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Two Decades of Conservation Agriculture Enhances Soil Structure, Carbon Sequestration, and Water Retention in Mediterranean Soils

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Abstract. Conservation agriculture offers a pathway for enhancing soil health with climate co-benefits in Mediterranean agricultural systems. This study examined long-term impacts of combining no-till management with cover cropping over 20 years in California's Central Valley, providing rare insights into soil system equilibrium under sustained conservation management. We assessed soil physical, chemical, and structural properties comparing reduced tillage with cover crops (CTCC) to standard tillage without cover crops (STNC), employing density fractionation and spectroscopic analysis to understand carbon protection mechanisms. After two decades, conservation agriculture achieved dynamic equilibrium characterized by fundamental shifts in carbon stabilization pathways. Water-stable aggregate analysis revealed the most pronounced management effects, with CTCC exhibiting 136% greater stability than STNC, indicating substantial improvements in soil structural integrity. These structural enhancements corresponded with a reorganization of carbon protection mechanisms: CTCC disproportionately enriched the occluded light fraction (44.1% vs. 35.4% of total recovered carbon in STNC), demonstrating that physical protection within aggregates becomes a dominant carbon stabilization pathway under long-term conservation management. Mineral-associated organic carbon saturation analysis revealed that both management systems remained well below theoretical maximum capacity (11.5% vs. 7.4% saturation for CTCC and STNC, respectively), indicating substantial remaining potential for carbon sequestration even after reaching equilibrium. Physical property improvements under CTCC included 15% lower bulk density and 13% greater water retention at field capacity, though benefits were concentrated in the surface horizon. Our findings demonstrate that two decades of conservation agriculture fundamentally transforms soil functioning through aggregate-mediated physical protection, while creating substantial improvements in soil structural integrity and water retention capacity. This mechanism shift represents a new soil system equilibrium that maintains enhanced functionality and continued carbon sequestration potential in Mediterranean agricultural systems.

1 Introduction

Modern agricultural systems have significantly contributed to global soil degradation through intensive tillage, continuous cultivation, and management practices that disrupt fundamental soil processes (Sanderman et al., 2017; Mitchell et al., 2025).

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These practices have resulted in widespread erosion, loss of soil organic matter, and deterioration of soil structure, with most erosion directly attributable to anthropogenic activities such as prolonged soil exposure and excessive mechanical disturbance (Blanco-Canqui and Ruis, 2018). The consequences extend beyond local productivity losses to global biogeochemical cycling, as soils represent the largest terrestrial carbon reservoir, containing approximately 3000 Pg of carbon—more than three times the amount stored in atmosphere and vegetation combined (Friedlingstein et al., 2025). Agricultural activities have created a substantial carbon debt of 116 Pg C (Sanderman et al., 2017), yet this same sector holds significant potential for carbon sequestration, with croplands capable of sequestering over 0.28 Pg of carbon annually (Lessmann et al., 2022). Understanding how management practices can simultaneously restore soil function while maintaining agricultural productivity remains a fundamental challenge, requiring mechanistic insights into how different practices alter soil physical, chemical, and biological processes.

Conservation agriculture addresses soil degradation through three fundamental principles: minimal mechanical soil disturbance, permanent soil cover through crop residues and cover crops, and diversification of crop species through rotations (Francaviglia et al., 2023). These practices work synergistically to enhance soil biological activity, reduce erosion, and improve soil structure (Six et al., 2004). No-till farming eliminates the mechanical disruption that destroys soil aggregates and exposes organic matter to accelerated decomposition (Blanco-Canqui and Ruis, 2018). This reduction in soil disturbance can decrease erosion by more than 70% while preserving soil pore network architecture (Lee et al., 2021). Cover crops provide continuous carbon inputs throughout the year, contributing organic matter that serves as both an energy source for soil organisms and a binding agent for aggregate formation (Dupla et al., 2022). The integration of these practices fundamentally alters soil development processes, yet our mechanistic understanding of how these changes manifest over extended timeframes remains incomplete. Most conservation agriculture research has focused on short-term responses or single management components, leaving critical knowledge gaps regarding the long-term, integrated impacts on soil functioning.

Central to understanding conservation agriculture impacts is that soil organic matter exists in multiple forms with vastly different stabilities and functions. Modern understanding distinguishes between particulate organic matter (POM) and mineral-associated organic matter (MAOC), representing fundamentally different carbon protection strategies with distinct temporal dynamics (Lavallee et al., 2020). MAOC, or the heavy fraction (HV), forms through sorption and complexation processes with clay and silt particles, creating relatively stable but potentially saturable carbon pools that develop rapidly following management changes (Stewart et al., 2009). POM comprises two carbon pools: free light fraction (FLF) and occluded light fraction (OLF). FLF consists predominantly of vegetative litter with high C:N ratios that is relatively unprotected (Leuthold et al., 2024). OLF occurs when organic matter becomes occluded within soil aggregates, where reduced oxygen diffusion and limited substrate accessibility slow decomposition rates (Christensen, 2001). Recent evidence demonstrates that row crops under conservation management in semi-arid climates can fundamentally shift carbon distribution from chemically-protected to physically-protected forms over multi-decade periods, following predictable temporal stages: initial rapid formation of MAOC during years 0-5, continued MAOC accumulation with enhanced microbial processing during years 5-15, and a mature phase beyond 15 years where MAOC approaches saturation limits and physical protection within aggregates becomes the dominant pathway for continued carbon sequestration (Tian et al., 2024). This temporal evolution reflects the finite capacity of mineral

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surfaces to bind organic matter, necessitating alternative protection mechanisms as soils mature under conservation management. The concept of carbon saturation, particularly for MAOC, suggests that soils may reach maximum storage capacity through certain pathways, potentially requiring shifts toward alternative protection mechanisms for continued sequestration (Georgiou et al., 2022).

Mediterranean agricultural systems provide an ideal environment for investigating conservation agriculture impacts due to their unique environmental constraints and management challenges. The Mediterranean climate, characterized by hot, dry summers and mild, wet winters, creates pronounced seasonal patterns of soil wetting and drying that intensify aggregate formation and destruction cycles (Bronick and Lal, 2005). These climatic conditions, combined with typically high clay content soils, create systems where aggregate stability becomes particularly critical for maintaining soil function. Water limitation places additional constraints on soil biological activity, potentially altering the relative importance of different carbon protection mechanisms compared to more mesic environments. The seasonal nature of precipitation and temperature patterns also influences the timing and magnitude of organic matter inputs, creating distinct pulses of substrate availability that may favor particular microbial communities and decomposition pathways. These environmental characteristics make Mediterranean systems particularly sensitive to management-induced changes in soil structure and organic matter dynamics, while simultaneously testing the resilience of conservation practices under climatic stress. California's Central Valley exemplifies these conditions while supporting intensive agricultural production, providing a representative system for understanding how conservation practices perform under real-world constraints.

Advances in soil analytical techniques have enabled increasingly sophisticated approaches to understanding soil organic matter dynamics and carbon persistence. Traditional measurements of total soil organic carbon, while providing important baseline information, offer limited insight into processes determining long-term carbon stability (Paustian et al., 2019). Density fractionation methods address this limitation by physically separating soil organic matter into functionally distinct pools based on association with mineral particles or occlusion within aggregates (Christensen, 2001). These approaches distinguish between FLF, OLF, and MAOC, each representing different levels of protection and turnover rates. Modern protocols employ sequential size and density separation procedures that have proven essential for detecting carbon protection mechanism shifts over decadal timescales (Poeplau et al., 2018). Complementing physical fractionation, spectroscopic techniques such as Fourier-transform infrared (FTIR) spectroscopy provide detailed information about organic matter molecular composition, enabling assessment of decomposition state and substrate quality (Margenot et al., 2023). Integrating these analytical approaches with traditional soil health indicators provides a comprehensive framework for evaluating conservation management impacts, bridging the gap between field-applicable measures and fundamental soil science principles.

Building on this foundation, our study addresses critical knowledge gaps regarding long-term impacts of integrated conservation practices on soil carbon protection mechanisms in Mediterranean agricultural systems. We examine how combining no-till management with cover cropping over 20 years alters soil carbon distribution among protection pathways, with particular focus on the shift between chemically-protected and physically-protected carbon pools. Recent research demonstrates that soil carbon systems under conservation agriculture reach dynamic equilibrium after 15-20 years (Caruso et al., 2018). We hypothesized that long-term conservation management enhances aggregate-mediated physical protection of soil organic matter while

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approaching saturation limits for mineral-associated carbon storage. After two decades, these conservation systems should approach equilibrium conditions where maximum benefits are realized, providing unique insights into the ultimate potential of these practices. We employ density fractionation, spectroscopic analysis, and comprehensive soil physical characterization to test whether conservation agriculture fundamentally shifts carbon stabilization mechanisms and whether these shifts correspond with measurable improvements in soil structural and hydraulic properties. These mechanistic insights are essential for predicting soil responses to management and environmental changes, while contributing to our broader understanding of soil functioning as a foundation for sustainable agricultural systems.

2 Materials and Methods

2.1 Research Location and Experimental Framework

This research was conducted at the Conservation Agriculture Systems Innovation (CASI) facility in Five Points, California ($36.34°N, 120.12°W$). The site features Panoche clay loam soil (fine-loamy, mixed, superactive, thermic Typic Haplocambids) with a textural gradient from clay loam to sandy clay loam. The Mediterranean climate averages 178 mm annual precipitation and temperatures ranging from 7.8-23.9°C. Complete site characterization is available in Veenstra et al. (2007). The experiment employs a randomized complete block design with four treatments (combining standard/reduced tillage with presence/absence of winter cover crops) replicated eight times in 10 m × 100 m plots. The treatments consisted of reduced or no-till with cover cropping (CTCC), no-till with winter fallowing (CTNC), standard tilling with cover crops (STCC), and standard tilling with winter fallowing (STNC) (Mitchell et al., 2015).

2.2 Management History

Tillage Practices

The standard tillage (ST) system represented conventional San Joaquin Valley operations, involving intensive soil disturbance through residue shredding, multiple disk passes to incorporate residues to 8-inch depth, subsoiling to 12-18 inches before tomato and cotton crops, additional disking to break up compacted layers, bed listing, and surface residue incorporation using a cultimulcher that created a fine, powdery seedbed (Mitchell et al., 2015; Veenstra et al., 2007). These operations completely broke down and re-established planting beds following each harvest, representing typical regional tillage intensity. In contrast, the no-tillage (NT) system emphasized soil disturbance reduction and permanent bed management (Mitchell et al., 2015). Controlled traffic farming restricted tractor movement to designated furrows, while planting beds remained undisturbed throughout the study period. The NT system evolved significantly: from 1999–2011, shallow cultivation was permitted for tomato crops with cotton management limited to root pulling or shallow root severing to 4-inch depth. By 2012, the system achieved true zero-tillage status with soil disturbance restricted exclusively to seeding and transplanting operations, reducing tillage passes by 40% or more compared to standard practices (Mitchell et al., 2017).

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Cover Crop Species and Management

Cover crop implementation followed a systematic approach to enhance soil health through increased plant diversity and year-round soil coverage (Mitchell et al., 2015). The initial cover crop mixture (1999–2009) consisted of Juan triticale (30%), Merced cereal rye (30%), and common vetch (40%) by seed weight, seeded at 80 lbs/acre using no-tillage disc openers. From 2010–2014, species diversity was enhanced to include pea (40%), fava bean (40%), radish (10%), and Phacelia (10%), reflecting evolving understanding of multi-species cover crop benefits (Mitchell et al., 2015). Cover crops were typically seeded in late October to mid-November at 1-inch depth with 7.5-inch row spacing, preceding winter rainfall patterns. Legume species received Rhizobium leguminosarum biovar viciae inoculation to enhance nitrogen fixation. The approximately 120-day growth period concluded with mid-March termination using contrasting methods: standard tillage plots received stalk chopping followed by disk incorporation, while no-tillage plots were terminated with 2% glyphosate solution after chopping, leaving residues on the surface as protective mulch. This differential termination created distinct surface residue environments, with NT cover crop plots achieving $>$90% residue cover compared to $<$20% in ST systems.

Crop Rotation and Species

The experimental design incorporated three distinct cropping phases to evaluate conservation practices across diverse crop types (Mitchell et al., 2017). Phase 1 (1999–2014) featured a cotton-tomato rotation with the field divided into two halves to ensure both crops were grown annually on separate sections (Veenstra et al., 2007). Processing tomatoes (variety 8892') were transplanted in early April at 21,581 plants/ha using modified commercial transplanters equipped with coulters for residue management. Cotton production utilized RoundUp Ready transgenic upland cotton Riata' (2000–2007) and experimental Pima variety 'Phy-8212 RF' (2008–2009), established with John Deere no-till planters at approximately 148,000 plants/ha (Mitchell et al., 2017). Phase 2 (2015–2018) transitioned to garbanzo-sorghum rotation to achieve greater crop diversity consistent with conservation agriculture principles (Gomes et al., 2023). This rotation change allowed evaluation of conservation practices under different rooting systems and residue characteristics. Phase 3 (2019) returned to tomato production, providing comparative data across the study timeline (Araya et al., 2022). This rotation diversity ensured that management impacts could be assessed across crops with varying growth habits, nutrient requirements, and soil interaction patterns.

Irrigation Methods and Equipment

Irrigation management evolved significantly, reflecting regional trends toward water use efficiency (Mitchell et al., 2017). From 2000–2012, furrow irrigation supplied water to tomato and cotton crops using evapotranspiration-based scheduling ($ET_c = K_c \times ET_o$), where crop coefficients were adjusted for growth stage and canopy development. Applied water averaged 71 cm/ha for tomatoes and 61 cm/ha for cotton, with scheduling based on reference evapotranspiration data from an on-site California Irrigation Management Information System (CIMIS) weather station (Mitchell et al., 2017). In 2013, the entire experimental site converted to subsurface drip irrigation, requiring a one-time tillage operation across all treatments for installation (Araya et al., 2022). The system utilized 1.34-inch diameter drip tape buried 12 inches deep in the center of each 5-foot-wide planting

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bed. This transition aligned with regional adoption trends and reduced surface water application. Cover crop irrigation remained minimal throughout the study, totaling only 10 inches over 20 years (4 inches in 1999, 2 inches each in 2012, 2017, and 2018), with cover crops primarily dependent on the 127 inches of natural precipitation received during the study period (Araya et al., 2022).

Additional Relevant Management Information

Fertilizer management maintained consistency across treatments to isolate tillage and cover crop effects (Mitchell et al., 2017). Tomato crops received pre-plant applications of 11-52-0 fertilizer at 89.2 kg/ha applied via shanks 15 cm below transplants, followed by side-dressed urea (111.5 kg/ha) four weeks post-transplanting, providing total nitrogen inputs of 51.3 kg N/ha (Veenstra et al., 2007). Cotton received pre-plant 11-52-0 fertilizer at 224 kg/ha applied at 20 cm depth, with application methods varying by tillage system (mixed through beds in ST, shanked in NT) (Mitchell et al., 2017). Pesticide applications remained similar across treatments. Surface residue management created dramatically different soil surface environments, with residue cover measurements showing stark contrasts: STNC plots maintained $<5\%$ cover, STCC achieved 10–20%, CTNC reached 40–70%, and CTCC exceeded 90% surface residue cover (Mitchell et al., 2017). These differences resulted from the interaction between tillage intensity and cover crop residue inputs, creating distinct microclimates for soil biological activity, water infiltration, and thermal regulation (Baker et al., 2005). The 20-year duration of consistent management makes this site particularly valuable for assessing long-term conservation agriculture impacts on soil physical, chemical, and hydrological properties in Mediterranean climate conditions (Araya et al., 2022).

2.3 Sampling Strategy and Protocols

Soil collection occurred in October 2019, with sampling strategies designed based on previous findings by Araya et al. (2022), which showed significant management effects on soil hydro-physical properties (porosity, pore size distribution, field capacity) in the 0-5 cm layer but not at 20-25 cm depth. Consequently, sampling focused on 0-5 cm and 5-10 cm depths.

Seven undisturbed cores were collected from each of the two treatments (CTCC and STNC) at both depths using standardized METER HYPROP cylinders ($250\ \mathrm{cm}^3$). Treatment selection emphasized the extreme management contrasts, as prior HYPROP measurements (Araya et al., 2022) showed no significant differences in intermediate treatments (tillage-only or cover crop-only modifications). Core locations were randomly selected within each plot in inter-row positions away from tillage operations and irrigation lines.

Complementary bulk samples (0-10 cm) were collected from all four treatments for aggregate stability and density fractionation analyses. A spade shovel was used to dig to approximately 10 cm deep. Then a representative sample of soil was transferred to a rigid plastic container. Upon arrival to the lab, sample containers were left open for 2 weeks to air dry and stored at room temperature.

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2.4 Aggregate Stability Analysis

Wet aggregate stability was determined using a wet sieving apparatus (Eijkelkamp North America Inc.) on air-dried bulk soil samples (Nimmo and Perkins, 2002). Samples were separated through a stack of sieves (8 mm, 4 mm, and 2 mm), yielding four size fractions: >8 mm, 8-4 mm, 4-2 mm, and <2 mm. Analysis focused exclusively on macro-aggregates (8-4 mm and 4-2 mm) because these size classes are most responsive to management-induced changes in soil structure through root and fungal hyphal binding, whereas micro-aggregate formation depends primarily on inherent soil properties (Tisdall and Oades, 1982). Additionally, micro-aggregates comprised 40-70% more material by volume but were concentrated at depths where treatment effects were minimal, making macro-aggregates more sensitive indicators of surface management impacts. Preliminary testing confirmed that air-dried samples provided optimal discrimination between treatments, as pre-saturated aggregates remained nearly 100% stable after wet-sieving regardless of treatment.

Two 4-g subsamples were collected from each size fraction, with one subsample used for analysis and the other for moisture content determination. Aggregates from the 8-4 mm fraction were placed in 2 mm mesh sieve cups, while the 4-2 mm fraction was placed in 1 mm mesh sieve cups. Samples underwent wet-sieving in tap water for 3 minutes, with the stable fraction manually dispersed through the sieve into a separate collection container. All fractions were oven-dried at 100 °C for 48 hours before final mass determination.

Wet aggregate stability (WAS) was calculated as the percentage of stable aggregates relative to total aggregate mass, corrected for moisture content and rock fragments:

$$\mathrm{WAS} = \frac{m_{\text{stable}}}{m_{\text{stable}} + m_{\text{unstable}}} \times 100 \quad (1)$$

where m_{stable} is the oven-dry mass of the stable fraction and m_{unstable} is the oven-dry mass of the unstable fraction.

2.5 Soil Physical Properties

Physical property characterization was conducted on intact soil cores through sequential analysis measuring hydraulic conductivity, water retention, and porosity.

Experimental Timeline and Setup: Cores underwent sequential analysis over approximately 14 days: (1) initial saturation (24 hours), (2) saturated hydraulic conductivity measurement, (3) continuous drying with water retention measurement (12 days), and (4) final moisture determination (24 hours). Cores were processed in batches of six containing samples from both treatments and depths to minimize batch effect. Additional methodological details are provided in Alvarez-Sagrero et al. (2025).

Saturated Hydraulic Conductivity: Saturated hydraulic conductivity (K_{sat}) was measured using a METER KSAT automated measurement system. Intact soil cores were prepared by carefully trimming ends to ensure flat surfaces perpendicular to core walls. Cores were gradually saturated from bottom up over 24 hours using 0.01 M $CaCl_2$ solution to minimize clay dispersion and maintain soil structure.

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Measurements employed the falling head method, with the METER KSAT instrument automatically recording water level changes using a pressure transducer and temperature sensors. Measurement concluded when three consecutive K_{sat} values were within 5% of each other, indicating stable flow conditions. K_{sat} values were calculated using Darcy's law:

$$K_{\text{sat}} = \frac{L}{t} \ln \frac{h_1}{h_2} \tag{2}$$

where L is sample length (cm), t is time (s), and h_1 and h_2 are hydraulic heads (cm) at the start and end of the measurement period, respectively.

Soil Water Retention and Total Porosity: Water retention characteristics were measured using the HYPROP system (METER Group, Inc.) employing the extended evaporation method. Cores were instrumented with precision tensiometers at 1.25 and 3.75 cm depths, recording soil water potential and mass loss at 10-minute intervals during drying from saturation ($\psi = 0$ kPa) to approximately -80 kPa.

To extend the measurement range, subsamples (5 g) from each core were analyzed with a WP4C dewpoint potentiameter (METER Group, Inc.) at lower water contents. Complete datasets were fitted with a bimodal van Genuchten-PID model (Peters et al., 2021):

$$\theta(\psi) = (\Phi - \theta_r)\frac{\Gamma(\psi) - \Gamma(\psi_0)}{1 - \Gamma(\psi_0)} + \theta_r \frac{\log(\psi/\psi_0) - b\log\left(1 + (\psi_a/\psi)^{1/b}\right)}{\log(\psi_0/\psi_a)} \tag{3}$$

where Φ represents total porosity, θ_r the maximum adsorbed or residual water content, and $\psi_0 = 10^{5.8}$ kPa water potential at oven dryness. The saturation function $\Gamma(\psi)$ uses a bimodal version representing textural and structural pore spaces:

$$\Gamma(\psi) = \sum_{i=1}^{2} w_i \Gamma_i(\psi) \tag{4}$$

with each pore-size class following van Genuchten (Van Genuchten, 1980):

$$\Gamma_i(\psi) = (1 + (\alpha_i \psi)^{n_i})^{1/n_i - 1} \tag{5}$$

Total porosity (Φ) was determined directly from model fitting, representing maximum volumetric water content at saturation. Field capacity was derived as volumetric water content at -33 kPa soil water potential.

Bulk Density: Following hydraulic measurements, soil cores were oven-dried at 105 °C for 24 hours to determine final oven-dry mass. Bulk density (ρ_b, g cm^{-3}) was calculated as $\rho_b = m_{\text{dry}}/V_{\text{core}}$, where m_{dry} is oven-dry mass (g) and V_{core} is core volume (cm^{-3}).

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2.6 Carbon and Nitrogen Analysis

Following physical measurements, soil cores were oven-dried at 105 °C (24 hours), gently disaggregated, and passed through a 2-mm sieve. A 5-g subsample from each core was finely ground with an agate mortar and pestle to pass a 150-µm mesh for elemental analysis. Acid reaction testing confirmed negligible inorganic carbonate content, indicating total carbon measurements effectively represented total organic carbon.

Total carbon (TC) and total nitrogen (TN) were determined using dry combustion in an Elemental Analyzer coupled with a Delta V Plus Continuous Flow Isotope Ratio Mass Spectrometer (Costech 4010). Samples (15 ± 0.1 mg) were weighed into tin capsules using a microbalance (Mettler Toledo XP6, precision ± 0.001 mg). Calibration employed certified reference materials USGS 40 and 41a with mean TC values of 40.30 ± 2.10% (n = 7) and 41.59 ± 1.13% (n = 4), and mean TN values of 9.40 ± 0.52% (n = 7) and 9.71 ± 0.26% (n = 4), respectively. Quality control standards were analyzed every 10 samples.

TC and TN stocks were quantified using analytical measurements, soil physical properties, and sample dimensions:

$$S_i = C_i \times \rho_b \times d \times (1 - f_c) \tag{6}$$

where S_i represents elemental stock (kg m^{-2}) for element i (TC or TN), C_i is measured elemental concentration (kg kg^{-1}), ρ_b denotes soil bulk density (kg m^{-3}), d is sampling interval thickness (m), and f_c represents volumetric coarse fraction (>2 mm). The coarse fraction was consistently minimal (<5%) across all analyzed samples.

2.7 Organic Matter Composition Analysis

Fourier-transform infrared spectroscopy (FTIR) analysis was conducted on surface soil samples (0-5 cm depth) to characterize functional group composition of soil organic matter and assess management-induced changes in organic matter quality.

FTIR spectra were collected using a FTIR spectrometer (Bruker Vertex 70) equipped with a Praying Mantis DRIFTS accessory. Pure potassium bromide (KBr) served as background reference material. Spectra were recorded in absorbance mode from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution, with each spectrum representing an average of 300 scans and atmospheric compensation enabled to minimize interference from water vapor and CO_2. Data collection occurred at room temperature under ambient conditions.

Spectral processing was performed using OPUS software (Bruker Cor.). This included baseline correction and smoothing to minimize noise while preserving spectral features. Peak assignments for soil organic matter functional groups were based on established literature values (Mainka et al., 2022). Key functional groups identified included: aliphatic C-H stretching of methyl groups (2976–2898 cm^{-1}) and methylene groups (2870–2839 cm^{-1}) associated with simple plant materials, aromatic C=C stretching representing complex plant compounds such as lignin (1550–1500 cm^{-1}), and C=O stretching of carbonyl groups indicative of microbial-derived organic matter (1660-1580 cm^{-1}). These spectral regions were selected as they exhibit minimal interference from the mineral component, allowing clearer interpretation of organic matter composition and bio-availability to soil microorganisms.

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2.8 Density Fractionation and Carbon Protection Mechanisms

Density fractionation was performed on air-dried bulk soil samples (0-10 cm depth) to separate soil organic matter into three functionally distinct fractions: free light fraction (FLF), occluded light fraction (OLF), and heavy fraction (HV) closely following (Golchin et al., 1994),(Swanston et al., 2005),(Berhe et al., 2012). Samples were passed through an 8 mm sieve and homogenized by hand mixing. Approximately 25 g of soil was weighed for fractionation, with a separate 10 g subsample collected for moisture correction (dried at 105 °C for 48 hours).

For FLF separation, soil samples were placed in Nalgene conical tubes with 90 mL of sodium polytungstate solution (SPT, density = 1.85 g cm^{-3}). Tubes were inverted by hand 10 times and shaken at 150 rpm for 1 hour, followed by centrifugation at 3,470 rpm for 1 hour. The floating FLF was collected by aspiration, filtered through 0.8 µm polycarbonate filter paper, and rinsed five times with 100 mL Milli-Q water before drying at 50 °C for 48 hours.

The remaining soil was mixed at 170 rpm for 1 minute using a stand mixer, then sonicated in an ice bath to achieve approximately 22,400 joules of energy input. After centrifugation at 3,470 rpm for 1 hour, the OLF was aspirated and purified through 4 repeated washing cycles with 150 mL Milli-Q water, including vortexing (1 minute), centrifugation (30-60 mins at 3,470 rpm), and decanting through 0.8 µm polycarbonate filter paper. The OLF palet formed at the bottom of the conical tube was collected and oven dried at 50 °C for 48 hours. The final fraction, HV, underwent identical washing procedures before collection and drying at 105 °C for 48 hours.

All fractions were weighed and manually ground for subsequent carbon and nitrogen analysis using the same protocols described for soil cores. The summation of final oven dried masses from the 3 fractions was assumed to be the total soil mass when calculating carbon and nitrogen recovery.

2.9 Mineral-Associated Organic Carbon Saturation

Mineral-associated organic carbon (MAOC) saturation was assessed using the heavy fraction obtained from density fractionation. The analysis employed the clay-silt model and maximum organic carbon estimates established by Georgiou et al. (2022), which proposes theoretical carbon saturation values of 86 ± 9 mg C g^{-1} mineral for high-activity minerals and 48 ± 6 mg C g^{-1} mineral for low-activity minerals.

Soil classification previously conducted at the site identified the soil series as Panoche clay loam (Typic Haplocambids, Aridisol order). Based on USDA soil survey data for this series (Soil Survey Staff), the surface horizon (0-18 cm) contains 28% clay with a cation exchange capacity of 22.2 cmol(+)/kg, confirming classification as high-activity minerals under the Georgiou et al. (2022) framework (CEC >15 cmol(+)/kg). Site-specific textural analysis determined clay content ranging from 23-32% and similar proportions for silt (23-32%) (Baker et al., 2005), consistent with the soil survey range of 25-28% clay for this series.

MAOC saturation percentage was calculated as:

$$\text{MAOC}_{\text{saturation}} = \frac{C_{\text{measured}}}{C_{\max}} \tag{7}$$

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where $C_{\max}$ represents theoretical maximum carbon storage capacity (mg C g^{-1} soil) calculated as the product of mineral (clay+silt) content and the appropriate mineral-specific maximum carbon storage value, and C_{measured} is measured carbon concentration in the MAOC fraction (mg C g^{-1} soil).

2.10 Data Validation and Quality Control

Assessment of data integrity followed a systematic three-phase protocol. Initially, all measurements underwent screening for instrument-specific artifacts according to established validation parameters for each analytical device. Subsequently, mass recovery criteria retained only data exhibiting recovery rates within $\pm 10\%$ of initial mass values, particularly relevant for density fractionation and aggregate stability determinations.

In the final phase, anomaly detection utilized an adjusted boxplot methodology calibrated for log-normal distribution characteristics of measured variables. Data points falling beyond 1.5 × interquartile range (IQR) thresholds were flagged for individual evaluation, with elimination from the final dataset only when definitive instrumental malfunctions or procedural inconsistencies could be identified (Yang et al., 2019).

2.11 Statistical Analysis

Statistical analysis protocols were designed to accommodate the diverse data types and distributions encountered. Prior to analysis, all datasets were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test in R using the car package.

When both normality and homogeneity of variance assumptions were satisfied, analysis of variance (ANOVA) was employed to compare treatments, depths, and their interactions. For variables where normality and homogeneity assumptions were not met, Welch's t-tests were applied for comparisons between two groups, while Mann-Whitney U tests were used for non-parametric comparisons when distributions were severely non-normal.

Statistical significance was denoted at levels of $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). All statistical analyses were performed in R version 4.2.0 (R Core Team, 2022). The analysis code and complete dataset are available in the public repository listed in the Data Availability statement.

3 Results

Extended application of conservation agriculture methods resulted in substantial alterations to soil physical characteristics, carbon and nitrogen content, and carbon protection mechanisms. These changes were most pronounced in the uppermost soil layer (0-5 cm), with notable trends extending to deeper depths (5-10 cm).

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3.1 Aggregate Stability Response to Conservation Management

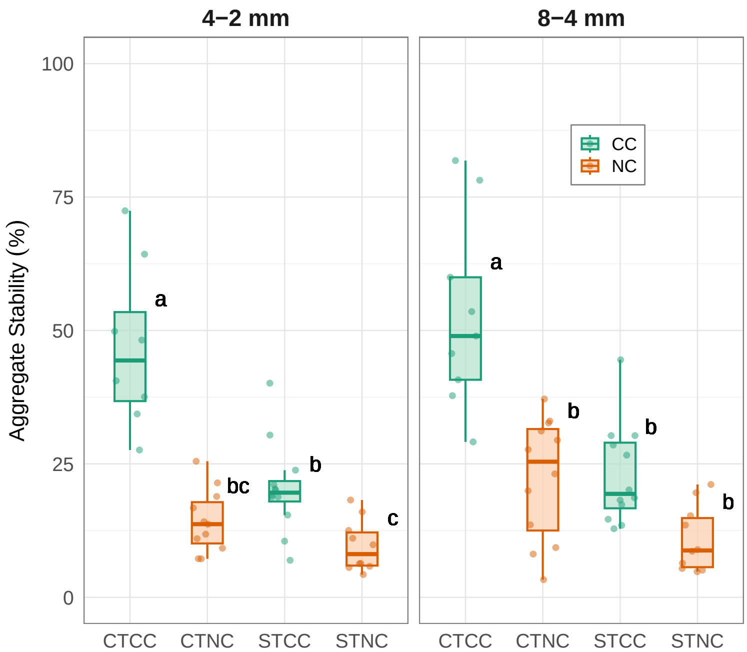

Figure 1. Water stable aggregates in systems with a combination of no-till (CT) or standard tilling (ST) and fallowing (NC) or cover cropping (CC) management at 0-10 cm depths for aggregate size of 2-4 mm and 4-8mm (n=7). The treatments consists of CTCC, CTNC, STCC, STNC. CTCC is significantly more stable than all other treatments in both aggregate class sizes. In the aggregates 2-4 mm, the addition of CC leads to greater stability in systems with ST. Error bars represent standard error of the mean. Statistical significance between treatments with-in size classes is indicated by different lower case letters (Tukey HSD t-test).

Conservation management (CTCC) significantly enhanced wet aggregate stability compared to all other treatments across both analyzed size fractions (**Fig.** 1). In the 4-2 mm aggregate fraction, CTCC exhibited 136% greater stability ($46.85 \pm 15.22\%$) than STNC ($9.60 \pm 4.79\%$, $p = 9.21 \times 10^{-10}$). Similarly, in the 8-4 mm size fraction, CTCC maintained $52.86 \pm 17.79\%$ stability compared to STNC at $10.88 \pm 6.12\%$ ($p = 8.12 \times 10^{-09}$).

Standard tillage with cover crops (STCC) showed significantly higher stability than STNC in the 4-2 mm aggregate fraction, with a 113% increase ($20.45 \pm 8.59\%$ vs. $9.60 \pm 4.79\%$, $p = 0.037$). Reduced tillage without cover crops (CTNC) demonstrated intermediate stability values, ranging from 9.60% to 22.96% across size fractions. STNC consistently exhibited the lowest aggregate stability values across all measured size classes.

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3.2 Soil Physical Properties Under Conservation Management

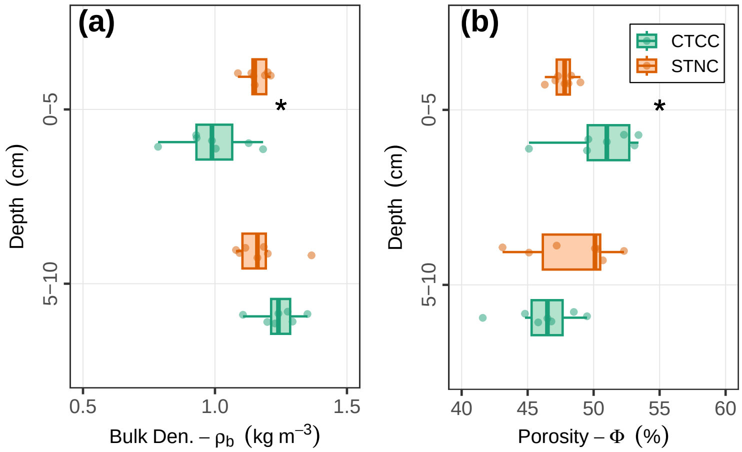

Figure 2. Physical properties in soils under conservation (CTCC, green, n=7) and standard (STNC, orange, n=7) management, measured on undisturbed cores from 0-5 and 5-10 cm depths. **(a)** Bulk density (mean ± SE) and **(b)** total porosity estimated from fitted water retention curves, calculated using **Eq. 3 and ??**. CTCC showed improved physical properties of both bulk density and porosity with statistical significance only in the 0-5 cm depth. Error bars represent standard error of the mean. Statistical significance between treatments is indicated by symbols: $***$ for $p < 0.001$, $**$ for $p < 0.01$, $*$ for $p < 0.05$, and $\circ$ for $p < 0.1$ (Welch's t-test).

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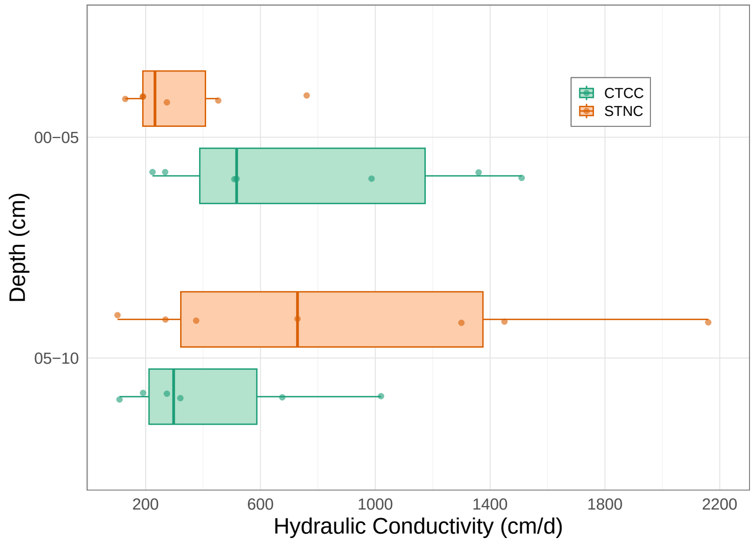

Figure 3. Hydraulic conductivity under conservation (CTCC, green, n=7) and standard (STNC, orange, n=7) management at 0-5 and 5-10 cm depths. Measurements were conducted on the same undisturbed cores used for physical property characterization. Hydraulic conductivity did not statistically differ between treatments in either depths. Error bars represent standard error of the mean. Statistical significance between treatments is indicated by symbols: $***$ for $p < 0.001$, $**$ for $p < 0.01$, $*$ for $p < 0.05$, and $\circ$ for $p < 0.1$ (Mann-Whitney U test).

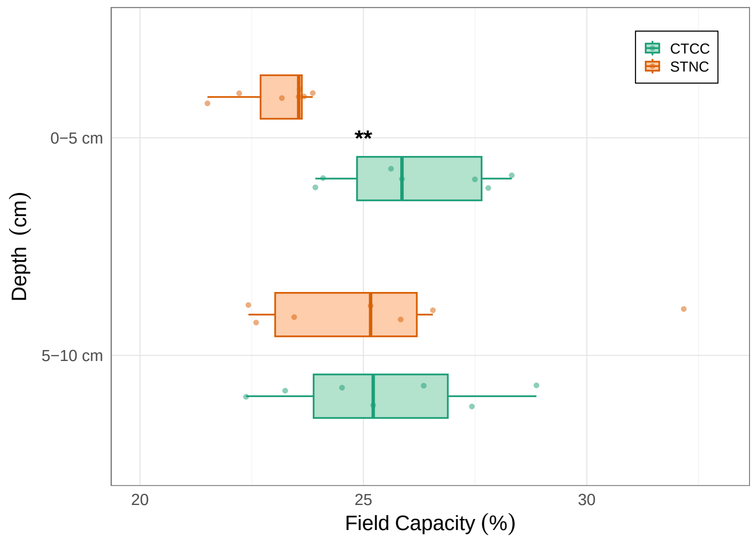

Figure 4. Field Capacity for conservation (CTCC, green, n=7) and standard (STNC, orange, n=7) management at 0-5 and 5-10 cm depths. Measurements were conducted on the same undisturbed cores used for physical property characterization. Like other physical properties, field capacity is significantly greater in CTCC in the 0-5 cm. Error bars represent standard error of the mean. Statistical significance between treatments is indicated by symbols: $***$ for $p < 0.001$, $**$ for $p < 0.01$, $*$ for $p < 0.05$, and $\circ$ for $p < 0.1$ (Wilcox Test).

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Physical property measurements on intact soil cores revealed significant differences between reduced tillage with cover crops (CTCC) and standard tillage without cover crops (STNC), with effects concentrated in the surface horizon.

Bulk Density: Bulk density values under CTCC were significantly lower in the 0-5 cm layer (0.99 ± 0.13 g cm^{-3}) compared to STNC (1.16 ± 0.04 g cm^{-3}, $p = 0.014$), representing a 15% reduction (**Fig.** 2). Total porosity under CTCC increased by 6% in the 0-5 cm layer ($50.57 \pm 2.87\%$) relative to STNC ($47.70 \pm 0.88\%$, $p = 0.030$). No significant differences in bulk density or porosity were detected in the 5-10 cm layer ($p = 0.162$ and $p = 0.198$ respectively).

Hydraulic Properties: Saturated hydraulic conductivity in the 0-5 cm layer was higher under CTCC (767.86 ± 520.42 cm d^{-1}, $p = 0.074$) compared to STNC (333.00 ± 238.03 cm d^{-1}) (**Fig.** 3). This pattern reversed in the 5-10 cm layer, where CTCC exhibited lower conductivity (431.83 ± 347.84 cm d^{-1}) relative to STNC (912.29 ± 751.42 cm d^{-1}, $p = 0.284$). High variability in measurements resulted in non-significant differences for both depth increments.

Volumetric water content at field capacity increased significantly under CTCC in the 0-5 cm layer ($26.16 \pm 1.77\%$) relative to STNC ($23.08 \pm 0.88\%$, $p = 0.003$), representing a 13.3% enhancement (**Fig.** 4). The 5-10 cm layer showed an inverse pattern with higher values under STNC, though differences were not statistically significant ($p = 0.987$).

3.3 Carbon and Nitrogen Stocks and Composition

Carbon and nitrogen accumulation patterns varied substantially between management systems and soil depths, with pronounced effects concentrated in the surface horizon.

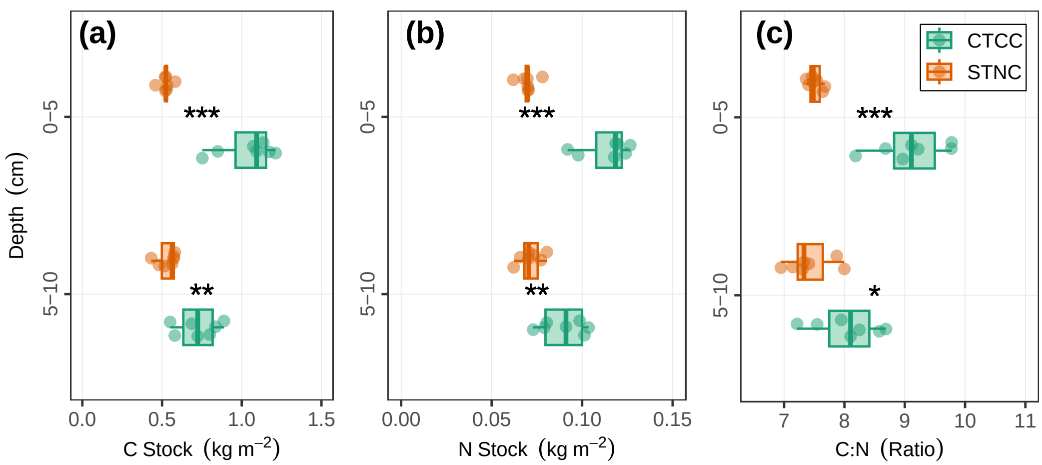

Figure 5. Carbon and nitrogen stocks under conservation (CTCC, green, n=7) and standard (STNC, orange, n=7) management at 0-5 and 5-10 cm depths. Measurements were conducted on the same undisturbed cores used for physical property characterization. **(a)** Total carbon stocks, **(b)** total nitrogen stocks (both determined via elemental combustion), and **(c)** C:N ratios. CTCC significantly improved soil chemical properties, with greater effect in the 0-5 cm. Error bars represent standard error of the mean. Statistical significance between treatments is indicated by symbols: $***$ for $p < 0.001$, $**$ for $p < 0.01$, $*$ for $p < 0.05$, and $\circ$ for $p < 0.1$ (Welch's t-test).

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Carbon Stocks: Total carbon stocks in the 0-5 cm layer under CTCC (1.04 ± 0.17 kg m^{-2}, $p = 0.0001$) were approximately double those measured under STNC (0.52 ± 0.03 kg m^{-2}) (**Fig.** 5). In the 5-10 cm layer, CTCC (0.72 ± 0.13 kg m^{-2}) increased carbon stocks by 35% compared to STNC (0.531 ± 0.06 kg m^{-2}, $p = 0.006$).

Nitrogen Stocks: Nitrogen stocks in the 0-5 cm layer increased by 83% under CTCC (0.11 ± 0.01 kg m^{-2}) relative to STNC (0.06 ± 0.004 kg m^{-2}, $p = 0.00005$) (**Fig.** 5). The 5-10 cm layer showed a 14% increase in nitrogen stocks under CTCC (0.090 ± 0.012 kg m^{-2})(0.071 ± 0.01 kg m^{-2}, $p = 0.006$).

Carbon to Nitrogen Ratios: C:N ratios in the 0-5 cm layer were significantly higher under CTCC (9.1 ± 0.57) than under STNC (7.5 ± 0.11, $p = 0.0002$)(**Fig.** 5). The 5-10 cm layer maintained elevated C:N ratios under CTCC (8.0 ± 0.53) compared to STNC (7.4 ± 0.37, $p = 0.029$).

Bulk Soil Analysis: Analysis of bulk soil samples (0-10 cm depth) showed no statistically significant differences between CTCC and STNC for carbon concentration ($1.63 \pm 0.468\%$ vs. $1.04 \pm 0.295\%$, $p = 0.23$), nitrogen concentration ($0.18 \pm 0.043\%$ vs. $0.12 \pm 0.026\%$, $p = 0.16$), or C:N ratios (9.19 ± 0.461 vs. 8.67 ± 0.907, $p = 0.80$) (**Fig.** 11).

3.4 Organic Matter Composition Analysis

Fourier-transform infrared spectroscopy analysis of surface soil samples (0-5 cm) revealed compositional differences in soil organic matter between management systems.

Simple plant-derived organic matter increased by 8.2% under CTCC ($26.78\% \pm 2.17$) compared to STNC ($24.74\% \pm 1.93$, $p = 0.087$), though this difference was not statistically significant (**Fig.** 6). Microbially-associated organic matter decreased significantly under CTCC ($48.22\% \pm 0.58$) relative to STNC ($50.41\% \pm 0.72$, $p = 0.00004$). Complex plant-derived organic matter showed similar proportions between CTCC and STNC (25.0 ± 1.67 vs $25.0\% \pm 2.08$, $p = 0.848$).

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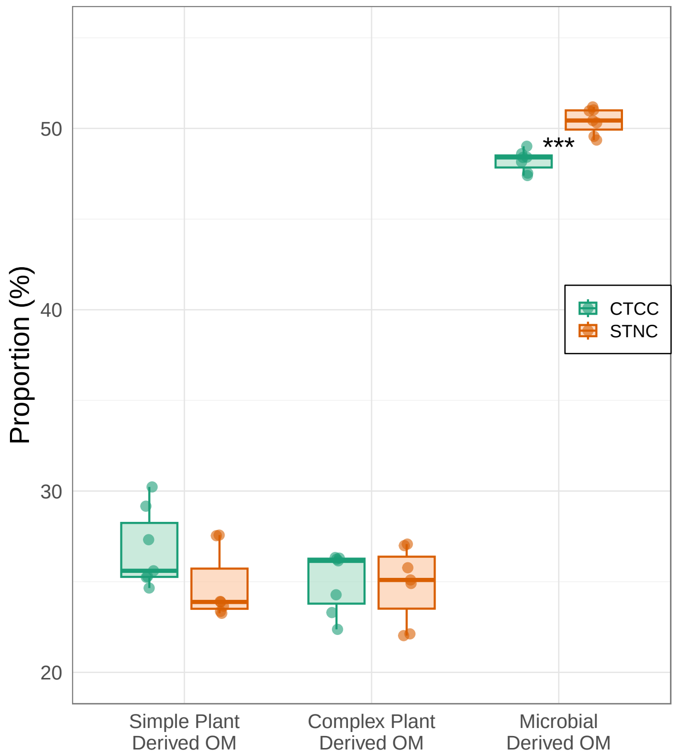

Figure 6. Proportions of SOM composition under conservation (CTCC, green, n=7) and standard (STNC, orange, n=7) management at 0-5 cm depth. Measurements were conducted on the same undisturbed cores used for physical property characterization. Simple Plant Derived OM, Complex Plant Derived OM, and Microbially Derived OM proportions we calculated via integration under their corresponding spectral peaks. The application of no-till and cover cropping yielded a statistically lower proportion of microbial derived OM, suggesting less mineralized OM. Error bars represent standard error of the mean. Statistical significance between treatments is indicated by symbols: $***$ for $p < 0.001$, $**$ for $p < 0.01$, $*$ for $p < 0.05$, and $\circ$ for $p < 0.1$ (Welch's t-test).

3.5 Carbon Protection Mechanisms

Density fractionation analysis of bulk soil samples (0-10 cm depth) revealed distinct patterns of carbon and nitrogen distribution among functionally different soil organic matter pools.

3.5.1 Carbon Distribution Among Density Fractions

The amount of free light fraction (FLF) carbon content showed no significant difference between CTCC ($0.26 \pm 0.204\%$) and STNC ($0.18 \pm 0.115\%$, $p = 0.94$) (**Fig.** 7). Occluded light fraction (OLF) carbon was substantially higher under CTCC ($0.64 \pm 0.175\%$) compared to STNC ($0.30 \pm 0.059\%$, $p = 0.03$). Heavy fraction (HV) carbon under CTCC ($0.56 \pm 0.113\%$) exceeded STNC values ($0.37 \pm 0.052\%$, $p = 0.05$).

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3.5.2 Nitrogen Distribution Among Density Fractions

FLF nitrogen content was $0.019 \pm 0.014\%$ under CTCC and $0.012 \pm 0.008\%$ ($p = 0.92$) under STNC. OLF nitrogen showed a
385 pronounced difference, with CTCC containing $0.051 \pm 0.015\%$ compared to STNC at $0.023 \pm 0.005\%$ ($p = 0.03$). HV nitrogen was highest in both treatments, measuring $0.09 \pm 0.014\%$ under CTCC and $0.07 \pm 0.005\%$ ($p = 0.115$) under STNC.

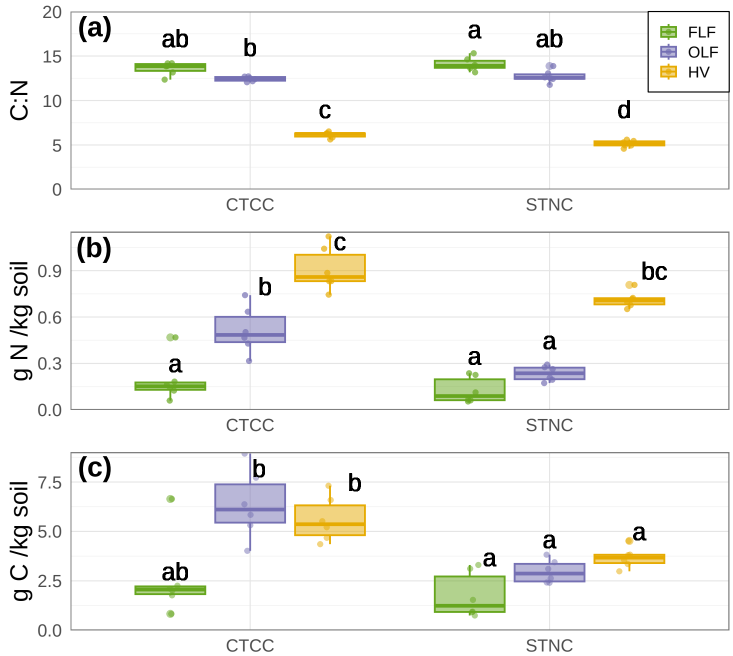

Figure 7. Distinct carbon and nitrogen pools in systems with treatments of CTCC and STNC at 0-10 cm depths (n=6). The 3 pools are free-light (green), occluded (purple), and heavy fraction (yellow). CTCC led to an increase in the quantity of C and N held in the occluded fraction and had slightly less mineralization of SOM in the heavy fraction. The Error bars represent standard error of the mean. Statistical significance within and between treatments is indicated by different lower case letters (Games-Howell Post-HOC).

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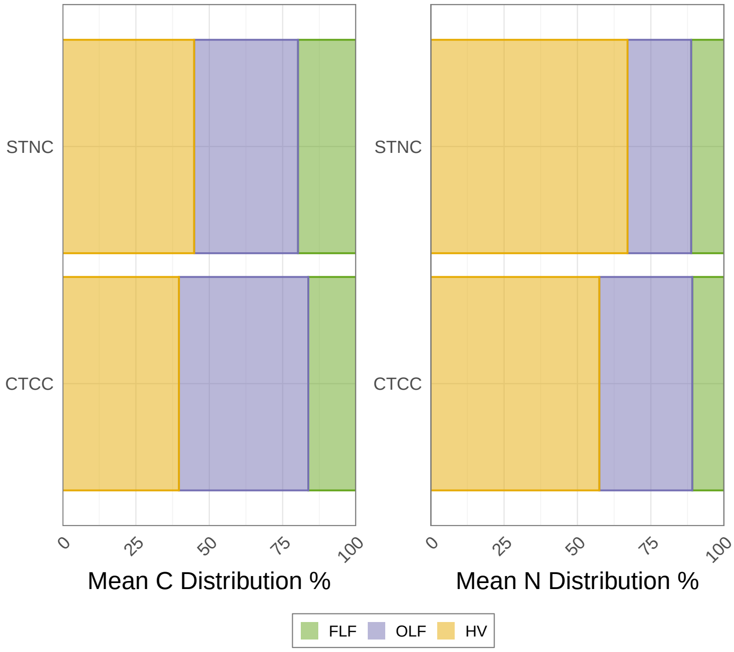

Figure 8. Distribution of carbon and nitrogen within functional SOM pools in systems with treatments of CTCC and STNC at 0-10 cm depths (n=6). The 3 pools are free-light (green), occluded (purple), and heavy fraction (yellow). CTCC increased in the quantity of C and N held in the occluded fraction.

3.5.3 Relative Carbon and Nitrogen Distribution

When expressed as proportions of total recovered carbon, CTCC showed the greatest percentage in the OLF ($44.07 \pm 3.93\%$), followed by HV ($39.67 \pm 5.85\%$) and FLF ($16.25 \pm 6.95\%$). In CTCC, FLF was significantly lower than OLF and HV ($p = 0.0003$ and $p = 0.001$)(**Fig.** 8). STNC exhibited the highest proportion in the HV ($44.85 \pm 9.23\%$), followed by OLF ($35.37 \pm 2.17\%$) and FLF ($19.78 \pm 10.32\%$). There was only statistical differences between FLF and HV ($p = 0.01$) for STNC. Between treatments, CTCC had significantly higher OLF proportions ($p = 0.01$).

For nitrogen distribution relative to total recovered nitrogen, CTCC showed $57.47 \pm 6.75\%$ in HV, $31.66 \pm 3.54\%$ in OLF, and $10.87 \pm 5.14\%$ in FLF. All fractions in CTCC were significantly different ($p < 0.0004$). STNC demonstrated $67.06 \pm 7.82\%$ in HV, $21.70 \pm 1.96\%$ in OLF, and $11.24 \pm 6.57\%$ in FLF. For fractions in STNC, HV was significantly higher than the rest ($p < 0.0001$). Between treatments, CTCC had significantly higher OLF proportions ($p = 0.003$).

3.5.4 Carbon to Nitrogen Ratios in Density Fractions

C:N ratios in FLF and OLF fractions ranged from 12 to 14 for both treatments, with no significant differences between management systems ($p < 0.07$) (**Fig.** 8). The HV fraction showed significantly higher C:N ratios under CTCC (6.14 ± 0.33) compared to STNC (5.14 ± 0.38, $p = 0.007$).

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3.6 Mineral-Associated Organic Carbon Saturation

Mineral-associated organic carbon saturation analysis revealed significant differences between management practices. CTCC exhibited substantially higher carbon saturation levels (11.46 ± 2.60%) compared to STNC (7.38 ± 0.82%), representing a 55% increase ($p = 0.004$) (**Fig.** 9). These saturation levels were calculated relative to the theoretical maximum carbon storage capacity of 86 ± 9 mg C g^{-1} mineral for high-activity minerals (Georgiou et al., 2022). Both treatments remained well below this theoretical maximum, indicating substantial remaining potential for carbon sequestration even after two decades of management.

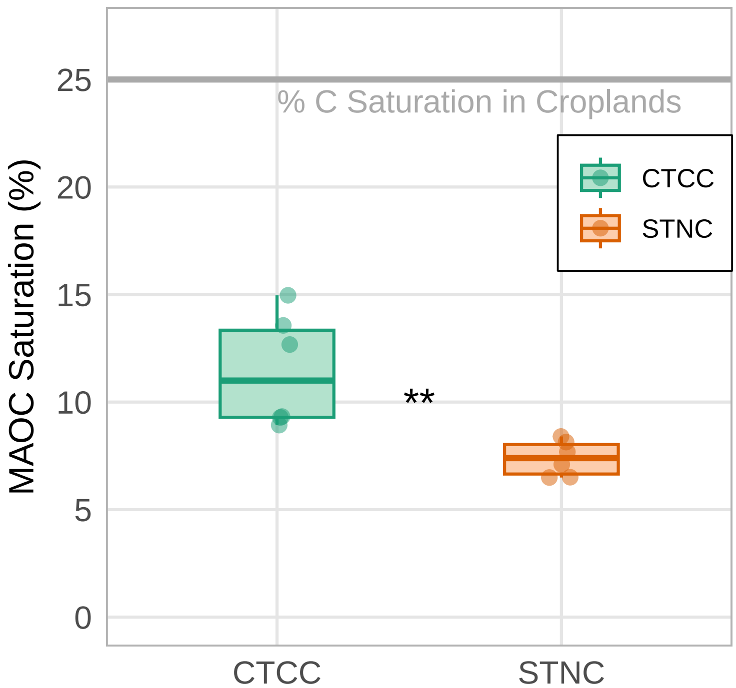

Figure 9. Carbon saturation of the mineral fraction under conservation (CTCC, green, n=6) and standard (STNC, orange, n=7) management at 0-10 cm depth. CTCC leads to higher C saturation in the mineral fraction, while both management types are well below their theoretical capacity. Error bars represent standard error of the mean. Statistical significance between treatments is indicated by symbols: $***$ for $p < 0.001$, $**$ for $p < 0.01$, $*$ for $p < 0.05$, and $\circ$ for $p < 0.1$ (Welch's t-test).

4 Discussion

Our findings demonstrate that two decades of conservation agriculture fundamentally alters soil functioning in Mediterranean agricultural systems, with changes extending beyond simple carbon accumulation to represent a reorganization of soil physical

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structure and carbon protection mechanisms. The magnitude and consistency of improvements across multiple soil health indicators demonstrate that conservation agriculture creates stable, self-reinforcing soil systems.

4.1 Aggregate Stability as the Foundation of Soil System Transformation

The dramatic improvement in aggregate stability under reduced tillage with cover crops (CTCC)—exhibiting 136% greater stability than standard tillage without cover crops (STNC)—represents the cornerstone of soil system transformation and aligns with global patterns from long-term conservation studies. Global meta-analyses consistently demonstrate enhanced aggregate stability as a characteristic response to conservation agriculture, with improvements driven by reduced soil disturbance and enhanced organic matter inputs (Liu et al., 2021). The pronounced differences in structural properties between treatments reflect underlying changes in soil biological activity and organic matter dynamics that serve as the foundation for understanding all subsequent improvements.

The substantial improvement in aggregate stability can be attributed to synergistic effects of increased carbon inputs from cover crop residues and reduced physical disturbance through no-till practices (Six et al., 2004; Bronick and Lal, 2005). Our findings reveal that the mechanisms driving these improvements extend beyond simple additive effects. Modern understanding of aggregate formation emphasizes the critical role of fungal networks in creating stable soil structure, with mycorrhizal associations showing enhanced development under integrated conservation management (Tian et al., 2024; Schmidt et al., 2019). The enhanced stability observed under CTCC likely reflects development of complex fungal-bacterial networks that create robust binding agents while simultaneously providing pathways for enhanced carbon protection within aggregate structures.

Our observation that standard tillage with cover crops (STCC) exhibited greater aggregate stability than reduced tillage without cover crops (CTNC) provides important insights into the relative importance of biological versus physical drivers of soil structure. This pattern aligns with global analyses showing that biological carbon inputs consistently outweigh tillage effects in determining long-term structural improvements (Liu et al., 2025). This finding supports the hypothesis that biological processes associated with continuous root activity and residue inputs are primary drivers of aggregate formation and stabilization (Liu et al., 2005; Tisdall and Oades, 1982).

The improved aggregate stability under CTCC creates the physical foundation for all subsequent improvements in soil functioning. Stable aggregates provide protected micro-sites for organic matter accumulation, create favorable conditions for microbial activity, and establish the pore network architecture governing water movement and retention.

4.2 Carbon Protection Mechanism Evolution: From Chemical to Physical Protection

Our density fractionation analysis reveals a fundamental reorganization of carbon protection strategies. The substantial enrichment of the occluded light fraction under CTCC (44.1% vs. 35.4% of total recovered carbon in STNC) demonstrates clear improvement in aggregate-mediated physical protection, confirming theoretical predictions about conservation agriculture's long-term impacts on soil carbon cycling.

This mechanism shift aligns with recent global analyses revealing predictable temporal patterns in carbon protection under conservation management. Global cropland analysis from 2000-2022 showed that particulate organic carbon increased 21.8%

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while MAOC remained stable with a 5.3% decrease across agricultural systems, with North American croplands implementing conservation policies for more than 20 years showing increases in both POC and MAOC (Liu et al., 2025). Our Mediterranean site results provide field-scale validation of these global trends, demonstrating that physically and chemically-protected carbon are the dominant protection mechanisms when conservation practices are sustained over multi-decade periods.

The temporal evolution of carbon protection mechanisms follows predictable stages that our 20-year study captures at equilibrium. Recent research demonstrates that conservation management creates distinct phases: initial rapid MAOC formation during years 0-5, continued MAOC accumulation with enhanced microbial processing during years 5-15, and a mature phase beyond 15 years where MAOC approaches saturation limits and physical protection becomes the dominant pathway for continued carbon sequestration (Tian et al., 2024). Our findings provide rare empirical evidence of this mature phase, where the soil system has reorganized to rely primarily on aggregate-mediated protection for continued carbon storage.

The shift toward physical protection has profound implications for carbon persistence. While MAOC is often considered more stable due to strong organo-mineral bonds, physically-protected carbon within stable aggregates can achieve similar longevity through reduced exposure to decomposer organisms and limited oxygen availability (Six et al., 2002). Modern understanding emphasizes that protection mechanism diversity enhances overall carbon stability, with aggregate-mediated protection providing resilience against environmental perturbations that might destabilize the MAOC pool (Lavallee et al., 2020).

Critically, FLF carbon content did not differ significantly between treatments despite substantial additional organic matter inputs under CTCC. This observation suggests rapid integration of fresh residues into more stable soil fractions under conservation management, indicating enhanced biological processing efficiency that quickly incorporates new inputs into protected pools (Mitchell et al., 2018). This rapid processing aligns with evidence that conservation agriculture enhances microbial carbon use efficiency over time (Tian et al., 2024).

The MAOC saturation analysis provides crucial context for future sequestration potential. Our finding that CTCC exhibited 55% higher carbon saturation (11.46%) compared to STNC (7.38%), while remaining well below theoretical maximum capacity, demonstrates that conservation management enhances the efficiency of mineral-association processes while maintaining substantial capacity for additional carbon accumulation (Georgiou et al., 2022). Mediterranean agricultural soils typically show lower MAOC saturation compared to other climatic regions due to high clay activity and seasonal moisture dynamics, suggesting that our observed values represent realistic targets for regional conservation efforts (Caruso et al., 2018).

4.3 Physical Property Transformation and Hydrological Implications

The comprehensive improvements in soil physical properties under CTCC—including 15% lower bulk density, 6% greater total porosity, and 13% enhanced water retention at field capacity—demonstrate practical benefits for agricultural sustainability. These changes reflect development of a more complex pore network that enhances both water storage and movement, addressing critical needs in Mediterranean systems where water availability often constrains productivity.

The magnitude of these improvements aligns with global patterns from mature conservation systems, where water retention improvements of 10-15% are typical in Mediterranean climates after 15-20 years (Caruso et al., 2018). Enhanced water reten-

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tion represents a valuable adaptation to increasing climate variability, potentially improving system resilience to drought while reducing irrigation requirements (Gomes et al., 2023; Araya et al., 2022).

Improved pore structure under CTCC reflects integration of biological and physical processes that create distinct functional zones within the soil profile. Recent research emphasizes that conservation agriculture creates hierarchical pore networks, where larger inter-aggregate pores facilitate rapid water infiltration during precipitation events, while smaller intra-aggregate pores retain water under stress conditions (Tian et al., 2024). This dual-porosity system provides both drainage during wet periods and water retention during dry periods, optimizing water availability throughout the growing season.

The pronounced vertical stratification of physical property improvements—with enhanced properties concentrated in the surface layer—reflects the surface-focused nature of conservation practices while creating distinct functional zones. This stratification is characteristic of mature conservation systems and represents efficient organization of soil functions, with the surface layer optimized for rapid infiltration and carbon storage, while deeper layers maintain structural integrity for root support (Bienes et al., 2021). The concentration of improvements in the surface horizon represents development of a functionally stratified soil system that optimizes resource use efficiency.

Hydraulic conductivity showed high variability that limited statistical significance, reflecting inherent spatial heterogeneity. However, directional trends align with expectations based on porosity and aggregate stability improvements. Advanced analytical approaches recognize that hydraulic properties in conservation systems exhibit complex relationships with pore network geometry that cannot be predicted from bulk density measurements alone (Poeplau et al., 2018). Future research employing high-resolution imaging techniques could provide valuable insights into pore network architecture governing water movement in these improved systems.

4.4 Carbon and Nitrogen Dynamics: Equilibrium Achievement and Quality Transformation

The substantial carbon accumulation under CTCC—with surface soil stocks approximately double those in STNC—demonstrates significant carbon sequestration potential while revealing important insights about retention efficiency and transformation processes. The 19% retention efficiency of total added cover crop carbon indicates substantial processing and transformation of organic inputs, with the majority being either mineralized or transported to deeper soil layers not captured in our sampling design.

Our carbon accumulation patterns align with evidence that conservation agriculture systems reach dynamic equilibrium after 15-20 years, where carbon gains from enhanced protection mechanisms balance losses from continued decomposition (Caruso et al., 2018). Achievement of this equilibrium state after two decades suggests that observed carbon stocks represent sustainable capacity under current management regimes, providing valuable targets for regional conservation planning.

The quality and composition of soil organic matter exhibited distinct differences that provide insights into transformation processes. Wider C:N ratios under CTCC (9.1 vs 7.5 in the surface layer) combined with FTIR evidence of reduced microbial-derived compounds indicate that conservation management preserves more plant-derived characteristics in the soil organic matter pool. This compositional shift challenges traditional assumptions about carbon stability, with recent evidence suggesting

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that physically-protected organic matter with relatively wide C:N ratios can achieve long-term persistence when embedded within stable aggregate structures (Kleber et al., 2011).

Nitrogen cycling patterns closely paralleled carbon dynamics but revealed distinct fractionation patterns. The disproportionate increase in OLF nitrogen under CTCC (150% increase compared to STNC) versus the more modest increase in HV nitrogen (29% increase) suggests fundamental modifications of nitrogen availability patterns (Jilling et al., 2020). This redistribution from predominantly chemically-protected to physically-protected forms may improve nitrogen synchrony with crop demand while reducing leaching losses, representing a key ecosystem service improvement (Tian et al., 2024).

The critical finding that significant treatment effects were detected in stratified surface sampling (0-5 cm) but not in bulk sampling (0-10 cm) emphasizes the importance of appropriate sampling strategies for detecting conservation agriculture impacts. Surface-focused sampling is essential for detecting management effects in conservation systems, where carbon accumulation is concentrated in the surface horizon due to reduced mixing and surface-applied organic inputs (Poeplau et al., 2018). This insight has important implications for monitoring and verification protocols in carbon sequestration programs.

The concentration of carbon accumulation in the surface horizon raises questions about potential carbon redistribution versus net profile accumulation. However, previous comprehensive sampling at this site by Araya et al. (2022) demonstrated that management effects were confined to the upper soil profile, with no significant differences detected below 10 cm depth. This pattern is consistent with conservation agriculture systems where carbon inputs from surface-applied cover crop residues and reduced mixing create distinct vertical stratification without depleting deeper soil carbon stocks (Baker et al., 2005). The absence of deeper carbon depletion distinguishes these mature conservation systems from recently converted no-till systems that may show temporary redistribution effects during transition periods (Blanco-Canqui and Ruis, 2018).

4.5 Mediterranean System Responses and Global Implications

Our findings from California's Central Valley provide important validation that conservation agriculture principles are effective across diverse climatic conditions, with Mediterranean systems showing particularly strong responses to integrated conservation practices. The Mediterranean climate, characterized by pronounced seasonal moisture patterns and high potential evapotranspiration, creates conditions that enhance conservation agriculture benefits through intensified aggregate formation during wet-dry cycles and increased water use efficiency.

The improvements in our Mediterranean system suggest that these environments may be particularly responsive to conservation management due to interactions between climatic constraints and management practices. Global analyses indicate that Mediterranean agricultural regions show some of the highest potential returns on conservation agriculture investments, with water limitation creating strong incentives for practices that enhance water retention and use efficiency (Liu et al., 2025). The
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The depth stratification patterns we observed—with pronounced improvements in surface layers and more modest changes at depth—reflect the unique challenges and opportunities of Mediterranean agricultural systems. Surface-concentrated benefits align with the seasonal nature of Mediterranean precipitation, where enhanced infiltration and surface water storage provide

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maximum value during the critical spring growing period (Tian et al., 2024). This stratification represents efficient adaptation to Mediterranean climatic constraints rather than management limitations.

The magnitude of aggregate stability improvements (136%) observed in our Mediterranean system exceeds many temperate region studies, suggesting that the combination of high clay content soils and pronounced wet-dry cycles creates particularly favorable conditions for aggregate development under conservation management. Global comparisons indicate that Mediterranean systems consistently show enhanced responsiveness to conservation practices, likely due to interaction between clay mineralogy, climate patterns, and biological activity cycles (Caruso et al., 2018).

4.6 Methodological Insights and Future Research Directions

Our integration of density fractionation, spectroscopic analysis, and comprehensive physical characterization provides a methodological framework that successfully captured multidimensional impacts of conservation agriculture on soil functioning. The combination of these approaches enabled detection of mechanism shifts that would have been missed by traditional total carbon measurements alone, demonstrating the value of mechanistic analytical approaches (Poeplau et al., 2016).

The success of our density fractionation approach in detecting carbon protection mechanism shifts validates recent methodological advances in soil carbon research. Standardized protocols for density fractionation enable consistent comparisons across studies, with our results contributing to a growing global database of carbon fraction responses to management (Lavallee et al., 2020). FTIR spectroscopy integration provided valuable insights into organic matter quality that complemented quantitative measurements, revealing compositional changes that explain observed differences in carbon cycling dynamics.

Limitations and Research Needs.

Several limitations suggest important directions for future research. The concentration of our sampling in the upper 10 cm, while appropriate for detecting management effects, limits understanding of whole-profile carbon dynamics. Recent research emphasizes the importance of deep soil carbon responses to conservation management, with evidence that carbon transport to subsoil layers may represent an important sequestration pathway not captured by surface-focused sampling (Liu et al., 2025).

The high spatial variability in hydraulic conductivity measurements highlights ongoing challenges in characterizing soil physical properties that exhibit extreme heterogeneity. Advanced approaches including X-ray computed tomography offer promising opportunities to characterize pore network architecture governing water movement in conservation systems (Poeplau et al., 2018).

Our focus on physicochemical properties, while comprehensive, represents only part of the soil health equation. Integration of microbial community analysis and enzymatic activity measurements would provide crucial insights into biological mechanisms driving the physical and chemical changes observed (Tian et al., 2024). Understanding these biological drivers would enhance predictive capacity and guide optimization of conservation practice implementation.

4.7 Implications for Sustainable Agriculture and Carbon Sequestration

The comprehensive improvements in soil health indicators demonstrated in our 20-year study provide strong evidence for conservation agriculture's effectiveness in enhancing agricultural sustainability while contributing to climate change mitigation.

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Achievement of dynamic equilibrium after two decades indicates that these benefits represent stable, long-term improvements rather than temporary responses to management changes (Caruso et al., 2018).

The shift from chemically-protected to physically-protected carbon dominance has important implications for carbon market protocols, as physically-protected carbon may respond differently to environmental perturbations compared to chemically-protected pools (Lavallee et al., 2020). Understanding these protection mechanism differences is crucial for developing accurate carbon accounting approaches and assessing permanence requirements for carbon credit programs.

The practical implementation of conservation agriculture based on our findings suggests several key principles for optimizing benefits. The synergistic effects of combining no-till management with cover cropping emphasize the importance of integrated practice adoption rather than isolated implementation (Tian et al., 2024). The substantial benefits observed even under conventional tillage with cover cropping provide encouragement for stepwise adoption approaches, while the superior performance of fully integrated systems demonstrates the value of comprehensive conservation implementation.

5 Conclusions

This 20-year study demonstrates that conservation agriculture achieves fundamental transformation of soil functioning in Mediterranean agricultural systems, reaching dynamic equilibrium characterized by enhanced aggregate stability (136% improvement), shifted carbon protection mechanisms (44% vs 35% in occluded fractions), and improved water retention (13% increase in field capacity). The achievement of stable equilibrium after two decades validates conservation agriculture as a scientifically sound approach for enhancing multiple soil functions while maintaining substantial carbon sequestration potential.

5.1 Key Scientific Insights

The increase in physically-protected carbon represents fundamental reorganization of soil carbon cycling, with aggregate-mediated protection becoming a dominant pathway for long-term carbon storage under mature conservation systems. Despite 20 years of enhanced management, mineral-associated carbon saturation remains well below theoretical capacity (11.5% vs 7.4% in standard systems), indicating continued potential for carbon sequestration. The concentration of benefits in surface soil layers reflects efficient functional stratification optimized for Mediterranean climatic conditions rather than management limitations.

5.2 Implementation and Policy Implications

Our findings support policy frameworks requiring long-term commitments to conservation agriculture, as fundamental soil transformations require sustained implementation over multi-decade periods. The superior performance of integrated conservation systems (no-till plus cover crops) emphasizes the importance of comprehensive practice adoption rather than incremental implementation. The substantial ecosystem service improvements documented—including carbon sequestration, enhanced water use efficiency, and improved soil structural integrity—provide strong justification for economic incentives supporting conservation agriculture adoption.

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The responsiveness of Mediterranean systems to conservation management suggests particularly high returns on conservation investments in water-limited agricultural regions. Monitoring protocols should focus on surface soil layers where management effects are most pronounced, while recognizing that continued carbon sequestration increasingly relies on aggregate stability enhancement rather than chemical protection. Our results provide essential evidence for carbon sequestration programs requiring permanence guarantees and demonstrate that conservation agriculture represents a viable strategy for addressing interconnected challenges of food security, climate change mitigation, and environmental sustainability.

Code and data availability. following acceptance of the final manuscript version, we will make available the raw data, processed datasets, and analytical code used to generate all figures presented in both the main text and supplementary materials through https://datadryad.org. Reviewers may currently access these materials via the following link: https://drive.google.com/drive/folders/1RC3dr7TVWH$_a 5fRAgP2w1HPwi-FqwqYg$

Author contributions. JA contributed to study conceptualization, investigation, formal analysis, and original draft preparation. AAB performed formal data analysis and participated in manuscript review and editing. SSC performed formal data analysis and participated in manuscript review and editing. JM contributed methodology development and formal data analysis. TAG participated in conceptualization, formal data analysis, and both original draft preparation and manuscript review. All contributing authors have provided consent for submission of this manuscript in its current form.

Competing interests. The authors report no competing interests or conflicts of interest.

Disclaimer. We employed Claude AI (Anthropic, INc.) during manuscript preparation to enhance grammar, improve flow, and ensure clarity of expression. All AI-generated suggestions were carefully reviewed and modified as appropriate, with the authors maintaining complete responsibility for the final content.

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6 Appendix Figures

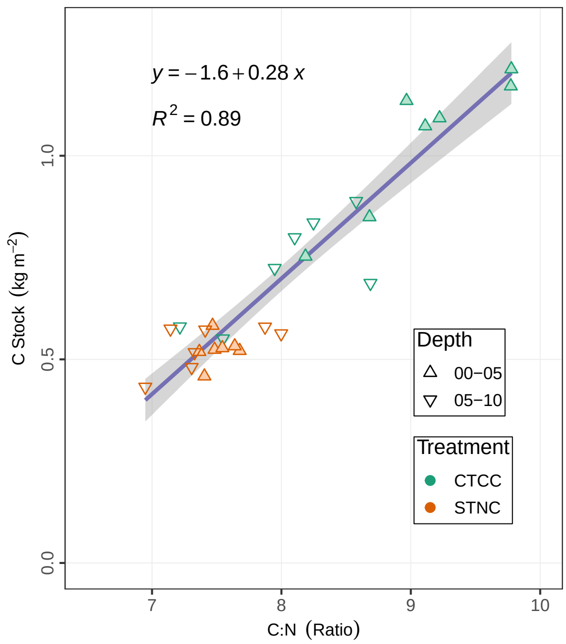

Figure 10. Relationship between carbon stocks and C:N ratios under conservation (CTCC, green, n=7) and standard (STNC, orange, n=7) management. Samples from 0-5 cm depth are shown with upward-pointing triangles and 5-10 cm with downward-pointing triangles, illustrating the coupling between carbon accumulation and organic matter processing. Error bars omitted for clarity; statistical comparisons between treatments are presented in Figure 5.

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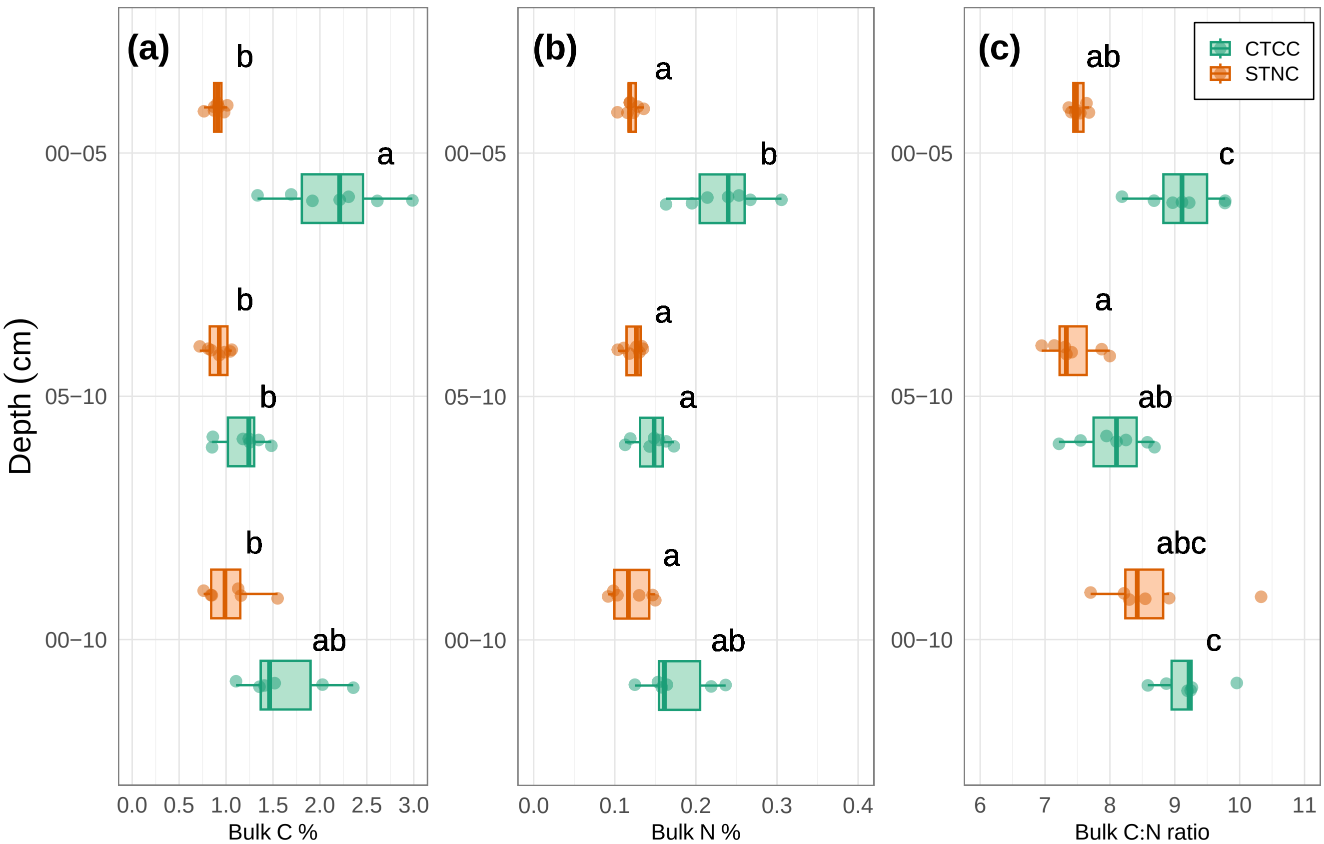

Figure 11. Carbon and nitrogen content under conservation (CTCC, green) and standard (STNC, orange) management from core (0-5 and 5-10 cm, n=7) and bulk soil samples (0-10 cm, n=6). **(a)** Carbon % weight, **(b)** nitrogen % weight (both determined via elemental combustion), and **(c)** C:N ratios. CTCC significantly improved soil chemical properties in the 0-5cm. Error bars represent standard error of the mean. Statistical significance between treatments is indicated by symbols: $***$ for $p < 0.001$, $**$ for $p < 0.01$, $*$ for $p < 0.05$, and $\circ$ for $p < 0.1$ (Games-Howell test).

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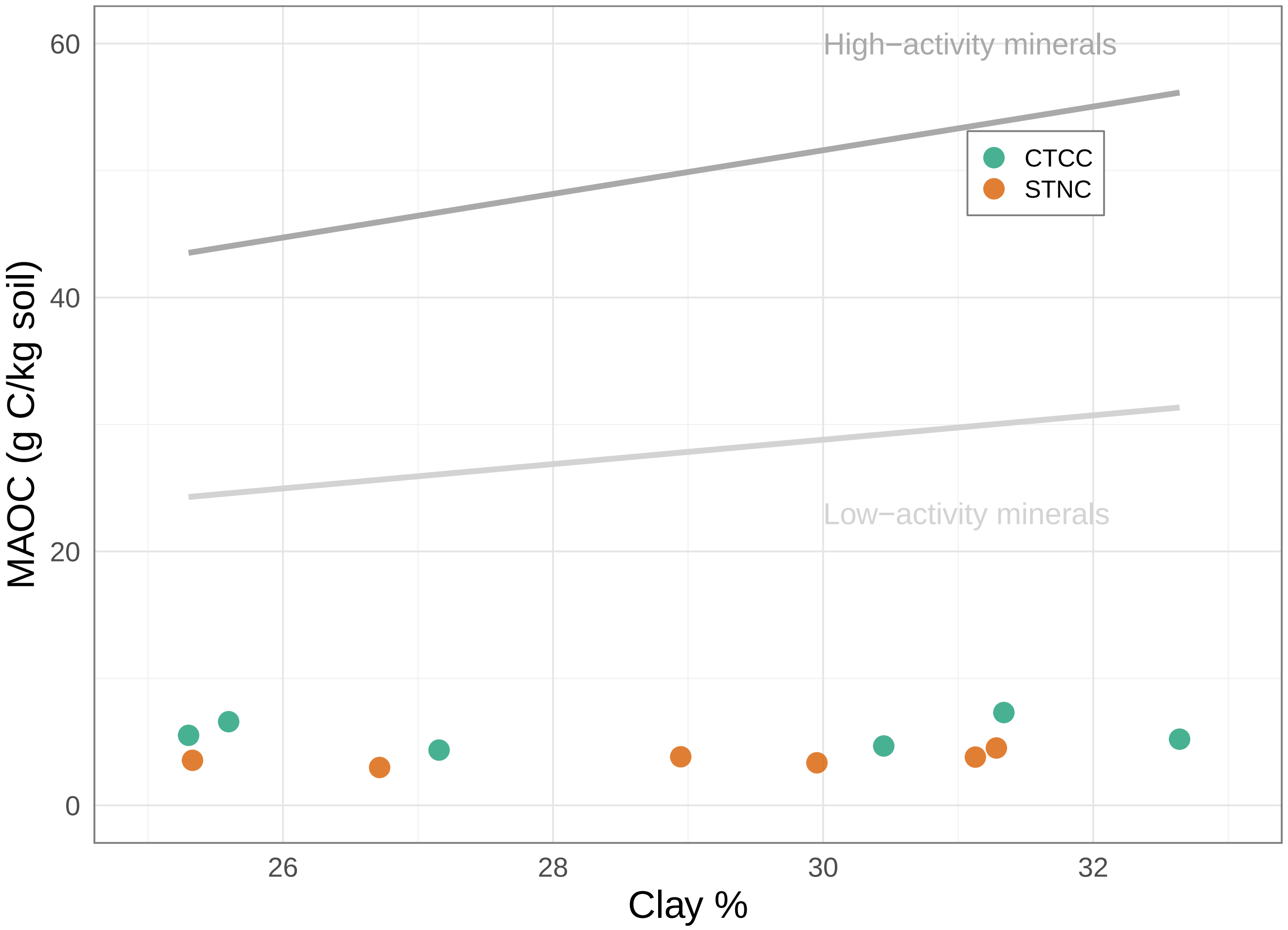

Figure 12. MAOC in systems Carbon saturation of the mineral fraction under conservation (CTCC, green, n=6) and standard (STNC, orange, n=6) management at 0-10 cm depth. CTCC leads to higher C saturation in the mineral fraction, while both management types are well below their theoretical capacity. Error bars represent standard error of the mean. Statistical significance between treatments is indicated by symbols: $***$ for $p < 0.001$, $**$ for $p < 0.01$, $*$ for $p < 0.05$, and $\circ$ for $p < 0.1$ (Welch's t-test).

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