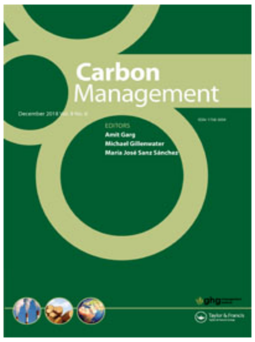


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# Soil organic carbon sequestration as influenced by long-term manuring and fertilization in the rice-wheat cropping system

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**ABSTRACT**

Fertilization is a feasible approach to increase the soil organic carbon. To investigate the effect of fertilization on crop biomass carbon, dynamics of soil organic carbon and soil carbon sequestration rate in the (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) cropping system under the middle reach of the Yangtze River, central China, a thirty-three years (1981 – 2013) long-term fertilizer experiment was conducted with nine treatments, including no amendment addition treatment (control), nitrogen (N), phosphorus (P), potassium (K) fertilizer treatments (N, NP, NPK), manure (M) and manure combined with chemical fertilizer treatments (MN, MNP, MNPK, hMNPK). The results indicated that average crop biomass carbon was increased by 39.9 – 77.2% compared to unfertilized control (4.43 t ha$^{-1}$ yr$^{-1}$) due to fertilizer application, the highest crop biomass carbon was 7.85 t ha$^{-1}$ yr$^{-1}$ in the hMNPK treatment and the lowest crop biomass carbon was 5.21 t ha$^{-1}$ yr$^{-1}$ in the N alone treatment. The annual total organic carbon input were 4.14 t ha$^{-1}$ yr$^{-1}$ in the M treatment and 5.80 t ha$^{-1}$ yr$^{-1}$ in the hMNPK treatment, which was 1.95 – 2.74 times of those in the NPK treatments (2.12 t ha$^{-1}$ yr$^{-1}$). Total organic carbon input of soil were increased by 10.2 – 23.3 kg C ha$^{-1}$ yr$^{-1}$, and increment rate in the appended manure treatments were much higher than those in the control and inorganic fertilizer treatments. Soil organic carbon retention in the topsoil (0 – 20 cm) decreased by 0.11 – 0.14 t ha$^{-1}$ yr$^{-1}$ in the control, N and NP treatments; nevertheless, soil organic carbon sequestration rates varied from 0.03 to 0.20 t ha$^{-1}$ yr$^{-1}$ in the NPK and appended organic manure treatments. These results demonstrate that organic manure use or integrated organic manure with chemical fertilizer application can be important strategies for increasing soil organic carbon sequestration and maintaining soil quality in the rice-wheat cropping system of China.

**KEY WORDS**
Crop biomass carbon; soil organic carbon; carbon sequestration rate; rice-wheat cropping system

## Introduction

The rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) cropping system is one of the largest agricultural production systems in the world, which occupy 24 million hectares cultivated land and is mainly distributed in southern and eastern Asia (Indo-Gangetic plains and South China) [1]. The 13.5 million hectares in South Asia are distributed in India, Pakistan, Bangladesh, Nepal, Myanamar and Bhutan [2]. The 10.5 million hectares in East Asia are widely spread in Jiangsu, Zhejiang, Hubei, Guizhou, Yunnan, Sichuan and Anhui provinces of China, which account for roughly half of the total rice cultivated area in China. It is crucial to keep sustainable crop yields, ensure China's food security, and protect the soil environment.

Soil organic carbon, as is closely linked with soil physical-chemical properties, nutrient cycling and plant-available nutrients uptake and release, is an important index of soil fertility and agricultural sustainability. Usually, a positive relationship exists between the soil organic carbon content and the crop yield, which can reflect the soil productivity [3]. The soil carbon retention is a complex process, which is affected by many factors, such as conservation tillage, cropping sequences, fertilizations, organic amendments, soil textures, climate conditions and anthropogenic factors [4, 5]. The application of the manure increases the soil organic carbon content and improves other soil physical-chemical properties; including soil tilth, water-holding capacity, aggregate stability, and the water infiltration rate [6]. In many cases, there is a positive relationship between carbon inputs from manures and crop residues and the corresponding soil organic carbon content [7]. Yadvinder-Singh

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et al. [1] observed that the regular incorporation of crop residues and farmyard manures slowly increased soil organic carbon contents in the rice-wheat cropping system in India.

Because of the high resilience of the soil organic carbon, effects of agricultural management on soil organic carbon should be observed through long-term field experiments [8]. Furthermore, the long-term fertilizer experiment can analyze the changeable discipline of the soil fertility and fertilizer benefit, crop growth and soil environments due to annual changes of climate in a systematic and scientific way [9]. Long-term field experiments are essential to evaluate the cropping management practices required to achieve desired yields and agricultural sustainability in terms of the crop production, soil fertility, and environmental impacts.

However, there were few investigations about the effects of long-term organic manure and chemical fertilizer application on soil organic carbon input, soil organic carbon concentration and soil carbon sequestration rate in the yellow-brown soil, central China. To fill this knowledge gap, the aims of this study are to: (1) evaluate the impacts of the long-term application of inorganic and organic fertilizers on the soil organic carbon and its sequestration in the yellow-brown soil and (2) estimate the relationship between soil organic carbon input and soil carbon sequestration rate in the rice-wheat cropping system. In this study, it is hypothesized that long-term organic manure application or manure combined with chemical fertilizer application can sequester carbon in soil, but soil organic carbon will be declined due to long-term chemical fertilizer application alone.

## Materials and methods

### Study site, climate and soil

A thirty-three years (1981 – 2013) long-term fertilizer experiment in a rice-wheat rotation system was initiated from rice cultivation in 1981, belonging to the National Fertilizer Experiment Monitoring Network at Nanhu experimental Station, Hubei Academy of Agricultural Sciences in Wuchang of China, located at latitude 30°28′N, longitude 114°25′E and altitude 20 m in the central of China. The experimental site lies in a subtropical monsoon zone, which is characterized by hot summers and severe winters, and occasional snowfall during winter. The mean annual temperature is 13 °C ranging from a minimum of 3.7 °C in January to a maximum of 28.8 °C in July. The mean annual precipitation is 1300 mm, most of which (about 60%) is received from April to July and the annual non-frost period is 240 days.

The soil at experimental site is a yellow-brown soil, belonging to Albic Luvisol in FAO classification, which has a clay loam texture with 15% sand, 36% silt, and 49% clay. Soil properties (in 1981) in anthraquic horizon depth (0 – 20 cm) were as follows: soil organic carbon 15.9 g $kg^{-1}$, total N 1.80 g $kg^{-1}$, total P 1.00 g $kg^{-1}$, total K 30.2 g $kg^{-1}$, alkaline hydrolysable N 151 mg $kg^{-1}$, available P 5.00 mg $kg^{-1}$, available K 98.5 mg $kg^{-1}$, pH 6.30 and the bulk density 1.29 g $cm^{-3}$.

### Experiment design and field management

A thirty-three years (1981 – 2013) long-term fertilizer experiment was conducted with nine treatments (in a randomized complete block design with three replicates), and each plot size was 40 $m^2$ (5 m width ×8 m length). The nine treatments were as follows: (i) no chemical fertilizer or manure application, Control; (ii) the inorganic N fertilizer treatment, N; (iii) the inorganic N and P fertilizer treatment, NP; (iv) the inorganic N, P and K fertilizer treatment, NPK; (v) the manure (pig dung compost) treatment, M; (vi) inorganic N fertilizer plus the manure treatment, MN; (vii) inorganic N and P fertilizer plus the manure treatment, MNP; (viii) inorganic N, P and K fertilizer plus the manure treatment, MNPK; (ix) the inorganic N, P and K fertilizer plus 1.67 times of manure treatment, hMNPK. Application doses for manure and chemical fertilizer and split times are shown in Table 1. The inorganic N, P, and K fertilizers were applied as annual rate of 150 kg N $ha^{-1}$, 75 kg $P_2O_5$ $ha^{-1}$, and 150 kg $K_2O$ $ha^{-1}$. The N, P, and K fertilizers were applied as urea, ammonium phosphate, and potassium chloride, respectively. The 22,500 kg $ha^{-1}$ of organic manure from pig dung compost every year were applied to M, MN, MNP and MNPK treatments, while 37,500 kg $ha^{-1}$ were applied to hMNPK treatment. The pig dung compost averagely contained 282 g $kg^{-1}$ C, 15.1 g $kg^{-1}$ total N, 20.8 g $kg^{-1}$ $P_2O_5$, 13.6 g $kg^{-1}$ $K_2O$, and 69% water. The 60% of inorganic fertilizers were applied during the rice growth season and the other 40% during the wheat growth season, while manure was applied equally (50:50) to the two crops. The 40% of the N fertilizer was applied as a basal fertilizer, 40% was applied during the tillering stage and 20% during the booting stage in the rice

**Table 1.** Experimental design and manure, chemical fertilizer of application doses and split times in a long-term fertilizer experiment.

| | Basal fertilizer | | | | | Supplementary fertilizer | |
|---|---|---|---|---|---|---|---|
| Treatment | Urea (kg N $ha^{-1}$) | Ammonium Phosphate (kg N $ha^{-1}$) | Ammonium Phosphate (kg $P_2O_5$ $ha^{-1}$) | Potassium chloride (kg $P_2O_5$ $ha^{-1}$) | Manure (t $ha^{-1}$) | First Urea ( kg N $ha^{-1}$) | Second Urea (kg N $ha^{-1}$) |
| Rice | | | | | | | |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N | 36 | 0 | 0 | 0 | 0 | 36 | 18 |
| NP | 27 | 9 | 45 | 0 | 0 | 36 | 18 |
| NPK | 27 | 9 | 45 | 90 | 0 | 36 | 18 |
| M | 0 | 0 | 0 | 0 | 11.25 | 0 | 0 |
| MN | 36 | 0 | 0 | 0 | 11.25 | 36 | 18 |
| MNP | 27 | 9 | 45 | 0 | 11.25 | 36 | 18 |
| MNPK | 27 | 9 | 45 | 90 | 11.25 | 36 | 18 |
| hMNPK | 27 | 9 | 45 | 90 | 18.75 | 36 | 18 |
| Wheat | | | | | | | |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N | 30 | 0 | 0 | 0 | 0 | 15 | 15 |
| NP | 24 | 6 | 30 | 0 | 0 | 15 | 15 |
| NPK | 24 | 6 | 30 | 60 | 0 | 15 | 15 |
| M | 0 | 0 | 0 | 0 | 11.25 | 0 | 0 |
| MN | 30 | 0 | 0 | 0 | 11.25 | 15 | 15 |
| MNP | 24 | 6 | 30 | 0 | 11.25 | 15 | 15 |
| MNPK | 24 | 6 | 30 | 60 | 11.25 | 15 | 15 |
| hMNPK | 24 | 6 | 30 | 60 | 18.75 | 15 | 15 |

Pig dung compost amount was wet weight and dry matter content of pig dung compost was 31%. Pig dung averagely contained 282 g $kg^{-1}$ C, 15.1 g $kg^{-1}$ total N, 20.8 g $kg^{-1}$ $P_2O_5$, 13.6 g $kg^{-1}$ $K_2O$, and 69% water.

growth season. The 50% of N fertilizer was applied as a basal fertilizer, 25% during the wheat seedling stage and 25% during the jointing stage in the wheat growth season. Every year, the P, K fertilizer and manure were applied as basal fertilizers, prior to plough. All basal fertilizers and manure were evenly sprinkled on the soil surface by hand and were incorporated into the plough layer by tillage as soon as possible. Tillage was done to 20 cm depth by plough and followed by harrow. The fertilized and unfertilized plots were tilled similarly.

The cropping system was rice and wheat rotations. The rice was transplanted in June and harvested in October, and then the wheat was directly sowed in November and harvested in May of the next year. The aboveground crops were harvested with sickle and removed, thus no straw returned into the soil in all plots. Nevertheless, rice or wheat stubbles and roots were incorporated into the soil with a plow before the subsequent rice and wheat. Besides the fertilizer treatments, all other agronomic managements were identical in fertilized and unfertilized plots.

Rice and wheat grains were separated from straws using a plot thresher. Straws were removed after threshing. Grains were weighed after sun-drying and recorded from the whole plot (14% water content by oven-dry basis).

## Soil and plant sampling

A total of twenty-seven composite soil samples from nine treatments by three replicates were collected from 0 – 20 cm soil layer in June or October every year after crops being harvested before soil plowing. Each composite soil sample consists of ten mixed cores. Each soil sample was stored in a sealed plastic bag, transferred to the lab as soon as possible, air-dried for 14 days at room temperature and then ground. Subsamples were sieved through a 1-mm screen, mixed, and analyzed the contents of N, available P and available K and soil pH. Other subsamples were ground through a 0.25 mm sieve to determine soil organic C, total N, total P and total K concentrations. Both of straws and grains were oven-dried at 65 °C for 72 h and then ground through a 0.5-mm sieve in order to analyze the carbons and total N, P, K concentrations.

## Laboratory analysis

The soil bulk density was measured with an iron ring method. The potassium dichromate external heating method was used to determine soil organic C concentration [10]. The semi-micro Kjeldahl method and the alkaline-hydrolysable diffusion method measured total N and alkaline-hydrolysable N concentrations, respectively [11]. Soil total P and soil total K were digested in a nickel crucible with sodium hydroxide at 750 °C. Soil total P were determined using the molybdenum colorimetric method at a wavelength of 880 nm [12]. Soil total K were determined using atomic absorption spectro-photometry [13]. Soil available P was extracted with 0.5 mol $L^{-1}$ $NaHCO_3$

(soil: solution = 1:20) and measured with the Olsen method [14]. Soil available K was extracted with 1 mol $L^{-1}$ $NH_4Ac$ (soil: solution = 1:10) and measured with the flame photometry method [15]. Soil pH was measured with 0.01 mol $L^{-1}$ $CaCl_2$ slurry (soil: solution = 1:2.5) with a glass electrode. Samples from plant tissues and pig dung composts digested in $H_2SO_4$ and $H_2O_2$ were analyzed for total N by a micro-Kjeldahl method [11]. The P concentration of plant tissues digested in $HNO_3$ and $HClO_4$ was determined by the ammonium molybdate method [16] and K concentration of plant tissues by a flame photometry [15]. Carbon contents of plant tissues and manures were determined by a wet oxidation method [17]. All the data were expressed on the basis of dry mass.

## Calculation of carbon input

The yields of each crop straws and grains were recorded from all plots every year. Crop biomass carbon is calculated according to aboveground crop biomass and carbon concentrations of harvestable crop grains or straws respectively. Namely, the rice biomass carbon is equal to the rice straw yield multiplied by its carbon concentration plus the rice grain yield multiplied by its carbon concentration. The wheat biomass carbon is calculated with the same method. Crop biomass carbon is calculated by the following equation.

$$C_{rice-biomass} = Yield_{rice-straw} \times C_{rice-straw} + Yield_{rice-grain} \times C_{rice-grain} \quad (1)$$

$$C_{wheat-biomass} = Yield_{wheat-straw} \times C_{wheat-straw} + Yield_{wheat-grain} \times C_{wheat-grain} \quad (2)$$

Where $C_{rice\text{-}biomass}$ (t $ha^{-1}$ $yr^{-1}$) and $C_{wheat\text{-}biomass}$ (t $ha^{-1}$ $yr^{-1}$) are biomass carbon of rice and wheat, $Yield_{rice\text{-}straw}$, $Yield_{rice\text{-}grain}$, $Yield_{wheat\text{-}straw}$ and $Yield_{wheat\text{-}grain}$ (t $ha^{-1}$ $yr^{-1}$) are straw and grain yields of rice and wheat. $C_{rice\text{-}straw}$, $C_{rice\text{-}grain}$, $C_{wheat\text{-}straw}$ and $C_{wheat\text{-}grain}$ (g $kg^{-1}$) are the carbon concentration of straw and grain of rice and wheat.

The annual total carbon input is measured based on belowground roots and stubbles incorporated into the topsoil, and applied organic manures. Crop carbon input is calculated by the following equation.

$$C_{input} = C_{belowground} + C_{stubbles} + C_{manure} \quad (3)$$

$$C_{belowground} = R_{rice-belowground} \times C_{rice-biomass} + R_{wheat-belowground} \times C_{wheat-biomass} \quad (4)$$

$$C_{stubbles} = R_{rice-stubbles} \times Yield_{rice-straw} \times C_{rice-straw} + R_{wheat-stubbles} \times Yield_{wheat-straw} \times C_{wheat-straw} \quad (5)$$

Where $C_{input}$ (t $ha^{-1}$ $yr^{-1}$) is annual total carbon input into soil, $C_{manure}$ (t $ha^{-1}$ $yr^{-1}$) is carbon concentration of applied organic manure into soil. $C_{belowground}$ (t $ha^{-1}$ $yr^{-1}$) is the underground biomass carbon mainly from rice or wheat roots. Both of $R_{rice\text{-}belowground}$ and $R_{wheat\text{-}belowground}$ have a fixed value of 30%, which is the ratio of annual underground biomass carbon of each crop to aboveground one. The 75.3% of wheat roots and almost 100% of rice roots are distributed in 0 – 20 cm soil layers. $C_{stubbles}$ (t $ha^{-1}$ $yr^{-1}$) is rice and wheat stubbles biomass carbon. $R_{rice\text{-}stubbles}$ is 5.6% and $R_{wheat\text{-}stubbles}$ is 15%, both of which are the ratio of each crop stubble biomass incorporated into soil to aboveground straw biomass [18, 19].

## Calculation of soil organic carbon stock and sequestration

Soil organic carbon stock is equal to soil organic carbon concentration multiplied by the bulk density and the depth. Soil organic carbon stock is calculated by the following equation.

$$C_{stock} = SOC \times BD \times D \times 10 \quad (6)$$

Where $C_{stock}$ is soil organic carbon stock (t $ha^{-1}$), SOC is soil organic carbon concentration (g $kg^{-1}$), BD is the soil bulk density (g $cm^{-3}$), and D is the depth of soil (m) [20].

Sequestered soil organic carbon of each treatment in the 0 – 20 cm soil depth is equal to the difference between the soil present organic carbon stock (2013) and the initial one (1981). The carbon sequestration is calculated by the following equation.

$$C_{sequestration} = C_{stock\ in\ 2013} - C_{stock\ in\ 1981} \quad (7)$$

Where $C_{sequestration}$ is carbon sequestration of each treatment in the 0 – 20 cm soil depth (t $ha^{-1}$), $C_{stock\ in\ 2013}$ is organic carbon stock of each treatment in 2013 (t $ha^{-1}$) and $C_{stock\ in\ 1981}$ is organic carbon stock in 1981 (t $ha^{-1}$) [21].

The carbon sequestration rate is equal to sequestered soil organic carbon of each treatment divided by the period of the experiment (thirty-three years). The carbon sequestration rate is calculated by the following equation.

$$C_{sequestration\ rate} = (C_{stock\ in\ 2013} - C_{stock\ in\ 1981})/T \quad (8)$$

Where $C_{sequestration\ rate}$ is soil carbon sequestration rate (t ha$^{-1}$ yr$^{-1}$), T is the period of the experiment, and thirty-three years for the present study [22].

### Statistical analysis

All data were subjected to statistical analysis of variance using the SPSS 11.5 software package and were used to evaluate differences between different treatments. Difference obtained at $P < 0.05$ level was considered statistically significant using the LSD (least significant difference) test.

## Results

### Crop biomass carbon

The harvestable aboveground crop biomass carbon was significantly influenced by fertilization ($P < 0.001$) and fertilization years ($P < 0.001$). Averagely, crop biomass carbon was increased by 39.9 – 77.2% compared to unfertilized control due to thirty-three years fertilizer application in the rice-wheat cropping systems. The unfertilized control had the lowest biomass carbon (averagely annual 4.43 t ha$^{-1}$), whereas crop biomass carbon were the highest in the hMNPK treatment (averagely annual 7.85 t ha$^{-1}$). Annual crop biomass carbon was generally higher with manure combined with chemical fertilizer compared to chemical fertilizer or manure alone treatments. Averagely, annual crop biomass carbon were 5 t ha$^{-1}$ in N treatment, approximate 6 t ha$^{-1}$ in NP, NPK, M treatments, and approximate 7 t ha$^{-1}$ in MN, MNP, MNPK, hMNPK treatments (Figure 1).

### Organic carbon input

Averagely, the lowest total carbon input was 1.44 t ha$^{-1}$ yr$^{-1}$ in the control treatment, whereas the highest one was 5.80 t ha$^{-1}$ yr$^{-1}$ in the hMNPK treatment (Figure 2). The average annual carbon inputs in the manure treatments (M, MN, MNP, MNPK, hMNPK) were 1.95 – 2.74 times of that in the NPK treatment (2.12 t ha$^{-1}$ yr$^{-1}$). The slopes of the linear regression equation in total organic carbon inputs indicated that 10.2 – 23.3 kg ha$^{-1}$ carbon were increased into soil every year, and slopes in the adding manure treatments were much

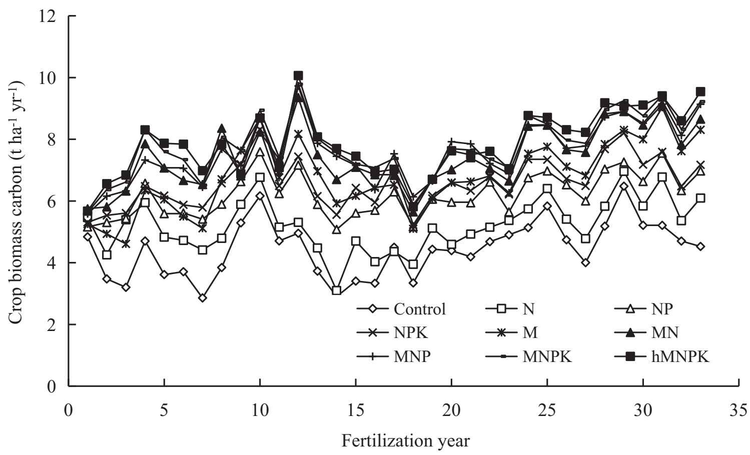


Figure 1. Dynamics of crop biomass carbon in different fertilization treatments during thirty-three years experimental periods.

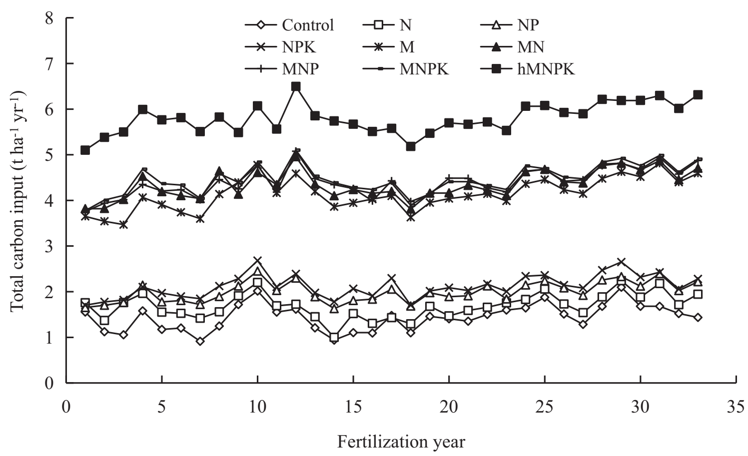


Figure 2. Annual total organic carbon input in different fertilization treatments during thirty-three years experimental periods.

Table 2. Slopes of the linear regression vs. time for total organic carbon input (kg $ha^{-1}$ $yr^{-1}$) in different fertilization treatments.

| | Total carbon input | | |
|---|---|---|---|
| Treatment | Slope | $R^2$ | *P*-value |
| Control | 10.2 | 0.14 | 0.036 |
| N | 10.6 | 0.13 | 0.037 |
| NP | 11.9 | 0.26 | 0.003 |
| NPK | 13.1 | 0.25 | 0.003 |
| M | 23.3 | 0.40 | <0.001 |
| MN | 17.6 | 0.30 | 0.001 |
| MNP | 20.2 | 0.38 | <0.001 |
| MNPK | 17.5 | 0.28 | 0.002 |
| hMNPK | 18.7 | 0.30 | 0.001 |

Values of $P < 0.05$ were considered significantly.

sharper than those in the control, N, NP, and NPK treatments during thirty-three years periods (Table 2). The slopes in the balanced application of the NPK fertilizer treatment were much sharper than those in the unbalanced application of inorganic fertilizer treatments (N, NP).

### *Dynamics of soil organic carbon*

During thirty-three years periods, soil organic carbon contents had decreasing tendency in the control, N and NP treatments, but had increasing tendency in the NPK, especially appended organic manure treatments. The rates of decline or increase were diverse in different fertilization treatments (Figure 3). The slopes of linear regression equations in soil organic carbon indicated that 128.4 – 162.4 mg $kg^{-1}$ carbon was accumulated in the appended organic manure treatments every year, whereas only 66.2 mg $kg^{-1}$ carbon in NPK treatment. Soil organic carbon declined at an rate of 30.1, 21.9, and 14.1 mg $kg^{-1}$ carbon in the control, N, and NP treatments every year, respectively. Applications of organic manures combined with inorganic fertilizers (MN, MNP, MNPK, hMNPK) significantly ($P < 0.05$) increased soil organic carbon contents compared to the corresponding application of inorganic fertilizers alone (N, NP, NPK). The balanced application of the NPK fertilizer increased soil organic C contents in comparison to the unbalanced application of inorganic fertilizers (N, NP).

### *Soil carbon sequestration*

Soil organic carbon stocks (0 – 20 cm) declined in the control (33.6 t $ha^{-1}$), N (33.5 t $ha^{-1}$) and NP (34.7 t $ha^{-1}$) treatments in contrast to the initial organic carbon stock (38.0 t $ha^{-1}$), whereas the organic carbon stocks ascended in the NPK (39.1 t $ha^{-1}$) and appended manure treatments (41.8 – 44.5 t $ha^{-1}$) in the topsoil during thirty-three years periods. Soil organic carbon sequestration rates (0 – 20 cm) in the MN (0.12 t $ha^{-1}$ $yr^{-1}$), MNP (0.18 t $ha^{-1}$ $yr^{-1}$), MNPK (0.20 t $ha^{-1}$ $yr^{-1}$) and hMNPK (0.19 t $ha^{-1}$ $yr^{-1}$) treatments were significantly ($P < 0.05$) higher than those in M (0.12 t $ha^{-1}$ $yr^{-1}$) and NPK (0.03 t $ha^{-1}$ $yr^{-1}$) treatments, and the sequestration rate in the M treatment was significantly ($P < 0.05$) higher than that in the NPK treatment (Figure 4).

### *Regression analysis*

There exists a significant non-linear regression between the soil carbon sequestration rates and the annual carbon inputs (Figure 5). According to the non-linear regression equation, parameters related to soil carbon sequestration were deduced in yellow-brown soil. The estimated decomposition rates of soil organic carbon were 0.46 t $ha^{-1}$ $yr^{-1}$ and the minimum rate of the carbon input to maintain the initial soil organic carbon level was 2.40 t $ha^{-1}$ $yr^{-1}$. There is a non-linear correlation between the soil carbon sequestration rates and the annual carbon inputs.

## Discussion

### *Effects of manuring and fertilization on soil carbon sequestration*

Soil organic carbon showed a slight decline trend in the control, N and NP fertilizer treatments for the duration of thirty-three years experiments. It was attributed that every year abundant crop biomass was removed from those treatments, but the carbon input from crops into soil could not maintain the soil carbon equilibrium. Soil carbon output through soil respiration was higher than soil input crop roots and stubbles in the control with no amendment addition [8, 23]. However, the soil organic carbon content slightly increased in the unfertilized control in a long – term experiment site in Italy [8] and also increased in the N and NP fertilizer treatments in the dryland of Loess Plateau in northwest China [24]. Increases of soil organic carbon contents in the balanced NPK application plots and organic manure application plots were similar to the other experiment sites. For example, adding farmyard manure significantly enhanced soil organic carbon in a dark loessial soil [25], in a wheat-maize cropping system of China [26, 27] and in a wheat-maize [28] and rice-wheat cropping

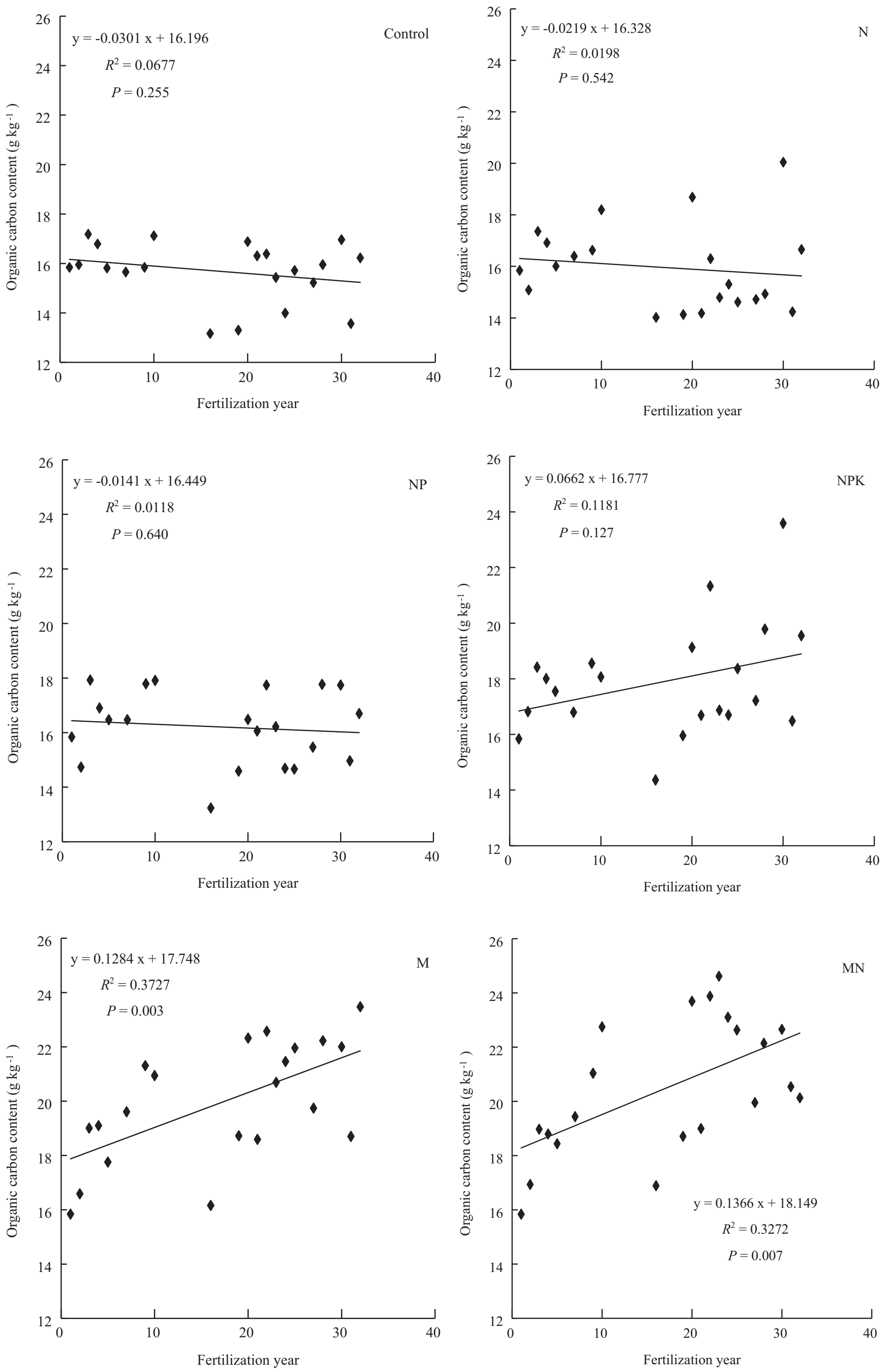


Figure 3. Change trend of soil organic carbon in different fertilization treatments during thirty-three years experimental periods.

system of India [29], as organic manure was an additional carbon source and its simultaneous application also increased the crop carbon input into soil [30].

Averagely, soil organic carbon sequestration (0 – 20 cm) was 1.06 t ha$^{-1}$ in the NPK fertilizer treatment, but was 3.80 – 6.47 t ha$^{-1}$ in the manure alone or manure combined with inorganic fertilizer treatments for the duration of the fertilizer experiment, which suggested that application of organic manure had greatly accelerated soil organic carbon accumulation since it was an additional carbon resource incorporated into soil [31]. The soil organic carbon sequestration rate (0 – 20 cm) was 0.03 t ha$^{-1}$ yr$^{-1}$ under the NPK fertilizers treatment in the present study. Every year, 0.08 – 0.25 t ha$^{-1}$ carbon was sequestered in soils due to balanced NPK fertilizer applications with

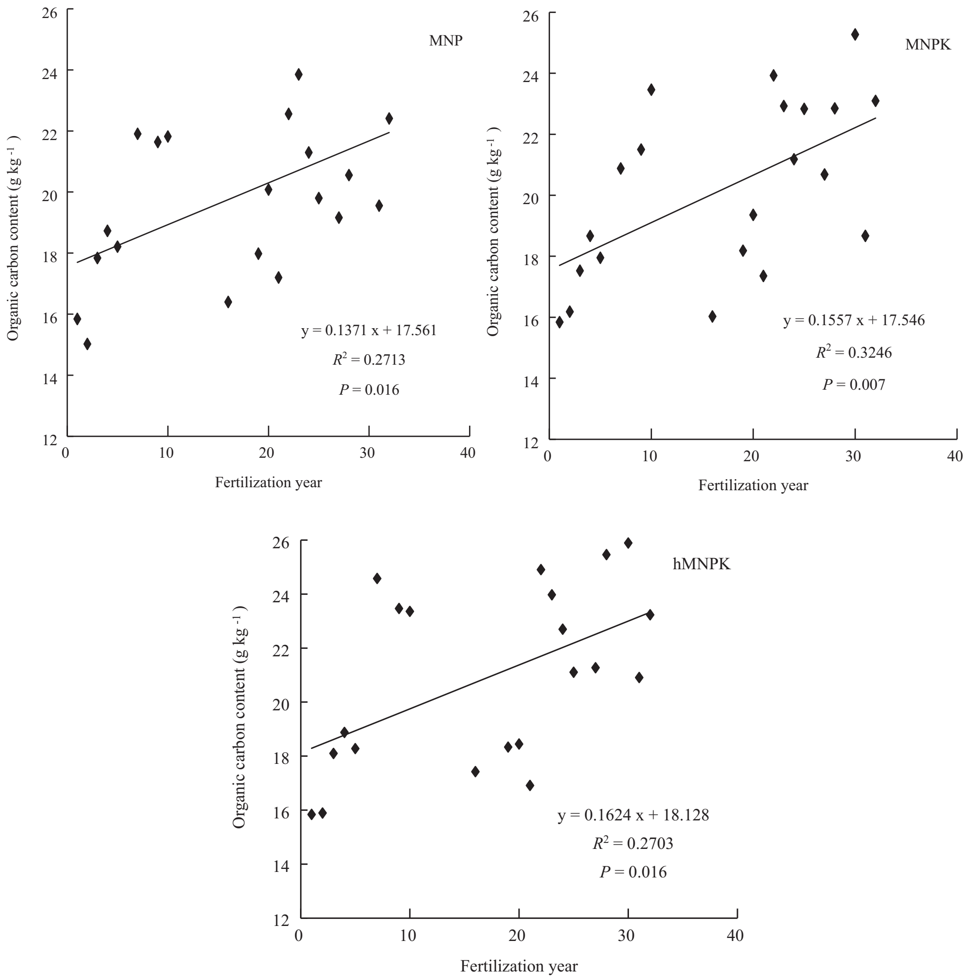


**Figure 3.** Continued.

the upland cropping systems [32]. The soil organic carbon sequestration rate (0 – 20 cm) was 0.12 – 0.20 t ha$^{-1}$ yr$^{-1}$ under the appended manure treatment in this study. In contrast to the other long-term fertilizer experiment sites, Su et al. [33] reported that changes in soil organic carbon stocks (0 – 20 cm) were 0.16 – 0.42 t ha$^{-1}$ yr$^{-1}$ in the middle of Hexi Corridor. Soil organic carbon sequestration rate (0 – 20 cm) was 0.02 – 0.37 t ha$^{-1}$ yr$^{-1}$ due to the application of the animal excretion in a semi – arid cropland [34] and 0.04 – 0.16 t ha$^{-1}$ yr$^{-1}$ under the arid region after the farmyard manure application in southeastern Norway [35]. The possible reason was that the soil organic matter was more rapidly degraded in a rice-wheat cropping system than that in an upland cropping system. The paddy-upland rotation could accelerate soil organic matter degradation due to high hydrothermal conditions.

The soil organic carbon usually rose with the increase of the carbon input before equilibrium [36]. A non-linear regression was observed between the soil carbon sequestration rate and the organic carbon input in this experimental site. The non-linear regression indicated that the soil carbon sequestration efficiency descended with the increasing gradient of the carbon input.

### *Factors affecting soil organic carbon sequestration*

The agricultural soil is an important carbon reservoir, which plays a key role in migrating carbon dioxide emission [8]. Soil organic carbon sequestration was mainly influenced by climates, soil types, tillage, fallows, rotations, fertilizations, and so on [37]. For example, Zhang et al. [32] reported that the organic carbon conversion rate decreased significantly with the increase in the annual active accumulative temperature and precipitation. Under normal conditions, the soil organic carbon accumulation rate tends to decrease with the higher soil temperature and moisture. There was usually a positive linear correlation between soil organic carbon levels and soil clay contents of surface soils [38]. No-tillage could increase the soil organic carbon stock compared with conventional tillage, because it could significantly reduce soil carbon release by reducing the turnover of soil aggregates and the exposure of young and labile organic matters to microbe decomposition [39], which had been considered as an effective and environment friendly soil carbon sequestration strategy [40]. The crop straw return to soil significantly increased soil carbon stocks [41, 42]. Some studies indicated that applications of fertilizers (including chemical fertilizer, organic manure and chemical fertilizer combined with organic manure) could increase soil organic carbon contents [43 – 46]. Follett et al. [47] found that the N fertilization increased soil organic carbon contents due to higher crop biomass inputs. However, other long-term fertilization studies reported different results. Triberti et al. [8] indicated that the NP fertilization did not affect the soil organic carbon in spite of the huge increase of the stover carbon input in Italy and Argentina, and Su et al. [33] found that N, NP and NPK fertilizer applications decreased the soil organic carbon contents in the wheat-maize cropping system. In all experimental sites in the world, long-term applications of organic manures increased soil organic carbon contents by adding carbon from the manure itself

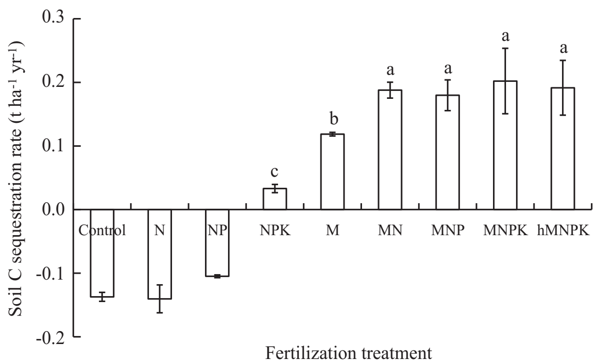


Figure 4. Soil organic carbon sequestration rates in different fertilization treatments during thirty-three years experimental periods. The bars are the standard deviation. Different letters (a, b, c) indicate significant differences ($P < 0.05$) among treatments according to LSD multiple comparison.

and increasing carbon from the crop residue due to the higher crop biomass in amended soil [43, 46, 48]. However, the field research demonstrated that different amendments applied at the same carbon rate had diverse effects on soil organic carbon sequestration [49]. Both of crop residues and cattle slurries, which were degraded more rapidly, had a lower carbon sequestration capacity compared with cattle dung due to different composition characteristics of organic matters [8]. According to the non-linear regression equation between the soil carbon sequestration rate and the annual carbon input, we deduced that the minimum amount of the carbon input to maintain the initial soil organic carbon level was 2.40 t ha$^{-1}$ yr$^{-1}$, while the carbon input in the control, N, NP fertilizer treatment was only 1.44 – 1.99 t ha$^{-1}$ yr$^{-1}$. In a word, the soil carbon sequestration rate was lower in rice – wheat cropping systems owing to higher frequency hydrothermal and water-upland alternation compared with other cropping systems. Therefore, the further increments of soil organic carbon sequestration in rice-wheat cropping system would be mainly attributed to extra carbon inputs (including straws returned into soil, cover crops, enhancing crop residues, planting green manures, applying farmyard manures, livestock and poultry dung, etc).

## Conclusion

Thirty-three years (1981 – 2013) manuring and fertilization increase crop biomass carbon, organic carbon input, however, only organic manure application can increase soil organic carbon concentration in the rice-wheat cropping system of China. Averagely, crop biomass carbon is increased by 39.9 – 77.2% compared to unfertilized control due to organic manure or chemical fertilizer application. Total organic carbon input into soil is

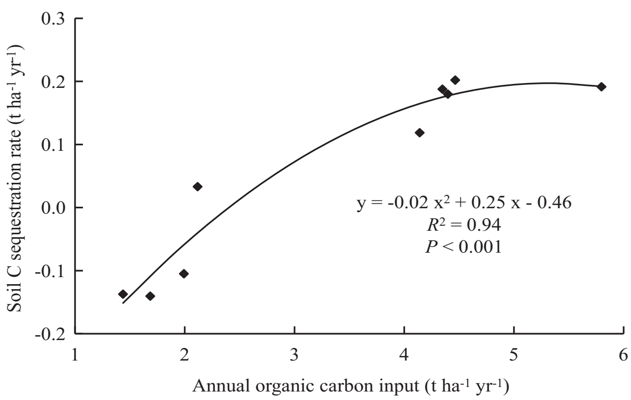


Figure 5. Non-linear regression between soil organic carbon sequestration rate and annual carbon input for duration of thirty-three years fertilizer experiments.

increased by $10.2-23.3\,kg\ C\ ha^{-1}\ yr^{-1}$ due to manuring or fertilization. Soil organic carbon concentration in the topsoil have decreasing tendency in the control, N and NP treatments; however, soil organic carbon concentration have ascending tendency in the NPK treatment and is significantly increased in the manure alone or manure appended chemical fertilizer treatments. These results demonstrate that organic manure application or integrated organic manure with chemical fertilizer application can be important strategies for increasing soil organic carbon sequestration and maintaining soil quality in the rice-wheat cropping system of China.

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