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Enhancing soil organic carbon sequestration at different depths: the role of chitin-rich organic amendment in salt-affected soils

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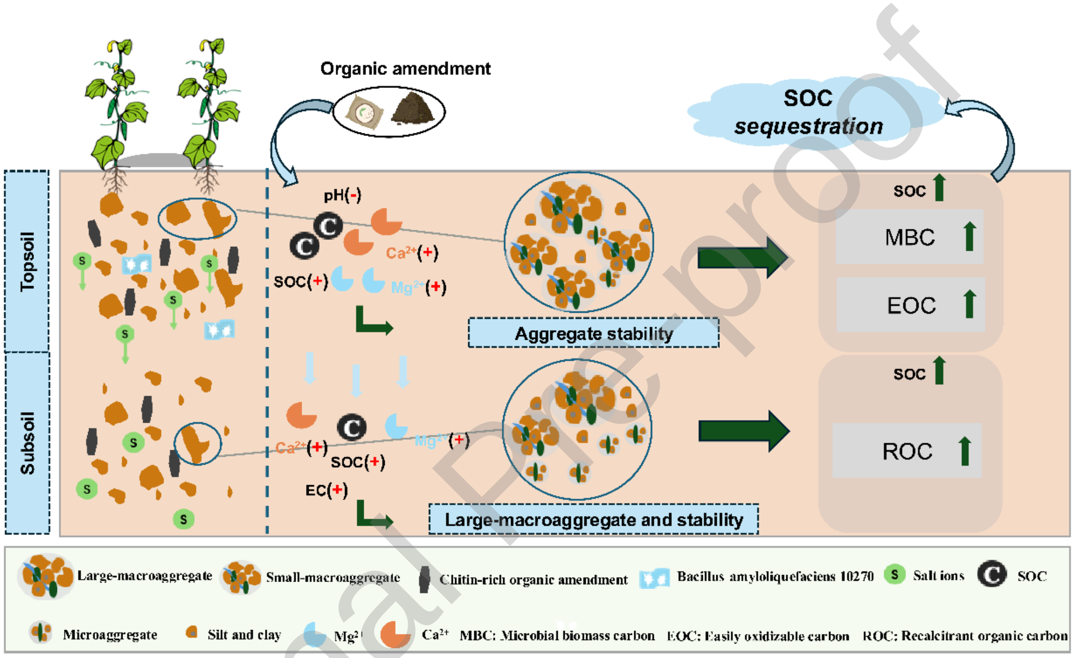
Abstract:

Chitin-rich organic amendments are known for enhancing soil aggregation and mitigating salinity, yet their effects on subsoil organic carbon (SOC) remains underexplored. An experiment was conducted in a greenhouse vegetable production system, employing three treatments: CK (no amendment), SC (chitin-rich organic amendment), and ST (bio-organic amendment). Soil samples were collected from both the topsoil (0–20 cm) and subsoil (20–40 cm) layers. The results revealed significant increase in soil electrical conductivity (EC), salt ion content, labile SOC fractions under organic amendment treatments in both soil layers. In the topsoil, a notable reduction in pH and enhanced desalination were observed. The bio-organic amendment notably increased the proportion of large macroaggregates and aggregate stability in both layers. In contrast, the chitin-rich organic amendment specifically enhanced small macroaggregates in the topsoil and large macroaggregates in the subsoil ($P<0.05$). Redundancy analysis identified soil pH as a crucial factor influencing topsoil aggregation, while EC, Mg^{2+}, and Ca^{2+} were critical for subsoil aggregation. Organic amendments increased SOC, with higher Ca^{2+} and Mg^{2+} levels under lower pH in the topsoil improving aggregate stability. In the subsoil, elevated EC, primarily driven by increased Ca^{2+} and Mg^{2+} (excluding Na^{+}), facilitated aggregate formation and accumulation of recalcitrant organic carbon, thereby contributing to SOC stabilization. These findings underscore the potential of chitin-rich and bio-organic amendments to improve soil structure and to enhance carbon sequestration in both topsoil and subsoil.

This has important implications for sustainable soil management in secondary saline greenhouse vegetable systems.

Graphical abstract



Keywords: Secondary saline soil, Greenhouse vegetable production, Organic amendment, Salt ions, Aggregate distribution and stability, Soil organic carbon

1. Introduction

Over the past two decades, greenhouse vegetable production in China has experienced rapid development, contributing to the stabilization of fresh vegetable supply (Zhang et al., 2021b). However, excessive fertilizer and frequent irrigation in greenhouse vegetable production systems usually lead to imbalance of soil nutrients

(Lv et al., 2020), salt accumulation and soil degradation (Li et al., 2019). Soil organic carbon (SOC), a key indicator of soil quality and productivity, is controlled by the balance between SOC input from plants or fertilizer and output from mineralization (Jackson et al., 2017). However, high concentration of soluble salts in greenhouse vegetable production systems could induce osmotic stress and ionic toxicity, which interfere with photosynthesis, carbohydrate metabolism, and nutrient absorption (Kumari et al., 2022; Wong et al., 2010). These stresses directly impair plant growth and productivity, ultimately diminishing carbon inputs to the soil (Setia et al., 2013). Meanwhile, sodium ions (Na^+) can replace multivalent cations such as Ca^{2+} and Mg^{2+} at cation exchange sites, leading to the dispersion of soil aggregates. This breakdown of soil structure exposes previously protected SOC to microbial decomposition, accelerating its mineralization (Haj-Amor et al., 2022; Xie et al., 2020). Therefore, under such conditions, it is imperative to develop and implement effective soil management strategies to mitigate salinity stress, maintain a balance between SOC inputs and outputs, enhance SOC sequestration, and promote sustainable practices in greenhouse vegetable production systems.

Globally, approximately 6–8 million tons of shrimp and crab shells wastes are generated annual, containing 15–40% chitin and other substances (i.e., protein and calcium carbonate) (Yan and Chen, 2015). Converting these organic wastes into chitin-rich organic amendments have gained interest in recent years, because the amendments not only offer a sustainable approach to recycle wastes but also provide an effective strategy for SOC sequestration in salt-affected soils due to their distinctive properties. Chitin-rich amendments are characterized by a low carbon-to-nitrogen (C:N) ratio (approximately 6 – 7) (Shamshina et al., 2019), which facilitates rapid decomposition of organic matter. This enhances the turnover of labile SOC fractions, thereby promoting SOC accumulation even under highly saline-sodic conditions (Ji et al., 2019). Additionally, these amendments improve crop productivity by enhancing nutrient retention through cation exchange and chelation, indirectly increasing SOC inputs via improved plant biomass (Aklog et al., 2016; Li et al., 2023; Rassaei, 2023). Moreover, the potential of chitin-rich amendments to enhance SOC sequestration also depends on their effects on soil structure. Several studies have demonstrated that they promote the formation of soil macroaggregates by binding organic matter to soil minerals through calcium bridges, which protects SOC from microbial decomposition (Zhang et al., 2021a). In parallel, *Bacillus amyloliquefaciens*, a well-known plant growth-promoting bacteria, can enhance soil nutrient availability and stimulate plant growth and root development through the secretion of phytohormones and organic compounds (Luo et al., 2025). Applying this bacteria with organic amendments offers additional potential for improving soil structure, boosting plant productivity, and thereby enhancing SOC inputs—making them an essential tool for promoting sustainable agriculture (Mao et al., 2022; Zhu et al., 2021). For instance, Peng et al. (2023) indicated that *Bacillus amyloliquefaciens*, when combined with organic amendment (frass), reduced salt concentrations by 23.54%, improved both soil macroaggregate and microaggregate, and enhanced SOC as well as other nutrient content. However, limited studies have explored the combined effects of chitin-rich organic amendment and plant growth-promoting bacteria (i.e., *Bacillus amyloliquefaciens*) in salt-affected soil, especially in terms of mitigating salt accumulation and influencing SOC sequestration in greenhouse vegetable production systems.

In addition, most studies related to the effect of organic amendments application on SOC improvement have focused on the topsoil (0–20 cm), as this depth corresponds to the typical zone for organic materials input (Bai et al., 2023; Yang and Zhang, 2022). Nevertheless, subsoil may offer greater potential for SOC sequestration due to oxygen limitation, which reduces microbial activity and slows carbon turnover rate, thereby increasing SOC stabilization (Balesdent et al., 2018). Previous studies have shown that organic amendments can significantly enhance subsoil SOC content, especially in below ploughing depth (20-40cm) (Kätterer et al., 2014; Hechmi et al., 2021). This enhancement can be explained by: (1) physical protection; (2) plant-derived carbon input (increased plant litter and root exudates due to increased crop yield under organic amendment application); and (3) the transport of dissolved and particulate organic carbon from topsoil (Rumpel and Kögel-Knabner, 2010). However, the input of organic amendments may also accelerate SOC mineralization in subsoil via positive priming effect, leading to a repaid decomposition of native SOC and reducing the residence time of SOC (Fang et al., 2020). Therefore, the consequences of organic amendments input for SOC sequestration in the subsoil is still uncertain. Furthermore, in salt-affected soil, the migration of salt ions between topsoil and subsoil alters soil chemical properties and structure, which further complicates SOC dynamics. Consequently, focusing on differences and underlying mechanisms in SOC sequestration across soil layers is essential for optimizing chitin-rich and bio-organic amendments management strategies, and enhancing the sustainability in greenhouse vegetable production systems.

This study aims to address existing knowledge gaps by elucidating the mechanisms underlying SOC sequestration at varying depths. Specifically, we investigated the impacts of chitin-rich organic and bio-organic amendments on soil salinity, aggregate characteristics, and SOC fractions in both topsoil (0–20 cm) and subsoil (20–40 cm) within a greenhouse setting in Shandong Province, China. We hypothesized that: 1) the application of these amendments significantly reduces electrical conductivity (EC) and enhances aggregation in both soil layers, with a more pronounced effect anticipated from the bio-organic amendment; and 2) salt ions influence SOC sequestration under these amendments by affecting aggregate formation, stability, and SOC fractions across the two soil depths. This research intends to provide effective strategies and a theoretical foundation for enhancing SOC sequestration in salt-affected greenhouse vegetable production systems.

2. Materials and method*s*

2.1 Site and amendment description

The experiment was conducted from August 2022 to August 2023 (covering two growing seasons) in a typical solar plastic greenhouse located in Jinan, Shandong Province, China (37°05′ N, 117°23′ E). The soil type is classified as silty clay loam, comprising of 27% clay, 48% silt, and 25% sand. The basic physicochemical properties of the 0-15 cm soil layer at the beginning of the experiment were as follows: pH, 7.26; total salt content, 4.63 g kg^{-1}; electrical conductivity (EC), 1.10 ds m^{-1}; bulk density (BD), 0.94 g cm^3; organic C content (OC), 72.5 g kg^{-1}; total N (TN), 4.23 g kg^{-1}; available potassium (AK), 489.91 mg kg^{-1}; available phosphorus (AP), 137.49 g kg^{-1}.The initial concentrations of salt ions are presented in Table 1.

Two types of amendments were applied in this study: chitin-rich organic amendment and bio-organic amendment. The chitin-rich organic amendment was primarily composed of shrimp head and crab shell waste. It underwent autolytic fermentation at 25 °C for 24 hours, followed by hydrolysis, and was mixed with other organic materials (i.e., straw and peanut shells) and composted at 30–70 °C for 25 days. This amendment was produced by Botai Biochemical Technology Industrial Co., Ltd. (Zhanjiang, China). The basic physicochemical properties of the amendment were as follows: pH, 7.75; OC, 85.7 g kg^{-1}; TN, 12.7 g kg^{-1}; total potassium (TK), 22.88 g kg^{-1}; total phosphorus (TP), 11.65 g kg^{-1} and 35 wt % calcium and magnesium.

The bio-organic amendment was produced using the same process as the chitin-rich organic amendment, with the addition of Bacillus *amyloliquefaciens* strain 10270 during application (6L ha^{-1}; diluted 100 times with water). This strain was inoculated at a concentration exceeding 1×10^8 CFU mL^{-1}. It was obtained from the Agricultural Culture Collection of China (Beijing, China) and selected based on its plant growth-promoting properties and salt tolerance, as demonstrated in laboratory experiments (Peng et al., 2025).

2.2 Greenhouse experiment

The experiment was conducted using a completely randomized block design with three treatments, each replicated three times, resulting in a total of nine plots (2 × 8 m each; Fig. 1). Each plot contained 60 cucumber plants. The experiment spanned two growing seasons per year, with cucumbers planted in early spring and early autumn, respectively. The cucumber variety was “Lvyou No.2”. To prevent lateral movement of fertilizers and water between plots, plastic films were buried to a depth of 1 m. The three treatments were as follows: (1) CK: no amendment (mineral fertilizer 255 kg ha^{-1} N, 111 kg ha^{-1} P, and 212 kg ha^{-1} K); (2) SC: chitin-rich organic amendment (15 t ha^{-1} yr^{-1}); (3) ST: bio-organic amendment (15 t ha^{-1} yr^{-1}). To ensure nutrient balance among treatments, any differences in nutrient input from the amendments were compensated using mineral fertilizers as needed. Both the mineral fertilizers and organic amendments were applied as base fertilizers before cucumber planting in each season and were incorporated into the top 0–20 cm of soil. Irrigation practices included both flood and drip irrigation, selected based on soil temperature. Each application delivered 0.4 tons of water per plot, as recorded by a water meter. Tillage followed conventional practices, with soil plowed to a depth of 20 cm after each harvest. Other agronomic practices, such as planting configuration and plastic film mulching, were consistent across all treatments and followed local greenhouse production standards.

2.3 Soil sampling and analysis

In July 2023, soil samples were randomly collected from the topsoil (0–20 cm) and subsoil (20–40 cm) for each treatment (a total of 18 soil samples) using a core sampler at five points along an S transect. All samples were separated into two sub-samples after the visible roots and stones were removed using tweezers. One subsample was naturally air-dried to measure soil and aggregate properties. The other subsample was stored at 4°C to estimate soil microbial biomass carbon (MBC).

The EC and pH (soil:water ratio 1:5) were measured using a conductivity meter (conductivity FE30, Mettler Toledo, China) and a PHS-3G digital pH meter (Shanghai Leici Instrument Factory, Shanghai, China), respectively. Soil salt ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and NO_3^-) were extracted from soil samples using deionized water (soil:water ratio 1:5). Soluble cations were measured via inductively coupled plasma optical emission spectrophotometry (ICP-OES) (Optima 8000, PerkinElmer, Waltham, MA, USA) and ion chromatography (HPIC, 930 Compact IC Flex, Herisau, Switzerland). The soil desalination rate (SDR,%) was calculated as follows (Fu et al., 2023):

$$\mathrm{SDR} = \frac{S0 - Si}{S0} \times 100\% \qquad (1)$$

where S0 represents the soil EC before planting (Table S3), and Si is the soil EC at harvest.

The SOC was quantified as described by Bao (2000). MBC was measured following the method described by Vance et al. (1987). After fumigation, soil samples were extracted using 0.5 M K_2SO_4 and the solution were measured on an Elementar TOC analyzer (Multi N/C 3100, Analytik Jena, Germany). The carbon (C) content was calculated as the difference in C content between the fumigated and non-fumigated samples. Particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) were measured using the method detailed by Cambardella and Elliott (1992). Briefly, a 10 g soil sample was placed into a 50 mL centrifuge tube, mixed with 30 mL of sodium hexametaphosphate solution (5 g L^{-1}), and shaken at 100 rpm for 18 hours to ensure complete dispersion. The resulting soil suspension was passed through a 53 μm sieve, washed with distilled water, and the retained and passing fractions were collected in aluminum pans to separate particulate organic carbon (POC; ≥ 53 μm) and mineral-associated organic carbon (MAOC; < 53 μm), respectively. The collected fractions were oven-dried at 50 °C for 48 hours, weighed, and subsampled (0.1 g) for carbon content analysis following the method of Bao (2000). Easily oxidizable carbon (EOC) was determined using the potassium permanganate ($KMnO_4$) oxidation method (Blair et al., 1995), and recalcitrant organic carbon (ROC) was calculated based on the variation between total SOC and EOC.

2.4 Water-stable aggregate analysis

Soil aggregates were separated into four sizes according to the wet-sieving method (Elliott et al., 1986; Chen et al., 2024): large macroaggregate (>2000 μm, LMA), small macroaggregates (250–2000 μm, MA), microaggregate (53–250 μm, MI), and silt and clay fractions (<53 μm, SAC). In detail, 50 g of air-dried soil (<8 mm) was placed on a nest of three sieves with mesh sizes of 2000, 250, and 53 μm, along with 30 glass beads (4 mm in diameter). The sieve stack was positioned in an automatic sieve shaker (Analysette 3, Fritsch, Germany), and deionized water was slowly added until the soil was fully submerged. The setup was left undisturbed for 10 minutes to allow pre-wetting. Subsequently, the sieves were vertically shaken 450 times over 15 minutes at an amplitude of 3 cm. After sieving, the aggregate fractions retained on each sieve were carefully collected and transferred to pre-weighed aluminum pans. The samples were oven-dried at 60 °C and weighed to determine aggregate size distribution. Soil aggregate stability was assessed using three indicators: mean weight diameter (MWD), geometric mean diameter (GMD), and the percentage of aggregates >0.25 mm in size ($WR_{0.25}$). These parameters were calculated as follows:

$$\mathrm{MWD} = \sum_{i=1}^{n} \mathrm{xi} \times \mathrm{wi} \tag{2}$$

$$\mathrm{GMD} = \mathrm{EXP}\ [(\sum_{i=1}^{n} \mathrm{wi} \times \mathrm{lnxi}) / \sum_{i=1}^{n} \mathrm{wi}] \tag{3}$$

$$\mathrm{WR0.25} = \sum_{i=1}^{2} \mathrm{w}i \times 100 \tag{4}$$

where xi is the mean diameter of aggregates in fraction i (mm), wi is the proportion of soil aggregate weights in fraction i (%), and i is equal to 1, 2, 3, or 4 representing aggregate sizes of >2000, 250–2000, 53–250, and <53 μm, respectively.

2.5 Statistical analysis

The differences of soil properties, salt ion content, aggregate distribution, and SOC fraction were evaluated using one-way analysis of variance (ANOVA, Duncan tests) using SPSS Statistics 26.0 (SPSS Inc., Chicago, IL, USA) and R software (version 4.3.2). Redundancy analysis (RDA) was conducted using the 'vegan' package (version 4.3.2) to address the relationship among soil properties, SOC fractions and aggregate characteristics constrained by amendment application. Linear regression analysis was conducted through the lm() function in R (version 4.3.2). To further expose the direct and indirect effects of soil salt ions, aggregate distribution, and SOC fractions on SOC sequestration at different soil layers, structural equation modeling (SEM) was conducted using AMOS 22.0 (SPSS Inc., Chicago, IL, USA). The maximum likelihood method was employed to test the fitness of the SEM model. The significance of chi-square test (p), chi-square (χ^2), goodness-of-fit index (GFI), and root-mean square errors of approximation (RMSEA) were the key indices.

3. Results

3.1 Impact of chitin-rich amendments on soil EC, pH, and salt ion dynamics in topsoil and subsoil

Soil EC values ranged from 532 to 595 μs cm^{-1} in the topsoil and from 340 to 537 μs cm^{-1} in the subsoil (Fig. 2). Compared to the control (CK), the EC values in the topsoil strongly increased by 11.84% and 3.13% under SC and ST treatments, respectively. In the subsoil, the EC values increased by 57.79% and 46.72%, respectively. The SDR values in the topsoil increased following organic amendment application, whereas a decrease was observed in the subsoil (Fig. 2c), which suggesting the organic amendment effectively decreased soil salinity in the topsoil. The pH values in topsoil decreased by 2.70% for SC and 1.11% for ST compared with CK treatment. However, no differences were detected among treatments in the subsoil ($P>0.05$, Fig. 2b).

In comparing the organic amendment treatments to CK, significant increases in the concentrations of Ca^{2+}, Mg^{2+}, and SO_4^{2-} were observed in the topsoil. Specifically, under the SC treatment, these increases were 30.24%, 36.11%, and 52.98% respectively, while under the ST treatment, they were 21.23%, 29.29%, and 30.03% respectively ($P<0.05$, as detailed in Table 2). In the subsoil, the concentrations of salt ions including Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, and SO_4^{2-} increased significantly under SC and ST treatments compared to the CK treatment. However, no significant changes were observed for Na^+ and K^+ ions ($P < 0.05$; Table 2).

3.2 Soil organic carbon and its fractions under chitin-rich amendments application

Compared to the CK, the SOC content showed a notable increase under SC and ST in the topsoil (Fig. 3a, $P<0.05$). The POC, EOC, and MBC under ST increased by

44.81%, 30.22%, and 13.73%, respectively. While the similar trends were found in the SC with the 43.20%, 45.15%, and 17.22% increase (Fig. 3b, d and f). Variations in ROC showed the opposite trends in the topsoil (Fig. 3e), decreasing by 7.04% in SC ($P>0.05$) and 16.82% in ST ($P<0.05$). In the subsoil, the POC and MBC increased 67.29% and 87.05% under ST ($P<0.05$), and 32.59% and 114.46% under SC ($P<0.05$), compared to CK (Fig. 4b and f).

3.3 Aggregate distribution and stability in topsoil and subsoil under chitin-rich organic amendments

The distribution of aggregates in the topsoil and subsoil is depicted in Fig. 5. In the topsoil, the dominant fraction was MA (accounting for 39 ~ 51% of total aggregate mass) across all treatments, followed by LMA (constituting 16 ~ 28% of total aggregate mass). Compared to the CK, the percentage of MA significantly increased by 31.40% under SC, and the LMA exhibited a significant increase under ST ($P<0.05$) in the topsoil. In the subsoil, the percentage of LMA was significantly higher in the SC (16.97%) and ST (19.82%) compared to the CK (8.41%) ($P<0.05$). Moreover, 50.87% of MI was detected in the CK of the subsoil, but the percentage of this fraction reduced by 39.44% and 38.74% ($P<0.05$) in the SC and ST, respectively.

Amendments input significantly improved soil aggregate stability in both soil layers (Fig. 5b, c and d). Under the ST treatment, the MWD, GMD, and $W_{R0.25}$ in the topsoil significantly increased by 51.65%, 105.50%, and 30.81% compared to the CK ($P<0.05$). Similarly, the ST treatment showed the striking improvement in the subsoil aggregate stability with the increased MWD, GMD, and $W_{R0.25}$ (53.81%, 46.73%, and 18.13%), compared to the CK.

3.4 Effects of soil salt properties on SOC fractions and soil aggregation

Salt properties such as EC, Ca^{2+}, and Mg^{2+} showed a significant positive correlation with SOC and its fractions in both soil layers (Fig.6a and b, Fig S3). Moreover, soil variables like TN, Ca^{2+}, and Mg^{2+} were positivity related to soil aggregate distribution and stability (i.e., LMA, MWD, and GMD) in the topsoil (Fig.6c). Among these, Mg^{2+} was the most crucial variable (R^2=0.80, P=0.014) (Fig. S4). In contrast, soil properties such as pH and Na^+ were negatively related to LMA, MWD, and GMD. In the subsoil, soil properties accounted for 93.30% of the variation in aggregate distribution and stability (Fig. 6d), in which EC (R^2=0.86, P=0.011) and Ca^{2+} (R^2=0.68, P=0.040) were the most important factors.

3.5 Contribution of soil salt properties, aggregate distribution and SOC fractions to SOC sequestration

In the topsoil, SOC exhibited a significantly positive correlation with Ca^{2+}, Mg^{2+}, EOC and MBC ($P<0.05$), while negatively associated with pH, ROC, MI and SAC ($P>0.05$; Fig. S5). However, in the subsoil, the SOC exhibited a higher correlation with Ca^{2+}, EC, Mg^{2+}, ROC, LMA, MWD and GMD, but negatively correlated with pH, MI and SAC (Fig. S5). Regression analysis further indicated that the SOC in the topsoil was more closely linked with Ca^{2+} (R^2=0.76, $P<0.01$, Fig. 7a) and EOC (R^2=0.75,

$P<0.01$, Fig. 7a), while the SOC in the subsoil was linearly linked with ROC across treatments ($R^2=0.85$, $P<0.01$, Fig. 7b).

Furthermore, the SEM model revealed that the impact pathways of amendments on SOC differed across soil layers. In the topsoil, the variation in SOC was driven by pH, Mg^{2+}, Ca^{2+}, aggregate stability, EOC and MBC (Fig. 8a). The EOC and MBC directly affected the SOC content, while pH indirectly influenced the soil organic C fractions (EOC and MBC) by affecting soil aggregate stability and salt ions (Mg^{2+} and Ca^{2+}), and consequently altering the soil SOC content. In the subsoil, the total standardized effects of EC, LMA, aggregate stability, MBC and ROC on SOC were 0.746, 0.698, 0.589, 0.2, and 0.799, respectively. Among these, the ROC and EC were the key influencing factors. The EC had a notable effect on soil LMA and stability and subsequently influenced the SOC content by altering the ROC content (Fig. 8c).

4. Discussion

4.1 Contradictory Impact of Organic Amendments on Soil Salinity in Greenhouse Environments

In contrast to our first hypothesis that the organic input may induce a strong decrease in EC, we observed an increase in the EC values in both layers under SC and ST treatments (Fig. 2a). This contradicts the results of our previous field study, which demonstrated that soil salinity decreases strongly in saline-sodic soils following chitin organic amendment (Ji et al., 2019). Previous research pointed out that climatic factors like temperature are the key factors determining the salt accumulation. For the greenhouse vegetable production system in the study, the high air temperature (the highest temperature is 44°C; Fig. S1) can draw more salt upward to the soil surface following water evaporation (Corwin 2021). Additionally, high temperature and humidity (the average air humidity is 72.6%, Fig. S2) in greenhouse can also enhance microbial biomass (Fig. 3f) and stimulate microbial activities (Wu et al., 2020), thus leading to the rapid degradation of organic amendments into organic acids and increasing the release of soluble salts, ultimately causing an increase in soil EC (Ravindran et al., 2022). Comparing two layers, EC in the top layer was higher than in the sublayer. The reason may be due to higher MBC in topsoil (Fig. 3f), which promoted faster decomposition of organic amendments and resulted in elevated soluble salts release. Interestingly, despite the higher EC, the desalination rate in the topsoil was higher than that in the subsoil under organic amendment input, especially in the ST treatment (Fig. 2c). These findings suggested that the organic amendment may enhance the infiltration rate and salt leaching from topsoil to subsoil following the growing season, thereby reducing the hazards of salt accumulation in topsoil.

4.2 Boosting Carbon Sequestration and Aggregate Stability Across Soil Layers

Labile SOC fractions such as POC, EOC and MBC, represent a transitional form of organic matter between fresh crop residues and stabilized SOC (Chen et al., 2016). Due to their high turnover rate, these fractions are commonly served as early indicators of soil carbon variation. Our study revealed significant increases in labile SOC fractions-POC, EOC, and MBC in the topsoil (Fig. 3), implying that organic amendment potentially accelerates SOC turnover and accumulation. In general, POC is largely derive from relatively undecomposed plant fragments (Lavallee et al., 2019), and its increase in our study can be attributed to both the direct input of incompletely decomposed organic substances and the physical protection offered by soil aggregates (Chen et al., 2024). This was evidenced by a positive relationship between POC content and aggregate stability (Fig. S5a). Similarly, the enhancement of EOC and MBC suggest that organic amendments stimulated microbial biomass and reproduction through supplying a large amount of available carbon sources. This accelerated the process of organic matter mineralization (Sun et al., 2023), and thus further enhancing labile SOC content and nutrient cycling, consistent with research reported by Li et al. (2018). In the subsoil, POC and MBC still significantly increased under the SC and ST treatments (Fig. 4b and f). This increase can be partly attributed to the migration of organic molecules into deeper soil layers through pores and fractures (Wang et al., 2024), providing more substrates for microbial growth and activity.

In this study, ST addition significantly increased the percentage of LMA and improved aggregate stability in the topsoil (Fig. 5). This enhancement can be attributed to the bio-organic amendment. According to our previous study, bio-organic amendment application stimulated the metabolic activity of indigenous microorganisms (Zhang et al., 2024), and enhanced extracellular polymeric substance production, such as protein and polysaccharide (Peng et al., 2025), which is crucial for cementing microaggregates into macroaggregates (Sarker et al., 2022). In addition, as bio-organic amendment gradually decomposes and transforms, components such as carbohydrates, polysaccharides and humic substances are released, which act as persistent binding agents through hydrophobic interactions and cation bridging, further contributing to the formation and stability of soil aggregate (Bronick and Lal, 2005). However, chemical aspects of soil organic matter associated with aggregation were not identified in this study, and further research is therefore needed to reveal the relationship. Moreover, a positive correlation was observed between the contents of Ca^{2+} and Mg^{2+} and both LMA and aggregate stability (Fig. 6c), with these cation levels being significantly higher in ST than in CK (Table 1), suggesting higher opportunity to form organic matter–mineral complexes via Ca^{2+} bridge (Yuan et al., 2023). These cations can also replace Na^{+}, which increases macroaggregate percentages and improving stability (Guo et al., 2019). Furthermore, a negative association was found between pH and LMA in the topsoil (Fig. 6c), with amendment application decreasing soil pH (Fig. 2b). This relationship may be attributed to the reduction in soil pH within the slightly alkaline salt-affected soil, and thus causing a more neutral soil environment that favors microorganism proliferation (Lauber et al., 2008) and microbial metabolite secretion, thereby increasing soil aggregation. Notably, ST induced a more pronounced LMA proportion and aggregate stability relative to SC treatment in the topsoil (Fig. 5). This difference may owing to the low C/N ratio (6–7) of chitin-rich organic amendment, which had strong but transient effects on aggregate formation and stability due to its rapid decomposition rate (Ma et al., 2024). However, in the subsoil, SC and ST exhibit similar positive effects on the percentage of LMA (Fig. 5a), suggesting that the soluble organic molecules and decomposed organic compounds migrated vertically from the top layer (0–20 cm) to deeper soils under irrigation. This influenced the activities of subsoil microorganisms and root growth, and thus increasing the percentage of LMA (Liu et al., 2013; Wang et al., 2024).

4.3 SOC sequestration affected by salt ions, aggregates, and SOC fractions in the topsoil and subsoil

The application of chitin-rich organic or bio-organic input strongly enhanced the SOC content in both soil layers (Fig. 3), mainly due to the direct C input and the improvements of soil physicochemical properties. Interestingly, the pathways of SOC sequestration were distinct in the topsoil and subsoil (Fig. 8). In the topsoil, SOC sequestration was influenced by pH, which directly affected soil salt ions (Ca^{2+} and Mg^{2+}) and aggregate stability, and indirectly influenced labile C fractions (MBC and EOC) (Fig. 8a). A lower pH caused a decline in negatively charged soil particles, resulting in decreased cation adsorption and increased release of soluble Ca^{2+} and Mg^{2+} into the soil (Bolan et al., 2023), which subsequently facilitated Na^+ replacement, aggregate formation and stability (Duan et al., 2021; Yuan et al., 2023). Furthermore, our study indicated that pH influences SOC sequestration by indirectly affecting MBC and EOC. The higher concentrations of these fractions with the effect of pH suggests that more organic matter can be decomposed, promoting nutrient cycling processes and crop yield (Dai et al., 2023; Yuan et al., 2021). This, in turn, indirectly contributes to higher C input via plant residue return and rhizodeposition (Liang et al., 2021), thus altering SOC sequestration.

Unlike the topsoil, the EC was the key factor in SOC sequestration in the subsoil by directly affecting LMA and indirectly affecting aggregate stability and ROC (Fig. 8b). Generally, higher EC decreased SOC by decreasing plant inputs caused by osmotic potential and ion toxicity effects (Setia et al., 2013; Wong et al., 2010). However, in this research, the EC was positively related to SOC content, likely due to its inhibitory effect on organic matter decomposition. The increasing EC in the subsoil suppressed soil respiration (Rath and Rousk, 2015), thereby reducing decomposition of SOM. In addition, our results suggest that higher EC can facilitate LMA formation in soil, enhance aggregate stability. These findings are in contrast to those of previous studies reporting that a high soil EC decrease soil aggregation by increasing soil electrolyte concentration, net negative surface charge, and soil clay dispersion (Chang et al., 2024; Feng et al., 2021). This difference may be explained by the lower EC values in the subsoil in this study, as shown by Li et al. (2024), who reported that when $EC_{1:5}$ values were lower than 3.188 dS m^{-1} with organic materials input, the aggregate stability increased with EC. SOC sequestration was also related to the ROC, which was directly or indirectly influenced by soil LMA, aggregate stability, and EC. Aggregate occlusion physically protect organic compounds from microbe and enzyme access (Stockmann et al., 2013), while restricting oxygen diffusion and water and nutrient flow, thereby limiting microbial mineralization (Xu et al., 2024) and increasing ROC and SOC sequestration.

5. Conclusion

Our study provides evidence that chitin-rich organic and bio-organic amendment enhance SOC content in both topsoil and subsoil, albeit through distinct mechanisms. Key soil properties, especially pH and EC, play a pivotal role in regulating SOC sequestration across these soil layers by influencing soil aggregate stability and the distribution of SOC fractions, which in turn are affected by salt ions. These findings contribute to a deeper understanding of SOC dynamics at various soil depths and emphasize the necessity of managing soil pH and EC when applying organic amendments in salt-affected soils. However, the significance of microbial activity in the SOC sequestration process should not be overlooked. Therefore, future research should focus on integrating measurements of soil salinity characteristics, SOC fractions, and microbial activity within both bulk soil and aggregates across different soil horizons to elucidate the complex interactions involved for SOC sequestration.

CRediT authorship contribution statement

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References

Aklog, Y., Egusa, M., Kaminaka, H., Izawa, H., Morimoto, M., Saimoto, H., Ifuku, S., 2016. Protein/CaCO3/Chitin Nanofiber Complex Prepared from Crab Shells by Simple Mechanical Treatment and Its Effect on Plant Growth. Int. J. Mol. Sci. 17(10). https://doi.org/10.3390/ijms17101600.

Balesdent, J., Basile-Doelsch, I., Chadoeuf, J., Cornu, S., Derrien, D., Fekiacova, Z., Hatté, C., 2018. Atmosphere–soil carbon transfer as a function of soil depth. Nature 559(7715), 599-602. https://doi.org/10.1038/s41586-018-0328-3.

Bai, X., Tang, J., Wang, W., Ma, J., Shi, J., Ren, W., 2023. Organic amendment effects on cropland soil organic carbon and its implications: A global synthesis. Catena 231. https://doi.org/10.1016/j.catena.2023.107343.

Bao, S., 2000. Soil and agricultural chemistry analysis. Beijing: China agriculture press.

Blair, G., Lefroy, R., Lisle, L., 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. Aust. J. Agric. Res. 46(7), 393-406. https://doi.org/10.1071/AR9951459.

Bolan, N., Sarmah, A.K., Bordoloi, S., Bolan, S., Padhye, L.P., Van Zwieten, L., Sooriyakumar, P., Khan, B.A., Ahmad, M., Solaiman, Z.M., 2023. Soil acidification and the liming potential of biochar. Environ. Pollut. 317, 120632. https://doi.org/10.1016/j.envpol.2022.120632.

Bronick, C.J. and Lal, R. 2005. Soil structure and management: a review. Geoderma 124(1-2), 3-22. https://doi.org/10.1016/j.geoderma.2004.03005.

Cambardella, C.A., Elliott, E.T., 1992. Particulate Soil Organic-Matter Changes across a Grassland Cultivation Sequence. Soil Sci. Soc. Am. J. 56(3), 777-783. https://doi.org/10.2136/sssaj1992.03615995005600030017x.

Chang, F., Zhang, H., Zhao, N., Zhao, P., Song, J., Yu, R., Kan, Z., Wang, X., Wang, J., Liu, H., Han, D., Wen, X., Li, Y., 2024. Green manure removal with reduced nitrogen improves saline-alkali soil organic carbon storage in a wheat-green manure cropping system. Sci. Total Environ. 926, 171827. https://doi.org/10.1016/j.scitotenv.2024.171827.

Chen, H., Hao, Y., Ma, Y., Wang, C., Liu, M., Mehmood, I., Fan, M., Plante, A.F., 2024. Maize straw-based organic amendments and nitrogen fertilizer effects on soil and aggregate-associated carbon and nitrogen. Geoderma 443. https://doi.org/10.1016/j.geoderma.2024.116820.

Chen, S., Dong, H., Hong, Y., Gao, F., Guo, S. and Mi, W. 2024. Long-term different fertilization practices restructured the functional carbon pools in a paddy soil through distinct mechanisms. Agric. Ecosyst. Environ. 374. https://doi.org/10.1016/j.agee.2024.109168.

Chen, S., Xu, C., Yan, J., Zhang, X., Zhang, X. and Wang, D. 2016. The influence of the type of crop residue on soil organic carbon fractions: An 11-year field study of rice-based cropping s ystems in southeast China. Agric. Ecosyst. Environ. 223, 261-269. https://doi.org/10.1016/j.agee.2016.03.009.

Corwin, D.L., 2021. Climate change impacts on soil salinity in agricultural areas. Eur. J. Soil Sci. 72(2), 842-862. https://doi.org/10.1111/ejss.13010.

Dai, W., Fan, Y., Cao, L., Cao, L., Sha, Z., 2023. Soil organic carbon dynamics shift by incorporating wheat straw in paddy soil in China. J. Environ. Qual. 52(5), 960-971. https://doi.org/10.1002/jeq2.20496.

Duan, M., Liu, G., Zhou, B., Chen, X., Wang, Q., Zhu, H., Li, Z., 2021. Effects of modified biochar on water and salt distribution and water-stable macro-aggregates in saline-alkaline soil. J. Soils Sediments 21(6), 2192-2202. https://doi.org/10.1007/s11368-021-02913-2.

Elliott, E., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50(3), 627-633. https://doi.org/10.2136/sssaj1986.03615995005000030017x.

Fang, Y., Singh, B.P., Farrell, M., Van Zwieten, L., Armstrong, R., Chen, C., Bahadori, M., Tavakkoli, E., 2020. Balanced nutrient stoichiometry of organic amendments enhances carbon priming in a poorly structured sodic subsoil. Soil Biol. Biochem. 145. https://doi.org/10.1016/j.soilbio.2020.107800.

Feng, H., Wang, S., Gao, Z., Pan, H., Zhuge, Y., Ren, X., Hu, S., Li, C., 2021. Aggregate stability and organic carbon stock under different land uses integrally regulated by binding agents and chemical properties in saline-sodic soils. Land Degrad. Dev. 32(15), 4151-4161. https://doi.org/10.1002/ldr.4019.

Fu, X., Wu, X., Wang, H., Chen, Y., Wang, R., Wang, Y., 2023. Effects of fertigation with carboxymethyl cellulose potassium on water conservation, salt suppression, and maize growth in salt-affected soil. Agric. Water Manage. 287. https://doi.org/10.1016/j.agwat.2023.108436.

Guo, Z., Zhang, J., Fan, J., Yang, X., Yi, Y., Han, X., Wang, D., Zhu, P. and Peng, X. 2019. Does animal manure application improve soil aggregation? Insights from nine long-term fertilization experiments. Sci. Total Environ. 660, 1029-1037. https://doi.org/10.1016/j.scitotenv.2019.01.051.

Haj-Amor, Z., Araya, T., Kim, D.G., Bouri, S., Lee, J., Ghiloufi, W., Yang, Y., Kang, H., Jhariya, M.K., Banerjee, A., Lal, R., 2022. Soil salinity and its associated effects on soil microorganisms, greenhouse gas emissions, crop yield, biodiversity and desertification: A review. Sci. Total Environ. 843, 156946. https://doi.org/10.1016/j.scitotenv.2022.156946.

Hechmi, S., Hamdi, H., Mokni-Tlili, S., Zoghlami, R.I., Khelil, M.N., Jellali, S., Benzarti, S., Jedidi, N., 2021. Variation of soil properties with sampling depth in two different light-textured soils after repeated applications of urban sewage sludge. J. Environ. Manage. 297, 113355. https://doi.org/10.1016/j.jenvman.2021.113355.

Jackson, R.B., Lajtha, K., Crow, S.E., Hugelius, G., Kramer, M.G., Piñeiro, G., 2017. The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. Annu. Rev. Ecol. Evol. Syst. 48(1), 419-445. https://doi.org/10.1146/annurev-ecolsys-112414-054234.

Ji, Z., Zhou, J., Zhang, H., Guo, K., Liu, X., Jiang, H., Yang, J., Li, G., Zhang, J., 2019. Effect of soil conditioners on the soil chemical properties and organic carbon pool of saline-sodic soil (Chinese). J. Agro-Environ. Sci. 38(8), 1759-1767. https://doi.org/10.11654/jaes.2019-0426.

Kätterer, T., Börjesson, G., Kirchmann, H., 2014. Changes in organic carbon in topsoil and subsoil and microbial community composition caused by repeated additions of organic amendments and N fertilisation in a long-term field experiment in Sweden. Agric. Ecosyst. Environ. 189, 110-118. https://doi.org/10.1016/j.agee.2014.03.025.

Kumari, R., Bhatnagar, S., Deepali, Mehla, N., Vashistha, A., 2022. Potential of Organic Amendments (AM fungi, PGPR, Vermicompost and Seaweeds) in Combating Salt Stress ... A Review. Plant Stress 6. https://doi.org/10.1016/j.stress.2022.100111.

Lauber, C.L., Strickland, M.S., Bradford, M.A. and Fierer, N. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol. Biochem. 40(9), 2407-2415. https://doi.org/10.1016/j.soilbio.2008.05.021.

Lavallee, J.M., Soong, J.L. and Cotrufo, M.F. 2019. Conceptualizing soil organic matter into particulate and mineral‐associated forms to address global change in the 21st century. Global Change Biol. 26(1), 261-273. https://doi.org/10.1111/gcb.14859.

Li, J., Wan, X., Liu, X., Chen, Y., Slaughter, L.C., Weindorf, D.C., Dong, Y., 2019. Changes in soil physical and chemical characteristics in intensively cultivated greenhouse vegetable fields in North China. Soil Till Res. 195. https://doi.org/10.1016/j.still.2019.104366.

Li, J., Wen, Y., Li, X., Li, Y., Yang, X., Lin, Z., Song, Z., Cooper, J.M. and Zhao, B. 2018. Soil labile organic carbon fractions and soil organic carbon stocks as affected by long-term organic and mineral fertilization regimes in the North China Plain. Soil Till Res. 175, 281-290. https://doi.org/10.1016/j.still.2017.08.008.

Li, L., Kaufmann, M., Makechemu, M., Van Poucke, C., De Keyser, E., Uyttendaele, M., Zipfel, C., Cottyn, B., Pothier, J.F., 2023. Assessment of transcriptional reprogramming of lettuce roots in response to chitin soil amendment. Front. Plant Sci. 14. https://doi.org/10.3389/fpls.2023.1158068.

Li, W., Yang, J., Yao, R., Wang, X., Xie, W., Xiao, P., 2024. Interactive effects of salinity and straw on the soil aggregate stability and organic carbon sequestration in saline soils in the Hetao area, China. Land Degrad. Dev. 35(5), 1685-1698. https://doi.org/10.1002/ldr.5014.

Liang, Y., Al-Kaisi, M., Yuan, J., Liu, J., Zhang, H., Wang, L., Cai, H., Ren, J., 2021. Effect of chemical fertilizer and straw-derived organic amendments on continuous maize yield, soil carbon sequestration and soil quality in a Chinese Mollisol. Agric. Ecosyst. Environ. 314. https://doi.org/10.1016/j.agee.2021.107403.

Liu, C., Li, F., Zhou, L., Zhang, R., Yu, J., Lin, S., Wang, L., Siddique, K., Li, F., 2013. Effect of organic manure and fertilizer on soil water and crop yields in newly-built terraces with loess soils in a semi-arid environment. Agric. Water Manage. 117, 123-132. https://doi.org/10.1016/j.agwat.2012.11.002.

Luo, L., Zhang, N., Wang, E., Zhao, C., Liu, Q., Pang, X. and Yin, C. 2025. Bacillus amyloliquefaciens application alleviated the stimulation of organic fertilizer on soil denitrification rate in acidic soils. Microbiol. Res., 128216. https://doi.org/10.1016/j.micres.2025.128216.

Lv, H., Zhao, Y., Wang, Y., Wan, L., Wang, J., Butterbach-Bahl, K., Lin, S., 2020. Conventional flooding irrigation and over fertilization drives soil pH decrease not only in the top- but also in subsoil layers in solar greenhouse vegetable production systems. Geoderma 363. https://doi.org/10.1016/j.geoderma.2019.114156.

Ma, S., Cao, Y., Lu, J., Ren, T., Cong, R., Lu, Z., Zhu, J., Li, X., 2024. Response of soil aggregation and associated organic carbon to organic amendment and its controls: A global meta-analysis. Catena 237. https://doi.org/10.1016/j.catena.2023.107774.

Mao, X., Yang, Y., Guan, P., Geng, L., Ma, L., Di, H., Liu, W., Li, B., 2022. Remediation of organic amendments on soil salinization: Focusing on the relationship between soil salts and microbial communities. Ecotoxicol. Environ. Saf. 239, 113616. https://doi.org/10.1016/j.ecoenv.2022.113616.

Peng, Y., Zhang, H., Lian, J., Zhang, W., Li, G., Zhang, J., 2023. Combined Application of Organic Fertilizer with Microbial Inoculum Improved Aggregate Formation and Salt Leaching in a Secondary Salinized Soil. Plants. 12(16). https://doi.org/10.3390/plants12162945.

Peng, Y., Zhang, H., Li, G. and Zhang, J. 2025. Microbial inoculum improved soil aggregate formation and increased cucumber yield in a greenhouse under secondary salinization conditions. J. Environ. Manage. 376. https://doi.org/10.1016/j.jenvman.2025.124576.

Rassaei, F., 2023. Chitosan as an organic amendment to improve soil properties and plant growth in the presence of polystyrene microplastics. Environ Prog Sustainable Energy. 43(2). https://doi.org/10.1002/ep.14301.

Rath, K.M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. Soil Biol. Biochem. 81, 108-123. https://doi.org/10.1016/j.soilbio.2014.11.001.

Ravindran, B., Awasthi, M.K., Karmegam, N., Chang, S.W., Chaudhary, D.K., Selvam, A., Nguyen, D.D., Rahman Milon, A., Munuswamy-Ramanujam, G., 2022. Co-composting of food waste and swine manure augmenting biochar and salts: Nutrient dynamics, gaseous emissions and microbial activity. Bioresour. Technol. 344(Pt B), 126300. https://doi.org/10.1016/j.biortech.2021.126300.

Rumpel, C., Kögel-Knabner, I., 2010. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant Soil 338(1-2), 143-158. https://doi.org/10.1007/s11104-010-0391-5.

Sarker, T.C., Zotti, M., Fang, Y., Giannino, F., Mazzoleni, S., Bonanomi, G., Cai, Y., Chang, S.X., 2022. Soil Aggregation in Relation to Organic Amendment: a Synthesis. J. Soil Sci. Plant Nutr. 22(2), 2481-2502. https://doi.org/10.1007/s42729-022-00822-y.

Setia, R., Gottschalk, P., Smith, P., Marschner, P., Baldock, J., Setia, D., Smith, J., 2013. Soil salinity decreases global soil organic carbon stocks. Sci. Total Environ. 465, 267-272. https://doi.org/10.1016/j.scitotenv.2012.08.028.

Shamshina, J.L., Kelly, A., Oldham, T., Rogers, R.D., 2019. Agricultural uses of chitin polymers. Environ. Chem. Lett. 18(1), 53-60. https://doi.org/10.1007/s10311-019-00934-5.

Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., Courcelles, V.d.R.d., Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unkno wns and unknowns of sequestration of soil organic carbon. Agric. Ecosyst. Environ. 164, 80-99. https://doi.org/10.1016/j.agee.2012.10.001.

Sun, H., Li, X., Jin, L., Su, X., Li, C., Kou, J., 2023. Effects of biological soil crusts on soil labile organic carbon of patchy alpine meadows in the Source Zone of the Yellow River, West China. Catena 220. https://doi.org/10.1016/j.catena.2022.106715.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19(6), 703-707. https://doi.org/10.1016/0038-0717(87)90052-6.

Wang, Y., Yin, Y., Joseph, S., Flury, M., Wang, X., Tahery, S., Li, B., Shang, J., 2024. Stabilization of organic carbon in top- and subsoil by biochar application into calcareous farmland. Sci. Total Environ. 907, 168046. https://doi.org/10.1016/j.scitotenv.2023.168046.

Wong, V.N.L., Greene, R.S.B., Dalal, R.C., Murphy, B.W., 2010. Soil carbon dynamics in saline and sodic soils: a review. Soil Use Manage. 26(1), 2-11. https://doi.org/10.1111/j.1475-2743.2009.00251.x.

Wu, G., Chen, X.M., Ling, J., Li, F., Li, F.Y., Peixoto, L., Wen, Y., Zhou, S.L., 2020. Effects of soil warming and increased precipitation on greenhouse gas fluxes in spring maize seasons in the North China Plain. Sci. Total Environ. 734, 139269. https://doi.org/10.1016/j.scitotenv.2020.139269.

Xie, W., Chen, Q., Wu, L., Yang, H., Xu, J., Zhang, Y., 2020. Coastal saline soil aggregate formation and salt distribution are affected by straw and nitrogen application: A 4-year field study. Soil Till Res. 198. https://doi.org/10.1016/j.still.2019.104535.

Xu, P., Wang, Q., Duan, C., Huang, G., Dong, K., Wang, C., 2024. Biochar addition promotes soil organic carbon sequestration dominantly contributed by macro-aggregates in agricultural ecosystems of China. J. Environ. Manage. 359. https://doi.org/10.1016/j.jenvman.2024.121042.

Yan, N. and Chen, X. 2015. Don't waste seafood waste. Nature 524(7564), 155-157. https://doi.org/10.1038/524155a.

Yang, Q., Zhang, M., 2022. Effect of bio-organic fertilizers partially substituting chemical fertilizers on labile organic carbon and bacterial community of citrus orchard soils. Plant Soil 483(1-2), 255-272. https://doi.org/10.1007/s11104-022-05735-4.

Yuan, G., Huan, W., Song, H., Lu, D., Chen, X., Wang, H., Zhou, J., 2021. Effects of straw incorporation and potassium fertilizer on crop yields, soil organic carbon, and active carbon in the rice–wheat system. Soil Till Res. 209. https://doi.org/10.1016/j.still.2021.104958.

Yuan, Y., Liu, Q., Zheng, H., Li, M., Liu, Y., Wang, X., Peng, Y., Luo, X., Li, F., Li, X., Xing, B., 2023. Biochar as a sustainable tool for improving the health of salt-affected soils. Soil Environ. Health 1(3). https://doi.org/10.1016/j.seh.2023.100033.

Zhang, H., Yang, J., Zhou, J., Li, G., Zhang, J., 2021a. Effects of organic and inorganic amendments on aggregation and crop yields in sandy fluvo-aquic soil (Chinese). J. Plant Nutr. Fertil. 27(5),791-801. https://doi.org/ 10.11674/zwyf.20576.

Zhang, H., Degré, A., De Clerck, C., Li, S., Lian, J., Peng, Y., Sun, T., Luo, L., Yue, Y., Li, G., Zhang, J., 2024. Changes in bacterial community structure and carbon metabolism in sandy soil under the long-term application of chitin-rich organic material and attapulgite. Appl. Soil Ecol. 194. https://doi.org/10.1016/j.apsoil.2023.105161.

Zhang, Z., Sun, D., Tang, Y., Zhu, R., Li, X., Gruda, N., Dong, J., Duan, Z., 2021b. Plastic shed soil salinity in China: Current status and next steps. J. Cleaner Prod. 296. https://doi.org/10.1016/j.jclepro.2021.126453.

Zhu, L., Jia, X., Li, M., Wang, Y., Zhang, J., Hou, J., Wang, X., 2021. Associative effectiveness of bio-organic fertilizer and soil conditioners derived from the fermentation of food waste applied to greenhouse saline soil in Shan Dong Province, China. Appl. Soil Ecol. 167. https://doi.org/10.1016/j.apsoil.2021.104006.

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Declaration of Competing Interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figure legends:

Fig. 1. Schematic of experimental plot design.

Fig. 2. Effect of chitin-rich organic and bio-organic amendments on the soil EC (a), pH (b), and soil desalination rate (c) in the topsoil and subsoil. Different letters above each bar indicate statistically significant differences between treatments in each soil layer, according to Duncan's test ($P < 0.05$). EC: electric conductivity, SDR: soil desalination rate, CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment.

Fig. 3. Soil organic carbon fractions under organic amendment applications in the topsoil. Different letters above each bar indicate statistically significant differences between treatments in each soil layer, according to Duncan's test ($P < 0.05$). CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment.

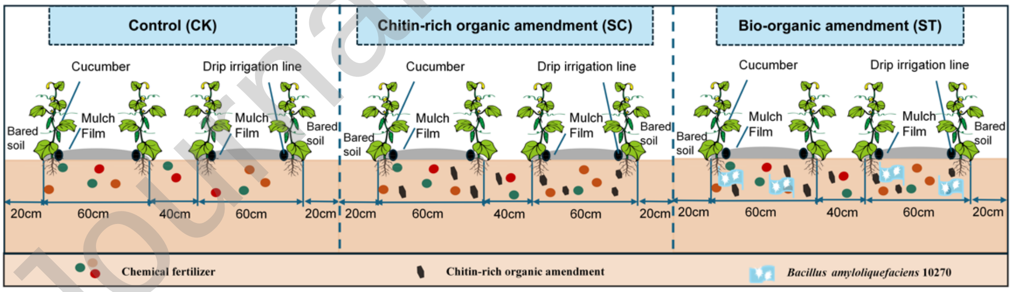
Fig. 4. Soil organic carbon fractions under organic amendment applications in the subsoil. Different letters above each bar indicate statistically significant differences between treatments in each soil layer, according to Duncan's test ($P < 0.05$). CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment.

Fig. 5. Aggregate size distribution (a) and aggregate stability (b, c, and d) in response to different organic amendment applications. CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment. Different letters above each bar indicate statistically significant differences between treatments in each soil layer, according to Duncan's test ($P < 0.05$).

Fig. 6. Redundancy analysis (RDA) for the various impacts of soil parameters on SOC fractions in the topsoil (a) and subsoil (b), as well as aggregate distribution and stability in the topsoil (c) and subsoil (d). SC: chitin-rich organic amendment, ST: bio-organic amendment.

Fig. 7. Liner regression analysis between SOC and key environmental factors both in the topsoil (a) and subsoil (b).

Fig. 8. Contribution of soil properties, aggregate characteristics, and organic carbon fractions to the SOC in the topsoil (a) and subsoil (c), and standardized total effects in the topsoil (b) and subsoil (d). Solid and dashed arrows indicate significant positive and negative relationships, respectively. The width of the arrow indicates the strength of the effect; the number next to the arrow is the standardized path coefficient, and the significance level is as follows: *, *P*< 0.05; **, *P*<0.01; and ***, P<0.001.



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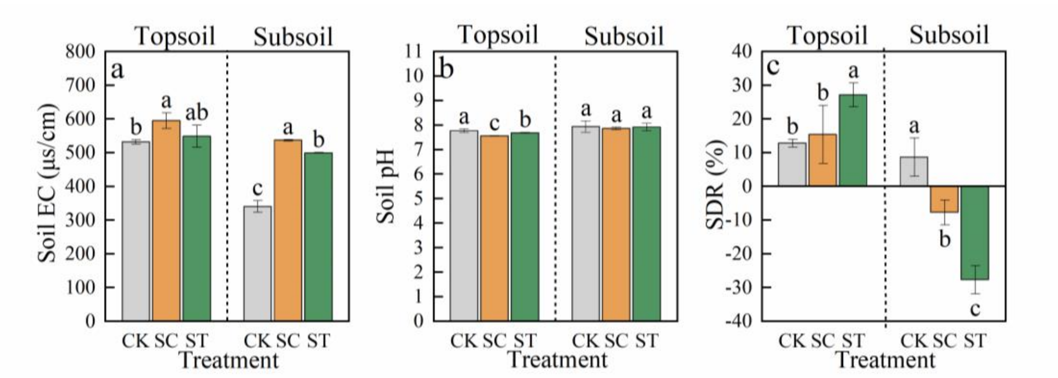

Fig. 2. Effect of chitin-rich organic and bio-organic amendments on the soil EC (a), pH (b), and soil desalination rate (c) in the topsoil and subsoil. Different letters above each bar indicate statistically significant differences between treatments in each soil layer, according to Duncan’s test ($P < 0.05$). EC: electric conductivity, SDR: soil desalination rate, CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment.

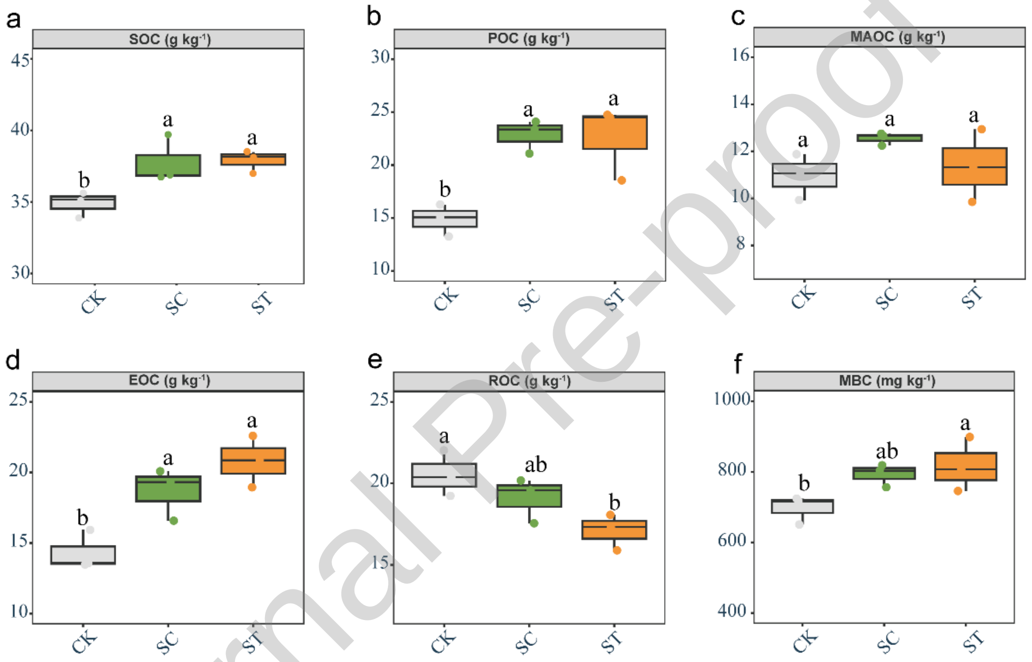

Fig. 3. Soil organic carbon fractions under organic amendment applications in the topsoil. Different letters above each bar indicate statistically significant differences between treatments in each soil layer, according to Duncan’s test ($P < 0.05$). CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment.

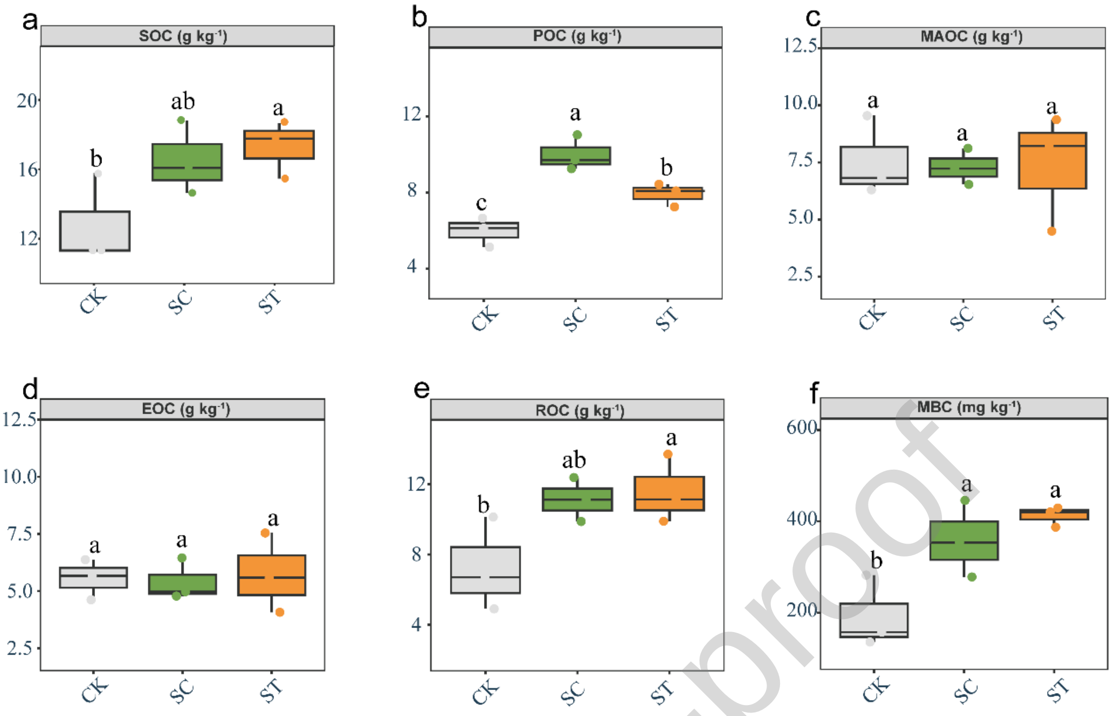

Fig.4. Soil organic carbon fractions under organic amendment applications in the subsoil. Different letters above each bar indicate statistically significant differences between treatments in each soil layer, according to Duncan's test (P< 0.05). CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment.

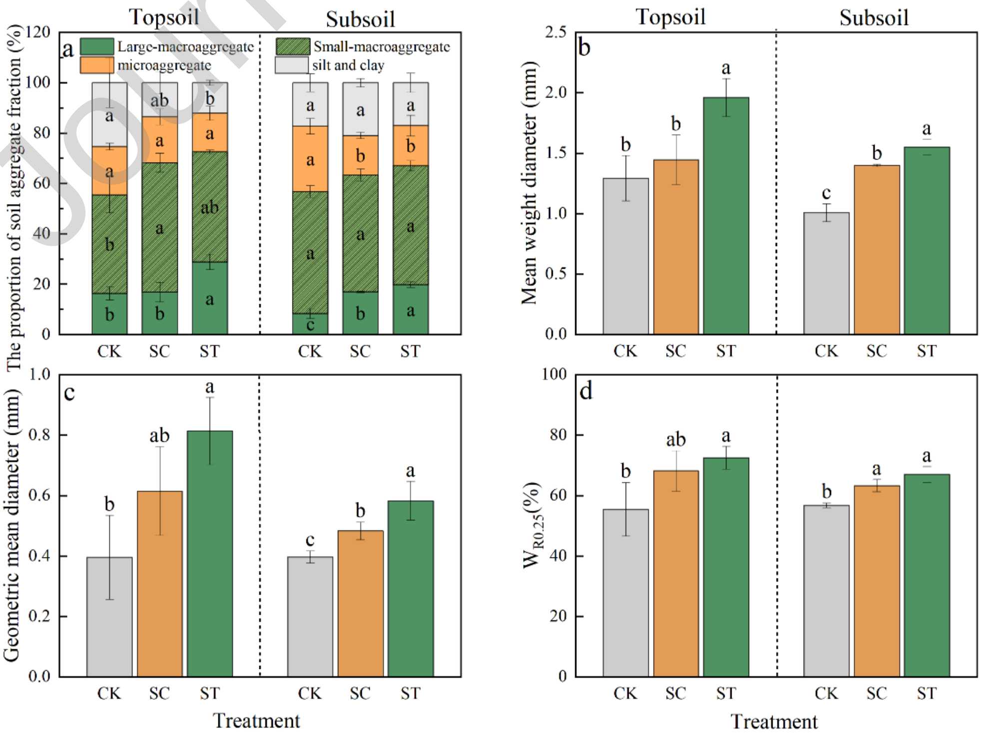

Fig. 5. Aggregate size distribution (a) and aggregate stability (b, c, and d) in response to different organic amendment applications. CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment. Different letters above each bar indicate statistically significant differences between treatments in each soil layer, according to Duncan's test ($P < 0.05$).

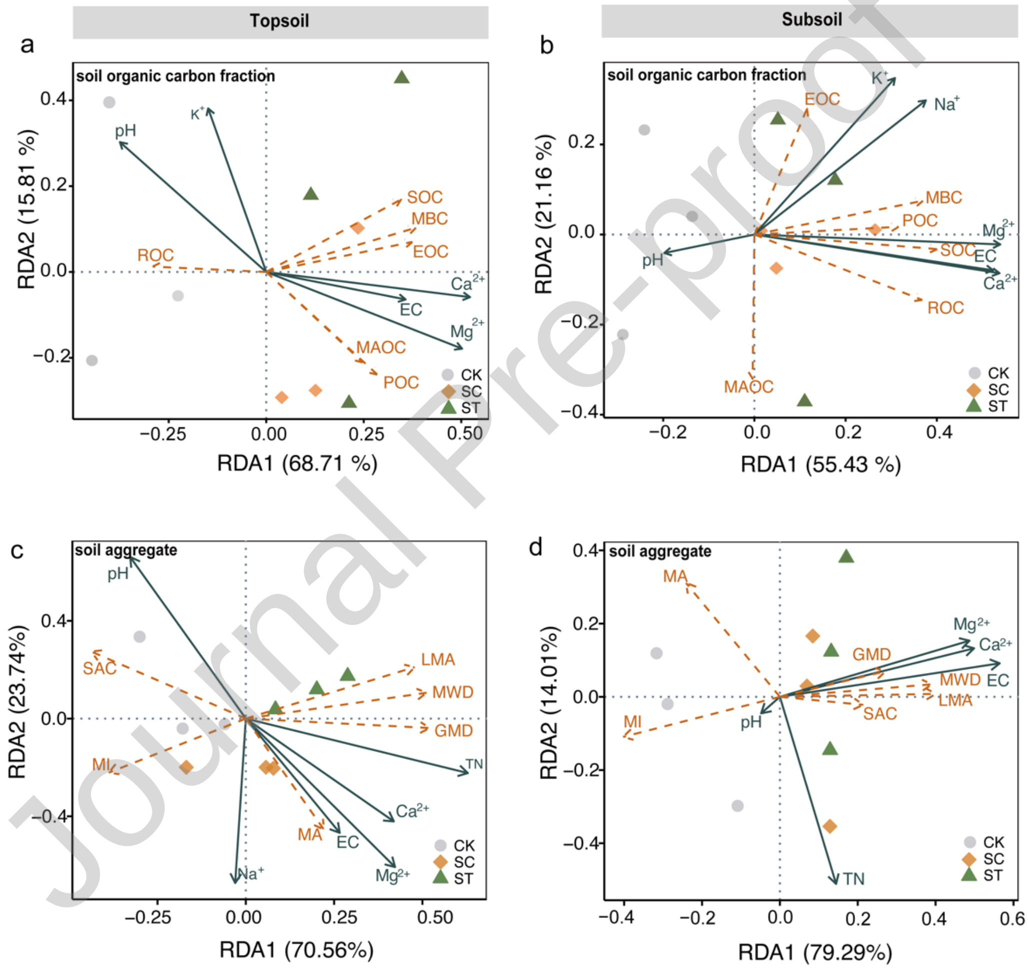

Fig. 6. Redundancy analysis (RDA) for the various impacts of soil parameters on SOC fractions in the topsoil (a) and subsoil (b), as well as aggregate distribution and stability in the topsoil (c) and subsoil (d). CK: no amendment, SC: chitin-rich organic amendment, ST: bio-organic amendment.

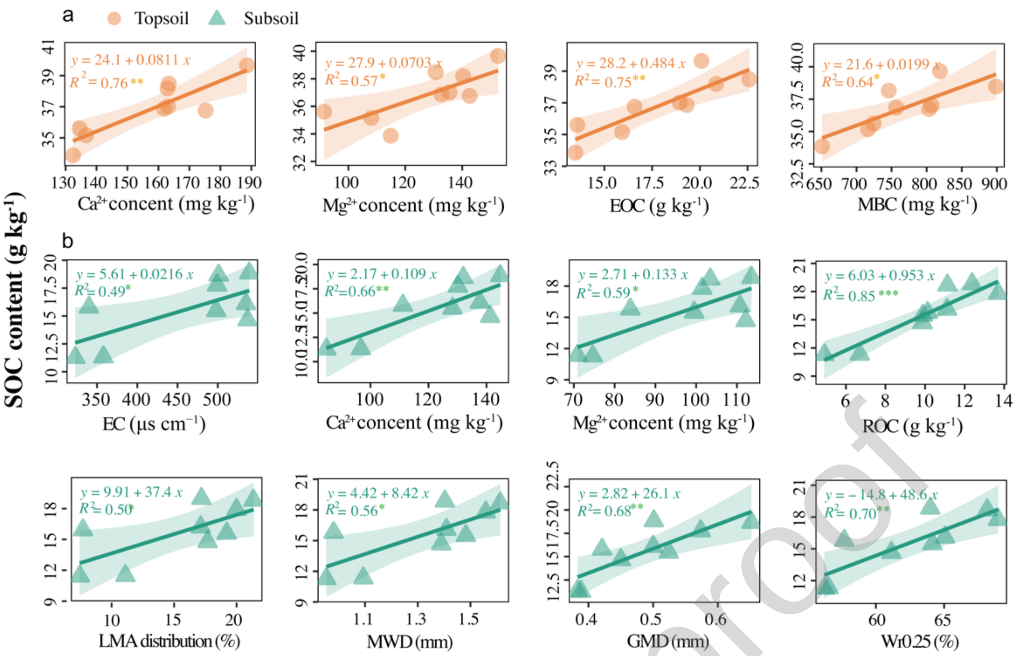

Fig.7. Liner regression analysis between SOC and key environmental factors both in the topsoil (a) and subsoil (b).

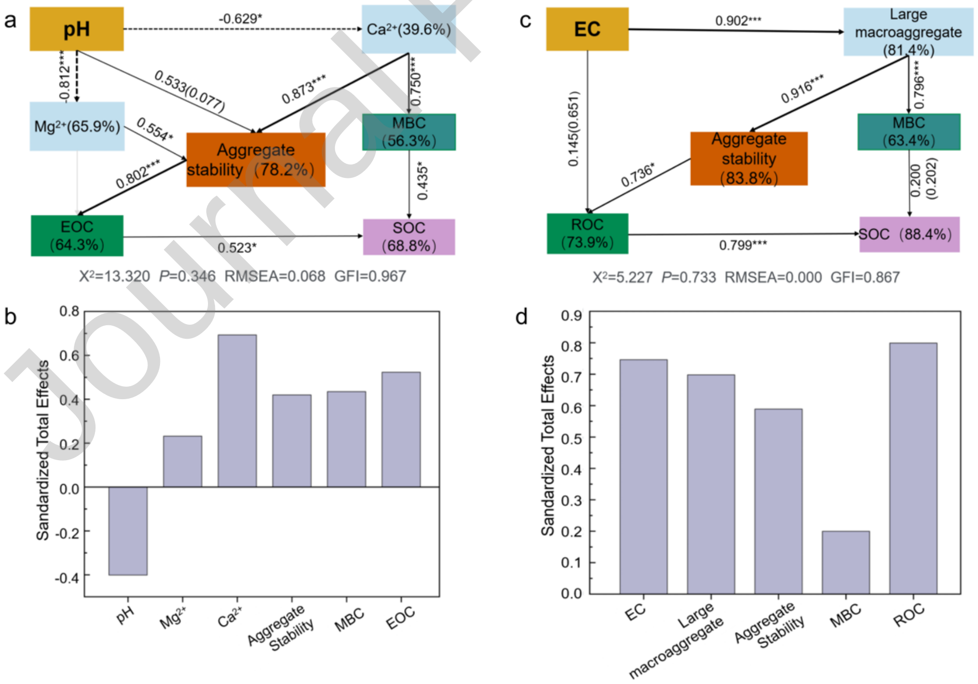

Fig. 8. Contribution of soil properties, aggregate characteristics, and organic carbon fractions to the SOC in the topsoil (a) and subsoil (c), and standardized total effects in the topsoil (b) and subsoil (d). Solid and dashed arrows indicate significant positive and negative relationships, respectively.

The width of the arrow indicates the strength of the effect; the number next to the arrow is the standardized path coefficient, and the significance level is as follows: *, $P< 0.05$; **, $P<0.01$; and ***, $P<0.001$.

Table:

Table 1 Salt ion content at the study site before the experiment.

Item	Ca^{2+} (mg kg^{-1})	Mg^{2+} (mg kg^{-1})	Na^{+} (mg kg^{-1})	K^{+} (mg kg^{-1})	SO_4^{2-} (mg kg^{-1})	Cl^{-} (mg kg^{-1})	NO_3^{-} (mg kg^{-1})	$CO_3^{2-}+HCO_3^{-}$ (mg kg^{-1})
value	188.4	169	374	130.8	510.8	280.8	279.2	18.5

Table 2 Salt ion content in the topsoil and subsoil under amendment application (mg kg^{-1}).

Salt ion	Topsoil			Subsoil		
	CK (n=3)	SC (n=3)	ST (n=3)	CK (n=3)	SC (n=3)	ST (n=3)
Ca^{2+}	134.56 ± 2.13b	175.25±13.3a	163.13±0.23a	97.57±13.23b	141.25±3.52a	130.14±1.86a
K^{+}	59.31± 8.25a	58.36±6.43a	55.01±5.35a	51.65±9.39a	55.81±7.68a	58.33±11.92a
Mg^{2+}	104.80±12.04b	142.64±9.99a	135.50±4.83a	76.46±6.67c	112.15±1.42a	101.48±1.98b
Na^{+}	227.32±3.70a	240.67±24.49a	224.66±46.03a	153.66±10.18a	193.08±12.62a	183.04±35.24a
Cl^{-}	167.58±23.12a	190.68±31.88a	185.03±40.64a	120.99±17.33b	168.90±1.93a	162.01±3.59a
NO_3^{-}	524.38±104.42a	532.73±11.58a	603.23±123.85a	365.18±38.05b	424.16±7.77a	467.66±11.69a
SO_4^{2-}	416.76±15.34c	637.58±3.48a	541.90±12.55b	298.69±11.275c	522.625±42.2a	452.45±5.025b

CK: no amendment, SC: chitin-rich organic amendment: ST- bio-organic amendment. Different lowercase letters within a row indicate significant differences between treatments in each soil layer, according to Duncan's test ($P< 0.05$)

Highlights:

1. Chitin-rich organic amendment accelerated soil aggregate formation, stability and soil organic carbon (SOC) sequestration through regulating Ca^{2+} and Mg^{2+}.
2. Soil properties (pH, Mg^{2+}, and Ca^{2+}) and aggregate stability increased microbial biomass carbon (MBC) and easily oxidizable carbon (EOC) to sequestrate SOC in topsoil.
3. Organic amendment sequestrated SOC in subsoil mainly via promoted aggregation and ecalcitrant organic carbon (ROC) by altering electrical conductivity (EC).