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Long-Term Fertilization Alters the Storage and Stability of Soil Organic Carbon in Chinese Paddy Soil

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Abstract: The storage of soil organic carbon (SOC) in cropland soils is an essential strategy that serves the dual purpose of enhancing soil fertility and mitigating climate change. However, how the stability of stored carbon is altered under long-term fertilization has not been well understood, especially in the double rice cropping system in Chinese paddy soils. In this study, we explored the SOC storage and consequent stability of SOC under long-term fertilization. The soil samples were fractionated chemically to isolate various fractions and constituent pools of SOC (i.e., very labile C/VLC, labile C/LC, less labile C/LLC, and non-labile C/NLC). The following treatments were tested: control (CK), recommended rate of inorganic fertilizer (NPK), double the amount of recommended rate of inorganic fertilizer (2NPK), and NPK combined with manure (NPKM). The results showed that, relative to the initial level, the application of NPKM significantly improved the SOC storage as compared to the control. The long-term NPKM increased the total SOC in the paddy soil and this increased SOC was mainly stored in LLC, as revealed by the highest increase (142%) over the control. Furthermore, the highest proportion of labile pool was associated with unfertilized CK, while the reverse was true for the recalcitrant pool, which was highest under NPKM. This supports the role of combining manure with NPK to improve the stability of SOC, further verified by the high recalcitrance index under NPKM (56.75% for 0–20 cm and 57.69% for 20–40 cm) as compared to the control.

Keywords: SOC stock; soil organic matter; sustainable agriculture; manure; inorganic fertilizers

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1. Introduction

Soil organic carbon (SOC) is the key contributor to soil fertility and crop productivity, potentially controlling the nutrient cycling and promoting physical, chemical, and biological soil health. The storage of SOC is the outcome of a net balance between carbon (C) accumulation (through the addition of amendments) and decomposition (through abiotic and microbial oxidation) in agro-ecosystems [1]. The accumulation of SOC is regulated by the type and intensity of soil management practices, land use types, climatic fluctuations, and edaphic factors [2]. The continuous and compositionally heterogeneous nature of SOC necessitates the identification and isolation of fractions with varying stability to facilitate a comprehensive understanding of the influence of long-term management practices on soil fertility [3–5]. Therefore, it becomes imperative to ascertain the impacts of management strategies on SOC dynamics.

The application of organic and inorganic fertilizers to arable soils is the most commonly reported management strategy for improving soil fertility and quality [6–8]. Manure addition in agricultural fields enhances C input and so does the SOC pool, while the inorganic fertilizer might have a positive, negative, or near neutral influence on SOC pools [9–12]. Moreover, the addition of organic and inorganic fertilizers enhances crop biomass and yields which ultimately increases crop residue inputs and hence SOC [13,14]. The storage and stability of SOC depends on C stabilization mechanisms which are categorized as: (a) inherent recalcitrance; (b) spatial inaccessibility; and (c) organo-mineral interactions [3,15,16]. To better describe these stabilization mechanisms, the SOC is separated into labile and recalcitrant pools with differing stability [12,17]. The labile pool is characterized by fast turnover rates representing products of a microbial origin and live microbes [1,18]. This labile pool changes quickly under the influence of management practices and derives the flux of nutrients in agro-ecosystems [2,19]. However, the less labile and non-labile C pool is characterized by the stabilized fractions of SOC which are protected against microbial degradation due to the formation of organo-mineral interactions [20]. Since this pool is considered stable, its evaluation provides the basis to understand and improve SOC sequestration [12,21]. It is well-established in the literature that organo-mineral associations stabilize organic C against biodegradation in a variety of ways, including: (1) adsorption of organic matter to mineral surfaces [22,23]; (2) formation of metal-organic matter complexes [24,25]; and (3) occlusion of organic matter within aggregates [15,26]. Thus, to understand the soil fertility change and C sequestration potential of fertilization, the labile and recalcitrant C pools are separated and quantified [2]. However, to date, the understanding of the influence of long-term fertilization on SOC stability by altering recalcitrant C pool has been limited. The SOC in subsoil depths is assumed to be stable [27,28] as it is found in association with soil minerals showing biochemical recalcitrance and physical protection in deep soil [2]. Moreover, almost half of the SOC is found below 30 cm [2,29] showing the potential of subsoil to sequester and stabilize SOC. Therefore, understanding the dynamics of SOC pools in subsoil provides deeper insights into the effects of long-term management practices. It is worth noting that the term subsoil refers to the layer immediately below the topsoil [30], however, different works define topsoil and subsoil depths based on their sampling strategies.

Red soils (Ultisols, as referred by the FAO soil classification system) represent 30% of Chinese arable soils, covering 2.04 million km^2 in tropical and subtropical areas of China [31,32]. These soils are a base for the high food demands for the growing population, especially fulfilling the need for rice [33,34] due to favorable climatic conditions. In general, these paddy soils have been characterized as having higher SOC stocks as compared to their counterpart upland soils in the same region [35,36]. Previously, it has been reported that the application of long-term fertilization has resulted in the enhanced accumulation of SOC [34,37]. However, most of the studies on these typical soils have explored the SOC dynamics of the surface soil. Moreover, the intensive cultivation (rice–rice) associated with higher chemical fertilization and enhanced soil erosion has resulted in the degradation and loss of soil fertility. Therefore, more studies are required to assess the actual benefits of long-term nutrient management practices on the stability of SOC and its inter-depth variations at (0–20 cm and 20–40 cm) depths. Therefore, we took advantage of a long-term fertilization experiment with red paddy soil to assess the changes in soil fertility at different depths. The specific aims were: (i) to assess changes in the accrual of SOC in the soil profile (0–40 cm); and (ii) to assess variations in SOC stability as revealed by pools separated by chemical fractionation and their sensitivity to long-term fertilization.

2. Materials and Methods

2.1. Site Description

The long-term experiment was commenced in 1981 at Jinxian (28°15′30′′ N, 116°20′24′′ E, elevation 30 m above sea level), managed by the Institute of Red Soil, Jinxian Town, Jiangxi, China. The soil is a loam and classified as a Stagnic Anthrosols (IUSS working group) [38] and a red paddy soil in the Chinese classification system, originally derived from Quaternary Red Clays. The soil sampled in 1981 had SOC 16.2 g kg^{-1}, TN 0.95 g kg^{-1}, TP 0.05 g kg^{-1}, TK 1.07 g kg^{-1}, C:N 10.5, pH of 6.9, and clay contents (<0.002 mm) of 26.6% [32]. The climate is subtropical humid monsoon, the mean annual precipitation is 1627 mm, and the mean annual temperature is 18.1 °C.

2.2. Experimental Design and Treatments

This long-term experiment had a double rice cropping system (rice–rice–winter fallow), which represents the most common cropping practice of this area. The early rice was grown from April to early July, and the late rice from the middle of July to late October. Rice seedlings, 30 days old, were transplanted manually in the experimental field plots. Crops were harvested manually close to the ground and all harvested biomass was removed from the plots. Each experimental plot had an area of 46.67 m^2 with each treatment replicated three times, in a randomized complete block design. The following treatments were the tested control (CK), recommended rate of inorganic fertilizer (NPK), double the amount of the recommended rate of inorganic fertilizer (2NPK), and NPK combined with manure (NPKM). The inorganic fertilizers were supplied as urea, calcium-magnesium phosphate, and potassium chloride, while for the early rice the source of manure was milk vetch (*Astragalus sinicus* L.) with the following composition: water contents 80%, organic carbon 39.24%, and organic nitrogen 3.01%; and for late rice it comes from pig manure having the following composition: water content 70%, organic carbon 41.38%, and organic nitrogen 2.09%. All of the P, K, and manure fertilizers were applied as a basal dose, while 60% of N fertilizer was applied as a basal dose before the rice seedling transplantation. The remaining 40% N fertilizer was applied in equal portions at the panicle initiation stage. The annual doses of arranged treatments in this site are presented in (Table 1).

Table 1. The amount of inorganic N, P, K fertilizer, and manure applied under various fertilization treatments.

Treatments	Early Rice Inorganic Fertilizers (kg ha^{-1})			Early Rice Manure (Milk Vetch) (Mg ha^{-1})	Late Rice Inorganic Fertilizers (kg ha^{-1})			Late Rice Manure (Pig) (Mg ha^{-1})
	N	P	K		N	P	K	
CK	0	0	0	0	0	0	0	0
NPK	90	20	62	0	90	20	62	0
2NPK	180	40	124	0	180	40	124	0
NPKM	90	20	62	22.5	90	20	62	22.5

2.3. Soil Sampling and Analyses

Composite soil samples were obtained in 2010 by collecting three auger samples from each of the replicated plots from 0–20 (topsoil) and 20–40 cm (subsoil) depth intervals after the crop harvest. Crop remains, dead roots, and pebbles were removed. Soil samples were brought to the lab after air drying and passed through a 2 mm sieve for further analysis. A portion of the soil samples were crushed and passed through a 0.15 mm sieve for the analysis of SOC, TN, and different fractions of C. The separate soil samples were collected from each plot and two depths following the ring method (volume of 100 g cm^{-1}) for measuring bulk density [39]. For SOC quantification, the samples were processed to remove carbonates and then ground. The content of SOC and TN was analyzed using a CHN analyzer (EA–3000, EuroEA Elemental Analyzer, EuroVector, Pavia, Italy).

2.4. Oxidizable Organic Carbon and Its Fractions

Different fractions of oxidizable organic carbon were determined following a modified Walkley–Black method [40]. This involved using different concentrated H_2SO_4 resulting in three acid–aqueous solution ratios corresponding 12, 18, and 24 *N* of H_2SO_4, respectively, and caused the oxidation of SOC by the produced heat [41]. The amount of oxidizable organic carbon was determined using 5, 10, and 20 mL of concentrated sulfuric acid, which allowed for the separation of total organic carbon into four fractions of decreasing oxidizability, as defined by Chan et al. [40].

In this regard, each chemically isolated SOC pool was calculated as the difference between the total SOC (TOC) and the SOC content after the treatment, as shown in (Table 2).

Table 2. Chemically isolated SOC fractions using different concentrations of H_2SO_4.

Very Labile C (VLC)	Difference between TOC and SOC Content Following 12 *N* H_2SO_4 Treatment
Labile C (LC)	Organic carbon content as a difference between 18 *N* and 12 *N* H_2SO_4-treated soils
Less labile C (LLC)	Organic carbon content as a difference between SOC in 24 *N* and 18 *N* H_2SO_4-treated soils
Non-labile C (NLC)	Difference between 24 *N* H_2SO_4-treated soil and total SOC as determined by the CHN analyzer

Subsequently, in line with the study objectives, the very labile and labile C were pooled as the labile pool, while the less labile and non-labile C were the recalcitrant pool [40]. Moreover, the recalcitrance index was measured as a ratio of recalcitrant pool/labile pool [42].

Furthermore, the SOC stock (Mg ha^{-1}) was calculated from the following equation:

$$\text{SOC stock (Mg ha}^{-1}) = C_{conc} \times B_d \times D \times 10 \quad (1)$$

where C_{conc} represents the SOC concentration (g kg^{-1}), B_d is the bulk density (Mg cm^{-3}), D is the soil depth interval, and 10 is the unit conversion factor.

2.5. Statistical Analyses

Data processing and statistical analysis were performed using MiniTab 21.3 (MiniTab Inc., State College, PA, USA, 2022) and Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA). Two-way ANOVA with separation of means by Tukey test ($p < 0.05$) was used to examine the impacts of treatments on soil BD, total SOC, SOC (NLC, VLC, LC, LLC) and corresponding pools, total N, C:N ratio, and RI. Additionally, linear regression as well as bivariate association analyses were conducted to evaluate the link between the content of selected organic C fractions to the total SOC. Pearson correlation (heat map) was plotted using R software version 4.2.3.

3. Results

3.1. Lability and Stability of SOC in Top and Subsoil

Considerable differences were examined for the distribution of C contents in fractions of SOC and its pools under the 29-years-long fertilization. Generally, higher SOC contents were observed in the top layer as compared to the deep layer (20–40 cm) for VLC, LC, LLC, and NLC (Figure 1a,b). The application of manure in combination with inorganic fertilizer (NPKM) significantly ($p < 0.0001$) increased the SOC contents of VLC, LLC, and NLC compared to the control treatment. However, the effect of NPKM on the content of LC was not statistically significant (Figure 1a). Moreover, this effect was only pronounced in the 0–20 cm layer. These findings for SOC fractions were patterned as 0–20 > 20–40 for soil depths, while for treatments it patterned as NPKM > 2NPK ≈ NPK > CK (Figure 1a,b). Furthermore, the highest increase in SOC contents under NPKM over control (142%) in

0–20 cm was observed in LLC, suggesting that these fractions are the most responsive in the accumulation of SOC (Figure 1b). These results were further verified by a two-way ANOVA showing the significant ($p < 0.001$) effects of treatment, depth, and their interaction on LLC (Supplementary Materials, Table S1).

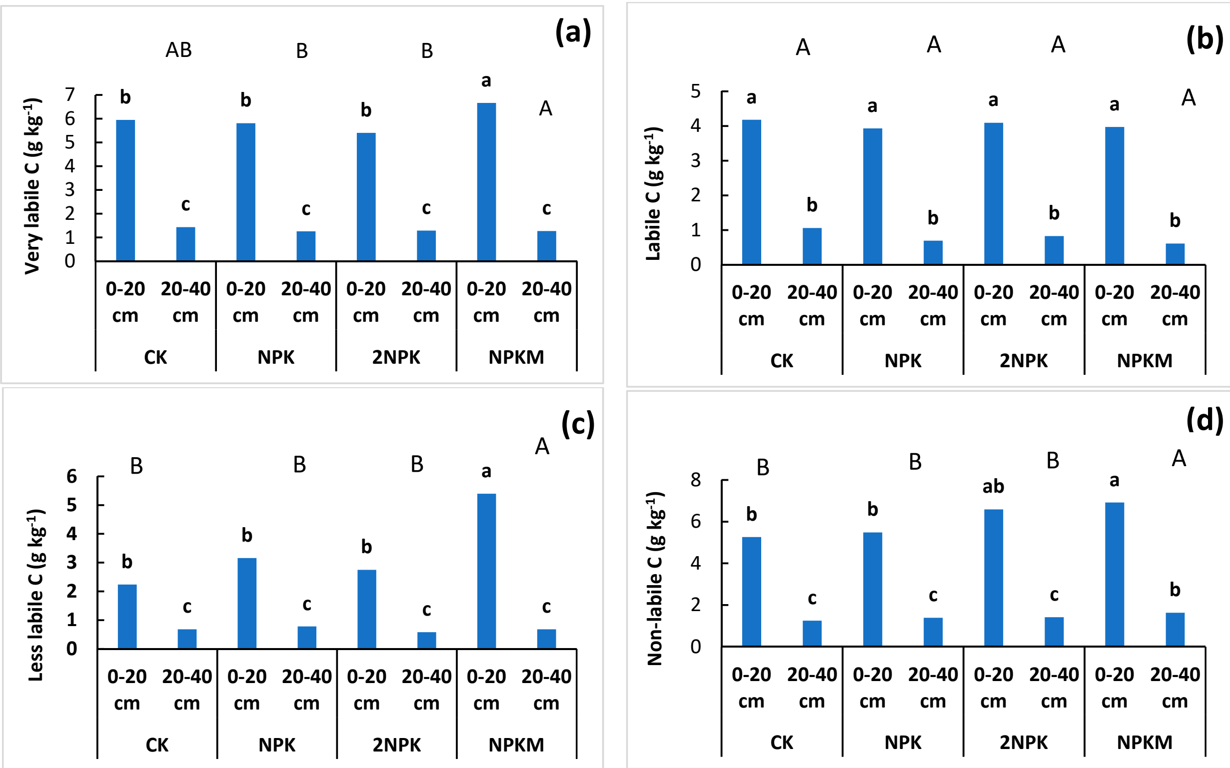

Figure 1. The distribution of SOC fractions under long-term sole and the integrated application of inorganic and organic fertilizers spanning surface and sub-surface soil depths. Dissimilar lowercase and uppercase letters represent statistical differences between soil depth intervals and treatments, respectively. Abbreviations are: CK, control; NPK, application of nitrogen, phosphorus and potassium; 2NPK, double the rate of NPK; NPKM, combined application of manure and NPK. Pools are: (**a**) VLC, very labile carbon; (**b**) LC, labile carbon; (**c**) LLC; less labile carbon; and (**d**) NLC, non-labile carbon, ($n = 3$).

In addition, the labile and recalcitrant pools mirrored the findings of relevant fractions. Specifically, the application of different fertilizer regimes for 29 years did not change the labile pool (LP) relative to the control (Figure 2a). The addition of two levels of inorganic fertilizers (NPK and 2NPK) non-significantly decreased the labile pool as compared to the control, while NPKM yielded higher values of LP but remained non-significant to the control (Figure 2a). Moreover, the labile pool considerably decreased in the following trend 0–20 > 20–40 (Figure 2a). Contrarily, the recalcitrant pool was considerably higher in the fertilized treatments as compared to the control and found in the order 0–20 > 20–40 (Figure 2). In this case, the application of NPKM and 2NPK yielded the highest (RP), ranging between 64.21 and 24.40%, respectively, in the topsoil layer (Figure 2a). Moreover, the treatments, soil depths, and their interaction had a significant effect on RP (Table S1). Furthermore, the proportion of LP and RP varied among the applied treatments and soil depths (Figure 2b). The highest proportion of LP was associated with the unfertilized CK soil while the lowest proportion was with the NPKM treatment. The reverse was true for RP, whereby the highest proportion was found under NPKM treatment, which was statistically significant as compared to CK (Figure 2b). This suggests the potential of combining manure with NPK fertilizers for stabilizing higher amounts of C in soil. This was further verified by the findings of the recalcitrance index which was significantly higher under NPKM (56.75%

for 0–20 cm and 57.69% for 20–40 cm) treatment as compared to the control (Figure 3a). In addition, a strong positive relationship of RI was found with total SOC for topsoil as compared to subsoil (Figure 3b).

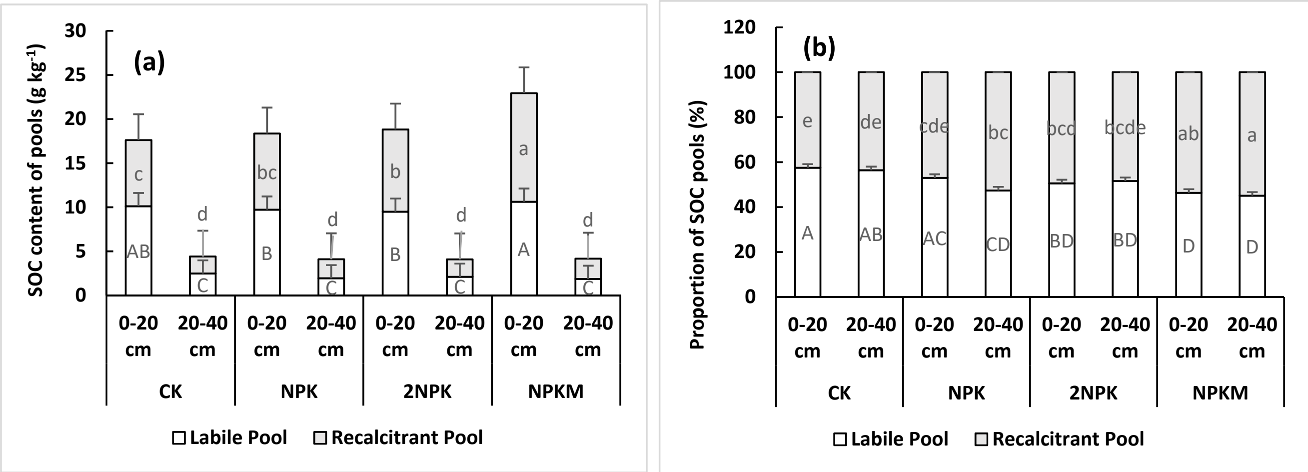

Figure 2. The contents of SOC pools (**a**) and proportions of pools (**b**) under long-term sole and the integrated application of inorganic and organic fertilizers spanning surface and sub-surface soil depths. Dissimilar lowercase letters represent statistical difference between treatments and soil depths. Abbreviations are: CK, control; NPK, application of nitrogen, phosphorus and potassium; 2NPK, double the rate of NPK; and NPKM, combined application of manure and NPK. ($n = 3$). In each panel, different letters denote significant difference between depth intervals for SOC pools at p-value < 0.05.

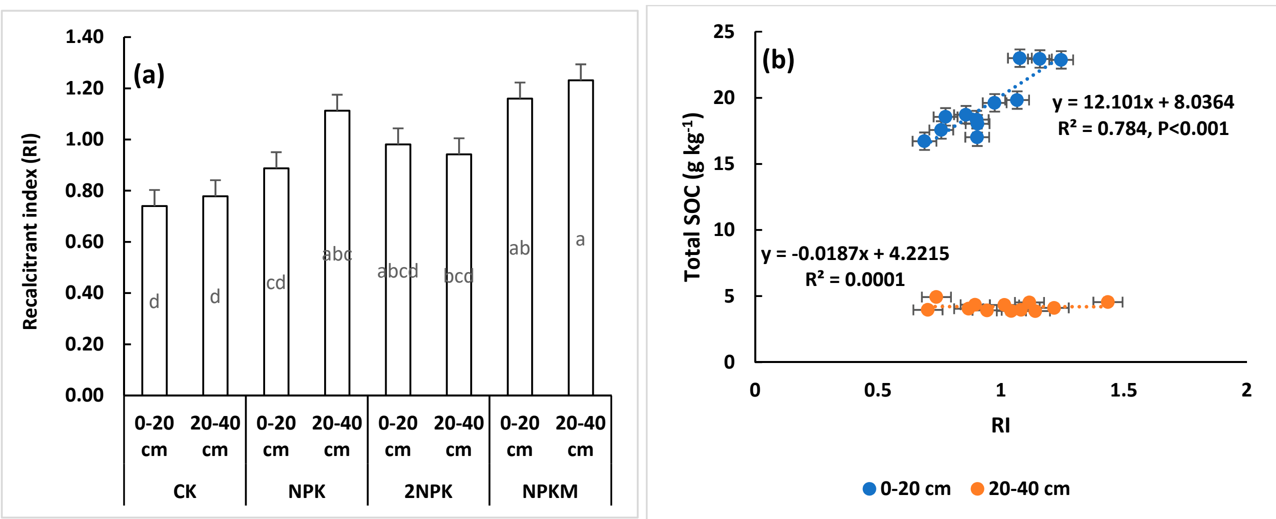

Figure 3. The recalcitrance index as a measure of SOC stability (recalcitrance pool/labile pool) under long-term sole and the integrated application of inorganic and organic fertilizers spanning surface and sub-surface soil depths (**a**) and relationship between RI and total SOC (**b**). Dissimilar lowercase letters (panel a) represent statistical differences between treatments and soil depths. Abbreviations are: CK, control; NPK, application of nitrogen, phosphorus and potassium; 2NPK, double the rate of NPK; and NPKM, combined application of manure and NPK.

3.2. Soil Basic Characteristics in Top and Subsoil

The soil basic properties exhibited significant differences following long-term fertilization, as expected. These properties vary greatly between both soil depths (Table 3). The soil BD was the most affected and increased with increasing depth intervals. The application of 2NPK and NPKM resulted in the highest values (1.57 and 1.56 Mg m^{-3}), respectively, in 20–40 cm which were significantly different than those in 0–20 cm (1.21 and 1.06 Mg m^{-3}, respectively). The highest SOC was observed under NPKM (22.94 g/kg), which was 30% higher than the control in the 0–20 cm layer (Table 3). In this case, the higher values were associated with the surface rather than the sub-surface soil layer. A similar SOC trend was observed for TN, whereby the application of NPKM significantly enhanced TN contents by 33.62% as compared to the control in the 0–20 cm soil layer. The same treatment significantly enhanced the SOC stocks by 15.4% in the 0–20 cm soil depth as compared to the control; however, no significant effect was observed for the 20–40 cm soil depth. The soil C:N, however, remained statistically unchanged under fertilization treatments with higher values observed for the unfertilized control (Table 3).

Table 3. Distribution of soil basic characteristics spanning surface (0–20 cm) and sub-surface (20–40 cm) soil depths under long-term fertilization in the Chinese red paddy soil.

Depth (cm)	Treatments	Bulk Density (Mg m^{-3})	SOC g kg^{-1}	SOC Stock Mg ha^{-1}	TN (g kg^{-1})	C: N Ratio
0–20	CK	1.2 ± 0.01 b	17.62 ± 0.92 b	42.29 ± 0.95 b	2.26 ± 0.1 b	7.81 ± 0.05 a
	NPK	1.21 ± 0.01 b	18.37 ± 0.34 b	44.57 ± 0.94 ab	2.39 ± 0.04 b	7.7 ± 0.01 a
	2NPK	1.21 ± 0.02 b	18.82 ± 1.56 b	45.38 ± 0.96 ab	2.49 ± 0.19 b	7.56 ± 0.05 a
	NPK + M	1.06 ± 0.03 c	22.94 ± 0.06 a	48.78 ± 0.97 a	3.02 ± 0.01 a	7.59 ± 0.03 a
20–40	CK	1.55 ± 0.02 a	4.41 ± 0.48 c	13.69 ± 0.93 c	0.75 ± 0.03 c	5.9 ± 0.37 b
	NPK	1.54 ± 0.03 a	4.12 ± 0.35 c	12.69 ± 0.94 c	0.68 ± 0.02 c	6.05 ± 0.27 b
	2NPK	1.57 ± 0.01 a	4.1 ± 0.21 c	12.91 ± 0.95 c	0.7 ± 0.01 c	5.88 ± 0.18 b
	NPK + M	1.56 ± 0.02 a	4.18 ± 0.34 c	13.06 ± 0.94 c	0.79 ± 0.13 c	5.37 ± 0.79 b

Comparison of means of basic soil characteristics among the whole soil dataset (i.e., different fertilization treatments and soil depth intervals). Different lowercase letters are significant at $p < 0.05$ following Tukey test.

3.3. Relationships among Observed Attributes

A significant ($p < 0.05$) linear relationship was found between labile pool and bulk SOC in both soil layers (0–20 and 20–40 cm) (Figure 4a). It can be seen that the slope of the relationship for the bottom soil layer is higher than that of the topsoil. A similar trend was observed for the recalcitrant–SOC relationship. However, no significant relationship was found for the SOC–recalcitrant contents in the 20–40 cm soil layer (Figure 4b). Linear regression analysis showed that there was a significant relationship between the recalcitrance index (RI) and the total SOC content for the topsoil layer only (Figure 3b). This means that a higher RI value (recalcitrant pool/labile pool ratio) led to higher total SOC content in the topsoil layer, suggesting that recalcitrant pool contributes significantly to the SOC content of the surface soil.

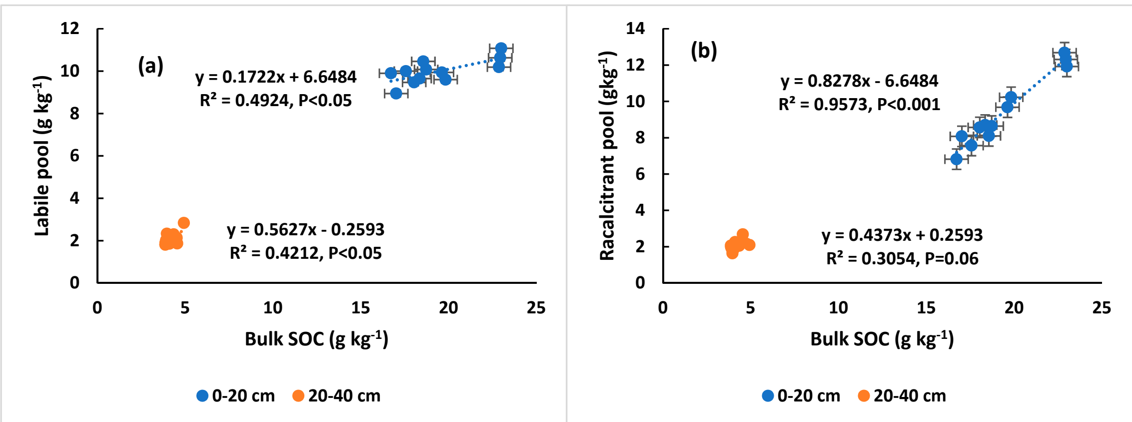

Figure 4. Relationships among SOC in bulk soil and (**a**) labile C pool, and (**b**) recalcitrant C pool (g kg^{-1}) under long-term sole and integrated application of inorganic and organic fertilizers spanning surface and sub-surface soil depths.

Pearson correlation analysis for the two soil layer datasets is shown in (Figure 5). The topsoil dataset (Figure 5) displayed a strong positive correlation between total SOC and its fractions (r = 0.702–0.978) and SOC-TN (r = 0.996 at $p < 0.0001$). However, SOC is not significantly correlated with labile C fractions.

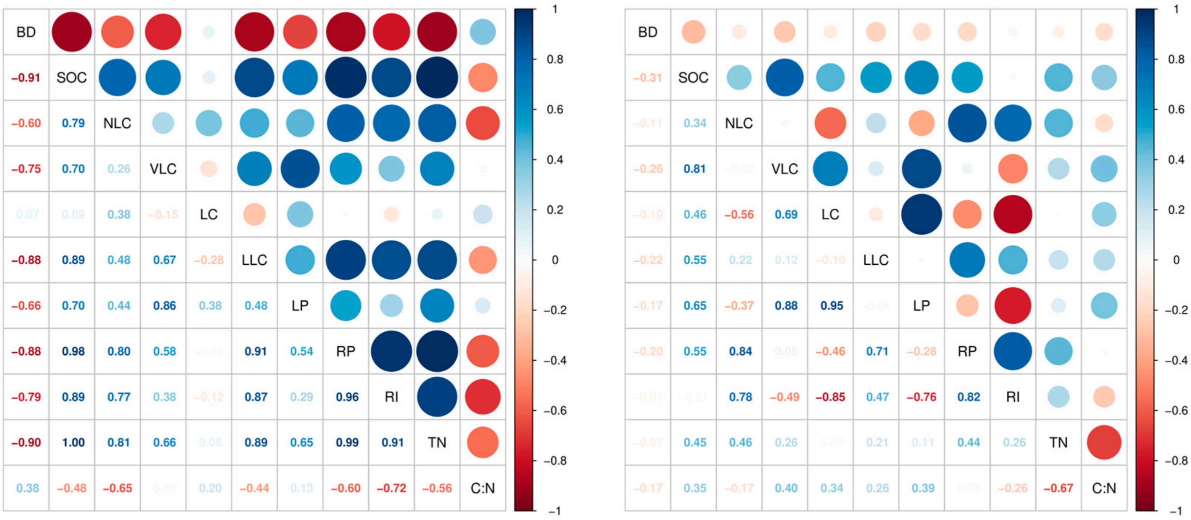

Figure 5. Pearson correlation (heat map) among basic soil characteristics, soil organic carbon sequestration and its stability indicators spanning surface (0–20 cm, left panel) and sub-surface (20–40 cm, right panel). The numbers in both panels represent the correlation coefficients, while colors denote the positive and negative correlations among observed soil characteristics. Abbreviations are: BD, bulk density; SOC, total soil organic carbon content; VLC, very labile carbon; LC, labile carbon; LLC; less labile carbon; NLC, non-labile carbon; LP, labile pool; RP, recalcitrant pool; RI, recalcitrance index; TN, total nitrogen content; and C:N, carbon to nitrogen ratio in the Chinese red paddy soil.

In contrast to the topsoil dataset, no significant correlation was found in the subsoil layer for the inter-organic C relationships (i.e., SOC with isolated fractions), except for the SOC very labile C (r = 0.886 at $p < 0.01$) and SOC labile pool (r = 0.649 at $p < 0.05$). This indicates that increasing SOC will result in an increase in labile C. No substantial correlation was found for SOC-TN, implying that the total N content in the bottom soil layers was not significantly regulated by the soil organic carbon content (Figure 5).

4. Discussion

Higher accumulation and distribution of SOC in VLC, LLC, and NLC pools in manure and NPK-treated soils can be attributed to the higher amount of decomposed organic manure (pig and or milk vetch) and a higher rate of its decomposition [43] which resulted in a greater amount of VLC in the present study. Moreover, the enhancement of crop yields and biomass under the combined application of NPK and manure has been well acknowledged [8]. The enhanced crop yield and root biomass would resultantly increase labile C inputs in the soil, ultimately increasing the contribution of labile C in soil [29,44]. These results are substantiated with the findings of Sun et al. [10] who found a similar pattern to the one observed in the present study. Additionally, our observations revealed that the highest accumulation of SOC occurred in the LLC (Figure 1a), indicating that this fraction was the most responsive to SOC accumulation. These findings are in line with Mandal et al. [17] who observed that organic amendments derived C accumulated more in LLC and NLC. Moreover, the enhanced LLC and NLC under NPKM addition might be associated with the fact that the manure might be partially decomposed with an increased recalcitrant compound [45]. These findings stand in agreement with Belay-Tedla et al. [46] who reported that the application of manure resulted in enhanced lignin (recalcitrant compound of decomposition). The correlations among different SOC fractions and bulk contents as well as labile and recalcitrant pool (Figures 4a,b and 5) demonstrate their interconversion. This shows that some of the labile C might become incorporated into NLC, or NLC into LC, through the microbially enhanced mineralization of manure [47]. Manure, combined with NPK, has been recognized to improve SOC stability [12,48]. We found higher recalcitrant pool in soils fertilized with either inorganic manure or combined with manure–NPKM treatments (Figure 2). These results are in accord with the findings of Zhou et al. [12] who found higher recalcitrant pool under long-term fertilization in four cropland soils in China. However, these results contrast with the findings of Chen et al. [49] who found higher labile pool under integrated fertilizer treatments. Such contradictions could be due to the quantity and quality of the added manure types [10]. Moreover, a higher proportion of the total SOC of recalcitrant pool was found in the sub-surface (20–40 cm) layer amounting (55%), with the higher proportion being observed for the NPKM treatment (Figure 2b). These results could be due to (i) the inherent recalcitrance of the applied manure and its interaction with soil minerals in the deep layer [50]; or (ii) the stimulatory effect of the combined nutrient treatment on crop root biomass [44] and reduced access of microbes to available SOC for decomposition [2]. Soil microbes in the sub-surface layers are more distant from the rhizosphere, resulting in decreased microbial activity and biomass, ultimately limiting the decomposition of SOC in the deeper soil layers [51,52]. Thus, this higher accumulation of C into recalcitrant pool explains higher values of recalcitrance index (RP/LP) (Figure 3), which might be due to the intercalation of OC in soil clay minerals, which ultimately increases the chemical recalcitrance of SOC [53,54].

Increasing SOC and TN contents under NPKM treatment (Table 3) in the present study are in line with several previous works that describe increased SOC and TN under long-term fertilization [55–58]. Bhattacharyya et al. [59] reported enhanced SOC contents in bulk soil through addition of manure combined with NPK fertilization in India. Moreover, Li et al. [60] and Mustafa et al. [48] found higher TN in soils subjected to long-term NPKM application in a Chinese Mollisol, which was related to higher OM input and improved soil physico-chemical properties. Furthermore, the higher SOC and TN in the surface soil layer (0–20 cm) might be associated with higher root and plant biomass coming to the topsoil and the reduced concentration of nutrients associated with the lower microbial biomass in the sub-surface layers [61,62]. The soil C and N dynamics in the topsoil are regulated by management practices [63] which corroborate well with the findings obtained in the present study, in which different fertilization treatments had different effects on SOC and TN and their effects were more pronounced in the topsoil as compared to the subsoil (Table 3). Interestingly, no significant correlation was found for the SOC–C:N ratio suggesting that the total SOC content of topsoil was not significantly controlled by SOC turnover. The long-term fertilization significantly enhanced SOC stocks (Table 3) which were higher in the surface than the sub-surface soil layers. These findings are substantiated with Abrar et al. [14] who reported the highest SOC storage in the topsoil layer under long-term fertilization in a Chinese Mollisol. In another study, Zhou et al. [11] found a depreciation in SOC stocks with increasing soil depths, a pattern similar to the one observed in the present study (Table 3). Furthermore, the highest SOC stocks were observed under manure combined with inorganic fertilizer application, which is directly linked to the higher OC derived from manure and its stabilization in soil as also revealed by a higher proportion of passive carbon pool under this treatment. The higher SOC stocks with manure application could also be due to the better soil aggregation and encapsulation of C in soil aggregates under manure addition [57,64]. Previous studies have reported that NPKM treatment could significantly enhance crop yields, resulting in higher C addition from crop biomass to soil [65,66], which ultimately enhances SOC stocks. The advantage of using green manures in addition to animal manure is the large increment in C input in soils. The advantage of using green manure in agricultural soils in improving SOC sequestration was also observed in previous studies [67,68]. Regarding the types of manures (green versus animal manure in this study), it should be noted that animal manure incorporation has the tendency to increase SOC stock, and such manures are efficient in increasing SOC stocks in the surface soil layers due to the fact that these manures are incorporated through tillage in the upper 0–20 cm soil layers [69,70]. On the other hand, the inclusion of green manures (milk vetch in this case) may increase the SOC stocks through improved C input by extensive root systems in deep soil profile, as noted by Guan et al. [71] in a previous study that including alfalfa promoted SOC stocks in addition to animal manure. Another study reported that adding green manure increased SOC down the soil profile up to 2 m [72]. As the deep soil depths have a great potential to sequester SOC, therefore, management practices that promote the translocation of manures to deeper soil depths are highly recommended. This study is an effort to promote SOC sequestration into the deep soil profile by including milk vetch in addition to animal manure in order to improve and synchronize C input under the double rice cropping system of southern China.

5. Conclusions

Long-term fertilization for 29 years improves SOC sequestration and its stability in Chinese red paddy soil under the double rice cropping system. Among various fractions, the highest increase was associated with less labile carbon (LLC) which suggests this fraction is the most robust in the accumulation of SOC. Among treatments, the manure combined with inorganic fertilizers (NPKM) yielded the highest recalcitrant pool (RP) which supports the role of combining manure with inorganic fertilization in enhancing the stability of sequestered carbon in red paddy soil. In conclusion, these findings suggest the role of long-term manure addition in improving sequestration and stability of SOC relative to initial conditions by modifying soil physico-chemical properties and distribution of SOC pools in the red paddy soil of China.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13061463/s1, Table S1: title; Analysis of variance (Two-Way ANOVA) for determined soil properties.

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