Effects of returning corn straw and fermented corn straw to fields on the soil organic carbon pools and humus composition

Yifeng Zhang[a], Sen Dou[a,*], Batande Sinovuyo Ndzelu[a], Rui Ma[a], Dandan Zhang[a], Xiaowei Zhang[a], Shufen Ye[a], Hongrui Wang[a]

[a]College of Resource and Environmental Science, Jilin Agricultural University, Changchun, Jilin Province 130118, China

Correspondence to: Sen Dou (dousen1959@126.com)

Abstract. In our previous studies, we filtered out fungus (*Trichoderma reesei*) to have the best ability to transform corn straw into a humic acid-like substance through laboratory incubation experiments. In order to further verify our former findings, we set up a 360 day-field experiment that included three treatments applied under equal carbon (C) mass: (i) corn straw returned to the field (CS), (ii) fermented corn straw treated with *Trichoderma reesei* returned to the field (FCS-T), and (iii) blank control treatment (CK). Soil organic carbon (SOC), soil labile organic C components, soil humus composition, and the management levels of SOC pools under the three treatments were analyzed and compared. The results showed that the SOC content of CS and FCS-T treatments increased by 12.71 % and 18.81 %, respectively, compared with CK at 360 d. The humic acid carbon (HA-C) content of the FCS-T treatment was 0.77 g kg^{-1} higher than in the CS treatment. Applying FCS-T appeared to promote a significant increase in SOC content, carbon pool activity index, and carbon pool management index through the accumulation of HA-C, humin carbon, and easily oxidizable organic carbon. Applying fermented corn straw treated with *Trichoderma reesei* (FCS-T) is more valuable and conducive to increasing soil easily oxidizable organic C (EOC) and humus C content than direct application of corn straw.

1 Introduction

Recycling and returning crop residues as soil amendments is an important prospect for increasing soil organic carbon (SOC) content and crop yield (Villamil et al., 2015), and managing crop straw residues. However, the decomposition process is slow when crop residues are directly applied to the soil (Zhang et al., 2019), and it is still not fully known how crop residues are transformed into stable SOC when applied to the soil (Cotrufo et al., 2013; Lehmann and Kleber, 2015; Zhang et al., 2015a). Information about the decomposition and stability of carbon (C) is needed for long-term soil C sequestration (Cotrufo et al., 2013; Ndzelu et al., 2020a). Contrary to direct crop residue application and conventional composting methods, pre-treatment of crop residues with microbial inoculants is an effective method for reusing crop residues as eco-friendly amendments to improve soil fertility and increase soil organic matter (SOM) (Bhattacharjya et al., 2021; Organo et al., 2022). This strategy accelerates crop residue degradation and humification (Vargas-Garcia et al., 2006; Ahmed et al., 2019; Nigussie et al., 2021; Sajid et al., 2022) by significantly halving the time needed for the compost to reach maturity (Organo

30   et al., 2022). The microbial inoculant-based fermentation product contains both labile organic substances from the degraded portion and relatively stable humic-like substances (Huang et al., 2008). This favours the accumulation and production of SOM when applied to the soil (Vargas-Garcia et al., 2006; Ahmed et al., 2019).

     The labile organic C components are sensitive and respond promptly to changes in soil management practices (Blair et al., 1995; Xu et al., 2011). Hence, the labile organic C fractions such as water extractable organic carbon (WEOC), easily

35   oxidizable organic carbon (EOC), and microbial biomass carbon (MBC) are effectively used to detect small changes in soil quality (Chen et al., 2009; Sainepo et al., 2018). Chen et al. (2017) and Ma et al. (2021) reported a significant increase in MBC and WEOC contents after crop straw residues were returned to the soil. In another study, Ndzelu et al. (2020b) also found that five years of corn straw application increased soil EOC, WEOC, and MBC contents by 34.09%, 41.38%, and 49.09% in the 0 – 20 cm depth, respectively. Therefore, assessing labile SOC fractions after crop straw applications may

40   provide information about the formation of SOC (Chen et al., 2009; Huang et al., 2018; Liu et al., 2019; Ma et al., 2021). The carbon pool management index incorporates carbon pool index and carbon pool activity index, and is an important and sensitive index widely used to determine changes in SOC and C sequestration in the soil (Blair et al. 1995; Duval et al. 2019). A high carbon pool management index indicates that soil management practices have agreater potential to promote soil Csequestration (Duval et al. 2019).

45   The humic substance is the most stable fraction of SOM and contributes the largest proportion to the total SOC (Olk et al., 2019; Dou et al., 2020). As a result, studying changes in soil humus components and the labile organic C fractions after corn straw application could inform about the formation and stabilization of SOC during crop residue decay. Over the years, extensive studies have been conducted to investigate the effects of crop residues on SOM and its pools (Atiyeh et al., 2002; Romero et al., 2007; Zhang et al., 2015a; Ng et al., 2016; Yang et al., 2020). However, there are still conflicting reports and

50   there is no consensus about the effects of crop residue application on the formation and composition of SOM. For instance, recent studies have found that corn straw application significantly increases soil humus content and enriches soil humic acid structure with aromatic compounds (Fan et al., 2018; Zhang et al., 2019). In contrast, other studies reported increased aliphatic compounds in soils amended with corn straw (Yang et al., 2020; Ndzelu et al., 2020a). These diverging reports indicate that the magnitude and the influence of corn straw residues on SOM composition are unclear and site-specific.

55   *Trichoderma*-mediated straw fermentation is gaining attention as a soil amendment and nutrient source (Gaind and Nain. 2006; Gaind and Nain. 2007; Siddiquee et al., 2017) because of the role *Trichoderma*-mediated straw play in improving crop yield (Islam et al., 2014), promoting plant development, and alleviating biotic and abiotic stresses in crops (Sarangi et al., 2021). In our previous studies (Yang et al., 2019; Zhang et al., 2020; Zhang et al., 2021), we observed in laboratory incubation experiments that the *Trichoderma reesei* (*T. reesei*) had the best ability to form humic acid-like during corn straw

60   decomposition when compared with other fungi (*Phanerochaete chrysosporium* and *Trichoderma harzianum*). However, there is limited knowledge on the potential effects of *T. reesei* fermented corn straw on SOM formation and accumulation. In particular, the dynamic change process of different SOC fractions has not been sufficiently reported after soils are amended with *T. reesei* fermented corn straw. In this study, we quantified differences in soil organic carbon pools and humus

composition between treatments. The purpose of this study was to verify whether the application of fermented corn straw treated with *T. reesei* is more effective in forming a relatively stable SOC fraction in a field experiment compared to the direct application of corn straw. We hypothesized that: (1) application of fermented corn straw treated with *T. reesei* (FCS-T) will be the most efficient in increasing soil humus content and soil C storage, due to the increase in aromatic C compounds; (2) application of FCS-T may also increase soil labile organic C components (WEOC, EOC, and MBC); and (3) application of FCS-T may increase carbon pool management index level more than direct corn straw application.

2 Materials and methods

2.1 Site description

A 360-day field experiment was conducted in a corn monocropping experimental field located at Jilin Agricultural University in Northeast China (N43°49′5″, E125°24′8″). Since 2005, monocropping of corn (*Zea mays* L.) has been the main cropping system in the region. The area is in a semi-humid region and receives a mean annual rainfall of 618 mm, with the highest precipitation occurring in the months of July and August. Soils in the study area are classified as Argiudolls according to the United States Department of Agricultural Soil Taxonomy (Soil Survey Staff. 2014). The basic soil characteristics are presented in **Table 1**.

2.2 Preparation and description of corn straw and fermentation of corn straw

Corn straw was collected from the adjacent cropland of corn (*Zea mays* L.) located at Jilin Agricultural University in Northeast China (N43°48′43.5″, E125°23′38.50″). The corn cultivar Zhongjin 368 type (Beijing Golden Grain Seed Co., Ltd.) was planted at the end of April 2018 and harvested in early October 2018. After harvest, the whole corn straw residue was cut at the bottom and air-dried, thereafter shredded into 0.5 cm segments. A portion of the shredded corn straw was regarded as CS material.

The fermentation of corn straw was prepared by the fungal strains (*Trichoderma reesei* (*T. reesei*) MCG77) which were purchased from the American Type Culture Collection. The strains of fungi were inoculated on a medium containing 30 mL of potato dextrose agar and placed in an incubator at 28 °C for 72 hours to obtain mature microbial spores (mycelium). This process was carried in a BIOTECH-30SS solid fermentation tank (Shanghai Baoxing Biological Engineering Equipment Co., Ltd). A KQ-C type automatic steam generator (Shanghai Fengxian Xiexinji Power Plant) was used to generate steam for sterilization, and 2 kg of air-dried corn straw (particle size = 0.5 cm) was sterilized in a solid fermenter. The sterilization process was conditioned for 25 min at 121 °C. After sterilization, the *T. reesei* liquid containing the spore mycelia (0.8 L) and a mineral salt solution (5 L) was mixed with sterilized corn straw. The spore solution and a mineral salt solution (pH = 5) used were prepared similarly as described by Zhang et al (2020), and the C/N ratio was adjusted to 25:1 using a mineral salt nutrient solution. The mineral salt nutrient solution (g L^{-1}) was prepared as a mixture of: KH_2PO_4 28 g, $(NH_4)_2SO_4$ 9.6 g, $MgSO_4$ 4.2 g, $CoCl_2$ 4.2 g, $(NH_2)_2CO$ 2.2 g, $FeSO_4·7H_2O$ 0.07 g, $CaCl_2$ 0.028 g, $MnSO_4$ 0.021 g, $ZnSO_4$ 0.019 g, and the pH

95 = 5. The fermentation process lasted 90 days and was carried out at 30 °C, 60% humidity, and 6.0 rpm. The final fermented product after 90 days was designated as fermented corn straw treated with *T. reesei* (FCS-T) material. The basic elemental properties of the CS and FCS-T materials are presented in **Table 2**, and were determined with an element analyzer (Vario-EL-III Hanau, Germany).

2.3 Field procedures and sampling

100 **2.3.1 Experimental layout: Field plot settings and specifications**

The field experiment was set up to have nine plots and three treatments, namely CS, FCS-T, and CK (as a control), applied under equal C mass. Each treatment was replicated three times and arranged in a completely randomized design. The size of each plot was 0.6 m × 0.6 m. The specific scheme of soil treatment is shown in **Figure 1**.

The CS return treatment was prepared by mixing 360 g of corn straw residues (equivalent to 1 kg m^{-2}) in the 0 – 20 cm
105 surface soil layer. Exactly 5.975 g CH_4N_2O was added to adjust the C/N ratio to 25:1 (suitable for soil microbial growth (Chapin III et al., 2011)). After that, the base fertilizer (17.68 g of CH_4N_2O and 7.92 g of KH_2PO_4) was applied to the 0 – 20 cm soil layer.

Preparation of FCS-T treatment was done by mixing 428 g (the same amount of C mass as the C of the CS material) of fermented corn straw treated with *T. reesei* material (equivalent to 1.189 kg m^{-2}) in the topsoil layer of 0–20 cm. The same
110 amount of base fertilizer as in the CS treatment (17.68 g of CH_4N_2O and 7.92 g of KH_2PO_4) was applied in the 0–20 cm depth of the FCS-T plots.

The blank control (CK) treatment was prepared by only mixing 17.68 g of CH_4N_2O and 7.92 g of KH_2PO_4 fertilizer to the 0–20 cm soil depth.

2.3.2 Soil sampling and analysis

115 Five topsoil samples (0 – 20 cm) were collected from each plot at 0 d, 30 d, 60 d, 90 d, 180 d, and 360 d using a stainless-steel soil auger (5 cm in diameter). For each soil sampling day, all visible corn straw materials in CS and FCS-T soils were picked out with tweezers and returned to their respective plots. The collected fresh soil was immediately divided into two sub-samples and passed through a 2 mm sieve. One subsample was then placed in a refrigerator (4 ∘C) to later analyze MBC in soil. The remaining subsample was air-dried to determine SOC, EOC, WEOC content, and humus composition.

120 **2.4 Analytical methods**

2.4.1 Labile soil organic carbon fractions

The SOC content was determined by the potassium dichromate oxidation method (Nelson and Sommers, 1982). The WEOC content was obtained by successively extracting 5 g of air-dried soil samples with distilled water in a 1:6 ratio of soil to water. The soil-solution mixture was shaken on a reciprocal shaker at 25 ∘C for 60 min, and then centrifuged at 4500 rpm for

20 min. The solution was filtered through a 0.45-μm filter membrane (Changtingny et al., 2010). The EOC content was determined using the $KMnO_4$ (333 mM) oxidation procedure (Lefroy et al., 1993). Fresh soil equivalent to 10 g of oven-dried soil was fumigated with $CHCl_3$ for 24 h, and the other 10 g of soil was not fumigated. Both fumigated and unfumigated soils were then extracted with 0.5 mol L^{-1} K_2SO_4. The MBC content was estimated from the increase in organic C in the 0.5 mol L^{-1} K_2SO_4 extracts of $CHCl_3$ fumigated soils as described by Vance et al. (1987). The soil WEOC and MBC contents were determined by a TOC analyser (Shimadzu TOC-VCPH, Japan). MBC was calculated as below Eq. (1):

$$MBC = \frac{F_c}{k_c} \tag{1}$$

where F_c is the difference between the amount of CO_2 released by fumigated and unfumigated soil (control) during the cultivation period; k_c is the conversion coefficient.

The carbon available ratio (CAR) of labile organic C contents (WEOC, EOC and MBC) were calculated as below Eqs. (2.3.4):

$$CAR_{(WEOC)} = \frac{WEOC \ (mg \ kg^{-1})/1000}{SOC \ (g \ kg^{-1})} \times 100\% \tag{2}$$

$$CAR_{(EOC)} = \frac{EOC \ (g \ kg^{-1})}{SOC \ (g \ kg^{-1})} \times 100\% \tag{3}$$

$$CAR_{(MBC)} = \frac{MBC \ (mg \ kg^{-1})/1000}{SOC \ (g \ kg^{-1})} \times 100\% \tag{4}$$

The carbon pool index (CPI), carbon pool activity (CPA), carbon pool activity index (CPAI) and carbon management pool index (CPMI) were calculated, according to Blair et al. (1995) and Jiang et al. (2021) as below Eq. (5):

$$CPI = \frac{SOC_{Treatment}}{SOC_{CK_0}} \tag{5}$$

where $SOC_{Treatment}$ represents the SOC content (g kg^{-1}) in soil of a given treatment (CS, FCS-T or CK), SOC_{CK_0} represents the SOC content (g kg^{-1}) in soil of CK at 0 d.

$$NLOC = SOC - EOC \tag{6}$$

NLOC represents the non-labile organic C content (g kg^{-1}), which is the difference between the SOC content and EOC content.

$$CPA = \frac{EOC}{NLOC} \tag{7}$$

$$CPAI = \frac{CPA_{Treatment}}{CPA_{CK_0}} \tag{8}$$

where $CPA_{Treatment}$ represents the CPA in soil of a given treatment (CS, FCS-T or CK), CPA_{CK_0} represents the CPA in soil of CK at 0 d.

$$CPMI = CPI \times CPAI \times 100 \tag{9}$$

2.4.2 Humus composition

Humus composition was sequentially analyzed following the International Humic Substances Society procedure (Kumada 1987) described in detail by Dou (2010). Briefly, 5 g of air-dried soil was extracted with a 30 mL mixture of 0.1 M alkali solution (NaOH + $Na_4P_2O_7$) under permanent shaking at 70 ∘C for 1 h and centrifuged. The remaining soil residue was humin, and the mixture, which is humus extract, was acidified with 0.5 M sulfuric acid to separate humic acid and fulvic acid. The carbon contents of the humus extract (HE-C), humic acid (HA-C) and humin (HM-C) were determined. Then the C content of fulvic acid (FA-C) was calculated as the difference between HE-C and HA-C. The humification degree (PQ) was calculated as HA-C/HE-C ratio (Sugahara and Inoko, 1981).

2.5 Statistical analysis

Microsoft Office Excel 2017 was used for data processing, and the statistical analysis was performed by SPSS Statistics 22.0 (IBM Statistics 21.0). Significant differences among treatment means were evaluated using the least significant difference test with TUKEYs adjustment at $P < 0.05$. Principal component analysis (PCA) was performed with Minitab 18 software (Pennsylvania, USA) to check for similarities between treatments. The graphs were compiled using the Origin 2019 software (OriginLab Corporation).

3 Results

3.1 Changes in SOC contents

At 0 d, the SOC content did not differ significantly between the three treatments but differed significantly from 30 d to 360 d among the three treatments (**Figure 2**). The FCS-T treatment showed significantly higher SOC content than all treatments, whereas the CK had significantly lower SOC content throughout the study period. The CS and FCS-T treatments showed the most significant increase in SOC content with the increase in the duration of the study. In contrast, SOC content in the CK treatment did not change significantly throughout the 360-day period. At the 360 d, the SOC content of CS and FCS-T was 12.71% and 18.81% higher than that of CK, respectively.

3.2 Changes in soil labile organic carbon fractions and carbon available ratios

In the 360-day field experiment, the WEOC, EOC, and MBC contents of CK, CS, and FCS-T treatments showed a similar changing trend (**Figure 3**). The content of these attributes increased from 0 d to 90 d, then gradually decreased to the 360th d in the CS and FCS-T treatments. Water extractable organic C, EOC, and MBC contents of CS and FCS-T treatments were highest at 90 d. The WEOC, EOC and MBC contents of CK appeared to decrease slightly with the duration of the experiment. Comparing all treatments, the contents of WEOC, EOC and MBC did not differ significantly at 0 d, 30 d, 180 d, and 360 d between CS and FCS-T treatments.

In terms of WEOC, the carbon available ratios of CS (1.19 %) and FCS-T (1.29 %) treatments was highest at 60 d, and lowest at 0 d (**Table 3**). Regarding EOC, the carbon available ratios of CS (9.25 %) and FCS-T (9.34 %) treatments was significantly higher at 90 d. With respect to MBC, the carbon available ratio of FCS-T (5.80 %) treatment was also higher at 90 d and that of CS (2.92 %) treatment was significantly higher at 60 d. Irrespective of sampling time, the carbon available ratios of WEOC, EOC, and MBC were always significantly higher under FCS-T and CS treatments compared with CK. These parameters did not always differ significantly between the CS and FCS-T treatments.

3.3 Soil carbon pool management index

The soil carbon pool management index was computed at the end of the experiment (i.e., day 360). At the 360th day, the CS and FCS-T treatments significantly increased the carbon pool management index and carbon pool activity index compared with the CK treatment. However, the carbon pool activity index of CS and FCS-T treatments did not differ significantly (**Table 4**). Applying CS and FCS-T significantly increased the carbon pool management index compared with CK, increasing the CPMI by 17.3 % and 31.7 %, respectively.

3.4 Humus composition and C content in soil under different treatments

At 0 d of the experiment, there was no significant difference in the relative content (**Table 5**) and composition of humus C among the three treatments (**Figure 4)**. With the application of CS and FCS-T, the HE-C and HM-C contents in the soil increased with the duration of the experiment. Compared with CK, the CS and FCS-T treatments increased HE-C content in the soil. At 360 d, the HE-C content of the FCS-T treatment was significantly higher than that of the CS treatment, and the HE-C of the FCS-T and CS treatments increased by 1.99 g kg^{-1} and 1.31 g kg^{-1}, respectively, when compared with that at 0 d. The HM-C content of the FCS-T treatment increased significantly when compared with other treatments throughout the experiment, with a cumulative increase of 0.79 g kg^{-1} at 360 d (**Figure 4**). Throughout the experiment, no significant difference was observed between CS and CK treatments with respect to HM-C content.

Compared with CK, the application of CS and FCS-T increased the FA-C content in the soil throughout the experiment (**Figure 5**). The highest FA-C content in the CS and FCS-T treatments was measured at 180 d, and the lowest FA-C content was recorded at 0 d. The content of HA-C under CS and FCS-T treatments increased with the duration of the experiment. The highest HA-C content in the CS and FCS-T treatments was measured at 360 d, and the lowest HA-C content was recorded at 0 d. The content of HA-C in the FCS-T treatment at 360 d was 0.77 g kg^{-1} higher than in the CS treatment.

3.5 Multivariate Analysis

The relationship between SOC parameters and humus components, shown according to PCA (**Figure 6**), was well confirmed by Pearson's correlation analysis (**Figure 7**). Figure 6 indicated that under all the treatments, the HA-C, HM-C, and EOC contents exhibited significant correlations with SOC content, carbon pool activity index, and carbon pool management index, whereas WEOC and MBC contents were significantly correlated with the FA-C content. The PCA clearly separated the three

treatments, implying that each treatment had a distinct influence on SOC content, CPMI, and humus component characteristics. The correlation between SOC and carbon pool management index was more pronounced under the CS and FCS-T treatments. The correlation between MBC and SOC was stronger under the CS treatment, and the correlations between WEOC, MBC, and FA-C were more pronounced under the FCS-T treatment.

4 Discussion

4.1 Effects of different treatments on SOC, soil labile organic carbon fractions, and humus fractions

A large number of studies have shown that the application of organic materials is beneficial to the accrual of SOC (Ros et al., 2006; Zhang et al., 2015b) and the distribution of labile organic C components (Blair, 2000; Chen et al., 2009; Sainepo et al., 2018). This is consistent with the results of our study, which showed that the application of CS and FCS-T increased SOC content (**Figure 2**), MBC, WEOC, and EOC contents (**Figure 3**). Although applied under equal C mass input, the FCS-T treatment sequesters more organic C in the soil than the CS treatment. This may be because the FCS-T used in the present study was produced by fermentation with *T. reesei* and had a lower C/N ratio (**Table 2**). Studies show that part of the organic matter input is converted into CO_2 and other substances during fermentation. The remaining residue is converted into the stable organic matter like humic substances (Atiyeh et al., 2002; Romero et al., 2007). The closer the substrate's C/N ratio is to the microorganisms' C/N ratio, the more significant the fraction of substrate C that remains in the soil (Hessen et al., 2004). Furthermore, according to Sprunger et al. (2019), a low C/N ratio of organic residues promotes the accumulation of SOM. Whereas organic inputs applied to the soil with a large C/N ratio (such as the CS treatment in the case of our study), may lose more C in turnover compared with organic amendments with a small C/N ratio (Dannehl et al., 2017). The C/N ratio of organic amendments and the C fate in the soil had a negative connection (Dannehl et al., 2017), which is consistent with the results of our study.

Our results further showed that after FCS-T and CS application, the concentrations of WEOC, EOC, and MBC in the soil increased at the initial stages of the experiments (i.e., 0 – 90 d) and then gradually decreased towards the end of the experiment (**Figure 3**). In contrast, the HE-C and HM-C contents appeared to increase with the duration of the experiment, with a greater increase reported in the FCS-T treatment (**Figure 4** and **5**). This result is consistent with the findings of Guan et al. (2015). The reason for this phenomenon may be because the WEOC and EOC are easily and are the first organic compounds utilized by soil microorganisms (Haynes et al., 2005). Corn straw contains aromatic C compounds (Roldán et al., 2011; Zhang et al., 2020), which are more difficult to decompose and tend to accumulate as humic substances (Kuzyakov et al., 2009; Pan et al., 2016; Dou et al., 2020).

Compared with other treatments, the FCS-T treatment appeared to have a significantly higher WEOC content, but the EOC content did not consistently differ significantly between the FCS-T and CS treatments during the duration of the experiment (**Figure 3**). Ma et al. (2021) reported similar findings where the WEOC content in the soil amended with barley treated with microbial inoculant was significantly higher than that of barley residue without microbial inoculant, but the EOC content

differed seldomly. The higher EOC content in FCS-T treatment than in CS treatment (**Table 3**) suggests that organic matter after microbial treatment is likely converted into EOC. During the entire duration of the present experiment, the MBC content of FCS-T treatment was also higher than that of CS treatment, but not always significant, which is consistent with Ng et al. (2016). This may be because crop residues treated with microbial inoculants are easily assimilated by soil microorganisms (Gaind and Nain, 2006; Vargas-Garcia et al., 2006; Pan et al., 2016), thereby promoting the sequestration of organic C in organic materials.

The WEOC and EOC of the soil largely depend on the SOC content (Guan et al., 2018). This was also confirmed by the results from the present study, which showed SOC content to be positively correlated with WEOC, EOC, and MBC contents (**Figure 7**). This means the WEOC, EOC, and MBC can be used as the best proxies to detect changes in SOC content, since these fractions respond promptly to changes in soil management practices. In the present study, the correlation of SOC content with MBC and EOC was more pronounced under FCS-T treatment than CS and CK treatments. This may be likely due to differences in the chemical composition of these treatments. Vanlauwe et al. (2005) and Mandal et al. (2007) found that changes in soil C are mainly influenced by the chemical composition of the applied organic matter.

Compared with other treatments, the FCS-T treatment significantly increased the content of HA-C and FA-C throughout the 360 d period (**Figure 5**). This is because the FCS-T material contains relatively higher alkyl, aromatic C contents, and humic-like substances (see Zhang et al., 2021); compounds resistant to direct microbial degradation. Accumulating microbial-resistant compounds promotes the formation of soil humic substances during the humification process (Huang et al., 2008; Roldán et al., 2011). During the humification process, parts of the humic-like substances in the FCS-T treatment gradually formed new FA and HA in the soil, possibly through microbial *ex vivo* modification and *in vivo* turnover (Liang et al., 2017). Zhang et al. (2019) further found that fermented corn straw was more conducive to the increase of HA-C, and the CS treatment was more conducive to the increase of FA-C when applied to the soil. In other studies, Gaind and Mathur (2001) and Gaind and Nain (2007) reported a significant increase in humus C content in the soil amended with wheat straw compost treated with *T. reesei*. Analyzing the changes of humic substances components in different periods in our study, we found that the FCS-T treatment significantly and continuously increased FA relative content for up to 180 days, while the increase in the HA relative content lasted up to 360 days (**Table 5**). Therefore, the application of FCS-T materials is more conducive to increasing humus C content and HA relative content (including more aromatic C compounds), which is important for the long-term storage of SOC.

4.2. Relationships between SOC, soil labile organic carbon fractions, humus components, and CPMI

The results of this study showed that the increase in SOC content was mainly due to the increase in EOC, HA-C, and HM-C contents, rather than the accumulation of WEOC and MBC. The possible explanation is that WEOC and MBC are more easily utilized by soil microorganisms, and their ratio in SOC is much lower (Blair, 2000; Haynes, 2005). The carbon pool management index is a comprehensive index to evaluate SOC variation rates in response to soil management practices. For instance, a high carbon pool management index indicates that the soil management practices have a stronger potential to

promote soil C sequestration (Blair et al., 1995). In the present study, the FCS-T treatment showed a significantly higher carbon pool management index and carbon pool index than CS and CK treatments (**Table 4**). This suggests that the FCS-T treatment was more conducive to the accumulation of organic C in the soil. This result may be due to the fact that soil C accumulation is mainly driven by increased plant residue input, which increases SOC content. The MBC was positively correlated with FA-C and SOC (**Figure 6** and **7**), providing evidence that the activity of microorganisms affects the accumulation of FA in the soil, thereby promoting the increase of SOC.

5 Conclusion

In this 360-day field experiment, we applied corn straw (CS) and fermented corn straw treated with *Trichoderma reesei* (FCS-T) under equal C input, and a blank control treatment (CK) for comparison. The following conclusions were drawn:

1. The change of SOC content mainly depends on the C content of the stable soil components, i.e., aromatic compounds (HA-C and HM-C). The FCS-T material has a lower C/N ratio, higher alkyl and aromatic C content, and humic-like substances. When the FCS-T material is applied to the soil, it is more advantageous in promoting the soil humification process and increasing soil HA-C and HM-C content. Compared with direct corn straw application (i.e., CS treatment), the FCS-T treatment increased the SOC content by 1.715 g kg^{-1} on the 360th day, and increased the PQ value to 74.1%. The application of FCS-T material with a lower C/N ratio sequestered more SOC than the application of CS, which supported the idea that the C/N ratio in the organic amendments is negatively correlated with SOC content.

2. The application of FCS-T significantly improves the release of soil labile carbon components. In particular, the WEOC content could maintain a high level for a long time, while the EOC and MBC contents of FCS-T treatment increased significantly more than CS treatment on the 60th and 90th days.

3. Compared to CS treatment, the FCS-T treatment significantly improved the level of carbon pool management to 13.95, primarily by promoting the simultaneous increase in the contents of EOC and stable organic carbon components (HA-C and HM-C).

The results confirmed our initial hypothesis that the application of FCS-T has a greater potential to increase soil carbon sequestration compared with direct application of CS. As a method of returning straw residues to the field, the application of FCS-T is a practice worthy of further exploration.

Data availability

The data generated in this study are available from the corresponding authors upon reasonable request.

Author Contribution

305 Yifeng Zhang: Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation; Sen Dou: Formal analysis, Funding acquisition, Supervision; Batande Sinovuyo Ndzelu: Validation, Writing-review & editing; Rui Ma: Supervision; Dandan Zhang: Data curation; Xiaowei Zhang: Supervision; Shufen Ye: Data curation; Hongrui Wang: Data curation.

Competing interests

310 The contact author has declared that neither they nor their co-authors have any competing interests.

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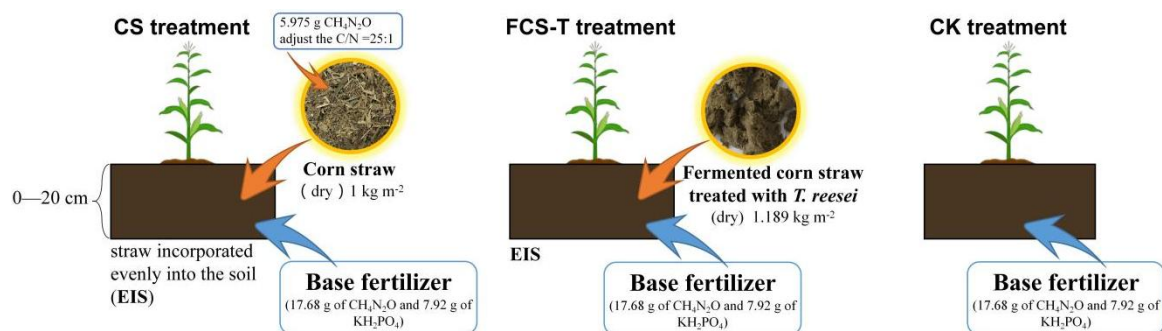
Figure 1: Schematic diagram of three different treatment methods in the field

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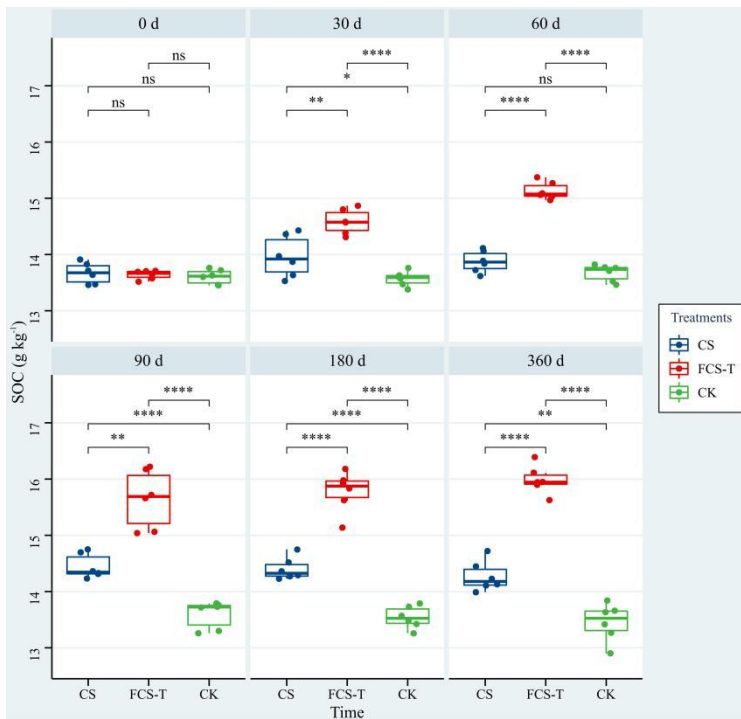
Figure 2: Soil organic carbon content of the three different treatments during the 360-d experimental period.
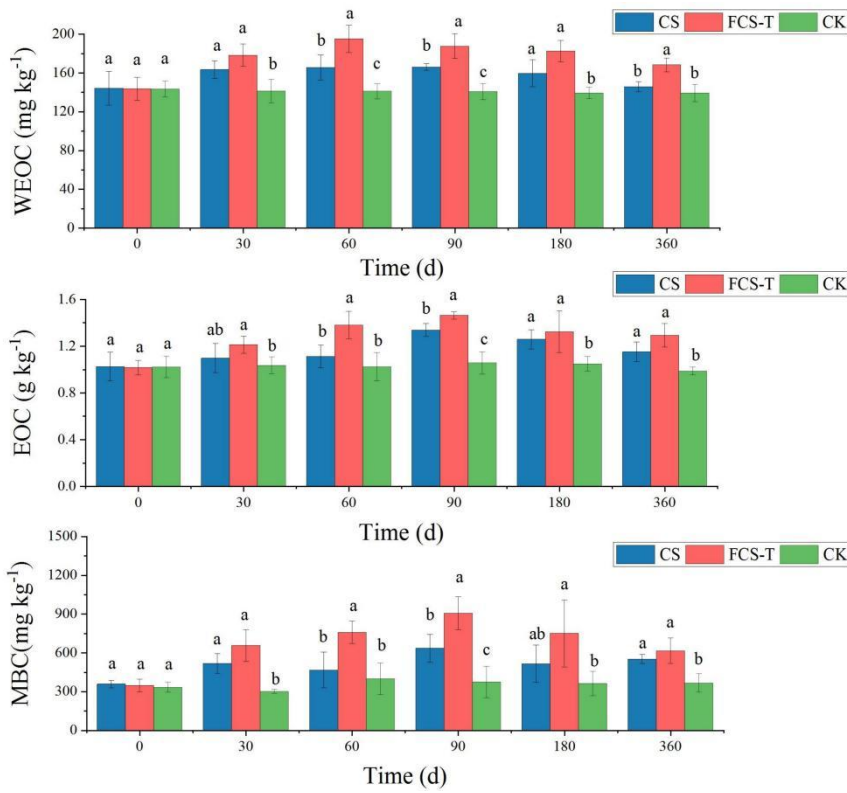
Figure 3: Effects of corn straw returned (CS), fermented corn straw treated with *T. reesei* returned to the field (FCS-T) and non-straw amended soil (CK) on soil labile organic carbon (WEOC, EOC and MBC) concentrations in the 0–20 cm soil depth.

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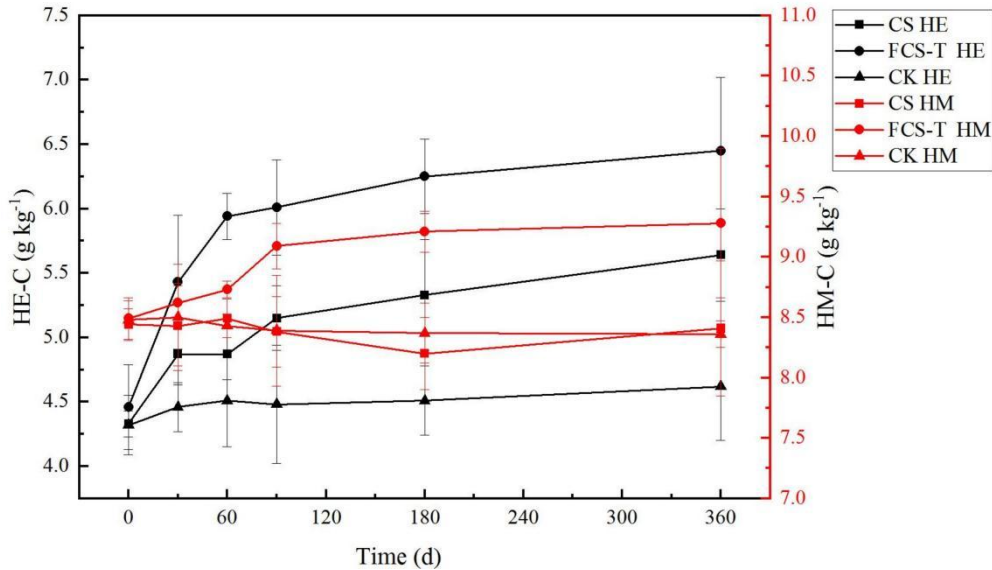


Figure 4: The carbon content of humus extracted (HE-C) and humin (HM-C) of the three different treatments during the 360-day period.

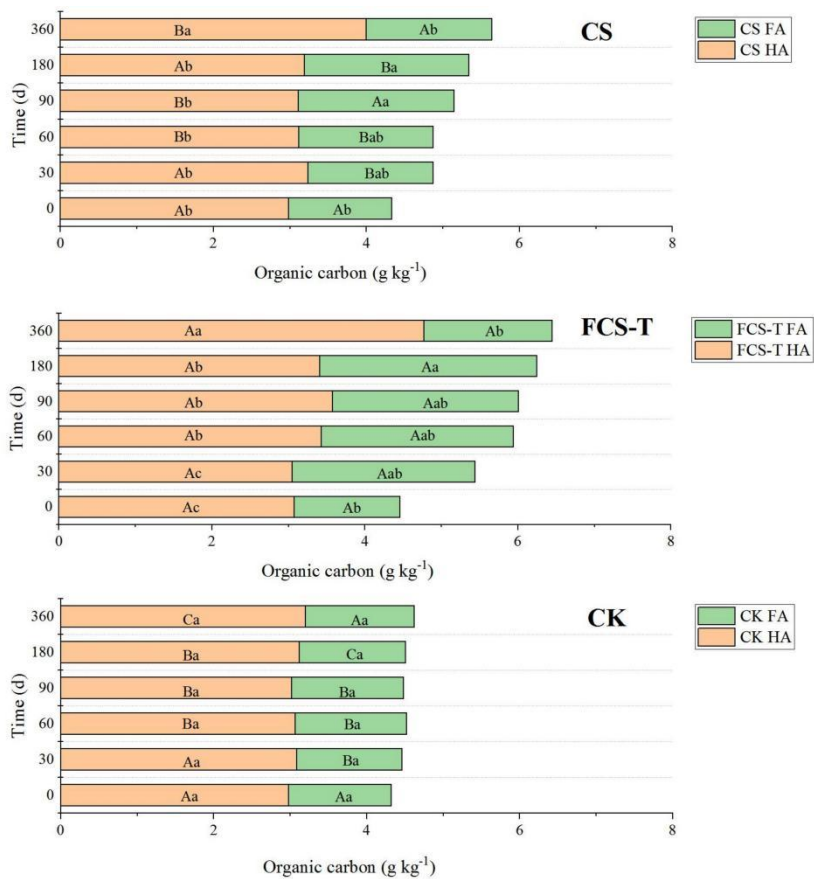



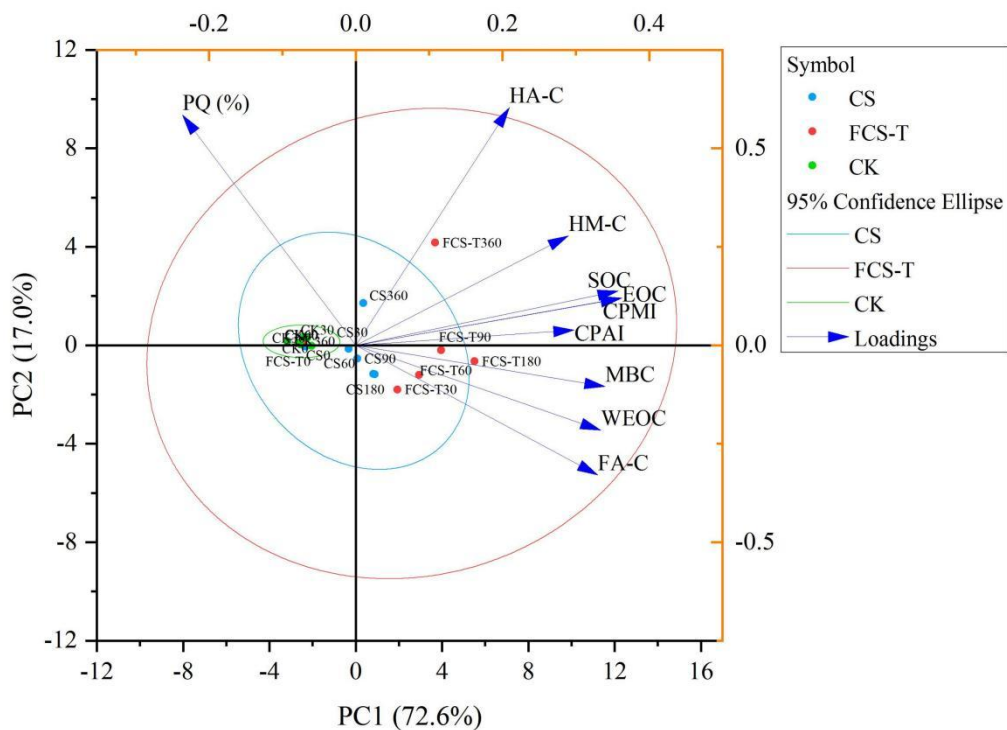
Figure 5: The carbon contents of humic acid (HA) and fulvic acid (FA) isolated from different treatments.

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Figure 6: Principal component analysis (PCA) of soil organic carbon parameters and humus components affected by different treatments during 360 d of the experiment.
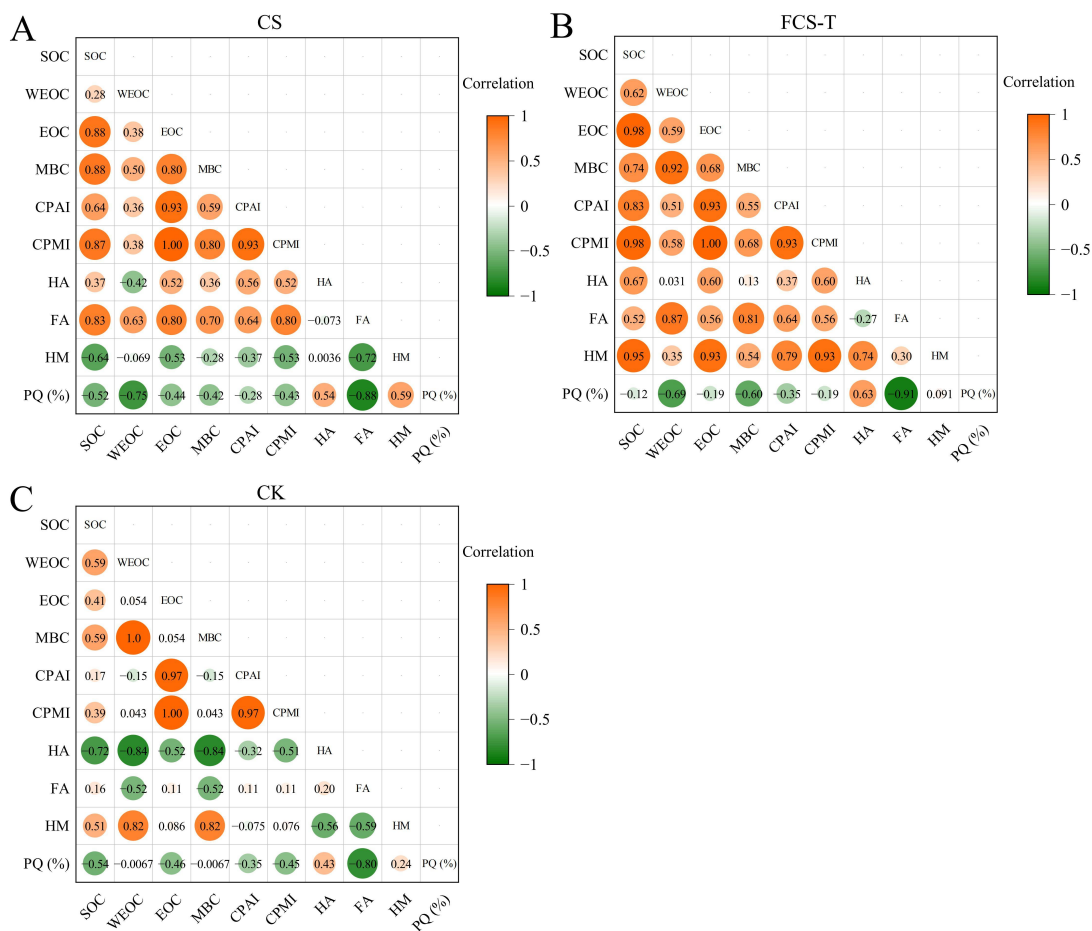
Figure 7: The heatmap of Pearson's correlations among soil parameters from different treatments soil during the 360 d of the experiment. (A, CS; B, FCS-T; C, CK).

Figure captions

Figure 1. Schematic diagram of three different treatment methods in the field.

Figure 2. Soil organic carbon content of the three different treatments during the 360-d experimental period. The upper, middle, and lower horizontal lines of the box represent the upper quartile, the median, and the lower quartile, respectively. The values represented by the upper- and lower-line segments refer to the maximum and minimum values of the data, and the points outside the box represent outliers. The symbol on the figure indicates the P value between two variables. The number of "*" indicates the degree of significance. For example: "*" means $P < 0.05$, "**" means $P < 0.01$, "***" means $P < 0.001$, "****" means $P < 0.0001$,and "ns" means no significance. CS, corn straw returned to the field; FCS-T, fermented corn straw treated with *T. reesei* returned to the field; CK, blank control treatment.

510 **Figure 3.** Effects of corn straw returned (CS), fermented corn straw treated with *T. reesei* returned (FCS-T) and non-straw amended soil (CK) on soil labile organic carbon (WEOC, EOC and MBC) concentrations in the 0−20 cm soil depth. Each bar represents the mean ± standard deviation in the figure (n=3). Different lowercase letters within the same time indicate significant differences among different treatments at $P < 0.05$ level.

Figure 4. The carbon content of humus extracted (HE-C) and humin (HM-C) of the three different treatments during the

515 360-day period. Error bar is the standard deviations of triplicate averages. CS, corn straw returned to the field; FCS-T, fermented corn straw treated with *T. reesei* returned to the field; CK, blank control treatment.

Figure 5. The carbon contents of humic acid (HA) and fulvic (FA) isolated from different treatments. Each bar represents the mean of HA and FA in the figure (n=3). Different uppercase letters mean significant difference ($P < 0.05$) for either HA or FA among the treatments in a particular time (d) of the experiment. Different lowercase letters mean significant difference

520 ($P < 0.05$) for either HA or FA among different experimental time (d) for a given treatment.

Figure 6. Principal component analysis (PCA) of soil organic carbon parameters and humus components affected by different treatments during 360 d of the experiment. PC, principal component; SOC, soil organic carbon; CPMI, carbon pool management index; CPAI, carbon pool activity index; WEOC, water extractable organic carbon; EOC, easily oxidizable organic carbon; MBC, microbial biomass carbon; HA, humic acid; FA, fulvic acid; HM, humin; PQ, humification degree.

525 CS, corn straw returned to the field; FCS-T, fermented corn straw treated with *T. reesei* returned to the field; CK, blank control treatment.

Figure 7. The heatmap of Pearson's correlations among soil parameters from different treatments soil during the 360 d of the experiment. (A, CS; B, FCS-T; C, CK).

Table captions

530 **Table 1.** Basic properties of the soil in field experiments.

Table 2. Elemental composition of materials used in field experiments.

Table 3. The carbon available ratio (CAR) of water extractable organic carbon (WEOC), easily oxidizable organic carbon (EOC), and microbial biomass carbon (MBC) under different treatments in the 0-360 d period.

Table 4. The carbon management indices under different treatments during the 360-d period.

535 **Table 5.** Changes of relative content of each humic substances component and the PQ values in different treatments during the 0-360 d period.

Table 1. Basic properties of the soil in field experiments.

Soil	pH	Organic matter (g kg^{-1})	Alkaline N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
Black soil	6.55±0.31	51.18±1.41	7.44±0.57	565.0±2.3	59.00±0.85

Note: Values (± standard deviation) were averaged over 3 replicates.

Table 2. Elemental composition of materials used in field experiments.

Materials	C (g kg^{-1})	H (g kg^{-1})	N (g kg^{-1})	O (g kg^{-1})	C/N
CS	376.4±1.0	51.18±0.33	7.44±0.03	565.0±0.8	50.57±0.08
FCS-T	319.4±1.4	43.87±0.25	29.50±0.12	607.2±1.5	10.83±0.09

Note: Values (± standard deviation) were averaged over 3 replicates. CS, corn straw; FCS-T, fermented corn straw treated with *T. reesei*.

Table 3. The carbon available ratio (CAR) of water extractable organic carbon (WEOC), easily oxidizable organic carbon (EOC), and microbial biomass carbon (MBC) under different treatments in the 0-360 d period.

CAR (%)	WEOC			EOC			MBC		
Time (d)	CS	FCS-T	CK	CS	FCS-T	CK	CS	FCS-T	CK
0	1.06a	1.05a	1.06a	7.50a	7.45a	7.51a	2.63a	2.55a	2.45a
30	1.17a	1.22a	1.04b	7.88b	8.31a	7.62b	3.73b	4.51a	2.22c
60	1.19b	1.29a	1.03c	8.02b	9.11a	7.48c	3.38b	5.01a	2.92c
90	1.15b	1.20a	1.04c	9.25a	9.34a	7.78b	4.40b	5.80a	2.76c
180	1.11a	1.15a	1.03b	8.76a	8.31b	7.75c	3.60b	4.72a	2.68c
360	1.02b	1.05a	1.04a	8.06a	8.08a	7.34b	3.88a	3.86a	2.73b

Note: Values are means that do not share the same letter for a given parameter and time (d) of experiment are significantly different ($P < 0.05$). CS, corn straw returned to the field; FCS-T, fermented corn straw treated with *T. reesei* returned to the field; CK, blank control treatment.

Table 4. The carbon management indices under different treatments during the 360-d period.

Indexes	Treatments	values
CPI	CS	1.049±0.029b
	FCS-T	1.175±0.028a
	CK	0.989±0.036c
CPA	CS	0.088±0.006a
	FCS-T	0.088±0.005a
	CK	0.079±0.004a
CPAI	CS	1.080±0.068a
	FCS-T	1.083±0.065a
	CK	0.977±0.054b
CPMI	CS	113.20±4.48b
	FCS-T	127.15±5.57a
	CK	96.51±3.47c

Note: Mean values ± SE that do not share the same letter within a column of indexes are significantly different ($P <$ 0.05). CS, corn straw returned to the field; FCS-T, fermented corn straw treated with *T. reesei* returned to the field; CK, blank control treatment. CPI, carbon pool index; CPA, arbon pool activity index; CPAI, carbon pool activity index; carbon pool management index.

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560 **Table 5.** Changes of relative content of each humic substances component and the PQ values in different treatments during the 0-360 d period.

Time (d)	Treatments	HA (%)	FA (%)	HM (%)	PQ (%)
0	CS	21.8±1.5a	9.9±1.1a	61.8±1.3a	68.9±3.7a
	FCS-T	22.6±0.9a	10.3±2.1a	62.3±1.3a	69.2±4.5a
	CK	21.9±0.3a	9.9±2.2a	62.3±1.5a	69.1±4.9a
30	CS	23.3±1.2a	11.7±1.9b	60.6±1.4a	66.7±4.7a
	FCS-T	20.9±0.8b	16.3±2.4a	59.1±1.8a	56.2±3.1b
	CK	22.8±0.9a	10.1±1.5b	62.6±4.1a	69.3±4.7a
60	CS	22.5±0.6a	12.6±1.6b	61.2±0.1a	64.2±3.6a
	FCS-T	22.7±0.6a	16.6±1.8a	57.7±1.0b	57.8±3.3b
	CK	22.4±0.8a	10.6±1.7b	61.7±1.2a	68.1±2.8a
90	CS	21.5±1.4a	14.1±2.3ab	58.0±1.6a	60.55±4.8b
	FCS-T	22.8±1.6a	15.5±1.8a	58.1±1.0a	59.54±4.4b
	CK	22.3±1.8a	10.8±1.4b	61.8±4.4a	67.42±1.0a
180	CS	22.2±0.7a	14.9±1.6b	57.1±2.5b	59.8± 2.4b
	FCS-T	21.4±1.6a	17.9±1.8a	57.9±1.4b	54.6± 3.1b
	CK	23.0±0.9a	10.3±0.5c	61.9±1.9a	69.2± 0.8a
360	CS	28.0±1.9a	11.5±1.3a	58.9±2.6b	71.0±1.4ab
	FCS-T	29.9±2.2a	10.5±1.7a	58.0±3.3b	74.1±1.8a
	CK	23.8±1.2b	10.6±1.1a	62.2±1.9a	69.2±1.7b

Note: Values are means ± SE. Means that do not share the same letter within a column of a given parameter and time (d) of the experiment are significantly different ($P < 0.05$). HA, humic acid; FA, fulvic acid; HM, humin; CS, corn straw returned to the field; FCS-T, fermented corn straw treated with *T. reesei* returned to the field; CK, blank control
565 treatment.