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Deep soil inventories reveal that impacts of cover crops and compost on soil carbon sequestration differ in surface and subsurface soils

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Abstract

Increasing soil organic carbon (SOC) via organic inputs is a key strategy for increasing long-term soil C storage and improving the climate change mitigation and adaptation potential of agricultural systems. A long-term trial in California's Mediterranean climate revealed impacts of management on SOC in maize-tomato and wheat–fallow cropping systems. SOC was measured at the initiation of the experiment and at year 19, at five depth increments down to 2 m, taking into account changes in bulk density. Across the entire 2 m profile, SOC in the wheat–fallow systems did not change with the addition of N fertilizer, winter cover crops (WCC), or irrigation alone and decreased by 5.6% with no inputs. There was some evidence of soil C gains at depth with both N fertilizer and irrigation, though high variation precluded detection of significant changes. In maize–tomato rotations, SOC increased by 12.6% (21.8 Mg C/ha) with both WCC and composted poultry manure inputs, across the 2 m profile. The addition of WCC to a conventionally managed system increased SOC stocks by 3.5% (1.44 Mg C/ha) in the 0–30 cm layer, but decreased by 10.8% (14.86 Mg C/ha) in the 30–200 cm layer, resulting in overall losses of 13.4 Mg C/ha. If we only measured soil C in the top 30 cm, we would have assumed an increase in total soil C increased with WCC alone, whereas in reality significant losses in SOC occurred when considering the 2 m soil profile. Ignoring the subsoil carbon dynamics in deeper layers of soil fails to recognize potential opportunities for soil C sequestration, and may lead to false conclusions about the impact of management practices on C sequestration.

KEYWORDS
carbon sequestration, compost, cover crops, irrigation, Mediterranean, organic amendments

1 | INTRODUCTION

Soil organic carbon (SOC) is a cornerstone of agroecosystem sustainability, as a driver of soil structure, nutrient cycling, water dynamics, microbial activity, and biodiversity. Increasing SOC reallocates atmospheric CO_2 to long-term organic pools, offsetting greenhouse gas emissions of CO_2, and increasing the resilience of agroecosystems and mitigating the global effects of climate change. SOC is also a common indicator of soil health, receiving considerable attention from growers, environmental advocates, and policymakers alike (California Department of Food and Agriculture (CDFA), 2018; Lal, 2010; Lehman et al., 2015). A recent international policy to mitigate

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CO_2 emissions aims to sequester C in agricultural soils (French Ministry of Agriculture & Food, 2018), attracting widespread investment, scrutiny, and criticism (Amundson & Biardeau, 2018; Chabbi et al., 2017; Minasny et al., 2017). Given the likelihood of future incentives to build soil C in agricultural soils, it is essential to understand and accurately estimate potential gains and losses associated with different management practices. Gaining this knowledge for rainfed and irrigated systems in a semiarid climate, and across a diversity of inputs, will be key for prioritizing management strategies that sequester soil C.

Crop management practices that increase long-term C include cultivation of perennial crops and/or cultivation of pastures (Armstrong, Millar, Halpin, Reid, & Standley, 2003; Follett & Reed, 2010; Lal, 2004; Sanford et al., 2012) and applications of organic amendments (e.g., manure, compost) (Brar, Singh, Dheri, & Kumar, 2013; Poulton, Johnston, Macdonald, White, & Powlson, 2018; Zhang et al., 2012). Net C losses, however, can result from excessive tillage, overgrazing and fallowing (Hernanz, Sanchez-Giron, & Navarrete, 2009; Maia, Ogle, Cerri, & Cerri, 2009). Increases of 0.3 – 4.0 Mg C ha^{-1} $year^{-1}$ (Lee, Owens, & Doolittle, 2007; Smith, Powlson, Glendining, & Smith, 1997; Su, Wang, Suo, Zhang, & Du, 2006) have been observed widely in manured systems. In semiarid rainfed systems, frequent fallowing resulted in no net soil C change, compared to gains of 0.44–1.32 Mg C ha^{-1} $year^{-1}$ in annually cropped systems (Curtin, Wang, Selles, McConkey, & Campbell, 2000; Peterson et al., 1998).

A global meta-analysis of 30 studies found that cover crops increase soil C stocks by 0.32 Mg C ha^{-1} $year^{-1}$ but was limited to the top 30 cm (Poeplau & Don, 2015). In contrast, Poulton et al. (2018) observed losses of 0.55 Mg C ha^{-1} $year^{-1}$ in temperate annual cropping systems with winter cover crops (WCC). Studies more commonly report increases rather than decreases in soil C with WCC. How and to what extent WCC influences C, especially throughout the soil profile, needs more study along a co-management gradient, considering different WCC species and climates.

While Mediterranean agroecosystems represent some of the most diverse, productive, and economically valuable systems in the world, we know surprisingly little about how management affects SOC in these systems (Aguilera, Lassaletta, Gattinger, & Gimeno, 2013; DeGryze et al., 2004; Suddick et al., 2010). These agroecosystems tend to be undersaturated in SOC and may have potential for sequestering additional C if water constraints can be overcome (Jones et al., 2005; Munoz-Rojas et al., 2012; Romanya & Rovira, 2011; West & Six, 2007). An estimated 75% of Mediterranean agroecosystems contain less than 2% soil organic matter (Van-Camp et al., 2004). Increasing SOC could increase the adaptive capacity of these regions, as they are particularly susceptible to rising temperatures and drought expected with climate change (Munoz-Rojas et al., 2012; Romanyà, Rovira, Duguy, Vallejo, & Rubio Sánchez, 2010).

A common assumption is that C in the surface 30 cm layer will be most affected by plant roots and agricultural management practices (Minasny et al., 2017). Few studies have examined soil C below 40 cm depths (Poeplau & Don, 2015). This overlooks much of soil's potential to sequester C, as soil below 30 cm holds between 30% and 75% of total soil C stocks (Chaopricha & Marin-Spiotta, 2013; Harrison, Footer, & Strahm, 2011; Jobbágy & Jackson, 2000; Rumpel & Kögel-Knabner, 2010). Radiocarbon dating showing increased mean residence times of SOC with depth suggests that deep soil C is inherently more resistant to decomposition (Chabbi, Kögel-Knabner, & Rumpel, 2009; Kaiser & Guggenberger, 2003; Paul et al., 1997; Rumpel, Eusterhues, & Kögel-Knabner, 2004). The subsoil generally contains greater reactive surface areas (von Lutzow, 2008) and soil organic matter exists there predominately in organo-mineral complexes, which are considered a key mechanism for long-term stabilization of soil organic matter (Kögel-Knabner et al., 2008; Rumpel et al., 2015). Moreover, deeper layers are not subjected to tillage, a physical disturbance that increases oxidation of SOC.

Long-term experiments provide unique opportunities for understanding C dynamics. Outcomes can be linked to well-documented management practices and evaluated for how they impact overall sustainability of different farming systems. The Century Experiment is a cropping systems trial initiated at the University of California, Davis, in 1993, which examines the long-term sustainability of soil-health building practices (such as WCC and compost), frequent fallow, and irrigation in maize (*Zea mays* L.)–tomato (*Lycopersicum esculentum* Mill.) and wheat (*Triticum aestivum* L.)–fallow crop rotations on 0.4 ha plots. This experiment is one of few long-term studies in irrigated Mediterranean agroecosystems, which represent a globally important ecotype for vegetable and grain production but are also under large threat from climate change (Davidson & Janssens, 2006; Potter, Klooster, & Genovese, 2012). Previous research at the Century Experiment found that after 10 years of consistent management, soil C stocks were greater in organic tomato–maize systems than conventional tomato–maize systems, with and without WCC (Kong, Six, Bryant, Denison, & Van Kessel, 2005). However, analyses were restricted to the surface 0–15 cm and evaluated only a small subset of the cropping systems under comparison.

Here we describe changes in SOC sequestration at the Century Experiment after 19 years of management to a 2 m depth, and across nine farming systems. Our principal questions were: (a) How do long-term inputs of different sources of carbon and management affect soil C sequestration in row crops? (b) Do patterns of C sequestration across different depths vary between crops and management practices? and (c) Can patterns of C sequestration observed in the top 30 cm of soil predict C sequestration throughout the deeper soil profile? We predicted that (a) intensive annual vegetable/grain systems with the highest organic C inputs—e.g., from WCC and/or compost—will show the greatest soil C gains; (b) low-intensity systems with fallow will lose soil C throughout the soil profile; and (c) the direction of soil C change will differ among soil layers throughout the 2 m profile, particularly between the disturbed cultivated layer (0–30 cm) and the undisturbed subsoil (60–200 cm).

2 | METHODS

2.1 | Experimental site and cropping system design

The Century Experiment (previously known as Long-Term Research on Agricultural Systems, LTRAS) is located at the Russell Ranch Sustainable Agriculture Facility near the University of California, Davis (38°32′24″N, 121°52′12″W), with an elevation of 16 m. The site is located in California's northern Central Valley in an alluvial plain of the Putah Creek watershed, which contains soil deposited from what is now the Berryessa Reservoir and includes the Great Valley Complex, Sonoma Volcanics, and Quaternary surface deposits (Shlemon, Horner, & Florsheim, 2000; Wagner et al., 2011; Wolf et al., 2018). The area was originally oak savannah and perennial grassland; ecotypes which have been mostly replaced by annual row crop agriculture. The climate is semiarid, Mediterranean, and characterized by wet winters and hot, dry summers.

The Century Experiment was established in 1993 to test the long-term impacts of wheat- and maize-based cash crop rotations common to northern California on productivity, profitability, resource-use efficiency, environmental impacts, and ecosystem services. The site has two soil types: (a) Yolo silt loam (Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents) and (b) Rincon silty clay loam (fine, smectitic, thermic Mollic Haploxeralfs). Detailed soil horizon information (classification and depths) can be found in the Century Experiment published dataset in Wolf et al. (2018). Prior to layout of the Century plots, the site was surveyed for soil characteristics and laid out in a randomized complete block design with three blocks. Two blocks are placed on the Rincon silty clay loam, and the third block is located on the Yolo silt loam. The experiment includes nine cropping systems in 2 year rotations (Table 1), on 0.4 ha (64 × 64 m) replicate plots. Each cropping system is replicated six times (two plots per block), with both crops present within a block every year (three crops within system replicates, one plot per block). Disking operations were restricted to 15–20 cm depths, and tillage conducted to a maximum depth of 25 cm.

2.2 | Maize-based systems management

Maize-based systems compared conventional versus organic approaches to crop and soil management, and consisted of (a) conventional maize–tomato with synthetic fertilizer, pesticides, and winter fallow (CONV); (b) certified organic maize–tomato with composted poultry manure and WCC (ORG); and (c) a hybrid system with synthetic fertilizer, pesticides, and WCC (CONV+WCC; Table 1).

In the ORG system, composted poultry manure was broadcast and incorporated in March at an average rate of 4 t/ha. Beds were rolled to prepare the seedbed. Maize was planted in two rows per bed in all maize–tomato systems in early April with 56 kg N/ha 8-24-6 starter fertilizer. Maize in the CONV and CONV+WCC systems was fertilized via sidedressing in one application, or two split applications, with ammonium sulfate at a total rate of 180 kg N/ha. Maize in all systems was furrow irrigated with an average of 33.6 mm per year, with a minimum of 17.6 mm in 1995 and a maximum of 43.5 mm in 2004. Maize was harvested with a full-scale combine in late September or early October. Stalks were chopped and disked to incorporate residues. In CONV, maize was followed by winter fallow, whereas in the CONV+WCC and ORG systems, a WCC mix of field pea (*Pisum sativum* L.) and hairy vetch (*Vicia villosa* Roth) was planted from 1994 through 2001, and in 2002 through 2012, field pea was replaced with faba bean (*Vicia faba* L.) and cereal oat (*Avena sativa* L.). WCC were planted in November on the top of the beds and terminated by mowing and incorporated with two to three disking operations in March.

Maize was followed by tomato in all rotations. Tomatoes were started in a commercial greenhouse and transplanted in April into

TABLE 1 Maize- and wheat-based cropping systems in the Century Experiment, and inputs, from which soil C was measured 1993 and 2012. "Supplemental flood" irrigation refers to the application of irrigation water to wheat when winter rainfall was insufficient to meet wheat water needs

Cash crop base	Abbreviation	Crop rotation	Irrigation	Fertilizer source	Annual N rate (kg/ha)
Maize	CONV	Maize–Tomato	Furrow	Synthetic N Fertilizer	168
	CONV+WCC	WCC/Maize–WCC/Tomato	Furrow	Synthetic N Fertilizer+WCC	168
	ORG	WCC/Maize–WCC/Tomato	Furrow	Poultry Manure Compost+WCC	150–200[a]
Wheat	RWF	Wheat–Fallow	None	None	0
	RWF+N	Wheat–Fallow	None	Synthetic N Fertilizer	146
	RWF+WCC	Wheat–WCC/Fallow	None	WCC	0
	IWF	Wheat–Fallow	Supplemental Sprinkler	None	168
	IWF+N	Wheat–Fallow	Supplemental Sprinkler	Synthetic N Fertilizer	168

Abbreviations: CONV, conventional; IWF, irrigated wheat–fallow; N, nitrogen fertilizer; ORG, organic; RWF, rainfed wheat–fallow; WCC, winter legume cover crop mix.
[a]Depending on N composition of poultry manure compost.

150 cm beds prepared by listing and rolling. A preplant herbicide was applied and incorporated in the CONV and CONV+WCC systems (Table 1) and tomatoes were planted with 56 kg N/ha 8-24-6 starter fertilizer. CONV and CONV+WCC tomatoes were sidedressed in one application, or two split applications, with ammonium sulfate to apply a total rate of 112 kg N/ha. In the ORG system, composted poultry manure was broadcasted prior to tomato transplanting and maize seeding in March or April at an average rate of 4 t/ha, incorporated, and rolled, with tomatoes transplanted in April. Tomatoes in all systems were furrow irrigated as described for maize. Tomatoes were mechanically harvested in August and green fruits and vine residues incorporated by shallow disking after harvest. Tomatoes in the CONV were followed by winter fallow. In the CONV+WCC and ORG systems, a WCC mix as previously described (above) was planted in November on top of the beds and was terminated by two disking operations in March.

No synthetic biocides were applied in the ORG. One cultivation was performed between beds in each crop phase of the conventionally managed systems and three to four cultivations in the ORG, as needed, to control weeds. In the CONV and CONV+WCC systems, metribuzin and glyphosate in maize and trifluralin in tomato were applied prior to planting.

2.3 | Wheat-based systems management

Wheat cropping systems were designed to represent dryland wheat–fallow systems on semi-marginal lands in the foothills of California mountain ranges, with varying capabilities for water and fertilizer inputs. The wheat systems compare the effect of N fertilizer, supplemental winter irrigation, and leguminous N inputs via WCC and include in five systems: (a) rainfed wheat–fallow control with no additional inputs (RWF), (b) rainfed wheat–fallow+N fertilizer (RWF+N), (c) rainfed wheat–fallow with WCC planted after wheat harvest and terminated before summer fallow (RWF+WCC), (d) irrigated wheat–fallow with winter supplemental irrigation and no fertilizer inputs (IWF), and (e) irrigated wheat–fallow with supplemental irrigation and N fertilizer (IWF+N).

Winter wheat was planted in November, harvested by combine in July, straw incorporated by two shallow disking operations, then fallowed from August to the following November with the exception of RWF+WCC (Table 1). Fertilized wheat in both rainfed and irrigated systems received 56 kg/ha 15-15-15 starter fertilizer at planting, and an additional 90 and 112 kg/ha urea in March was broadcast in the rainfed and irrigated systems, respectively. Rainfed systems received an average of 366.1 mm precipitation from 1993 to 2012 (minimum of 101.6 and maximum of 615.7 mm) (Table S1). Irrigated wheat systems received supplemental irrigation of 80 mm per year except in 1995, 1998, 2000, 2005, and 2008–2012 due to sufficient precipitation. During fallow, weeds were managed with one herbicide application and four disking operations, beginning after wheat harvest. Wheat in the RWF+WCC system (Table 1) only received N from WCC, which were planted in November following wheat harvest. The WCC mix included hairy vetch (*Vicia villosa* Roth.) and “Magnus” pea (*Pisum sativum* L.) from 1993 to 2006, and faba bean (*Vicia faba* L.), hairy vetch, and “Montezuma” oat (*Avena sativa* L.) from 2007 to 2012. In March or April, WCC were terminated with two to three diskings, as necessary. Soils remained fallow until planting of wheat in November.

2.4 | Plant and compost sampling and analysis

After machine harvest, aboveground plant biomass was measured by cutting crop residues at the soil surface at two locations per plot (1.5 m^2 in maize and tomato and 1.0 m^2 in wheat). WCC incorporation was measured by cutting aboveground biomass at the soil surface in a 4.5 m^2 area. Root biomass was not measured during the study period. Crop residues and WCC biomass were dried for 4 days at 60°C and ground to 2 mm. Total C and N of incorporated aboveground biomass and composted manure were determined each year, using dry combustion analysis on an ECS 4010 Costech Elemental Analyzer (Costech Analytical Technologies). Total C and N incorporated was calculated by multiplying percent C and N by total harvest biomass. Total aboveground C inputs were calculated by summing crop residue C, WCC C, and compost C incorporated per plot per year.

2.5 | Soil sampling

At the onset of the experiment in September 1993, 3 cm inner diameter soil cores were collected from all six replicates in all nine cropping systems. Samples were composited from 10 random locations within plots in depth increments of 0–15, 15–30, 30–60, 60–100, and 100–200 cm layer. Sampling by depth layers, rather than soil horizons, was chosen because soils at this site are very young (<6,000 years), and horizons are relatively homogeneous compared to more highly weathered soils. Horizon boundaries are gradual and diffuse, changing over vertical distances >15 cm. In September 2012, 3 cm diameter soil cores were collected from all six replicates of the nine cropping systems. Samples were composited from six random locations per plot in similar depth increments, then air-dried, sieved to <2 mm, and archived in glass vials at room temperature.

Bulk density samples were collected with a Giddings hydraulic probe in both the 1993 and 2012 soil samplings. In 1993, bulk density was collected in 0–25 , 25–50 , 50–100 , and 100–200 cm depth layers with an 8.25 cm diameter probe. In 2012, bulk density was collected in 0–15 , 15–30 , 30–60 , 60–100 , and 100–200 cm depth layers, with a 4.7 cm diameter probe. In both 1993 and 2012, cores were collected from four random locations within each plot. Bulk densities were determined using mass of oven-dried soil (105°C, 24 hr) and total volume of the core averaged for each depth increment (Blake & Hartge, 1986). Soils were void of rock fragments (Batjes, 1996). Bulk density depths from 1993 were adjusted to 2012 depths through the calculation of weighted averages using the two adjacent 1993 depth layers to 2012 depth layers.

2.6 | Soil total C and N analysis

In 2015, subsamples were collected from well-homogenized archived soils from 1993 and 2012. All visible plant materials were removed and samples were oven-dried at 60°C for 72 hr and ground via ball mill for 12 hr. Total C and N were determined by dry combustion (ECS 4010 Costech Elemental Analyzer). The pH of all plots and depths was measured to estimate potential contribution of inorganic C to total C measurements. The pH of all samples was measured prior to C/N analysis, where pH measured above 7.4 (Table S2) suggested the presence of inorganic carbon, which was leached out using 2 M HCl until no effervescence was observed, as described in Carnell et al. (2018). Total soil C and N at each depth layer was calculated on both a concentration and mass basis, converting concentrations to stocks, by the depth weighted sum (Equation 1):

$$C_i = BD_i \times d_i \times [\%]_i, \tag{1}$$

where C_i is the total mass of soil C (Mg/ha) for depth increment i, BD is bulk density of the soil (Mg/m^3), d indicates the length of depth increment i (m), and [%] indicates the percent C in the sample. Change in SOC concentrations and stocks from 1993 to 2012 (ΔC concentration and ΔC stock, respectively) was calculated by subtracting C_{1993i} from C_{2012i}, for each depth increment i, for each plot. Positive values indicate a gain in SOC, whereas negative values indicate loss. Total C to N ratios were calculated for each plot in 1993 and 2012 by dividing SOC concentration by total N concentration for each depth increment i, for each plot. Change in soil C:N ratio (ΔC:N) was calculated by subtracting $C{:}N_{1993i}$ from $C{:}N_{2012i}$.

2.7 | Statistical analysis

Maize- and wheat-based systems (Table 1) were analyzed separately. Both linear and quadratic regression curves were fitted to cumulative C inputs across 19 years within each system using mixed effects models in the R statistical package *nlme* (Pinheiro, Bates, DebRoy, & Sarkar, 2018) with cropping system and year as fixed effects and replicate as a random effect. Regression models were compared using Akaike information criterion values to indicate the best model for each cropping system. The linear regression model provided the best fit in all cases and was used to compare the rate of cumulative C inputs across systems. Statistical significance was determined using $\alpha = 0.05$.

Change in soil bulk density was analyzed using mixed effects models in the R package *nlme*. Cropping system was treated as a fixed effect and replicate was treated as a random effect. Treatment by block interaction effects on change in SOC concentrations were examined to check for differences among soil types. Change in soil C concentration and stocks data met assumptions of normality and homoscedasticity. A statistically significant change in soil C concentrations and stocks was determined using t tests, with the null hypothesis that soil C change = 0 from year 0 to year 19. T tests were performed for each independent cropping system, within each depth layer, and 95% confidence intervals were computed for C change variables. Significant change (where change >0) was determined with a t test where $p < .05$, and confirmed using examination of 95% confidence intervals, where the intervals did not overlap with zero. Differences in change in SOC among cropping systems were determined using 95% confidence intervals, according to the visual inference methods described in Cumming (2009) and Brennan and Acosta-Martinez (2017). Linear regression models were used to analyze change in SOC concentration and cumulative C inputs and evaluated using Pearson's correlation coefficients (r) and p values, where significance was determined at $p < .05$, using the R package *Hmisc* (Harrell & Dupont, 2018). The change in soil C:N ratio (ΔC:N) from 1993 to 2012 among cropping systems was analyzed similarly. A positive change (increase) in ΔC:N indicates that soil C increased relative to soil N, whereas a negative change (decrease) in ΔC:N indicates that soil C decreased relative to soil N, over the 19 year period. Linear regression curves were fitted to soil ΔC concentration versus ΔC:N from 1993 to 2012 across all cropping systems in the R package *nlme*.

3 | RESULTS

3.1 | Baseline soils

At the start of the experiment, average SOC content was 9.46 g/kg in the surface 0–15 cm and decreased in concentration moving down in the soil profile (Table 2). Compared to the surface layer, soil C content was 34% and 60% lower at 60–100 and 100–200 cm, respectively (Table 2). Bulk density was similar between 0 and 60 cm, and was greater by 0.1 Mg/m^3 on average in the 60–200 cm layers

TABLE 2 Initial soil organic carbon (SOC) concentrations, soil C to N ratios, bulk densities, and clay content among depth increments in 1993 at Russell Ranch, at the initiation of the century experiment

Depth increment, cm	SOC concentration, g/kg	Soil C:N	Bulk density, Mg/m^3	Clay content, %	pH
0–15	9.46 a	10.1 a	1.49 a	18.1 a	7.17 a
15–30	8.56 b	10.3 a	1.48 a	18.2 a	7.14 a
30–60	7.27 c	10.3 a	1.49 a	18.6 a	7.18 a
60–100	6.24 d	10.2 a	1.59 b	20.0 b	7.22 a
100–200	3.87 e	9.1 b	1.57 b	20.2 b	7.49 b

Note: Different letters within a column represent statistically significant differences at $\alpha = 0.05$.

(Table 2). Clay content was similar among the depth layers in the top 0–60 cm, and was 10% greater in the 60–100 and 100–200 cm layers (Table 2). Clay content was not correlated with SOC in 1993 or 2012.

3.2 | Bulk density

Bulk density in the maize-based systems declined from 1993 to 2012 ($p < .001$; Table S3). There was no interaction between cropping system and year ($p = .179$) or cropping system, year, and depth ($p = .816$); however, there was an interaction between year and depth ($p < .001$). Bulk density declined on average by 0.31 Mg/m^3 and 0.032 Mg/m^3 in the 0–15 and 15–30 cm layers, respectively, and did not change in the 30–60, 60–100, and 100–200 cm layers (Table S3).

In the wheat-based systems, bulk density declined from 1993 to 2012 ($p < .001$; Table S3), with no interaction between cropping system and year ($p = .179$), year and depth ($p = .165$), or year, cropping system, and depth ($p = .912$). There was an interaction between cropping system and depth ($p < .0001$). Soil bulk density on average declined by 0.24 Mg/m^3 and 0.35 Mg/m^3 from 0 to 15 and 15 to 30 cm, respectively, but increased by 0.39 Mg/m^3 in the 30–60 cm layer. Bulk density did not change in the 60–100 and 100–200 depths (Table S3).

3.3 | Aboveground cumulative C inputs

Of the maize-based systems, the ORG had the greatest aboveground C input ($p < .001$), with an average C input of 7.27 Mg C ha^{-1} year^{-1} (Table 3). The CONV+WCC had lower C inputs than the ORG but greater C inputs than the CONV ($p = .024$), with an average input of 5.05 Mg C ha^{-1} year^{-1}. Carbon inputs from WCC and crop residues were similar between the CONV+WCC and ORG systems ($p = .696$), but poultry manure compost in the ORG added in an additional 40.4 Mg C/ha over 19 years (Figure 1a). Of the cumulative aboveground C inputs in the CONV+WCC, 30% was from WCC, 24% from tomato residues, and 46% from maize residues. Without a WCC, the CONV received 0.75 Mg C ha^{-1} year^{-1} less aboveground inputs (33% from tomato residues, 67% from maize stover) (Figure S1a). While tomato residue C inputs were similar in the conventional systems ($p = .115$), maize stover yields were generally greater following winter fallow than WCC ($p = .027$), leading to a cumulative stover-C input that was 7.1 Mg C/ha greater in the CONV than the CONV+WCC (Figure S1a).

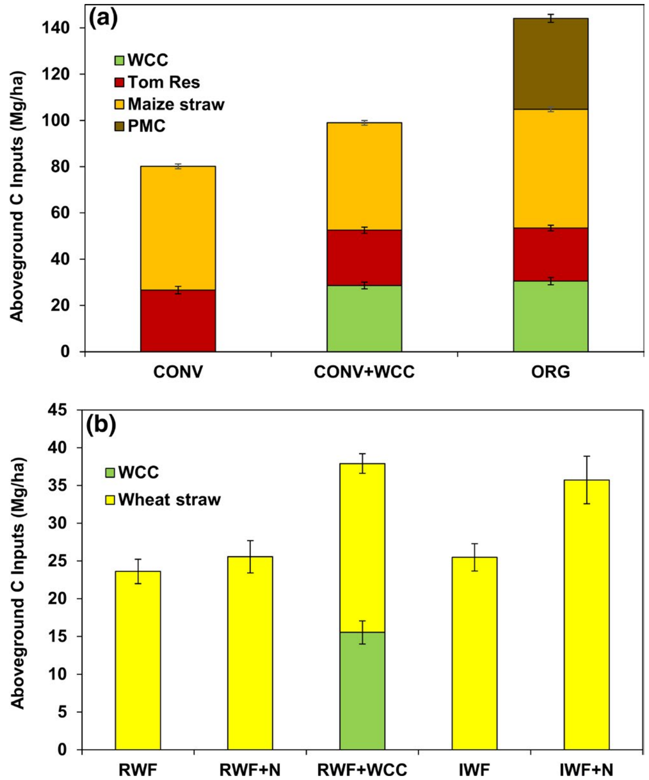

FIGURE 1 Source of cumulative aboveground C inputs incorporated into the soil in maize-based systems (a), and in wheat-based systems (b), with 95% confidence interval bars, over 19 years of cropping system management

TABLE 3 Average annual aboveground C input linear model parameters, and 95% confidence intervals, derived from regressing cumulative aboveground C inputs versus management year, for maize- and wheat-based systems, from 1993 to 2012

System	Intercept	Annual C input (Mg C ha^{-1} year^{-1})	2.5%	97.5%	R^2
Maize-based					
CONV	−1.9766	4.3042	4.1649	4.4435	0.9713
CONV+WCC	0.1590	5.0540	4.9084	5.1995	0.9771
ORG	4.4155	7.2736	7.1682	7.3790	0.9941
Wheat-based					
RWF	1.0250	1.1986	1.1357	1.2614	0.9610
RWF+N	−2.8546	1.4784	1.4117	1.5508	0.9659
RWF+WCC	−0.1663	1.9934	1.9106	2.0348	0.9535
IWF	0.9669	1.3377	1.2578	1.4177	0.9499
IWF+N	−2.2878	1.6219	1.5355	1.7084	0.9591

Abbreviations: CONV, conventional; IWF, irrigated wheat–fallow; N, nitrogen fertilizer; ORG, organic; RWF, rainfed wheat–fallow; WCC, winter legume cover crop mix.

In wheat-based systems, the greatest cumulative aboveground C inputs were in the RWF+WCC ($p < .001$), followed by IWF+N ($p = .047$), and were similar and lowest among the RWC, RWF+N, and IWC (Figure 1b). The RWF+WCC had the greatest cumulative C inputs (22.3 Mg C/ha from straw, 15.5 Mg C/ha from cover crops), despite higher crop yields and wheat straw C inputs (37.8 Mg C/ha; $p = .043$) in the IWF+N. Supplemental irrigation in the absence of N fertilizer did not increase cumulative C inputs (Figure 1b).

3.4 | Soil C changes: Maize-based systems

There was no interaction between treatment and block effects ($p = .537$), indicating SOC changes among treatments were not significantly different among soil types present on the site (as the experiment was blocked according to soil types present at the site). The greatest increases in SOC were observed in the ORG, especially in surface layers, where SOC increased by 4.20 g/kg in the top 15 cm layer ($p < .001$) and 2.59 g/kg at 15–30 cm ($p = .006$; Figure 2a). SOC concentration also increased in the CONV+WCC system by 2.03 g/kg in the top 15 cm ($p < .001$), and by 1.28 g/kg in the 15–30 cm layer ($p = .018$; Figure 2a). In the ORG, SOC stocks increased by 5.31 Mg C/ha (0.266 Mg C ha^{-1} $year^{-1}$; $p = .015$) in the 0–15 cm layer and by 2.59 Mg C/ha in the 15–30 cm layer ($p = .010$; Figure 2b). In the CONV+WCC, SOC stocks did not change in the 0–15 cm ($p = .556$) or the 15–30 cm ($p = .082$; Figure 2b) layers.

Decreases in bulk density in the 0–30 cm layer of the CONV+WCC due to high surface organic matter inputs and/or the disking operations to incorporate the WCC (Table S3) offset gains in soil C concentration. No changes in SOC concentration were observed in the CONV in the 0–15 cm ($p = .380$) or the 15–30 cm ($p = .231$) layers. However, decreases in bulk density without adequate gains in SOC concentration resulted in SOC stock declines of 3.57 Mg C/ha (−0.179 Mg C ha^{1} $year^{-1}$; $p = .003$) in the 0–15 cm layer (Figure 2b).

No changes in SOC concentrations or stocks were observed in the 30–60 cm layer except in the ORG. In the 60–100 cm layer, there was no change in SOC concentration or stocks in the CONV ($p = .975$) or ORG ($p = .454$; Figure 2a,b) In the CONV+WCC, SOC concentration (−0.57 g/kg) and stocks (−3.80 Mg C/ha) trended toward declines; however, negative changes were not significant ($p = .067$ and .070, respectively; Figure 2a,b).

In the 100–200 cm layer, significant changes in SOC concentrations were not observed in any system. Changes in SOC concentrations and stocks trended negative in the CONV+WCC ($p = .109$ and

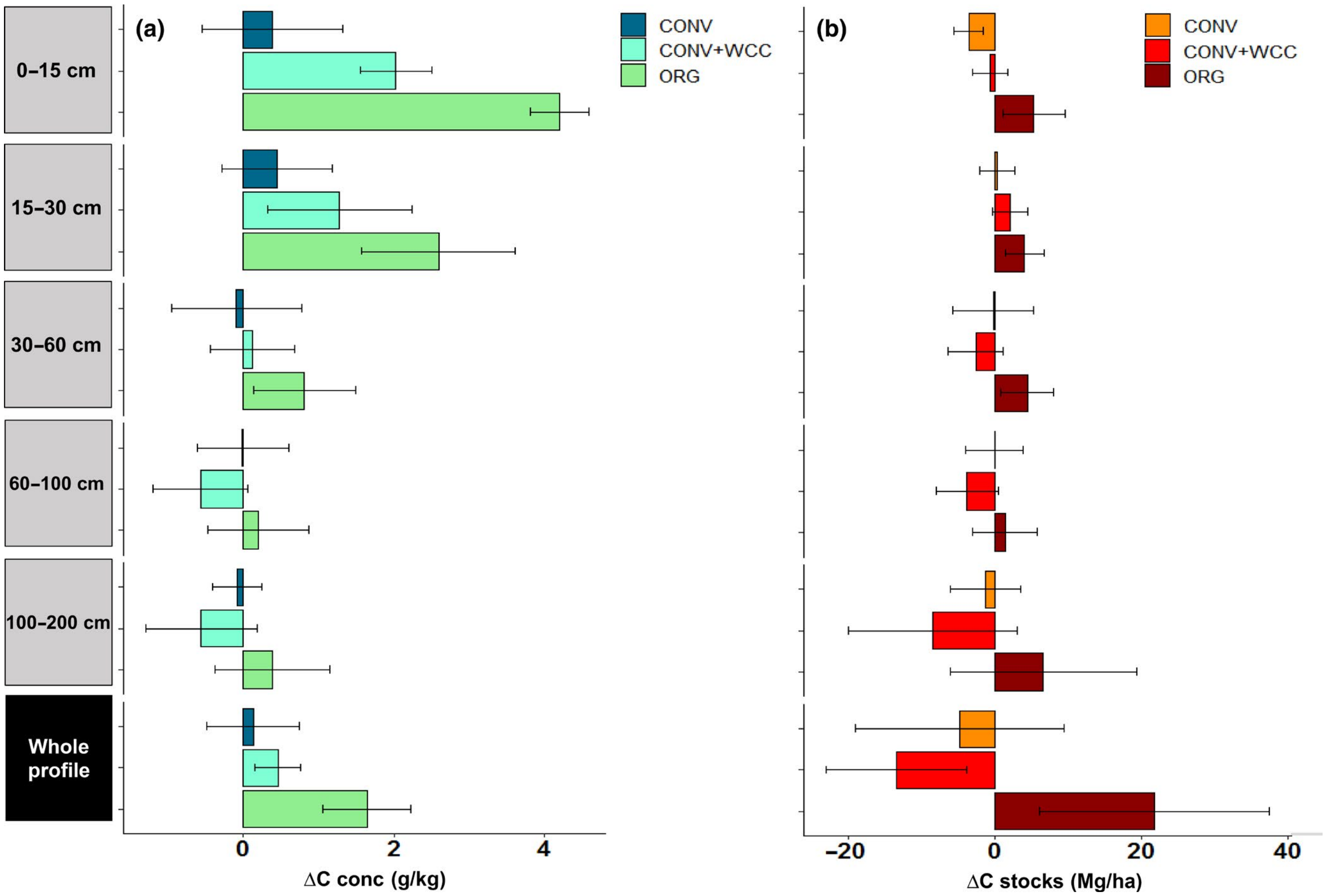

FIGURE 2 Soil organic carbon (SOC) changes in maize-based systems from 1993 to 2012, expressed as (a) change in SOC concentration (ΔC conc) and (b) change in SOC stocks (ΔC stocks). Whole profile data indicate the averages of SOC concentrations, and the sums of SOC stocks, across all five depths. Error bars indicate 95% confidence intervals

p = .116, respectively; Figure 2a,b) and positive in the ORG. No net change in SOC was observed throughout the 2 m profile in the CONV (p = .424). In the CONV+WCC, soil C concentration increased on average by 0.46 g/kg (p = .012) across the soil profile, while SOC stocks decreased by 13.4 Mg C/ha (−0.670 Mg C ha^{-1} $year^{-1}$; p = .016). In the ORG, however, average SOC concentrations and stocks increased across the entire soil profile by 1.64 g/kg (p < .001) and 21.8 Mg C/ha (1.09 Mg C ha^{-1} $year^{-1}$; p = .016), respectively (Figure 2a,b).

There was no relationship between the change in SOC concentration from 1993 to 2012 and the cumulative maize and tomato aboveground residue C inputs, nor WCC-C inputs, at any depth (Figure S1). However, change in SOC was positively correlated with cumulative poultry manure compost C inputs for the ORG at 15–30 cm (r = 0.88; p = .019), 30–60 cm (r = 0.84; p = .038), and 100–200 cm (r = 0.80; p = .047) (Figure S1).

3.5 | Soil C changes: Wheat-based systems

In the surface 15 cm, SOC concentration did not change in the RWC (p = .275), RWF+N (p = .105), RWF+WCC (p = .304), or the IWF (p = .251), and increased in the IWF+N (0.91 g/kg; p = .038; Figure 3a,b). SOC stocks did not change in the IWF (p = .265), and declined by 4.82 Mg C/ha (p = .007) in the RWF, by 3.09 Mg C/ha (p = .020) in the RWF+N, by 3.02 Mg C/ha (p = .021) in the RWF+WCC, and by 1.66 Mg C/ha (p = .032; Figure 3a,b). In the 15–30 cm layer, no changes were observed in SOC concentrations or stocks in any of the systems (Figure 3a,b).

In the 30–60 and 60–100 cm layers, neither SOC concentrations nor stocks changed significantly in any of the wheat systems (Figure 3a,b). In the 100–200 cm layer, both SOC concentration (−0.037 g/kg; p = .036) and stocks (−5.85 Mg C/ha, p = .032) decreased significantly in the RWF and did not change significantly in the other four systems (Figure 3a,b). SOC concentration and stocks trended toward increases in the IWF+N (Figure 3a,b), but changes were not significant due to high variation among replicates (e.g., 95% confidence interval for IWF+N stocks ranged from −7.89 to 24.2 Mg C/ha).

Across the entire soil profile (0–200 cm), SOC concentration increased by 3.5% (0.25 g/kg; p = .048) in the RWF+WCC and did not change in the other systems (Figure 3a). SOC stocks declined by 9.52 Mg C/ha (−0.476 Mg C ha^{-1} $year^{-1}$) in the RWF (p = .002; Figure 3b). SOC stocks across the entire profile of the IWF+N increased by 17.5 Mg C/ha on average, but the change was not statistically significant (p = .680) due to high variation among plots, with

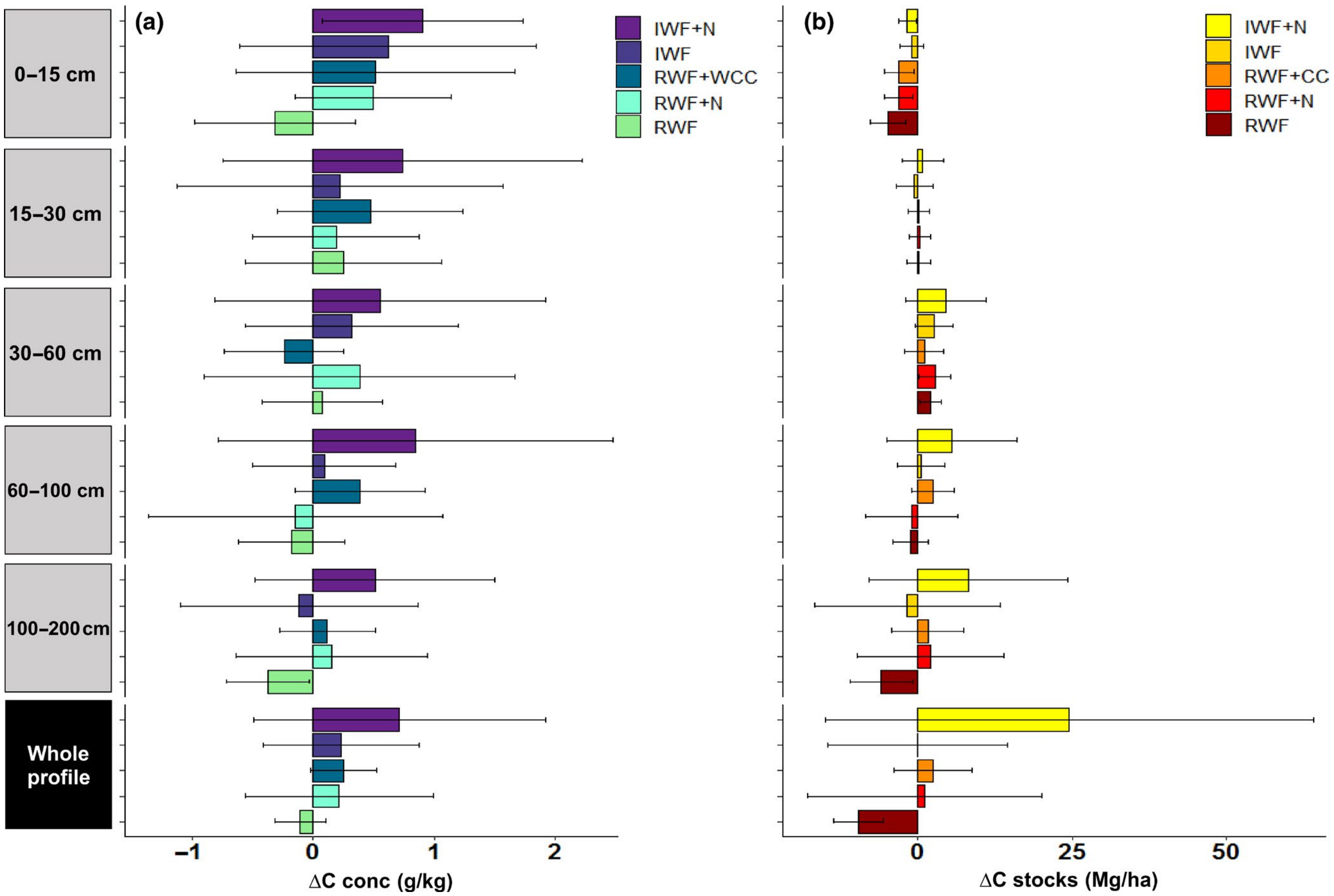

FIGURE 3 Soil organic carbon (SOC) changes in wheat-based systems from 1993 to 2012, expressed as (a) change in SOC concentration (ΔC conc) and (b) change in SOC stocks (ΔC stocks). Whole profile data indicate the averages of SOC concentrations, and the sums of SOC stocks, across all five depths. Error bars indicate 95% confidence intervals

soil C stock changes ranging from −4.74 to 59.0 Mg C/ha. SOC stocks did not change in the RWF+N, IWF, and the RWF+WCC (Figure 3). There was no relationship between SOC concentration change and cumulative wheat C inputs (p = .453), nor with cumulative WCC-C inputs (p = .899), throughout the soil profile.

3.6 | Soil C:N

In 1993, soil C:N ratios ranged from 9.0 to 11.3 in the top 100 cm, and 6.3–11.5 in the 100–200 cm layer (data not shown). These ratios generally increased across plots after 19 years of management.

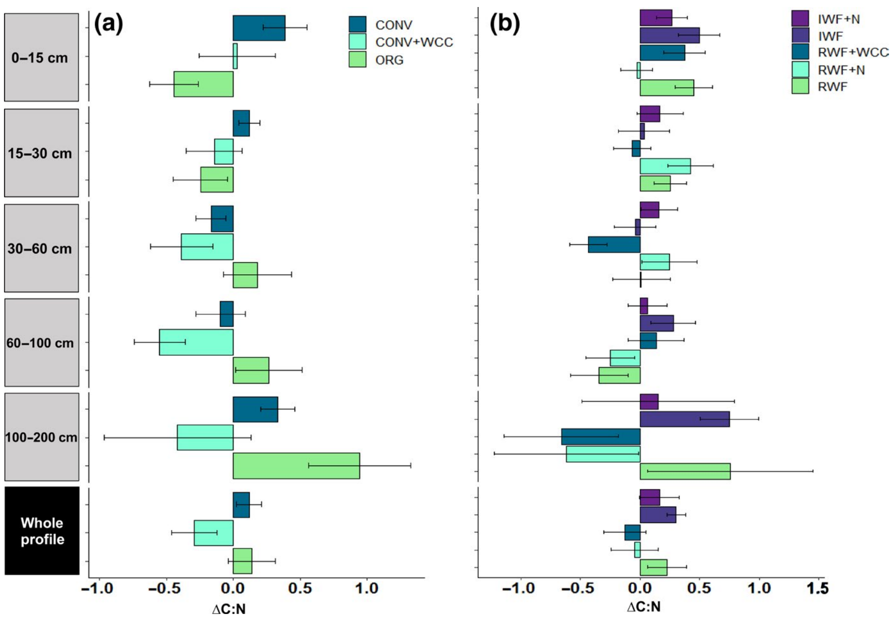

FIGURE 4 Change in soil C:N ratio from 1993 to 2012, and 95% confidence intervals, in maize-based (a) and wheat-based (b) cropping systems

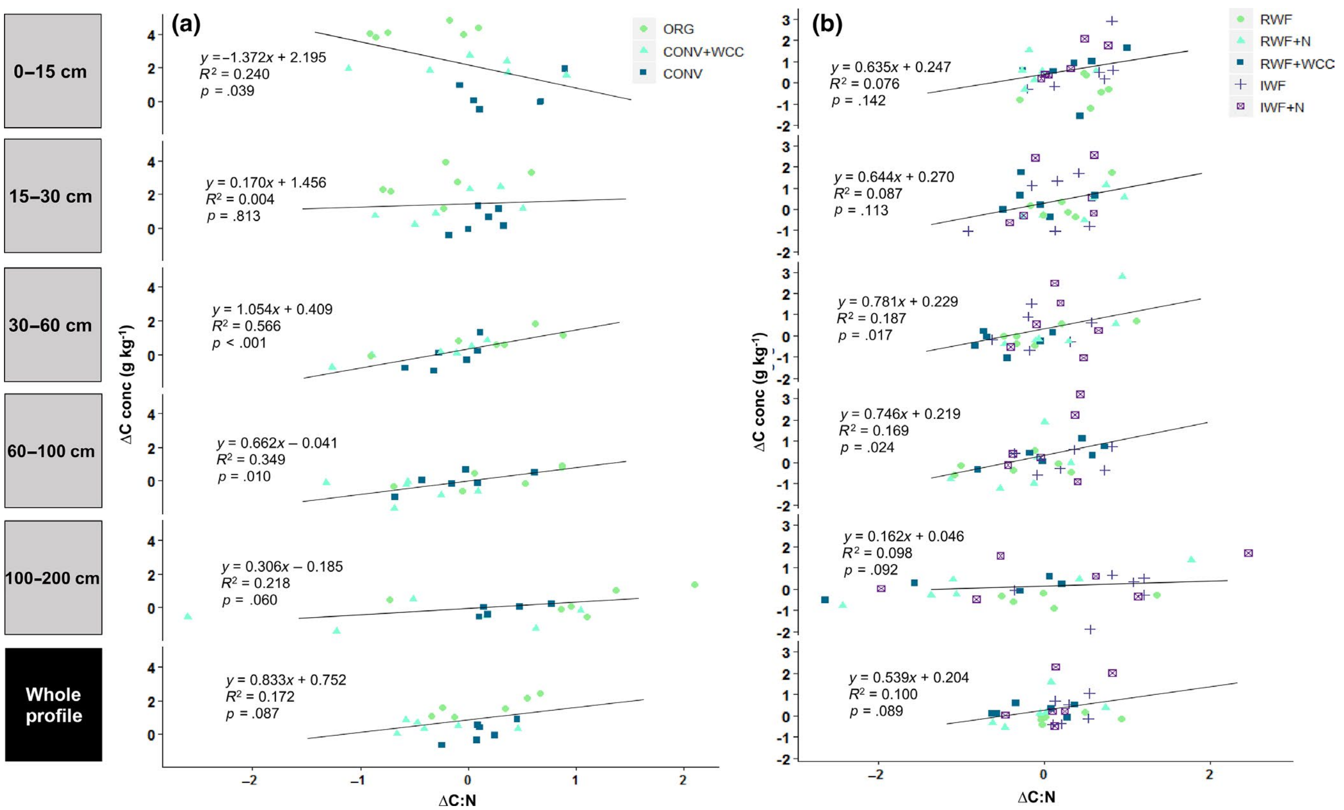

FIGURE 5 Change in soil organic carbon concentration versus change in soil C:N ratio (ΔC:N) from 1993 to 2012 in maize-based (a) and wheat-based (b) rotations at five depth layers, and average change across the whole profile, depicted with fitted linear regression models and coefficients

Between 1993 and 2012, change in soil C:N ratio varied substantially among maize-based systems. In the CONV, soil C:N increased in 0–15, 15–30, and 100–200 cm layers. In contrast, C:N declined in the 30–60 cm layer and showed no change in the 60–100 cm layer. In the ORG, soil C:N showed opposite trends from that observed in conventional, where C:N in the ORG decreased in the surface layers but increased in the lower two depths (Figure 4a). In the CONV+WCC, C:N only decreased in the 30–60 and 60–100 cm depths, where N increased 2 – 2.5 times relative to C (data not shown). Averaged across the entire 200 cm profile, soil C:N increased in the CONV and ORG, and decreased in the CONV+WCC (Figure 4a).

In the rainfed wheat-based systems, addition of synthetic N had no impact on soil C:N at most depths, or across the entire soil profile. The only exception was the 100–200 cm layer, where soil C increased relative to N in the RWF, while N increased relative to C in the RWF+N (Figure 4b). Irrigating wheat during dry winters did not substantially alter soil C:N compared to the RWF; however, N fertilizer inputs combined with supplemental irrigation generally increased ΔC:N across the 0–200 cm soil profile (Figure 4b). Inclusion of WCC increased soil C:N in the top (0–15 cm) layer but decreased C:N in 30–60 and 100–200 cm layers. The RWF+WCC was the only wheat-based cropping system that exhibited enrichment of soil N relative to soil C across the soil profile (Figure 4b).

The relationship between change in SOC concentration and change in C:N ratio differed among depth layers in maize-based systems (Figure 5). Change in SOC concentration decreased with increasing C:N ratio in the top 15 cm, which was the only layer in any systems where an inverse relationship between these parameters was observed. At 15–30 cm, no relationship was observed between changes in SOC and C:N ratio, whereas from 30 to 200 cm, changes in soil C concentration were positively correlated with changes in C:N ratio (Figure 5). In wheat-based systems, soil C:N ratio increased with increases in SOC concentration in 30–100 cm but showed no relationship in either the 0–30 or 100–200 cm layers (Figure 5).

4 | DISCUSSION

Our study represents one of few long-term efforts to track soil carbon changes throughout both the surface and subsoil layers in an agricultural system, and highlights the importance of including deep soil measurements in soil carbon accounting. Of the nine cropping systems observed in our 19 year study, only one system (ORG) showed increases in SOC stocks throughout the entire 0–200 cm soil profile. While three other systems displayed an increase in SOC concentration overall, these gains did not translate into SOC gains on a mass basis due to declines in bulk density offsetting gains in SOC concentration and/or declines in some layers offsetting gains in others. Bulk density likely declined in the surface layers of most systems due to the cumulative addition of organic matter, which built up as bulky or particulate organic matter; others have observed an inverse relationship between soil organic matter and bulk density (Périé & Ouimet, 2008). Poulton et al. (2018) also observed declines in bulk density in long-term cropping system experiments.

In maize-based systems, annual inputs of 9 t/ha of composted poultry manure resulted in the addition of 2.22 Mg ha^{-1} year^{-1} more C to the ORG compared to the CONV+WCC. This difference in C input was associated with an SOC concentration that was 3.5 times greater in the ORG system. The 21.8 Mg C/ha gain in SOC stocks observed in the organic system over this 19 year period translated to a rate of 6.6‰ increase in soil C per year, exceeding the benchmark of 4‰ increase in soil C per year targeted by the 4 per 1,000 initiative (French Ministry of Agriculture & Food, 2018). Relying primarily on poultry manure compost as an input to promote carbon sequestration in agriculture soil, either statewide or globally, may not be feasible because of limited supplies and the economic and environmental costs of transportation. Alternative feedstock should be evaluated for their efficacy in C sequestration along with a life cycle assessment of compost to estimate total greenhouse gas emissions and footprint. However, replacement of synthetic fertilizers with compost has high potential to reduce greenhouse gas emissions. For example, Alluvione, Bertora, Zavattaro, and Grignani (2010) observed a 49% reduction in CO_2 emissions from soils following compost amendment, compared to amendment with synthetic urea fertilizer. Emissions of N_2O, a potent greenhouse gas, were lower in organic systems fertilized with compost than in conventional systems with synthetic fertilizers, especially in Mediterranean croplands (Aguilera et al., 2013). Furthermore, in a global meta-analysis of greenhouse gas emissions from organic and synthetic soil amendments, Charles et al. (2017) found that compost had an N_2O emissions factor of 0.27% of total N applied, compared to 1.34% of total N applied in synthetic fertilizers. Our results demonstrated that substantial increases in soil C are achievable even in semiarid climates and compost-C inputs may be effective in increasing soil C and decreasing greenhouse gas emissions on decadal time scales. More research is needed to correlate particular compost characteristics (e.g., C to nutrient ratios) with soil C sequestration potentials.

It is rare that soil C is measured at depths below 30–40 cm (Poeplau & Don, 2015), despite the knowledge that carbon is more likely to be protected from biotic and abiotic losses in the subsoil (Hicks Pries et al., 2018; Jobbágy & Jackson, 2000). Considering SOC changes across the entire 2 m soil profile strongly impacts C sequestration inventories, both in terms of distribution of SOC among depths, and cumulatively across the entire soil profile. Had our study only measured SOC in the top 30 cm, gains of 12.39 Mg C/ha observed in deeper layers (30–200 cm) of the organic poultry manure-composted system would not have been accounted for, grossly underestimating soil C sequestration in that system by 57%.

In contrast, focusing only on the surface layer of soil could result in grossly overestimated SOC gains. In the CONV+WCC, constraining SOC measurements to the top 30 cm would have overestimated SOC gains, as gains of 1.44 Mg C/ha (0.072 Mg C ha^{-1} year^{-1}) were observed in that layer, compared to cumulative losses of 14.86 Mg C/ha (0.74 Mg C ha^{-1} year^{-1}) in the 30–200 cm profile. While SOC losses were not statistically significant within individual depth layers (60–100 and 100–200 cm) of the CONV+WCC, net declines in SOC stocks across the entire 2 m profile were significant. Even where gains were observed in the top 30 cm, the rate of C sequestration observed with WCC here falls well short of the 0.32 Mg C ha^{-1} $year^{-1}$ rate estimated globally by Poeplau and Don (2015), possibly due to the lower C input from WCC in this study (1.43 Mg C ha^{-1} $year^{-1}$) compared with the 1.87 Mg C ha^{-1} $year^{-1}$ estimated by Poeplau and Don (2015). Use of legumes such as vetch and pea in the WCC mix for the first 8 years may have decreased biomass production and thereby C input of WCC in this study compared to global estimates of C input from cover crops including more grass species, which tend to produce more biomass than legumes. Nonetheless, WCC did increase soil C and soil organic matter in the top 30 cm layer, despite frequent disturbances from tillage. While these findings suggest that WCC may not contribute to soil C stocks throughout the entire soil profile, they do confirm WCC's ability to increase soil organic matter in the plow layer.

The surface layer has been almost exclusively the zone of attention in studies examining relationships between soil microbial activity and soil organic C gains (Kallenbach & Grandy, 2011; Poeplau & Don, 2015; Tiemann, Grandy, Atkinson, Marin-Spiotta, & McDaniel, 2015). Microbial utilization of cover crop-C is an important pathway for increasing soil organic C (Kallenbach, Grandy, Frey, & Diefendorf, 2015), and indeed we observed this in the 0–30 cm layer where WCC increased both soil C and microbial biomass (K. Scow, unpublished data) compared to conventional maize–tomato with winter fallow. Increased soil available N provided by the leguminous WCC may have increased the microbial use efficiency of WCC-C inputs, leading to greater incorporation of WCC-C into microbial bodies and ultimately greater soil organic C pools (Lange et al., 2015). Strong relationships observed between microbial biomass and soil C gains may not be as clear in the subsurface where C inputs and microbial biomass are much lower and impacts of physical processes, such as occlusion and sorption, are more evident. Subsurface losses may be due to soil organic matter degradation from priming (Dignac et al., 2017) of resource-limited deep microbial communities and/or low soil moisture conditions decreasing occlusion and adsorption mechanisms (Blankenship & Schimel, 2018; Jardine, Weber, & McCarthy, 1989; Jones et al., 2018); however, more research is needed to elucidate soil C dynamics in this zone. Considering the entire 2 m-deep soil profile, WCC incorporated without additional nutrient application may have decreased soil C at depths >60 cm, resulting in net declines in soil C across the soil profile in terms of stocks. By comparison, application of 700–800 kg C ha^{-1} $year^{-1}$ via compost in the ORG drove soil C gains of 12% over 19 years in maize–tomato systems. Indications of possible C loss in the WCC root zone were unexpected and research is ongoing to understand potential mechanisms involved, as well as interactions of cover crops with compost in stabilizing soil C.

Other studies have observed that adding additional phosphorus and sulfur at the time of residue C incorporation increased SOC throughout a 1.6 m soil profile (Frossard et al., 2016; Kirkby, Richardson, Wade, Conyers, & Kirkegaard, 2016). The SOC increases we observed throughout the 2 m profile may have been similarly facilitated by the relatively large amounts of P, S (>25 kg/t) and other nutrients applied in the poultry manure compost alongside compost-C inputs. Conversely, WCC have been observed to decrease soil P and K levels compared to winter fallow (N. Tautges, unpublished data). Alternatively, soil C sequestered in the composted system may have been due to the addition of more stabilized C from recalcitrant compounds in the compost, compared to the more labile C in the crop and WCC residues.

Significant loss of SOC in terms of mass was observed in only one of the farming systems, the unfertilized RWF. This was likely due to low levels of C fixation into wheat biomass, supporting observations that increased crop productivity and fixed C in intensive agriculture (with inputs of irrigation and N) increases C sequestration relative to unmanaged plots (Haynes & Naidu, 1998; Lal, 2002). Inclusion of a fallow phase in the wheat-based systems did not necessarily contribute to SOC losses, as the wheat–fallow systems receiving either supplemental irrigation or N fertilizer alone displayed neither loss nor gain of soil C. Rather, limitation of nutrient (most likely N) availability likely decreased biomass yields and C input to soils via wheat straw, as the two wheat systems not receiving N fertilizer (RWF and RWF+WCC) produced the least amount of wheat straw.

To conclude, if only the surface soil (0–30 cm) had been analyzed—a typical practice in monitoring soil C sequestration—we would have jumped to false conclusions about soil C benefits of adding WCC to our conventionally managed annual row crop systems. Similarly, measuring C to 2 m deep revealed the organic system had substantially greater capacity to sequester C than what would have been thought based on just surface soil sampling. Our results provide concrete examples of the importance, particularly for carbon crediting, of performing a full accounting of soil C changes throughout the entire soil profile when recommending crop management practices to optimize soil C sequestration.

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