

Article

Long-Term Manure Application Increases Organic and Inorganic Carbon Accumulation in Saline-Sodic Soil

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Abstract

Organic manure application is considered an effective strategy for enhancing soil fertility and increasing carbon sequestration in saline-sodic soil. The contribution of manure-derived soil organic carbon (SOC) under long-term application remains unclear, as do its concurrent effects on SOC and soil inorganic carbon (SIC) pools. Empirical evidence addressing both aspects is scarce. This study was based on a long-term field experiment established in saline-sodic soil, with four manure application durations (14, 19, 25, and 30 years) and an unfertilized control, each with three replicates. Soil samples were collected from a 0–200 cm profile to investigate the vertical distributions of SOC, permanganate-oxidizable organic carbon (POXC), $\delta^{13}\text{C}$ values, SIC and its carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) fractions. The effects of manure application on the sources of SOC and the fractions of SIC were investigated using one-way analysis of variance (ANOVA) and the widely applied binary equations. The results revealed that long-term manure application significantly increased the SOC, POXC, SIC, and HCO_3^- contents, whereas the CO_3^{2-} content decreased. The effects were most notable within the 0–60 cm soil layer. The $\delta^{13}\text{C}$ values of SOC increased significantly with increasing fertilization duration, which indicates increased incorporation of exogenous carbon. The results of a binary mixing model revealed that after 30 years of manure application, manure-derived carbon contributed 31.64% (7.44 g kg^{-1}) to SOC, maize-derived carbon contributed 22.42% (5.27 g kg^{-1}) to SOC, and the remaining fraction originated from native SOC. Vertically, the SOC content decreased with soil depth, whereas the SIC content fluctuated and became dominant below 60 cm. Long-term manure application altered both SOC sources and SIC dynamics, thereby promoting the accumulation and redistribution of carbon within the soil profile. Overall, long-term manure application increased both organic and inorganic carbon content. These findings highlight the importance of organic fertilization as a sustainable management practice for increasing soil carbon storage and mitigating land degradation in saline-sodic agroecosystems.



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1. Introduction

Saline-sodic soils are extensively distributed across China, covering an estimated area of 3.70×10^7 ha, approximately 6.6% of the national total arable land area [1,2]. These soils are concentrated primarily in four major geographical regions: Northeast, North, East, and Northwest China. The Songnen Plain constitutes a primary hotspot for saline-sodic

soils in China, where such degraded land covers an area of approximately 3.84×10^6 ha. Approximately 1.28×10^6 ha is under cultivation, representing approximately 10% of the total arable land area in the region [3]. Saline-sodic soils are characterized by high salinity, high alkalinity, low soil organic matter (SOM) content, and insufficient nutrient levels, all of which negatively affect crop yields [4,5].

The soil carbon pool, as the largest terrestrial carbon reservoir, contains an estimated 2–3 times more carbon than the atmosphere does [6]. The soil carbon pool comprises two principal pools, namely, the soil organic carbon (SOC) pool and the soil inorganic carbon (SIC) pool. Globally, the size of the SOC pool is estimated at approximately 1500 Gt C [7], constituting 67–75% of the total terrestrial carbon stock [8]. The SIC pool is larger and more spatially heterogeneous, with estimates ranging from 695 to 1700 Gt C [9]. This pool is stored primarily in arid and semiarid regions where saline-sodic soils prevail. Consequently, research on saline-sodic soil carbon pools must address the dynamic changes in both organic and inorganic carbon components.

Manure application represents a dual-benefit strategy for enhancing soil fertility and promoting carbon sequestration in saline-sodic soils. As a substantial source of organic carbon, manure plays a pivotal role in sustaining the soil carbon pool equilibrium, enhancing soil fertility, and increasing crop yields [10]. Extensive research has demonstrated that manure application increases the SOC content in saline-sodic soils [11–13]. However, relatively little attention has been given to changes in SOC sources and their fractions. Permanganate-oxidizable organic carbon (POXC) in soil is a critical indicator of soil labile organic carbon, and can detect changes in SOC more sensitively. Yu et al. [14,15] reported increases ranging from 4% to 499.3% in labile organic carbon fractions following manure incorporation. Wang et al. [16] reported that maize's contribution to SOC increased from 32% to 39.2%, highlighting the influence of environmental conditions on crop-derived carbon incorporation into soil organic matter. Zhang et al. [17] found that, in Northwest China's arid region, long-term organic fertilization resulted in manure- and maize-derived carbon contributions of 30% and 15%, respectively. The magnitude of the response is notably modulated by regional soil properties, fertilizer type, and application rate. Therefore, quantifying SOC fractions and their source partitioning (i.e., between manure application and in situ crop residues) in saline-sodic soils is of paramount importance for carbon cycling studies, yet these issues remain poorly understood.

Moreover, the influence of manure application on the SIC content in saline-sodic soils has received limited attention. For example, Song et al. [18] reported that the application of humic acid organic fertilizer increased the SIC content in the 0–40 cm soil layer by 18%. Similarly, Liu et al. [19] reported that organic fertilizer application increased the SIC content by 12–18% in saline-sodic soils. In contrast, Li et al. [14] reported a significant reduction in the SIC content within the 20–100 cm soil layer under low-phosphorus conditions when organic fertilizer was applied. Notably, the influence of organic fertilizer application on the SIC content varies considerably across different geographical regions, soil layer depths, and fertilization regimes [20]. Additionally, existing research on SIC relies primarily on short-term experimental studies, with sampling depths often confined to within 1 m. However, studies have revealed that substantial SIC reserves exist below depths of 1 m [21]. Therefore, it is critically essential to investigate the effects of manure application on SIC in saline-sodic soils at deeper soil depths.

To address the uncertainties that, after manure application to saline-sodic soils, SOC increases but its source remains unclear, and that the trend of SIC change is undetermined, we propose the following hypotheses: (1) long-term manure application promotes the accumulation of both SOC and SIC throughout the soil profile; (2) prolonged manure application increases the relative contributions of maize-derived and manure-derived

carbon to SOC while reducing the proportion of native SOC; and (3) the responses of soil carbon accumulation to long-term manure application differ between surface and deep soil layers, with stronger effects expected in the surface soil. This study aims to test these hypotheses through a long-term field experiment in saline-sodic soils. Cultivated saline-sodic soils with different durations of manure application were selected. Soil samples were collected from the 0–200 cm profile. We quantified the sources of SOC using stable carbon isotope analysis in conjunction with a widely adopted binary mixing model, and assessed the effects of long-term organic fertilization on SOC, SIC and their fractions. The results of this study could advance our understanding of carbon accumulation processes in saline-sodic soils and provide a systematic basis for carbon sequestration and soil fertility improvement.

2. Materials and Methods

2.1. Study Area

The long-term experimental station of saline-sodic soil of Northeast Agricultural University is located in Taifeng village, Yongle town, Zhaozhou County, Heilongjiang Province, China (125.06° E, 45.40° N; 135 m above sea level). The site is located in the first accumulated temperature zone of Heilongjiang Province. The climate is a temperate continental monsoon climate. The annual accumulated temperature sum ($\geq 10^{\circ}\text{C}$) is 2800 $^{\circ}\text{C}$, the annual precipitation is 427.5 mm, and the annual evaporation level is 1635 mm (according to the local meteorological station). The soil type is classified as a solonetz on the basis of the World Reference Base for Soil Resources, and the soil texture is clay (26% sand, 22% silt, and 52% clay). The typical natural vegetation is *Leymus chinensis* and *Phragmites australis*. Before reclamation, the basic soil properties were as follows: pH, 9.56; electrical conductivity, 6.23 dS m^{-1} ; SOC, 6.36 g kg^{-1} ; total nitrogen, 0.37 g kg^{-1} ; alkali-hydrolyzable nitrogen, 39 mg kg^{-1} ; available phosphorus, 12 mg kg^{-1} ; and available potassium, 125 mg kg^{-1} ; soil bulk density, 1.35 g cm^{-3} .

2.2. Experimental Design

To evaluate the effect of manure application duration, five treatments representing different lengths of continuous manure application were included: 30 years (Y95, initiated in 1995), 25 years (Y00, initiated in 2000), 19 years (Y06, initiated in 2006), 14 years (Y11, initiated in 2011), and an unfertilized control (CK). All treatments were implemented in the same field plot, with only the reclamation year varying; cattle manure was applied upon reclamation, and maize was planted subsequently. Each treatment consisted of three replicated plots arranged in a randomized complete block design. Each plot was 65 m^2 in size (6.5 m $\times$ 10 m). The experimental organic manure was well-decomposed cattle manure. The nutrient contents were as follows: SOM, 334.21 g kg^{-1} ; total nitrogen, 11.21 g kg^{-1} ; C:N ratio, 17.29; total phosphorus, 10.67 g kg^{-1} ; total potassium, 13.85 g kg^{-1} ; and pH, 8.79. Cattle manure was applied at a rate of 45 $\text{m}^3 \text{ ha}^{-1}$ each year before autumn tillage was conducted. The test crop was maize (Longdan 288). Urea was applied at a rate of 450 kg ha^{-1} at the jointing stage. In each year of the experimental period, all treatments were managed under the same cropping system and field management practices, including the application rate of mineral fertilizers, crop cultivation, and residue return, except for the duration of manure application.

2.3. Soil Sampling and Preparation

Soil samples were collected in April 2025 before maize was sown. Soil samples were collected with a soil auger. For each treatment, four sampling sites were randomly selected, and at each site, five boreholes for each soil layer were combined to form a composite soil

sample. Soil samples were collected from depths of 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, 80–100 cm, 100–120 cm, 120–140 cm, 140–160 cm, 160–180 cm, and 180–200 cm in each plot. The soil samples were spread into thin layers with a thickness of approximately 2 cm and gently crushed and mixed, after which any plant residues and other impurities were removed. After natural air drying in a cool and ventilated indoor environment, some of the samples were ground and passed through 1- and 0.25-mm sieves for further analysis.

2.4. Analytical Methods

Soil organic carbon was determined via the potassium dichromate oxidation method with external heating. Soil inorganic carbon was measured through the gas volumetric method. POXC was measured as an indicator of the labile and biologically active fraction of SOC, which is highly responsive to long-term fertilization practices. The stable carbon isotope ($\delta^{13}\text{C}$) was determined to estimate the relative contributions of native, maize-derived, and manure-derived carbon to SOC using an isotope mixing approach. The permanganate-oxidizable organic carbon (POXC) content was quantified with a potassium permanganate spectrophotometric method, and soil carbonate and bicarbonate contents were determined via a double-indicator acid–base titration method [22].

The $\delta^{13}\text{C}$ values of SOC (Vienna Pee Dee Belemnite (VPDB)) were measured with a Finnigan MAT Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Notably, before the measurement, the samples were pretreated with HCl for 12 h to remove inorganic carbon interference and then combusted at 1020 °C in a pure oxygen environment to ensure the complete oxidation of organic matter.

2.5. Calculation of SOC Sources

Following the reclamation of saline-sodic soil for maize cultivation, maize residues (roots and stubble) are subsequently introduced into the soil. At this stage, SOC comprises both the inherent SOC pool of the original soil and SOC derived from maize residues. Given the substantial difference in $\delta^{13}\text{C}_{\text{SOC}}$ values between the native vegetation in pristine saline-sodic soil areas and maize, the cultivation of maize in saline-sodic soil effectively serves as in situ labeling for SOC. On the basis of the principle of mass conservation, Equation (1) can be used to estimate the relative contributions of the inherent SOC pool of saline-sodic soil and SOC derived from maize residues to the total SOC pool.

$$\delta_S = f\delta_A + (1 - f)\delta_0 \quad (1)$$

where δ_S is the $\delta^{13}\text{C}$ value of SOC after reclamation and maize cultivation in saline-sodic soil, δ_A is the $\delta^{13}\text{C}$ value of maize, and δ_0 is the $\delta^{13}\text{C}$ value of the original saline-alkaline soil. Moreover, f denotes the proportion of maize-derived carbon in the SOC pool, and $(1 - f)$ denotes the proportion of the original SOC derived from saline-sodic soil. For treatments without organic amendments, the maize-derived SOC content can be calculated as follows:

$$\text{SOC}_{\text{maize}} = \text{SOC} \times f \quad (2)$$

$$\text{SOC}_{\text{original}} = \text{SOC} \times (1 - f) \quad (3)$$

where $\text{SOC}_{\text{maize}}$ and $\text{SOC}_{\text{original}}$ denote the maize-derived SOC and original SOC contents, respectively, and SOC denotes the total soil organic carbon content. For treatments with organic amendments, the manure-derived SOC content can be calculated as the difference between the total SOC content and the sum of the maize-derived SOC and original SOC contents [23].

2.6. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 27.0 (v27.0; IBM Corp., Armonk, NY, USA). Before conducting one-way analysis of variance (ANOVA), the normality of data distribution and homogeneity of variances were assessed using the Shapiro–Wilk test and Levene’s test, respectively. The data met the assumptions required for ANOVA. Differences among manure treatments within each soil depth were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected, means were separated using Duncan’s multiple range test at the $p < 0.05$ significance level. Results are expressed as the mean $\pm$ standard deviation (SD) of three replicates.

3. Results

3.1. Soil Organic Carbon

Manure application significantly influenced the SOC content, which varied systematically ($p < 0.05$) with increasing duration of fertilization. As shown in Figure 1, within the 0–60 cm soil layer, the SOC content increased significantly with increasing duration of manure application. Compared with that in the CK treatment, the SOC content in the Y95, Y00, Y06, and Y11 treatments increased by 4.55–11.63 g kg^{−1}, 4.07–7.09 g kg^{−1}, 4.07–4.35 g kg^{−1}, and 2.72–3.78 g kg^{−1}, respectively. In the 60–200 cm soil layer, the differences in SOC among treatments were relatively minor. Significant increases were observed only in the Y06 treatment at the 60–80 cm depth and the Y95 treatment at the 120–140 cm depth, which exceeded those in the CK treatment by 3.17 and 2.46 g kg^{−1}, respectively. Overall, the SOC content increased with increasing fertilization duration and decreased with increasing soil depth, with manure application exerting the most notable effect within the 0–60 cm soil layer.

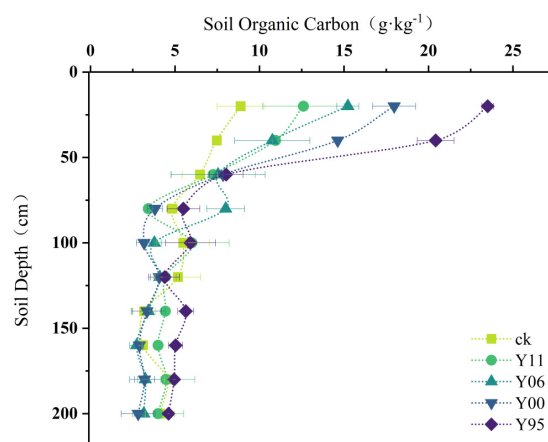


Figure 1. Vertical distribution of SOC in the 0–200 cm soil layer.

3.2. Permanganate-Oxidizable Organic Carbon

Organic fertilizer application significantly affected the content of POXC in the soil. As shown in Figure 2, within the 0–40 cm soil layer, the amount of POXC increased significantly in all the manure treatments compared with that in the CK treatment. Among them, the increase in the Y95 treatment was the greatest, reaching 67.18%, which was significantly greater than that in the other manure application treatments. Within the 40–60 cm soil layer, compared with that in the CK treatment, only the Y95 treatment resulted in a significantly higher content of POXC, while no significant differences were observed among the other treatments. Within the 60–200 cm soil layer, there were no significant differences in the content of POXC among all the treatments. Overall, the content of POXC increased with

increasing fertilization duration and decreased with increasing soil depth, and manure application mainly affected the content of POXC within the 0–60 cm soil layer.

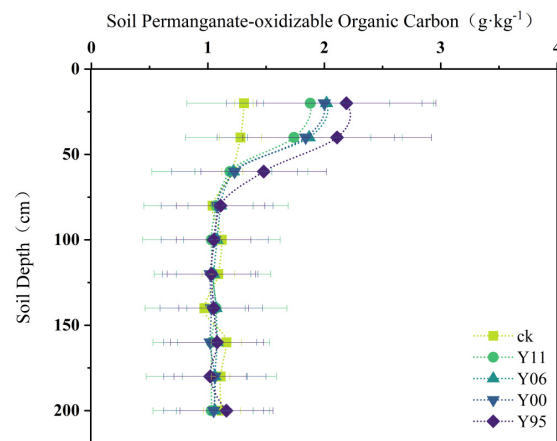


Figure 2. Vertical distribution of POXC in the 0–200 cm soil layer.

3.3. $\delta^{13}\text{C}$ Values of SOC

The $\delta^{13}\text{C}$ value of SOC ($\delta^{13}\text{C}_{\text{SOC}}$) was altered following organic fertilizer application and the cultivation of C_4 plants. As shown in Figure 3, manure application significantly affected the $\delta^{13}\text{C}_{\text{SOC}}$ values at different soil depths. Within the 0–60 cm soil layer, the $\delta^{13}\text{C}_{\text{SOC}}$ values increased in the order of $\text{CK} < \text{Y11} < \text{Y06} < \text{Y00} < \text{Y95}$, and the differences became significant after 19 years of organic fertilization (Y06 treatment). Within the 60–200 cm soil layer, the $\delta^{13}\text{C}_{\text{SOC}}$ values also increased overall with increasing duration of manure application, but the increase was less pronounced than that within the 0–60 cm soil layer. Moreover, compared with the other treatments, only the Y95 treatment resulted in significant differences. Vertically, the $\delta^{13}\text{C}_{\text{SOC}}$ value decreased with increasing soil depth across all the treatments.

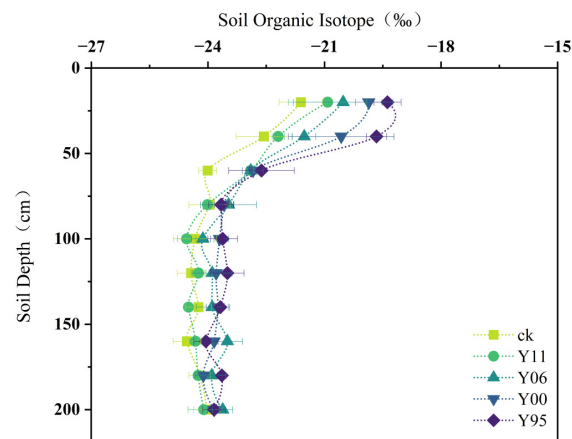


Figure 3. Vertical distribution of $\delta^{13}\text{C}_{\text{SOC}}$ in the 0–200 cm soil layer.

3.4. Sources of SOC

Manure application affected the $\delta^{13}\text{C}$ values of SOC. As shown in Figure 4, with increasing organic fertilization duration, the original SOC pool was increasingly replaced by maize- and manure-derived carbon fractions. The proportion of the original SOC in all the organic fertilizer treatments decreased from 63.15–78.40% to 36.57–58.73%. The proportion of maize-derived carbon increased from 0.95–6.92% to 8.20–23.87%, and the proportion of manure-derived carbon increased from 14.68–35.90% to 31.64–39.56%.

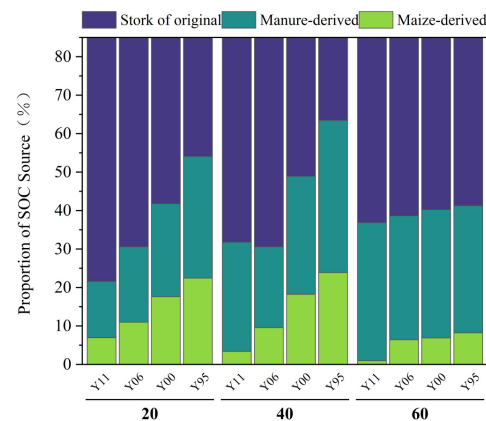


Figure 4. Allocation of SOC sources across the different soil layers.

With increasing soil depth, the proportion of maize-derived carbon decreased from 3.37–23.87% in the 0–40 cm layer to 0.95–8.20% in the 0–60 cm layer. In contrast, the proportion of manure-derived carbon increased from 14.68–39.56% within the 0–40 cm layer to 32.22–35.90% within the 0–60 cm layer. These results indicate that within the 0–60 cm soil layer, the content of maize-derived carbon increased with increasing manure application duration and decreased with increasing soil depth, whereas the content of manure-derived carbon increased with both the manure application duration and soil depth.

3.5. Soil Inorganic Carbon

Organic fertilization affected not only the SOC content but also the SIC content. As shown in Figure 5, organic fertilization increased the SIC content. Within the 0–80 cm soil layer, the SIC content in all the organic fertilizer treatments was significantly greater than that in the CK treatment and increased with increasing organic fertilization duration. The highest SIC content was observed in the Y95 treatment, with a value of 14.92 g kg^{-1} . Among all the soil layers, the greatest increase in the SIC content occurred within the 20–40 cm soil layer. In the 80–140 cm soil layer, the rate of increase in the SIC content decreased. Compared with the CK treatment, only the Y95 treatment provided a significant increase in the SIC content. In addition, the SIC content in the Y11 treatment fluctuated within the 100–140 cm layer and was lower than that in the CK treatment. In the 140–180 cm soil layer, the SIC content increased again under all the fertilization treatments and was generally higher than that in the CK treatment. Within the 180–200 cm soil layer, no significant differences were observed among the treatments.

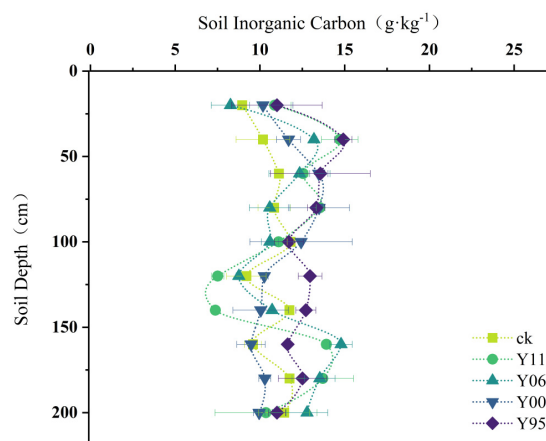


Figure 5. Vertical distribution of SIC in the 0–200 cm soil layer.

Vertically, the SIC content exhibited a fluctuating distribution pattern characterized by an initial increase, followed by a decrease, a slight subsequent increase, and a final decrease. This trend differed from the pattern observed for the SOC content, which increased initially before decreasing and stabilizing. Overall, SOC dominated in the 0–40 cm soil layer, whereas SIC predominated ($>10 \text{ g kg}^{-1}$) over SOC ($<6 \text{ g kg}^{-1}$) in the 60–200 cm soil layer.

3.6. Carbonate Ion Content

Carbonate ions (CO_3^{2-}) can serve as critical diagnostic indicators for distinguishing saline from alkaline soils. Organic fertilization significantly reduced the CO_3^{2-} content in the soil. As shown in Figure 6, within the 0–140 cm soil layer, compared with the CK treatment, all the manure application treatments exhibited significantly lower carbonate ion contents. The most notable reduction was observed in the 0–40 cm layer, where no carbonate ions could be detected in the Y95, Y00, and Y06 treatments. Within the 140–200 cm layer, except for the Y11 treatment, all the other fertilization treatments yielded lower carbonate levels than that in the CK treatment, but the differences were not statistically significant. Vertically, the carbonate content increased with soil depth within the 0–120 cm layer. Except for the Y11 treatment, a decreasing trend was observed with increasing depth in the 120–200 cm soil layer.

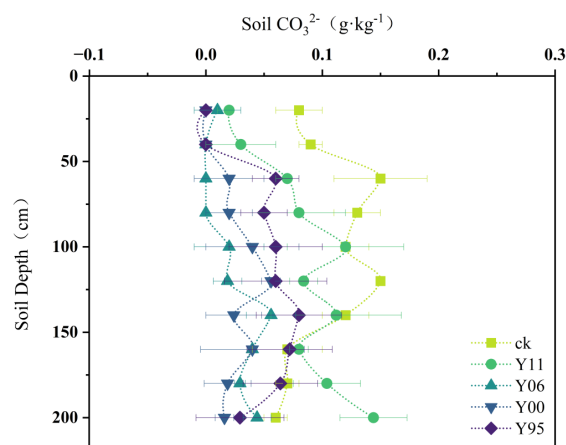


Figure 6. Vertical distribution of CO_3^{2-} in the 0–200 cm soil layer.

3.7. Bicarbonate Ion Content

Long-term organic fertilization influenced the soil HCO_3^- content. As shown in Figure 7, within the 0–120 cm soil layer, the HCO_3^- contents in the CK treatment ranged from 0.01 to 0.07 g kg^{-1} . Organic fertilization increased the soil HCO_3^- content. Compared with that in the CK treatment, the HCO_3^- contents in the Y95, Y00, Y06, and Y11 treatments increased by $0.02\text{--}0.07 \text{ g kg}^{-1}$, $0.01\text{--}0.07 \text{ g kg}^{-1}$, $0.01\text{--}0.11 \text{ g kg}^{-1}$, and $0.01\text{--}0.11 \text{ g kg}^{-1}$, respectively. However, manure application did not significantly influence the HCO_3^- content in deeper soil layers. No statistically significant differences were observed among the treatments in these layers. Notably, within the 140–160 cm and 180–200 cm soil layers, the HCO_3^- content in the CK treatment was slightly greater than that in the other treatments.

In terms of the vertical distribution, the bicarbonate content in the 0–120 cm soil layer progressively increased with increasing manure application duration. In the 120–200 cm soil layer, however, no consistent trend in the bicarbonate content was observed compared with that in the CK treatment.

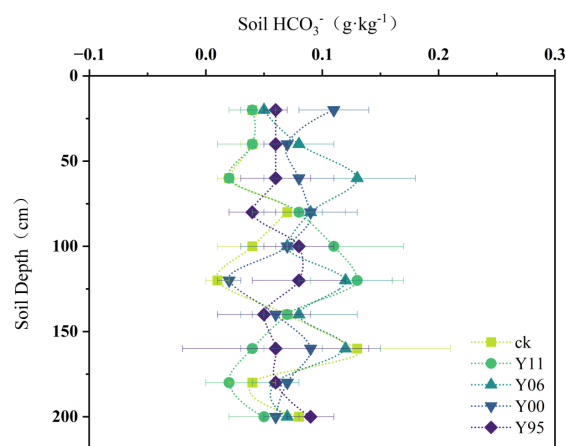


Figure 7. Vertical distribution of HCO_3^- in the 0–200 cm soil layer.

3.8. Correlation Analysis of Soil Carbon Components

Pearson correlation analysis was conducted to evaluate the associations among SOC, POXC, SIC, CO_3^{2-} , and HCO_3^- in the 0–60 cm layer and the whole 0–200 cm soil profile (Table 1). In the 0–60 cm layer, SOC was strongly and positively correlated with POXC ($r = 0.911^{**}$, $p < 0.01$), indicating that labile organic carbon was closely associated with total SOC accumulation. SOC and POXC were both negatively correlated with CO_3^{2-} , with correlation coefficients of -0.628^* ($p < 0.05$) and -0.677^{**} ($p < 0.01$), respectively. In addition, CO_3^{2-} was significantly negatively correlated with HCO_3^- ($r = -0.664^{**}$, $p < 0.01$), suggesting a potential transformation between carbonate and bicarbonate ions in the surface soil.

Table 1. Correlation coefficients of various indicators in the 0–200 cm soil layer ($n = 4$).

Soil Depth	Treatment	SOC	POXC	SIC	CO_3^{2-}	HCO_3^-
0–60 cm	SOC	1	0.911 **	−0.064	−0.628 *	0.195
	POXC	0.911 **	1	−0.024	−0.677 **	0.158
	SIC	−0.064	−0.024	1	−0.2	0.117
	CO_3^{2-}	−0.628 *	−0.677 **	−0.2	1	−0.664 **
	HCO_3^-	0.195	0.158	0.117	−0.664 **	1
0–200 cm	SOC	1	0.945 **	0.056	−0.427 **	−0.084
	POXC	0.945 **	1	0.079	−0.497 **	−0.066
	SIC	0.056	0.079	1	−0.164	−0.129
	CO_3^{2-}	−0.427 **	−0.497 **	−0.164	1	−0.349 *
	HCO_3^-	−0.084	−0.066	−0.129	−0.349 *	1

Note 1: * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

Across the 0–200 cm profile, SOC remained highly positively correlated with POXC $r = 0.945^{**}$, $p < 0.01$. SOC and POXC were also significantly negatively correlated with CO_3^{2-} , with correlation coefficients of -0.427^{**} and -0.497^{**} , respectively ($p < 0.01$). A significant negative correlation was also observed between CO_3^{2-} and HCO_3^- ($r = -0.349^*$, $p < 0.05$). However, SIC showed no significant correlation with SOC, POXC, CO_3^{2-} , or HCO_3^- in either soil layer. These results indicate that SOC accumulation was closely linked to the increase in labile organic carbon, whereas the relationships between organic carbon indicators and carbonate ions were stronger in the 0–60 cm layer than in the whole 0–200 cm profile.

4. Discussion

4.1. Effects of Long-Term Manure Application on SOC and Its Fractions

The SOC content serves as a critical indicator for evaluating both soil carbon storage and soil fertility [7]. Saline-sodic soils generally contain low SOC levels, and manure application has been demonstrated to increase the SOC content in such soils [24]. In this experiment, long-term manure application significantly increased the SOC content, with the extent of increase positively correlated with the fertilization duration, which is consistent with findings reported by Hao and Liang et al. [25,26]. This phenomenon can be attributed to the following two primary mechanisms: first, the application of cattle manure enhances the carbon input into the soil, thereby supplying nutrients that stimulate microbial activity. This increased input, in turn, accelerates the decomposition of organic residues and the transformation of certain inorganic compounds, thereby promoting the accumulation of total SOC [24]. Second, the applied cattle manure facilitates the conversion of microaggregates into macroaggregates within the soil matrix, which increases the capacity of the soil for carbon sequestration [27].

The influence of manure application on the SOC content in saline-sodic soils exhibited distinct vertical stratification characteristics. Manure application primarily influenced the SOC content within the 0–60 cm soil layer but minimally influenced that within the 60–200 cm layer. This pattern occurs because long-term manure application significantly mitigates salt stress, reduces the surface soil pH and total salt content, and enhances microbial activity. Additionally, organic fertilizers contain substantial amounts of organic carbon inputs, which, upon incorporation into the topsoil, are partially converted into stable SOC through microbial processes. Consequently, prolonged organic fertilization promotes the accumulation of SOC and microbial biomass carbon within the 0–60 cm layer [28]. Furthermore, as cattle manure is primarily applied to the soil surface and as plant roots are densely distributed within the 0–40 cm layer, root activities reinforce the benefits of organic amendments by improving the soil structure, supplying nutrients, and stimulating root growth [29]. In contrast, the 60–200 cm soil layer is characterized by oxygen deficiency, an elevated pH, salt accumulation, and stable temperature conditions, which notably inhibit microbial activity. Moreover, the limited downward transport of exogenous carbon may further restrict SOC accumulation in deep soil [30].

Soil labile organic carbon refers to the fraction of SOC characterized by high availability, rapid transformation, and high microbial activity. This fraction actively participates in soil nutrient cycling and can effectively reflect short-term fluctuations in SOC levels [31,32]. In this study, within the 0–60 cm soil layer, compared with the CK treatment, all the organic fertilization treatments yielded a significantly higher content of POXC, and the content increased progressively with increasing fertilization duration. On the one hand, organic fertilizers themselves are rich in easily decomposable active organic carbon components [33]. Hence, the continuous annual application of organic fertilizers leads to the continuous input of these permanganate-oxidizable organic carbon sources into the soil. On the other hand, long-term application of organic fertilizers can facilitate the mobilization of existing soil carbon pools, stimulate soil microbial activity, and accelerate the decomposition and transformation of both newly added and preexisting organic materials, thereby contributing indirectly to the expansion of the POXC [34]. In this experiment, no significant differences were observed in the content of POXC among treatments within the 60–200 cm soil layer. This observation may be attributed to the decline in microbial activity with increasing soil depth, which is associated with lower biological activity and slower carbon turnover in deeper soil layers, thereby reducing the decomposition rate of organic compounds. Consequently, POXC contents decrease with depth. Furthermore, deeper soil layers are characterized by longer residence times and lower bioavailability of SOC. Thus, during

the study period, POXC accumulation was predominantly observed in surface soils, while changes in deeper layers remained comparatively limited [35].

4.2. Effects of Long-Term Manure Application on SIC and Its Fractions

Previous studies have indicated that long-term application of organic fertilizers can increase the SIC content. However, such increases occurred primarily in the 0–60 cm soil layer [24,36]. Other studies revealed an increasing trend in SIC across the 0–160 cm soil profile following prolonged organic fertilizer application, but the changes were not statistically significant. In this study, the distribution pattern of SIC differed slightly, fluctuating across soil depths. Specifically, the SIC content was significantly greater in all the manure application treatments than in the CK treatment within the 0–80 cm and 140–180 cm layers, with the largest increase observed in the 0–80 cm layer. Within the 80–140 cm layer, although the SIC content increased across manure application treatments, compared with that in the CK treatment, only the Y95 treatment (which represents 30 years of exclusive manure application) provided a significantly increased SIC content. The observed increase in the SIC content may be attributed to the abundance of Ca^{2+} and Mg^{2+} ions in the applied manure, as well as the substantial release of CO_2 from manure decomposition and root respiration. The dissolved CO_2 forms carbonate species in soil solution, which combine with Ca^{2+} and Mg^{2+} to precipitate as stable carbonate minerals, thereby facilitating long-term carbon sequestration [24]. Alternatively, manure application may increase root biomass and activity levels, thereby creating a biochemical environment conducive to SIC accumulation [37]. Concurrently, the application of organic manure promotes the transformation of microaggregates into macroaggregates. These aggregates can encapsulate carbonate minerals, inhibit dissolution, and contribute to the accumulation of SIC [26,38]. Furthermore, the microbial biomass significantly increases under organic fertilization regimes [17], leading to increased respiratory CO_2 production. The elevated partial pressure of CO_2 in soil induces partial carbonate dissolution within the surface layer. However, the dissolved inorganic carbon does not simply disappear. Instead, it migrates downward as ions with soil water. Upon reaching deeper soil layers, changes in environmental conditions (e.g., pH, water evaporation, and reduced CO_2 partial pressure) may favor carbonate precipitation. Consequently, the observed increase in SIC may reflect the potential redistribution and accumulation of inorganic carbon within the soil profile and may also suggest possible secondary carbonate formation [39,40].

SIC refers primarily to carbonate minerals formed during the weathering of soil parent rocks, including primary and secondary carbonates [41]. While HCO_3^- in the soil solution and CO_2 in soil air account for a relatively small proportion of the total SIC pool, both play crucial roles in the formation of secondary carbonates. The HCO_3^- and CO_3^{2-} in the soil solution constitute key components of the soil dissolved inorganic carbon (SDIC) fraction, accounting for nearly its entire content [42]. In this experiment, manure application resulted in a significant decrease in the CO_3^{2-} content and an increase in the HCO_3^- content within the 0–120 cm soil layer. The transformation of SIC is controlled by carbonate equilibrium. On one hand, in saline-sodic soils, carbonate speciation is strongly influenced by soil pH. Application of organic fertilizers reduces the soil pH, shifting the chemical equilibrium toward the generation of HCO_3^- . On the other hand, organic fertilizers (such as manure and straw) generate various organic acids (e.g., humic acid and fulvic acid) and CO_2 during decomposition, thereby shifting the carbonate equilibrium toward HCO_3^- [43]. Furthermore, the microbial decomposition of organic fertilizers is associated with the release of substantial amounts of CO_2 , which dissolves in soil water to form carbonic acid. This acid then reacts with salts such as calcium carbonate (CaCO_3) in the soil, thereby converting them into HCO_3^- . Meanwhile, dissolved inorganic carbon can be redistributed through

soil water movement, and its accumulation is further influenced by leaching and seasonal hydrological conditions. Owing to limited precipitation infiltration and intense evaporative pumping in the semi-arid area, secondary carbonates undergo only minor downward migration, while SIC transformation and reprecipitation, mainly fueled by roots and organic acids, remain largely concentrated in the upper horizon. In semi-arid saline-sodic soils of the Songnen Plain, seasonal alternation between rainfall infiltration and strong evaporation may facilitate the vertical redistribution of dissolved inorganic carbon. Moreover, shallow groundwater dynamics may further influence carbonate equilibrium and SIC accumulation. Therefore, the observed changes in CO_3^{2-} and HCO_3^- are more likely to reflect dynamic geochemical equilibrium processes than a simple one-way transformation [39].

Studies have demonstrated that secondary carbonates may form through the dissolution and reprecipitation of primary carbonates, as well as through reactions between Ca^{2+} or Mg^{2+} and CO_2 in soil [19]. Carbonate dissolution is induced under conditions such as low Ca^{2+} and HCO_3^- contents in the soil solution, low pH levels, elevated CO_2 partial pressures, and high soil moisture contents. Conversely, under the opposite environmental conditions, soil carbonate precipitation occurs, thereby facilitating the formation and transformation of secondary carbonates [44].

4.3. Effects of Long-Term Organic Manure Application on SOC Sources

Cultivation and fertilization practices significantly influenced the $\delta^{13}\text{C}_{\text{SOC}}$ value of SOC ($\delta^{13}\text{C}_{\text{SOC}}$). In this study, following the implementation of maize cultivation and organic fertilizer application, the soil $\delta^{13}\text{C}_{\text{SOC}}$ value increased with increasing duration of both cropping and organic fertilization. This increase could be attributed to several factors. First, the shift in vegetation type plays a critical role in this process. The native vegetation in the saline-sodic soil area comprises C3 plants, whereas maize is a C4 plant. As the cultivation system transitioned to C4 crops and cultivation progressed, the proportion of C4-derived carbon in the SOC pool increased, consequently increasing the $\delta^{13}\text{C}_{\text{SOC}}$ value [45]. Furthermore, the application of organic fertilizer contributed to the increase in $\delta^{13}\text{C}_{\text{SOC}}$, which is consistent with the findings of Hao [25] and Jin [46]. Long-term organic fertilization increases the alkyl-carbon content and modifies the chemical structure of SOC [47]. Additionally, microbial fractionation plays a significant role. Microorganisms preferentially use lighter ^{12}C during the decomposition of SOM, leading to the relative enrichment of ^{13}C in microbial metabolites and residues. This isotopic fractionation persists throughout SOM decomposition and transformation processes, further contributing to the increase in soil $\delta^{13}\text{C}_{\text{SOC}}$ [48].

The variation in soil $\delta^{13}\text{C}_{\text{SOC}}$ values corresponded to shifts in the sources of SOC. Under natural saline-sodic soil conditions, in which sparse aboveground vegetation occurs and the degree of plant coverage is low, plant-derived carbon constitutes a relatively small proportion of SOC, with the majority originating from the intrinsic SOC. However, with increasing duration of maize cultivation and organic fertilizer application, the contributions from maize- and manure-derived carbon progressively increase. Zhang et al. [49] reported that after nine years of organic fertilization in saline-sodic soil, the contribution of manure-derived carbon to the SOC pool was 25.92%, whereas maize root residue contributed 14.36%. In this study, after 14–30 years of organic fertilization, the proportions of manure-derived carbon ranged from 14.68% to 39.56%, and maize root residue-derived carbon contributed 6.91% to 14.69% to SOC, which is relatively similar to prior findings. The change in SOC sources was closely associated with the input of exogenous carbon, such as organic manure and crop residues. As a crucial source of SOC, the contribution of organic manure may surpass that of crop residues. This occurs because the addition of high-C/N ratio materials such as straw stimulates microbial activity [50], leading to the

formation of substantial amounts of labile organic carbon, which serve as the core carrier of short-term carbon sinks but exhibit relatively rapid turnover [51]. In contrast, organic manure (e.g., decomposed cattle dung) initially decomposes during composting, and upon application to the soil, it exhibits greater potential for generating mineral-associated organic carbon. This carbon fraction, which is bound to soil minerals, demonstrates enhanced stability [52]. Consequently, the proportion of manure-derived carbon is greater than that of maize-derived carbon.

Furthermore, with increasing soil depth, the proportion of plant-derived organic carbon in saline-sodic soils gradually decreased. Generally, plant litter, root exudates, and fine roots are distributed mainly within the 0–30 cm surface layer, and their contents decrease rapidly at greater depths. The high pH and salinity of saline-sodic soils inhibit deep root growth, which results in minimal plant-derived carbon input into deeper soil layers [51]. This phenomenon contributes to the reduction in plant-derived carbon in deep soils. In this study, both the total SOC and POXC contents decreased significantly with soil depth. As a component of SOC, the absolute and relative amounts of plant-derived carbon also decreased, yielding a reduced contribution of maize-derived carbon [52].

Overall, long-term manure application significantly altered the SOC sources. This modification primarily involved the input of exogenous organic carbon, which became the dominant contributor to SOC while simultaneously promoting the transformation and renewal of preexisting SOC. It should be noted that the contributions estimated by the binary mixing model represent relative estimates, and the uncertainty associated with source partitioning was not quantitatively assessed because the $\delta^{13}\text{C}$ value of manure was unavailable.

4.4. Relationships Among Organic Carbon Fractions and Carbonate Transformation

Correlation analysis further indicated a close linkage between SOC accumulation and POXC transformation in saline-sodic soil. This result is consistent with the findings of Qu et al. [53]. The consistently positive correlation between SOC and POXC suggests that POXC is a sensitive indicator of SOC changes under long-term manure application and reflects changes in the active fraction of SOC [54].

The negative correlations of SOC and POXC with CO_3^{2-} suggest that increased organic carbon input may influence carbonate speciation, particularly in the 0–60 cm layer, where manure incorporation and biological activity are greatest [55]. Concurrently, organic matter decomposition releases CO_2 and organic acids, which can reduce soil alkalinity and facilitate the conversion of CO_3^{2-} to HCO_3^- . The negative correlation between CO_3^{2-} and HCO_3^- further supports this carbonate-bicarbonate transformation [53].

In contrast, soil inorganic carbon (SIC) showed no statistically significant correlations with SOC, POXC, CO_3^{2-} , or HCO_3^- in either depth interval, indicating that SIC accumulation is not directly governed by organic carbon content alone. This observation is consistent with Naorem et al. [56]. Rather, SIC dynamics appear to be predominantly regulated by physicochemical drivers, including Ca^{2+} and Mg^{2+} availability, soil water flux, carbonate mineral dissolution kinetics, and secondary carbonate precipitation [57]. Moreover, the attenuated strength of correlations in the 0–200 cm profile implies that the influence of surface-derived organic inputs on carbonate speciation diminishes with depth, while subsurface physicochemical processes, including dissolved inorganic carbon transport, solute leaching, and possible carbonate precipitation, may become relatively more important [39]. Collectively, manure application enhances SOC content and indirectly influences the formation of SIC by regulating carbonate transformation processes. The formation of SIC is also regulated by multiple interacting biogeochemical processes.

5. Conclusions

Organic fertilization substantially increased the carbon sequestration capacity of saline-sodic soil. Following manure application, both the SOC and POXC contents increased greatly, with the most notable effects occurring in the 0–60 cm soil layer. Moreover, the SIC content increased across the entire profile, particularly within the 0–80 cm layer. Concurrently, the CO_3^{2-} content decreased, whereas the HCO_3^- content increased in the fertilized soils. Long-term manure application combined with maize cultivation led to a significant increase in $\delta^{13}\text{C}_{\text{SOC}}$ ($p < 0.01$), which continued to increase with increasing fertilization duration. After three decades of manure application, maize residue-derived carbon and native SOC constituted 22.42% and 45.94% of total SOC, respectively; manure-derived carbon, estimated indirectly, accounted for the remaining 31.64%, indicating that its contribution to SOC accumulation exceeded that of maize residue-derived carbon. With increasing soil depth, the proportion of maize-derived organic carbon decreased, whereas that of manure-derived carbon increased. Longer manure application durations were associated with a larger decrease in maize-derived carbon and a smaller increase in manure-derived carbon.

However, this study mainly evaluated carbon content changes and source dynamics, while profile-scale carbon stocks were not quantified because reliable soil bulk density measurements could not be obtained due to the influence of a high groundwater table at the study site. In addition, some manure-related parameters (e.g., $\delta^{13}\text{C}$ signature, moisture content, and annual carbon input) were unavailable, which limited further quantitative evaluation of carbon input and source partitioning. Future research integrating assessments of soil carbon stocks, characterization of secondary carbonate formation, and long-term environmental monitoring is required to rigorously evaluate the role of manure management in advancing carbon-neutral agricultural systems.

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