GRAIN AND FORAGE CROPPING SYSTEMS TYPICAL OF THE UPPER MIDWEST EXCEPT GRAZED PASTURE LOSE SOIL ORGANIC CARBON OVER 30 YEARS

by

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Abstract

Climate change, driven by anthropogenic greenhouse gas emissions, is undermining human health, livelihoods, food security, water supply, and biodiversity. Agricultural soil carbon sequestration, and markets based on payments for sequestration, are touted and incentivized as a strategy to help mitigate climate change. But our understanding about long-term effects of agricultural management on soil organic carbon (SOC), particularly in the carbon-rich and intensively managed Mollisols that dominate the US Corn Belt, does not provide enough certainty for reliable C markets to flourish. Accounting for bulk density changes, we evaluated SOC stocks across the entire 90-cm soil profile under 6 common Midwestern cropping systems (3 cash-grain, 3 dairy-forage) in a southern Wisconsin Mollisol over 30 years. Rotationally grazed perennial pasture was the only system to stabilize SOC. While other forage systems (maize-alfalfa rotations) accrued SOC in surface horizons, these gains were offset by losses at depth. Losses were observed in all cash-grain systems. Average SOC debt after 30 years for cash-grain and dairy-forage systems (excluding pasture) were -19.5 and -13.0 Mg ha^{-1}, respectively. Sensitivity analyses were performed to evaluate the adequacy of ‘space-for-time’ methods (i.e., ignoring baseline data), depth-based sample comparison (i.e., no correction for bulk density change), and using only the surface 30-cm to assess SOC change (common in carbon market protocols). All three methods resulted in overestimation of SOC stocks, including apparent increases in the pasture system under ‘space-for-time’ and surface sampling methods, which highlights the significant implications of accurate carbon accounting methods for future carbon markets and climate mitigation efforts. Our findings support others demonstrating the need for well-managed perennial grasslands to stabilize SOC in the Mollisols that dominate the landscape of the Upper Midwest.

Introduction

Climate change is undermining human health, livelihoods, food security, water supply, and biodiversity (IPCC, 2022). Drawing carbon dioxide from the atmosphere via carbon sequestration, alongside substantial reductions in greenhouse gas emissions, may help mitigate the more extreme consequences of climate change. According to a major review released following the fifth report of the Intergovernmental Panel on Climate Change (IPCC), sequestering carbon in soils via improved management can remove 2.3 to 5.3 Gt CO_2 yr^{-1} until 2050, a number comparable to or greater than the sequestration potential of afforestation and reforestation (0.5 to 3.6 Gt CO_2 yr^{-1}), direct air carbon capture and storage (0.5 to 5 Gt CO_2 yr^{-1}), and bioenergy with carbon capture and storage (0.5 to 5 Gt CO_2 yr^{-1})(Fuss et al., 2018). However, agricultural soils have historically been a source of carbon (C) to the atmosphere (Sanderman, Hengl, & Fiske, 2017). Disturbances such as tillage, tile drains, removal of native vegetation, and the comparably small C inputs of agricultural residues lead to less carbon in the soil and more carbon in the atmosphere. Many have proposed that because agricultural soils are now well below their theoretical maximum SOC storage capacity, they are now capable of serving as important SOC sinks (Wenzel et al., 2022) with the greatest potential in soils with greater mineralogical carbon capacity (i.e. lower C saturation of mineral surfaces (Georgiou et al., 2022)). Projects such as the “4 per 1,000” initiative and emerging agricultural carbon trading schemes seek to leverage this potential to mitigate climate change or offset current CO_2 emissions (Soussana et al., 2019).

In contrast to these hopes, other studies (Knotters, Teuling, Reijneveld, Lesschen, & Kuikman, 2022; Sanderman & Baldock, 2010) have documented ongoing widespread soil organic carbon (SOC) losses in grassland and agricultural soils, especially in deeper soil horizons. This may be due in part to warming soils (Rocci, Lavallee, Stewart, & Cotrufo, 2021; Soong et al., 2021) or the ongoing effects of past land use change (e.g. disequilibrium). Furthermore, rising demand for biofuels and grain-fed animal products drives conversion of native ecosystems to annual cropland, a process known to release large amounts of SOC in the form of CO_2 to the atmosphere (Anderson-Teixeira, Davis, Masters, & Delucia, 2009; Davidson & Ackerman, 1993; Salemme, Olson, Gennadiyev, & Kovach, 2018). Thus, while agricultural soils possess potential as a carbon sink, they have an equal potential to continue to be a carbon source, releasing large amounts of CO_2 (Sanderman et al., 2017). Furthermore, many high-input strategies to increase soil organic carbon (SOC) stocks such as fertilizer applications and irrigation generate emissions of their own, reducing their true net sequestration potential (Schlesinger, 2022).

SOC stocks are the balance between C inputs from photosynthesis, and C outputs mostly from heterotrophic respiration. We can increase C inputs from plant biomass by growing more productive crops, reducing the amount to biomass removed, growing perennials with extensive and fibrous root systems, and maximizing the distribution of living cover throughout a growing season (e.g. via cover crops or perennials). Conversely, heterotrophic respiration of CO_2 may be limited when SOC becomes occluded within soil aggregates (Or, Keller, & Schlesinger, 2021; Sheng, Han, Zhang, Long, & Li, 2020) or adsorbed to mineral surfaces (Kan et al., 2022). An increase in microbial carbon use efficiency (CUE) via changes in input quality (Wang et al., 2022) or other practices (C. M. Kallenbach, Grandy, Frey, & Diefendorf, 2015; Rui et al., 2022) can also increase the amount of C inputs that persist in a system because much of the carbon that is either occluded or adsorbed has first undergone microbial degradation and is often in the form of microbial necromass (Deng & Liang, 2022; Yang et al., 2022).

Many factors impacting SOC stocks are largely beyond farmer control, including climate, soil mineralogy (Wang et al., 2022; Zhao et al., 2020), and socioeconomic factors such as limited access to transition resources or protection against short-term yield losses. Rates of plant biomass accumulation and microbial respiration are temperature- and moisture-dependent (Cates, Jilling, Tfaily, & Jackson, 2022; Rocci et al., 2021). Soil pH and moisture availability dictate whether exchangeable calcium or iron- and aluminum-oxyhydroxides enable SOC adsorption to minerals, and soil mineralogy determines how much of those materials are present (Rasmussen et al., 2018). Lastly, while changes in management can affect SOC stocks, subsistence farmers with limited access to resources cannot sustainably change their practices to increase SOC if such changes result in short-term yield losses that threaten their ability to feed themselves and their communities (Ng'ang'a, Jalang'o, & Girvetz, 2020; Powlson et al., 2014).

The impacts of agricultural management on SOC are complex and not fully understood. However, some general trends emerge from decades of research with regard to C inputs, tillage, and annual versus perennial crop-based systems. All else being equal, reducing C inputs to a system results in loss of SOC. Theoretically, this happens because soil microbes, deprived of their previous source of C and nitrogen (N), consume and respire extant SOC (Chowdhury et al., 2021). Paradoxically however, increasing the C input to a system can also decrease SOC in a process termed "priming" (Kan et al., 2022; Kuzyakov, Friedel, & Stahr, 2000). Microbial populations boom with the arrival of new C and N sources and consume native SOC to sustain their enlarged numbers. However, sufficiently large, sustained additions of C potentially overcome this paradox. A metanalysis by Jian et al. (2020) shows that cover crops increase SOC in surface soils (0 to 30 cm) relative to rotations without cover crops, potentially because of sufficiently increased C inputs from the cover crops' root exudates and biomass when the cover crops are terminated (Jian, Du, Reiter, & Stewart, 2020). Increased surface soil SOC stocks, like those observed by Jian et al. (2020) may not be sufficient to overcome losses in deeper soils (Tautges et al., 2019).

In annual cropping systems, tillage often results in a deeper distribution of C inputs in the soil profile, at the expense of soil aggregation and carbon accumulation at the surface (Powlson et al., 2014). According to a meta-analysis by Nicoloso and Rice, long-term cessation of tillage (no-till) results in greater SOC stocks compared to conventional tillage (Nicoloso & Rice, 2021). To achieve actual C sequestration however, no-till systems require leguminous cover crops and double cropping to generate C inputs sufficient to offset losses at depth (Nicoloso & Rice, 2021; Powlson et al., 2014). In perennial cropping systems such as pasture, even infrequent tillage can cause significant, long-lasting losses (Syswerda, Corbin, Mokma, Kravchenko, & Robertson, 2011). Numerous studies show that the return of cropping systems to perennial-based grassland in the form of rotationally grazed pasture (Becker, Horowitz, Ruark, & Jackson, 2022; Rui et al., 2022; Sanford et al., 2012) or other perennial agroecosystems (Diederich, Ruark, Krishnan, Arriaga, & Silva, 2019; Syswerda et al., 2011) results in larger SOC stocks than their annual-crop counterparts, possibly stemming from deeper, constantly present root systems, along with greater soil aggregation and subsequent SOC occlusion. As with the previous examples of management induced relative differences, these larger SOC stocks observed in perennial systems may not represent absolute or “true” C sequestration.

To quantify absolute, rather than relative, SOC changes and therefore C sequestration and climate change mitigation potential, different methods may be required. These include; 1) evaluation of SOC change over time, 2) evaluation of the complete soil profile, and 3) comparisons of SOC stocks based on equivalent mineral soil mass or another coordinate system that is stable over time (Sanderman & Baldock, 2010; Sanford et al., 2012; von Haden, Yang, & DeLucia, 2020). While private organizations have already begun building agricultural soil carbon markets, many of the frequently used carbon accounting methods are prone to over- or under-estimate SOC sequestration and undermine efforts to properly mitigate the most disastrous effects of climate change and the credibility of present and future agricultural carbon accounting (Oldfield, Eagle, Rubin, Rudek, & Gordon, 2022). Many studies claiming to demonstrate carbon sequestration with improved management lack baseline SOC stock data. Instead, they compare present SOC stock differences between treatments and attribute the difference to carbon sequestration. However, unless the SOC stocks of the "control" treatment in such "space-for-time" studies remained constant over time (an unlikely situation if temperatures are warming (Soong et al., 2021)), these studies can only show relative changes in SOC stocks. Differences between treatments could be equally attributed to differential gains or losses in both the control and "improved" management scenario (Rocci et al., 2021; Sanderman & Baldock, 2010).

Further exacerbating these accounting issues, many studies do not sample the entire soil profile. While increasing SOC in surface soils has many important benefits beyond SOC sequestration such as improved grain yields (Lal, 2006; Pan, Smith, & Pan, 2009), decreased erosion, and improved nutrient cycling (Jian et al., 2020), if a practice increases surface SOC while losing SOC at deeper, unsampled depths, it may not actually translate into carbon sequestration on an areal basis (Olson & Al-Kaisi, 2015). For example, meta-analyses by Luo, Wang, and Sun (2010) and Powlson et al. (2014) demonstrate that while no-till systems increase SOC in the surface 10 cm of soil, these gains are partially or completely offset by losses at lower depths, likely driven by decreased C inputs at those depths (Luo, Wang, & Sun, 2010; Powlson et al., 2014). Similarly, Rocci et al. (2021) explored the effects of global changes such as warming, nitrogen and carbon dioxide fertilization, and increased precipitation on SOC, finding that the depth measured in each study significantly altered the magnitude and sometimes direction of SOC responses.

Changes in soil bulk density over time can also have significant impacts on SOC stock estimates. Many studies treat depth-from-soil-surface as a constant through time and between treatments, despite potential soil expansion and/or compaction with differing management. These bulk density changes can also affect how much of the soil profile is sampled (von Haden et al., 2020). For example, Guillaume et al. (2022) investigated the impacts of grass rotations in cropping systems and found a 16% underestimation of treatment effect when changes in soil bulk density were ignored (Guillaume et al., 2022). To account for these changes, additional analysis is required to convert linear depth measurements to an unchanging reference system, such as equivalent soil mass (ESM). Institutions such as the IPCC and FAO have emphasized the importance of ESM for SOC stock assessment and tracking (FAO, 2018; Intergovernmental Panel on Climate Change (IPCC), 2019), but standardization remains lacking in SOC crediting methods (Oldfield et al., 2022).

Few studies combine these three critical methodological considerations (change from baseline, deep sampling, ESM corrections) when estimating absolute or “true” rates of SOC stock changes. When they do, they often demonstrate different magnitudes or even directions of SOC changes compared to studies using alternative methods. One such 8-year study by Liu et al. (2022), investigating the effects on SOC of straw retention in conservation tillage and no-till wheat-maize systems, found that tillage redistributed carbon through the soil profile but did not lead to significant changes in SOC stocks, while straw retention increased SOC stocks regardless of tillage (Liu et al., 2022). These results are inconsistent with those of Al-Kaisi and Kwaw-

Mensah (2020) using linear depth methods or Powlson et al. (2024) evaluating surface soils. Both reported potential for SOC gains in no-till systems.

Furthermore, for SOC gains to be considered in climate change mitigation, their permanence needs to be reliable on a multidecadal or longer timescale in realistic farming conditions. This highlights the need for long-term, systems-level experiments such as the Wisconsin Integrated Cropping System Trial (WICST) which allow us to measure the long-term impacts of regionally appropriate cropping systems managed like production farms. Experiments like WICST are more likely to represent real-world conditions, and thus provide results that are more widely and directly applicable. When considering pressing issues such as climate change mitigation, accurate and applicable results are increasingly necessary.

To address the existential threat of climate change and assess the feasibility of carbon sequestration in the carbon-rich agricultural soils of the United States' Upper Midwest, we need to understand management's long-term impact on absolute, not relative, SOC stocks. Do common cropping systems in the Upper Midwest emit or sequester SOC? Do estimates of SOC stocks change when different analysis methods are used? To address these questions, we quantified SOC changes over 30-years under 6 common Upper Midwestern agricultural systems (3 cash-grain, 3 dairy-forage) at WICST. We hypothesized that; 1) the three cash-grain systems would lose more SOC than the dairy-forage systems, and that 2) the pasture system would stabilize and perhaps gain SOC. We also hypothesized that; 3) methodologies such as the use of "space for time", omitting ESM corrections, and limiting analysis of SOC stocks to surface soil horizons would result in significant inaccuracies in SOC sequestration potential estimates for each system, as other studies have found (Guillaume et al., 2022; Powlson et al., 2014; Tautges et al., 2019).

To test these hypotheses, we calculated temporal changes in ESM-corrected SOC stocks for the entire soil profile (0 to 90 cm) for each cropping system. We then subset the full dataset to produce results as though we had used space-for-time, linear depth, or shallow sampling methods. Long-term experiments like WICST are invaluable in our efforts to leverage agricultural best management practices to mitigate the most severe impacts of climate change. They allow us to evaluate true SOC dynamics in cropping systems and soils that are relevant and applicable to the broader US Corn Belt.

Methods

Site description and history

We conducted this work at the University of Wisconsin-Madison's Agricultural Research Station in Arlington, WI (43^{o}18'N, 89^{o}20'W) on soils classified as Plano Silt loam (fine-silty, mixed, superactive, Mesic Typic argiudolls). Mean annual temperatures at the site were 8.1 deg C between 1981 and 2000 and 9.3 deg C between 2001 and 2020, while mean annual precipitation was 901 mm between 1981 and 2000, and 882 mm between 2001 and 2020 (NOAA, 2021).

Conversion of tallgrass prairie vegetation at the site, primarily for the continuous cultivation of wheat, began in the 1840s (Posner, Casler, & Baldock, 1995). In the 1860s, crops for dairy cattle feed predominated. From the 1960s until the establishment of WICST, maize (*Zea mays* L.), alfalfa (*Medicago sativa* L.), and soybeans (*Glycine max* (L.)Merr.) were the main crops in rotation with dairy manure applied for fertility (Posner et al., 1995).

In 1989, Posner et al. planted maize across the 24-ha field where WICST was to be established to provide a "uniformity year", homogenizing the fields crop history and allowing baseline measurements (crop yield and soil parameters), which were then used to determine the boundaries of each block in the core experiment's four-block randomized complete block design. In 1990, six cropping systems were initiated at the trial with the first legume phase of the rotation. To reduce the influence of yearly weather variation on the results, the starts of the rotations were staggered. By 1992, every phase of every treatment was present every year (Posner et al., 1995). These rotations are grouped by enterprise type with three cash-grain and three dairy-forage systems common to the Upper Midwest, varying in crop diversity, tillage intensity, and input levels. The three cash-crop systems include high-input continuous maize (Maize), moderate-input no-till maize-soybean (MS), and organically managed maize-soybean-winter wheat interseeded with red clover (MSW). The three forage-crop systems include high-input maize-alfalfa (MaAA), organic maize-oats/alfalfa-alfalfa (Mo/aA), and management-intensive rotationally grazed pasture (MIRG) (Table 1). Posner et al. (2008) provide additional details on the design and operation of WICST.

Within each of WICST's four blocks, every phase of each system is present. Continuous maize (Maize) therefore has one plot per block for example, while MaAA has four plots per block. Each 0.3-ha plot within WICST is sized so that work can be done with standard farming equipment.

Soil sampling

In 1989, prior to the creation of WICST, soil samples were collected across the entire 24-ha field by points on a 27 x 27-m grid. At each sampling point, four cores were taken and divided and homogenized by depth (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm). The first two depths were collected using a 3.2-cm diameter probe and the deeper two depths were taken using a 1.9-cm diameter probe. After initial analysis in 1989, the samples were dried, ground, and archived. In 2009, these dried homogenized samples were cleaned of visible plant material and analyzed for C content following the same methods as outlined below (Sanford et al., 2012).

In June 1989, cores for bulk density (ρ_B) were collected at two sampling depths (0 to 15 cm and 15 to 30 cm) on the same 27 x 27-m grid, using a 7.5-cm diameter hammer core. Detectible change in ρ_B below 30 cm between 1989 and 2009 was unlikely, given the well-structured grassland soils and the exclusion of excessively heavy equipment use at WICST. Comparison of the 2009 ρ_B and ρ_B of the most recent sampling at WICST in 2019 showed no significant difference between the two timepoints below 30 cm, supporting our initial assumption. Therefore, ρ_B values for the 30 to 60- and 60 to 90-cm depths from 2009 were used for 1989 calculations of soil carbon on a mass basis.

To align the original sampling grid with the 2009 and 2019 data, which were collected by plot, we georeferenced a map of the original sampling grid using GPS data and determined the location of the original sampling points relative to plot-level sampling points using ArcGIS. We then used ordinary kriging to spatially interpolate the 1989 data and digitally resample at the plot-level sampling points using R package gstat.

Between 22 April and 17 July 2009 soil samples were collected from each plot at WICST using a tractor mounted hydraulic soil sampler with a 3.2-cm diameter probe. Nine cores were taken from each plot, three from the middle of three 18 x 52-m sections (north (N), center (Ct.) and south (S)). In each section, cores were collected 19 cm from each other, so that in-row and between-row soils in the maize plantings were equally represented. The three cores from each section were then divided and homogenized by the four depth increments (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm). Field-moist samples were sieved to 2 mm, picked free of visible plant matter, and dried in a 45 °C oven. Aliquots of these dried homogenized soils were ground to a fine powder in a ball mill and analyzed for soil C content. The same process was repeated in autumn 2019.

Cores for 2009 ρ_B measurements were collected in 2007 using a 3.7-cm diameter hammer core and in 2008 using a 5.4-cm diameter hydraulic core, to depths of 90 cm, divided into 0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm to align with carbon concentration measurements. Additional sampling was conducted in 2008 with the 7.5-cm diameter hammer core used in 1989 to test equivalence to the smaller diameter tools (data not shown). For sampling points missing ρ_B values in 2009 (n=518 out of 2376 total), values from within that system, depth, and block were averaged to estimate ρ_B.

We collected cores for 2019 ρ_B in autumn 2019 using a 5.4-cm diameter hydraulic core. Missing or damaged cores were recollected in October 2020. Similar to 2009, we divided cores into 0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm sections. Prior to weighing, we dried the sections in a 50-°C oven until weights stabilized. After weighing, we sieved the 60 to 90 cm sections to 2 mm to remove rock fragments. We subtracted the weight and estimated volume of these fragments from the total weight and volume prior to ρ_B calculation, so that ρ_B estimations represent the same soil as the SOC measurements. We calculated ρ_B by dividing the weight of the dried section by the original volume of its section of the soil core.

Organic carbon determination

Because inorganic carbon in these soils is negligible (~0.005%)(Paul, Collins, & Leavitt, 2001) we used total C interchangeably with SOC. To confirm this assumption and that routine liming of soils had not significantly increased inorganic C, we measured organic C of samples from all recently limed plots using our CN analyzer’s manufacturer’s recommended method (OEA Team, 2009) finding it to be insignificantly different from total C (Figure 1).

Finely powdered subsamples of the dried soils from each depth (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm) at each point in the 1989 grid or in each section (N, Ct., S) in each plot were packed into 5 x 9-mm tin capsules. For the 1989 and 2009 samples, 8 to 10 mg of soil was used. For the 2019 samples, 15 mg, 18 mg, 30 mg, and 50 mg of soil were used for the 0 to 15, 15 to 30, 30 to 60, and 60 to 90-cm depths, respectively, to better remain within the instrument's range of detection. Total percent C for the encapsulated samples was then determined by dry combustion using a Flash EA 111d CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy).

Calculation of carbon stocks

To determine carbon stocks, we used mass of mineral soil from surface, rather than linear or fixed depth from surface, as a reference system that remains stable between time points. This *equivalent soil mass* method accounts for compaction, expansion, and additions or losses of organic matter as a result of different treatments. This ensures the same section of the soil profile is considered each time. Fixed-depth measurements were converted to ESM using R code provided in von Haden et al., 2020 (von Haden et al., 2020) (Figure 2).

After we converted $\rho_B \; and \; \%C$ to ESM, the SOC stock was calculated:

$$SOC_{stock} = \frac{SOC_{\%} \, \rho_B V}{A}$$

Here, SOC_{stock} is the SOC stock in Mg C ha^{-1}, $SOC_{\%}$ is percent SOC, ρ_B is the bulk density in Mg soil cm^{-3}, V is the volume of the bulk density core in cm^3, and A is the upper surface area of the bulk density core in hectares.

Analysis of SOC trends over time

General linear mixed effects models (PROC GLIMMIX, SAS 9.4) were used to estimate the rate of carbon change over time within a system as well as compare the average change since 1989 (ΔSOC) in between systems in 2009 and 2019. Analysis of covariance (ANCOVA) was used to estimate rate of carbon change within system. In this analysis year (1989, 2009, 2019) is treated as a continuous covariate, system (Maize, MS, MSW, MaAA, Mo/aA, MIRG, categorical variable) is treated as a categorical fixed effect, and block (1 to 4) is treated as a random effect. Because of expected spatial and temporal correlations in the data, and to enable variance heterogeneity between cropping systems we chose a variance-covariance matrix with first-order heterogenous autoregressive structure (subject = plot, group = system). See the Code section for complete model details.

System comparison analysis

Analysis of variance (ANOVA) was then used to evaluate ΔSOC for each depth separately. In this model, system is treated as a fixed effect while block is treated as a random effect, using the same variance-covariance structure as the ANCOVA. Outliers for each subset of data were removed using SAS PROC UNIVARIATE.

Methods sensitivity analysis

To test the influence of methodology used in estimating SOC stocks (space-for-time, fixed-depth, shallow sampling), analysis was conducted for each method to produce three alternative datasets that could be compared to the dataset generated using longitudinal measurements, ESM corrections, and the full 90-cm soil profile. In the space-for-time dataset, only data from 2019 were used. MaAA was chosen as the “baseline” scenario for calculating ESM conversions and change-from-baseline since it was most similar to the crop rotation present on the site prior to the establishment of WICST, being the treatment closest to historical land use at WICST. Equivalent soil mass and change since 1989 were determined using an average of MaAA plots in each block in 2019 as the baseline. For the shallow-sampling dataset, data were converted to ESM to account for ρ_B changes. SOC data below 30 cm were removed. For the linear-depth dataset, the full 90-cm SOC stock was included, but not translated to ESM coordinates.

For each method, outliers within each system were identified visually using SAS PROC UNIVARIATE and removed. We used t-tests (base R function “t.test”) to compare each alternative dataset with the dataset generated using longitudinal measurements, ESM corrections, and the full 90-cm soil profile for each cropping system. Then we used the ANOVA described above to analyze change in SOC stocks between 1989 and 2019 as a function of cropping system (PROC GLIMMIX, SAS 9.4). Estimates and standard errors for each cropping system were determined using SAS lsmeans for the entire profile in 2019.

Results

In the ANCOVA analysis of SOC stocks, year, system, and their interaction term were all statistically significant (p = 0.0001, 0.02, and 0.02, respectively) indicating that while there was a linear trend, it differed between systems in slope, magnitude, or both. Of the six cropping systems evaluated, MIRG showed a positive, though non-significant (p=0.39), gain in SOC over the 30-year period indicating that the system was able to maintain SOC stocks over time (Figure 3). All other cropping systems had significant negative slopes indicating a long-term net loss of SOC (Figure 4). Of these five cropping systems, the dairy-forage systems lost less SOC than the cash-grain systems with an annual loss rate of 0.36 $\mathrm{Mg\ ha^{-1} yr^{-1}}$ for MaAA and 0.62

$\mathrm{Mg\ ha^{-1}yr^{-1}}$ for the organic Mo/aA (Table 2). In the cash-grain rotations, Maize and the organic MSW lost carbon at similar rates ($0.71\ \mathrm{Mg\ ha^{-1}yr^{-1}}$ and $0.76\ \mathrm{Mg\ ha^{-1}yr^{-1}}$, respectively) while MS, the minimum-till maize soybean rotation lost the most carbon annually (1.3 Mg $\mathrm{ha^{-1}}$ $\mathrm{yr^{-1}}$)(Table 2).

In 2019, SOC stocks in MIRG were significantly larger than all other cropping systems (Table 3) because significant increases in surface soils were not offset by losses in the 15 to 60-cm depths as they were in most other cropping systems (Table 4). The other forage systems (MaAA and Mo/aA) had the next largest SOC stocks, maintaining surface-soil carbon while losing at depth, although they were not significantly different from Maize (Table 4). Maize lost SOC in the 0 to 15-cm and 60 to 90-cm depths but maintained SOC in the middle depths compared to 1989 (Figure 6). MSW and MS had the lowest SOC stocks (110.32 and 98.13 Mg $\mathrm{ha^{-1}}$, respectively) and were not significantly different.

No significant changes in total SOC were detected within a given cropping system between 2009 and 2019 ($\alpha = 0.1$). However, Mo/aA did accumulate a small though significant amount of SOC in the surface 0 to 15 cm (2.14 ± 1.11 Mg $\mathrm{ha^{-1}}$) (Figure 7). Between 30 and 60 cm, both Maize and MIRG gained SOC (9.50 ± 2.08 and 3.95 ± 1.30 Mg $\mathrm{ha^{-1}}$, respectively), but Maize lost SOC (-2.09 ± 0.38 Mg $\mathrm{ha^{-1}}$) between 60 and 90 cm.

The alternative analysis methods (space-for-time, linear depth, and shallow-sampling) all estimated higher 2019 SOC stocks compared to their longitudinal, ESM-corrected, deep sampling counterpart (Figure 5). This difference, however, was only significant in the shallow-sampling dataset for CS, CSW, and Co/aA. All methods showed the same general trends between cropping systems with SOC stocks greatest in MIRG > dairy-forage systems > cash-grain systems. The space-for-time and shallow sampling results, however, showed SOC stock maintenance in the forage systems (MaAA, Mo/aA) and gains in MIRG, which contrasted with linear-depth findings of maintenance in MIRG and losses in all other systems (Table 5).

Discussion

We found that all cropping systems except for MIRG lost SOC over a 30-year period. While dairy-forage rotations maintained SOC in the surface 0 to 15 cm, they failed to compensate for the loss of SOC from deeper soils. However, we found very few changes in SOC between the 2009 (year 20) and 2019 (year 30) analysis of deep carbon from WICST, which suggests that losses were not linear (see Sanford et al. 2012 for 20-y analysis). Below, we outline how some of these findings might be related to methodological artifacts and compare our findings to other studies evaluating SOC change in common cropping systems.

SOC losses likely from agricultural soils without perennial grassland

The maintenance of SOC in MIRG is likely the result of high C inputs (above and belowground, see Sanford et al. 2012) and a lack of tillage, providing an ideal environment for fungal hyphae which are known to improve aggregation and subsequently increase occlusion of SOC (King et al., 2019; Six, Elliott, Paustian, & Doran, 1998). Even occasional tillage may disrupt fungal networks, breaking up aggregates and delaying future aggregate formation, leaving SOC vulnerable to respiration (Helfrich, Ludwig, & Potthoff, 2008; Wilson, Rice, Rillig, Springer, & Hartnett, 2009). Microbial, and particularly fungal necromass, is responsible for much of the C found in mineral-associated organic matter (MAOM-C), one of the most stable SOC pools (Kallenbach, Frey, & Grandy, 2016). In a study investigating MAOM-C at WICST (0 to 30 cm), Rui et al. (2022) found that MIRG had a significantly higher amount of MAOM-C than the other systems (Rui et al., 2022). Rui et al. (2022) also report greater carbon use efficiency (CUE) in MIRG than the other systems, suggesting that a greater proportion of the carbon metabolized by the microbial communities in MIRG is assimilated into microbial biomass rather than respired.

It is clear from our results that a lack of tillage alone is insufficient to build or maintain SOC stocks in systems with low C inputs. For example, the minimum-tillage MS cropping system lost a significant amount of SOC despite a lack of soil disturbance. We believe this stems from the limited amount of biomass returned to the system during the soybean phase. This finding agrees with other studies showing that no-till systems require leguminous cover crops and double cropping to generate C inputs sufficient to offset losses at depth (Nicoloso & Rice, 2021; Powlson et al., 2014).

Significant losses in the 60 to 90-cm depth of all our systems suggest that not all of the observed SOC change is a result of differences between present cropping system. Soil warming due to climate change may be responsible for the release of this deeper SOC, as laboratory and in situ studies have shown that warming soils can induce SOC loss (Rocci et al., 2021; Soong et al., 2021). Globally, loss of subsoil SOC has been observed in both agricultural and natural systems in the past several decades, suggesting a cause such as warming rather than a change in management (Knotters et al., 2022; Sanderman & Baldock, 2010). Another potential cause may be the field conversion from a dairy-forage rotation prior to 1989 to the six new cropping systems, half of which do not receive dairy manure. While MaAA and Mo/aA still receive manure, losses may result from a transition from historical semi-solid (~15% dry matter) manure application to dairy slurry (~4% dry matter) applications in the mid-90s. Under this hypothesis, SOC losses would result from continued respiration-as-usual without high manure C inputs to offset the losses. This transition likely represents a regional transition as manure management has evolved and, as with warming, we'd expect similar trends across the Upper Midwest.

Dairy-forage rotations maintained SOC in surface soils

Compared to the SOC losses observed in the cash-grain cropping systems (Maize, MS, MSW), the dairy-forage systems (MaAA, Mo/aA, MIRG) maintained or accumulated SOC in the 0 to 15-cm depth. This likely stems from a combination of lower tillage levels in the dairy-forage rotations improving soil aggregation (Cates, Ruark, Hedtcke, & Posner, 2016) and providing opportunities for SOC occlusion as well as greater above ground and below ground inputs from root and shoot biomass as well as dairy manure. While alfalfa taproots can grow to depths below 1 m (Pietola & Smucker, 1995), a majority of its root growth and senescence occurs in the surface 15-cm (Bolinder, Angers, Béanger, Michaud, & Laverdière, 2002). Despite the theoretical subsoil C-sequestering promise of alfalfa as a perennial nitrogen-fixing crop (Peixoto et al., 2022), at WICST at least, the alfalfa-based forage systems lost a significant amount of SOC over the 30-year period despite surface-soil stability. Indeed, MaAA and Mo/aA did not significantly differ from MS when the entire soil profile was considered. A long-term, whole-profile study on Iowa Mollisols found similar results comparing a maize-soy rotation to a maize-maize-oats/alfalfa-alfalfa rotation. Sampling to 90 cm, they found no significant differences between the total SOC stocks of both rotations (Poffenbarger et al., 2020).

Total SOC stocks not different between year 20 and year 30

While lack of significant changes in whole-profile SOC stocks between 2009 and 2019 may suggest the cropping systems are approaching equilibrium, some changes in each depth appear significant (figure 7). SOC is a large and variable stock relative to the small changes we seek to detect. It is possible that, despite ongoing SOC fluxes, 10 years is insufficient to accrue detectable changes on these carbon rich Mollisols, an important consideration for those seeking to capitalize on carbon market schemes. A 2020 study by Cusser et al. (2020) found that at least 15 years of data were necessary to detect changes in both crop yield and soil water that aligned with larger observed trends (Cusser, Bahlai, Swinton, Robertson, & Haddad, 2020). Furthermore, two time points are not sufficient to determine equilibrium.

The appearance of SOC losses in 60 to 90-cm from 2009 to 2019 in the Maize system and SOC gains in 30 to 60-cm in Maize and MIRG may be an artifact of the depths measured in this study. The depth of the A horizon of Plano silt loams varies from 23 to 36 cm. If portions of the A horizon are only sometimes included in the 30 to 60-cm depth, this may lead to high variability in SOC estimates. This artifact is most likely to appear in the Maize and MIRG systems, as they occupy only one plot per block compared to the other cropping systems, which occupy up to four plots per block, since each phase of each system occupies one plot within each block. With fewer datapoints, Maize and MIRG are more likely to be influenced by noise.

Sensitivity to alternative methods

The datasets generated by the alternative methods were not significantly different ($p > 0.1$) from the primary dataset, except for MS, MSW, and Mo/aA in the shallow-sampling analysis. However, when used with generalized linear mixed models to estimate SOC stocks, the alternative methods did estimate larger SOC stocks. The larger estimated SOC stocks in the linear-depth method come from an increase in bulk density across cropping systems in the surface soils, which leads to more soil and thus more SOC sampled. Space-for-time also resulted in larger SOC stocks. The space-for-time analysis relies on the assumption that the reference system is at equilibrium. However, MaAA lost SOC below 15 cm. This resulted in the space-for-time analysis overestimating SOC in all systems by an amount roughly equal to the true losses in

MaAA. The SOC stocks from the shallow-sampling method are larger and more precise, due to having the same number of datapoints as the complete dataset without the variability and losses of subsoil SOC.

This sensitivity to methods aligns with findings from Guillaume et al. (2022) investigating the impacts on SOC of grass rotations in cropping systems showed a 16% underestimation of treatment effect when using linear depth and 58% without accounting for subsoil stocks response (Guillaume et al., 2022). When using linear depth and shallow sampling to compare the difference in SOC between Maize and MIRG, we observed a 16.8% and 62.4% change in difference in SOC, respectively.

Relevance of SOC storage to climate change mitigation

The common row crop systems evaluated in this study represent sources, not sinks, of CO_2. This observation is likely applicable across the C-rich, prairie-derived soils prevalent in the Upper Midwest. Prior to 1989, all WICST soils were under a dairy-forage, livestock-integrated row cropping system. The change from this to cash-grain systems like Maize and MS is largely representative of changes experienced across the Upper Midwest as farms have increased in size and specialization, separating livestock and livestock feed production.

Regarding the alfalfa-based forage systems, while increases in surface SOC have multiple benefits which may improve resilience to climate change, including reduced erosion, improved water retention, and nutrient cycling (Jian et al., 2020; Lal, 2006; Pan et al., 2009) these benefits do not include climate change mitigation when overall, SOC is lost to the atmosphere.

For all systems, losses of SOC below 60 cm may represent a blind spot in climate models and carbon market schemes, many of which assume SOC stocks are stable or increasing on agricultural land with “improved” management such as no-till (MS), organic management

(Mo/aA), and/or cover crops (MSW)(Tautges et al., 2019). In contrast, we observed overall SOC losses for those systems. Overlooking these losses may lead to an underestimation of the urgency of the need to reduce emissions from fossil fuels and land use conversion or to mitigate these losses by conversion to systems like MIRG.

Sensitivity of the results to alternative, commonly used methods, especially shallow sampling, demonstrates the importance of long-term, temporal, compaction-adjusted studies that measure the entire soil profile. Relying solely on alternative methods may lead to inaccurate or imprecise estimations of SOC sequestration, further undermining efforts to mitigate and adapt to climate change.

Conclusions

The changing SOC stocks measured at WICST illustrate the importance of agricultural management and assessment methods in determining the status of soils as sources or sinks of CO_2. We hypothesized that the cash-grain systems would lose more SOC than the dairy-forage systems, while the pasture system would stabilize and perhaps gain SOC. This study provides strong evidence that our hypothesis was correct, with significant losses in all cropping systems except for MIRG, which had stable overall SOC stocks. MIRG's lack of increased SOC and the other systems' losses of SOC were both partially driven by losses at depth. While MIRG significantly increased surface-soil SOC stocks, the alfalfa-based dairy-forage systems maintained and the cash-grain systems lost surface SOC.

Most observable changes in total SOC stocks occurred in the first 20 years of the experiment, but statistical considerations and the smaller amount of time between year 20 and year 30 prevent definitive conclusions about the status of ongoing SOC changes or equilibrium. Furthermore, we found that common methods used in SOC accounting, such as space-for-time, linear depth, and shallow sampling, would have obscured the magnitude of SOC losses in these soils, and in the case of space-for-time and shallow sampling, both would suggest SOC gains in MIRG. Accurate accounting of SOC changes is imperative for the success of climate models, carbon offset programs, and policy making. While this study suggests emission offsets using SOC sequestration in highly productive, carbon-rich agricultural soils may not be possible, our work does agree with other studies in suggesting that, of these common Upper Midwestern cropping systems, well-managed pasture represents the best option for SOC-based climate mitigation.

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Tables

Table 1. WICST cropping system descriptions.

Cropping system	System code	System description	Species
Maize	CS1	Conventional continuous maize	*Zea mays* L.
MS	CS2	Conventional maize-soybean with minimum tillage.	*Zea mays* L., *Glycine max* L. Merr.
MSW	CS3	Organic maize-soybean-winter wheat with interseeded red clover	*Zea mays* L., *Glycine max* L. Merr., [WINTER WHEAT], *Trifolium pratense* L.
MaAA	CS4	Conventional maize followed by three years of conventional alfalfa	*Zea mays* L., *Medicago sativa* L.
Mo/aA	CS5	Organic maize followed by oats/alfalfa followed by alfalfa	*Zea mays* L., *Medicago sativa* L., *Avena sativa* L.
MIRG	CS6	Rotationally grazed pasture seeded to red clover, timothy, smooth bromegrass, and orchardgrass	*Trifolium pratense* L., *Phleum pratense* L., *Bromus inermis* L., *Dactylis glomerata* L.

Table 2. Modeled change in SOC stocks at WICST between 1989 and 2019 showing the significant losses in all systems except MIRG. Values and significance determined using SAS PROC GLIMMIX Estimate using the ANCOVA described in the Methods section.

Cropping system	SOC change (Mg $\mathrm{ha}^{-1}\mathrm{yr}^{-1}$)	$P_r > \lvert t \rvert$
CS1: CC	-0.7131	0.0326
CS2: CS	-1.3028	<0.0001
CS3: CSW	-0.7623	0.0034
CS4: CAAA	-0.3568	0.0577
CS5: CoAA	-0.6219	0.0077
CS6: MIRG	0.3338	0.3906

Table 3. Modeled SOC stocks at WICST in 2019. Systems with the same letter are not significantly different ($\alpha = 0.1$). Values and significance determined using SAS PROC GLIMMIX lsmeans using the ANCOVA described in the Methods section.

Cropping system	**System code**	**Mean SOC ($Mg\ ha^{-1}$)**	**Standard Error ($Mg\ ha^{-1}$)**
Maize	CS1	123.7^{ab}	12.0
MS	CS2	98.1^{c}	10.7
MSW	CS3	110.3^{ac}	11.2
MaAA	CS4	135.4^{b}	10.1
Mo/aA	CS5	124.2^{ab}	10.7
MIRG	CS6	158.8^{d}	13.8

Table 4. Modeled change in SOC stocks between 1989 and 2019 by depth increment for different cropping systems at WICST. Values and significance determined using SAS PROC GLIMMIX lsmeans on subsets of the data by depth, using the ANOVA described in the Methods section. Outliers for each system and depth were removed using SAS PROC UNIVARIATE.

Cropping system	**System code**	**Depth (cm)**	**ΔSOC (Mg ha^{-1})**	**Standard Error (Mg ha^{-1})**	**Pr > |t|**
Maize	CS1	0-15	-3.3	0.7	***0.028***
		15-30	-3.5	1.7	0.112
		30-60	-3.5	2.5	0.205
		60-90	-7.3	1.3	***0.002***
		cumulative	-21.9	6.4	***0.01***
MS	CS2	0-15	-1.9	0.8	***0.067***
		15-30	-7.9	2.4	***0.008***
		30-60	-2.9	1.6	***0.098***
		60-90	-5.7	1.3	***0.004***
		cumulative	-16.9	4.8	***0.012***
MSW	CS3	0-15	-1.5	0.7	***0.053***
		15-30	-7.6	2.0	***0.008***
		30-60	-5.1	1.5	***0.004***
		60-90	-7.2	1.3	***0.001***
		cumulative	-19.7	4.8	***0.002***
MaAA	CS4	0-15	-0.3	0.8	0.662
		15-30	-4.5	1.9	***0.053***
		30-60	-3.4	1.6	***0.052***
		60-90	-6.3	1.2	***0.002***
		cumulative	-12.1	4.9	***0.03***
Mo/aA	CS5	0-15	0.8	1.0	0.422
		15-30	-3.4	2.3	0.169
		30-60	-3.2	1.6	***0.062***
		60-90	-7.2	1.2	***0.001***
		cumulative	-13.9	4.7	***0.013***
MIRG	CS6	0-15	7.6	2.4	***0.023***
		15-30	-2.1	2.8	0.466
		30-60	1.3	3.7	0.731
		60-90	-4.8	1.0	***0.016***
		cumulative	1.4	10.4	0.899

Table 5. Change in SOC of the different cropping systems if the SOC stocks had been collected and calculated using different methods.

System	*Complete dataset* $(Mg\ C\ ha^{-1})$			*Space-for-time* $(Mg\ C\ ha^{-1})$			*Linear depth* $(Mg\ C\ ha^{-1})$			*Surface 0-30 cm* $(Mg\ C\ ha^{-1})$		
CS1	-21.9	±	6.4	-4.8	±	4.3	-14.5	±	5.4	-6.4	±	2.4
CS2	-16.9	±	4.8	-14.8	±	9.3	-12.3	±	4.1	-11.5	±	3.1
CS3	-19.7	±	4.8	-16.9	±	9.4	-12.4	±	5.0	-10.0	±	1.7
CS4	-12.1	±	4.9	-3.2	±	4.5	-6.7	±	4.9	-4.8	±	2.1
CS5	-13.9	±	4.7	-2.2	±	5.1	-5.7	±	4.7	-2.7	±	2.7
CS6	1.4	±	10.4	17.9	±	7.2	5.2	±	11.6	5.8	±	5.6

Figures

Figure 1. Comparison of organic carbon (OC) and total carbon (TC) for limed soils at WICST. Lack of significant difference between these analyses indicates insignificant inorganic carbon in these soils.

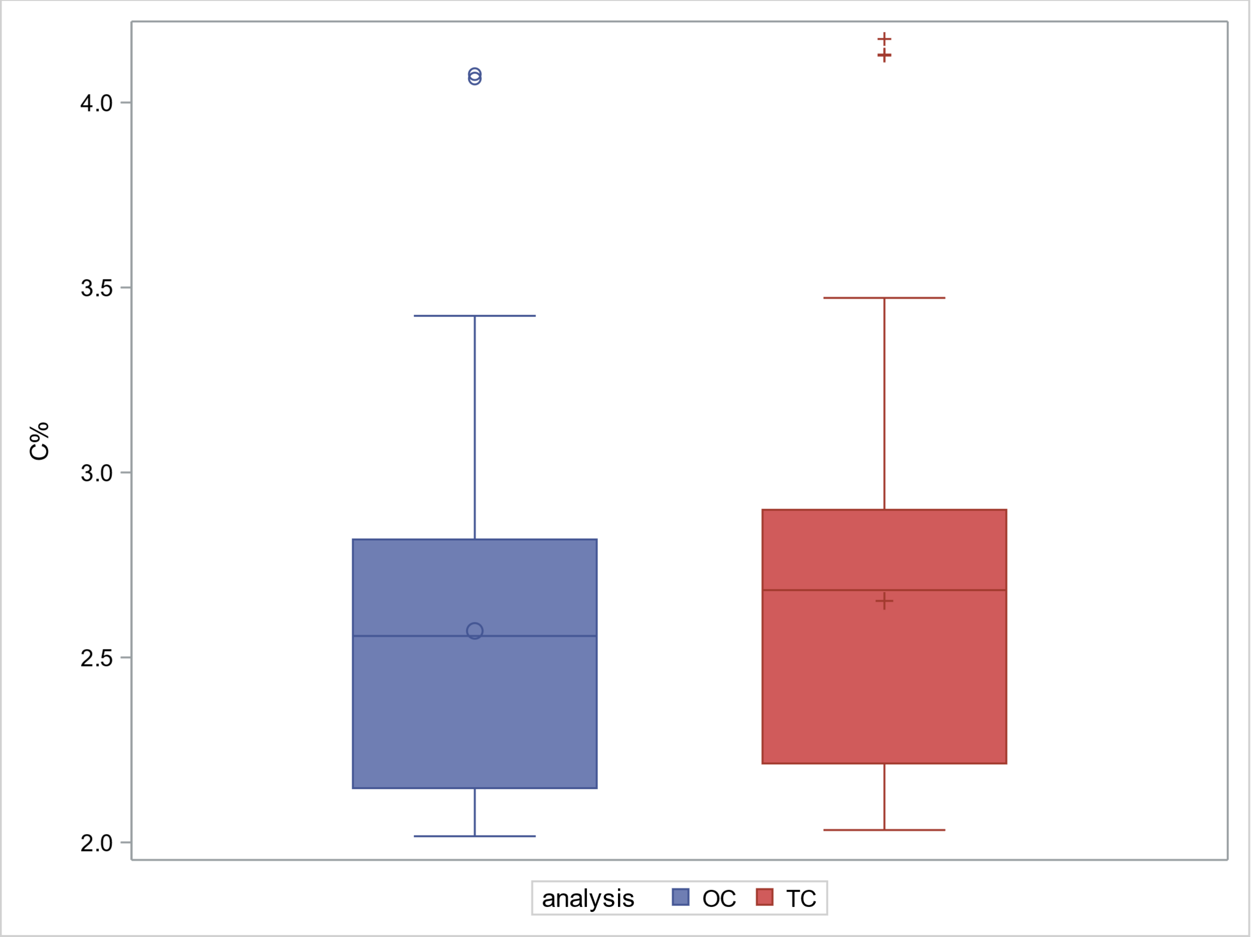


Figure 2. A hypothetical scenario demonstrating ESM corrections where SOC stocks remained stable over 30 years but surface soils were compacted. Adapted from von Haden et al. 2020 Fig. 3**.**

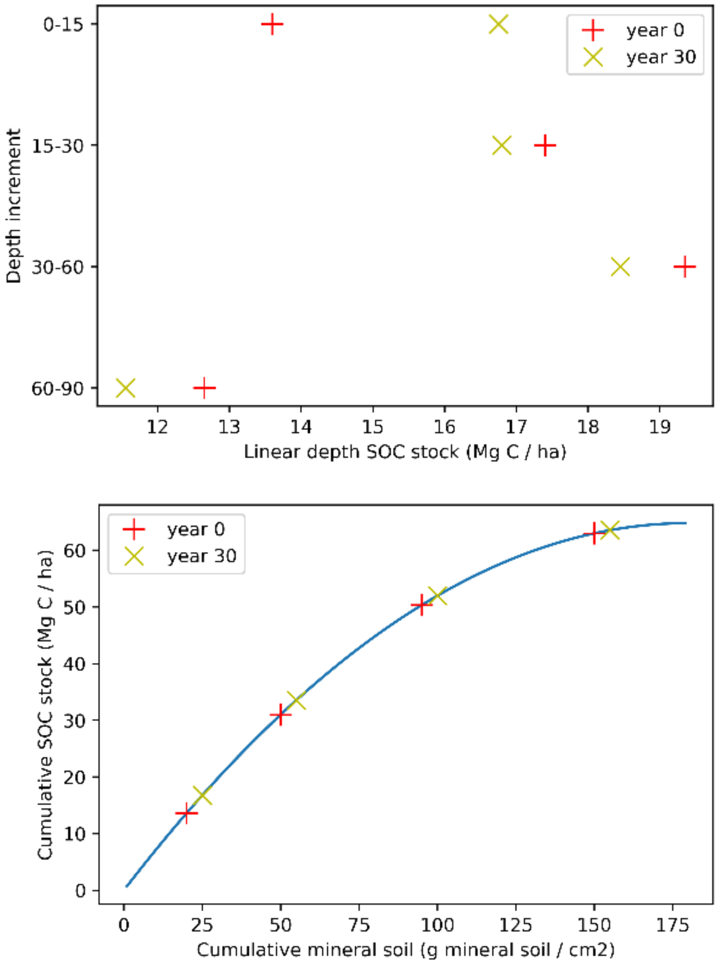


Figure 3. Change in total SOC in each cropping system. Center bar represents estimated change in SOC since 1989. Boxes represent +/- the standard error. Whiskers represent upper and lower 90% confidence limits. If a whisker overlaps with the zero line, that group has not changed significantly since 1989.

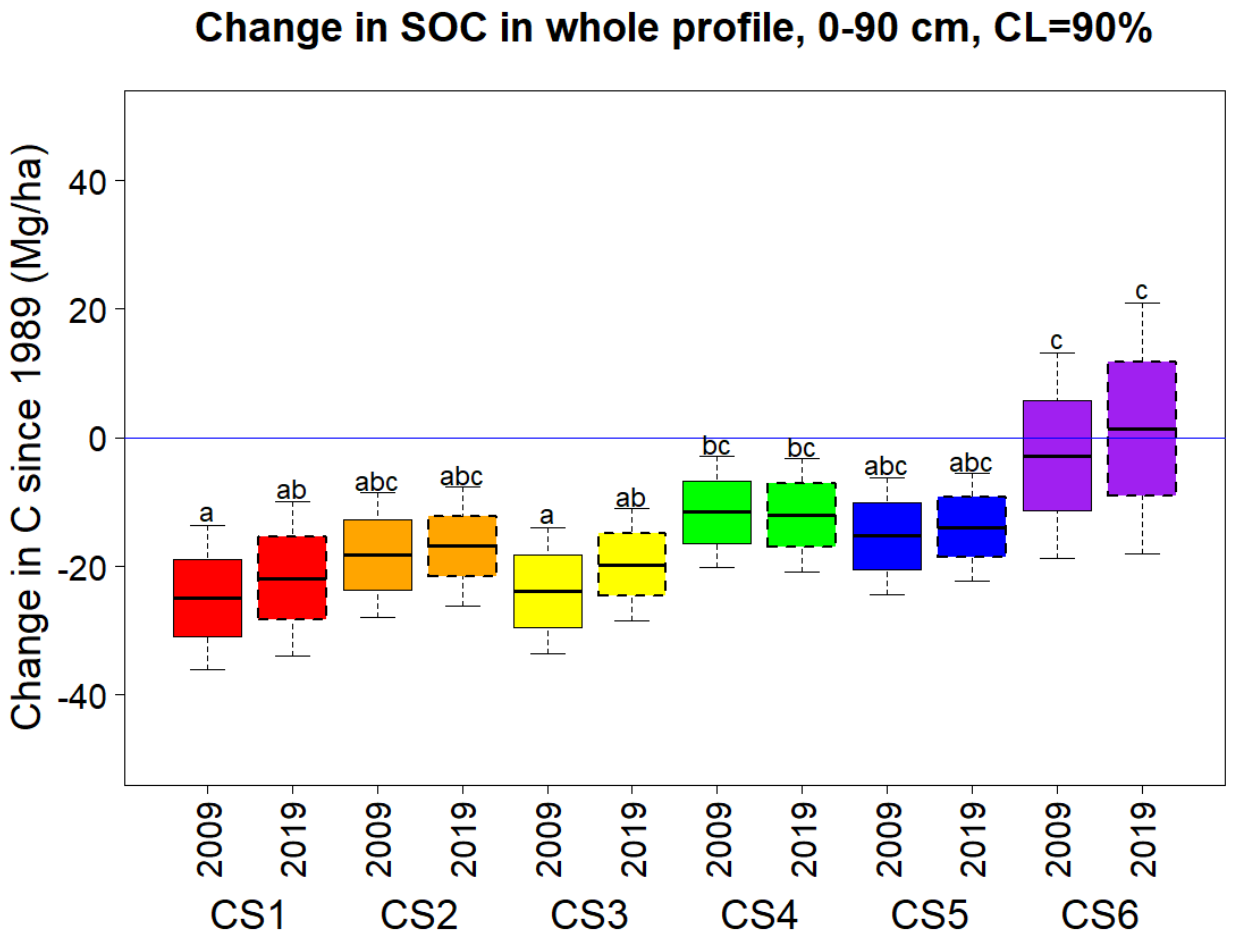


Figure 4. SOC change over time for the WICST cropping systems. Lines represent estimated SOC stocks from the ANCOVA described in the Methods section. Error bars and shading represent standard error. A solid line indicates a p-value less than 0.1.

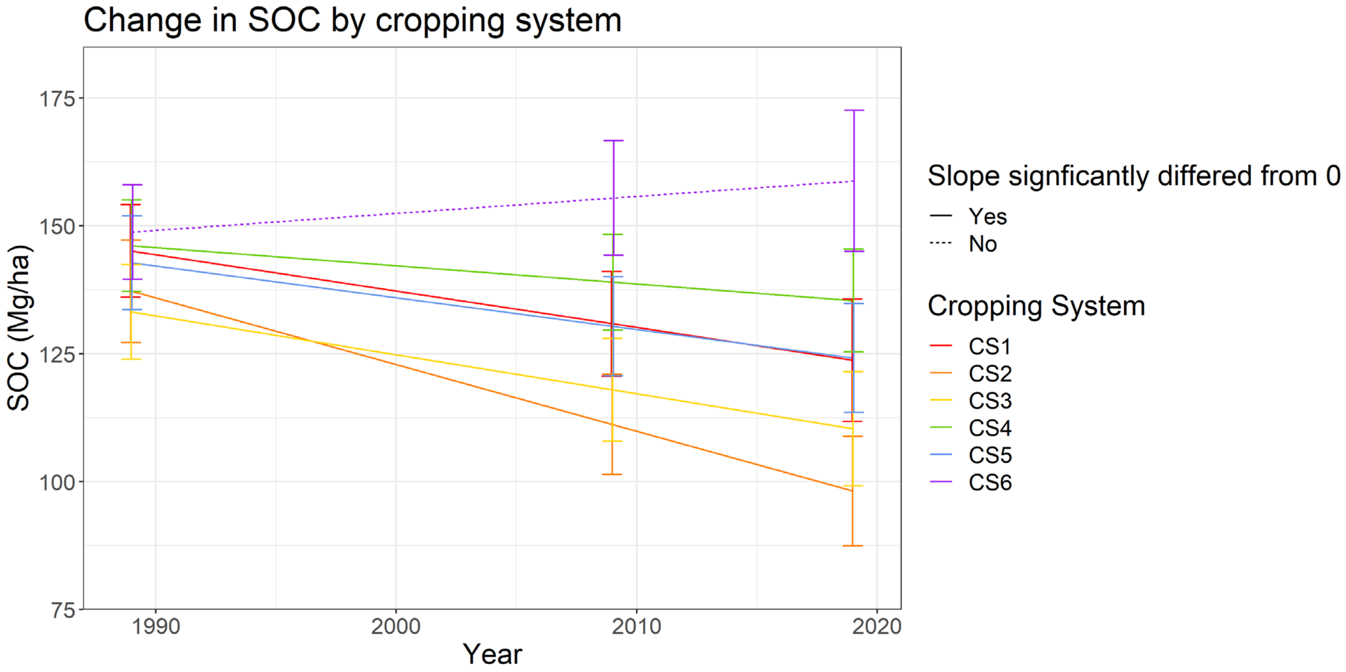


Figure 5. Modeled change in SOC of the different cropping systems if the SOC stocks had been collected and calculated using different methods. Error bars represent standard error. "**" indicates a p-value < 0.05 and "*" indicates a p-value < 0.1 from a t-test between that alternative-analysis dataset and the temporal, ESM-corrected, full-soil-profile dataset.

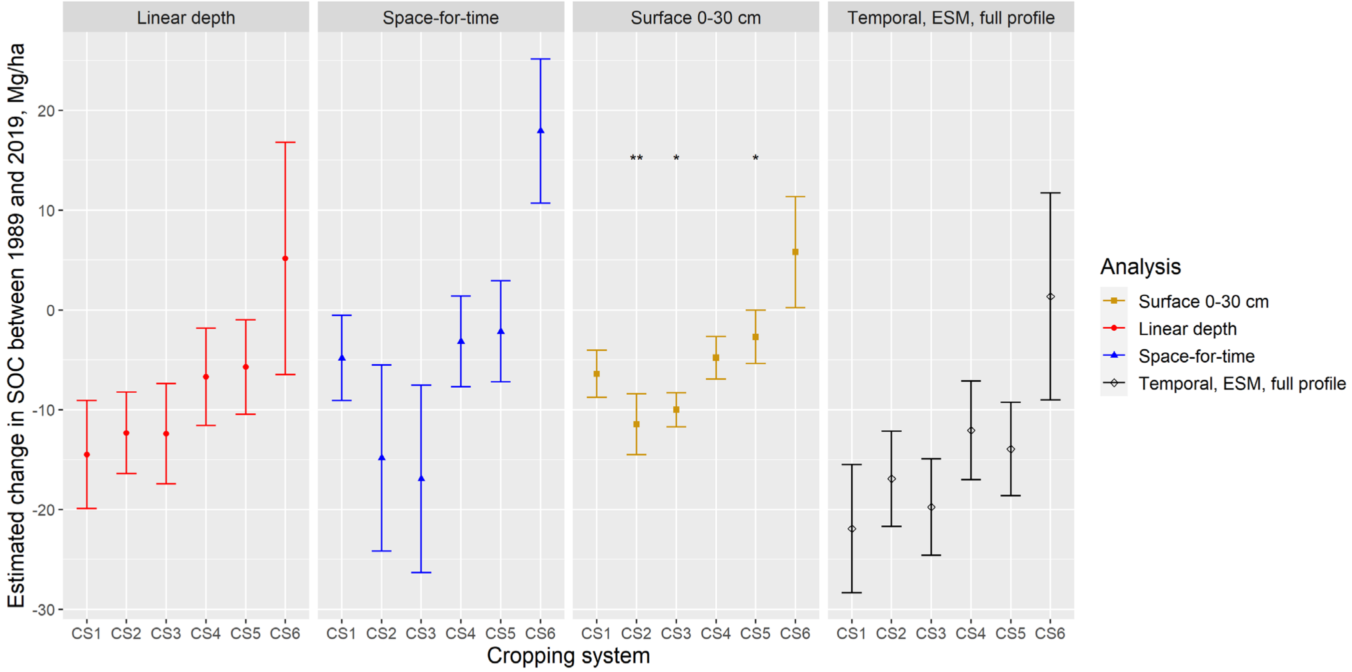
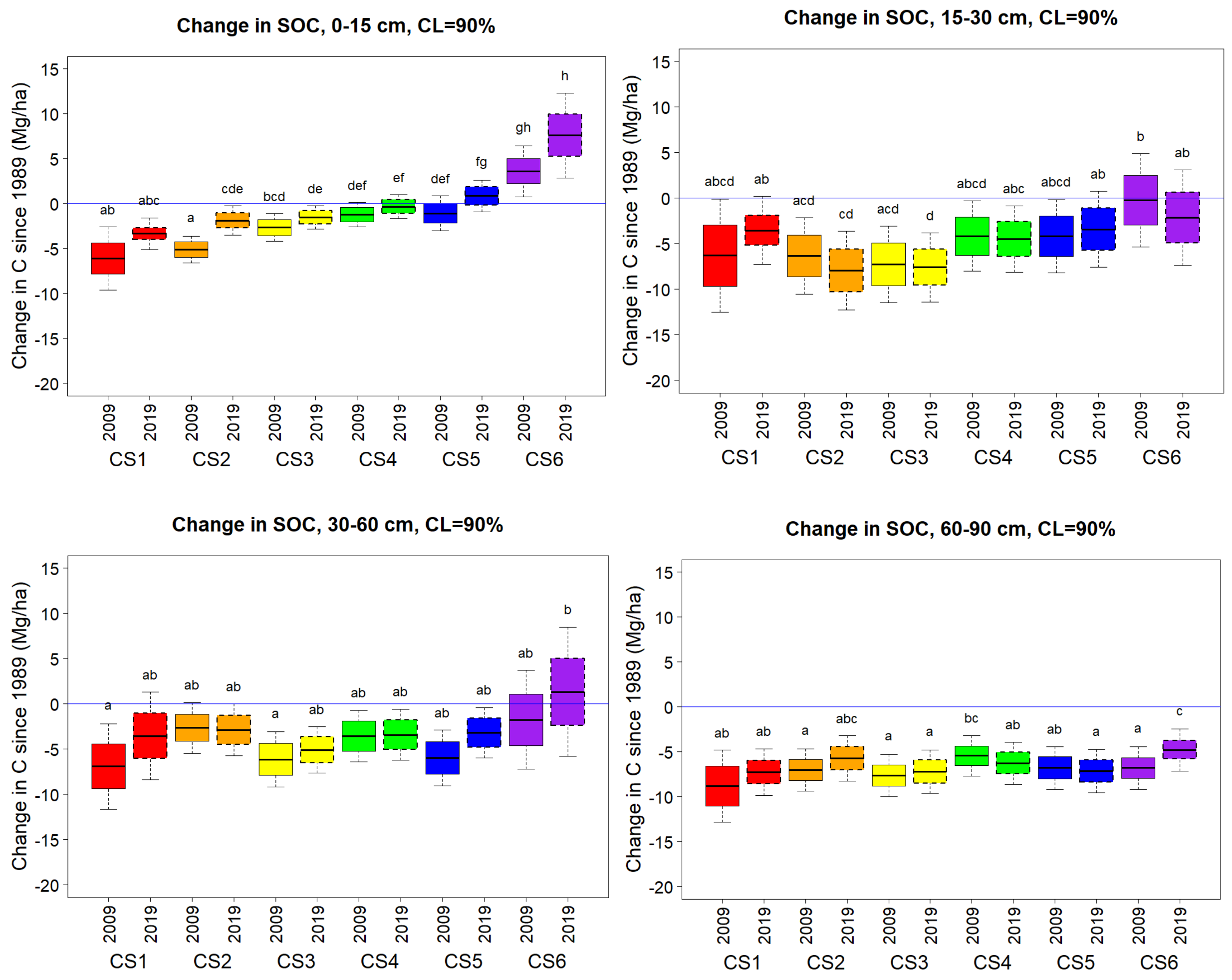


Figure 6. Change in SOC by depth in each cropping system. Center line represents estimated change in SOC since 1989. Boxes represent +/- the standard error. Whiskers represent upper and lower 90% confidence limits. If a whisker overlaps with the zero line, that group has not changed significantly since 1989.



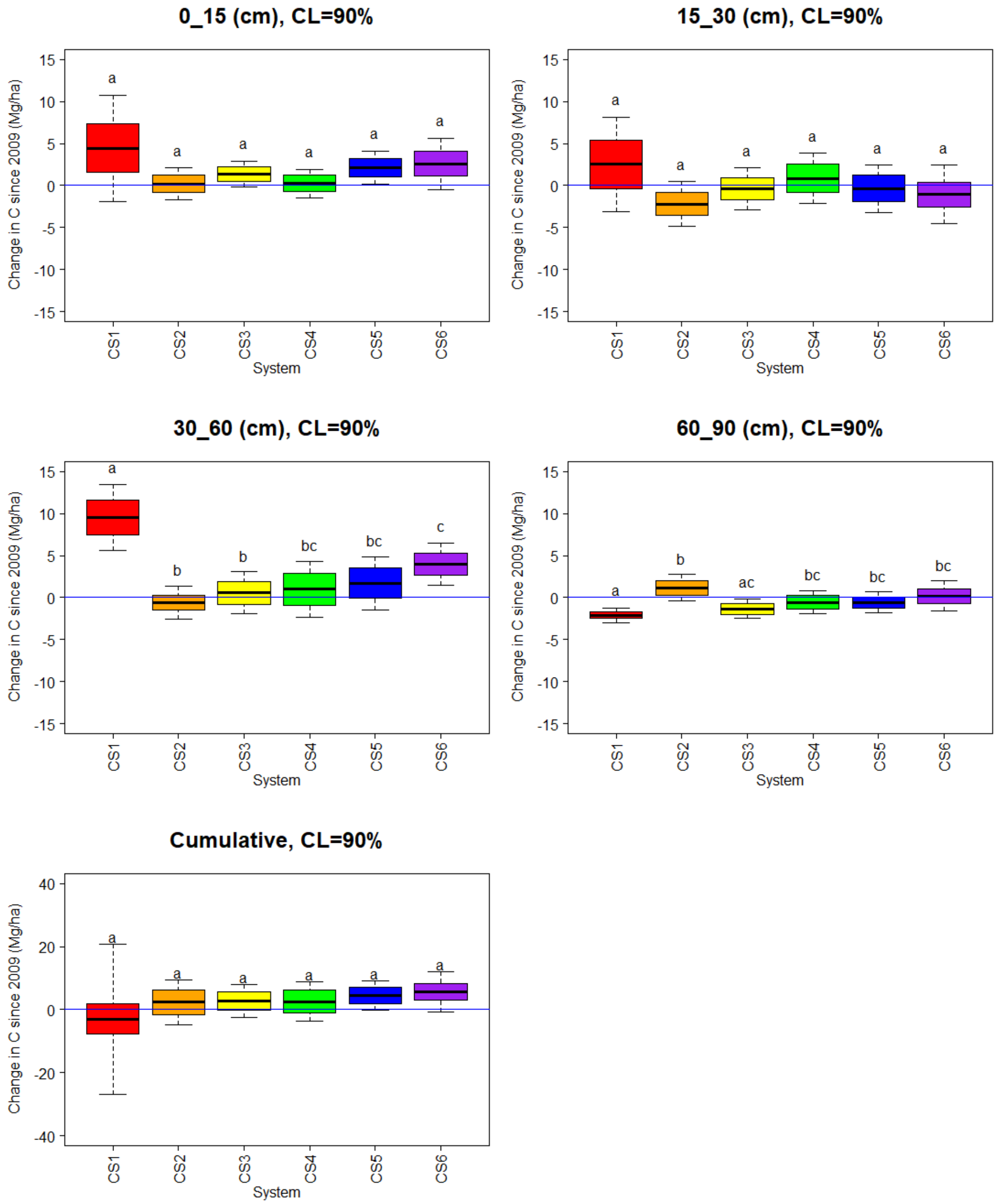
Change in SOC, 0-15 cm, CL=90%

Change in SOC, 15-30 cm, CL=90%

Change in SOC, 30-60 cm, CL=90%

Change in SOC, 60-90 cm, CL=90%

Figure 7. Change in SOC between 2009 and 2019. Center bar represents estimated change in SOC between 2009 and 2019. Boxes represent +/- the standard error. Whiskers represent upper and lower 90% confidence limits. If a whisker overlaps with the zero line, that group has not changed significantly since 2009.



Code

SAS code for ANCOVA

This is the code used to analyze the complete dataset.

```
proc glimmix data = whole_profile plots=studentpanel;
nltoptions gconv=0;
class block system plot;
model SOC_Mg_ha = system year year*system / ddfm=kr;
random block;
random _residual_ / subject = plot group = system type=arh(1);
run;
```

SAS code for ANOVA

This is the code to generate the dataset for the 0 to 15 cm depth and analyze it using an ANOVA. Prior to this step, “delta_SOC_Mg_ha” was calculated by subtracting the baseline SOC from the 2009 and 2019 SOC, respectively, for each sampling point (North, Center, and South) within each plot. Then outliers were removed visually using PROC UNIVARIATE.

```
data delta_0_15;
set WICST_30y_SOC;
if horizon = "0_15";
*options are '0_15', '15_30', '30_60', '60_90', and 'profile';
if year ^=1989;
systyr = cats(system,y);
proc print;
run;

proc glimmix data = delta_0_15 plots=studentpanel;
nltoptions gconv=0;
class block systyr plot;
model delta_SOC_Mg_ha = systyr / ddfm=kr;
random block;
random _residual_ / subject = plot group = system type=arh(1);
run;
```