CHANGES IN SOIL ORGANIC CARBON AND ITS FRACTIONS AFTER 13 YEARS OF CONTINUOUS STRAW RETURN IN A SOYBEAN-MAIZE CROPPING SYSTEM

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Abstract. Straw return to the soil is proposed as an effective practice to increase soil organic carbon (SOC) storage in croplands. Based on a 13-year field experiment with soybean-maize cropping system, we studied the changes of total SOC and SOC fractions under no fertilizer (NF), mineral fertilizers (NPK) and mineral fertilizers with straw return (NPKS). Compared with the initial soil, SOC storage in the bulk soil significantly increased by 7.19% in the NPKS treatment, with an annual increase of 0.32 Mg ha^{-1}; while the SOC storage significantly decreased by 3.47% in the NF treatment, and no significant change was recorded in NPK treatment after 13 years. The NPKS treatment significantly increased the storage of free light fraction carbon (fLFC), occluded light fraction carbon (oLFC), heavy fraction (HFC), humic acid carbon (HAC) and fulvic acid carbon (FAC) by 44.4, 31.8, 5.47 10.5 and 3.92%, respectively. The HAC contributed the highest percentage (47.0%) of carbon to the improvement of SOC after 13 years of straw return. Therefore, straw return was conducive to the accumulation of labile fractions (fLFC and oLFC) which were in favor of soil fertility. Simultaneously, the increased HAC after straw return are beneficial to carbon sequestration.

Keywords: *labile fraction carbons, Mollisol, soil density fractionation, long term fertilization, soil organic carbon storage*

Introduction

Soil organic carbon (SOC) is an important pool in the global carbon (C) cycle, and has the function of mitigating climate change (Stockmann et al., 2013). Elevated levels of SOC have been positively related to crop productivity by enhancing soil fertility and plant nutrient supply (Lal, 2010; Singh et al., 2020). Therefore, management strategies that increase the net SOC of agricultural soils are considered soil health improvement strategies and promoted worldwide (Oliveira et al., 2019). However, this requires implementing agricultural practices adapted to local conditions that will increase the net soil C input, with outputs remaining stable or increasing, thus maximizing the soil C storage. In recent times, the impact of agricultural management on SOC storage has attracted much attention (Poeplau and Don, 2015; Chenu et al., 2019).

Crop straw return to the soil has been regarded as an environmentally friendly approach for straw utilization due to its positive effect on SOC storage in croplands (Liu et al., 2014), and widely adopted in diverse cropping systems. A number of studies have demonstrated that the duration of straw return to the soil has linearly increased SOC word wide in agroecosystem (Zhang et al., 2017; Jian et al., 2020). However, some studies have

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also reported a slight or no increase in SOC storage in response to crop straw return (Niu et al., 2011; Guo et al., 2015; Poeplau and Don, 2015). These differences might occur due to the duration of experiment. Jian et al. (2020) have reported that SOC could be significantly increased under the treatment experiencing straw return of more than 10 years, which depends on climate conditions, soil types, and agronomic practices. A meta-analysis has demonstrated that SOC could reach saturation under continuous straw return after 12 years (Liu et al., 2014). Therefore, medium- to long-term experiments are necessary to explore the dynamics of SOC under continuous straw return in agroecosystem.

SOC is heterogeneous and composed of several functional pools with different stability, which results in their turnover rates ranging from a few months to hundreds of years (von Lützow et al., 2007). To estimate the changes in SOC, it is crucial to quantify and understand the sensitivity of the different functional SOC pools to agricultural practices, for example straw return (Poeplau and Don, 2013). In view of SOC stabilization mechanisms, SOC can be separated to the following fractions: (1) unprotected fractions; (2) physically protected fractions by soil aggregates; (3) chemically or biochemically protected (Six et al., 2002; Yang et al., 2018). Various physical and chemical fractionation methods have been developed to separate SOC fractions with distinct degradability and turnover times. Density fractionation method highlights the observation that the physical location of SOC within the soil matrix is a key factor determining its turnover (Llorente et al., 2010). The obtained fractions are free light fraction (FLF), occluded light fraction within aggregates (OLF) and heavy fraction (HF). The FLF and OLF representing labile SOC pools with a rapid turnover time are both sensitive indicator of agronomic practices (Golchin et al., 1994; Tamn et al., 2005; Llorente et al., 2010). In contrast, HF is considered as stable pool, with turnover times ranging from decades to centuries. Generally, crop straw return has a positive effect on soil light fraction C (Nayak et al., 2012; Chen et al., 2019). The HF could be further chemically fractionated into three humic fractions (humic acid, fulvic acid and humin). Humic substances are operationally defined by a standardized extraction procedure, but previous studies have demonstrated that they are a heterogeneous pool of substances with distinct turnover rate (von Lützow et al., 2007). Crop straw is one of the major sources of humic substances in cropland soil (Guimarães et al., 2013). Several studies have addressed the effect of straw return on the size and composition of different humic fractions (Zhang et al., 2017, 2019a; Mi et al., 2019). Zhang et al. (2017) observed that continuous maize straw return increased humic and fulvic acid C concentrations in Mollisol. However, Mi et al. (2019) found that application of rice straw increased the concentrations of humic acid C only. Despite the widely researched response of different SOC fractions to straw return, the relative contributions of various functional SOC fractions to SOC change related to long-term straw return are still unclear.

Mollisols are one of the most important soil resources for food production and climate change mitigation due to their high organic C (Liu et al., 2012; Sanford et al., 2012). However, the SOC content of Mollisols has decreased rapidly over the past decades in the Northeast China, which is caused by long-term intensive cultivation and lower crop residue return (Li et al., 2016; Xu et al., 2020). In recent times, returning crop straw into soil is encouraged and widely applied in Northeast China aimed at improving soil fertility instead of burning the straw in the field (Wang et al., 2018). Previous studies have reported, that straw return could improve C sequestration by physically protecting soil macroaggregates and the occluded microaggregates (Guan et al., 2019), and also increase the soil recalcitrant C content (Zhang et al., 2019b). However, those studies were mainly conducted in continuous maize cropping systems in the south of the Mollisol region in Northeast China. Soybean-maize rotation is the primary cropping system in the north of the Mollisol region of China. Yet, a detailed examination of the changes in SOC and its fractions after straw return has not been considered in this region. The objectives of this study were to 1) identify the changes in SOC in the bulk soil and various fractions (light fractions, heavy fraction and humic fractions) after 13 years of continuing straw return under soybean-maize rotation, 2) assess the relative contributions of different SOC fractions to SOC change in relation to continue straw return. We hypothesize that continuous straw return would increase SOC storage both in the bulk soil and its fractions. Alternatively, the rate of increase of different SOC fractions caused by straw return would be different.

Materials and Methods

Site description

The field experiment was located at the National Field Observation and Research Station of Hailun Agroecosystems, Chinese Academy of Sciences (47°27′ N, 126°55′ E), in the central region of Mollisol in northeast China (*Fig. 1*). The region has a typical temperate continental monsoon climate with an average annual temperature of 1.5 °C. The lowest mean monthly temperature is −23 °C in January and the highest monthly mean temperature is 21 °C in July. The mean annual precipitation of the region is 550 mm, with more than 80% occurred from May to September. The frost-free period of the region is about 120 days. The soil of the study area is classified as Mollisol according to the USDA Soil Taxonomy System (Soil Survey Staff, 2010), which developed from sedimentary materials of loamy loess. The study site is a flat plain and had been under native prairie before the land was reclaimed for cropping about 120 years ago (Song et al., 2007). Before 1993, the cropping system was inter-annual rotation between wheat (*Triticum aestivum* L.) and soybean (*Glycine max* (L.) Merrill.), and between wheat, maize (*Zea mays* L.) and soybean rotation in 1993-2003.

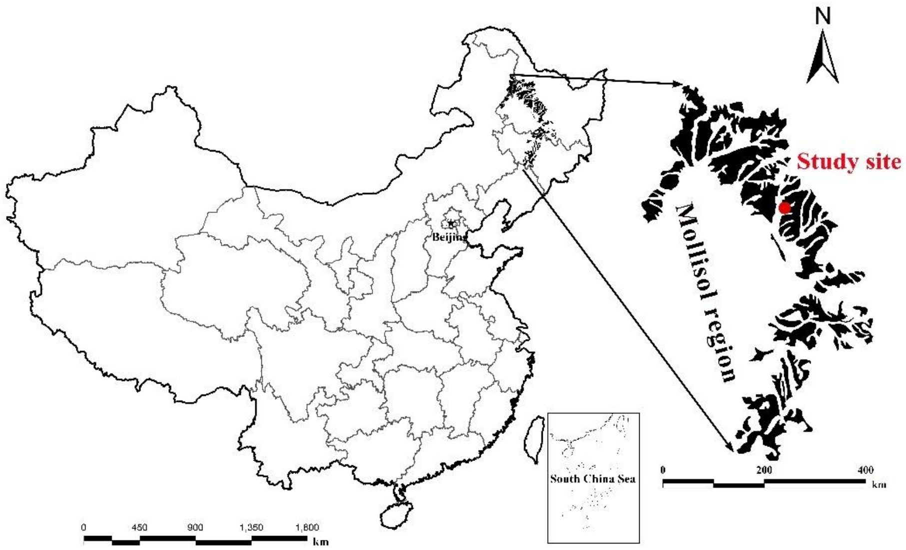

Figure 1. *Geographical distribution of study site in northeast China*

Experimental design

The long-term experiment was established in 2004. The cropping system was inter-annual rotation between soybean and maize. Before 2004, all crop residues were removed from the field after harvest. Three treatments were set up in 2004, including no fertilizer (NF), mineral fertilizers (NPK) and mineral fertilizers with straw return (NPKS). The area of each treatment was 1800 m^2 (width 30 m × length 60 m). Our experimental design was pseudo-replicated for NF, NPK and NPKS treatments, from which six composite soil samples were collected from each treatment. Our specific interest was assessing the SOC and SOC fractions in site-specific soil that had been treated for 13 years, representing the long-term agronomic outcomes. We presumed that any significant difference among those plots could be attributed to the effects of long-term treatment based on the random selection and low spatial variability of the soil characteristics. In the NF and NPK treatments, all aboveground straw was removed after harvest. In the NPKS treatment, maize or soybean straw was chopped to 3-4 cm in length after harvest, spread evenly in the treatment plot. All maize or soybean straws were plowed into the topsoil (0-20 cm depth) by rotary tillage. In the NPK and NPKS treatments, the mineral fertilizers were applied at rates of 64 kg N ha^{-1}, 70 kg P_2O_5 ha^{-1} and 20 kg K_2O ha^{-1} for soybean, and 138 kg N ha^{-1}, 70 kg P_2O_5 ha^{-1} and 20 kg K_2O ha^{-1} for maize. For the soybean, the mineral fertilizers were applied once as basal fertilizer at sowing. For maize, P and K fertilizers, and one third (33%) of the N fertilizer were applied as basal fertilizer at sowing, and the remainder two thirds (67%) was applied as topdressing at jointing stage. Mineral fertilizers were applied as urea (46% N), ammonium hydrogen phosphate (18% N; 46% P_2O_5) and potassium sulphate (51% K_2O). In all treatments, soils were subjected to conventional tillage, and were ridged by rotary tillage to a depth of 20 cm after harvest in autumn.

Plant-derived C input estimates

Every year, maize and soybean grain were manually harvest from five randomly selected sub-plots (6 m^2) of each treatment plot to estimate grain yield at crop maturity. Organic matter derived from crop straw, roots, stubble and rhizodeposition in this study, was estimated by the average ratios of crop yields to the above residues from 2004 to 2017. The ratios were estimated by harvesting 10 individual plants of soybean and maize in September every year. Each plant was separated into grain, straw, stubble and root (0-20 cm soil depth), and oven-dried at 60 °C to constant weight and weighed to estimate the dry matter mass. The C concentration of each tissues was determined using a CN elemental analyzer (EA3000, Euro Vector， Italy). The average ratios of grain to straw, grain to stubble and grain to root were 1:0.73, 1:0.05 and 1:0.14 for soybean, respectively, and were 1:1.2, 1:0.13 and 1:0.26 for maize, respectively during experimental years. The C input derived from rhizodeposition was assumed to be equal to root biomass C (Bolinder et al., 1999).

Soil sampling

Soil samples were collected from the NF, NPK and NPKS treatments after harvest in each experimental year. Thirty randomized soil cores (depth 0-20 cm and diameter 5 cm) were collected in each plot and every five soil cores were mixed as six composite soil samples. After removing visible plant fragments and roots, the fresh soil samples were sieved to pass through a 10-mm sieve by gently breaking soil clods along the natural failure surfaces of the soil, air-dried, and then stored in glass bottles. A portion of the air-dried soils was ground to pass through a 2-mm sieve for analyses of SOC and its fractions. During soil sampling, five randomized point were selected at each sampling plot for the determination of soil bulk density. At each point, four soil cores (100 cm^3) were sampled, and soil bulk density was measured after drying the soil cores at 105 °C for 48 h.

SOC fractions analysis

Soil density fractionation

Density fractionation of SOC was carried out following Llorente et al. (2010). 10 g air-dried soil sample (<2 mm) was placed in a 100 mL centrifuge tube with 50 mL sodium iodide (NaI) solution (d=1.8 g cm^{-3}). The tube was gently turned upside down 5 times by hand. After centrifugation for 30 minutes (min) at 4000 revolutions per minute (rpm), the supernatant was passed through a 0.45 μm membrane filter into a millipore vacuum unit. The separation method was repeated three times. The soil particles on the membrane were collected, washed with deionized water and considered as the free light fraction (fLF, d<1.8 g cm^{-3}). The residue remaining in the tube was then added with 50 mL NaI. The tube was placed in an ice bath and sonicated at 300 J ml^{-1} for 15 min with a probe-type ultrasonic disintegrator. The floating material was the occluded light fraction (oLF, d<1.8 g cm^{-3}) protected by soil aggregates, then recovered by centrifugation, filtered and washed in the same way as the fLF. The leftover soil in the centrifuge tube was washed with distilled water until the water became clear and used as the heavy fraction (HF, d>1.8 g cm^{-3}). All fractions were dried at 50 °C, weighed, ground in a mortar, and analyzed for C.

Humic substance extraction

The extraction of humic substance was preformed according to the method described by Stevenson (1994). Briefly, 50 mL 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} $Na_4P_2O_7$ solution (50:50, v/v) was added into the heavy fraction, and the tubes were then placed in a water bath at 70 °C for 1 h. The supernatant solution was centrifuged at 3500 rpm for 15 min and collected as the humic extractable substances (HE). The HE was acidified to pH 1.0 with 0.5 mol L^{-1} H_2SO_4, the precipitated fraction was acid-insoluble humic acid (HA), and the solution was fulvic acid (FA), the two fractions were separated by centrifugation at 3500 rpm for 15 min. The HA was re-dissolved by 0.05 mol L^{-1} NaOH. The residue soil was humin (HM) fraction. The C contents of HE (HEC) and HA (HAC) was determined by the $K_2Cr_2O_7$ oxidation method. Carbon content of FA (FAC) and HM (HMC) were calculated using the following formulas:

$$\mathrm{FAC} = \mathrm{HEC} - \mathrm{HAC} \quad \text{(Eq.1)}$$

$$\mathrm{HMC} = \mathrm{SOC} - \mathrm{fLFC} - \mathrm{oLFC} - \mathrm{HEC} \quad \text{(Eq.2)}$$

where, fLFC and oLFC are the C contents of fLF and oLF.

Total C contents in bulk soil and light fractions were determined using the CHN elemental analyzer (EA3000, Euro Vector， Italy).

Statistical analysis

Statistical analyses were performed by SPSS V19.0. One-way ANOVA with Tukey test was conducted to analyze the differences of SOC and SOC fractions among treatments at 5% level of significance. Homogeneity of variance and normality assumption were tested using Levene's Test. The date differences between 2004 and 2017 were compared with paired t-test. Regression analysis was performed to determine the relationships between SOC content and experimental year.

Results

Plant-derived C input

During the 13-year experimental period, the cumulative C input in the NF, NPK and NPKS treatments were 10.92, 15.12 and 45.96 Mg ha^{-1}, respectively (*Table 1*). In the NPKS treatment, the cumulative C input was 321% and 204% higher than NF and NPK, respectively, and 66.2% was derived from straw return, and more than 80% of cumulative C input were derived from root and rhizodeposition in the NF and NPK.

Table 1. *Plant-derived C input in different treatments in 2004-2017 (Mg ha^{-1}). NF, No fertilizer; NPK, mineral fertilizers; NPKS, mineral fertilizers with straw return*

Treatment	**Straw C** [a]		**Root C**		**Stubble C**		**Rhizodeposition C** [b]		**Cumulative C input**
	Soybean	**Maize**	**Soybean**	**Maize**	**Soybean**	**Maize**	**Soybean**	**Maize**	
NF	0	0	0.88	3.52	0.31	1.81	0.88	3.52	10.92
NPK	0	0	1.03	5.05	0.37	2.59	1.03	5.05	15.12
NPKS	5.99	24.42	1.09	5.17	0.39	2.65	1.08	5.17	45.96

[a] Soybean straw including the pod husk. [b] Carbon input from rhizodeposition was assumed to be equal to root biomass C (Bolinder et al., 1999)

SOC storage in bulk soil

Different agronomic practices had a significant impact on both SOC storage. After 13 years of different fertilizer treatments, significant differences in the storage of SOC were observed among treatments, shown as NPKS > NPK > NF (*Fig. 2*). Compared with the initial soil, the SOC storage significantly increased by 7.19% in the NPKS treatment ($P < 0.05$), with an annual average increase of 0.32 Mg ha^{-1}. In the NF treatment, SOC storage significantly decreased by 3.47%, and without any significant change in the NPK treatment over the past 13 years. The changes of SOC in the NPKS treatment, NPK and NF treatments were 4.10, 0.11 and -2.02 Mg ha^{-1}, respectively (*Fig. 2*).

Temporal changes of SOC content

Compared with the initial soil, SOC content in the NPKS treatment significantly increased ($P < 0.001$), and no significant changes ($P > 0.05$) in the NF and NPK treatments over the 13 years (*Fig. 3*). The SOC content in the NPKS treatment was significantly higher ($P < 0.05$) than those in the NF and NPK treatments in 2008, four years after the experiment was established (2008). Subsequently, the significant differences among three treatments were recorded in the thirteenth year of the experiment with the highest SOC content in the NPKS treatment (28.37 g kg^{-1} soil), followed by the NPK treatment (26.35 g kg^{-1} soil), and the lowest in the NF treatment (25.16 g kg^{-1} soil). Compared with the initial soil, SOC content increased by 19.5% for NPKS, but decreased by 6.64% for NF. It is worth noting that there were relatively high standard deviation values in the NPK treatment in 2013 and 2014, this might be caused by the variation of sample plots.

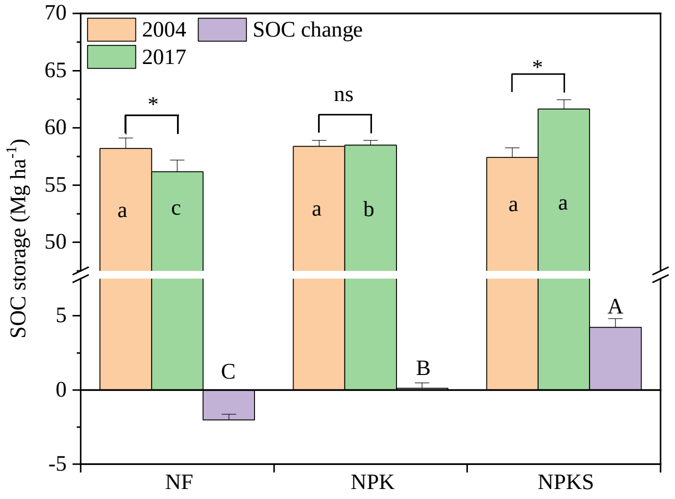

Figure 2. *Soil organic carbon (SOC) storage change in bulk soils (0-20 cm) after 13 years of different treatments. NF, No fertilizer; NPK, mineral fertilizers; NPKS, mineral fertilizers with straw return. Different lowercase letters indicate significant differences (P < 0.05) in SOC storage among treatments in the same year. Different uppercase letters indicate significant differences (P < 0.05) in SOC storage change among treatments. *Indicate significant differences (P < 0.01) between 2004 and 2017. Bars represent the standard deviations (n = 6)*

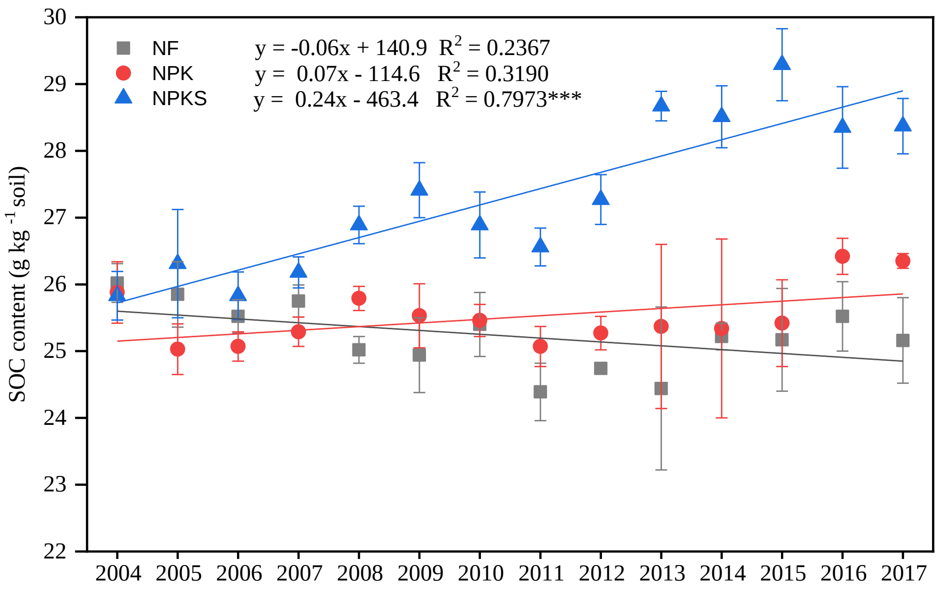

Figure 3. *Changes in soil organic carbon (SOC) content (0-20 cm) in different treatments during the period 2004-2017. NF, No fertilizer; NPK, mineral fertilizers; NPKS, mineral fertilizers with straw return. ***Indicate significance level at P< 0.001. Bars represent the standard deviations (n = 6)*

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SOC storage in density and humic fractions

Different agronomic practices have impacted the storage of SOC fractions in this study (*Fig. 4*). Compared with the initial SOC, treatment with continuous straw return with mineral fertilization significantly increased ($P < 0.05$) the storage of fLFC, oLFC, HFC, HAC and FAC by 44.4%, 31.8%, 5.47%, 10.5% and 3.92%, respectively (*Fig. 4a, b, c, d, e*). However, all of the above mentioned SOC fractions significantly decreased by 17.7%, 11.2%, 2.76%, 7.21% and 3.41% for fLFC, oLFC, HFC, HAC and FAC, respectively, under NF treatment. In the NPK treatment, there were no significant differences in all the SOC fractions between the soil samples taken in 2004 and 2017. After 13 years of experiment, the significant differences ($P < 0.05$) in the storage of fLFC, oLFC, HFC and HAC were recorded among the three treatments in the following order of NPKS > NPK > NF (*Fig. 4a, b, c, d*). However, there was no significant difference in the storage of HMC among three treatments (*Fig. 4f*).

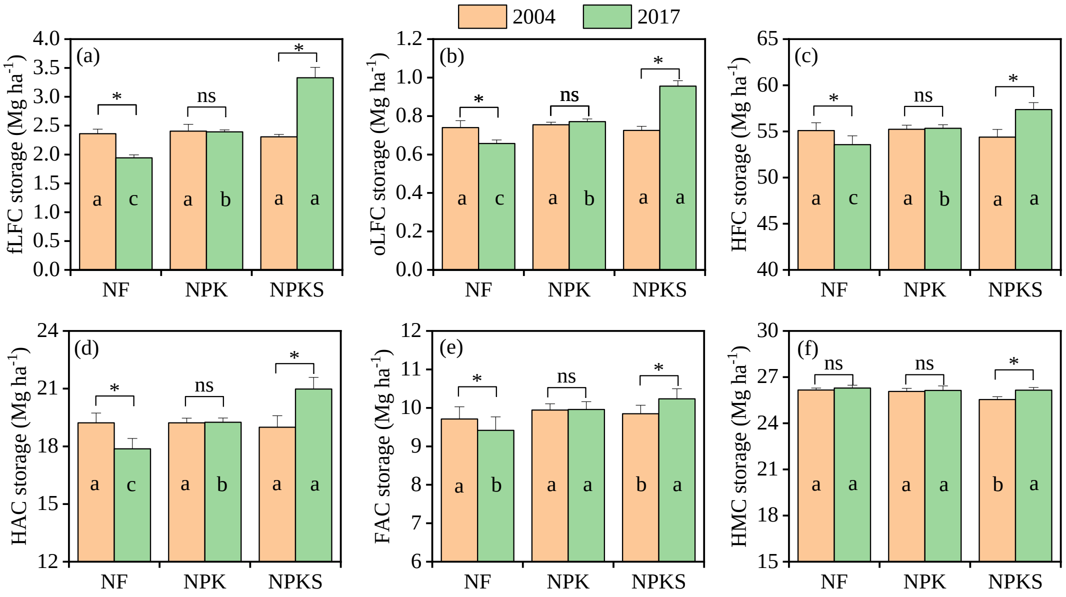

Figure 4. *Carbon storage of soil organic carbon (SOC) fractions (0-20 cm) in different treatments in 2004 and 2017. NF, No fertilizer; NPK, mineral fertilizers; NPKS, mineral fertilizers with straw return. fLFC, free light fraction carbon; oLFC, occluded light fraction carbon; HFC, heavy fraction carbon; HAC, humic acid carbon; FAC, fulvic acid carbon; HMC, humin carbon. Different lowercase letters indicate significant differences ($P < 0.05$) among treatments in the same year. * indicate significant differences ($P < 0.01$) between 2004 and 2017. Bars represent the standard deviations ($n = 6$)*

HMC, HAC, FAC, fLFC and oLFC storage accounted for 44.4%-46.8%, 33.1%-34.0%, 16.6%-17.2%, 3.46%-5.40% and 1.17%-1.55% of total SOC, respectively (*Fig. 5*). The NPKS treatment significantly increased ($P < 0.05$) the proportion of fLFC, oLFC and HAC by 34.5%, 22.7% and 2.88%, respectively, but decreased the proportion of HMC by 4.66%. The proportions of fLFC, oLFC and HAC under NF treatment were significantly decreased. Meanwhile, the different agronomic practices had a significant influence on the HA/FA ratio of humus, the largest value was recorded in the NPKS treatment with 2.05, followed by NPK treatment with 1.93 and NF treatment with 1.90, NPKS of which were higher than the initial soil with 1.93 (*Table 2*).

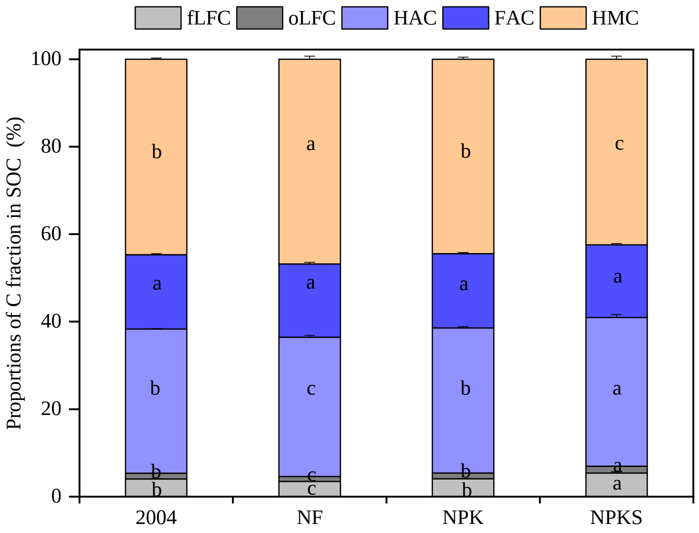

***Figure 5.** Proportion of carbon fractions in bulk soil organic carbon (SOC) (0-20 cm) in 2004 and 2017.The proportions of carbon fractions in 2004 are the mean value of the three treatments. NF, No fertilizer; NPK, mineral fertilizers; NPKS, mineral fertilizers with straw return. fLFC, free light fraction carbon; oLFC, occluded light fraction carbon; HFC, heavy fraction carbon; HAC, humic acid carbon; FAC, fulvic acid carbon; HMC, humin carbon. Different lowercase letters indicate significant differences (P < 0.05) among treatments. Bars represent the standard deviations (n = 6)*

***Table 2.** The HA/FA ratio of humus in different treatments in 2004 and 2017*

Treatment	2004	2007
NF	1.98 ± 0.03 Aa	1.90 ± 0.04 Bb
NPK	1.93 ± 0.03 Ab	1.93 ± 0.04 Ab
NPKS	1.93 ± 0.03 Bb	2.05 ± 0.02 Aa

NF, No fertilizer; NPK, mineral fertilizers; NPKS, mineral fertilizers with straw return. Different lowercase letters indicate significant differences (*P* < 0.05) among treatments in the same year. Different uppercase letters indicate significant differences (*P* < 0.05) between 2004 and 2017. The results are shown as the mean ± SD (n=6)

Contribution of C fractions on the improvement of SOC after straw return

The increased amount of SOC fractions under NPKS treatment shown as the order of HAC > fLFC > HMC > FAC > oLFC (*Table 3*), and HAC accounting for 47.0% of SOC change was significantly higher than the other SOC fractions. Although the light fraction C accounted for only about 5% of SOC (*Fig. 5*), its contribution to the improvement of SOC was as higher as 29.8%, including the increase of 24.3% from fLFC and 5.5% from oLFC, respectively (*Table 3*). However, the contribution of HMC to the improvement of SOC in the NPKS treatment was only 14.3%, even though the HM accounted for more than 40% of SOC.

Table 3. *The change amount and the percentage contribution of different organic carbon fraction in SOC (0-20 cm). NF, No fertilizer; NPK, mineral fertilizers; NPKS, mineral fertilizers with straw return*

C fraction	**Change of C fractions (Mg ha^{-1})**			**Proportion to SOC change (%)**		
	NF	**NPK**	**NPKS**	**NF**	**NPK**	**NPKS**
SOC	-2.02	0.11	4.23	-	-	-
fLF	-0.42	0.00	1.02	21.1	-29.9	24.3
oLF	-0.08	0.02	0.23	4.2	-11.3	5.5
HA	-1.35	0.12	1.99	66.4	45.7	47.0
FA	-0.29	0.06	0.39	15.1	-63.8	9.0
HM	0.13	0.19	0.60	-6.9	159.3	14.3

SOC, soil organic carbon; NF, No fertilizer; NPK, mineral fertilizers; NPKS, mineral fertilizers with straw return; fLFC, free light fraction carbon; oLFC, occluded light fraction carbon; HFC, heavy fraction carbon; HAC, humic acid carbon; FAC, fulvic acid carbon; HMC, humin carbon

Discussion

Change in bulk SOC in response to continuous straw return

Our results clearly support the hypothesis that straw return to the soil has a positive impact on SOC storage and sequestration. SOC storage increased by 7.19% after 13 years of straw return to soil, with an annual increase of 0.32 Mg ha^{-1} in this study (*Fig. 2*). This increase in SOC was within the range presented by Zhang et al. (2010), who found that the rate of SOC sequestration ranged from 0.07 to 1.46 Mg ha^{-1} year^{-1} under different upland cropping systems across northern China. Similarly, Wang et al. (2018) also reported a SOC sequestration rate of 0.28 Mg ha^{-1} yr^{-1} in a 21-year straw return experiment in the same pedoclimatic region as this study. Generally, a significantly linear relationship occurs between straw C input and SOC sequestration rate when the soils do not reach the C-saturated point (Kong et al., 2005; Duval et al., 2016; Jiang et al., 2017). The average C input was 3.5 Mg ha^{-1} yr^{-1} under NPKS treatment in this study (*Table 1*), which was 318% and 206% higher than the NF and NPK treatments. Consequently, the highest storage of SOC was found under NPKS treatment after 13 years (*Fig. 2*).

A meta-analysis has demonstrated that the effects of straw return on SOC content were not evident in short-term field experiments (1-3 years), but significantly increased in medium-term experiments (3-15 years). SOC contents showed a significantly increasing trend with the duration increase of straw return (Liu et al., 2014), which was consistent as our result. Although a significantly linear relationship between SOC content and experimental years of continuous straw return was found (*Fig. 3*), there was no significant change of SOC content in the first 3 years of straw return, which could be attributed to the relatively lower input of straw return under the soybean-maize rotation system. Different with our results, Liu et al. (2019) reported that the maize monoculture had continuously increased SOC content during the 8-year field experiment with straw return. The response of SOC to organic matter input depends on the initial SOC content (Chenu et al., 2019). When the initial SOC content is high, it is difficult to measure the change of SOC within a shorter period of time (Campbell et al., 1991). Berhane et al. (2020) has also described that soils with lower initial SOC have a higher potential to store and sequester SOC than soils with higher initial SOC following combined application of chemical fertilizer and straw. At the same study site, You et al. (2017) studied SOC changes during the early stages of the development of Mollisol with excessively low SOC (4.79 g kg^{-1} soil), and demonstrated that the return of maize and soybean straw induced a steady increase in SOC storage in the early years of the experiment with an annual SOC sequestration of 0.80 Mg C ha^{-1}, which was relatively higher than that in this study (0.32 Mg ha^{-1}) (*Fig. 3*). In addition, the effect of straw return on SOC accumulation was affected by straw return approaches and climate (Liu et al., 2014; Han et al., 2020; Jian et al., 2020). In general, crushing and incorporating straw into soil increased SOC more than mulching straw on soil surface (Han et al., 2020). Straw incorporation by tillage increases the contact between soil and straw, and thus promotes more straw C was sequestrated by soil. The decomposition rate of crop residue in this study area is substantially lower than other areas due to the low temperature in northeast of China (Xu et al., 2017). Therefore, incorporating straw in to the plough layer is an effective approach to accelerate the decomposition of straw in northeast of China (Han et al., 2020).

It has been widely accepted that SOC content could not be growing constantly with C input because of the potential upper limit of SOC concentration called the SOC saturation point, where there is an equilibrium between the C inputs and outputs (Stewart et al., 2007). Prior research has reported that due to the high base status of SOC, Mollisols can appear C saturation after many years of organic material input (Chung et al., 2010). In this study, a significantly linear relationship was found between SOC content and experimental years under the treatment with straw return (*Fig. 3*), which is an indication that the tested Mollisol has not reached an upper threshold of C sequestration over the experimental period. This could be explained by the high clay content of Mollisols (>40%) that could enhance the capacity of soil to store more C (Ding et al., 2012). However, we cannot conclude from this study that SOC saturation will occur with increasing straw-C inputs. Therefore, further long-term research is needed in this region to establish that conclusion of SOC saturation.

The above-ground biomass under the NF and NPK treatments was removed from filed and more than 80% of C input was derived from root (root biomass and rhizodeposition) in this study. Moreover, the root-derived C input is relatively lower under the NF treatment. Consequently, the cumulative C input was significantly lower in NF as compared with the NPK and NPKS treatments (*Table 1*), which contributed lower SOC storage under NF treatment (*Fig. 2*). Similar results have been reported in different climate and soil conditions (Lee et al., 2009; Ding et al., 2014; Yang et al., 2018). Applying mineral fertilizer alone for 13 years did not change SOC concentration and storage (*Figs. 2, 3*), indicating the SOC was in equilibrium and the input of C could compensate for the decomposition of SOC. The result from a 35-year field trial demonstrated that SOC would maintain the balance under the treatment with annual C input of 1.4 Mg ha^{-1} in northeast China (Hao et al., 2016), which is very close to the value under NPK treatment of our study. However, some studies showed that application of mineral fertilizer alone decreased SOC in Northeast China (Yan et al., 2007; Wang et al., 2013; Li et al., 2016). This reduction in SOC from the application of mineral fertilizer alone, could be attributed, in part, to the serious soil erosion in this area from slopes in fields due to hills in the landscape (Liu et al., 2010). The slope at this study site, is less than 2° and the effects of soil erosion can be ignored. In this case, incorporating crop stubble and root biomass into soils consequently can compensate for the loss from the SOC mineralization. Including legumes in crop rotations has been introduced as a sustainable alternative to nitrogen fertilizer-based systems, due to the increased N availability to following crops, particularly when residues are added to the soil (Oliveira et al., 2019). In addition, legume crops have a positive effect on soil biology, promote the stabilization of soil aggregates, which protect native SOC from microbial decomposition, leading to increased soil C storage (Franke et al., 2018). Hence, rotation system including soybean is an effective practice to maintain the level of SOC in Mollisols.

Effect of straw return on SOC density fractions

Light fractions of SOC, mainly consisted of fresh plant-derived materials, represented labile SOC pools with rapid turnover rates (Golchin et al., 1994; Tamn et al., 2005; Llorente et al., 2010). In general, the light fractions of SOC is closely associated with soil nutrient cycling and soil fertility owing its impacts on soil food webs (Chen et al., 2017). The combined application of straw return and mineral fertilizer significantly increased not only the storage of fLFC and oLFC but also both proportion in SOC (*Figs. 4, 5*), which could be attributed to large straw inputs provided abundant source for the formation of light fractions under NPKS treatment. Moreover, the increase rate in light fraction C (44.4% for fLFC, and 31.8% for oLFC) was much higher than that in bulk SOC (7.19%), indicating that the light fraction C was more sensitive to straw return than bulk SOC. Previous studies have also reported that straw return significantly increased the light fraction C compared to no straw return (Nayak et al., 2012; Chen et al., 2017; Guan et al., 2019; Yan et al., 2020). After plant residue was incorporated into soil, part of residue directly existed in the soil as unprotected fLF, and part was protected by aggregates, which then slowly decomposed and utilized by soil microorganisms (Six et al., 2002). Although the light fraction C accounted for only 5% of SOC (*Fig. 5*), its contribution to the improvement of SOC was as high as 29.8% after 13 years of experiment with straw return to soil, including the increase of 24.3% from fLFC and 5.5% from oLFC, respectively (*Table 2*). Similar result was reported by Yan et al. (2020), who found that more than 34% of the gain in SOC storage was stored in light fractions. Thus, light fraction C may be of greater importance in defining SOC turnover. Previous studies have indicated that the decrease of root biomass caused by the changes of soil management were the main factor that induced the decrease of light fraction C (Angst et al., 2018). In addition, higher amounts of root residue and exudates could promote the formation of soil aggregate, and thereby increase the oLFC that was physically protected within aggregates (Yamashita et al., 2006). Root-derived C in no fertilizer treatment was obviously lower than that in the fertilizer treatment (*Table 1*), which resulted in a significant decline of light fraction C in NF treatment compared with the initial soil (*Fig. 4a, b*).

The heavy fraction (HF) organic C is mineral associated fractions with a slower turnover rate and a higher degree of chemical protection (Llorente et al., 2010). More than 90% of organic C in Mollisol was stored in HF (*Fig. 5*). Although the storage of HFC increased by 5.47% after 13 years with continues straw return, it accounts for more than 70% of all increased SOC in the bulk soil. Likewise, Liu et al. (2008) found that the contribution rate of HFC to SOC improvement was 70.7% in the treatment with maize straw return in a 10-year experiment in Northeast China. This indicates that a larger proportion of the SOC storage derived from external C inputs was sequestered in HFC pool. Therefore, the response of HFC to soil management was slow, but it played a crucial role in maintaining the stability and quantity of SOC (Llorente et al., 2010).

Effect of straw return on SOC humic fractions

In order to clarify the stable characteristics of SOM, we further fractionated HF to different humic fractions (i.e. HA, FA and HM). The C storage in humic substances were characterized as HM>HA>FA (*Fig. 4d, e, f*), which is consistent with published results in Mollisol (Zhang et al., 2019a). Crop residue is one of the major sources of humic substances in farmland soil (Guimarães et al., 2013). Hence all of the three humic fractions were significantly increased by continuous straw return to soil. Humic-like substances in organic amendments have been documented to contribute to the accumulation of native soil humic substances (Brunetti et al., 2007). Crop straw contained a certain amount of humic-like substances (Adani and Ricca, 2004). During the straw decomposition process, this humic-like substance could be adsorbed by soil minerals (Simonetti et al., 2012), meanwhile, some released labile biomolecules (e.g., polysaccharides, peptides and aliphatic compounds) could be incorporated into native soil humic substances throw chemical protection. When the soil was in a net loss of SOC, its humic fractions was always in a decreasing state (Guimarães et al., 2013). Therefore, the treatment with no mineral fertilization significantly decreased the C storage in HA and FA (*Fig. 4d, e*). On the other hand, mineral fertilizers alone did not significantly affect the C storage in humic fractions (*Fig. 4d, e, f*), which implies that the humic composition of Moillsol is always in a stable state under the conventional management.

The ratio of HA/FA could provide information on the humification rate and accumulation regularity of SOC under different management (Guimarães et al., 2013). The HA/FA ratio significantly increased after 13 years of combined application of straw return and mineral fertilizers compared with initial soil, mineral fertilizers alone and no fertilizers in this study, indicating that long-term straw application was more conducive to the accumulation of HAC. This could be further proved by the highest contribution rates of HAC (47%) to the SOC improvement (*Table 3*). Previous studies conducted in Fluvisol (Shindo et al., 2006), Inceptisol (Zhang et al., 2011) and Hapludoll (Song et al., 2019) also reported that application of straw or organic manure in combination with mineral fertilizers could increase the HA/FA ratio of SOM. A plausible explanation is that FA was more soluble and reactive than HA (Dou et al., 2020). In the early stage of crop residues decomposition, the formation of FA fractions is rapid, however, the FA later would be easily transferred into more stable HA fractions under microbe’s activity (Ingelmo et al., 2012; Dou et al., 2020). Thus, our result suggested that medium-term application of straw is favorable for the formation of HA fraction. The HA fraction would play a major “sink” role in the process of SOC sequestration.

Conclusions

A 13-year field experiment demonstrates that, due to the higher C input by straw, the combined application of straw return and mineral fertilizer significantly increased SOC in the top 0-20 cm layer of a Mollisol under soybean-maize cropping system. There was a positive linear relationship between SOC content and increasing years with straw return, which indicates that Mollisols do not reach saturation point in C sequestration over the experimental period. The storage of organic C in density (i.e. fLF, fLF and HF) and humic fractions (i.e. HA, FA and HM) were also generally higher after the returning of straw. Moreover, straw return clearly increased the proportion of fLFC, oLFC and HAC, but decreased the proportion of HMC, resulting in the HA/FA ratios being higher than the application of no fertilizer and mineral fertilizers. The HA fractions contributed the highest rates to the SOC improvement after 13 years straw return. The storage of SOC and its fractions did not change under treatment with application of mineral fertilizers alone, indicated an equilibrium state of SOC. It was concluded that SOC fractions in response to straw return were different. In addition, this study was only over a medium-term period of 13 years, a long-term study is needed to verify the effects of crop straw return on SOC fractions in a soybean–maize rotation system in Northeast China.

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