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# Effects of long-term fertilisation on soil organic carbon sequestration after a 34-year rice-wheat rotation in Taihu Lake Basin

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**Abstract:** To evaluate the long-term effects of fertilisation on soil organic carbon (SOC) sequestration in rice-wheat cropping ecosystems, SOC dynamics, stocks and fractionation were determined. The treatments included no fertilizer, mineral N and P, mineral N, P and K, organic fertiliser (OF), OF plus NP and OF plus NPK. The results showed that the average carbon inputs that derived from crop stubble, root residues and organic fertilisers were between 1.47 and 4.33 t/ha/year over the past 34 years. The average SOC stocks measured in the samples collected in 2011–2013 ranged from 31.20 to 38.52 t/ha. The range of the SOC sequestration rate was 0.11–0.40 t/ha/year with a SOC sequestration efficiency of 6.3%. Overall, organic fertilisation significantly promoted C-input, SOC and the sequestration rate compared to mineral fertilisation. The "active pool" (very labile and labile fractions) and "passive pool" (less labile and recalcitrant fractions) accounted for about 71.0% and 29.0% of the SOC fractions, respectively. Significant positive relationships between C-inputs and SOC fractions indicated that SOC was not saturated in this typical rice-wheat cropping system, and fertilisation, especially organic amendment, is an effective SOC strategy sequestration.

**Keywords:** carbon input estimation; soil quality; terrestrial ecosystem; long-term experiment; paddy soil

Soil organic carbon (SOC) is widely used as an important index of soil quality and fertility (Arshad and Martin 2002), and the enrichment of SOC is crucial to sustaining the good health of soils (Powlson et al. 2011). Furthermore, as an important C-reservoir in terrestrial ecosystems, SOC sequestration helps mitigate the effects of atmospheric CO<sub>2</sub> (Lal 2004) and reduces greenhouse gas emissions by approximately 90% (Smith et al. 2007).

SOC sequestration in agricultural soil mainly depends on carbon inputs (C-inputs); most of these C-inputs are respired, and only a small fraction remains, leading to a slow and gradual accumulation or depletion of SOC. Numerous studies have been

conducted to assess the effects of long-term fertilisation on SOC sequestration. For example, Xu et al. (2010) and Zhang et al. (2012) separately reported that long-term organic amendments significantly enhanced SOC sequestration, and paddy soil with a high clay content had a greater potential to sequester more C. However, there is still a lack of information about the SOC fractions that are primarily sequestered under the condition of different long-term fertilisation in a typical rice-wheat rotation system.

Chan et al. (2001) developed a method of fractionating SOC. In this method, the SOC is separated into labile and stable fractions. The labile part is the active pool with a more rapid turnover rate, which plays an impor-

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tant role in maintaining soil quality and productivity (Majumder et al. 2008). The stable part is the passive pool that is significantly attributed to the build-up of the SOC stock (SOCS) (Srinivasarao et al. 2014). This method is thus able to provide an insight into the mechanisms underlying whether SOC sequestration remains active or not. In the present study, we aimed to estimate the effect of C-inputs on SOCS, SOC sequestration rate/efficiency and fractionation after 34 years of the application of different types of fertiliser.

## MATERIAL AND METHODS

**Basic information.** This long-term fertilisation experiment (since 1980) is located at the National Soil Quality Observation Experiment Station in Xiangcheng District, Suzhou city, China (31°32'45"N, 120°41'57"E). The soil of this study is classified as Hydragric Anthrosols according to the World Reference Base for Soil Resources of FAO (IUSS Working Group WRB 2015), with hydromica and smectite as the dominant clay minerals. Initially, the soil contained 14.0 g/kg SOC, 1.43 g/kg total-N, 8.4 mg/kg Olsen-P, and 127 mg/kg available-K (extracted by  $\text{CH}_3\text{COONH}_4$ ), with a pH of 6.8 (ratio of soil and  $\text{CO}_2$ -free water, weight/volume, 1:2.5) and the bulk density (0–15 cm) of 1.26 g/cm<sup>3</sup>.

The experiment had a randomised triplicate design. Each treatment plot size was 20 m<sup>2</sup> (4 m × 5 m), and plots were separated by 35- to 40-cm-deep cement plates. The treatments in this study were as follows: no fertiliser (CK); mineral N and P (NP); mineral N, P, and K (NPK); organic fertiliser (OF); OF plus NP (OFNP) and OF plus NPK (OFNPK). Urea (46% N), calcium superphosphate (5.3% P) and potassium chloride (49.8% K) were used as the sources of N, P and K, respectively. Pig manure and oil rape cake were sepa-

rately applied before and after 1997, respectively. The depth of organic fertiliser was approximately 15 cm. The dry pig manure contained an average of 38.9% C, 2.2% N, 1.3% P, and 1.4% K, while the dry oil rape cake contained 33.4% C, 5.3% N, 0.8% P and 1.0% K. The fertilisation management is shown in Table 1.

Rice and wheat were cultivated one season per year in rotation. Rice seedling (18–20 days of age) was transplanted by hand in early June, and wheat was directly sown after rice harvest, usually in October.

**Soil sampling and analysis.** Soil samples were collected from the 0–15 cm layer after the rice harvest. In each plot, five replicates were randomly collected using a stainless-steel soil sampler. Soil samples were transported to the laboratory and air-dried; visible crop residues were removed, and the soil was ground. SOC was measured by the wet combustion method (Nelson and Sommers 1996). Total soil carbon was determined using the dry combustion method on an elemental analyser (Analytic Jena Co. Ltd, Jena, Germany). Soil bulk density (BD) was determined at a 0–15 cm depth using the metallic core method in 1980 and 2011–2013.

SOC fractionation was performed on the samples collected in 2011–2013 using a modified Walkley-Black method (Chan et al. 2001). Each soil sample (< 0.5 mm; 0.5000 g) was placed in a 500 mL Erlenmeyer flask (replicated nine times), to which 10 mL 0.167 mol/L  $\text{K}_2\text{Cr}_2\text{O}_7$  was added. These nine replicates were split into three equal groups, and the concentrated  $\text{H}_2\text{SO}_4$  (5, 10 or 15 mL) was added to the flasks of each group. The final concentrations of  $\text{H}_2\text{SO}_4$  in these groups were 6, 9 and 12 mol/L, respectively. After complete mixing and reaction, 1.0 mol/L  $\text{FeSO}_4$  was used to titrate the excess dichromate. The amount of oxidizable carbon was calculated through the amount of dichromate consumed by

Table 1. Summary of the fertilisation application rate

Treatment	Nitrogen	Phosphorus	Potassium	Dry organic fertiliser (t/ha/year)	
	(kg/ha/year) – 1980–2013			1980–1996	1997–2013
CK	–	–	–	–	–
NP	225–300	55.8	–	–	–
NPK	225–300	55.8	137.5	–	–
OF <sup>#</sup>	–	–	–	7.4	2.2
OFNP	225–300	55.8	–	7.4	2.2
OFNPK	225–300	55.8	137.5	7.4	2.2

<sup>#</sup>OF at the average rate equivalent to 103.1 kg N/ha/year; 82.7 kg P/ha/year and 70.1 kg K/ha/year; CK – no fertiliser; OF – organic fertiliser

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the soil based on the theoretical value that 1.0 mL 0.0167 mol/L  $K_2Cr_2O_7$  oxidizes 3 mg carbon. The fractions were defined and calculated as follows:

- The very labile fraction was oxidizable under 6 mol/L  $H_2SO_4$ .
- The labile fraction was the difference between the 9 mol/L  $H_2SO_4$ - and 6 mol/L  $H_2SO_4$ -oxidizable fractions.
- The less labile fraction was the difference between the 12 mol/L  $H_2SO_4$ - and 9 mol/L  $H_2SO_4$ -oxidizable fractions.
- The recalcitrant fraction was calculated by subtracting the SOC oxidised by 12 mol/L  $H_2SO_4$  from the total soil carbon.

**C-input estimation.** C-inputs derived from crop stubble, roots residues and organic fertilisers were estimated as follows (Zhang et al. 2012):

$$C_{input} = \left( (Y_s + Y_g) \times R_r \times D_r + R_s \times Y_s \right) \times C_{plant} + C_{OF}$$

where:  $Y_s$  and  $Y_g$  – dry biomass of straw and grain, respectively;  $R_r$  – ratio of roots to aboveground biomass that was estimated as 0.3 (Chander et al. 1997, Kundu et al. 2007).  $D_r$  and  $R_s$  – ratio of root biomass in the plough horizon (0–15 cm) to the total root biomass and the ratio of stubble to straw biomass, respectively, were separately determined in 2006 and 2007.  $C_{plant}$  and  $C_{OF}$  are the carbon contents of dry plants and organic fertiliser, respectively, which were determined annually.

The parameters mentioned above are shown in Table 2. The SOC stock of the 0–15 cm layer in 2011, 2012 and 2013 were calculated as follows (Poeplau et al. 2017):

$$SOCS = SOC \times BD \times \text{depth}$$

Where: SOC – SOC content (%) in the fine soil; BD – soil bulk density ( $g/cm^3$ ), depth – respective soil layer in this study (15 cm). The SOC sequestration rates (SSR) in 2011, 2012 and 2013 were determined as follows:

$$SSR = \frac{SOCS_{year} - SOCS_{1980}}{Year - 1980}$$

Table 2. Parameters used to estimate C-inputs derived from roots and stubble

Crop	$Y_s/Y_g$	$R_r$	$D_r$	$R_s$	$OC_{crop}$ (%)
Rice	0.9:1	0.3	0.851	0.056	44.40
Wheat	1.1:1	0.3	0.753	0.150	39.90

$Y_s/Y_g$  – biomass ratio of straw to grain;  $R_r$  – biomass ratio of roots to aboveground components;  $D_r$  – biomass ratio of roots in the plough layer to total roots;  $R_s$  – biomass ratio of stubble to straw;  $OC_{crop}$  – carbon content

**Statistical analysis.** Statistical analysis was performed in the R environment (<https://cran.r-project.org>). Analysis of variance and Tukey's *HSD* (honestly significant difference) method were used to test significance and for multiple comparisons, respectively, and the  $P < 0.05$  level was considered statistically significant unless otherwise noted. The SOC dynamics were fitted using a logarithmic model ( $\log_{10}$ ), and the relationships between C-inputs and SSR and SOC fractions were analysed by a general linear model.

## RESULTS AND DISCUSSION

**Dynamics of C-input and SOC.** Fertilisation, especially organic amendments, effectively increased C-inputs (Figure 1A), and the highest average C-input was from OFNPK (4.33 t/ha/year) followed by OFNP (4.14 t/ha/year), OF (3.78 t/ha/year), NPK (2.29 t/ha/year), NP (2.16 t/ha/year) and CK (1.47 t/ha/year). Organic fertiliser amendment significantly increased C-inputs, similar to other reports (Zhang et al. 2012, Hua et al. 2014, Wang et al. 2015). Notably, the average C-input in CK was higher than that in other studies (0.45–0.53 t/ha/year) (Yan et al. 2013, Hua et al. 2014), probably due to the seasonal eutrophication of Taihu Lake Basin (Qin et al. 2007).

The SOC contents, including those of CK, exhibited a significant increase ( $P < 0.01$ ) from 1980 to 2013 and fitted well with the logarithmic model (Figure 1B). The consistent increase in SOC was the result of the equilibrium between C-inputs and C-losses. To accurately assess SOC, we calculated the average total SOC from 2011 to 2013. The highest average SOC was measured in the OFNPK treatment (22.03 g/kg) followed by OFNP (20.61 g/kg), OF (19.51 g/kg), NPK (17.47 g/kg), NP (17.28 g/kg) and CK (15.89 g/kg). These data indicated that organic and balanced mineral fertilisation resulted in an obviously higher SOC. A similar net increase in SOC in CK was found in another paddy soil (Zhang et al. 2012), mainly due to that flooding that restricts soil carbon turnover and organic matter decomposition (Devêvre and Horwáth 2000, Sahrawat 2004). The logarithmic model indicated that the soil had low to moderate C levels ( $< 5\%$ ) and had not reached a plateau (Six et al. 2002). Furthermore, the logarithmic model indicated that the SOC contents more quickly increased when using pig manure (before 1997) compared to oil rape cake (Figure 1B). The probable reason was that more C-inputs were derived from pig manure (Figure 1A), and a similar relationship between C-inputs and the

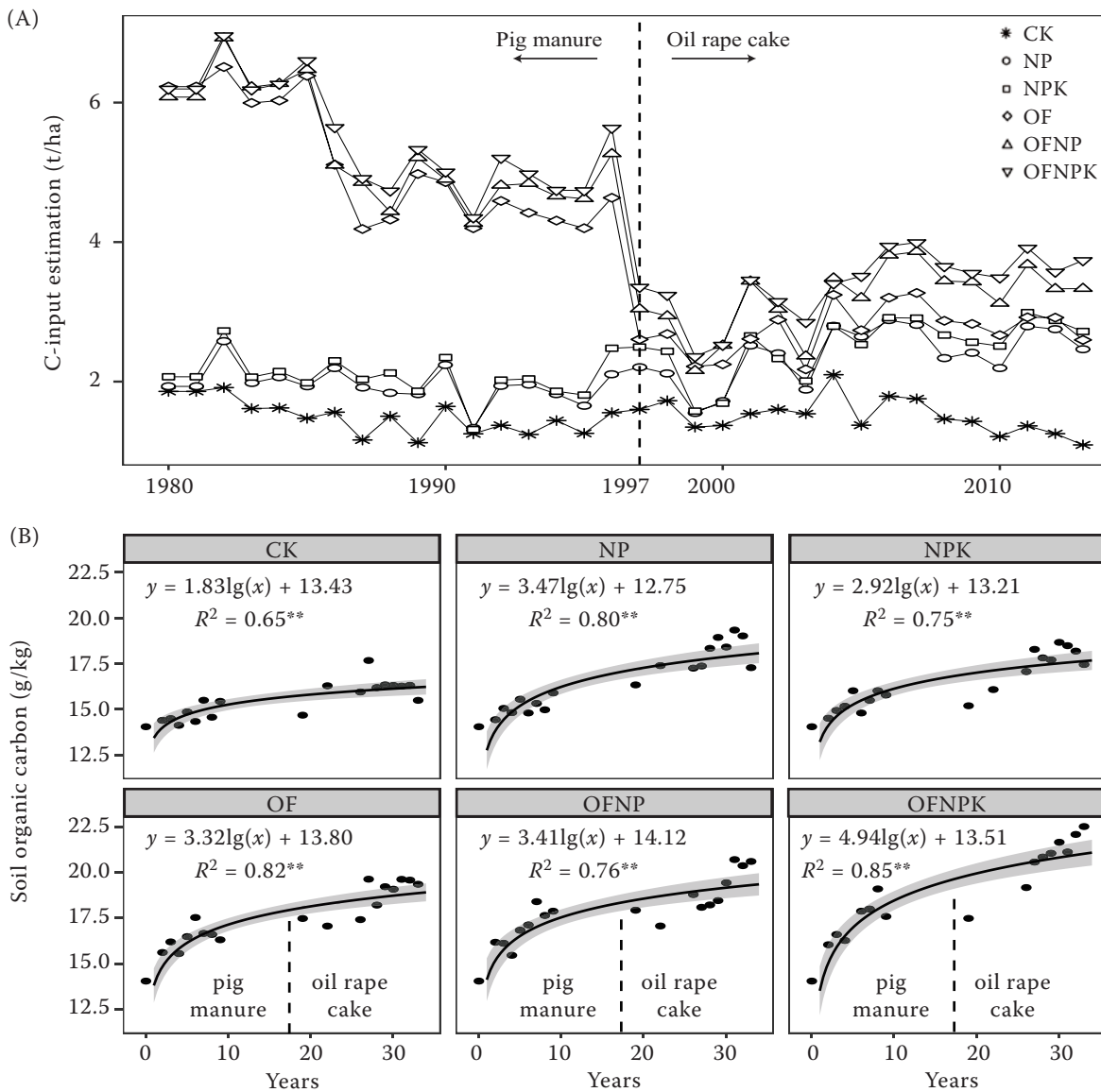


Figure 1. (A) Annual average C-inputs from 1980 to 2013. The vertical line indicates the shift in organic fertiliser and (B) soil organic carbon (SOC) dynamics from 1980 to 2013.  $^{**}P < 0.01$ ; grey shading represents the 95% confidence interval; CK – no fertiliser; OF – organic fertiliser

increase of SOC contents or stocks had been widely reported (Chen et al. 2016).

**SOCS, SSR and SOC sequestration efficiency.** BD is an important factor that affects SOCS (Walter et al. 2016). Long-term fertilisation significantly decreased BD compared to CK, but relatively higher BD was measured in OF than in other fertilisation treatments (Table 3). Yu et al. (2020) found similar results, i.e., long-term manure fertilisation increased BD by altering the soil microstructure. The SOCS of all treatments were estimated by averaging the consecutive years (2011–2013), which ranged from 31.20 to 38.52 t/ha (Figure 2), while the SOCS in the

initial soil was only 26.50 t/ha. Significant differences in SOCS were detected among treatments, and the highest SOCS was measured in OFNPK, nearly 1.2-fold higher than that in CK (Figure 2). The difference in SOCS between NPK and OFNPK was 6.40 t/ha, suggesting that organic fertilisation steadily increased SOCS. Hua et al. (2014) reported approximate results that organic amendments increased SOCS by 6.48 t/ha in a 29-year long-term fertilisation experiment.

The SSR of all treatments ranged from 0.11 to 0.40 t/ha/year, and there was a significant correlation between SSR and the average C-input (Figure 3). Furthermore, the SOC sequestration efficiency over

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Table 3. Soil bulk density (g/cm<sup>3</sup>) determined in 1980 and from 2011 to 2013

Year	CK	NP	NPK	OF	OFNP	OFNPK
1980	1.26					
Average <sup>#</sup>	1.31 <sup>a</sup>	1.18 <sup>c</sup>	1.22 <sup>bc</sup>	1.24 <sup>b</sup>	1.15 <sup>c</sup>	1.16 <sup>c</sup>

<sup>#</sup>Average represents the mean from 2011 to 2013. Different lowercase letters in the same rows represent significant differences among treatments at the 0.05 level; CK – no fertiliser; OF – organic fertiliser

34 years was 6.3% estimated by the slope of the linear model (Figure 3), while the efficiency of this soil was relatively lower than that in other reports. For example, the SOC sequestration efficiency was 20% in Wuchang (Zhang et al. 2012) and 8.0–35.7% in red soil (Tong et al. 2014). The SOC sequestration efficiency estimation is an integrated and conditional response, which is thought to be related to the clay content, nutrient reserves and so on (Lal 2018). SOCS cannot increase indefinitely, and a low SOC sequestration efficiency indicates that soil could be approaching but has not yet reached C saturation (Six et al. 2002).

**SOC fractionation.** Organic and mineral fertilisation significantly increased the C contents of the different fractions, and a consistent decreasing tendency (OFNPK, OFNP, OF, NPK, NP and CK) was observed for the four fractions (Table 4). Chan et al. (2001) classified these four fractions into two pools, where very labile and labile fractions belong to the "active pool", and less labile and recalcitrant fractions belong to the "passive pool". The average proportions of the active and passive pools of total SOC were 71.0% and 29.0%, respectively. Our values for the active pool

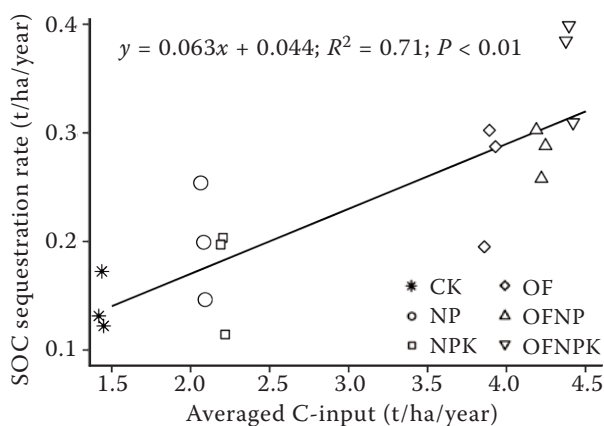


Figure 3. The relationship between the soil organic carbon (SOC) sequestration rate (SSR) and average C-input; CK – no fertiliser; OF – organic fertiliser

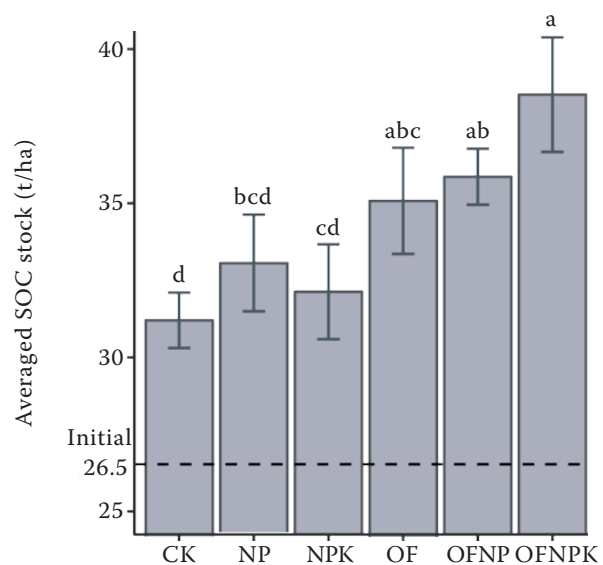


Figure 2. The average soil organic carbon (SOC) stocks in the different treatments from 2011 to 2013. The dashed line represents the value in 1980. The same lowercase letters above the bars indicate that the treatments are significantly different (honestly significant difference,  $P < 0.05$ ); CK – no fertiliser; OF – organic fertiliser

were similar to the results presented by Chan et al. (2001), much higher than those presented by Mandal et al. (2008) but lower than those presented by Sun et al. (2013). This is because the major C-inputs in this study were derived from organic fertilisers, crop exudates and residues, which are easily decomposed by microbes. However, Mandal et al. (2008) applied compost with a higher content of lignin-derived phenolic compounds, which generally resulted in a large passive pool (Olk et al. 2002, Majumder et

Table 4. Influence of the different treatments on soil organic carbon (SOC, g/kg) fractionation

Treatment	Active pool		Passive pool	
	very labile	labile	less labile	recalcitrant
CK	8.12 <sup>c</sup>	3.70 <sup>c</sup>	2.15 <sup>c</sup>	1.91 <sup>b</sup>
NP	8.36 <sup>c</sup>	4.32 <sup>b</sup>	2.53 <sup>c</sup>	2.06 <sup>b</sup>
NPK	8.40 <sup>c</sup>	4.35 <sup>b</sup>	2.60 <sup>c</sup>	2.10 <sup>b</sup>
OF	8.50 <sup>bc</sup>	4.97 <sup>a</sup>	3.36 <sup>b</sup>	2.67 <sup>a</sup>
OFNP	8.97 <sup>b</sup>	5.12 <sup>a</sup>	3.71 <sup>ab</sup>	2.79 <sup>a</sup>
OFNPK	9.51 <sup>a</sup>	5.43 <sup>a</sup>	4.10 <sup>a</sup>	2.98 <sup>a</sup>

The values in the table are the average from 2011 to 2013. Different lowercase letters represent significant differences at the 0.05 level; CK – no fertiliser; OF – organic fertiliser

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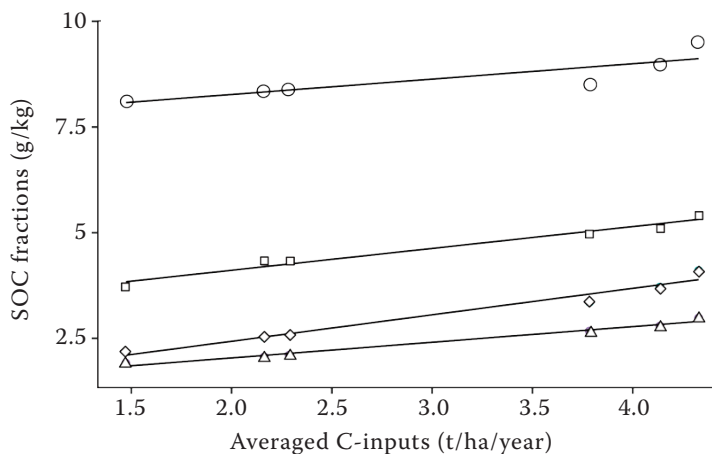


Figure 4. The linear regression analysis between soil organic carbon (SOC) fractions and average C-inputs from 2011 to 2013

- very labile:  
 $R = 0.86$ ;  $P = 0.029$ ;  $y = 0.36x + 7.54$
- labile:  
 $R = 0.98$ ;  $P < 0.01$ ;  $y = 0.52x + 3.08$
- ◇ less labile:  
 $R = 0.99$ ;  $P < 0.01$ ;  $y = 0.63x + 1.18$
- △ recalcitrant:  
 $R = 0.99$ ;  $P < 0.01$ ;  $y = 0.37x + 1.30$

al. 2008, Mandal et al. 2008). Sun et al. (2013) used green manure with more degradable organic matter, which resulted in a large active pool.

The relationships between the average SOC and C-inputs were analysed by a linear model, and significant positive correlations were found for the four fractions (Figure 4). The increase in the SOC content per unit of C-input, i.e., the slope of the linear model, could be an index of SOC saturation. Gulde et al. (2008) proposed that as C-inputs increase, the passive pool of SOC becomes saturated, and consequently, additional C-inputs will only accumulate in the active pool of SOC. The largest increase in the SOC content per unit of C-input occurred in the less labile fraction of the passive pool (Figure 4), suggesting that the passive pool of SOC was not saturated.

In summary, long-term fertilisation, especially organic fertilisation, significantly enhanced SOC sequestration with more C-inputs, higher SOCS and SRR. The SOC sequestration efficiency was relatively low (6.3%), and C-inputs were more effectively sequestered into the less labile fraction, suggesting that SOC was slowly approaching but had not yet reached saturation. Overall, organic fertilisation is an effective strategy to improve SOC sequestration in a typical rice-wheat rotation system.

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