Soil organic and inorganic carbon interactions under tillage and cover cropping determine potential for carbon accumulation in temperate, calcareous soils

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Abstract

The global soil carbon pool comprises soil organic carbon (SOC), found in almost all soils, and soil inorganic carbon (SIC), in calcareous soils. Despite their agricultural significance, calcareous soils, mostly prevalent in drylands and often alkaline, are historically understudied. Using soils obtained from a decade-long, fully factorial field experiment located on temperate, near neutral pH, calcareous soils, this study examined the influence of cover crops (no-cover *vs* radish) and three levels of tillage intensity: shallow (10 cm) and deep (20 cm) non-inversion, and plough (25 cm inversion) on SOC and SIC stocks. Further, considering recent experimental and observational evidence indicating the interactions of SOC and SIC pools and their likely microbial control, we also investigated how SOC, the soil microbial biomass pool, and SIC are correlated. For SOC stock, there were significant interactions with total SIC and SOC:SIC ratio that differed by tillage intensity. Across the whole soil profile (0-60 cm), there was a significantly positive relationship between SOC content and SIC stock that was only present with ploughing. Further, at low SOC:SIC ratios (~0.5-3.0), while SOC stock was marginally lower under plough, at higher SOC:SIC ratios (~3.1–10.0), SOC stock was predicted to be up to ~4–fold greater (4 kg m^{-2}) with ploughing than the lower intensity tillage treatments. This result highlights a critical SOC-SIC interaction that, depending on tillage intensity, may offset anticipated disturbance-related loss of SOC. SOC stock was also ~40% (0.42 kg m^{-2}) greater at 0-10 cm and ~30% (0.2 kg m^{-2}) greater at 30-40 cm under radish cover crop than without. SIC stock differences were determined by SOC content, tillage intensity and cover cropping . SIC stock was strongly correlated with SOC, with a predicted ~0.3–1 kg m^{-2} increase in SIC stock for ~1% increase in SOC. Under radish cover crops and with ploughing, there was ~0.7 kg m^{-2} more SIC than under all other conditions. Microbial biomass was positively correlated with SIC stock suggesting a causality that needs experimental testing. Given that reduced tillage is a frequently recommended practice to increase soil carbon storage and given the limited attention that has been paid to the influence of cover cropping on the SIC pool, our results indicate the need for further investigation around the dynamics of SOC and SIC interactions and stabilization processes in calcareous soils and highlights the pitfalls of a one-size-fits-all approach to soil carbon management.

Introduction

A global transition towards sustainable farming practices, that support food production for a growing population, is urgently needed to prevent the degradation of arable soils. This will be challenging, but simple recommended best management practices, like tillage intensity reduction and cover cropping, have emerged as potential solutions. Reduced tillage has been proposed as one pathway to increase carbon sequestration, as it preserves soil structures that protect soil organic carbon (SOC) (Sun et al., 2020), while cover cropping provides an additional input of soil organic matter and may offset a portion of the losses of SOC that naturally occur with farming; simultaneously improving soil stability, increasing biological activity, and reducing nutrient losses (Abdalla et al., 2019).

Historically, the focus of soil carbon research for climate change mitigation and agricultural ‘carbon farming’ has been on SOC. It is arguably one of the most important indicators of soil fertility, and because of its central role in a range of soil functions, is also the most measured soil parameter (Karlen et al., 2001; Stockmann et al., 2015). Only recently has the other soil carbon – soil inorganic carbon - become of interest to researchers, land managers, and policy makers (Raza et al., 2024). This is likely because the majority of calcareous, soil inorganic carbon (SIC) containing soils are found in drylands (Amiotte Suchet et al., 2003; Gaillardet et al., 2019). The chemistry of calcareous soils is poorly understood compared with soils without SIC, making soil carbon pools more operationally difficult to quantify (Apesteguia et al., 2018; Ramnarine et al., 2011). Further, the SIC pool has been erroneously considered to be relatively stable and non-dynamic and therefore less vulnerable to perturbation with agricultural management. However, recent research indicates that not only is the SIC pool highly dynamic and susceptible to human disturbance (Bughio et al., 2017), but it is likely that the SOC and SIC pools interact to influence each other's stability and persistence (Fernández-Ugalde et al., 2011; Virto et al., 2011).

SOC stabilization is understood to occur through additive structures (Oades and Waters, 1991). First, plant-derived organic matter (OM) is decomposed by microbial processes (Dynarski et al., 2020; Kallenbach et al., 2015), followed by microbial necromass binding to minerals to create stable mineral-associated organic matter (Wang et al., 2024), and by the formation of microaggregates and macroaggregates bound by microbially derived OM (Rabbi et al., 2020), fungal hyphae (Lehmann et al., 2020; See et al., 2022), plant roots (Poeplau et al., 2021), and abiotic processes (Yu et al., 2019). Further, existing SOC begets more SOC when organic molecules attach to the 'tails' of mineral-adsorbed organic compounds (Kleber et al., 2007). Conversely, SOC loss occurs via the physical, plant and microbially-mediated breakdown of these additive structures (Guidi et al., 2021; Kan et al., 2022). Thus, by reducing soil disturbance and increasing organic matter inputs, at least some of the losses of SOC from cropping may be minimized (Abdalla et al., 2019; Ball et al., 2019; Kan et al., 2022; Steenwerth and Belina, 2008). However neither technique offers a definitive solution (Chaplot and Smith, 2023). While fine silt and clays - the primary mineral surfaces for initial OM stabilization - remain a main driver of SOC stabilization (Matus, 2021), there is a need to look 'beyond clay' to other climate- and ecosystem-specific soil physicochemical properties mediating SOC stabilization (Rasmussen et al., 2018). This is especially important in calcareous soils given recent experimental evidence for calcium-mediated stabilization of organic matter (Rowley et al., 2021, 2020, 2018), and the role of carbonates in soil aggregation (Fernández-Ugalde et al., 2014, 2011; Virto et al., 2011).

The overlooked soil carbon pool – soil inorganic carbon - originates from lithogenic and pedogenic sources (secondary carbonates) (Zamanian et al., 2016). The former is inherited from soil parent material, and the latter is formed mainly through the following two reactions:

$$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+ \qquad (1)$$

$$Ca^{2+} + 2HCO_3^- \Leftrightarrow CaCO_3 + CO_2 + H_2O \quad (2)$$

Pedogenic carbonate formation is influenced by soil carbon dioxide (CO_2), pH, Ca^{2+} content and soil moisture and evaporation. An increase in soil pH (i.e., a decrease H+) would drive the reaction (1) to the right, resulting in production of HCO_3^-. When there is no limitation of soluble Ca^{2+}/Mg^{2+}, calcium carbonate precipitation occurs. On the other hand, an increase in soil CO_2 or a decrease in soil pH would drive the reaction (2) to the left (Zamanian et al., 2016). Therefore, acidic conditions could lead to carbonate dissolution, causing a decrease in SIC stock with leaching, whereas an alkaline environment would favor pedogenic carbonate formation. SIC formation binds calcium as $CaCO_3$, whereas SIC dissolution contributes Ca^{2+} to the soil, which has been shown to promote SOC stabilization through sorptive processes and cation-mediated bridging (Bob and Walker, 2001; Kalinichev and Kirkpatrick, 2007; Rowley et al., 2021). While in arid, alkaline, Ca^{2+} bearing soils pedogenic processes can dominate, the pH threshold for carbonate formation is not strictly defined (Slessarev et al., 2016), with evidence for pedogenic carbonate formation (Huang et al., 2021) and carbonate presence (Rowley et al., 2020) at near neutral pH. Further, carbonates may persist in soils seemingly out of balance with climate conditions because of contributions from underlying calcareous parent material, or biological processes (Bindschedler et al., 2016; Hasinger et al., 2015). In arid soils, the limiting factor governing the potential for SOC-SIC interaction is likely to be organic matter inputs, but in temperate, acidic-neutral pH soils with higher primary productivity and where carbonate dissolution is more likely to occur, SOC-SIC interactions may be upregulated.

While SIC by itself is an important storage form of soil carbon, its interaction with, and influence on the storage of SOC is poorly understood, especially in soils that contain higher organic matter inputs. SIC likely moderates the biogeochemical processes that influence physical stabilization of SOC in the soil mineral matrix *via* products of dissolution and reprecipitation of carbonates (Lopez-Sangil et al., 2013; Martí-Roura et al., 2019). These processes can form SIC-organo-mineral structures through cation-bridging and carbonate cementation. In dissolution, calcium ions increase cation-mediated bridging of organic matter (Rowley et al., 2021), and in re-precipitation, carbonate cementations can 'encase' organic matter (Fernández-Ugalde et al., 2014, 2011). Therefore, while SOC-aggregate hierarchy theory has been successfully applied in non-calcareous soils, in soils containing even small amounts of carbonates, the relationship between organic matter and aggregation can become decoupled (Fernández-Ugalde et al., 2011; Setia et al., 2010). In calcareous agricultural soils, management practices like tillage and cover cropping can directly alter soil organic carbon accumulation and mineralization and enhance secondary carbonate pedogenesis (Bughio et al., 2017; Mehra et al., 2019; Mikhailova and Post, 2006), and where these processes co-occur over time, a shift in the balance of SOC-SIC pools is foreseeable. There is increasing evidence that unique processes of soil C mineralization and stabilization are present in calcareous soils, where the addition of new organic matter can stimulate the precipitation and loss of SIC (Mehra et al., 2019; Setia et al., 2011). Increases in carbonate pedogenesis that occur in response to organic matter inputs and soil disturbance are partly attributable to increased CO_2 and reduced pH in soil pore spaces from both root respiration (Gocke and Kuzyakov, 2011) and soil mixing, but microbial processes likely also play a role. Microbial respiration can increase carbonic acid in soil pores (Philippot et al., 2023) and contribute to biological carbonate weathering (Sánchez-Cañete et al., 2018), and in the presence of Ca^{2+}, bacteria and fungi can rapidly accumulate carbonates (Dupraz et al., 2009; Hammes and Verstraete, 2002; Monger et al., 1991). Biological processes that contribute to carbonate dissolution and formation both indirectly through modification of the soil environment, and *via* direct precipitation (Dupraz et al., 2009) require more attention to understand the contribution of these elements to the global SIC cycle.

Here, we aim to quantify the impact of cover cropping and tillage (shallow non-inversion, deep non-inversion, and deep inversion plough) on SOC and SIC pools in temperate, carbonate-containing soils. While the amount of carbonate in these soils is low, the soils exhibit SOC:SIC ratios between 0.4 – 9.0, which in combination with the other data elements allowed examination of the potential for SOC-SIC interactions and how SOC-SIC balance in soils is influenced by management. We hypothesized that cover crops, by increasing organic matter inputs, and tillage by increasing organic residue incorporation, CO_2, and solute movement in pore spaces, increase carbonate dissolution and carbonate precipitation. If these conditions co-occur, it was expected that SOC-SIC interactions would be enhanced, potentially leading to higher SOC stocks, contrary to what is normally expected with soil tillage.

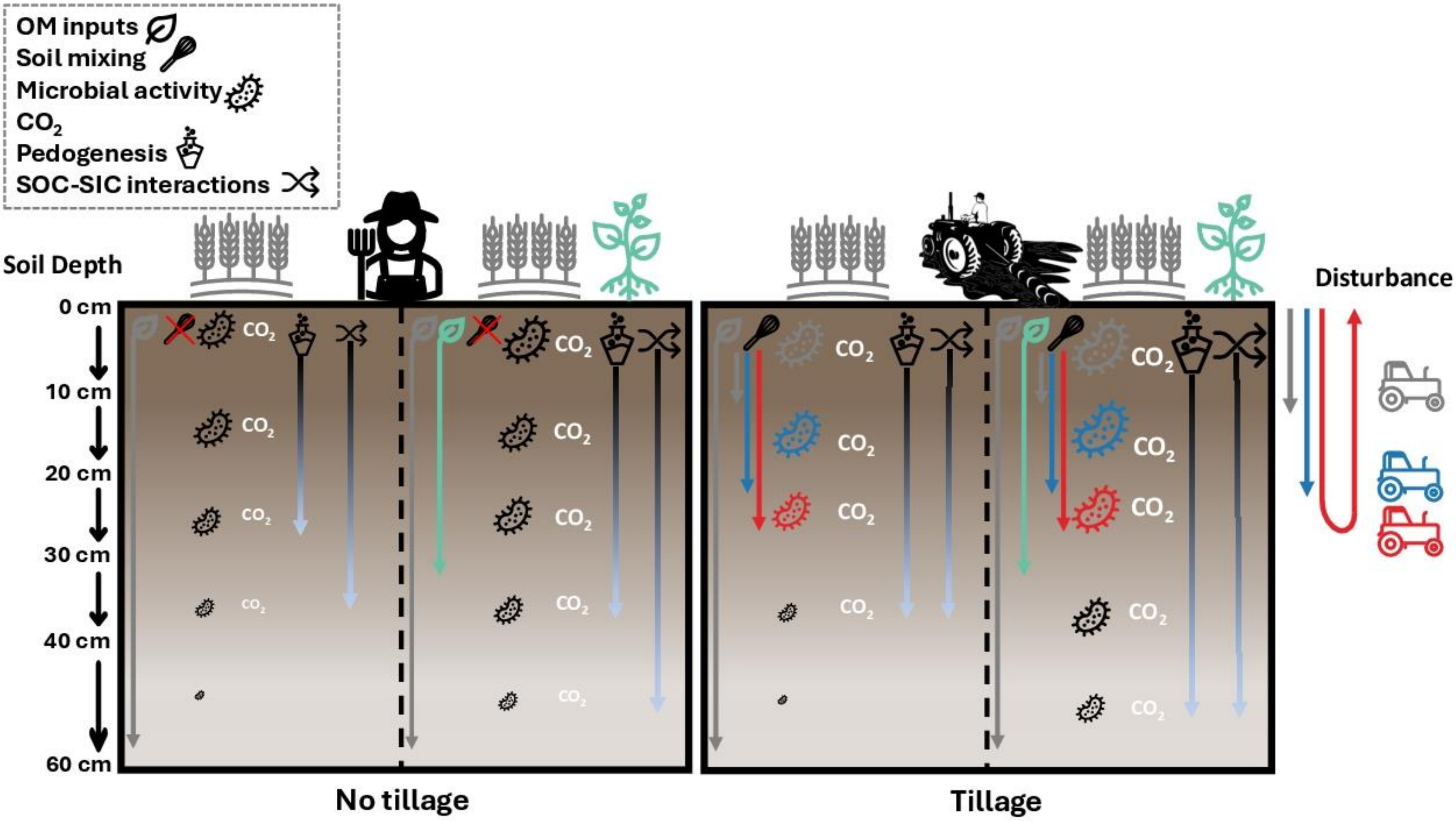

Figure 1: Conceptual diagram showing hypothesized influences of cover cropping and tillage by depth on soil organic matter inputs, soil mixing and residue incorporation, microbial activity, soil CO_2, carbonate pedogenesis, and SOC-SIC interactions. Grey organic matter inputs indicate those from the cash crop, while green indicate those from the radish cover crop. Soil mixing/disturbance (from tillage) is shown in grey for shallow non-inversion, blue for deep non-inversion, and red for inversion plough. Plant inputs from active root biomass and incorporated residues are expected to increase microbial biomass and CO_2 in soil pore spaces. Additionally, tillage will increase the incorporation of surface residues into deeper soil layers, while increasing CO_2 and solute/water transport in pore spaces. Where there is more organic matter, microbial biomass, and CO_2 in pore spaces, pedogenic processes are expected to be upregulated. Upregulated pedogenic processes combined with OM inputs may increase the stabilization of soil organic carbon and build more soil inorganic carbon. Conditions under no-tillage are shown for *comparison purposes only*.

Materials & Methods

Study area and soil sampling

The “New Farming Systems” (NFS) experiment is in Morley, Norfolk, UK (N 52°33’14’’, E 1°01’44’’) on a sandy loam soil classified as an Endostagnic Luvisol and is non-irrigated. The field trial was established in 2007 and is supported by The Morley Agricultural Foundation (TMAF) and the JC Mann Trust. During this period, the cash crop differed by year (**Table 1**), but the cover crop (radish) remained consistent; established in early autumn and terminated typically in February to April prior to the spring cash crop sown in March or early April. The tillage treatments included *shallow*, non-inversion to 10 cm, *deep*, non-inversion to 20 cm, and inversion *plough* to 25 cm. The non-inversion treatments used a Sumo Trio cultivator. During March 2018, 24 soil profiles were sampled for a total of 144 samples across six treatments: in the three tillage practices (shallow, deep, and plough), two crop residue management methods (with or without cover crop), and from 5 soil depths (0–10, 10–20, 20–30, 30–40, and 40–60 cm).

Table 1: Summary of the New Farming Systems field trial crop rotations by year. WW = winter wheat, SOSR = spring oilseed rape, WOSR = winter oilseed rape, SO – spring oats, SBN = spring bean, SBR = spring barley, WBR = winter barley, ± CC (cover crop: radish).

2007/08	2008/09	2009/10	2010/11	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18
Yr. 1	Yr. 2	Yr. 3	Yr. 4	Yr. 5	Yr. 6	Yr. 7	Yr. 8	Yr. 9	Yr. 10	Yr. 11
WW	SOSR ± CC	WW	SBN ± CC	WW	SBR ± CC	WOSR	WW	SO ± CC	WW	WBR

Soil analyses

Bulk density, soil water content, pH, and electrical conductivity (EC)

Soil cores with 6 cm diameter x 4 cm height were carefully inserted vertically into the soil at desired depths. Wet soil samples were placed in a drying oven for 48 h at 105 °C, and the dried soil weights recorded, and gravimetric water content and bulk density calculated. At each sampling location and depth, 5 grams (± 0.1 g) of field moist soil was weighed into 50 mL centrifuge tubes and 25 mL of 0.01M $CaCl_2$ was added to the tubes. The suspension was swirled for 1 hour, and then left to equilibrate for 30 minutes. After this period, soil pH and EC were measured using a pH and Conductivity Meter (HI5521 & HI5522), respectively.

Soil texture and soil organic matter

Soil texture data were obtained at the block level, from the radish cover crop treatment at each level of tillage, and by soil depth (**Table S1**). Over the entire range of each depth increment, a bulk sample was taken. Moist soil samples (~20 g) were dried (30 ºC in the oven for 48 hours) and sieved to pass a 2 mm screen. To remove organic matter from soils prior to texture analyses, five grams of sieved dry soil was weighed into 50 mL centrifuge tubes and ~ 1m dH_2O and 5 mL of 30 % v/v H_2O_2 (hydrogen peroxide) (VWR, AnalaR) added to the tubes (W_1) and left to react in the cold overnight. Then, 30% v/v H_2O_2 was added, and the solution heated to 90°C to decompose H_2O_2 – this was repeated until frothing had subsided. Once cool, centrifuge tubes and dry soil were weighed again (W_2).

After organic matter removal, 5 ml of 50 g L^{-1} sodium hexametaphosphate solution (Acros Organics, Fisher Scientific) was added, and the samples shaken on an orbital shaker for 3 hours at 150 rpm. This solution was then passed through a 63 μm sieve to allow subsequent separation of the clay and silt fractions. All material, separated into >63 μm and <63 μm, were placed into the oven and dried for 48 hours at 105 °C and then weighed as separate soil samples ($W_{>63}$ and $W_{<63}$).

1 ml dH_2O and 1 ml of 50 g L^{-1} sodium hexametaphosphate solution (Acros Organics, Fisher Scientific) were added to 0.6 g of sieving to 63 μm dried soil samples. This was shaken for 2-3 hours at 150 rpm on an orbital shaker and then passed through a Laser Particle Analyzer (LS 13 320). For each soil depth, soil organic matter (W_M, g) was calculated by the following equation:

$$W_M = W_1 - W_2 \tag{3}$$

Exchangeable calcium (Ca^{2+}) and magnesium (Mg^{2+})

Approximately 10 g of 2 mm sieved dry soil was weighed into 50 mL centrifuge tubes and shaken for 30 minutes with 20 ml of the extracting solution (1 M ammonium acetate salt-glacial acetic acid buffered at pH 7) and then left to stand overnight. The extract solution was filtered through Whatman No.42 filter paper before being kept at 4ºC for analysis. The filtered extract solution was measured by using flame-atomic absorption spectroscopy (AAS).

Total carbon, soil organic and inorganic carbon, and SOC:SIC ratio

Representative sub-samples were ball milled for total carbon (TC), soil organic carbon (SOC) and soil inorganic carbon (SIC) measurements. The contents of total soil carbon and SOC were measured using a CNS analyzer (CE NA2500 Elemental Analyser). For SOC, 10 mg soil was pretreated with 40 µl of 10% Hydrochloric acid for 12 h to remove carbonate, rinsed and dried at 40-50 °C. The pretreated sample was combusted at 1020 °C with a constant helium flow carrying pure oxygen to ensure completed oxidation of organic materials. Production of CO_2 was determined by a thermal conductivity detector. Soil inorganic carbon was calculated as the difference between total soil carbon and SOC.

For each soil profile, densities of TC, SOC and SIC stocks (X, kg m^{-2}) were calculated from carbon content ($\mathrm{X_i}$, g kg^{-1}), bulk density (E_i, g cm^{-3}) and depth increment in the soil profile (D_i, cm):

$$X = \sum_{i=1}^{\mathrm{n}} \mathrm{X_i} \times \mathrm{D}_i \times \mathrm{E}_i / 100, \qquad (4)$$

The SOC:SIC ratio represents the relative proportions of soil organic carbon (SOC) to soil inorganic carbon (SIC) stocks. An underused ratio in the field of biogeochemistry, this metric was used in this study to investigate the interactions between these carbon pools, with the hypothesis that their relative abundances influence the magnitude and nature of their effects on various soil response variables.

Soil Microbial Biomass Carbon

Chloroform fumigation extraction was used to obtain a measure of soil microbial biomass carbon following published methods (Dawson et al., 2007; Vance et al., 1987). Briefly, 20 g (± 2 g) of field moist soil was weighed into two glass containers – one in a small beaker for fumigating and one in a conical flask for immediate extraction. Soil samples in beakers for fumigation were placed in the desiccator, along with a 50 ml beaker containing 25 ml of acid-washed chloroform and anti-bumping granules and left to fumigate for 24 hours. Meanwhile, for non-fumigated samples, soil samples in the flasks were shaken with 50 ml 0.5 M K_2SO_4 for 30 minutes and then left to equilibrate for another 30 minutes. Both fumigated and non-fumigated sample solutions were filtered through Whatman No.42 filter and stored at 5 °C. All filtered solutions were measured using LabTOC.

Statistical analysis

Statistical analyses and data presentation were performed in R version 4.2.3 (R Core Team, 2020). Simple linear models using the ‘lm’ function in base R were used to understand differences between key variables at each factor level, and ANOVA’s for these models are presented within the ‘summary statistics’ section. The suitability of ‘lm’ for this purpose was confirmed through examination of residual *vs* fitted plots (linearity), histograms and qqplots (normality of residuals), scatter plots (homoscedasticity), and by performing a Durbin-Watson test for independence of residuals from the package “lmtest”.

Then, to examine the interactive effects of cover cropping, tillage, soil depth, soil cations, pH, percent SOC/SIC (depending on the response variable), SOC:SIC ratio, and microbial biomass carbon, on SOC and SIC stocks, the same workflow was employed for the development of 2 individual generalized linear models. For each generalized linear model, initial variables were included for consideration based on hypothesized interactions, and singular terms were always included where they featured in an interaction (Montesinos López et al., 2022). The response variable was log transformed to improve the distribution of model residuals, and all predictor variables were z-score standardized (Scaled value = (X – mean (X)) / standard deviation (X)) to make the variables consistently comparable and interpretable. Prior to fitting, homogeneity of variance was tested at each factor level using the “leveneTest” function, and if heterogeneous variance was detected, a weighted variance structure was fitted to address this model assumption and reduce the likelihood of Type 1 errors (Gastwirth et al., 2009).

Models were fitted using the “glm” gaussian family ‘identity’ link function from the ‘stats’ package in R. Following fitting of the full model, iterative model updating was performed starting with stepwise Akaike information criterion backward selection using the R package “MASS” (Ripley et al., 2013) and after checking for multicollinearity *via* variance inflation examination using the “car” package (Fox et al., 2007). StepAIC helped select for a model that minimized the AIC to provide a good trade-off between model fit, and simplicity, and the final model was fitted ensuring a maximum of 1 variable/interaction per 10 observations to avoid overfitting. If significant collinearity was detected, variables were removed from the analysis. Once the final model was derived, residuals vs. fitted value plots were examined to ensure linearity and mean-centeredness of residuals, quantile-quantile (Q-Q) plots to assess the normality assumption of residuals, and scale-location plots to investigate the homoscedasticity assumption.

The full model, prior to stepwise updating was as follows, where SOC or SIC as an independent variable were interchanged depending on the response:

y = Depth + Tillage + Cover crop + Calcium + Magnesium + SOC + SOC:SIC + MicBio + SOC:SIC*Tillage + pH + SOC*Tillage + Calcium*Tillage + Depth*Tillage + pH*Tillage + pH*Cover crop + Depth*Cover crop + Tillage*Cover crop + MicBio*Tillage + MicBio*Cover crop, data = data, family = gaussian (link = "identity", weights = “Factor”))

Finally, bootstrap resampling with n = 1000 iterations was performed on the original dataset using a custom loop function, and the final model iteratively refit to obtain robust parameter estimates (Harrell, 2017, 2015). Significance testing and reporting of the final, bootstrapped models were performed using type “III” ANOVA from the “car” package. This type of ANOVA was selected as it calculates the sums of squares for each factor while considering all other factors in the model, making it preferable for models with a high number of predictors and interactive terms. Therefore, where model predictions are presented, these estimates were obtained using the mean of the bootstrapped coefficient (± 95% CI) for each parameter and were generated using the “effects” package in R (Pinheiro et al., 2017). Model statistics from type III analysis of deviance are presented in tables as *Chisq*, p-value.

RESULTS

Summary statistics

Summary statistics for key modelled variables SOC kg m^{-2}, SIC kg m^{-2}, SOC:SIC ratio, pH, calcium mg g^{-1}, magnesium mg g^{-1}, and microbial biomass µg g soil^{-1} are displayed by depth, tillage, and cover crop levels (**Fig 2**), and ANOVA results testing these variables by factor level are in **Table 2**. The raw data figure for magnesium mg g^{-1} is presented in Supplementary Materials (**Fig S1**). **Table S2** summarizes bulk density statistics by depth, tillage, and cover crop levels.

Table 2: Results of ANOVA for key modelled variables tested at the individual factor level. Statistics from left to right are degrees of freedom, f-statistic, and p-value. *** = p<0.001, ** = p<0.01, * = p< 0.05.

	Variable						
	SOC (kg m^{-2})	SIC (kg m^{-2})	SOC:SIC	pH	Calcium (mg g^{-1})	Magnesium (mg g^{-1})	Microbial biomass (µg g^{-1})
Factor							
Depth	4, 12.31, ***	4, 22.31, ***	4, 1.61, 0.17	4, 4.34, **	4, 0.68, 0.14	4, 2.38, 0.05	4, 26.74, ***
Tillage	2, 6.20, **	2, 3.69, *	2, 1.09, 0.33	2, 3.00, 0.05	2, 1.67, 0.19	2, 0.85, 0.43	2, 2.75, 0.06
Cover crop	1, 10.77, **	1, 0.61, 0.16	1, 0.04, 0.82	1, 0.97, 0.32	1, 0.00, 0.95	1, 2.38, 0.05	1, 0.08, 0.77

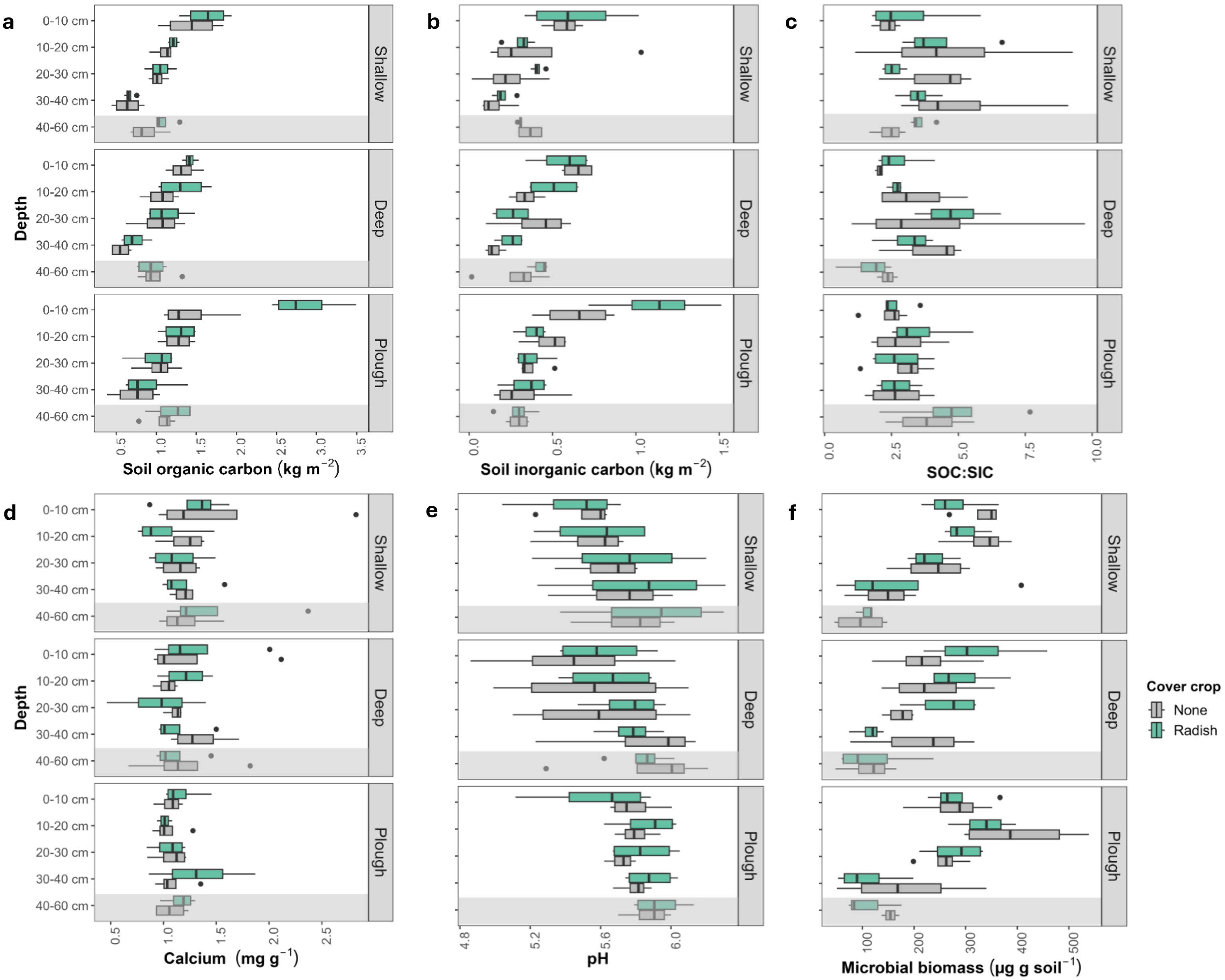

Figure 2: Raw data for the key modelled variables in tillage and cover crop treatment, and their pattern over 10 cm soil depth increment. Shaded grey areas in the 40-60 cm depth highlight the sampled 20 cm depth increment. **a)** Soil organic carbon stock, **b)** soil inorganic carbon stock, **c)** SOC:SIC ratio, **d)** extractable calcium, **e)** pH, **f)** microbial biomass. 'Shallow': shallow non-inversion tillage to 10 cm; 'Deep': deep non-inversion tillage to 20 cm; 'Plough': deep inversion tillage to 25 cm. Grey indicates no cover, while green indicates radish cover crop treatment.

Soil organic carbon stock

The final model for soil organic carbon stock (kg m^{-2}) explained 73% of the variation in the data (R^2 = 0.73; **Table 3**). Tillage and soil depth exerted the greatest influence on SOC stock (**Fig 3a**). Tillage was not associated with changes to SOC stocks between 0-30 cm. Below the plough layer, SOC stock under plough (inversion tillage) was ~ 42% greater than the non-inversion tillage treatments. SOC content at 40-60 cm did not differ across the tillage treatments. It is important to note that this increment covers a depth of 20 cm and should therefore be interpreted carefully in relation to the 10 cm increments above.

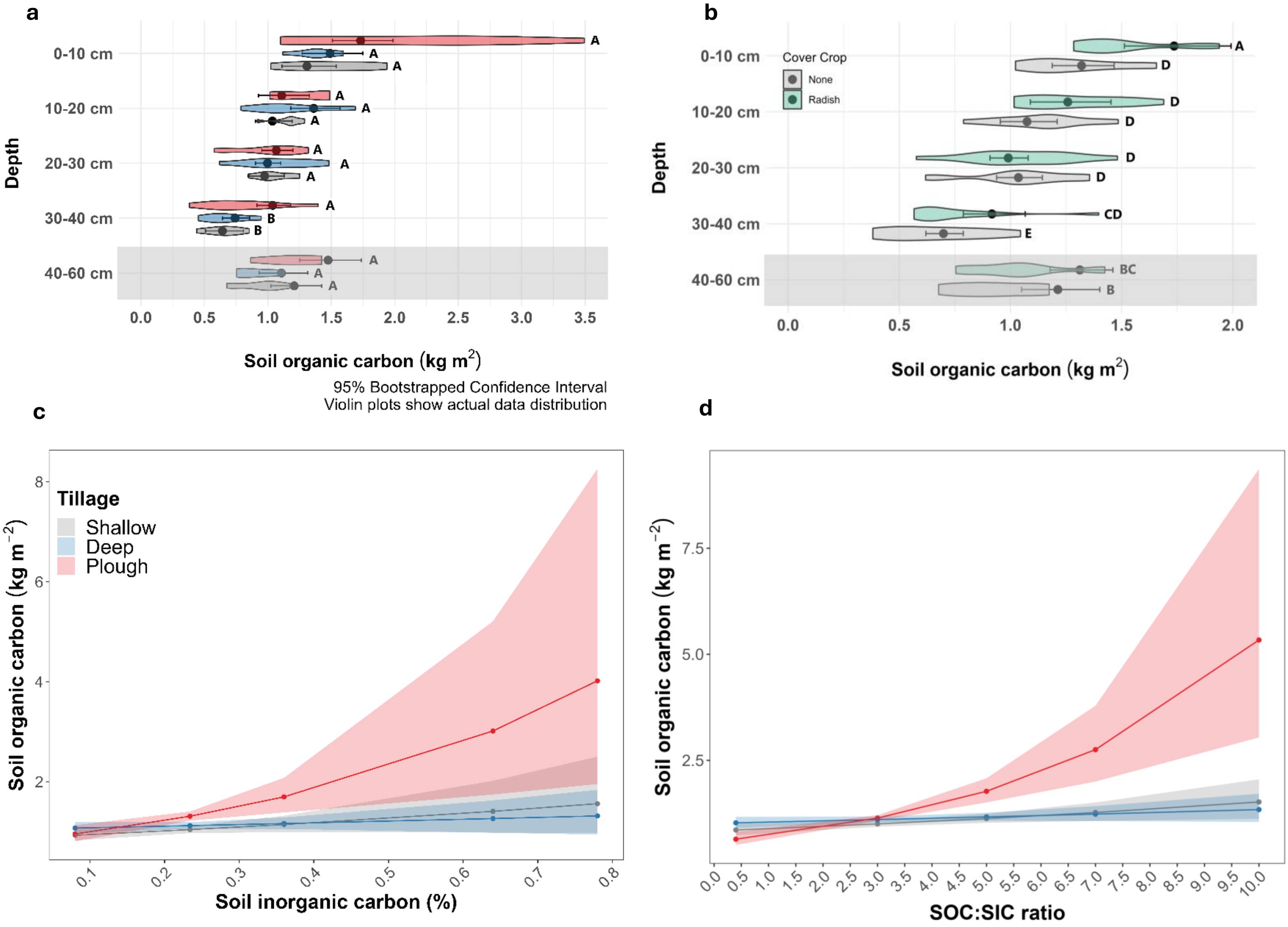

Figure 3: Significant drivers of SOC stocks in order of effect size from top left to bottom right: **a)** Depth*Tillage, **b)** Depth*Cover crop, **c)** Tillage*SIC, **d)** Tillage*SOC:SIC. For factorial variables, differing letters indicate significant differences between groups, for continuous effects, non-overlapping error bars indicate significant differences between groups using the 95% bootstrapped confidence interval. Grey 'Shallow': shallow non-inversion to 10 cm; Blue 'Deep': deep non-inversion to 20 cm; Red 'Plough': deep inversion plough to 25 cm.

Grey: no cover; green: radish cover crop treatment. Shaded grey areas in the 40-60 cm depth highlight the 20 cm depth increment.

Depth and cover crop were significant predictors of SOC stock (**Fig 3b**). There was a general trend of more SOC under radish cover than without: at 0-10 cm, SOC stock was ~35 % greater under radish, and at 30-40 cm, SOC stock was ~15 % greater under radish than without cover crop. SIC concentration was also a significant predictor of SOC stock, with the strength of the relationship changing dependent on tillage treatment: SIC was more strongly correlated with an increase in SOC stock under plough compared with the less intensive, non-inversion tillage treatments (**Fig 3c**). Across the whole soil profile (0-60 cm) at low SOC:SIC ratios (~0.5-3.0), SOC stock was marginally lower under plough, but at higher SOC:SIC ratios (~3.1 – 10.0), SOC stock was predicted to be up to 10-fold greater with ploughing (**Fig 3d**). There were no significant differences between the less intensive tillage treatments when regressed against SOC:SIC ratio. Also, as pH increased, there was a greater increase in SOC stock without cover crops, than with (**Fig S2a**). There was also a significant positive relationship between soil organic carbon, and microbial biomass (**Fig S2b**). Neither calcium, magnesium nor their interactions were chosen in the best fitting models for SOC stock.

Table 3: Type III ANOVA table showing the individual predictors for the SOC stock model. Chisq = Chi-square statistic, Df = degrees of freedom, P-value = statistical significance is determined at α = <0.05 (*** = p<0.001, ** = p<0.01, * = p< 0.05). Singular effects are not interpreted where higher level interactions occur.

Predictor	Chisq	Df	P-value
Depth	38.33	4	<0.001***
Tillage	7.191	2	0.02*
Cover crop	0.879	1	0.34
SIC (%)	3.33	1	0.06
SOC:SIC	6.80	1	0.009**
Microbial biomass	6.72	1	**0.009****
pH	5.52	1	0.01*
Tillage*SOC:SIC	18.04	2	**<0.001*****
Tillage*SIC	6.67	2	**0.03***
Depth:*Tillage	22.63	8	**0.003****
Cover crop*pH	4.85	1	**0.02***
Depth*Cover crop	13.73	4	**0.008****

Soil inorganic carbon stock

The final model for soil inorganic carbon stock (kg m^{-2}) explained 94% of the variation in the data ($R^2 = 0.94$; **Table 4**). Soil organic carbon exerted the greatest influence on SIC stock (**Fig 4a**), with an ~0.3-1.0 kg m^{-2} increase in SIC stock for 1% increase in soil organic carbon. Under radish cover crops and under plough, SIC stock was predicted to be ~15% higher than under all other conditions (**Fig 4b**). A similar stock of inorganic carbon was detected at 0-10 cm under plough, as at 40-60 cm (**Fig 4c**). There was also a positive correlation between SIC stock and microbial biomass, with a ~10 % increase of inorganic carbon per 100 µg of microbial biomass carbon (**Fig 4d**).

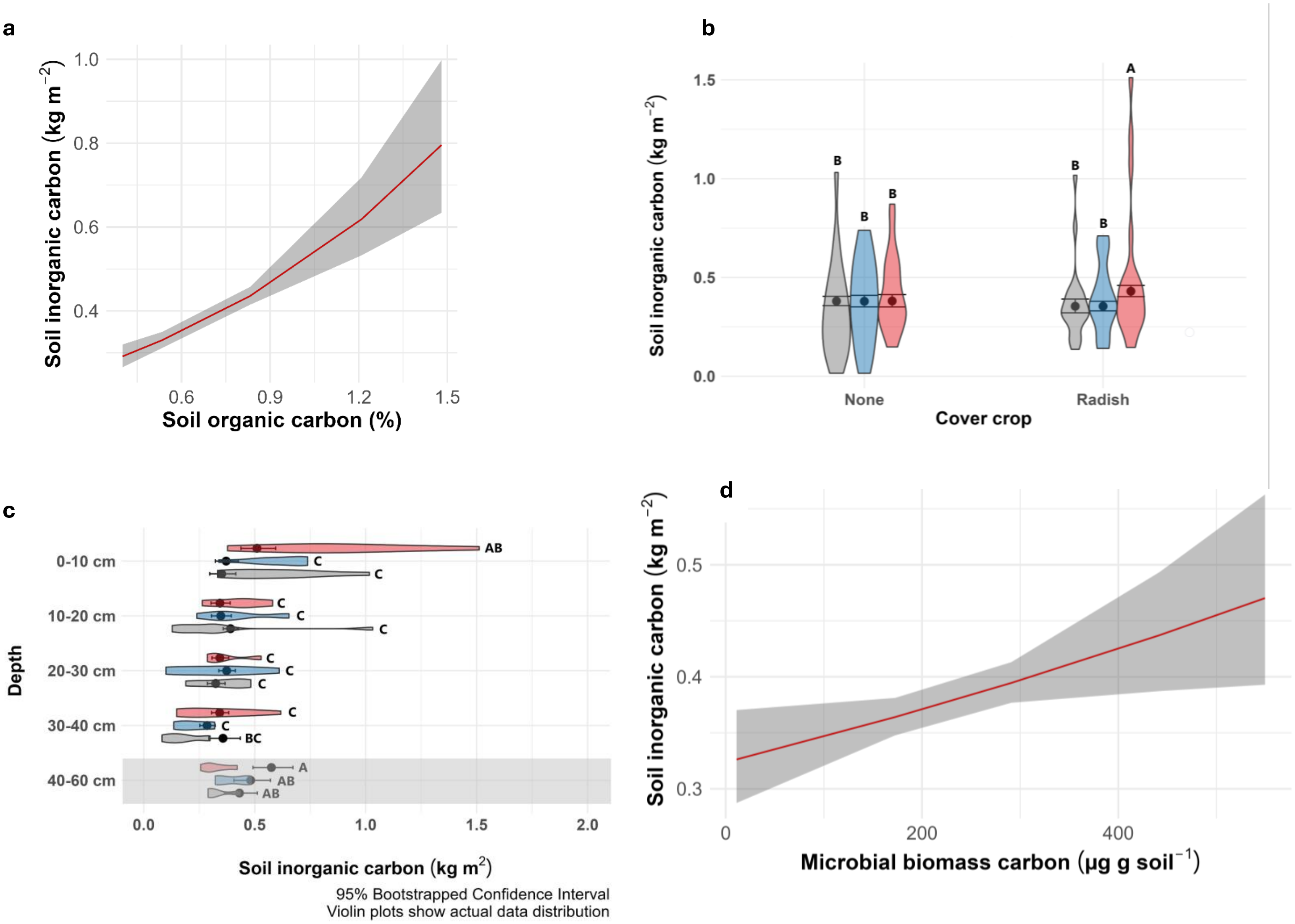

Figure 4: Significant drivers of SIC stocks in order of effect size from top left to bottom right: **a)** SOC**, b)** Tillage*Cover crop, **c)** Tillage*Depth, **d)** microbial biomass. For factorial variables, differing letters indicate significant differences between groups, for continuous effects, non-overlapping error bars indicate significant differences between groups using the 95% bootstrapped confidence interval. Grey 'Shallow': shallow non-inversion to 10 cm; Blue 'Deep': deep non-inversion to 20 cm; Red 'Plough': deep inversion plough to 25 cm.

Grey: no cover; green: radish cover crop treatment. Shaded grey areas in the 40-60 cm depth highlight the 20 cm depth increment.

SIC stock was also correlated with pH, a relationship that was dependent on tillage (**Fig S3**). Across the whole soil profile, under deep non-inversion and ploughing, soil inorganic carbon was predicted to decrease as pH increased. Under the shallow tillage treatment, SIC increased with pH. Finally, there was a significant negative relationship between SOC:SIC ratio and SIC stock, with a steeper decline in SIC stock occurring when the SOC:SIC ratio was between 0.5 – 5.0 (**Fig S4**).

Table 4: Type III ANOVA table showing the individual predictors for the SIC stock model. Chisq = Chi-square statistic, Df = degrees of freedom, P-value = statistical significance is determined at α = <0.05 (*** = p<0.001, ** = p<0.01, * = p< 0.05). Singular effects are not interpreted where higher level interactions occur.

Predictor	**Chisq**	**Df**	**P-value**
Depth	11.42	4	0.02*
Tillage	2.84	2	0.24
Cover crop	0.08	1	0.77
SOC %	40.97	1	**<0.001*** **
SOC:SIC	374.86	1	**<0.001*** **
Microbial biomass	4.45	1	**0.03***
pH	3.34	1	0.06
Tillage*SOC:SIC	5.99	2	0.05
Depth*Tillage	42.50	8	**<0.001*** **
Tillage*pH	16.42	2	**<0.001*** **
Depth*Cover crop	9.06	4	0.05
Tillage*Cover crop	9.86	2	**0.007*** **
Cover crop*Microbial Biomass	0.06	1	0.80

Discussion

This study aimed to quantify how cover cropping (radish) and tillage (shallow non-inversion to 10cm, deep non-inversion to 20 cm, and inversion plough to 25 cm) affect SOC and SIC pools in temperate, carbonate-containing soils. Contrary to established understanding, our findings challenge the notion that tillage diminishes SOC stocks within the top 30 cm of soil. Instead, like (Brown et al., 2021) on the same field, we found that tillage had no effect on SOC stocks in surface soils, and that inversion plough increased SOC stocks at depths of 30 – 40 cm, possibly due to the deeper incorporation of surface SOC. The results support the theory that SOC and SIC interactions are important for soil carbon storage; a relationship that is mediated by the intensity of soil disturbance. Notably, with SOC:SIC ratios above 3, intensive tillage may promote SOC accumulation. This result highlights a critical SOC-SIC interaction that, depending on tillage intensity, may offset anticipated disturbance-related loss of SOC. Additionally, our study supports the hypothesis that increased organic inputs from cover crops, combined with tillage, could enhance carbonate pedogenesis and affect SIC development; evidenced by the significant increase in SIC stocks under conditions of cover cropping and intensive tillage (**Fig 5**). Moreover, the research provides compelling indications of microbial involvement in the accrual of SIC, an important result given that the microbial contribution to SIC development usually goes unquantified. Taken together, our results not only contribute significantly to a growing body of evidence that has highlighted the potential for both SOC and SIC pools to be affected by agricultural management (Ball et al., 2023; Bughio et al., 2017; Sanderman, 2012), but also the importance of considering the interaction of SOC and SIC pools.

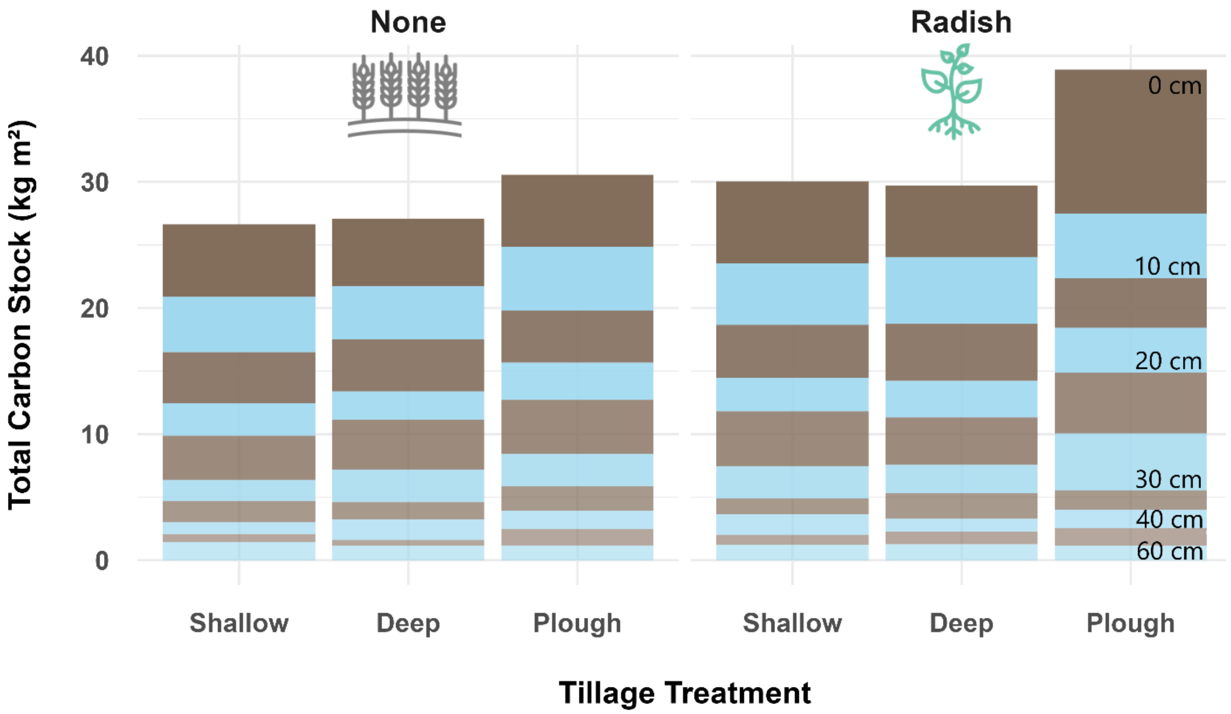

Figure 5: Raw data for total carbon stock (kg m^2) by soil depth under no cover crops and radish cover crop, by tillage treatments: 'Shallow': shallow non-inversion to 10 cm; 'Deep': deep non-inversion to 20 cm; 'Plough': deep inversion plough to 25 cm. Brown represents the organic carbon fraction, and blue the inorganic carbon fraction.

Tillage-related SOC losses may be buffered by carbonates in calcareous soils

It is generally accepted that tillage-induced soil disturbance has been a major contributor to the historical depletion of SOC on a global scale, and that transitioning from traditional plowing to less intensive, conservation tillage practices can lead to significant sequestration of SOC worldwide (Kan et al., 2022; Lal, 2004, 2003; Lal et al., 2003; Peterson et al., 1998). The success of reduced tillage in increasing SOC storage is heavily reliant on a few important assumptions: 1) that surface crop residues protect against erosion and water runoff (Lee et al., 2021), 2) that surface residues will readily decompose and enter the soil to become new organic matter (Man et al., 2021), and 3) that reduced soil disturbance helps maintain existing SOC protected in aggregate structures largely mediated by organic residues (Tang et al., 2011; Yu et al., 2019). But what is missing from this framework of understanding the primary benefits of tillage reduction, is that stabilization of organic materials in soils is also determined by the soil mineral fraction. Namely, the chemical composition of and existence of mineral surfaces capable of associating organic materials, the presence of multivalent cations, and the structure and heterogeneity of the soil matrix (Baldock and Skjemstad, 2000). While in the studied soils organic matter inputs were consistent and considerable, SOC stocks were ~50 % lower than expected in the top 30 cm of arable soils in this region of the UK (Bradley et al., 2005). However, in this study, it was not possible to determine whether SOC content was negatively affected by tillage given lack of a no-till comparison. Regardless, the general and widely applied concept that 'no-till and cover cropping lead to enhanced SOC storage' faces criticism given the inconsistency of positive results (Chaplot and Smith, 2023; Ogle et al., 2012; Powlson et al., 2014), and the limited capacity for *building* soil carbon *vs protecting* what is already there (Baker et al., 2007; Bossio et al., 2020). Importantly, most studies that have shown positive effects of reduced tillage and cover cropping on SOC stocks have not been conducted in calcareous soils. This lack of representation in the literature does not allow the assessment of potential for interactions between SOC and SIC pools, which might allow SOC to become 'protected' from decomposition through such interactions (Mehra et al., 2019). Carbonate minerals in soils may be contributing significantly to the stability of soil organic carbon, in addition to SIC being a significant and dynamic pool in and of itself.

Carbonate pedogenesis and SOC stabilization processes can be accelerated in cropping systems where water and organic acids are introduced, and that use tillage which disturbs the soil profile increasing CO_2, water, and ion movement through pore spaces (de Soto et al., 2017; Kim et al., 2020a). When $CaCO_3$ dissolves in the presence of H_2O or organic acids, bicarbonate (HCO_3^-) ions react with available cations to precipitate into carbonates. The release of complexed ions during carbonate dissolution can promote cation bridging between organic matter and soil minerals (Rowley et al., 2021, 2018); high concentrations of soil Ca^{2+} are positively correlated with SOC storage in both arid and temperate climates (Rasmussen et al., 2018; Rowley et al., 2020). While we did not detect a statistically significant interaction between Ca and SOC in the current study, the method by which Ca was measured (as an exchangeable pool) may partially explain this result. If most of the Ca in the soil was complexed as $CaCO_3$ rather than on exchange sites, there may be no detectable difference across treatments, unless the measurement was captured at a dynamic point of carbonate dissolution. Other studies have questioned the potential role of a more tightly-bound reactive Ca pool to influence SOC stabilization (Iskrenova-Tchoukova et al., 2010; Rowley et al., 2020, 2018); this was not tested in the current study, but is an important consideration for future work aimed at understanding of SOC-SIC interactions.

SOC-SIC interactions determine capacity for SOC storage

The SOC:SIC threshold value of 3.0, above which inversion ploughing diverges from the shallow and deep non-inversion tillage, indicates that SOC accumulation in this system is mediated by intensive soil disturbance. Therefore, given similar plant inputs, soils under inversion plough accumulated SOC to a greater extent than soils with less intensive tillage. This effect may be attributed to surface mixing effect by tillage that incorporates and therefore helps preserve new OM inputs in soil long enough to contribute to additive structures like organo-mineral complexes. It would also explain why more SOC was detected under inversion plough at depth. At SOC:SIC ratios below 3.0, it is possible that there is insufficient SOC relative to SIC to promote formation of these structures, and in this case SIC-stabilization processes are dominant drivers of total SOC in soil. This relative unimportance of the SOC:SIC ratio regarding total SOC storage may be explained by an enhanced retention of SIC-mediated aggregates (Pihlap et al., 2021). Pedogenic processes may also support the physical stabilization of SOC: as in the same pore spaces bicarbonate encounters calcium, $CaCO_3$ re-precipitation is promoted (Lal and Kimble, 2000) and carbonate coatings can form around organo-mineral compounds causing a ‘cementation’ effect (Virto et al., 2011), although this phenomenon is mostly attributed to dryland soils (Virto et al., 2011). If occurring, existing SIC-mediated aggregates left undisturbed by tillage and in the absence of an OM-mediated additive effect may reduce soil surface area available for sorption. It is unclear whether SIC dominated systems lend themselves to the accumulation of ‘new’ SOC (Virto et al., 2011), and if not, new plant-derived C could be rendered vulnerable to decomposition and liberation from the soil. Given the current system contains only small amounts of carbonates, and that high temperatures and evaporation are not dominant factors, a cementation effect is less likely to explain the enhanced SOC storage capacity under heavy disturbance, but we suggest that this mechanism be investigated further in both dryland and temperate soils.

Intensive tillage, cover cropping, and microbial biomass are positively correlated with higher SIC stocks

While SOC has received extensive attention for its role in carbon cycling, the SIC pool, SOC’s lesser-studied cousin, plays a critical role in global carbon cycle but is frequently disregarded in agricultural management studies. Agricultural management influences SIC stocks and emission dynamics through practices that alter soil structure and composition and pH, such as tillage (Bughio et al., 2017; Mehra et al., 2019; Plaza-Bonilla et al., 2015), crop rotation (Kim et al., 2020b) and irrigation (Ball et al., 2023; de Soto et al., 2017; Sanderman, 2012), and the application of fertilizers and amendments (Ahmad et al., 2015; Perrin et al., 2008). Evidence for tillage and cover cropping effects on SIC stocks are sparse (Mehra et al., 2019) and mostly conducted in dryland soils, therefore this study provides some of the first evidence that cover cropping and intensive tillage may increase SIC stock in temperate agricultural soils, and that the microbial biomass may also influence SIC development, despite the soil pH in this study being theoretically less conducive to carbonate precipitation (Raza et al., 2024; Zamanian et al., 2016). As previously discussed, we hypothesized that tillage may accelerate carbonate dissolution as it increases water and CO_2 in pore spaces, thereby altering pH. And in fact, we detected evidence to support the potential dissolution of carbonates despite increasing pH under more intensive tillage. But, in addition to the soil pH changes that occur with mechanical disturbance of soil, processes of biogenic carbonate formation may have enhanced pedogenic processes, *via* biologically-induced carbonate mineralization (Dupraz et al., 2009). For plants, regulation of the biochemical conditions conducive to carbonate precipitation includes modification of the soil's carbonic acid content during respiration, root exudation which introduces CO_2 and organic acids to directly alter soil pH and influence mineral solubility, selective ion uptake which changes cation balance, and plant-mediated modifications to soil moisture (Cailleau et al., 2004; Gocke and Kuzyakov, 2011). Microbial pathways involving biologically-induced mineralization often include processes like CO_2 absorption, sulphate reduction, and ammonification (Dupraz et al., 2009, 2004). Here, the microbial community can metabolize organic and inorganic substances, creating conditions that favor the precipitation of carbonates. In the case of biologically-influenced mineralization, microbial cells, or the extracellular polymeric substances (EPS) they secrete act as nucleation sites for carbonate formation. EPS, particularly when produced by fungi, binds calcium ions and promotes the growth of carbonate crystals without altering the soil's chemical properties, thereby indirectly contributing to carbonate mineral formation (Bindschedler et al., 2016). Additionally, and most often leveraged for soil structure improvement in the field of bioremediation (Liu et al., 2023), microbially-mediated carbonate precipitation can manifest as 'biologically-controlled' mineralization, where organisms orchestrate the nucleation and growth of minerals within or on their cells, often utilizing specific organic matrices as scaffolds. While this dataset did not allow for explicit testing of the biological mechanisms underpinning SIC formation, the observed correlations between agricultural practices and increased SIC stocks are promising. These findings contribute to the expanding body of evidence underscoring the significance of biological factors in SIC formation (Ball et al., 2023; Batool et al., 2024; Calmels et al., 2014; Zeng et al., 2023) and highlight the potential for refined management strategies to exploit these biological pathways, suggesting substantial opportunities for enhancing SIC storage through informed agricultural practices.

This study highlights how agricultural practices significantly impact the dynamic interplay between soil's organic and inorganic carbon pools. The findings not only challenge traditional views on the effects of tillage on organic carbon stocks, but also emphasize the potential of strategic agricultural management to enhance both SOC and SIC storage in calcareous soils. Given the biological elements intrinsic to SIC formation and storage, it becomes evident that there may be untapped opportunities to manipulate agricultural systems to enhance SIC accumulation. By strategically adjusting farming practices to boost biological processes that favor carbonate precipitation—such as selecting specific cover crops, optimizing tillage routines, and tailoring fertilizer and irrigation applications—agricultural managers can not only improve soil health but may also increase sequestration of inorganic carbon. Future research should aim to deepen understanding of SOC-SIC relationships in the context of agriculture where they are most likely to be enhanced, potentially leading to refined management strategies that capitalize on these interactions for increased soil carbon storage.

Acknowledgements and funding

The field trial upon which this study was conducted is supported by The Morley Agricultural Foundation (TMAF) and the JC Mann Trust.

Kirsten Ball is supported by a UKRI postdoc guarantee. Ref: EP/X022498/1

Yang Guo received funding for her PhD project from the China Scholarship Council (CSC) and the University of Aberdeen

Author contributions (CRediT)

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Smith: Conceptualization, Funding acquisition, Project administration, Resources, Writing (review and editing), Supervision

Hallett: Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Writing (review and editing), Supervision

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Malik: Conceptualization, Project administration, Writing (review and editing), Supervision

Data availability

All data and code used for subsequent analyses will be made available upon request

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Soil organic and inorganic carbon interactions under tillage and cover cropping determine potential for carbon accumulation in temperate, calcareous soils

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SUPPLEMENTARY MATERIALS

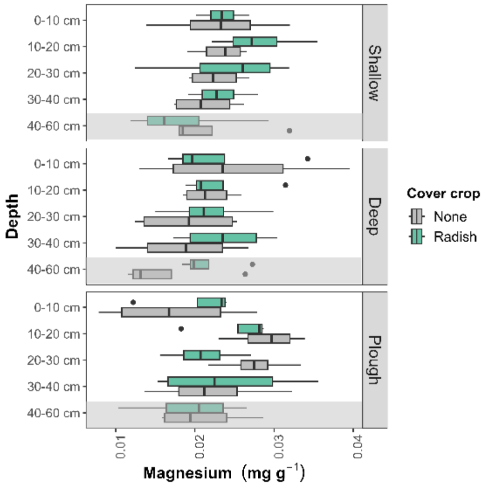

Figure S1: Summary data for magnesium mg g^{-1} by the factor levels "Soil depth, Tillage, and Cover crop". The shaded grey area in the 40-60 cm depth highlight the 20 cm depth increment.

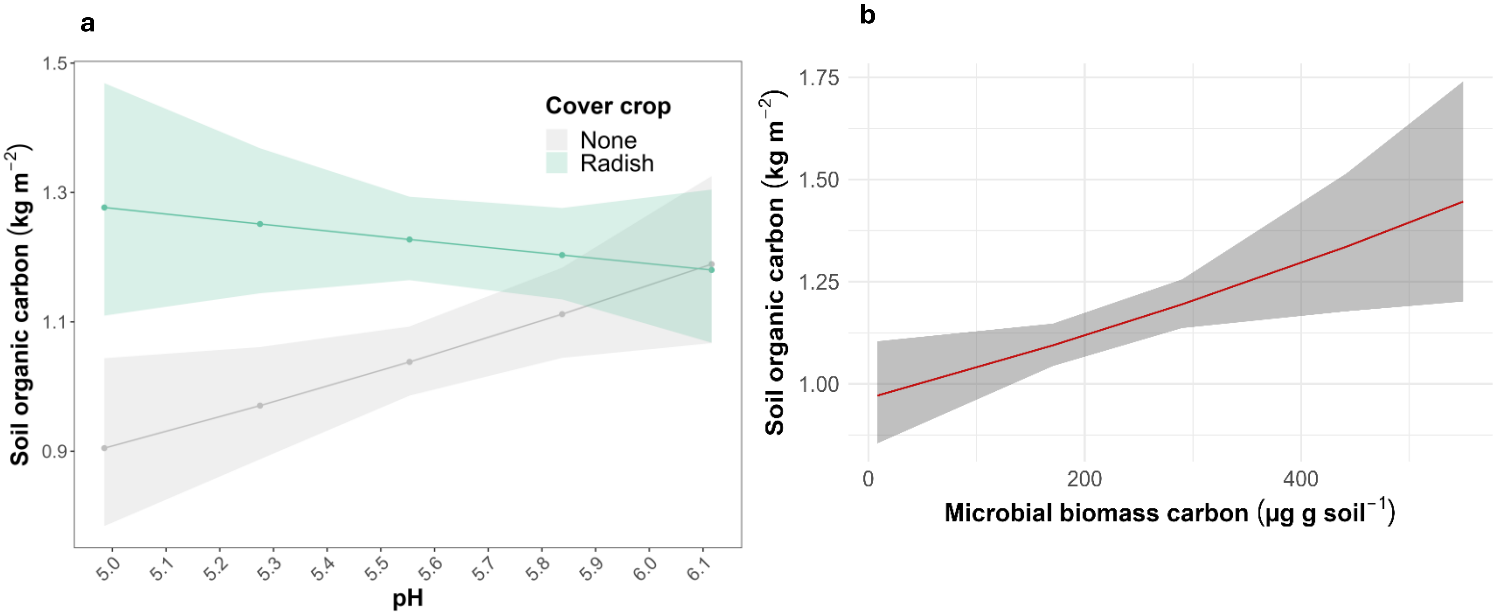

Figure S2: Significant drivers of SOC stocks in order of effect size from left to right. Non-overlapping error bars indicate significant differences between groups using the 95% bootstrapped confidence interval: **a)** pH*Cover crop effect, **b)** microbial biomass effect.

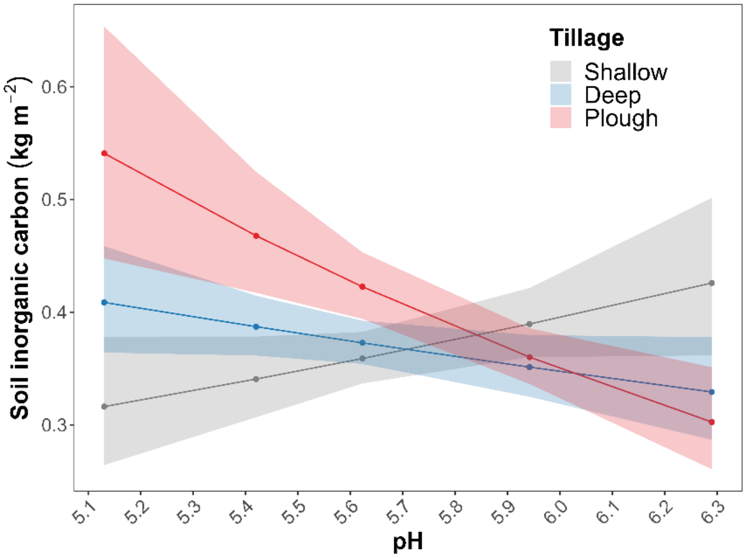

Figure S3: The tillage*pH effect. Non-overlapping error bars indicate significant differences between groups.

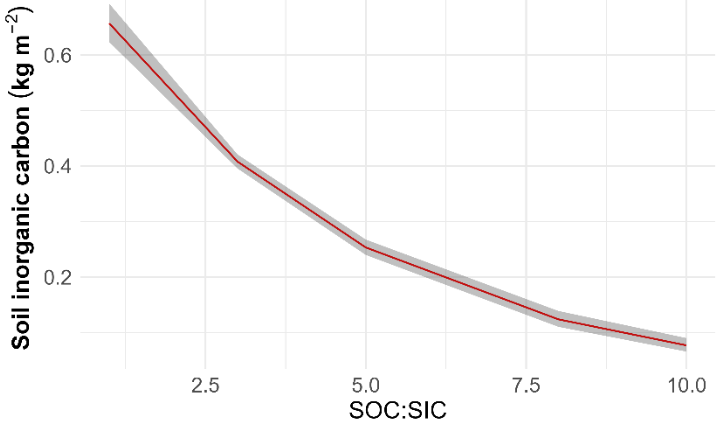

Figure S4: SOC:SIC effect on soil inorganic carbon stock showing the 95% bootstrapped confidence interval.

Table S1: Soil texture statistics (%) obtained at the block level (not replicated) by each level of tillage and soil depth.

	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
Depth	**Shallow (Non-inversion)**			**Deep (Non-inversion)**			**Plough (Inversion)**		
0-10 cm	75.00	18.40	6.55	71.50	17.90	10.70	72.00	18.20	9.50
10-20 cm	73.36	18.73	7.91	73.25	19.13	7.62	74.29	19.11	6.60
20-30 cm	73.40	17.00	9.60	73.03	16.02	10.95	71.89	19.07	9.03
30-40 cm	73.71	16.16	10.13	70.44	18.34	11.22	73.54	17.14	9.32
40-60 cm	73.04	15.59	11.37	54.54	26.22	19.25	78.09	13.17	8.74

Table S2: Mean bulk density values (g cm^3) (± SE) for shallow (non-inversion), deep (non-inversion), and plough (inversion) and with or without cover crop (radish) by depth.

	Shallow (Non-inversion)		**Deep (Non-inversion)**		**Plough (Inversion)**	
Depth	No cover	Cover	No cover	Cover	No cover	Cover
0-10 cm	1.48 (0.01)	1.49 (0.02)	1.54 (0.01)	1.56 (0.05)	1.63 (0.08)	1.59 (0.02)
10-20 cm	1.62 (0.05)	1.63 (0.11)	1.64 (0.14)	1.68 (0.10)	1.65 (0.03)	1.63 (0.08)
20-30 cm	1.59 (0.05)	1.62 (0.05)	1.69 (0.05)	1.64 (0.09)	1.56 (0.04)	1.56 (0.04)
30-40 cm	1.53 (0.12)	1.61 (0.09)	1.67 (0.04)	1.64 (0.08)	1.57 (0.12)	1.63 (0.07)
40-60 cm	1.57 (0.14)	1.64 (0.05)	1.63 (0.04)	1.55 (0.11)	1.59 (0.04)	1.62 (0.08)