Chen *et al. Chem. Biol. Technol. Agric.* (2023) 10:97
https://doi.org/10.1186/s40538-023-00469-6

Chemical and Biological Technologies in Agriculture

RESEARCH

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Differences of SOC storage and stability between soil layers influenced by long-term fertilization in a typical paddy soil of Southern China

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Abstract

Background The contribution of long-term fertilization to soil organic carbon (SOC) storage has been of great concern. To assess the effects of long-term fertilization on SOC storage and stability in top and sub-soil layers, soil samples were collected from a 29-year field experimental station in a typical paddy soil in southern China. The SOC storage of whole soil and SOC fractions was quantified at three soil depths (0–20, 20–40, 40–60 cm) under four treatments: no fertilization (Control), a combination of nitrogen, phosphorus and potassium (NPK), double the rates of NPK (2NPK), NPK plus manure (NPKM).

Results The increase of $C_{\text{input-total}}$ was significantly higher than that of SOC storage among different treatments ($p < 0.05$), indicating that soil fixation of exogenous carbon is limited. Besides, the SOC accumulation and sequestration rates patterned as NPKM > 2NPK > NPK, and these rates were higher at 0–20 cm depth as compared to other depth intervals. Furthermore, for the whole profile, the SOC storage of active pool was higher in the Control (39.6 t C ha^{-1}) than in other treatment (36.2 t C ha^{-1}, $p < 0.05$). Whereas, fertilization increased the SOC storage of passive pool, ranked as NPKM > 2NPK≈NPK > Control ($p < 0.05$), indicating that fertilization, especially organic combined with inorganic fertilization, improved SOC stability. From the perspective of soil layers, the difference of SOC storage among treatments for passive pool was mainly resulted from the difference at surface soil, and for active pool were the deeper layers. Additionally, manure application increased the difference among soil layers.

Conclusion This study concluded that non-fertilized treatment could improve the SOC storage of active pool especially in deep soil layers, while fertilization especially manure application could improve the SOC storage and stability in surface soil and increased the difference among soil layers.

Keywords SOC storage, Carbon fractions, SOC stability, Manure application, Soil layer

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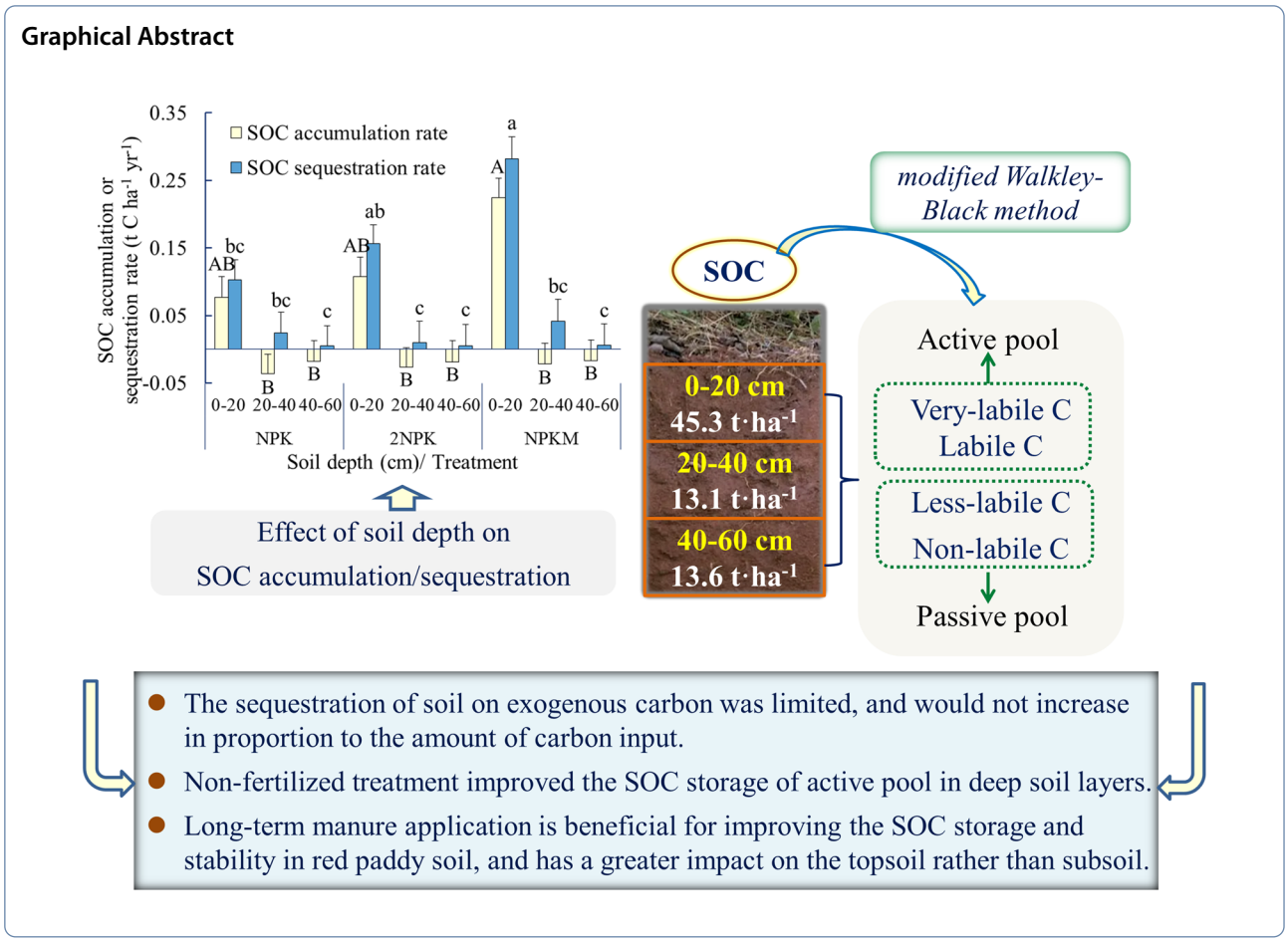

Introduction

Soil is a complex system with characteristic physical, chemical and biological properties vital for nutrient cycling, biodiversity conservation and climate change mitigation [1] particularly through carbon sequestration. Being the largest carbon pool in terrestrial ecosystem, the soil organic carbon (SOC) is a key parameter of soil quality and its ecosystem functioning [2]. Enrichment of SOC storage in soil is, therefore, important for maintaining soil quality and mitigating climate change. Long-term fertilization is one of the most efficient approaches for promoting carbon sequestration and sustaining soil fertility. The regular and sustained application of mineral fertilizers or manure has the potential to enhance SOC accumulation [3–5]. Studies conducted on paddy soils in China have demonstrated that combining manure application with mineral fertilizers enhances SOC levels to a greater degree than solely using mineral fertilization [6–8], suggesting that manure has a significantly positive effect on soil fertility. However, the majority of studies on this topic has primarily focused on the dynamics of bulk soil SOC and has been limited to the topsoil layer. Additionally, the actual disparities in SOC increase could be determined by analyzing the dynamics of SOC fractions in both topsoil and subsoil layers, which have been overlooked in previous research.

Since different SOC fractions do not make equal contributions to soil carbon accumulation [9, 10], a better understanding of carbon distribution among SOC fractions and how this distribution is affected by long-term fertilization are essential to carry out a valid assessment of total organic carbon sequestration. In this regard, wet oxidation, a chemical fractionation method which is widely used in describing SOC lability and stability, can separate SOC into fractions with a gradient of oxidizability (very-labile C [C_{frac1}], labile C [C_{frac2}], less-labile C [C_{frac3}] and non-labile C [C_{frac4}]) [9, 11, 12]. The $C_{frac1}+C_{frac2}$ represent the active pool and serves as an early indicator of how management influences SOC, while $C_{frac3}+C_{frac4}$ represent the passive pool which is used as an indicator for describing the long-term effect of field management on SOC sequestration. However, there

has been inconsistent results regarding the long-term fertilization effects on SOC enhancement in subtropical climate region [9, 12–14], which prompted a need for further studies to clarify how SOC storage occurs in SOC fractions in the long run.

It has been well established that, the SOC storage between soil layers is influenced primarily by crop root distribution and cultivation practices, and usually decreases with soil depth. To unveil these differences, stratification ratio (SR), defined as the ratio of a measurable property of the surface soil to that of deeper soil, can be used as an indicator of soil quality dynamics under various management practices [15–17]. Previous studies mainly focused on stratification of total SOC as affected by cropping systems or tillage practices [17, 18]. However, the heterogeneity of SOC in different soil layers and its response to long-term fertilization are still not clear, and no explicit conclusions have been drawn on the SR of soil active and passive pools which constitutes the further novelty of this work.

As one of the most important rice-producing soils, red paddy soils have attracted much attention with respect to SOC dynamics in China, whereas our knowledge about SOC storage and stability at different soil depths under long-term fertilization managements has been still limited. This study was an attempt to investigate (i) the effects of long-term inorganic/organic fertilization on the SOC storage in different soil depths, (ii) assess the stability of SOC, and (iii) assess the rate of change of SOC accumulation or sequestration under applied treatments. The findings obtained would be beneficial to the management of farmland fertilization and the sustainable use of soil.

Materials and methods

Site description

The experiment was introduced at the Jiangxi Research Institute of Red Soil, Jinxian, in the southern province of Jiangxi, China. The experimental site is located in a subtropical climate region at coordinates 28° 35′ 24″N and 116° 17′ 60″ E, with an altitude of 26 m above sea level. The mean annual rainfall and temperature of the region are 1537 mm and 18.1 °C, respectively. The soil at the site is red paddy soil, derived from quaternary red clay parent material. Soil samples collected from a depth of 0–20 cm at the beginning of the experiment indicated the presence of 16.3 g kg^{-1} soil organic carbon, 1.49 g kg^{-1} total nitrogen, 0.49 g kg^{-1} total phosphorus, 144 mg kg^{-1} alkaline hydrolyzed nitrogen, 9.50 mg kg^{-1} Olsen phosphorus, and 81.2 mg kg^{-1} exchangeable potassium (i.e., ammonia acetate extractable K), with an initial pH of 6.9 [19].

Experimental design

The experiment conducted was a long-term study of a rice-rice cropping system that included a winter fallow period since 1981. The early rice was cultivated from April-July, while the latter rice was grown from the middle of July to late October. Crops were harvested manually close to the ground and all harvested biomass was removed from the plots. The size of each plot was 46.67 m^2. There were four treatments: (1) Control: no fertilization, (2) NPK: the application rates for N (urea), P (calcium-magnesium phosphate), K (potassium chloride) fertilizers were 90 kg N ha^{-1}, 45 kg P_2O_5 ha^{-1}, 75 kg K_2O ha^{-1}, respectively, during both growing seasons. (3) 2NPK: double the fertilization rates of NPK treatment, and (4) NPKM: NPK plus organic fertilizers—Chinese milk vetch (*Astragalus sinicus L.*) for the early rice and pig manure for the latter rice, the application rates of organic fertilizers were both 22.5 t ha^{-1}. The experiment followed a completely randomized block design, with three replicates for each treatment. For both the early and late rice crops, all calcium–magnesium phosphate and organic fertilizers were applied as basal fertilizers prior to the transplantation of rice seedlings, urea and potassium chloride were fertilized as top-application after the regreening stage (50% urea and 100% potassium chloride) and at the tillering stage (50% urea). Other field management practices such as rice transplanting, irrigation, and plant protection identical to local farmers were adapted. The rice stubbles were removed after harvest.

Soil sampling and measurement

After 29 years of setting up the field experiment, soil samples were collected in November 2010, 7 days after the late rice harvest. The samples were collected from three different soil layers, namely 0–20 cm, 20–40 cm, and 40–60 cm depths. To create each sample per layer per plot, 10 soil cores were pooled together. Soil samples were air-dried and sieved through a 2 mm sieve for analyses. Soil bulk density (B_d) in the layers of 0–20, 20–40 and 40–60 cm was determined by taking three soil core samples from each depth in each plot using the ring-knife with a diameter of 5 cm and volume of 100 cm^3, the average of three soil cores was taken as the soil B_d of one depth in each plot. After collecting soil cores of 0–20 cm, soils above 20 cm were removed; then collected the soil cores of 20–40 cm, and so on, to collect the samples of 40–60 cm soil layer. Gravel and residue larger than 2 mm were removed from the samples, then the soil samples were weighed after overnight drying at 105 °C. The soil B_d was calculated by dividing the dry weight of each soil sample by the volume of ring-knife. The data of soil B_d are shown in Table 1.

The SOC fractions were separated by a modified Walkley–Black method as described by Chan et al. [11]. Specifically, 0.5 g soil samples were added into 10 mL 0.167 mol·L^{-1} $K_2Cr_2O_7$ solution, then added 5 mL

Table 1 Soil bulk density (B_d) in three soil depths in different treatments

Treatment	Depth		
	0–20 cm	20–40 cm	40–60 cm
CK	1.20 ± 0.01	1.55 ± 0.02	1.54 ± 0.01
NPK	1.21 ± 0.02	1.54 ± 0.03	1.51 ± 0.04
2NPK	1.21 ± 0.02	1.57 ± 0.02	1.55 ± 0.03
NPKM	1.06 ± 0.03	1.56 ± 0.02	1.50 ± 0.02

concentrated H_2SO_4 (18 mol L^{-1}) to produce reaction heat; after 30 min reaction, 1.0 mol L^{-1} $FeSO_4$ was titrated to determine the excess dichromate. The amount of dichromate consumed by the soil was used to calculate the amount of oxidizible carbon, this amount of oxidized carbon was defined as the 6 mol L^{-1} H_2SO_4 oxidized SOC. To obtain the 9 mol L^{-1} H_2SO_4 oxidized SOC and 12 mol L^{-1} H_2SO_4 oxidized SOC, the amounts of concentrated H_2SO_4 added were changed to be 10 mL and 20 mL, respectively. Finally, this resulted in four fractions in decreasing order of oxidizability: (1) Very-labile C: 6 mol L^{-1} H_2SO_4 oxidized SOC; (2) Labile C: 9 mol L^{-1} H_2SO_4 oxidized SOC—6 mol L^{-1} H_2SO_4 oxidized SOC; (3) Less-labile C: 12 mol L^{-1} H_2SO_4 oxidized SOC—9 mol L^{-1} H_2SO_4 oxidized SOC; (4) Non-labile C: Total SOC—12 mol L^{-1} H_2SO_4 oxidized SOC. These four fractions were grouped into the active pool [Frac1 + Frac2] and the passive pool [Frac3 + Frac4] according to Chan et al. (2001). Soil samples for total SOC measurement were pretreated using 36% HCl-fumigation method to remove carbonates, and then were determined with an elemental analyzer (Elementar, Vario Max, Germany). Due to different testing methods, the SOC content at the beginning of the experiment was corrected for comparison [12].

Calculations

The SOC storage (t C ha^{-1}) and carbon input (t C ha^{-1}) were calculated from the following equations:

$$\text{SOC storage}\left(\text{t C ha}^{-1}\right) = C_{\text{conc}} \times B_{\text{d}} \times D \times 10 \tag{1}$$

where C_{conc} represents SOC concentration (g kg^{-1}) at each soil depth, B_d is bulk density (g cm^{-3}) at each soil depth, and D is soil depth (cm), 10 is unit conversion factor. Total SOC storage is the sum of SOC storages at three soil depths.

$$C_{\text{input-root}} = (\text{YS} + \text{YR}) \times R_r \times C_{\text{rice}} \times 10^{-6} \tag{2}$$

$$C_{\text{input-stubble}} = \text{YR} \times R_s \times C_{\text{rice}} \times 10^{-6} \tag{3}$$

$$C_{\text{input-organic fert}} = m \times (1 - W) \times C_{\text{organic fert}} \times 10^{-3} \tag{4}$$

where $C_{\text{input-root}}$, $C_{\text{input-stubble}}$, $C_{\text{input-organic fert}}$ are carbon inputs from root (assume that root-carbon all distributed within 0–60 cm soil depth), stubble, organic fertilizers, respectively, t C ha^{-1}; YS and YR are the yields of rice seed and residue, respectively, kg·ha^{-1}; R_r is the proportion of carbon entering into underground part by photosynthesis, %; C_{rice} is the carbon content of the aboveground part of rice, %; R_s is the proportion of rice residue left in the fields after harvest, 5.6% in this study; m is the application rate of organic fertilizer, 22.5 t ha^{-1} for both milk vetch and pig manure; W is the water content of organic fertilizers, %; $C_{\text{organic fert}}$ is the carbon content of organic fertilizers, %. Total external carbon input ($C_{\text{input-total}}$) is the sum of $C_{\text{input-root}}$, $C_{\text{input-stubble}}$, and $C_{\text{input-organic fert}}$. Data of carbon input presented are average of 29 years, t C ha^{-1} $year^{-1}$.

The annual change rate of SOC storage (ACR_{soc}, t C ha^{-1} $year^{-1}$) was calculated using the equation below:

$$\text{ACR}_{\text{soc}} = (\text{SOC}_{\text{t}} - \text{SOC}_0)/T \tag{5}$$

where SOC_0 and SOC_t represent the SOC storage at the beginning and the end of the experiment, respectively; T is the duration of this experiment, 29 years. Calculations were made at 0–60 cm depth.

Moreover, the SOC accumulation and sequestration rates were calculated based on following equations;

$$\text{SOC accumulation rate}\left(\text{t C ha}^{-1}\ \text{year}^{-1}\right) = (\text{SOC}_{\text{t}} - \text{SOC}_{\text{c}})/T \tag{6}$$

where, SOCt is SOC stocks (t ha^{-1}) in treatments, whereas, SOCc is SOC stocks in control and T is experimental time.

$$\text{SOC sequestration rate}\left(\text{t C ha}^{-1}\ \text{year}^{-1}\right) = (\text{Rec.SOC}_{\text{t}} - \text{Rec.SOC}_{\text{c}})/T \tag{7}$$

where, Rec.SOCt and Rec.SOCc are recalcitrant carbon stocks (t C ha^{-1}) of a given treatment and unfertilized control and T is the experimental time.

Statistical analysis

Primary calculations of data were performed in Excel 2010 and statistical analyses were performed using SAS 9.4. A two-way analysis of variance (ANOVA) model was used to determine the main effect of treatments and soil depth. Multiple comparisons were made using the least

significant difference (LSD) at $p = 0.05$. Figures were made using Excel 2010, Origin 8.0.

Results

Carbon input and SOC storage

Table 2 shows that the total carbon inputs ($C_{\text{input-total}}$) were consistent with the soil organic carbon (SOC) storage levels in the 0–60 cm soil depth. The carbon input from both roots and stubble ($C_{\text{input-root}}$, $C_{\text{input-stubble}}$) was higher in the fertilized treatments compared to the Control ($p < 0.05$). When combined with the carbon input from organic fertilizer ($C_{\text{input-organic fert}}$), the $C_{\text{input-total}}$ showed a ranking of NPKM > 2NPK > NPK > Control ($p < 0.05$). Increasing $C_{\text{input-total}}$ led to a larger SOC storage, but not in the same proportion, the $C_{\text{input-total}}$ increased by 184% while SOC storage increased by 7.33% in the NPKM treatment comparing to Control.

The SOC storage was 66.28 t C ha^{-1} along the profile at the beginning of this experiment. After 29 years, the differences of annual changing rate of SOC storage (ACR_{soc}) among the four treatments showed the same pattern as the SOC storage (Table 2). The correlation coefficient between $C_{\text{input-total}}$ and annual changing rate of SOC (ACR_{soc}) was 0.952.

Carbon storage in SOC fractions at the whole soil profile

The data of carbon storage in the SOC fractions (Very-labile *C*, Labile *C*, Less-labile *C* and Non-labile *C*) at the soil profile of 0–60 cm are shown in Fig. 1A. The SOC storage of Very-labile *C* fraction showed no significant difference among four treatments. Organic fertilization lowered the SOC storage of the Labile *C* fraction by 19.1% and 15.3% comparing with the Control and 2NPK treatments, respectively ($p < 0.05$), but accreted the SOC storage of Less-labile C fraction by 46.7% compared to other treatments on average. Compared to other treatments, the largest SOC storage of the Non-labile *C* fraction was observed in the 2NPK (25.0 t C ha^{-1}) treatment, followed by NPKM (23.8 t C ha^{-1}).

Total SOC storage at different soil depths

The SOC storage was much higher at 0–20 cm depth (45.3 t C ha^{-1}, average of the four treatments) than those at depths of 20–40 cm (13.1 t C ha^{-1}) and 40–60 cm (13.6 t C ha^{-1}) ($p < 0.001$). The NPKM treatment showed the larger SOC storage than the Control and NPK treatments at 0–20 cm depth ($p < 0.05$), no significant difference among the four treatments was observed for both 20–40 cm and 40–60 cm layers (Fig. 1B). Moreover, in line with the SOC storage results, the SOC accumulation as well as sequestration rates were higher at 0–20 cm depth as compared to other depth intervals (Fig. 2). The highest SOC accumulation and sequestration rates were associated with NPKM treatment and patterned as NPKM > 2NPK > NPK.

Carbon storage in SOC fractions at different depths

In terms of different soil layers, the amounts of SOC stored in the four SOC fractions at 0–20 cm depth were all significantly higher than those at deeper layers (Fig. 1C–F). Irrespective of the soil layers, the SOC storage of Very-labile C fraction showed no significant difference among four treatments (Fig. 1C). The influences of fertilization on SOC storage of Labile C and Less-labile C fractions were mainly reflected in the topsoil (Fig. 1D–F). Compared to other SOC fractions, the Less-labile C fraction was most affected by organic fertilization, especially in topsoil. The SOC storage of Less-labile C fraction at 0–20 cm depth increased by 113%, 49.5% and 72.8% under NPKM compared to that in the Control, NPK and 2NPK treatments, respectively ($p < 0.05$). The SOC increment in Non-labile C fraction after long-term organic fertilization was not only in the surface soil but also in the sub-soil (20–40 cm) (Fig. 1F).

Carbon storage in SOC pools at different depths

By analyzing the proportion of active and passive pools of SOC under different fertilization treatments (Fig. 3) and redundancy analysis (Fig. 4), it can be seen that the Labile, Less-labile and Non-labile C stocks were related to whole depth interval as well as surface soil layer. On the other hand, deeper soil layers were linked to lower soil carbon fraction and pools. Axis two (F2) was also related to surface soil depth (0–20 cm) as well as to the fertilization treatment category, characterized by SOC sequestration rate, which implies that NPKM treatment

Table 2 Carbon input and SOC storages of 0–60 cm soil depth in different treatments

Treatment	$C_{\text{input-root}}$ (t C ha^{-1} year^{-1})	$C_{\text{input-stubble}}$ (t C ha^{-1} year^{-1})	$C_{\text{input-organic fert}}$ (t C ha^{-1} year^{-1})	$C_{\text{input-total}}$ (t C ha^{-1} year^{-1})	SOC storage (t C ha^{-1})	ACR_{soc} (t C ha^{-1} year^{-1})
CK	2.13 ± 0.47 c	0.15 ± 0.05 c	——	2.29 ± 0.52 d	69.9 ± 2.08 b	0.126 ± 0.072 b
NPK	2.44 ± 0.55 bc	0.24 ± 0.08 b	——	2.68 ± 0.63 c	70.2 ± 0.33 b	0.134 ± 0.012 b
2NPK	2.88 ± 0.68 a	0.29 ± 0.11 a	——	3.17 ± 0.78 b	72.4 ± 1.21 ab	0.212 ± 0.042 ab
NPKM	2.75 ± 0.71 ab	0.28 ± 0.12 ab	3.48	6.51 ± 0.82 a	75.2 ± 3.85 a	0.307 ± 0.133 a

Different lowercase letters indicate significant differences among treatments, $p < 0.05$

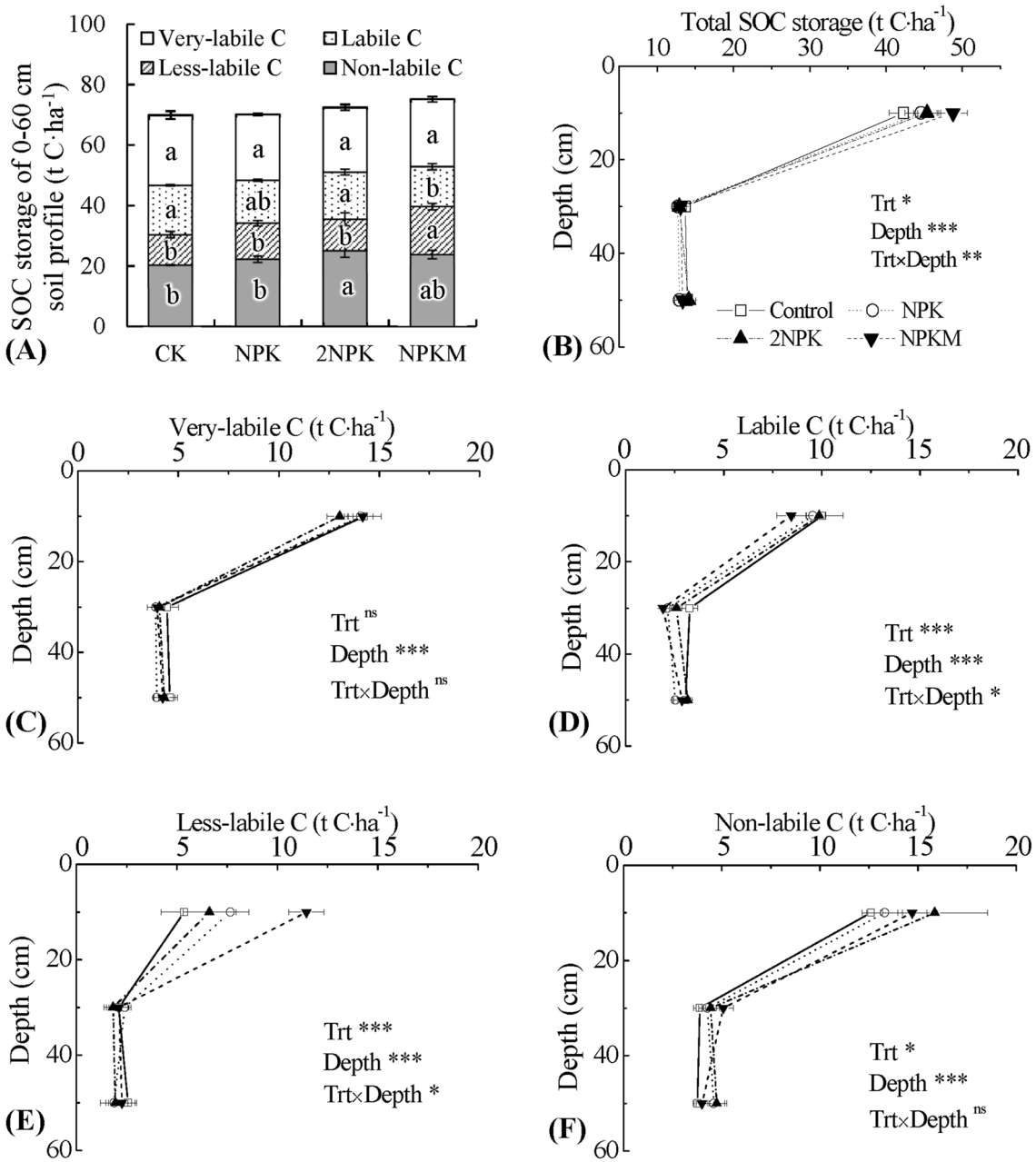

Fig. 1 The SOC storage of whole soil and SOC fractions under different treatments. Note: Different lowercase letters in (**A**) indicate significant differences among treatments within each SOC fraction ($p < 0.05$), bars represent mean ± standard error. In (**B**–**F**), "Trt" refers to the four treatments: Control, NPK, 2NPK, NPKM; "Trt × Depth" is the interaction between treatment and depth. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **C**–**F** shared the same legend with (**B**)

led to increased SOC sequestration rate in the top layer (0–20 cm) of the soil.

From the perspective of carbon pools with different stability, the SOC storage of active pool was higher in the Control (39.6 t C ha^{-1}) than in other treatment (36.2 t C ha^{-1} on average), and the passive pool showed the opposite scenario (Fig. 5). Fertilization increased the SOC storage of passive pool ($p < 0.05$), and the value in the NPKM treatment was 30.7%, 16.1%, 11.8% higher than in the Control, NPK, 2NPK treatments, respectively. The difference of SOC storage among treatments for passive pool was mainly resulted from the difference at surface soil, and for active pool were the deeper layers.

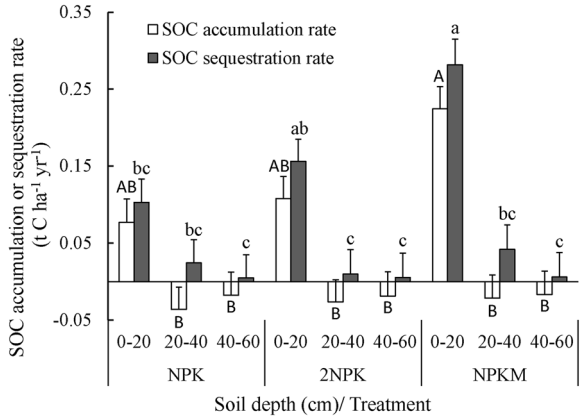

Fig. 2 Total SOC accumulation rate (t C ha^{-1} year^{-1}) and SOC sequestration rate (t C ha^{-1} year^{-1}) at three soil depths under different fertilization treatments (NPK and 2NPK and NPKM). In each panel, different uppercase letters denote significant difference between accumulation rates and lowercase letters show difference for sequestration at $p < 0.05$

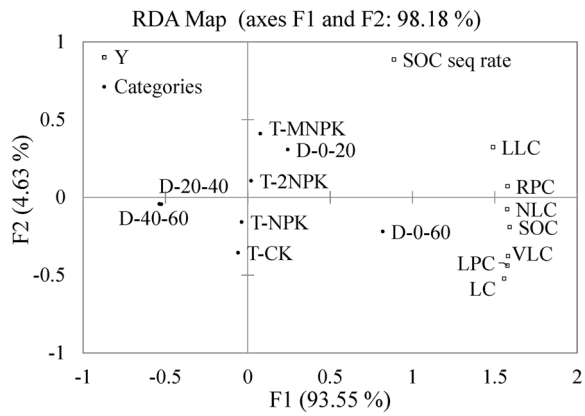

Fig. 4 Redundancy analysis of soil organic carbon (SOC) fraction and pool stocks constrained by soil depth interval and fertilization treatment. Note: T, treatment; D, depth; VLC, very labile carbon; LC, labile carbon; LLC, less-labile carbon; NLC, non-labile carbon; LPC, labile pool = active pool; RPC, recalcitrant pool = passive pool.

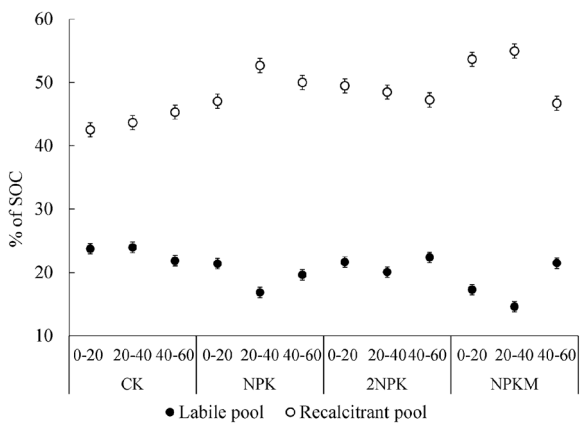

Fig. 3 Proportion of labile and recalcitrant pools of soil organic carbon (SOC) under different fertilization treatments

Stratification characteristics

The SR values of total SOC storage (Fig. 6) for both surface and deep soil layers were significantly greater in the NPKM treatment (3.74 and 3.68) than in the Control (3.12 and 3.03) ($p < 0.05$). The SR of both the active pool and the passive pool for 0–20 cm: 20–40 cm showed no significant difference among the four treatments, while the SR of the passive pool for 0–20 cm: 40–60 cm was higher under NPKM (4.24) than that in other treatments (3.29 averagely) ($p < 0.05$).

Discussion

Fertilization impacts on SOC storage and sequestration

Long-term fertilizer application is believed to affect crop yields and biomass [7, 20, 21], thus alter the amount of exogenous carbon input. This increase in carbon input is concomitantly linked to the SOC concentration [7, 22]. This long-term study showed that applying standard rate of inorganic fertilizer or double the standard amount of fertilizer can significantly increase crop biomass and carbon input, but their effects on SOC storage of the 0–60 cm soil profile were very limited (Table 2). The sole application of organic fertilizer can effectively improve the SOC storage. This was not completely consistent with previous works which was done in the surface soil [7], probably because the change of SOC storage in 0–60 cm soil layer was slower and more insensitive than in the surface layer. Besides, whether the fertilizer application rate doubled or not did affect the carbon input, but did not show significant impact on SOC storage, suggesting that the SOC concentration did not increase proportionally with increasing NPK fertilizer rate, which agrees with Li et al. [23]. However, there was a great difference observed for SOC storage for different soil depths, whereby, the top soil (0–20 cm) showed significantly highest SOC storage as compared to other depths. This is consistent with Abrar et al. [24] who observed the highest SOC storage in top soil layer under long-term fertilization in a Chinese Mollisol. Moreover, the manure combined with NPK had the highest SOC storage along the soil profile (Fig. 1). This is due to the higher organic carbon input from manure and its stabilization in the soil as further revealed by higher carbon input (Table 2)

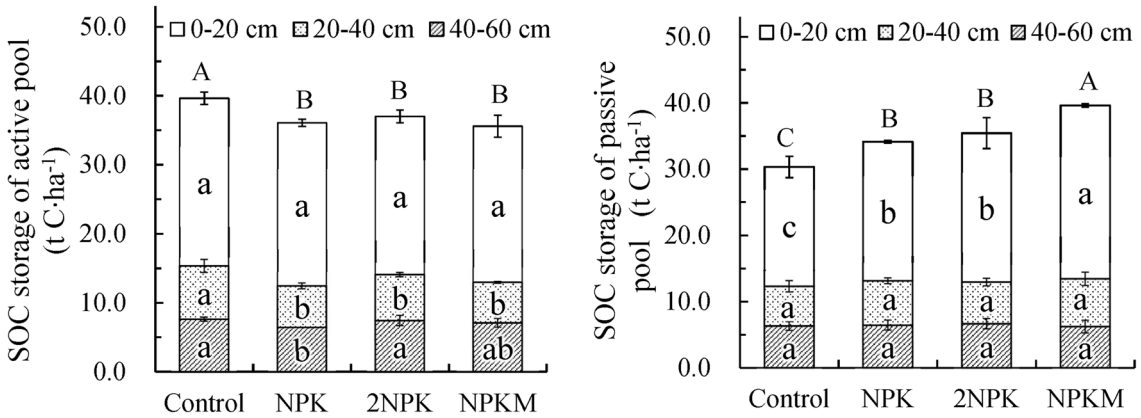

Fig. 5 The SOC storage of active and passive C pools at three soil depths in different treatments. Note: Different lower-case letters indicate significant differences among treatments at each soil depth ($p < 0.05$), different upper-case letters indicate significant differences among treatments at the whole soil profile of 0–60 cm ($p < 0.05$)

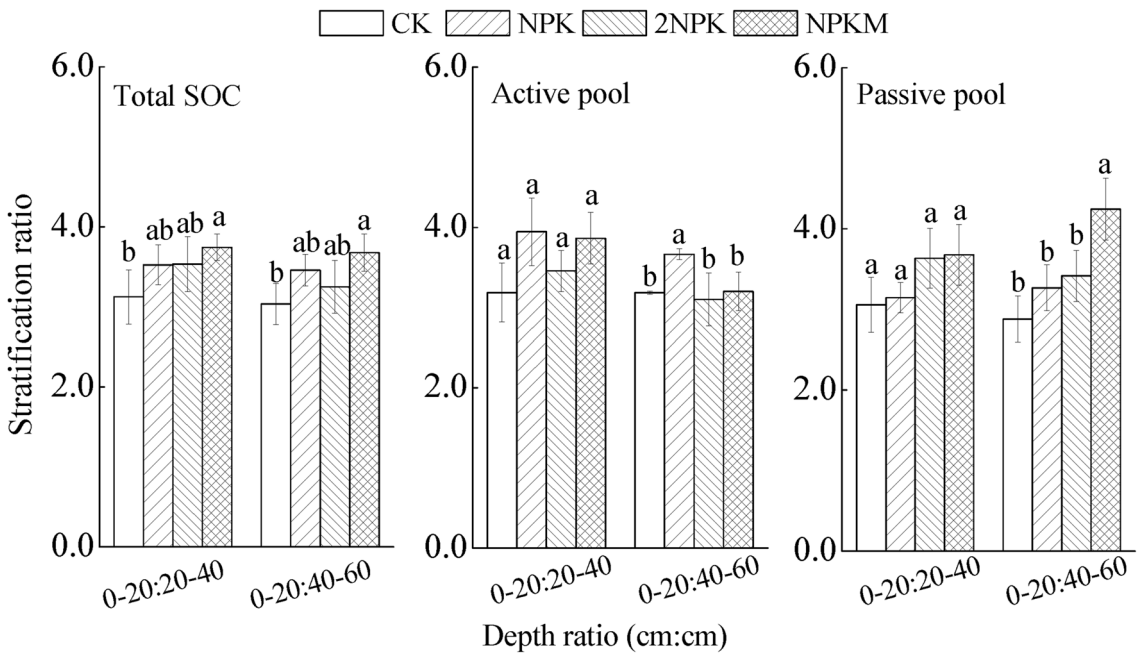

Fig. 6 Stratification ratios of total SOC, active pool and passive pool. Note: Different lower-case letters indicate significant differences among treatments for each depths ratio in each sub-figure ($p < 0.05$), bars represent mean ± standard error

and higher proportion of passive carbon observed in this study (Fig. 1). These findings were further substantiated by the observation that the accumulation rate of SOC storage during 29-year experimentation was significantly positively correlated with the amount of external carbon input, which suggested the potential of the red paddy soil in sequestrating exogenous carbon. The highest SOC accumulation and sequestration observed in topsoil under NPKM treatment (Fig. 2) further verify these findings. Taken together, these findings suggested that combining manure with balanced NPK could be an attractive strategy in improving SOC sequestration, however, which is further governed by annual carbon input and soil depths.

Fertilization impacts on SOC stratification characteristics

In red paddy soil, the distribution of carbon in SOC fractions showed variation with depth, with a higher level of SOC storage found in the surface soil compared to the deeper soils. This was mainly caused by carbon

accumulation from stubble and root residue in the surface soil. Zhang et al. [25] reported that, about 80–90% of the roots of rice crop are distributed in the 0–10 cm soil layer and little in deeper layers which might be due to soil compaction in deeper layers limiting root growth [16]. This implies that there is a wide gap in external carbon input between the surface soil and the deeper soils (Table 2). Thus, the SOC would be stratified in surface soil layers with higher stratification ratios being regarded as good indicators of dynamic soil quality [15]. In this study, the enhancement of stratification ratio of total SOC under manure application was mainly reflected in the passive pool rather than active pool (Fig. 6), indicating that manure application could efficiently improve carbon stability in surface soil. This could be associated with the properties of manure resulting in higher recalcitrant compounds, which consequently enhanced SOC stability [26].

Fertilization treatments influenced total SOC storage in surface soil and showed no significant effect on deeper layers except for the non-labile *C* fraction at 20–40 cm soil layer. Green manure or animal manure can improve root biomass [27] and the external carbon input from roots would accumulate not only in the top soil but also in the subsoil due to leaching or the increased soil microbial activity [28]. However, the impact of fertilization on root biomass is mainly reflected in the surface layer [29], and the amounts of root biomass distributed in the 20–60 cm soil layers were usually very low, about 10–20% of the total. Thus, the increase in root biomass in the deeper soil layers caused by fertilization was limited. Additionally, non-fertilized treatment relatively improved the SOC storage of active pool especially in deep soil layers comparing with fertilized treatments, which might be also related to root distribution, root exudates and rhizosphere microbial environment since the root growth and distribution in different soil layers vary between fertilized and non-fertilized conditions [29].

Fertilization impacts on SOC stability

The SOC storage of active pool was greater under no fertilization than in fertilized treatments, and fertilization increased the SOC storage of passive pool (Fig. 5). This was associated with the increased SOC storage of less-labile C fraction in the surface soil, hence the SOC storage of passive pool. This suggested that fertilization derived SOC accumulated in the passive pool and improved soil carbon stability, whereby the highest values were found under combined organic fertilization with inorganic fertilization. These findings corroborate with Yanardag et al. [30] stating that pig manure increased the amount of recalcitrant carbon in Luvisol. Contrarily, Mandal et al. [9] found that the increase in SOC due to manure application was not limited to the less-labile C fraction, but also involved the very-labile C and non-labile C fractions; and Sun et al. [12] showed that manure application increased total SOC by increasing SOC accumulation only in very-labile C fraction. These inconsistent results were probably due to variability in manure quality and quantity [30]. The differences in the findings of the studies by Mandal et al. [9], Sun et al. [12], and the current study could be attributed to variations in the types and quality of the organic amendments used. The manure used in Mandal et al. [9] was highly decomposed and had high amounts of lignin and polyphenol, while the manure used in Sun et al. [12] and the current study were fresh pig manure and legume and fresh pig manure, respectively. These variations could lead to differences in the proportions of WSC, biodegradable organic compounds, and non-decomposable organic compounds, and thus affect the distribution of SOC fractions in response to different organic amendments [31, 32], and this could lead to differences in the carbon decomposition, immobilization and distribution characteristics of SOC fractions which ultimately affects SOC stabilization. In nutshell, our results highlighted the positive influence exerted by organic amendments in intensive double-rice cropping in terms of increasing the stability of SOC.

Conclusions

This study systematically concluded that the sequestration of SOC per unit exogenous carbon input was limited. Fertilization especially manure application could improve the SOC storage and stability in surface soil and increased the difference among soil layers with more SOC being stratified in surface soil. Therefore, fertilization, if possible, combined with deep ploughing might be conducted practically to promote stability of SOC in deep soil, and be beneficial to the sustainability of soil fertility. This long-term manure application is beneficial for improving the SOC storage and stability in red paddy soil, and it has a greater impact on the topsoil rather than subsoil.

Acknowledgements
We thank the experimental station staff for managing the experimental fields.

Author contributions
XC, MX, QH, KL Design of the work, performed the research, data analysis; MX Supervision, project administration, conceptualization; XC, HX, NS, A Mustafa: Wrote the manuscript; MX, A Mustafa: Revised the manuscript.

Funding
The work was supported by National Natural Science Foundation of China (42177341).

Availability of data and materials
All data generated or analyzed during this study are included in this article, and be available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors listed have read the complete manuscript and have approved submission of the paper.

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

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Received: 29 June 2023 Accepted: 2 September 2023
Published online: 19 September 2023

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