

Article

# Corn and Rice Cultivation Affect Soil Organic and Inorganic Carbon Storage through Altering Soil Properties in Alkali Sodic Soils, Northeast of China

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**Abstract:** Soil organic carbon (SOC) and soil inorganic carbon (SIC) play essential roles in carbon cycling in terrestrial ecosystems; however, the effects of crop cultivation on them are still poorly understood, especially in alkali sodic soils widely distributed in semiarid regions. Alkali sodic soils from cornfields and paddies with cultivation years of 5, 15, and 25 were analyzed here to assess the response of soil properties and soil carbon pools to crop cultivation. Soil pH and exchangeable sodium percentages decrease in accordance with cultivation years, while enzyme activity (amylase, invertase, and catalase) shows a contrary trend. Soil pH and exchangeable sodium percentages are negatively correlated with SOC, but positively correlated with SIC. Redundancy analysis reveals an obvious relationship between SOC and invertase activity. The percentage of  $\delta^{13}\text{C}_{\text{SOC}}$  found here is approximately  $-24.78\text{‰}$  to  $-22.97\text{‰}$  for cornfields and approximately  $-26.54\text{‰}$  to  $-23.81\text{‰}$  for paddies, suggesting that crop cultivation contributes to SOC sequestration and stocking, increasing with cultivation years. The percentage of  $\delta^{13}\text{C}_{\text{SIC}}$  found here is approximately  $1.90\text{‰}$  to  $3.73\text{‰}$ , proving that lithogenic inorganic carbon is the major SIC, where the stock decreases with increasing cultivation years. Significant total carbon stock loss is observed in cornfields, while it is preserved at  $120 \text{ Mg ha}^{-1}$  in paddies. We conclude here from the results that corn and rice cultivation reduce alkali sodic conditions in soil, thereby improving soil enzymes and favoring SOC stocking, but reducing SIC stocks.

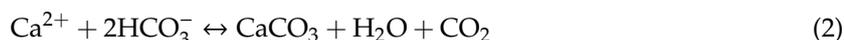
**Keywords:** soil organic carbon; soil inorganic carbon; crop cultivation;  $^{13}\text{C}$  isotope; alkali sodic soil

## 1. Introduction

Global warming is receiving more and more attention because of the series of threats it poses, like sea-level rise, land area decline, extreme climates, and food supply shortages, which challenge the sustainable development of eco-environments and social economy [1]. Greenhouse gas (GHG) emissions, including carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ), and nitrous oxide ( $\text{N}_2\text{O}$ ), are responsible for global warming, owing to the greenhouse effects. Carbon (C) pools in soil are the carbon source for  $\text{CO}_2$  and  $\text{CH}_4$  production and emission. Overall, 52% of global anthropogenic  $\text{CH}_4$  is emitted from agricultural land [2]. Holding vast reserves of C, the C variance in agricultural lands impacts the atmospheric GHG concentration and global climatic conditions. Cultivation practices regulate GHG emission and the global C cycle through altering the distribution and transformation of C in soil.

Soil organic carbon (SOC) is one of the main focuses of studies because of its vital role in regulating ecosystem function and greenhouse effects. SOC mainly is produced from root exudates and the

decomposition of litter and crop residues [3–5]. The progress is susceptible to cultivation practices, which adjust soil environments, i.e., the soil pH, soil water content, enzyme activities, and soil microbes [6–9]. Kuzyakov and Domanski (2000) noted that wheat and barley transport about 30% of the assimilated C production into soil [10]. Muller et al. (2016) reported that corn increased SOC content, especially in topsoil [11]. Rice cultivation gathers C in soil, while the C input in paddies is even lower (or higher) than that in upland areas [12,13]. However, few studies have taken the soil inorganic carbon (SIC) pool into account, which is the most common form of carbon in semiarid regions, representing the storage of ~695–1738 Pg (1 Pg = 10<sup>15</sup> g) when assessing soil C sequestration [14,15]. SIC is classified as two central units stored in soil, namely, lithogenic inorganic carbon (LIC), inheriting from the parent material, and pedogenic inorganic carbon (PIC), resulting from the precipitation of carbonate ions. The formation of pedogenic carbonate, shown in Equations (1) and (2), can either be a source or sink of C [16]. In the input or output of soil, C is transformed in the process of precipitation. One unit of CO<sub>2</sub> is consumed when a carbonate is dissolved, while, on the other hand, one unit of CO<sub>2</sub> is released when a carbonate is redeposited. Cultivation regulates carbonate dissolution and precipitation by altering the soil pH, soil water, ion activity (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Al<sup>3+</sup>, K<sup>+</sup>, and Fe<sup>3+</sup>), and CO<sub>2</sub>, either from the atmosphere or via microbial respiration [17–19]. Studies have reported that soil pH decreases or increases during rice cultivation, while increased pH is found for soybean cultivation [20,21]. Jaiyeoba (2003) noted that cation exchange capacity (CEC) declines after crop cultivation [22]. Cultivation and its management, i.e., fertilization and irrigation, governs SIC dynamics in soils through regulating the soil environment, SOC contents, and hydrological conditions [23,24]. Dong et al. (2017) noted that long-term fertilizer significantly decreased SIC content in a two-year crop rotation system, that is, winter wheat, summer corn, spring corn, and winter fallow for each two-year period [25], while Bughio et al. (2016) reported that irrigation and fertilization in a wheat–maize rotation system increased SIC content when compared with bare field [17]. Changes in the stock of SIC, induced by crop cultivation and its management, are still unclear. Thus, an accurate estimate, including both SOC and SIC, is required to assess the dynamics of soil C pools during cultivation in agricultural lands.



Soil natural isotope analysis ( $\delta^{13}\text{C}$ ) is used to analyze the dynamics of SOC in terrestrial ecosystems because it provides the isotope signatures of soil composition [26,27]. As is well known, the  $\delta^{13}\text{C}$  value of SOC for a C<sub>4</sub> plant (Hatch–Slack pathway during photosynthesis) is approximately −15‰ to −7‰, while it is approximately −35‰ to −20‰ for C<sub>3</sub> plants (Calvin cycle during photosynthesis) [28,29]. The  $\delta^{13}\text{C}$  value is a sensitive indicator to clarify the contribution of plants or crops to SOC sequestration. Chen et al. (2018) noted that, in restored mangrove forests, SOC relies on mangrove organic matter, using the  $\delta^{13}\text{C}$  values [30]. Chen et al. (2018) also reported that maize mulch increases SOC by 22% in topsoil [31]. Likewise, LIC and PIC are distinguished by  $\delta^{13}\text{C}$ , which is 0‰ for LIC, while for PIC it is related to the  $\delta^{13}\text{C}$  of SOC [17,32]. Jin et al. (2014) noted that the  $\delta^{13}\text{C}$  of SIC in grassland is lower than the forest land of the Loess Plateau, showing that grasslands produce more PIC than forest land [33]. An et al. (2019) reported that the desertification of grassland contributes to PIC formation in topsoil [34]. Therefore, soil  $\delta^{13}\text{C}$  analysis can be of great assistance in analyzing soil C pool transformations and evaluating the influence of different crops on soil C pools in agricultural lands.

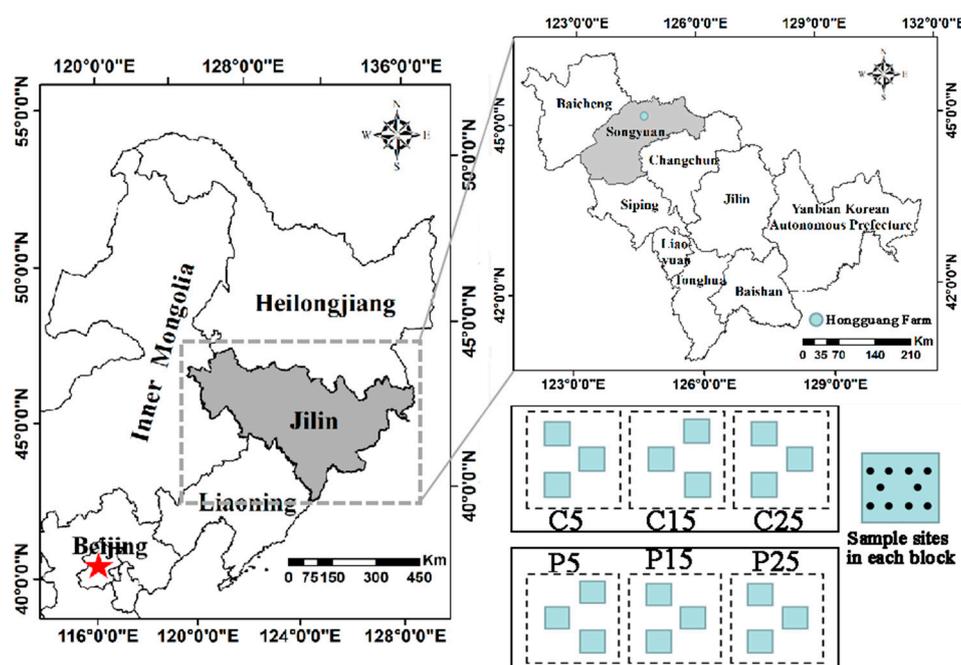
Soil desertification is one of the most severe land degradation problems and has occurred on a global scale for centuries [35]. Alkali sodic soil mitigates C sequestration and adjusts the global C cycle through reducing microbial activity and microbial biomass, microbial community structure, and soil respiration [36–38]. Because of land degradation, alkali sodic soils are widely distributed in the northeast of China, which includes arid and semiarid regions. To ease food shortages, the local government has carried out large-scale crop cultivation in saline–alkali soils since the 1950s. Grasslands have been converted into agriculture lands, cultivating corn and rice in large amounts,

supplying fresh organic material in soils, and increasing microbial and faunal activities [39]. However, the impacts of long-term crop cultivation on soil carbon pools in alkali sodic agricultural lands are still poorly understood. We hypothesize that crop cultivation alters soil properties and enzyme activities, affecting SOC and SIC stocks in cornfields and paddies. Thus, the objectives of the present study are to (i) analyze the alterations in soil physical and chemical properties, as well as enzyme activities in cornfields and paddies with different cultivation years, (ii) assess the response of SOC and SIC content, natural isotopes, and carbon stocks on corn and rice cultivation, and (iii) to evaluate the influence of crop cultivation on SOC and SIC, as affected by soil properties and enzyme activities.

## 2. Materials and Methods

### 2.1. Site Description

The study site was located in Songyuan, Jilin province, China (Figure 1), and is southeast of the Songnen Plain, where the area of salinized land is  $1.1 \times 10^6$  ha [40]. The region has a semiarid and subhumid continental monsoon climate, with an annual average temperature of  $5.6^\circ\text{C}$  and an annual precipitation of 446 mm. Soils have been cultivated regularly there since the 1950s, before which they were covered with grass, *Leymus chinensis*, *Suaeda glauca*, and *Puccinellia distans* being the primary forms of vegetation.



**Figure 1.** The location of the study area (Songyuan) in Jilin province, in the northeast of China.

Two tillage systems with one crop per year, planting in May and harvesting in October, have been investigated in the experiment. Three neighboring cornfields and three neighboring paddies were selected and studied, with areas of 1 ha ( $100 \times 100$  m) per field, in Hongguang farm, Songyuan, with an elevation of 140 m above the mean sea level. The main landform here is plain, and the soil type is meadow soil (Eutric Vertisol [41]). The fields were covered with native degraded grass vegetation (*Leymus chinensis*, C3 plant) before crop cultivation, without any prior cultivation or fertilization. Here, the grass cover level was less than 50% in 1991, when C25 and P25 were cultivated, and it has decreased steadily due to climate change, soil desertification, and human activities. Three rain-fed cornfields have been cultivated for 5 (C5), 15 (C15), and 25 years (C25), respectively, under the same conventional tillage management method. Maize seeds (*Zea mays* L., C4 plant) and base fertilizer were applied together by a planter machine. A blended fertilizer (16% N, 16%  $\text{P}_2\text{O}_5$ , and 16%  $\text{K}_2\text{O}$ ) was

applied with an application rate of  $200 \text{ kg ha}^{-1}$ , and  $180 \text{ kg ha}^{-1}$  urea was applied in the jointing stage. Three paddies, cultivated for 5 (P5), 15r (P15), and 25 years (P25), respectively, were under continuous irrigation, where the soil was covered with water at a depth of 5 cm. The soils were irrigated sufficiently before planting, and then rice (*Oryza sativa* L., C3 plant) was planted by a planter machine. The base fertilizer, a blended fertilizer (17% N, 18%  $\text{P}_2\text{O}_5$ , and 19%  $\text{K}_2\text{O}$ ) with a rate of  $200 \text{ kg ha}^{-1}$ , was applied after planting, while  $150 \text{ kg ha}^{-1}$  urea was applied in the tillering stage manually. Weed control was carried out manually with broad-spectrum herbicides. Corn and rice were harvested by machines. Crop straws were collected and processed into fertilizer, fodder, and fuel. All fields were plowed by a machine with a depth of 20 cm before planting. The irrigation water originates from Nen River and the groundwater depth ranges from 1.0 to 2.5 m.

## 2.2. Soil Sampling

Soils with the depth of 0–20 cm (surface layer) and 20–40 cm (subsurface layer) were sampled on September 25, 2016. We established three blocks ( $15 \times 15 \text{ m}$ ) in each field to collect soil subsamples (ten per layer) randomly (Figure 1), using a soil auger (7 mm). Subsamples from the same block and layer were homogeneously mixed to generate a composite sample. A total of 36 samples were collected and sent to the lab. After removing visible roots and plant material with tweezers, the soil samples were air-dried at room temperature and sieved to  $<2 \text{ mm}$  for further analysis. Soil cores ( $100 \text{ cm}^3$ ) in three duplicates from each layer in each field were collected for soil bulk density (BD) analysis when sampling.

## 2.3. Soil Chemical Analysis

Core samples were heated in an oven at  $105 \text{ }^\circ\text{C}$  for 48 h to identify the soil BD. A laser particle diameter analyzer (Bettersize 2000, Liaoning, China) was employed to identify the soil texture (i.e., the sand, silt, and clay proportion). Soil pH and electrical conductivity (EC) were tested at a ratio of 5 to 1 (water to soil). The exchangeable sodium percentage (ESP) was calculated by Equation (3) after measuring the content of exchangeable sodium ( $C_{\text{Na}^+}$ ) and the cation exchange capacity (CEC) [42]. Briefly, 2.0 g of soil, mixed with 80 mL EDTA-ammonium acetate, was centrifuged for 5 min at 3000 rpm. The clear liquid, with 3 mL aluminum sulfate solutions (0.1 M), was diluted to test  $C_{\text{Na}^+}$  using an atomic absorption spectrophotometer. The sediment was rinsed with ethyl alcohol, distilled, and then titrated by HCl (0.05 M) to determine the CEC. A TOC analyzer (Shimadzu TOC-V SSM-5000, Japan) was employed for total carbon (TC), soil organic carbon (SOC), and SIC analysis. Briefly, two sets of soils with a mass of 50 mg were put in sample boats separately, which were closed in the TOC analyzer. One sample for total carbon (TC) was burned at the temperature of  $900 \text{ }^\circ\text{C}$ , while the other, for inorganic carbon (IC), was burned at  $200 \text{ }^\circ\text{C}$  immediately after adding phosphoric acid. TC and IC were computed automatically with the  $\text{CO}_2$  emitted from the samples, while SOC was calculated from the TC and IC.

$$\text{ESP} = C_{\text{Na}^+} / \text{CEC} \times 100 \quad (3)$$

## 2.4. Carbon Isotope Analyses

The isotope analyses of SOC and SIC were carried out with a stable isotope mass spectrometer (Isoprime 100, Langensfeld, Germany). For the former, 5.0 g of soil was reacted with an overdosed HCl (1 M) solution for 24 h to remove SIC. They were washed with deionized water until the pH was less than 5, and then dried at  $40 \text{ }^\circ\text{C}$ . Then, the samples were combusted in an evacuated sealed quartz tube in the presence of silver foil and cupric oxide for 2 h at  $850 \text{ }^\circ\text{C}$ . The  $\text{CO}_2$  emitted from the reaction was extracted and analyzed with the spectrometer [43]. SIC isotope composition was determined from the  $\text{CO}_2$  gas emitted from air-dried soils reacting with  $\text{H}_3\text{PO}_4$  for 2 h at  $80 \text{ }^\circ\text{C}$  [34]. The  $\delta^{13}\text{C}$  values

of SOC ( $\delta^{13}\text{C}_{\text{SOC}}$ ) and SIC ( $\delta^{13}\text{C}_{\text{SIC}}$ ) were calculated with Equation (4) [44]. Here, Vienna Pee Dee Belemnite (VDPB) was the standard used. Three multiple samples were tested for each sample.

$$\delta^{13}\text{C} = (\text{R}_s + \text{R}_c) / \text{R}_c \times 1000\text{‰} \quad (4)$$

where  $\delta^{13}\text{C}$  is the natural abundance of SOC or SIC for the samples (‰),  $\text{R}_s$  is the stable C isotope compositions of SOC or SIC, and  $\text{R}_c$  is the isotopic composition of the standards.

### 2.5. Soil Enzyme Assays

Enzyme activities were measured following the method described by Zhang [45]. Amylase and invertase activities were determined by colorimetric assays. Briefly, 5.0 g of air-dried soil was reacted with a 10 mL substrate (1% starch solution) and 10 mL phosphate buffer at pH 5.5 for 24 h at 37 °C. One mL of the liquid filtrated from the mixture was pipetted into a 50 mL flask to react with 2.0 mL of 3,5-dinitrosalicylic acid in boiling water for 5 min. After cooling, it was diluted with deionized water to volume and quantified at 508 nm using a spectrophotometer (UV-2100, Beijing, China). For invertase, the substrate was a 15 mL sucrose solution (8%), the volumes of the phosphate buffer and 3,5-dinitrosalicylic acid were 5.0 mL and 3.0 mL respectively. Controls without a substrate or soil were processed to obtain the amylase and invertase activities, expressed as  $\mu\text{g glucose g}^{-1} \text{ soil h}^{-1}$ . In order to determine the catalase activity, 2.0 g of air-dried soil was mixed with 40 mL of deionized water and reacted with 5 mL of  $\text{H}_2\text{O}_2$  (0.3%) for 20 min, then filtrated and titrated with  $\text{KMnO}_4$  ( $0.02 \text{ mol L}^{-1}$ ). Catalase activity was expressed as  $\text{mL KMnO}_4 \text{ g}^{-1} \text{ soil h}^{-1}$ .

### 2.6. Statistical Analysis

Total carbon stocks ( $\text{Mg ha}^{-1}$ ) were calculated as the sum of SOC and SIC densities, which were calculated using Equation (5) [46]:

$$\text{SD} = \sum_{i=1}^2 \text{C}_i \times \text{BD}_i \times \text{H} \times 0.1 \quad (5)$$

where SD is the SOC or SIC density ( $\text{Mg ha}^{-1}$ ),  $\text{C}_i$  and  $\text{BD}_i$  are the SOC or SIC concentration ( $\text{g kg}^{-1}$ ) and bulk density ( $\text{g cm}^{-3}$ ) in the layer  $i$ , respectively, and H is the thickness of soil (here,  $\text{H} = 20 \text{ cm}$ ).

Statistical analysis was performed with the SPSS 19.0 software package (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) with separation of means by a least significant difference (LSD) test at  $p < 0.05$  was computed to assess the significant differences of soil properties, enzyme activities, and C pools among fields with different cultivation years, while the t-test was employed to assess the significant differences between soil layers. The redundancy analysis (RDA) was conducted via CANOCO 4.5 (Microcomputer Power, Inc., Ithaca, NY, USA) to assess the relationships between soil carbon pools and soil physical–chemical properties and enzyme activities. SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA) was used for the figures.

## 3. Results

### 3.1. Soil Physical–Chemical Properties

The soil physical and chemical indicators are summarized in Table 1. Soils were alkaline for pH values ranging from 7.43 to 10.04 in cornfields and from 7.74 to 9.16 in paddies, despite the fact that their values were reasonably high in the subsurface layer. The EC in cornfields was 0.18–0.45  $\text{ms cm}^{-1}$ , and the largest value appeared in C5. They ranged from 0.22 to 0.46  $\text{ms cm}^{-1}$  in paddies, where relatively large values appeared in the surface layer. Soils in the study area were slightly sodic, with ESP <15% indicating strongly sodic soil, with ESP = 24.32% in C5. The texture of the cornfield and paddy was of silt loam soil, although the silt content was higher in paddies compared with the cornfields. The soil

bulk density ranged from 1.27 to 1.41 g cm<sup>-3</sup> in cornfields, while it ranged from 1.30 to 1.66 g cm<sup>-3</sup> in paddies, where larger values appeared in the subsurface layer.

**Table 1.** Soil physical and chemical properties (n = 3).

|     |          | pH                 | EC<br>(ms cm <sup>-1</sup> ) | ESP<br>(%)         | Sand<br>(%)        | Silt<br>(%)        | Clay<br>(%)        | BD<br>(g cm <sup>-3</sup> ) |
|-----|----------|--------------------|------------------------------|--------------------|--------------------|--------------------|--------------------|-----------------------------|
| C5  | 0–20 cm  | 9.04 ± 0.26<br>Ab  | 0.42 ± 0.03<br>Aa            | 17.51 ± 1.14<br>Ab | 37.29 ± 2.58<br>Aa | 58.98 ± 1.18<br>Aa | 4.39 ± 0.41<br>Ba  | 1.30 ± 0.33<br>Aa           |
|     | 20–40 cm | 10.04 ± 0.05<br>Aa | 0.46 ± 0.05<br>Aa            | 24.32 ± 2.49<br>Aa | 36.63 ± 1.54<br>Aa | 57.98 ± 2.48<br>Bb | 4.74 ± 0.12<br>Ca  | 1.35 ± 0.11<br>Aa           |
| C15 | 0–20 cm  | 8.07 ± 0.07<br>Bb  | 0.29 ± 0.03<br>Bb            | 7.13 ± 0.16<br>Bb  | 36.18 ± 1.87<br>Aa | 59.68 ± 1.69<br>Ab | 4.14 ± 0.21<br>Bb  | 1.34 ± 0.09<br>Aa           |
|     | 20–40 cm | 8.92 ± 0.32<br>ABa | 0.45 ± 0.01<br>Aa            | 14.38 ± 1.02<br>Ba | 29.12 ± 0.45<br>Bb | 63.69 ± 0.48<br>Aa | 7.19 ± 0.31<br>Aa  | 1.39 ± 0.13<br>Aa           |
| C25 | 0–20 cm  | 7.43 ± 0.02<br>Cb  | 0.18 ± 0.01<br>Cb            | 6.12 ± 0.34<br>Bb  | 33.46 ± 1.47<br>Aa | 61.19 ± 1.36<br>Ab | 5.35 ± 0.12<br>Aa  | 1.27 ± 0.22<br>Ab           |
|     | 20–40 cm | 8.86 ± 0.10<br>Ba  | 0.30 ± 0.04<br>Ba            | 7.72 ± 0.72<br>Ca  | 29.32 ± 0.97<br>Bb | 65.13 ± 0.87<br>Aa | 5.55 ± 0.12<br>Ba  | 1.41 ± 0.32<br>Aa           |
| P5  | 0–20 cm  | 8.84 ± 0.01<br>Ab  | 0.46 ± 0.01<br>Aa            | 12.4 ± 1.08<br>Ab  | 20.43 ± 1.20<br>Aa | 69.74 ± 1.37<br>Ab | 9.83 ± 0.51<br>Aa  | 1.30 ± 0.11<br>Cb           |
|     | 20–40 cm | 9.16 ± 0.07<br>Aa  | 0.31 ± 0.03<br>Ab            | 14.88 ± 0.32<br>Aa | 9.18 ± 1.98<br>ABb | 81.52 ± 2.34<br>Ba | 9.31 ± 0.36<br>Aa  | 1.38 ± 0.46<br>Ca           |
| P15 | 0–20 cm  | 8.63 ± 0.09<br>Ab  | 0.42 ± 0.01<br>Ba            | 10.64 ± 0.55<br>Ab | 13.99 ± 4.92<br>Aa | 75.87 ± 4.43<br>Aa | 10.15 ± 0.49<br>Aa | 1.54 ± 0.12<br>Ab           |
|     | 20–40 cm | 8.80 ± 0.04<br>Ba  | 0.26 ± 0.01<br>Bb            | 13.59 ± 0.41<br>Ba | 16.17 ± 3.46<br>Aa | 73.38 ± 2.96<br>Aa | 10.46 ± 0.50<br>Aa | 1.66 ± 0.15<br>Aa           |
| P25 | 0–20 cm  | 7.74 ± 0.15<br>Ba  | 0.27 ± 0.01<br>Ca            | 5.87 ± 0.41<br>Bb  | 12.78 ± 3.48<br>Aa | 76.55 ± 2.93<br>Ab | 10.68 ± 0.71<br>Aa | 1.38 ± 0.31<br>Bb           |
|     | 20–40 cm | 8.26 ± 0.06<br>Ca  | 0.22 ± 0.02<br>Bb            | 7.11 ± 0.30<br>Ca  | 6.00 ± 0.47<br>Ba  | 84.46 ± 2.46<br>Ba | 9.55 ± 1.99<br>Aa  | 1.50 ± 0.25<br>Ba           |

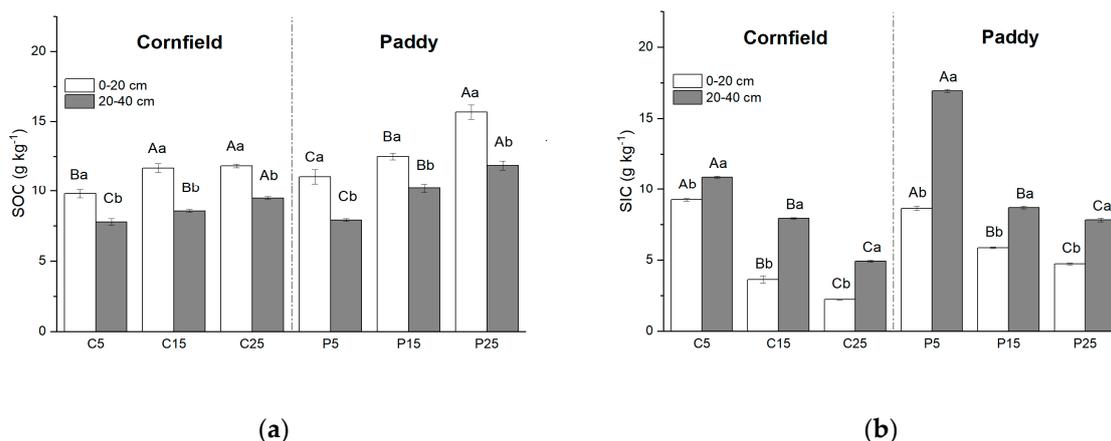
Mean ± standard errors. EC: Electrical conductivity; ESP: Exchangeable sodium percentage; BD: Bulk density; C: Cornfield; P: Paddy. Capital letters indicate the significance among plots with different cultivation years, while lowercase letters indicate the difference between layers ( $p < 0.05$ ).

### 3.2. Soil Enzyme Activities

In cornfields and paddies, enzyme activities in the surface layers were always larger than in the subsurface layers, and larger values were detected in longer cultivation years. Amylase activities in the surface layer were 1.61 and 1.64 times larger than in subsurface layers of the cornfields and paddies, respectively. Amylase activities in C25 (58.05 µg glucose g<sup>-1</sup> h<sup>-1</sup>) and P25 (80.87 µg glucose g<sup>-1</sup> h<sup>-1</sup>) were significantly higher than others. Amylase activities in paddies were always higher than cornfields with the same cultivation year. Invertase activities ranged from 18.27 to 279.44 µg glucose g<sup>-1</sup> h<sup>-1</sup> in cornfields and 73.35 to 462.14 µg glucose g<sup>-1</sup> h<sup>-1</sup> in paddies. Differing from amylase, the activities of invertase in the surface of C5 (158.62 µg glucose g<sup>-1</sup> h<sup>-1</sup>) and C15 (227.47 µg glucose g<sup>-1</sup> h<sup>-1</sup>) were lower than in P5 (134.04 µg glucose g<sup>-1</sup> h<sup>-1</sup>) and P15 (235.28 µg glucose g<sup>-1</sup> h<sup>-1</sup>). Catalase activities in the cornfields and paddies had mean values of 4.64 mL KMnO<sub>4</sub> g<sup>-1</sup> and 5.65 mL KMnO<sub>4</sub> g<sup>-1</sup>, respectively. Higher catalase activities were observed in paddies than in cornfields, except for the subsurface layer of P5 (2.31 mL KMnO<sub>4</sub> g<sup>-1</sup>).

### 3.3. Soil Carbon Distribution

SOC decreased with increasing soil depth in both the cornfields and paddies (Figure 2a). It was approximately 1.3 times larger than subsurface soil, varying from 9.78 to 11.83 g kg<sup>-1</sup> in the surface layer and from 7.77 to 9.46 g kg<sup>-1</sup> in the subsurface layer in cornfields. SOC increased with cultivation years, such that SOC in P25 (C15) was the largest, while the least appeared in P5 (C5). Larger SOC contents were always observed in paddies rather than cornfields, i.e., they were 11.67 g kg<sup>-1</sup> and 12.67 g kg<sup>-1</sup> in the surface layer of C15 and P15, respectively.

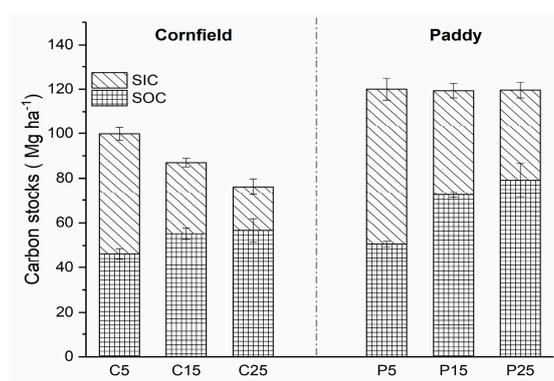


**Figure 2.** Soil carbon distribution in cornfields and paddies with different cultivation years: (a) Soil organic carbon (SOC); (b) soil inorganic carbon (SIC). Capital letters indicate the significance among plots with different cultivation years, while lowercase letters indicate the difference between layers ( $p < 0.05$ ).

The vertical distribution of SIC increased with soil depth, with an opposite trend of SOC (Figure 2b). Larger SIC content was observed in subsurface soil, ranging from 4.72 to 10.88 g kg<sup>-1</sup>, and 7.81 to 16.92 g kg<sup>-1</sup> in cornfields and paddies, respectively. SIC declined with increasing cultivation years. SIC in the surface layer of C25 (9.23 g kg<sup>-1</sup>) was more than four times that of C5 (2.23 g kg<sup>-1</sup>). Paddies had larger SIC content when compared to cornfields of the same cultivation year.

### 3.4. Soil Carbon Stocks

SOC and SIC density changed with increasing cultivation years in both cornfields and paddies, affecting the soil carbon stocks. The total organic carbon stock at a depth of 40 cm was 76.14 Mg ha<sup>-1</sup> in cornfields after 25 years of cultivation, while it was maintained at 120 Mg ha<sup>-1</sup> in paddies (Figure 3). SIC stocks contributed to nearly half of total carbon stocks in C5 and P5, but they declined to 25.59% and 33.92% in C25 and P25, respectively. The SIC stocks were lower, up to 33.87 Mg ha<sup>-1</sup> and 28.76 Mg ha<sup>-1</sup> in the cornfields and paddies after 20 years of cultivation (from 5 to 25 years), respectively. SOC stocks showed an opposite trend with SIC for the stocks in C25 and P25, which were 1.22 and 1.56 times that of C5 and P5, respectively.

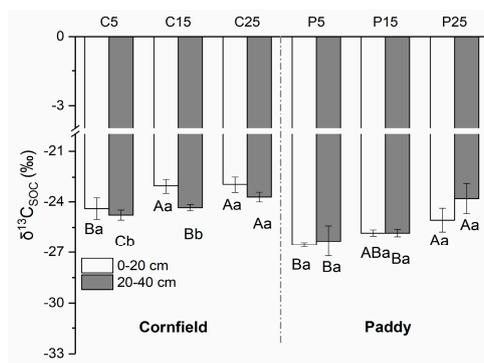


**Figure 3.** Carbon stocks in cornfields and paddies (0–40 cm) with different cultivation years.

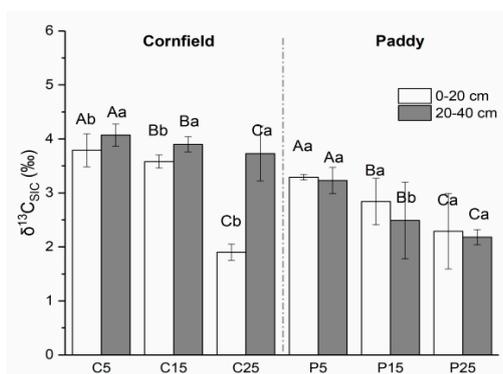
### 3.5. The $\delta^{13}\text{C}$ Values of SOC and SIC

The  $\delta^{13}\text{C}$  of SOC in cornfields, with a mean value of  $-23.88\text{‰}$ , was higher than in paddies ( $-25.57\text{‰}$ ) (Figure 4). Higher values were observed in the surface layer as compared with the subsurface soils in cornfields, varying from  $-24.40\text{‰}$  to  $-22.97\text{‰}$ . The  $\delta^{13}\text{C}_{\text{SOC}}$  value in C25 was

the highest, followed by C15 and C5. A similar trend was detected in paddies, where the  $\delta^{13}\text{C}_{\text{SOC}}$  values were in the range of P25, P15, and P5, although the values in the subsurface were larger than that in the surface soil. The  $\delta^{13}\text{C}$  of SIC ranged from approximately 1.90‰ to 4.07‰ in cornfields, where small values appeared in the surface layer (Figure 5). C5 had significantly higher  $\delta^{13}\text{C}_{\text{SIC}}$  values (3.79‰ and 4.47‰) than C15 (3.25‰ and 3.90‰) and C25 (1.90‰ and 3.73‰). The  $\delta^{13}\text{C}_{\text{SIC}}$  value was approximately 2.01‰ to 3.29‰ in paddies, where larger values appeared in the surface layer. The  $\delta^{13}\text{C}_{\text{SIC}}$  in the surface layer of P25 was 2.29‰, which was significantly lower than other paddies.



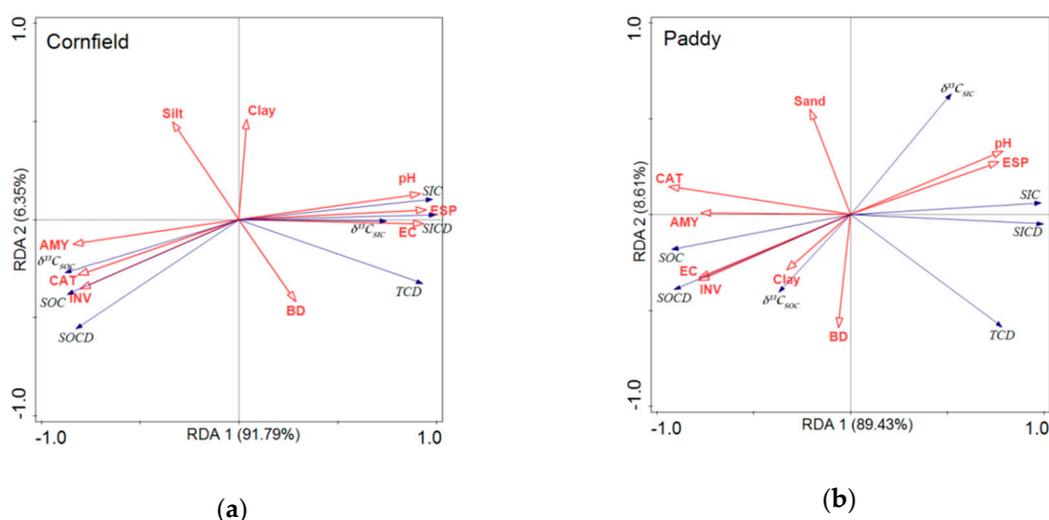
**Figure 4.** The natural abundance of SOC isotopes ( $\delta^{13}\text{C}_{\text{SOC}}$ ) in cornfields (C) and paddies (P). Capital letters indicate the significance among plots with different cultivation years, while lowercase letters indicate the difference between layers ( $p < 0.05$ ).



**Figure 5.** The natural abundance of SIC isotopes ( $\delta^{13}\text{C}_{\text{SIC}}$ ) in cornfields (C) and paddies (P). Capital letters indicate the significance among plots with different cultivation years, while lowercase letters indicate the difference between layers ( $p < 0.05$ ).

### 3.6. Redundancy Analysis between Soil Properties, Enzyme Activities, and Soil Carbon Pools

The relationship between soil properties, including the physical, chemical, and biological properties (enzyme activities), and soil carbon pools, was analyzed by RDA (Figure 6). The first two component axes explain approximately 89.43% to 91.97% and 6.35% to 8.61% of the variance of soil carbon pools, respectively. Soil pH, EC, ESP, invertase, catalase, and amylase played a bigger role than the soil physical properties, i.e., the BD, sand, silt, and clay content, in terms of explaining soil carbon pools. Soil organic carbon pools, SOC,  $\delta^{13}\text{C}_{\text{SOC}}$ , and SOC density, were positively correlated with enzyme activities, i.e., invertase, catalase, and amylase, while they showed significantly negative correlations with pH, ESP, and EC in the cornfields (Figure 6a). SIC was positively correlated with pH, ESP, and EC. In the paddies, SOC was significantly positively correlated with EC and invertase and was negatively correlated with pH and ESP, which was contrary to SIC (Figure 6b). Soil amylase, catalase, and invertase activities showed negative correlations with soil pH and ESP in both fields.



**Figure 6.** Redundancy analyses of soil carbon pools, soil properties, and soil enzyme activities. (a) Cornfield. (b) Paddy. AMY, CAT, and INV represent the activities of amylase, catalase, and invertase, respectively. BD, EC, and ESP represent the bulk density, electrical conductivity, and exchangeable sodium percentage, respectively. SOC D, SIC D, and TCD represent the stock of SOC, SIC, and TC, respectively.

## 4. Discussion

### 4.1. Effects of Crop Cultivation on Soil Properties and Enzyme Activities

Crop cultivation affects soil physical and chemical properties, as well as enzyme activities in both cornfields and paddies, through altering the soil structure, aggregation, porosity, strength, and infiltration with tillage practices [47]. Continuous irrigation and fertilization induces a reduction in soil pH [48]. In our study, the pH in the surface soil was significantly lower than in the subsurface layer, and a lower soil pH was observed in fields with longer cultivation years (Table 1). Consistent with Luo et al. (2017), maize cultivation in the Songnen Plain significantly improved soil quality [49], where EC and ESP decreased with cultivation years for long-term cultivation, and fertilization induced a reduction of  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ , and soil salinity [50]. Awe et al. (2020) suggested that crop cultivation reduces soil BD [51]; however, it was only observed in cornfields that BD in C25 was lower than C5 (Table 1). In paddies, soil BD and clay content increased with increasing cultivation years, which was related to the continuous flood irrigation [52].

Soil enzymes, mainly derived from soil microorganisms, crops, and root exudates [53], were sensitive to crop cultivation. They were active in the surface soil and their activities increased with cultivation years (Table 2). Long-term N fertilization increased enzyme activities associated with hydrolytic C for sufficient N supply in both the cornfields and paddies, not only directly stimulating soil microbes to produce more enzymes, but also favoring corn and rice production to increase root exudation [54]. The soil physical and chemical properties, altered by corn and rice cultivation, affected soil enzyme activities. Invertase, amylase, and catalase activities were negatively correlated with soil pH and ESP in our study (Figure 6), because high soil pH and ESP restricted the growth and development of corn and rice, limiting the metabolic process of soil microbes, and affecting the release of root exudates, limiting soil enzyme activities [55]. The soil C and N contents were closely related to soil enzyme activities [56] and sufficient SOC content, as well as long-term fertilization, which can explain the higher enzyme activities observed in fields with longer cultivation years in our study (Figure 2a).

**Table 2.** Soil enzyme activities under cornfields (C) and paddies (P) with depths of 0–20 cm and 20–40 cm.

|     |          | Amylase<br>( $\mu\text{g glucose g}^{-1} \text{h}^{-1}$ ) | Invertase<br>( $\mu\text{g glucose g}^{-1} \text{h}^{-1}$ ) | Catalase<br>( $\text{mL KMnO}_4 \text{g}^{-1} \text{h}^{-1}$ ) |
|-----|----------|---|---|--|
| C5  | 0–20 cm  | 39.97 $\pm$ 1.02 Ba <sup>1</sup>                          | 158.62 $\pm$ 2.53 Ca  | 4.84 $\pm$ 0.02 Ba   |
|     | 20–40 cm | 19.01 $\pm$ 0.54 Ab                                       | 18.27 $\pm$ 1.02 Cb   | 3.58 $\pm$ 0.01 Cb   |
| C15 | 0–20 cm  | 40.00 $\pm$ 1.61 Ba                                       | 227.47 $\pm$ 7.38 Ba  | 4.96 $\pm$ 0.2 Ba  |
|     | 20–40 cm | 31.87 $\pm$ 0.17 Ca                                       | 61.91 $\pm$ 4.05 Bb   | 4.23 $\pm$ 0.11 Bb   |
| C25 | 0–20 cm  | 58.05 $\pm$ 4.06 Aa                                       | 279.44 $\pm$ 13.43 Aa                                       | 5.61 $\pm$ 0.23 Aa   |
|     | 20–40 cm | 39.42 $\pm$ 0.32 Bb                                       | 83.29 $\pm$ 2.28 Ab   | 4.59 $\pm$ 0.16 Ab   |
| P5  | 0–20 cm  | 58.90 $\pm$ 0.64 Ba                                       | 134.04 $\pm$ 19.05 Ca                                       | 6.31 $\pm$ 0.17 Ba   |
|     | 20–40 cm | 41.85 $\pm$ 2.92 Ab                                       | 73.35 $\pm$ 3.40 Cb   | 2.31 $\pm$ 0.06 Cb   |
| P15 | 0–20 cm  | 75.74 $\pm$ 5.28 Aa                                       | 235.28 $\pm$ 14.63 Ba                                       | 6.46 $\pm$ 1.93 Ba   |
|     | 20–40 cm | 43.47 $\pm$ 3.02 Ab                                       | 114.75 $\pm$ 7.82 Bb  | 4.24 $\pm$ 0.12 Bb   |
| P25 | 0–20 cm  | 80.87 $\pm$ 0.41 Aa                                       | 462.14 $\pm$ 28.92 Aa                                       | 7.58 $\pm$ 0.13 Aa   |
|     | 20–40 cm | 45.76 $\pm$ 0.47 Ab                                       | 276.29 $\pm$ 14.63 Ab                                       | 6.98 $\pm$ 0.46 Aa   |

<sup>1</sup> Capital letters indicate the significance among plots with different cultivation years, while lowercase letters indicate the difference between layers ( $p < 0.05$ ).

#### 4.2. Effects of Crop Cultivation on SOC

Crop cultivation affected the vertical distribution of SOC in our study. SOC in the surface soil was significantly higher than that in the subsurface layer, agreeing with previous studies [11,17,57]. The  $\delta^{13}\text{C}$  values of SOC were closely related to the  $\delta^{13}\text{C}$  values of the plant material it originated from. In our study, the mean values of  $\delta^{13}\text{C}_{\text{SOC}}$  in the surface layer were  $-23.48\text{‰}$  for the cornfields and  $-25.82\text{‰}$  for the paddies (Figure 4), which were closer to the  $\delta^{13}\text{C}_{\text{SOC}}$  value of corn (approximately  $-15\text{‰}$  to  $-17\text{‰}$ ) and rice (approximately  $-35\text{‰}$  to  $-20\text{‰}$ ), inferring that crop cultivation performed sound effects on SOC sequestration and the fixation of surface soil. The crops fixed atmospheric carbon into soils by photosynthesis, which transforms  $\text{CO}_2$  from the air and releases it to the soil through the huge living root systems, which were well developed in the surface layer [58,59]. Fertilizer application in soil facilitates crop growth, thus improving C accumulation in the surface layer [17]. As reported by Jian et al. (2016) and Lu et al. (2011), N fertilization increases SOC content [54,60]. Moreover, the decayed roots also attribute to the SOC in soil [27,61–64].

SOC increased with cultivation years, and a larger value of  $\delta^{13}\text{C}_{\text{SOC}}$  appeared in fields with longer cultivation years in both layers (Figures 2a and 4), suggesting that long-term crop cultivation is valuable for SOC sequestration in the studied alkali sodic soils. Long-term fertilization, irrigation, and the decomposition of litters and roots increased SOC content, and the infiltration and transformation of soil particle fractions, as well as dissolved OC, increased the SOC content in subsurface soils [65,66]. The soil environment was altered by continual cultivation practice, contributing to SOC storage. Soil pH was the most sensitive indicator that regulated the SOC cycle and sequestration for the influence on crop growth, soil microorganisms, and enzyme activities [67–69]. The optimum pH for bacteria is 6.5–8, and 5–6 for fungi [70,71]. The intense alkali conditions, i.e., soil pH in C5 and P5, was 9.04 and 8.84, limited soil microbial activities, as well as the humification progress of root and litters, preventing the input and accumulation of SOC [72]. ESP significantly correlated with soil pH, showing a negative correlation with SOC in our study (Figure 6), implying that lower ESP conditions favor SOC and its storage. Wang et al. (2019) noted that SOC increased with cultivation years for native grassland converted into cropland, because decreasing soil salinity alleviates salt stress, as well as N fertilization, which is beneficial to plant growth and SOC accumulation [73]. Du et al. (2019) reported that grassland ecosystems have higher C losses than farmland when following desertification [74], which could also attribute to the SOC increment with increasing cultivation years. Soil desertification in the Songnen Plain has decreased the area of grassland, as well as grass vegetation coverage, resulting in a lower input of above- and below-ground biomass, reducing the SOC stock and increasing soil erosion from wind and water [75]. For instance, SOC losses induced by the 20-year coverage of degraded grasses in

C5 (and P5) were related to the low SOC stocks as compared with C25 (and P25) (Figure 3). Soil enzymes participate in the metabolism of organic matter, catalyze biochemical processes, and affect soil nutrient accumulation and mineralization [76,77]. In the present study, invertase had significant impacts on SOC content in both the cornfields and paddies (Figure 6). Some studies have argued that a high clay content stabilizes SOC physically by forming organo-mineral complexes and micro-aggregates to protect SOC from decay [28,78], whereas the correlation between SOC and clay was only detected in paddies here (Figure 6).

#### 4.3. Effects of Crop Cultivation on SIC

Our results are consistent with Dong et al. (2019), following that SIC content increases with the soil depth [79]. The SIC in subsurface soil was more extensive than that in the surface layer (Figure 2b). This could be explained by the primary geological parent materials, as displayed by the  $\delta^{13}\text{C}_{\text{SIC}} > 0$  relationship, where LIC was the primary unit of SIC in the study (Figure 5). Lettens et al. (2004) detected a strong relationship between soil geological conditions and SIC distribution [80], which is proven by our study, where SIC is positively correlated with soil pH and ESP (Figure 6). Worse soil alkali sodic status in the subsurface soil, higher pH, and ESP (Table 1), was favorable to SIC sequestration and storage. The loose soil structure induced by tillage practice and adequate SOC content in surface soil facilitates transforming and infiltrating dissolved carbonates (DIC) [81]. Natural rainfall and irrigation promote the dissolved carbonates that originally exist in the surface soil, flowing and moving downstream, re-precipitating in deeper soil [82].

Long-term cultivation decreased SIC content in both layers in the cornfields and paddies (Figure 2b), which is in line with Wu et al. (2009), who observed SIC loss in the east of China [83]. Changes in the soil environment induced by fertilization, tillage practice, and irrigation affect SIC stocking. Dong et al. (2017) and Eshel et al. (2007) noted that cultivation practices decrease SIC, where cultivation practice disturbs calciferous soils and transports SIC into rivers and lakes, deeper soils, and groundwater through irrigation and rainfall [4,84]. Ma et al. (2013) and Zamanian et al. (2016) argued the improvement in soil sodic and alkali conditions after long-term crop cultivation has triggered SIC loss [32,85]. High contents of soil cations and salt, coupled with the alkaline environment, are harmful to decomposing carbonates but conducive to SIC sequestration [86,87]. In our study, SIC was significantly positively related to pH and ESP (Figure 6), so long-term cultivation hastens SIC loss for the low alkali conditions. Besides, Bai et al. (2017) declared that SIC leaching is a vital factor controlling SIC sequestration and fixation [88]; thus, the leaching of DIC may be assigned to the SIC loss with cultivation years in both layers (0–40 cm).

#### 4.4. The Effects of Crop Types on Soil C Stocks

Total C stocks varied with cultivation years, where they significantly decreased in cornfields while they upheld a stock of  $120 \text{ Mg ha}^{-1}$  in paddies (Figure 3), implying that corn and rice cultivation was disadvantageous to C sequestration and storage in the study area. SOC stocks (0–40 cm) increased with cultivation years in both crop types, inferring that both corn and rice cultivation are beneficial to SOC sequestration. Crop cultivation lowers soil alkali sodic conditions, improving crop growth and improving soil microbial activity, thus promoting carbon transformation and sequestration in soil [89]. Liu et al. (2019) argued that rice cultivation generates more SOC than in cornfields [12]. Likewise, more massive SOC stocks were observed in paddies than cornfields in our study (Figure 3), owing to the continuous flooding irrigation practice, where the anaerobic conditions limit soil mineralization and soil organic matter decomposition, raising C stores [3,90]. SIC stocks (0–40 cm) decreased with increasing cultivation years, while the SIC stocks in paddies were larger than in cornfields (Figure 3). The groundwater in the arid and semiarid region contained high concentrations of the  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations [91], which hasten the formation of PIC [92]. Rice flooding irrigation dissolves soluble ions into the water, accelerating the carbonate weathering and erosion, as proven by the lower  $\delta^{13}\text{C}_{\text{SIC}}$  values in paddies (Figure 5). Higher silt and clay content and the larger BD in paddies (Table 1) restricted the

infiltration of DIC, thus reducing SIC loss in paddies. Surface and subsurface soils (0–40 cm) are the most sensitive soil layers that are directly or indirectly disturbed by crop cultivation and tillage practice, like ploughing, fertilization, and irrigation. In our study, total C stock losses at the depth of 40 cm in the alkali sodic cornfields and paddies were detected. Advanced studies could consider deeper soil layers to assess the “carbon source” (or “carbon sink”) function of alkali sodic agricultural soils.

## 5. Conclusions

Corn and rice cultivation improved soil alkali sodic conditions by lowering soil pH, EC, and ESP, thus increasing enzyme activities (amylase, invertase, and catalase). SOC and its stocks were negative correlated with soil pH and ESP, while SIC showed significant correlations with pH and ESP. Obvious positive relationships between SOC and enzyme activities were detected in the redundancy analysis carried out in this study. SOC,  $\delta^{13}\text{C}_{\text{SOC}}$ , and SOC stocks increased with cultivation years, while SIC,  $\delta^{13}\text{C}_{\text{SIC}}$ , and SIC stocks decreased with increasing cultivation years. A total carbon stock loss was observed in the cornfields with increasing cultivation years, while, in paddies, the stocks remained steady at  $120 \text{ Mg ha}^{-1}$ . Corn and rice cultivation in the studied alkali sodic region was advantageous to improve soil alkali sodic conditions but disadvantageous to soil total carbon storage at the depth of 0–40 cm.

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