacted, with the summit and side slopes generally having more soil SIC, particularly in Sterling and Stratton, where the side slope showed highest values (Fig. S6). Walsh, however, had highest amounts of SIC at the summit position. Site and depth also interacted, with Walsh consistently having more SIC at all depths until the 40-80 cm depth, where Sterling had the most.

Soil organic C stocks

Analysis of SOC stocks in the surface layers to 20 cm revealed simple effects of site, rotation, slope, and depth (Table 3). The GRASS treatment had 24% greater SOC in the top 20 cm, while OPP and Fs-HM were marginally greater (9% and 4%, respectively) than the WF rotation (Fig. 6). Stratton had greater SOC stocks than the other sites (Fig S3). We observed interactions between site and depth ($p < 0.001$ Table 3, Fig. S3), with Sterling showing a sharper decline in SOC at deeper layers than Stratton, and Walsh having lower and more uniform SOC distribution across all depths across all depths. Slope and depth ($p < 0.001$) interacted similarly to concentration where the toe slope had higher in SOC in surface layers. The summit and side slope became higher in stocks than the toe position in the 15-20 cm layer (Fig. S3). Site and slope interacted because Walsh had more C in the side slope than toe slope. At the same time, Sterling accumulated similar amounts of SOC in the side and toe, and Stratton accumulated most in the side and toe (Fig. S3).

When analyzing the data by grouping depths into two levels (0-40 and 40-80 cm), site and slope influenced SOC stocks (Table 3), and the rotation effect was weaker than in the surface (0-20 cm) soils ($p = 0.076$). The GRASS treatment continued to have higher SOC stocks down to 40 cm relative to the other rotation systems (Fig. S4). SOC stocks were higher in the summit and side slopes across both depths than the toe slope ($p = 0.002$; Fig. S5). The site by depth interaction revealed that Stratton had higher SOC in the 0-40 cm layer, while Sterling, had greater stocks in the 40-80 cm layer, and Walsh had the lowest SOC stocks across both layers ($p = 0.011$; Fig. 7)

Considering SOC stocks across the whole soil profile (sum of 0-80 cm), rotation no longer showed an effect ($p=0.123$). The most pronounced effect was site, ($p<0.001$) as Stratton had the highest C stocks, followed by Sterling and Walsh, respectively (Table 3).

DISCUSSION

SOC and C Stocks after 36 years

After 36 years, dryland, no-till, crop rotation effects on SOC were strongest in the top 20 cm and decreased with depth. Perennial grass strips had the highest SOC, and while cropping systems did not significantly differ from one another, the more intensively cropped systems (OPP and Fs-Hm) were the only two annual cropping systems with intermediate levels of SOC that did not differ from GRASS in the top 20 cm, aligning with findings from previous studies at these Dryland Agroecosystem Project sites. For example, Wood et al. (1990) at the 3.5-years after study establishment, Peterson et al. (1998), and Sherrod et al. (2003, 2005, 2018) at the 12 and 24-year marks also found that the conversion from WF to more intensified rotations increased SOC. Intensification effects were less pronounced at the 24 year mark after periods of intense drought (Sherrod et al., 2018), underscoring the importance of considering long-term precipitation patterns. In the past 12 years (2009- 2021), from the trial's 24-year to 36-year time frame, all sites have experienced drier springs, wetter falls, and a warming trend. Average temperatures, especially during summer months, have increased. For example, Sterling and Stratton have seen more frequent days with temperatures reaching or above 32 °C (Colorado State University, Colorado Climate Center). Our findings indicate that transitioning from WF to more intensified systems leads to a higher SOC. However, these intensified systems are not as effective as perennial grass systems in supporting SOC over longer periods with more variable weather patterns. Our study results also agree with the modeled predictions of SOC changes under future climate scenarios using similar rotation treatments and soil C data from the DAP plots (Robertson et al., 2018). Perennial grass systems were predicted to continue gaining SOC well into the future while annual cropping systems all decreased under future climate scenarios.

The more intensive OPP system was predicted to sustain SOC longer into the future than WF (Robertson et al., 2018).

The subtle rotation effects on SOC may also be due to the establishment of a new steady-state SOC levels following the initial transition from the previously tilled, wheat-fallow systems. Stewart et al. (2007) discuss the concept of a new equilibrium for SOC where consistent carbon inputs lead to a balance over time. Considering that the DAP plots have had roughly consistent C inputs in the cropped treatments for 36 years, it is plausible that the soil C content of the top 20 cm has approached a new steady state level within the confines of annual crop productivity and associated residue return to the soil (Stewart et al., 2007). The comparison to the perennial grass treatment tells us that the soils are capable of higher SOC levels. However, given the combination of climate (i.e., water) limitations, soil type, local agricultural practices, and farmer economic considerations, the SOC values reported may reflect the upper limit of what these cropped systems can realistically achieve.

While the differences in SOC among annual cropping systems are not statistically different, the more intense rotations (Fs-HM, W-Fs-HM, OPP) generally supported more SOC in the surface 20 cm than rotation systems with fallow (WF and WCF). Engel et al. (2017) observed similar effects in the top 30 cm of the profile when comparing SOC accumulation across eight cropping systems, with a cropping intensity spectrum ranging from traditional WF to a grass mix in CRP under no-till management near Bozeman, Montana, over 10 years. The more intense systems (e.g., wheat-pulse-grain) had 30% higher net primary productivity and higher SOC than tilled wheat-fallow due to the greater C inputs. Similar to our research, the first decade after establishment had the largest differences in SOC, followed by a stabilization, highlighting the

value of long-term trials for understanding management impacts and limitations in agricultural systems.

The continuous forage rotations in our study were incorporated in the last 12 years of the long-term trial. These treatments had previously been in a continuous wheat-wheat-corn-millet rotation during the first 24 years of the study. The decision to integrate forage rotations into the experiment was due to their potential to reduce the risks of crop failure relative to summer grain crops. Intensifying the rotation by incorporating a summer crop like forage sorghum or hay millet not only adds C inputs to the soil via live roots but also diversifies the rotation, using a crop with greater precipitation use efficiency compared to cereal grains (Holman et al., 2020). Continuous cropping with summer forages represents an opportunity for farmers to diversify their income and incorporate a crop that is not as vulnerable to failures in dry years. According to the USDA, the US sorghum industry has seen significant growth, with production surging 69% in 2023 compared to 2022. While that number reflects both grain and forage, the expanding adoption of sorghum suggests it is an increasingly popular crop farmers can integrate to manage risk and intensify their systems. Our results indicate that the integration of summer forage crops, where most of the aboveground biomass is removed with harvest, can maintain SOC levels similar to annual grain crops in dryland systems.

Cropping intensification has also provided a wide range of soil health benefits beyond SOC. For example, Rosenzweig et al. (2018) found a 17% increase in SOC in surface soils between WF and OPP rotations that corresponded to 2 to 3-fold increases in soil aggregate stability and microbial biomass. Kelly et al. (2020) found greater wet aggregate stability and macrofauna densities in surface soils with greater cropping system intensity at the Sterling and Stratton sites. Similar to our study, they found that the perennial grass treatment outperformed

the cropping strips in most soil health metrics. Greater organic matter input from intensified cropping systems supports increased biological activity (i.e., macrofauna, fungi, and bacteria) that, in turn, supports improved soil aggregation and related soil properties (Rosenzweig et al., 2018). Aggregates can physically protect OM, slowing its decomposition and supporting SOM accrual. The enhanced soil structure and hydraulic properties resulting from increased SOM and surface aggregation improve access to water for crops due to lower bulk density, higher porosity, and enhanced water capture (Shaver et al., 2002; Franzluebbers et al., 2002; Farahani et al., 1998). This improved water capture supports higher plant productivity, adding more OM to the system while reducing the potential for surface runoff and water erosion, thus creating a positive feedback loop for soil health. Intensified cropping systems may not contribute as much SOC compared to grass due to increased disturbance, shallower root systems, and the removal of biomass during harvest, but partnered with no-till they play a crucial role in improving soil health by increasing ground cover, limiting soil erosion, and increasing more biomass into the system than the traditional WF (Peterson et al., 1998).

The rotation effects on SOC in the surface layers of the soil did not extend to deeper soil depths, with the exception of the GRASS treatment strips. Deep soil carbon is thought to account for up to 50% of global terrestrial stabilized carbon but seems to be less affected by management practice below 30 cm compared to other pedogenic drivers, as mean annual temperature and soil type are some of the best predictors of SOC distribution (Rumpel and Kögel-Knabner., 2011; Morari et al., 2019). While plant production and decomposition also determine SOC input, these effects are often concentrated in surface soils, impacting the distribution of soil carbon with depth (Jobágyy and Jackson., 2000). Rooting depth is an important contributor to the vertical distribution of SOC because roots have a higher conversion efficiency to SOC than aboveground

C inputs (Rasse et al., 2005; Berhongaray et al., 2019). Given that the majority of root biomass in annual cropping systems occurs within the top 30 cm; it makes sense the benefit of intensification may not persist into deeper layers. This limits deep SOC in annual cropping systems compared to systems with perennial vegetation that have year-round and deeper root infrastructure (Lorenz and Lal., 2005). This can explain why we observed most SOC accrual in the top 20 cm which is the dominant rooting depth, and where surface residues are deposited for annual crops. The GRASS had more SOC in deeper depths, which could be attributed to the deeper and long-term persistence of the roots that is characteristic of perennial plants. More research on incorporating perennials or similar alternative crops like Kernza could help elucidate the maximum potential for dryland agroecosystems to sequester carbon in deeper layers.

Site and topographic impacts

While management effects on SOC were more pronounced in the surface layers, site and slope were more important drivers of SOC distribution deeper in the soil profile. The SOC in the toe slope position decreased more quickly with depth than the other slope positions, such that the average SOC values in the toe slope were well below that observed for the summit and side slope positions below 20 cm. In contrast, SOC was more evenly distributed across the whole profile for the summit and side slope positions. We suspect that the lower SOC in deeper layers of the toe slope could be due to greater water and sediment accumulation in the toe slope associated with runoff during heavy precipitation events. Greater water inputs results in more growth and C return from aboveground biomass, while sediments tend to come from SOC rich surface layers, thus further enriching the surface SOC concentrations in the toe slope. Fissore et al. (2017) found that moderate slopes (<15%) with concave curvature led to more SOC accumulation across sites

sampled in Southern California grasslands due to the contribution of eroded upslope materials. The high SOC in surface soils at the toeslope may also be related to shallower roots as water is more available in the surface layers. With roots being the primary contributors to organic material in the soil through exudates and litter material for decomposers (Jobbágy and Jackson 2000; Jackson et al., 2017), this could explain the decrease of SOC beyond 20 cm in the toe slope position.

We observed large differences in SOC concentrations and stocks across the three sites. Walsh had the lowest SOC overall, and we suspect that this is due to at least in part to the coarser textured soil at this site and higher potential evapotranspiration. Sandy soils tend to have larger pores that lead to quicker drainage, less organic matter retention and lower surface area to form mineral associated soil organic matter (SOM) (Six et al., 2002; Plante et al., 2006). The high PET at Walsh increases the rate of soil moisture loss, further reducing favorable conditions for crop growth. Thus low SOC content at Walsh is also likely to be associated with lower organic matter input due to lower productivity. Sherrod et al. (2003) report that annualized stover production is 50% less in the high PET site (Walsh) compared to low PET site (Sterling). The decomposition of OM is less efficient with lower plant productivity and quality and quantity of litter partnered with increased temperature (Conant et al., 2011; Kleber 2010). Results at the Stratton and Sterling sites likely reflect important interactive controls of climate and soil texture on SOC dynamics. Stratton's higher SOC down to deeper depths may be related to the higher clay content compared to Sterling (Sherrod et al., 2003). Higher clay content in soils generally allow for better nutrient and moisture retention which in return promotes SOM accrual. Silt and clay not only protect microbial carbon from decomposition but also enhance and stabilize SOC in dryland soils (Mao et al., 2024; Six et al., 2002). Climate influences various biogeochemical

processes in the soil and in turn will affect organic matter input, accumulation and decomposition rates (Cotrufo and Lavelle 2022; Paustien et al 1996). Our results further align with Balesdent et al. (2018) reporting subsoil carbon response to PET is stronger than mean annual temperature and land use alone. Grey et al. (2016) studied factors controlling SOC stocks with depth across different climate regimes in Eastern Australia, and similarly found that beyond the 30 cm depth, climate appears to be the primary controller of deep SOC, with parent material and vegetation cover having less effect. They found uniform trends of increasing SOC stock with increasingly moist climate and vegetative cover. Stratton being the medium PET site combined with high clay content may cause for more favorable deep SOC accumulation. However, Stratton trended to have most SOC only down to the ~50 cm depth, where then, Sterling, the lowest PET site accumulated more SOC at the deepest depths. Mirroring that finding, our main effect of SOC stocks for the full soil profile (0-80 cm) was also site. These results imply that SOC accumulation through the profile (0-80 cm) is primarily driven by abiotic factor of climate (PET) over, soil type and mineralogy.

The summit and side slope positions had higher concentration and stocks of SIC than the toe slope. As mentioned above, the hydrology varies across the slope positions, which can have a large influence on SIC precipitation and dissolution reactions. Our research supports the understanding that the summit slope position tends to have less available soil moisture, contributing to reduced leaching that can lead to greater carbonate accumulation (Nyachoti et al., 2019; Manning et al., 2024). This could also explain the site differences in SIC where Walsh, the lowest PET site, contained the highest proportion of SIC of the total C stock containing 24% and Sterling and Stratton containing 20% and 22% respectively. The summit slope also experiences higher wind erosion forces that could also enhance carbonate precipitation and SIC formation

(Xin et al., 2023). The large contribution of SIC to total soil C stocks in semi-arid systems is an often-overlooked component of the soil C cycle and the stability of SIC stocks in semiarid ecosystems, are essential to consider in discussions of soil C sequestration as a climate mitigation strategy.

CONCLUSION

The benefits of transitioning from WF to more intensified rotations have persisted over time but not increased significantly. The long-term effects of crop rotation on SOC accumulation might be subtle and less pronounced over time and in deeper depths, however the conversion from WF to more intensified systems offers benefits of increased water infiltration, aggregate stability, biological activity, C sequestration and less soil erosion, cannot be overlooked in a region that is severely sensitive to mismanagement. Furthermore, when considering future adaptations to climate change, it is paramount to consider the limitations in SOC accumulation agroecosystems have at depth and when compared to perennial cover. Our results along with previous research from the same experiment, confirm management has the strongest effects on SOC in the surface soils and highlight the complex relationship between climatic factors and topography in determining SOC distribution and accumulation beyond 20 cm in no-till dryland cropping systems.

Various environmental factors dominate the influence of C dynamics. For instance, slope position affects water availability and root penetration, and carbonate exposure leading to varying levels of SOC and SIC across different soil depths. However, the role of soil texture, as evidenced in Walsh's sandier soils and Stratton's higher clay content, cannot be overstated. It plays a crucial role in the retention and stability of both SOC and SIC at deep depths. Climatic conditions and topography position impact C dynamics, influencing the biogeochemical and soil hydrologic properties. Continuous cropping shows potential in enhancing SOC, but this potential could become more limited in the face of more extreme weather patterns. In a changing climate, quantifying the complete C stock in deep depths for carbon accounting or mitigation strategies

necessitates a holistic process-level approach that considers the weighted effect of climate and soil mineralogy.

Overall, our findings suggest that while intensifying cropping rotations and adopting no-till practices can offer significant benefits for surface soil health and C sequestration, more than these practices are needed to enhance SOC storage in deeper soil depths. This calls for a more nuanced approach to sustainable land management that is tailored to specific site conditions when considering the potential for C sequestration. This study emphasizes the importance of continued long-term research to fully understand and maximize the benefits of management practices on SOC, thereby supporting agricultural productivity and farm resilience in the Great Plains region. By contributing to a well-established and continually expanding body of knowledge, this research informs the development of sustainable agricultural practices critical for maintaining the long-term health, productivity, and resilience of vulnerable semi-arid ecosystems.

TABLES

Table 1. Site characteristics and soil properties for three long-term research sites across eastern Colorado, that form part of the Dryland Agroecosystem Project. Data is based on information provided by Peterson et al. (1993) and Sherrod et al. (2005)

Site	Classification	Pedon, Texture	Longitude	Latitude	Potential Evapo- transpiration (mm yr ⁻¹)	Mean Annual Temp. (°C)	Precip. (mm yr ⁻¹)
Sterling <i>Low PET</i>	Fine-loamy, mixed,mesic Aridic Argiustoll	Weld silt loam	40.37° N	103.13° W	1015	9.3	440
Stratton <i>Medium PET</i>	Fine-silty, mixed, mesic Aridic Argiustoll	Norka, silt loam	39.18° N	102.26° W	1270	10.8	415
Walsh <i>High PET</i>	Fine- loamy,mixed mesic Aridic Ustoll	Ascalon, sandy loam	37.23° N	102.17° W	1900	12.2	395

Table 2. ANOVA model P-value results testing the effects of site, slope position, cropping intensity, and soil depth on SOC and SIC concentrations (g kg^{-1}) in 2021 after 36 yrs under no-till management.

Parameter	Organic C Concentration		Inorganic C Concentration
	0-5, 5-10, 10-15, 15-20 cm	0-5, 5-10, 10-15, 15-20, 20-40, 40-80 cm	0-5, 5-10, 10-15, 15-20, 20-40, 40-80 cm
	<i>P</i> values		<i>P</i> values
Site	0.005	0.003	0.346
Slope	<0.001	0.250	<0.001
Depth	<0.001	<0.001	<0.001
Rotation	<0.001	<0.001	0.618
Site:Slope	0.027	0.050	0.157
Site:Depth	<0.001	<0.001	<0.001
Slope:Depth	<0.001	<0.001	<0.001
Site:Rotation	0.546	0.625	0.923
Slope:Rotation	0.884	0.814	0.695
Depth:Rotation	0.051	0.117	0.231
Site:Slope:Depth	0.168	0.062	<0.001
Site:Slope:Rotation	0.998	0.997	0.916
Site:Depth:Rotation	0.947	0.625	0.045
Slope:Depth:Rotation	0.307	0.275	0.650
Site:Slope:Depth:Rotation	0.312	0.559	0.603

Table 3. ANOVA model results testing the effects of site, slope position, cropping intensity, and soil depth on SOC cumulative stocks ($Mg\ C\ ha^{-1}$) and bulk density ($g\ cm^{-3}$) in 2021 after 36 yrs under no-till management.

	C Stocks			Bulk Density
	$Mg\ C\ ha^{-1}$			$g\ cm^{-3}$
	0-5, 5-10, 10-15, 15-20 cm	0-40, 40-80 cm	0-80 cm	0-5, 5-10, 10-20, 20-40, 40-80 cm
Parameter	P values			P values
Site	0.005	<0.001	<0.001	<0.001
Slope	<0.001	0.002	0.148	<0.001
Depth	<0.001	0.102	†	<0.001
Rotation	0.004	0.076	0.123	<0.001
Site:Slope	0.042	0.955	0.946	<0.001
Site:Depth	<0.001	0.012	†	<0.001
Slope:Depth	<0.001	0.418	†	<0.001
Site:Rotation	0.500	0.613	0.746	0.017
Slope:Rotation	0.959	0.404	0.983	0.890
Depth:Rotation	0.158	0.690	†	<0.001
Site:Slope:Depth	0.121	0.128	†	<0.001
Site:Slope:Rotation	0.997	0.749	0.977	0.750
Site:Depth:Rotation	0.934	0.406	†	<0.001
Slope:Depth:Rotation	0.405	0.960	†	0.999
Site:Slope:Depth:Rotation	0.804	0.987	†	0.989

†: value not available as depth was not present in the model when analyzing the sum of the profile.

Table 4. Mean SOC concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Sterling site.

Sterling (Low PET)							
	WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass	
	<i>Soil Organic Carbon g kg⁻¹ ± SE</i>						
	(cm)						
Summit	0-5	9.78±0.97	11.81±0.55	10.95±1.73	11.57±0.84	9.82±0.18	17.24±0.64
	5-10	7.31±0.27	6.97±0.018	9.20±1.43	7.09±0.19	7.66±0.24	9.91±0.13
	10-15	6.93±0.19	6.99±0.29	7.51±0.66	7.04±0.26	6.95±0.28	8.18±0.27
	15-20	6.63±0.42	7.15±0.42	7.51±0.74	7.08±0.21	7.58±2.07	7.60±0.32
	20-40	8.16±1.02	10.70±1.51	11.20±3.08	11.35±1.51	10.81±2.02	8.51±1.37
	40-80	13.15±1.62	11.51±1.39	11.53±0.80	13.90±1.60	8.88±1.60	15.69±0.52
Side	0-5	13.14±2.32	11.42±0.96	13.50±1.74	12.87±1.34	14.53±1.66	18.26±0.08
	5-10	8.10±0.33	9.57±1.70	9.51±1.22	9.12±0.96	11.84±3.64	13.56±1.31
	10-15	9.10±0.76	9.76±1.54	11.77±2.67	11.86±4.06	11.02±2.22	12.18±2.40
	15-20	9.96±1.54	11.47±1.90	12.37±2.96	9.90±1.71	12.18±3.36	10.78±1.50
	20-40	12.03±2.00	15.56±3.36	10.32±1.16	12.42±2.25	8.20±0.01	10.15±1.79
	40-80	14.83±1.87	10.30±0.71	10.72±0.91	11.98±1.41	8.62±1.21	11.72±1.86
Toe	0-5	14.01±1.99	15.89±1.78	12.59±2.44	16.80±0.67	19.85±0.85	28.14±5.26
	5-10	10.20±0.64	9.86±0.65	11.98±0.73	10.54±0.32	10.05±0.89	14.40±0.40
	10-15	8.74±0.60	9.09±0.17	9.52±0.25	8.93±0.10	9.25±0.43	11.47±0.23
	15-20	8.22±0.41	8.53±0.47	9.06±0.34	8.26±0.35	8.72±0.19	9.86±0.52
	20-40	8.02±0.52	8.13±1.30	7.19±0.19	6.65±0.27	7.43±0.14	7.44±0.42
	40-80	9.37±1.64	9.54±1.14	8.49±0.80	8.66±0.37	8.42±0.25	9.83±0.09

WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat-Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix

Table 5. Mean SOC concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Stratton site.

Stratton (Medium PET)							
		WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass
	(cm)	<i>Soil Organic Carbon g kg⁻¹ ± SE</i>					
Summit	0-5	12.93±1.58	15.16±1.63	12.36±1.93	14.10±1.25	15.85±2.95	27.05±1.65
	5-10	9.40±0.21	9.45±0.49	9.82±0.36	11.24±0.67	11.20±1.60	12.35±0.35
	10-15	10.08±1.83	8.93±0.83	9.84±0.70	10.09±0.83	8.38±0.67	9.77±0.73
	15-20	14.01±4.02	10.87±1.29	12.85±0.99	12.34±1.85	7.98±0.18	13.59±2.09
	20-40	12.07±3.58	14.24±1.36	11.98±0.42	14.18±1.84	14.59±3.11	21.11±1.61
	40-80	10.55±3.11	9.92±1.45	7.78±0.40	10.89±1.20	15.08±0.88	15.23±0.17
Side	0-5	12.06±2.06	12.63±1.33	13.71±2.50	15.66±1.20	18.89±3.39	13.19± NA
	5-10	10.03±2.00	9.40±1.19	9.68±1.35	10.15±0.80	14.15±5.71	19.29± NA
	10-15	12.80±4.70	9.16±1.29	8.46±1.72	9.26±1.12	12.91±6.25	16.29±4.49
	15-20	12.65±4.03	10.73±2.07	7.75±1.68	8.05±1.07	12.31±6.64	17.42±5.72
	20-40	12.65±3.01	10.92±1.49	7.61±1.07	7.45±1.37	11.06±5.60	17.37±3.77
	40-80	11.22±1.95	9.48±1.09	12.10±2.24	7.78±1.43	9.79±3.77	14.92±2.42
Toe	0-5	20.97±2.56	22.34±2.16	16.30±1.32	21.57±3.57	27.90±5.30	35.8± NA
	5-10	15.03±0.99	14.63±0.93	13.50±1.48	15.13±1.41	18.50±3.20	20.50±1.90
	10-15	11.56±0.48	11.24±0.73	11.18±0.76	12.78±1.52	12.84±2.44	12.00± NA
	15-20	8.88±0.48	8.34±0.38	10.42±0.92	10.23±1.15	11.80±2.90	9.06± NA
	20-40	8.80±0.73	7.91±0.37	8.70±0.84	8.33±0.70	9.87±1.03	7.91±1.47
	40-80	9.59±1.55	7.04±0.45	7.68±0.93	8.62±0.68	11.07±3.53	6.36±0.42

WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat- Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix

NA: not applicable (No standard error due to n=1 from outlier exclusion)

Table 6. Mean SOC concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Walsh site.

Walsh, CO (High PET)							
	WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass	
	<i>Soil Organic Carbon g kg⁻¹ ± SE</i>						
	<i>(cm)</i>						
Summit	0-5	5.50±0.89	6.89±1.21	6.20±0.31	6.87±1.41	6.83±2.57	10.09±0.78
	5-10	6.82±1.62	5.50±0.93	7.50±1.61	5.79±0.51	8.37±5.32	10.02±0.13
	10-15	7.62±0.65	5.97±0.95	8.52±1.11	5.49±0.49	5.31±0.49	7.92±2.07
	15-20	8.20±1.03	6.86±1.74	9.44±0.97	6.57±0.94	3.89±2.22	7.41±0.29
	20-40	7.22±1.76	8.11±1.48	8.68±1.38	8.63±0.77	6.70±0.83	9.81±0.69
	40-80	6.37±1.43	5.56±1.16	6.42±1.29	7.16±0.87	6.57±0.23	6.66±0.32
Side	0-5	5.34±0.34	6.65±0.40	8.14±0.50	6.21±0.82	4.63±0.40	11.05±0.42
	5-10	6.28±0.83	9.80±3.37	6.29±1.08	7.03±0.65	4.68±2.36	8.35±1.34
	10-15	8.05±0.71	6.17±1.18	9.10±1.17	6.95±0.36	7.86±0.14	8.31±1.56
	15-20	8.69±1.31	7.04±1.32	9.79±1.56	7.65±0.50	7.17±0.37	7.07±0.58
	20-40	8.78±1.75	5.70±1.29	9.01±1.46	5.77±0.79	7.72±1.13	8.98±0.78
	40-80	8.37±1.25	6.12±0.97	6.81±1.13	5.71±0.74	9.91±2.56	6.11±0.43
Toe	0-5	8.60±1.19	9.58±1.67	10.16±1.89	8.04±0.75	12.14±4.04	10.04±1.19
	5-10	7.23±0.29	7.49±1.45	7.95±0.18	6.93±0.37	6.28±0.19	8.02±0.16
	10-15	6.14±0.41	7.21±1.59	6.75±0.40	6.05±0.28	9.27±3.84	10.33±0.35
	15-20	5.76±0.24	6.52±0.80	5.99±0.43	5.47±0.29	9.09±3.04	10.30±3.00
	20-40	5.02±0.22	5.37±0.32	5.14±0.07	4.93±0.33	3.84±0.24	8.94±0.87
	40-80	5.62±0.26	5.09±0.33	4.67±0.47	6.24±1.10	7.24±1.06	6.92±0.15

WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat- Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix

FIGURES

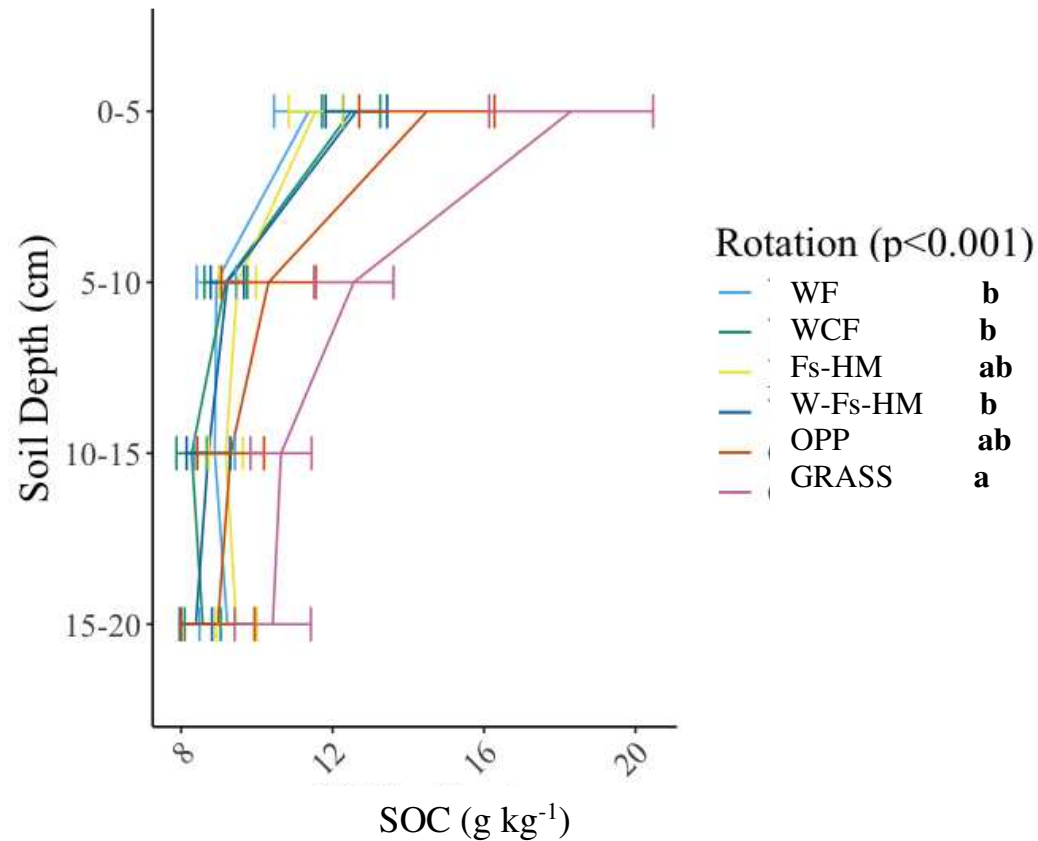


Figure 1. Mean SOC concentrations by cropping system (WF = Wheat-Fallow, WCF= Wheat-Corn-Fallow, Fs-HM = Forage Sorghum- Hay Millet, W-Fs-HM = Wheat- Forage Sorghum- Hay Millet, OPP = Opportunity crop; no fallow, GRASS = Perennial grass) and top soil layers (0-20 cm), averaged across three slope positions and three sites in Eastern Colorado. Samples were collected in May and July of 2021. Error bars represent the standard error of the mean. There is no interactive effect between rotation and depth ($p < 0.05$; Table 3) and post hoc Tukey letters for the rotation effect across the whole 0-20 cm depth are displayed in the legend.

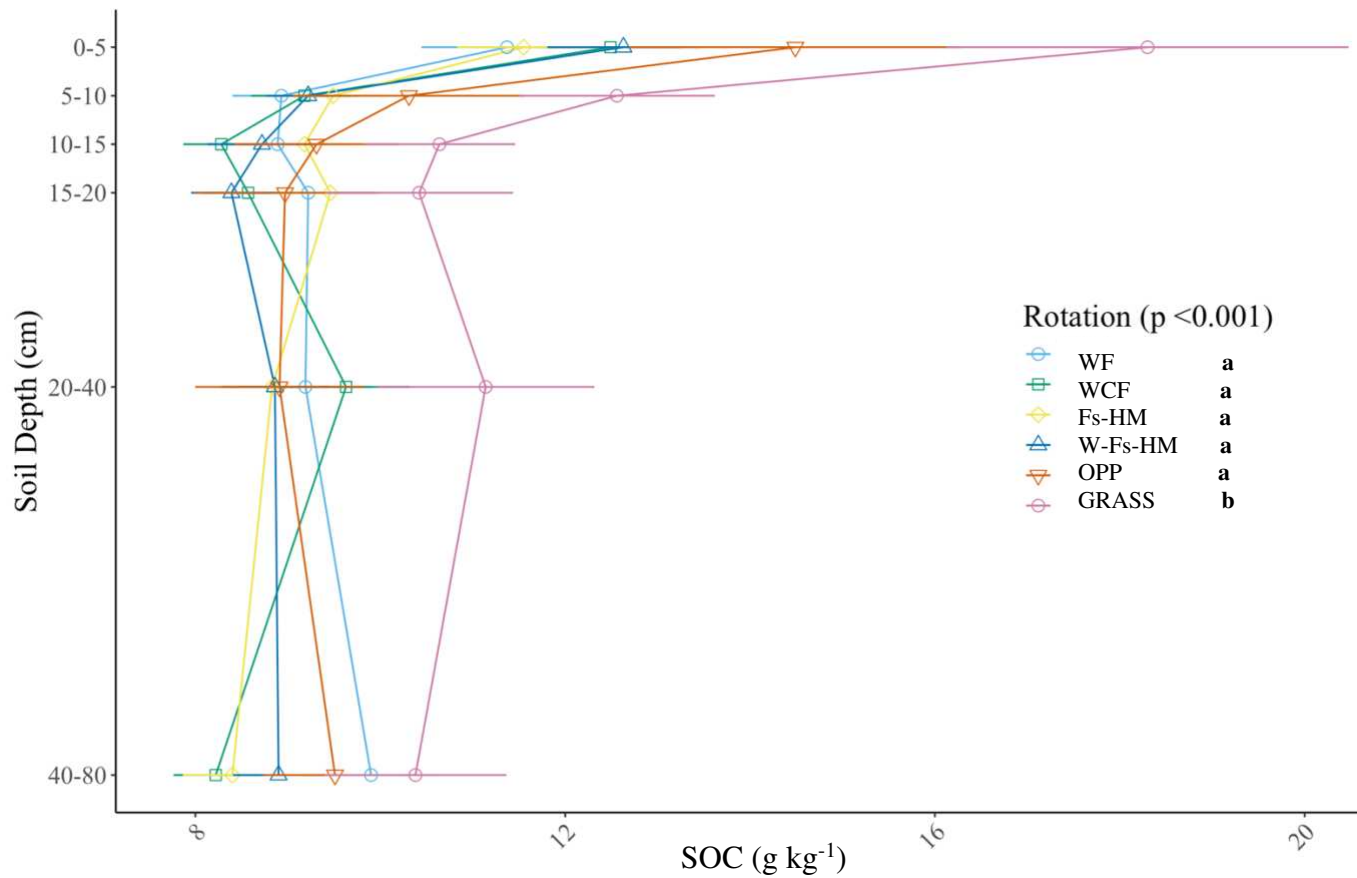


Figure 2. Mean SOC concentrations by cropping system (WF = Wheat-Fallow, WCF= Wheat-Corn-Fallow, Fs-HM = Forage Sorghum- Hay Millet, W-Fs-HM = Wheat- Forage Sorghum- Hay Millet, OPP = Opportunity crop; no fallow, GRASS = Perennial grass) and all soil layers (0-80 cm), averaged across three slope positions and three sites in Eastern Colorado. Samples were collected in May and July of 2021. Error bars represent the standard error of the mean. There is no interactive effect between rotation and depth ($p < 0.05$; Table 3) and post hoc Tukey letters for the rotation effect across the whole 0-80 cm depth are displayed in the legend.

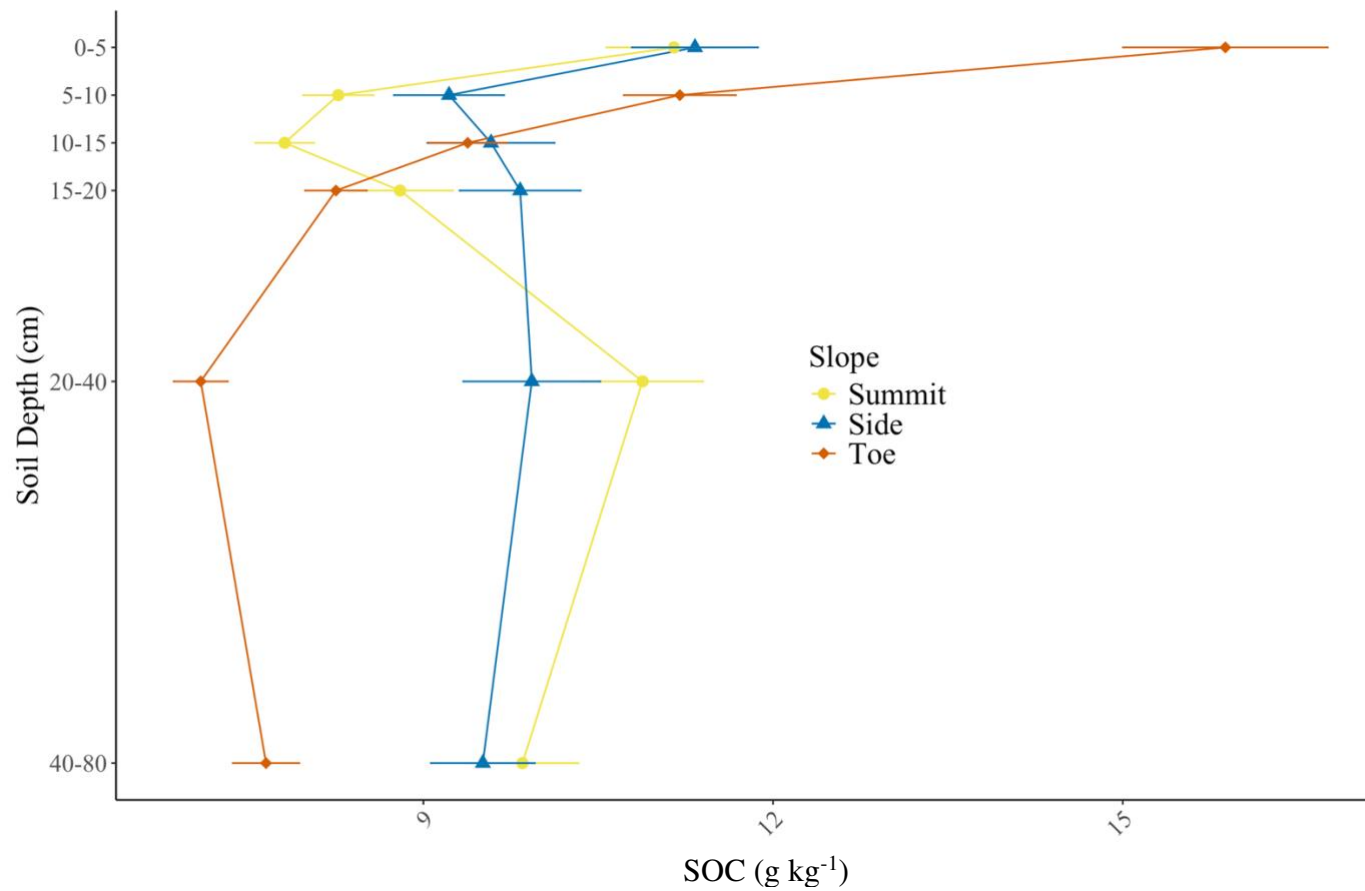


Figure 3. Mean SOC concentrations by three slope positions and all soil layers (0- 80 cm), averaged across six cropping systems and three study sites in Eastern Colorado. Samples were collected in May and July of 2021. Error bars represent the standard error of the mean. Interactive effect ($p < 0.001$, Table 2)

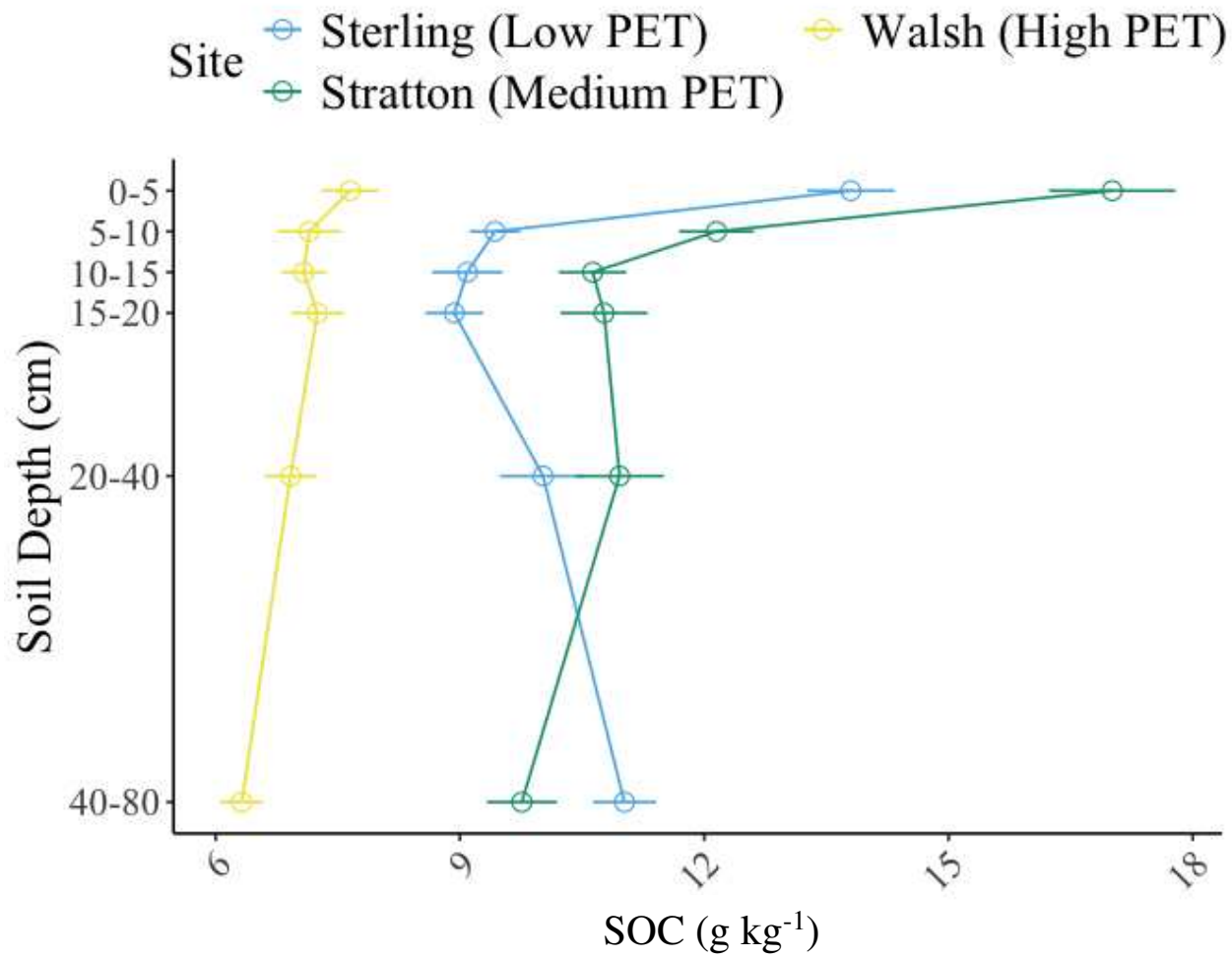


Figure 4. Mean SOC concentrations by three sites in Eastern, CO and all soil layers (0- 80 cm), averaged across six cropping systems and three slope positions. Samples were collected in May and July of 2021. Error bars represent the standard error of the mean. Interactive effect ($p < 0.001$, Table 2)

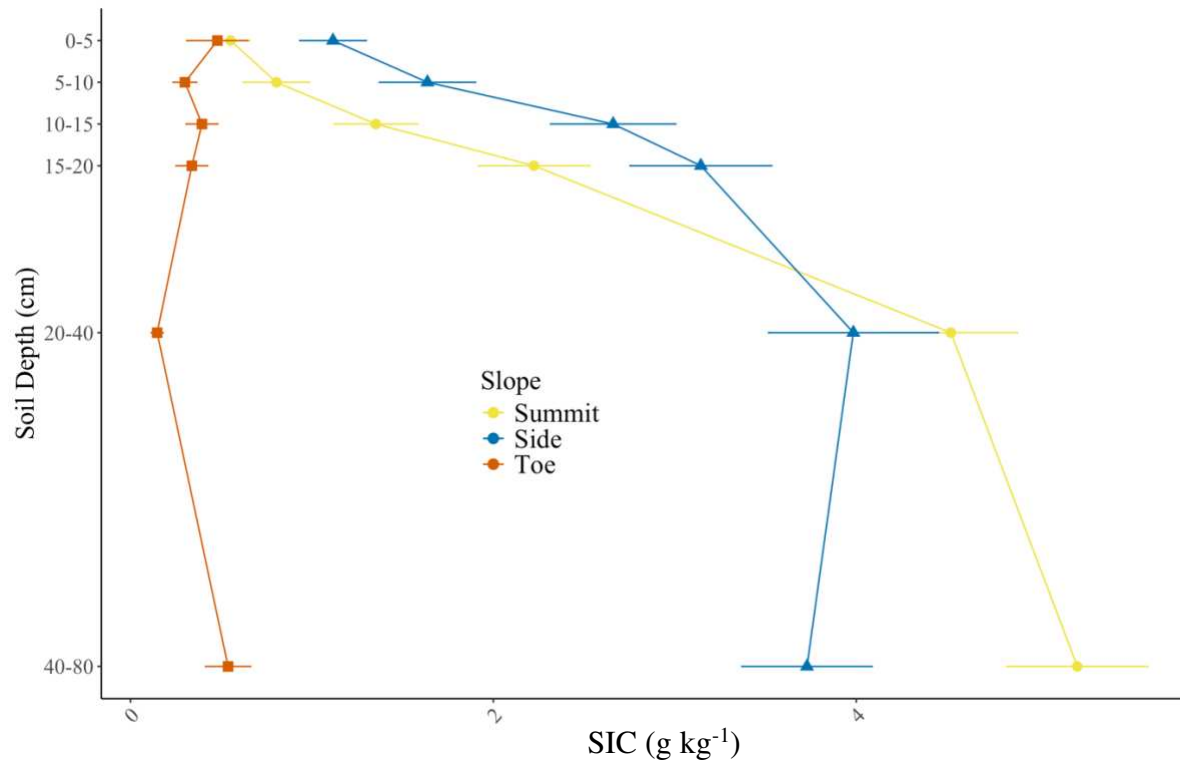


Figure 5. Mean SIC concentrations by three slope positions and all soil layers (0- 80 cm), averaged across six cropping systems and three study sites in Eastern Colorado. Samples were collected in May and July of 2021. Error bars represent the standard error of the mean. Interactive effect ($p < 0.001$, Table 2)

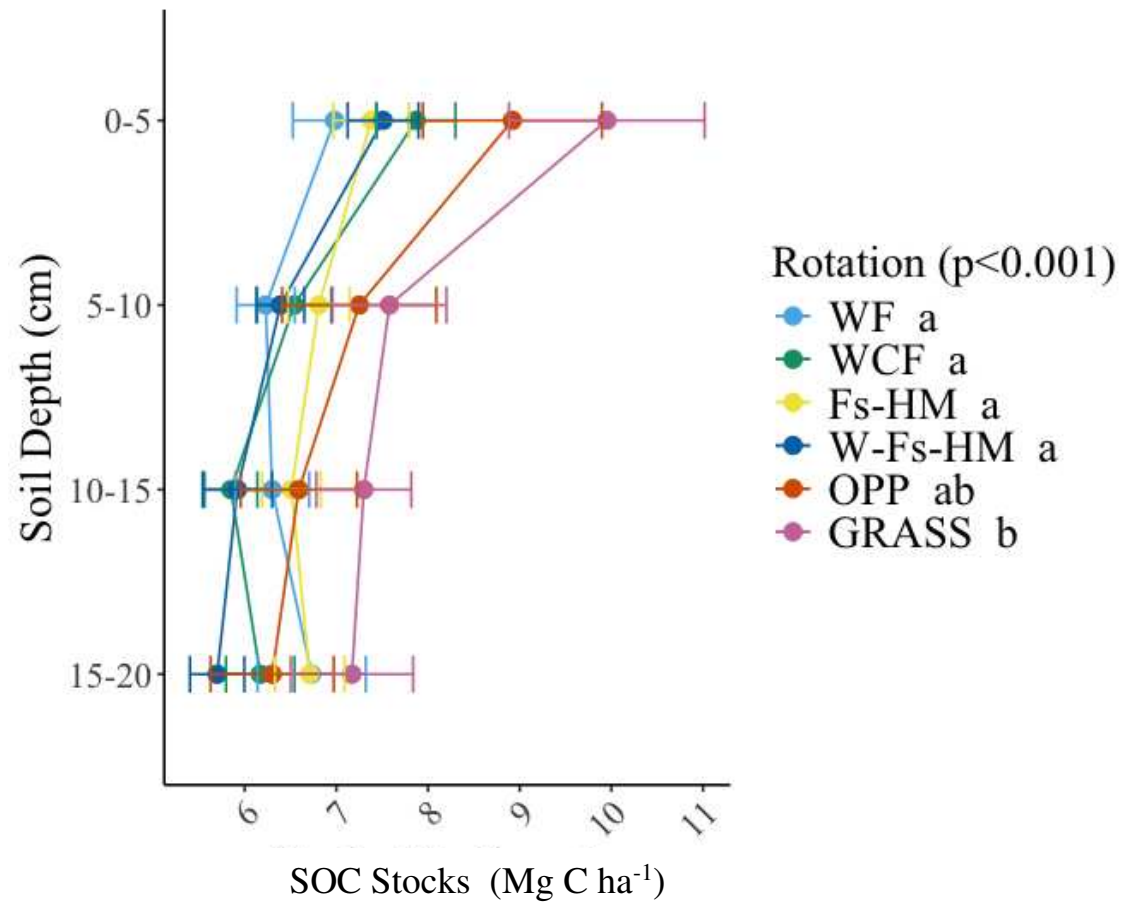


Figure 6. Mean SOC stocks by cropping system (WF = Wheat-Fallow, WCF= Wheat-Corn-Fallow, Fs-HM = Forage Sorghum- Hay Millet, W-Fs-HM = Wheat- Forage Sorghum- Hay Millet, OPP = Opportunity crop; no fallow, GRASS = Perennial grass) and top soil layers (0-20 cm), averaged across three slope positions and three sites in Eastern Colorado. Samples were collected in May and July of 2021. Error bars represent the standard error of the mean. There is no interactive effect between rotation and depth ($p < 0.05$; Table 3) and post hoc Tukey letters for the rotation effect across the whole 0-20 cm depth are displayed in the legend.

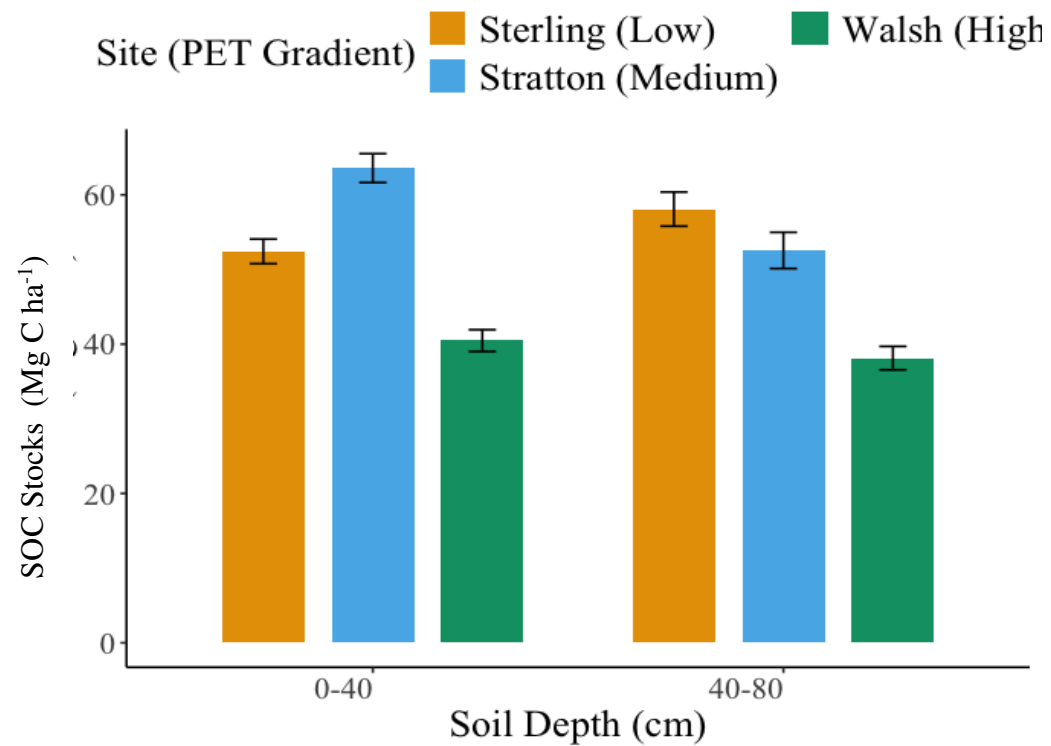


Figure 7. Mean SOC stocks by two soil layers (0- 40 cm, 40- 80 cm) and three sites in Eastern CO, averaged across six cropping systems and three slope positions. Samples were collected in May and July of 2021. Error bars represent standard error of the mean. Interactive effect ($p=0.012$, Table 3)

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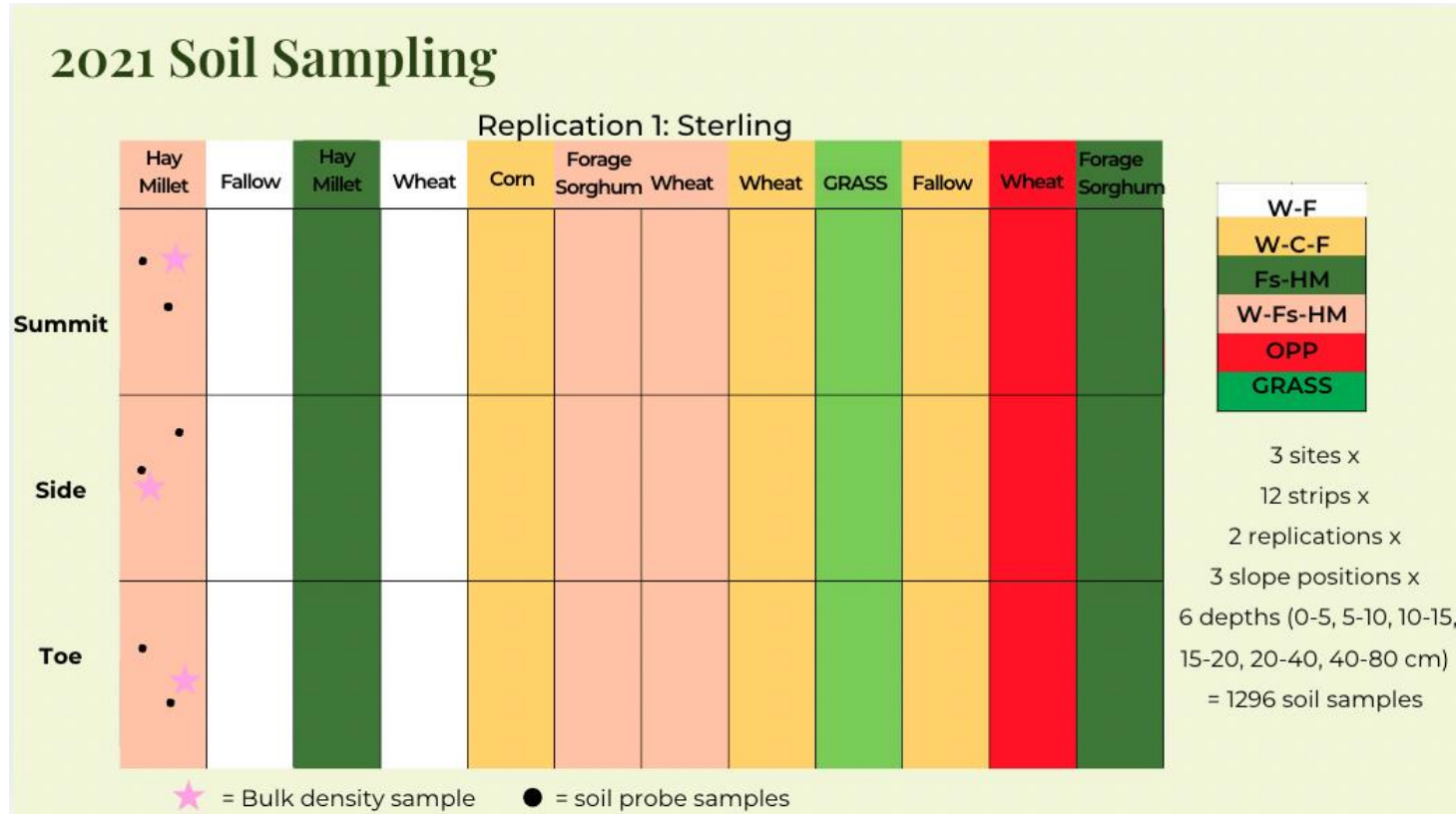
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APPENDICES

Supplementary Table 1. Map of soil sampling done in July 2021 in the first replication of 12 treatment strips at the Sterling site location.



Supplementary Table 2. Mean SIC concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Sterling site.

Sterling (Low PET)		WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass
	(cm)	<i>Mean Inorganic Carbon g kg⁻¹ ± SE</i>					
Summit	0-5	0.13±0.09	0.05±0.04	0.07±0.06	0.00±0.00	0.00±0.00	0.16±0.04
	5-10	0.23±0.23	0.02±0.01	0.04±0.03	0.01±0.01	0.00±0.00	0.05±0.00
	10-15	0.05±0.02	0.02±0.01	0.05±0.05	0.01±0.01	0.03±0.03	0.03±0.03
	15-20	0.07±0.04	0.03±0.02	0.01±0.01	0.01±0.01	1.05±1.00	0.03±0.03
	20-40	1.49±1.22	1.40±0.78	4.73±1.65	2.28±1.08	4.59±1.78	1.83±1.78
	40-80	5.70±2.02	6.06±1.61	3.97±2.30	8.18±2.30	6.32±1.30	7.56±7.53
Side	0-5	0.04±0.03	1.42±0.89	0.01±0.01	0.12±0.05	2.67±2.54	2.94±2.38
	5-10	0.22±0.17	2.58±1.72	0.13±0.09	0.03±0.02	2.93±2.79	0.89±0.84
	10-15	0.71±0.68	3.68±1.92	0.54±0.22	0.06±0.03	3.33±3.33	2.74±2.69
	15-20	0.77±0.71	5.08±2.23	1.35±0.52	2.12±1.44	4.78±4.78	3.14±3.09
	20-40	2.57±2.41	6.19±3.28	2.94±0.80	5.53±2.69	3.00±3.00	3.46±3.20
	40-80	3.94±2.28	4.39±1.20	5.78±1.79	6.53±1.88	3.73±3.73	3.83±3.71
Toe	0-5	0.13±0.12	0.01±0.01	2.33±2.28	0.03±0.02	0.00±0.00	2.32±2.20
	5-10	0.15±0.15	0.01±0.01	0.02±0.01	0.08±0.05	0.03±0.03	0.03±0.03
	10-15	0.13±0.12	0.06±0.05	0.00±0.00	0.10±0.07	0.06±0.06	0.05±0.00
	15-20	0.12±0.07	0.01±0.01	0.01±0.01	0.01±0.01	0.03±0.03	1.75±1.70
	20-40	0.07±0.07	0.02±0.01	0.01±0.01	0.01±0.01	0.03±0.03	0.03±0.03
	40-80	0.41±0.27	0.12±0.04	0.26±0.20	0.31±0.15	0.49±0.14	0.05±0.00

WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat- Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix

Supplementary Table 3. Mean SIC concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Stratton site.

Stratton (Medium PET)							
	WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass	
(cm)	<i>Mean Inorganic Carbon g kg⁻¹ ± SE</i>						
Summit	0-5	0.43±0.24	0.43±0.16	0.20±0.17	0.71±0.27	0.01±0.01	0.04±0.04
	5-10	0.32±0.17	0.62±0.28	0.38±0.21	0.65±0.25	0.04±0.04	0.01±0.01
	10-15	1.05±0.54	1.80±1.01	1.16±0.39	1.35±0.47	0.03±0.03	0.03±0.02
	15-20	2.74±1.17	5.07±1.53	4.92±1.05	3.42±0.94	0.08±0.08	2.54±0.98
	20-40	6.52±1.47	8.53±0.63	7.94±0.71	6.39±0.87	4.54±1.51	8.08±1.12
	40-80	6.79±0.50	6.61±0.23	6.97±0.36	6.09±0.47	6.24±0.47	6.41±0.20
Side	0-5	1.95±1.01	1.84±0.82	0.75±0.40	1.42±0.76	2.34±2.32	2.74±NA
	5-10	2.4±1.47	2.35±1.15	2.23±1.53	1.73±0.99	2.83±2.82	2.67±NA
	10-15	5.16±2.62	3.44±1.66	2.88±1.97	2.31±1.24	3.40±3.38	2.86±1.94
	15-20	4.88±2.17	3.60±1.58	3.03±1.90	1.49±1.04	3.72±3.70	4.67±2.69
	20-40	5.29±1.93	5.00±1.37	3.32±1.70	2.77±1.33	3.43±3.42	5.86±2.06
	40-80	3.69±1.46	5.54±0.63	2.65±1.47	3.93±1.38	3.40±2.54	5.86±1.56
Toe	0-5	0.37±0.28	0.33±0.16	0.45±0.35	1.7±0.97	0.42±0.34	0±NA
	5-10	0.51±0.31	0.28±0.10	0.54±0.45	0.89±0.47	1.24±1.22	0.04±0.04
	10-15	0.80±0.39	0.45±0.17	0.71±0.66	1.01±0.58	1.88±1.79	0±NA
	15-20	0.71±0.39	0.21±0.12	0.63±0.59	1.12±0.65	0.22±0.22	0±NA
	20-40	0.14±0.10	0.14±0.07	0.28±0.13	0.48±0.28	0.78±0.55	0.08±0.08
	40-80	0.03±0.01	0.05±0.03	0.05±0.03	0.12±0.04	0.07±0.02	0.17±0.16

WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat- Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix

NA: not applicable (No standard error due to n=1 from outlier exclusion)

Supplementary Table 4. Mean SIC concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Walsh site.

Walsh (High PET)								
	WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass		
	<i>Mean Inorganic Carbon g kg⁻¹ ± SE</i>							
	(cm)							
Summit	0-5	2.03±0.94	1.48±0.70	0.56±0.33	0.94±0.29	0.90±0.30	1.11±0.38	
	5-10	3.54±1.72	2.34±1.19	1.31±0.82	1.67±0.54	0.34±0.33	0.93±0.42	
	10-15	4.05±1.50	2.60±0.99	2.47±1.49	2.59±0.68	4.48±2.10	1.33±0.38	
	15-20	3.62±1.23	3.11±1.28	3.04±1.75	2.57±0.89	4.68±2.04	1.65±0.34	
	20-40	2.91±0.97	4.14±1.58	2.45±1.41	4.85±0.59	4.10±0.33	4.74±1.16	
	40-80	2.41±0.81	4.1±1.08	1.76±1.03	2.85±0.39	1.76±1.74	4.14±0.02	
Side	0-5	0.78±0.49	0.86±0.25	0.35±0.21	1.27±0.44	1.39±0.34	0.40±0.03	
	5-10	2.66±1.23	1.80±0.51	0.63±0.37	1.51±0.40	3.20±0.57	0.40±0.11	
	10-15	3.30±1.15	4.18±0.67	1.78±1.03	3.16±0.43	4.69±0.59	0.73±0.30	
	15-20	2.05±1.18	4.90±1.26	2.29±1.33	3.50±0.64	4.63±0.87	1.45±0.81	
	20-40	1.79±1.03	4.60±1.29	2.14±1.24	3.71±0.26	3.28±1.43	4.37±0.53	
	40-80	0.81±0.80	2.92±0.65	1.55±0.91	2.20±0.47	2.18±0.45	3.21±0.06	
Toe	0-5	0.15±0.09	0.12±0.05	0.11±0.007	0.16±0.06	0.12±0.00	0.04±0.04	
	5-10	0.20±0.12	0.38±0.14	0.20±0.15	0.32±0.08	0.20±0.20	0.15±0.00	
	10-15	0.18±0.11	0.55±0.17	0.17±0.17	0.39±0.06	0.10±0.10	0.22±0.00	
	15-20	0.09±0.05	0.33±0.14	0.27±0.20	0.28±0.05	0.27±0.00	0.15±0.15	
	20-40	0.04±0.02	0.15±0.04	0.11±0.07	0.14±0.02	0.10±0.10	0.22±0.07	
	40-80	1.50±0.87	1.96±0.93	1.03±0.74	0.99±0.42	0.90±0.90	0.25±0.11	

WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat- Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix

Supplementary Table 5. Mean BD concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Sterling site.

Sterling (Low PET)								
	WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass		
	(cm)	<i>Mean Bulk Density g cm⁻³ ± SE</i>						
Summit	0-5	1.35±0.04	1.32±0.03	1.31±0.03	1.29±0.01	1.32± 0.02	1.16±0.02	
	5-10	1.41±0.05	1.47±0.04	1.45±0.00	1.43±0.03	1.48±0.00	1.29±0.00	
	10-20	1.20±0.05	1.36±0.04	1.30±0.03	1.18±0.04	1.28±0.04	1.34±0.06	
	20-40	1.36±0.05	1.39±0.03	1.42±0.04	1.41±0.04	1.22±0.03	1.28±0.02	
	40-80	1.32±0.07	1.29±0.07	1.21±0.06	1.31±0.08	1.52±0.05	1.34±0.08	
Side	0-5	1.22±0.03	1.31±0.02	1.34±0.05	1.23±0.03	1.26±0.03	1.15±0.04	
	5-10	1.45±0.04	1.42±0.03	1.48±0.03	1.42±0.04	1.35±0.03	1.30±0.05	
	10-20	1.24±0.05	1.27±0.03	1.27±0.04	1.14±0.06	1.23±0.04	1.38±0.05	
	20-40	1.34±0.04	1.31±0.05	1.34±0.03	1.36±0.02	1.26±0.04	1.27±0.03	
	40-80	1.40±0.04	1.38±0.06	1.25±0.07	1.31±0.04	1.42±0.04	1.34±0.05	
Toe	0-5	1.21±0.06	1.14±0.02	1.17±0.05	1.09±0.05	1.11±0.03	1.05 ±0.02	
	5-10	1.36±0.01	1.34±0.03	1.41±0.02	1.36±0.03	1.20±0.01	1.13±0.00	
	10-20	1.34±0.05	1.31±0.03	1.24±0.04	1.22±0.05	1.34±0.06	1.35±0.01	
	20-40	1.35±0.03	1.30±0.03	1.41±0.02	1.37±0.03	1.28±0.02	1.28±0.01	
	40-80	1.38±0.01	1.33±0.02	1.30±0.04	1.35±0.03	1.34±0.08	1.32±0.01	

WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat- Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix

Supplementary Table 6. Mean BD concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Stratton site

Stratton (Medium PET)		WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass
	(cm)	<i>Mean Bulk Density g cm⁻³ ± SE</i>					
Summit	0-5	1.16±0.05	1.17±0.03	1.18±0.01	1.09±0.04	1.18±0.07	1.01±0.06
	5-10	1.39±0.05	1.33±0.02	1.33±0.04	1.31±0.03	1.43±0.06	1.19±0.16
	10-20	1.45±0.03	1.40±0.03	1.42±0.03	1.39±0.02	1.48±0.01	1.41±0.03
	20-40	1.37±0.04	1.36±0.02	1.28±0.02	1.33±0.02	1.51±0.04	1.33±0.01
	40-80	1.25±0.08	1.26±0.04	1.26±0.04	1.22±0.04	1.37±0.08	1.38±0.03
Side	0-5	1.32±0.03	1.36±0.05	1.31±0.03	1.23±0.06	1.26±0.05	1.24±NA
	5-10	1.46±0.05	1.45±0.03	1.41±0.03	1.44±0.02	1.40±0.04	1.31±NA
	10-20	1.46±0.01	1.49±0.02	1.45±0.02	1.47±0.03	1.47±0.04	1.38±0.02
	20-40	1.44±0.03	1.41±0.03	1.43±0.03	1.43±0.03	1.44±0.06	1.40±0.09
	40-80	1.41±0.09	1.39±0.06	1.44±0.04	1.36±0.07	1.47±0.09	1.42±0.01
Toe	0-5	1.04±0.09	1.18±0.02	1.22±0.05	1.04±0.05	1.14±0.04	1.03±NA
	5-10	1.29±0.10	1.39±0.04	1.46±0.05	1.27±0.04	1.41±0.04	1.13±0.16
	10-20	1.50±0.03	1.47±0.04	1.53±0.03	1.46±0.03	1.58±0.01	1.41±00
	20-40	1.55±0.01	1.48±0.03	1.47±00	1.50±0.01	1.56± 00	1.44± 0.09
	40-80	1.30±0.05	1.38±0.05	1.35 ± 0.07	1.33±0.05	1.47±0.03	1.44±00

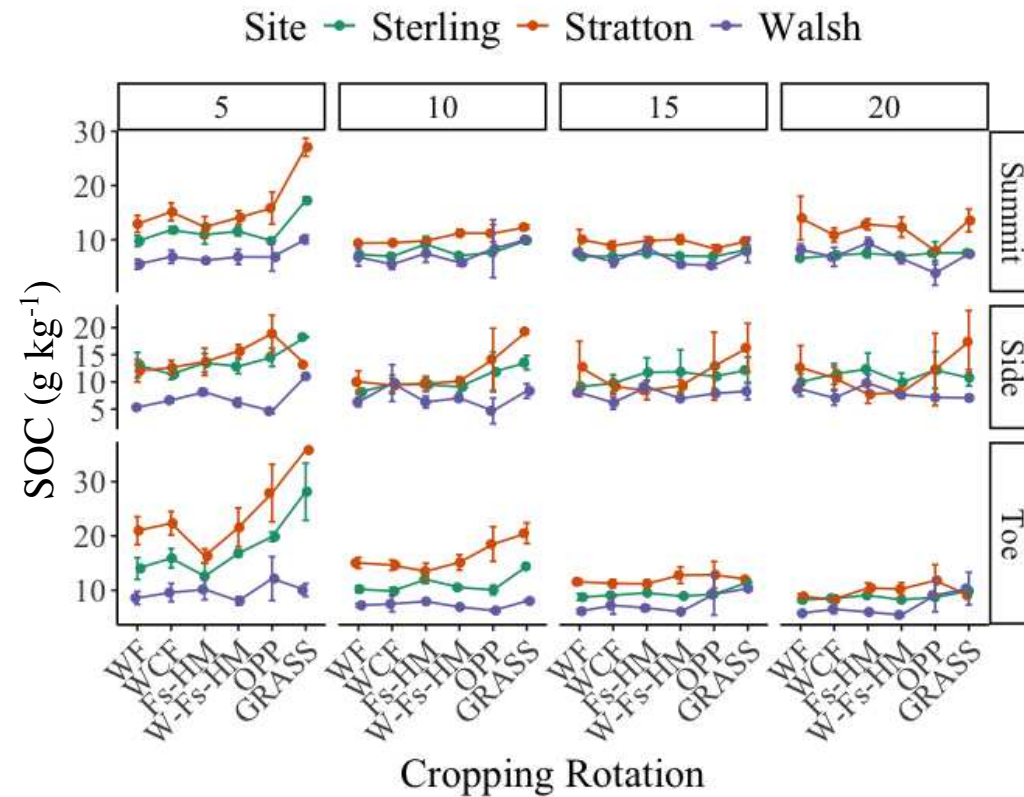
WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat- Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix

NA: not applicable (No standard error due to n=1 from outlier exclusion)

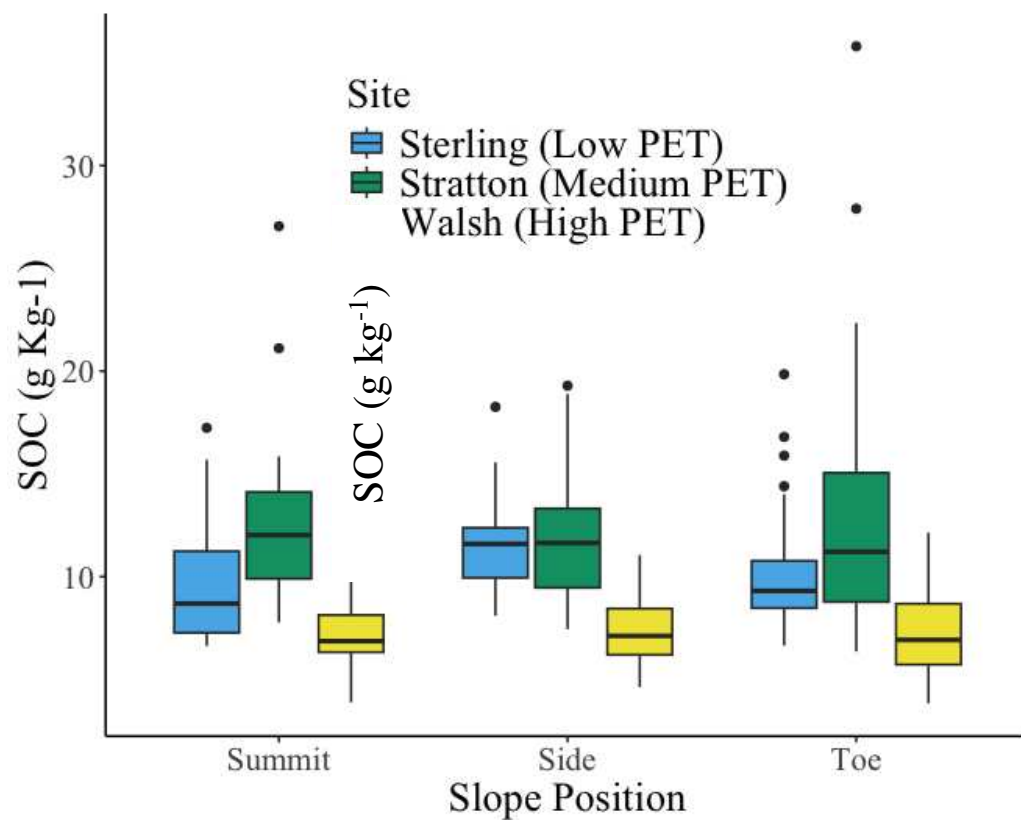
Supplementary Table 7. Mean BD concentrations in 2021, after 36 yrs under no-till management as affected by slope position, crop rotation and soil depth for Walsh site

Walsh (High PET)								
	WF	WCF	Fs-HM	W-Fs-HM	OPP	Grass		
	<i>Mean Bulk Density g cm⁻³ ± SE</i>							
	(cm)							
Summit	0-5	1.50±0.03	1.44±0.02	1.46±0.01	1.45±0.02	1.48±0.01	1.15±0.03	
	5-10	1.45±0.06	1.54±0.02	1.41±0.06	1.54±0.04	1.56±0.00	1.15±0.12	
	10-20	1.45±0.03	1.57±0.01	1.58±0.00	1.55±0.01	1.61±0.02	1.51±0.03	
	20-40	1.37±0.04	1.51±0.03	1.53±0.02	1.53±0.01	1.57±0.03	1.54±0.02	
	40-80	1.25±0.08	1.51±0.04	1.53±0.03	1.54±0.01	1.55±0.06	1.53±0.05	
Side	0-5	1.40±0.07	1.40±0.01	1.42±0.03	1.47±0.04	1.54±0.01	1.02±0.02	
	5-10	1.45±0.05	1.54±0.03	1.57±0.01	1.47±0.08	1.57±0.01	1.17±0.09	
	10-20	1.55±0.02	1.58±0.02	1.59±0.01	1.47±0.04	1.57±0.02	1.43±0.09	
	20-40	1.48±0.01	1.47±0.04	1.51±0.01	1.50±0.02	1.50±0.07	1.52±0.01	
	40-80	1.47±0.01	1.49±0.03	1.5±0.03	1.50±0.02	1.50±0.09	1.51±0.05	
Toe	0-5	1.34±0.02	1.34±0.07	1.29±0.04	1.36±0.02	1.31±0.05	1.18±0.01	
	5-10	1.49±0.04	1.48±0.04	1.46±0.03	1.49±0.03	1.49±0.05	1.29±0.02	
	10-20	1.39±0.05	1.37±0.06	1.41±0.05	1.45±0.03	1.31±0.04	1.27±0.05	
	20-40	1.53±0.01	1.47±0.04	1.54±0.02	1.54±0.02	1.57±0.03	1.38±0.04	
	40-80	1.56±0.02	1.48±0.04	1.48±0.04	1.42±0.10	1.52±0.06	1.44±0.03	

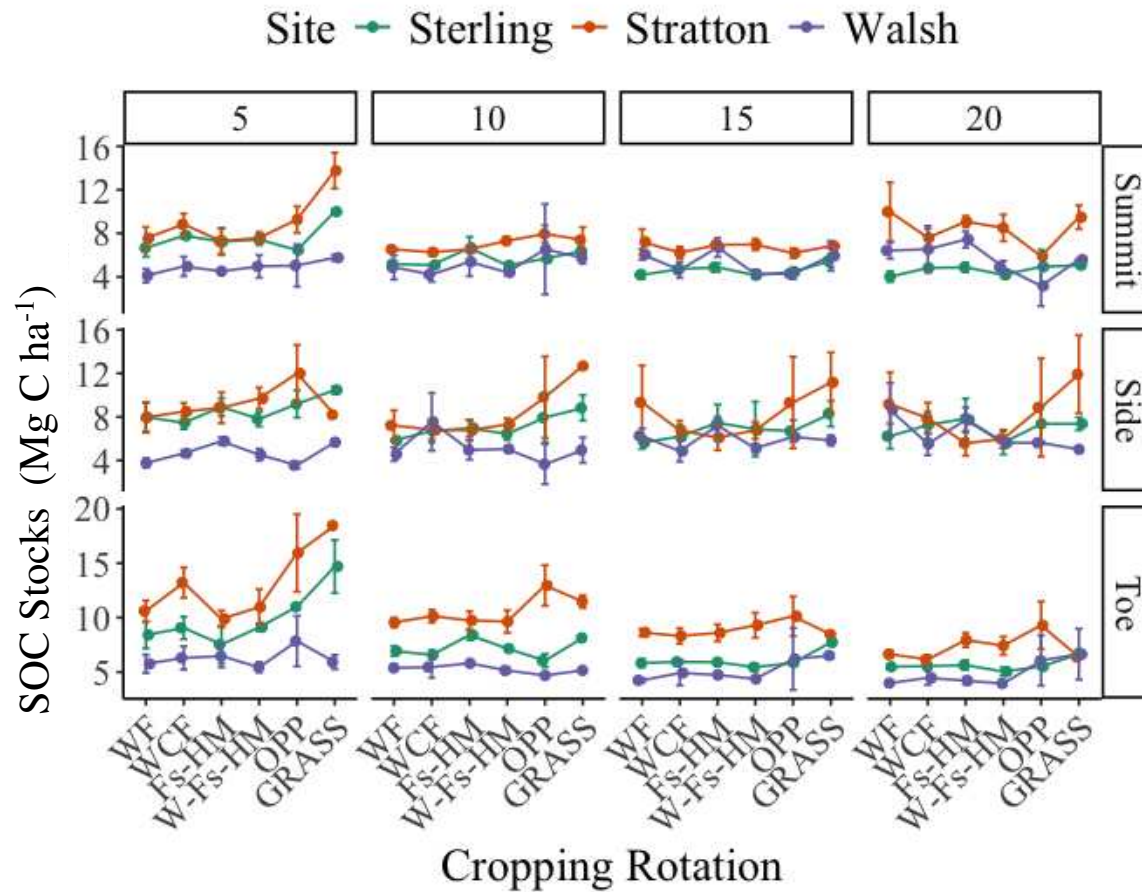
WF: Wheat- Fallow, WCF: Wheat- Corn- Fallow, Fs-HM: Forage Sorghum – Hay Millet, W-Fs-HM: Wheat- Forage Sorghum- Hay Millet, OPP: Opportunity, GRASS: Perennial Grass Mix



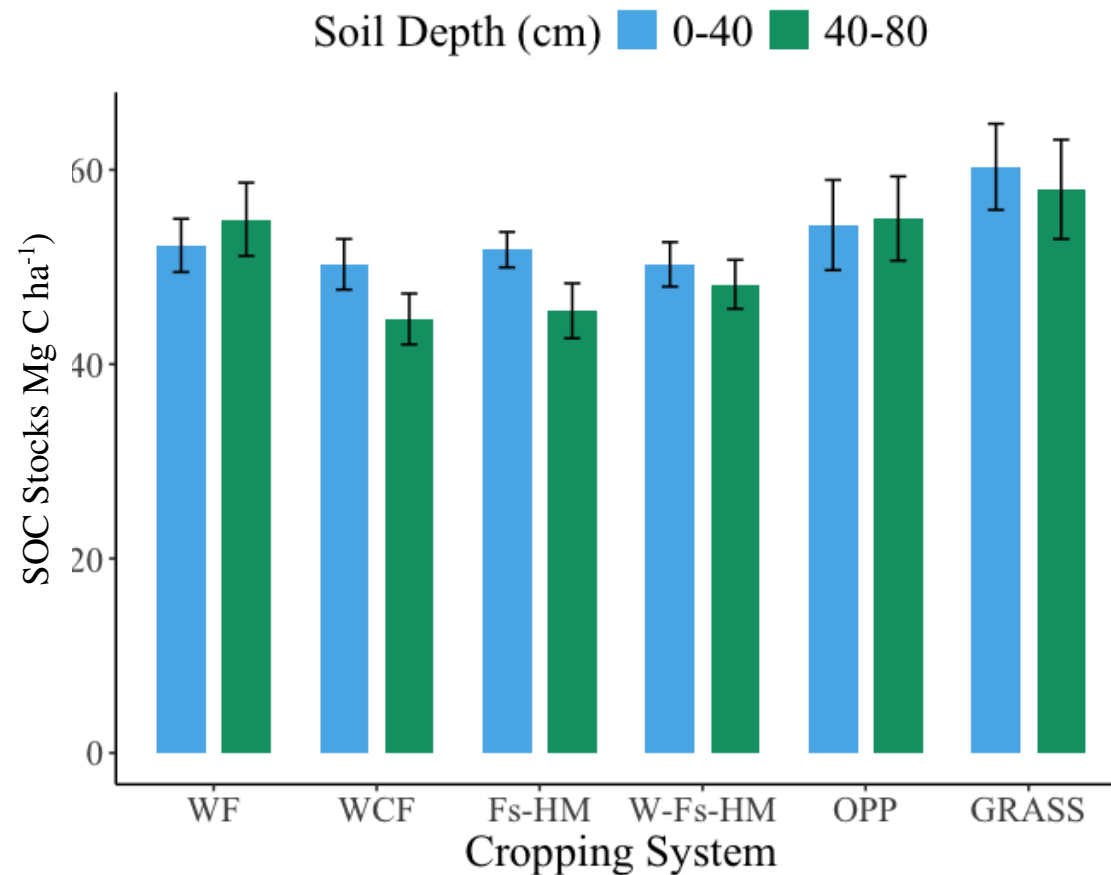
Supplemental Figure 1. Mean SOC concentration by cropping system (WF = Wheat-Fallow, WCF= Wheat-Corn-Fallow, Fs-HM = Forage Sorghum- Hay Millet, W-Fs-HM = Wheat- Forage Sorghum- Hay Millet, OPP = Opportunity crop; no fallow, GRASS = Perennial grass) and top soil layers (0-20 cm), three slope positions and three sites in Eastern Colorado. Samples were collected in May and July of 2021. Error bars represent the standard error of the mean.



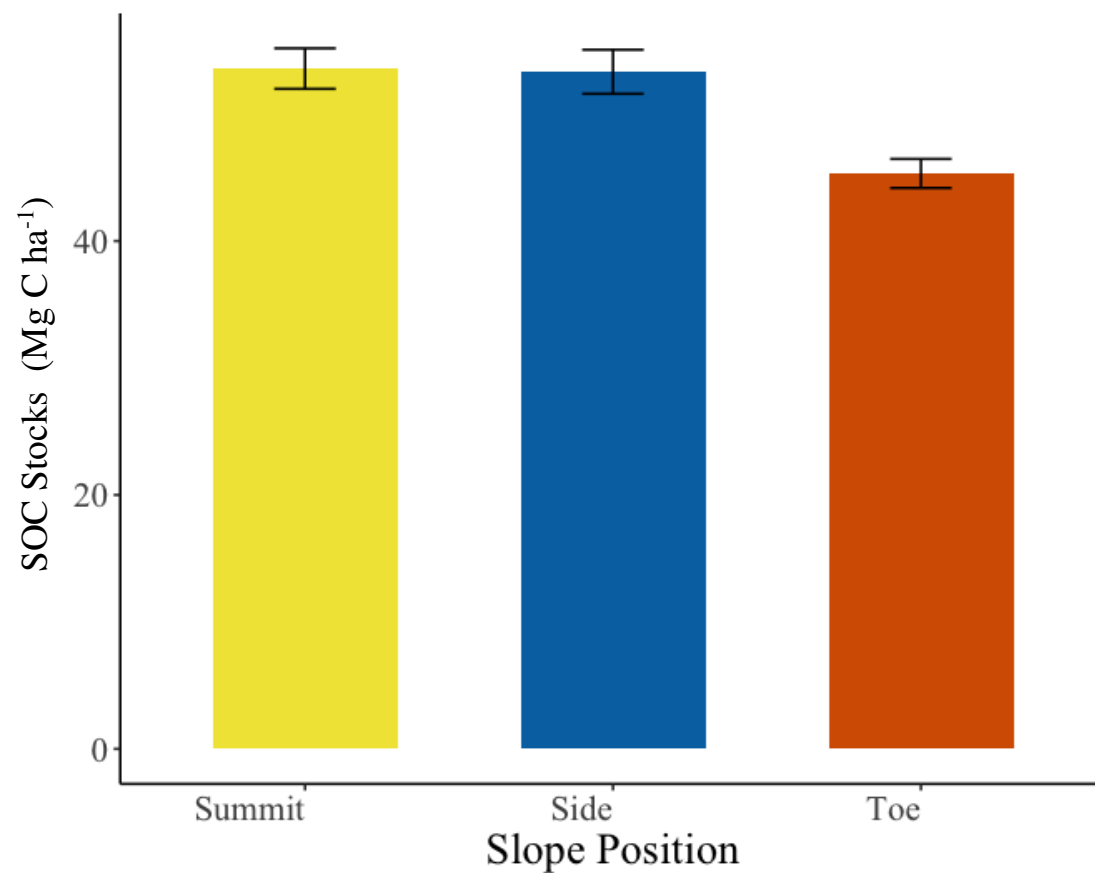
Supplementary Figure 2. Mean SOC concentrations by slope position and three study sites in Eastern Colorado, averaged by six cropping systems and six soil depth increments. Thick line in the boxplot represents median SOC value for each group and the whiskers represent the interquartile range between 25th and 75th percentiles.



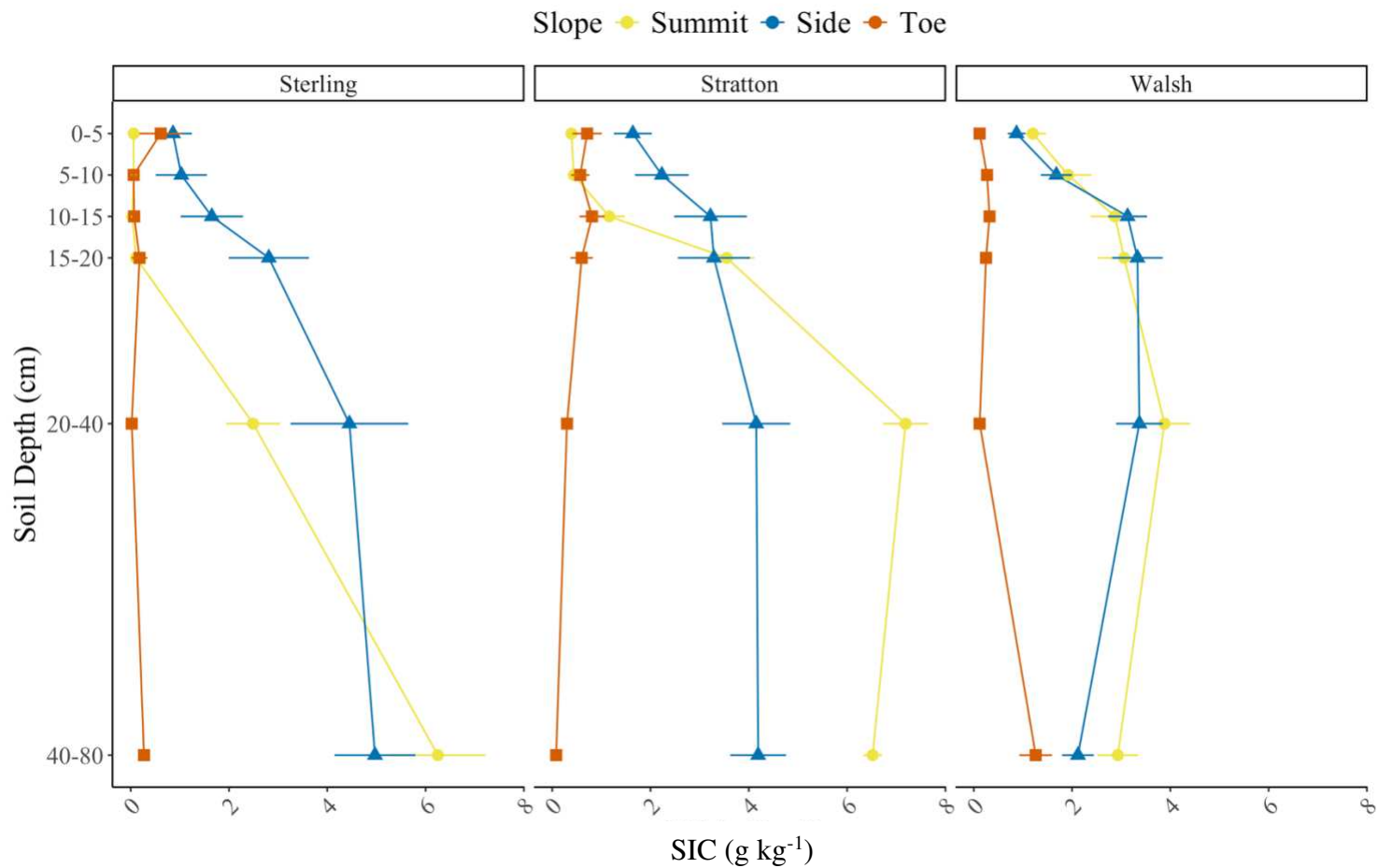
Supplementary Figure 3. Mean SOC stocks by cropping system (WF = Wheat-Fallow, WCF= Wheat-Corn-Fallow, Fs-HM = Forage Sorghum- Hay Millet, W-Fs-HM = Wheat- Forage Sorghum- Hay Millet, OPP = Opportunity crop; no fallow, GRASS = Perennial grass) and top soil layers (0-20 cm), three slope positions and three sites in Eastern Colorado. Samples were collected in May and July of 2021. Error bars represent the standard error of the mean.



Supplementary Figure 4. Mean SOC stocks by two soil depths and six cropping systems (WF = Wheat-Fallow, WCF= Wheat-Corn-Fallow, Fs-HM = Forage Sorghum- Hay Millet, W-Fs-HM = Wheat- Forage Sorghum- Hay Millet, OPP = Opportunity crop; no fallow, GRASS = Perennial grass), averaged across three slope positions and three sites in Eastern Colorado. Samples were collected in May and July of 2021 Error bars represent the standard error of the mean.



Supplemental Figure 5. SOC stocks by slope position, averaged across six cropping systems, two soil depths (0-40, 40-80cm) and three sites in Eastern CO. Samples were collected in May and July of 2021. Error bars represent standard error of the mean.



Supplemental Figure 6. Mean SIC concentrations by three slope positions, three sites in Eastern, CO, all soil layers (0-90 cm), averaged across six cropping systems. Samples were collected in May and July of 2021. Error bars represent standard error of the mean.