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

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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 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 higher than in the CS treatment. Application of FCS-T appeared to promote the significant increase of SOC, carbon pool activity index (CPAI) and carbon pool management index (CPMI) through accumulation of HA-C, humin carbon (HM-C), and easily oxidizable organic carbon (EOC) contents. Application of fermented corn straw treated with *Trichoderma reesei* (FCS-T) is more valuable and conducive to increasing soil EOC and humus C content than direct application of corn straw.

1 Introduction

Recycling and returning crop residues as soil amendments has proven to be an important prospect for increasing soil carbon (C) content and increasing crop yield (Villamil et al., 2015) and for managing crop straw residues. Ma et al (2019) found that soils amended with wheat straw and with wheat straw-decomposing microbial inoculants had average annual soil organic carbon (SOC) sequestration rates of 0.77 and 1.67 t C ha^{-1} yr^{-1} higher than those of no straw amended soils in the 0 – 20 cm depth, respectively. However, how plant residues are converted into stable SOC is still not fully understood (Cotrufo et al., 2013; Lehmann and Kleber, 2015; Zhang et al., 2015a). Information about the stability of C is needed for long-term soil C sequestration (Cotrufo et al., 2013; Ndzelu et al., 2020a) and for reducing carbon dioxide (CO_2) emissions into the atmosphere (Chatterjee, 2013; Guan et al., 2018).

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Soil organic carbon is considered a good indicator of soil quality, but a suite of labile organic C components, such as water extractable organic carbon (WEOC), easily oxidizable organic carbon (EOC), and microbial biomass carbon (MBC) are effectively used to detect small changes in soil quality (Blair et al., 1995; Chen et al., 2009; Sainepo et al., 2018). This is because these labile organic C compounds are sensitive and promptly respond to changes in soil management practices (Blair et al., 1995; Xu et al., 2011), and they are also essential for the formation of the more stable SOC (Cotrufo et al., 2013). The labile SOC fractions are reported to be significantly affected by the application of organic amendments. Recently, Ma et al. (2021) showed that soils amended with wheat straw or with wheat straw-decomposing microbial inoculants had higher WEOC, MBC and SOC contents than non-amended soils. In another study, Ndzelu et al. (2020b) also found that the application of corn straw 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 provide information about the formation of SOC. Another important index to monitor the effects of agricultural management practices on soil C sequestration is the Carbon Pool Management Index (CPMI) (Tang et al., 2018; Ma et al., 2021). This index has been widely used as a sensitive tool for calculating changes in soil C content (Blair et al., 1995).

Humic substances (HS) constitute the main component of soil organic matter (SOM), representing the most stable fraction of organic matter in soils (Stevenson 1994; Santos et al., 2010; Olk et al., 2019). The HS are composed of humic acid (HA), fulvic acid (FA) and humin (HM) (Stevenson 1994), which are different from the chemical composition and structure of their precursors (Dou et al., 2020). Although the existence and validity of HS in soils have been questioned recently (Kleber and Lehnman, 2015), the HS contributes to the largest proportion to soil organic matter (Olk et al., 2019; Dou et al., 2020). As a result, paying attention to the changes in humus components and labile organic C fractions of the soil after corn straw application, could inform about formation and stabilization of SOC during litter decay. Recently, researchers have found that soil humus composition and structure are influenced by crop straw management strategies (Fan et al., 2018; Zhang et al., 2020a). Therefore, assessing the transformation of corn straw residues into SOC can be important in managing crop straw residues and agricultural lands.

In our previous studies (Yang et al., 2019; Zhang et al., 2020b; 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 (HAL) substances during corn straw decomposition when compared with other fungi (*Phanerochaete chrysosporium* and *Trichoderma harzianum*). Gaind and Nain (2006) also found that incorporation of paddy straw and *T. reesei* into the soil increased soil C and humus content, which is due to the increase in the alkyl and aromatic C contents (Zhang et al., 2021). The objective of this study was to verify whether *T. reesei* can equally be effective in field trials to form relatively stable SOC fractions after corn straw application. We assumed 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; (2) application of FCS-T may also increase soil labile organic C components (WEOC, EOC, and MBC); and (3) application of FCS-T may also increase CPMI level more than direct corn straw application. These assumptions are based on that *T. reesei* inoculant has strong humification ability compared with direct application of corn straw.

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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 (43°49'5" N, 125° 24' 8" E). 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. 1999). 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 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 as described by Zhang et al (2020b). 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

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), which were 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.

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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 surface soil layer, and exactly 5.975 g CH_4N_2O was added to adjust the C/N (mol) ratio to 25:1 (which is suitable for soil microbial growth (Chapin III et al., 2011)). Thereafter, 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 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

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.

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 (Liang et al., 1998). The EOC content was determined using the KMnO4 (333 mM) oxidation procedure (Lefroy et al., 1993). The MBC content was extracted with 0.5 mol/L potassium sulfate based on the method 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):

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

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 (0.45).

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

$$\mathrm{CAR}_{(\mathrm{WEOC})} = \frac{\mathrm{WEOC\ (mg/kg\ )/1000}}{\mathrm{SOC\ (g/kg)}} \times 100\% \tag{2}$$

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$$CAR_{(EOC)} = \frac{EOC\ (g/kg)}{SOC\ (g/kg)} \times 100\% \quad (3)$$

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

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

$$CPI = \frac{SOC_{Treatment}}{SOC_{CK_0}} \quad (5)$$

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

$$NLOC = SOC - EOC \quad (6)$$

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

$$CPA = \frac{EOC}{NLOC} \quad (7)$$

$$CPAI = \frac{CPA_{Treatment}}{CPA_{CK_0}} \quad (8)$$

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. $CPMI = CPI \times CPAI \times 100$

$$CPMI = CPI \times CPAI \times 100 \quad (9)$$

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 (HM), and the mixture, which is humus extract (HE), was acidified with 0.5 M sulfuric acid to separate HA and fulvic acid (FA). The C contents of the humus extract (HE-C), HA-C and humin (HM-C) were determined. Then the C content of FA (FA-C) was calculated as the difference between HE-C and HA-C. The PQ = HA-C/(HE-C) was used to calculate the humification degree (PQ) (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).

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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**). Comparing all treatments, the FCS-T treatment showed significantly higher SOC content, whereas the CK had significantly lower SOC content throughout the study period. The CS and FCS-T treatments showed the largest increase in SOC content with the increase in the duration of the study. Whereas, 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 compared with that of CK, respectively.

3.2 Changes in soil labile organic carbon fractions and CAR

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 firstly increased from 0 d to the 90 d, and then gradually decreased to the 360 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 slightly decrease 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 CAR of CS (1.19%) and FCS-T (1.29%) treatments was highest at 60 d, and lowest at 0 d (**Table 3**). In terms of EOC, the CAR of CS (9.25%) and FCS-T (9.34%) treatments was significantly higher at 90 d. With respect to MBC, the CAR 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 CAR of WEOC, EOC and MBC was 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 CPMI

The soil CPMI 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 CPI and CPAI compared with the CK treatment, but the CPAI of CS and FCS-T treatments did not differ significantly (**Table 4**). Applying CS and FCS-T significantly increased the CPMI 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 the HE-C content

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in 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 and 1.31 g/kg, respectively, when compared with that at 0 d. The HM-C content of the FCS-T treatment increased significantly when compared with other treatments over the duration of the experiment, with a cumulative increase of 0.79 g/kg at 360 d (**Figure 4**). Throughout the duration of the experiment, there was no significant difference observed between CS and CK treatments with respect to HM-C content.

Compared with CK, application of CS and FCS-T increased the FA-C content in the soil with the duration of 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 higher than that 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, CPAI, and CPMI, whereas WEOC and MBC contents were significantly correlated with the FA-C content. The PCA clearly separated the three treatments, which implies that each treatment had a distinct influence on SOC content, CPMI, and humus component characteristics. The correlation between SOC and CPMI 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 and soil labile organic carbon fractions

A large number of studies has shown that application of organic materials is beneficial to the accrual of SOC (Ros et al., 2006; Zhang et al., 2015b; Lin et al., 2020) and 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 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 appeared to sequester 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 composting with *T. reesei*. During the composting process, studies show that part of the organic matter input is converted into carbon dioxide and other substances, and the remaining residue is converted into stable organic matter similar to HS (Atiyeh et al., 2002; Romero et al., 2007).

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

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experiment (**Figure 3**). In contrast, the HE-C and HM-C contents appeared to increase with the duration of the experiment, with the 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 that the WEOC and EOC are easily and the first organic compounds to be utilized by soil microorganisms (Haynes et al., 2005). Corn straw contains aromatic C compounds (Roldán et al., 2011; Zhang et al., 2020b), which are more difficult to decompose and tend to accumulate as HS (Kuzyakov et al., 2009; Pan et al., 2016; Dou et al., 2020).

Comparing all treatments, the FCS-T treatment appeared to have significantly higher WEOC content than CS, but the EOC content did not always differ significantly between the FCS-T and CS treatments during the duration of the experiment (**Figure 3**). Ma et al. (2021) reported similar findings with barely treated with microbial inoculant, in which the WEOC content was significantly higher than that of barely residue without microbial inoculant, but the EOC content differed seldomly. The higher EOC content in FCS-T treatment than that 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. Ng et al. (2016) reported similar observations, and this may be due to the fact that crop residues treated with microbial inoculants are easily assimilated by soil microorganisms (Gaind and Nain, 2006; Vargas-Garcia et al., 2007; 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, 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 is mainly influenced by the chemical composition of the applied organic matter.

4.2 Effects of different treatments on humus composition

The application of different organic materials has varying degrees of influence on the HS composition of the soil (Table 5). (**Table 5**). In the present study, the contents of HA-C and FA-C in the soil amended with CS and FCS-T materials were significantly higher than that of CK treatment, throughout the 360 d period (**Figure 5**). This is probably because both CS and FCS-T materials contain alkyl and aromatic C contents, and composted FCS-T contain relatively higher aromatic C contents and humic-like substances (see Zhang et al., 2021). The aromatic compounds are resistant to decomposition and gradually promote the formation of soil HS during the process of humification (Roldán et al., 2011; Puttaso et al., 2013). The increase in HA-C and FA-C after amending soils with crop residues is commonly reported in other studies (Dou et al., 2008; Zhang et al., 2020a). Zhang et al. (2020a) further found that application of fermented corn straw (i.e., FCS-T treatment in the present study) was more conducive to the increase of HA-C, and CS was more conducive to the increase of FA-C. In other studies,

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Gaint and Mathur (2001) and Gaind and Nain (2006) also found that treating crop straw compost with *T. reesei* significantly increased soil humus content. Our results suggest that application of FCS-T materials is more conducive to increasing humus C content, which is important for long-term storage of SOC.

4.3. 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 CPMI is a comprehensive index to evaluate SOC variation rates in response to soil management practices. For instance, a high CPMI 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 significantly higher CPMI and CPI 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 (Zhao et al., 2018). 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 FCS-T treatment was more effective than CS treatment in terms of soil carbon storage. The SOC content of CS and FCS-T treatments increased by 12.71% and 18.81%, respectively, when compared with CK at the 360-d. Contrasted with direct application of CS, the FCS-T treatment increased SOC reserve by 1.715 g/kg.
2. In terms of soil humus content and humification, FCS-T treatment was more effective than CS and CK treatment in accelerating the accumulation of HA-C and FA-C content in the soil. The relative content of HA-C in the FCS-T treatment was 1.9% higher than that in CS treatment at 360 days, and the PQ value of FCS-T increased to 74.1%. The FCS-T treatment was more conducive to the accumulation of stable HM-C component in the soil, with a cumulative increase of 0.79 g/kg at day 360.
3. The application of FCS-T in the soil resulted in an increase in the labile organic carbon fractions and carbon pool management index (CPMI), which was more pronounced on the 60th and 90th days. Amongst all labile soil organic carbon fractions, the FCS-T treatment is more conducive with the increase of WEOC content. The CS and FCS-T treatments had similar effects on the carbon available ratio of the soil labile organic carbon fractions. The FCS-T treatment was more advantageous with increasing the content of HA-C, HM-C and WEOC, which resulted to the overall increase in SOC and EOC, as well as the CPMI.

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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.

Author Contribution

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.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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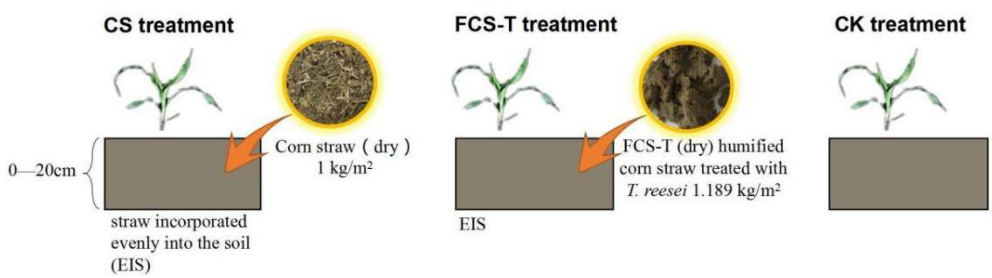

Figure 1: Schematic diagram of three different treatment methods in the field

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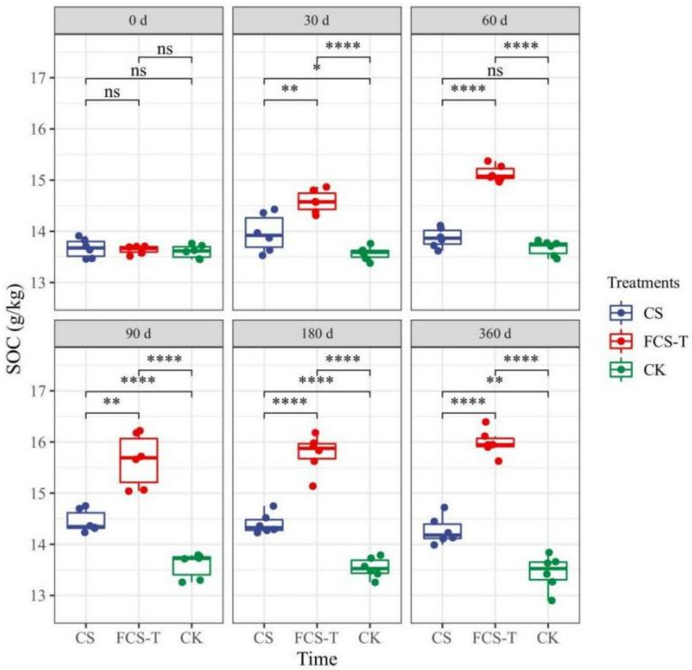

435 **Figure 2: Soil organic carbon content of the three different treatments during the 360-d experimental period.**

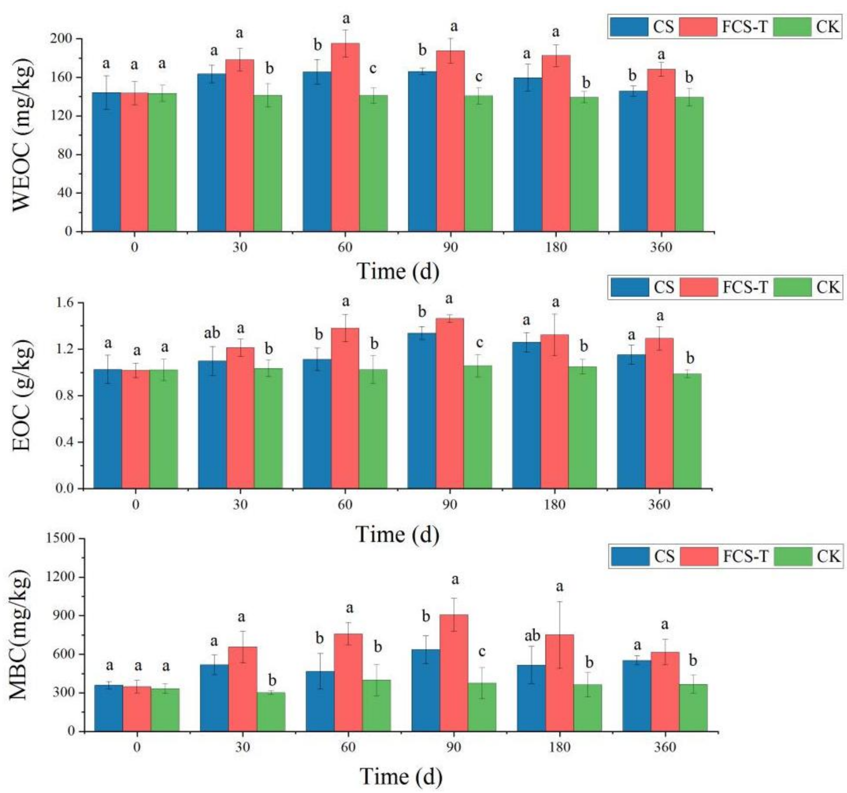

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Figure 3: Effects of corn straw returned (CS), fermented corn straw treated with *T. reesei* returned to the field (FCS-T) and non-straw amended soil (CK) on soil labile organic carbon (WEOC, EOC and MBC) concentrations in the 0–20 cm soil depth.

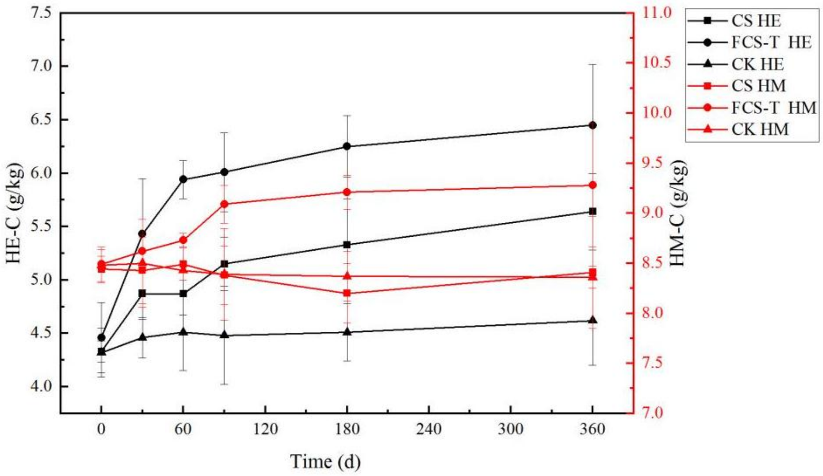

Figure 4: The carbon content of humus extracted (HE-C) and humin (HM-C) of the three different treatments during the 360-day period.

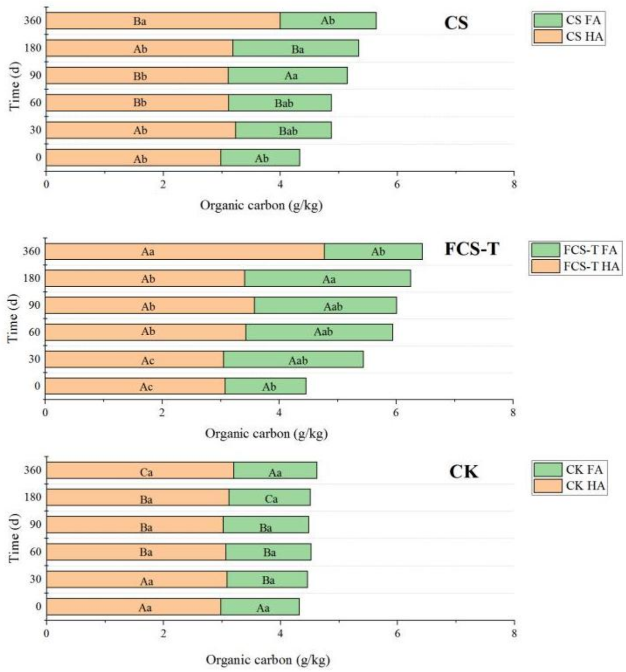

Figure 5: The carbon contents of humic acid (HA) and fulvic (FA) isolated from different treatments.

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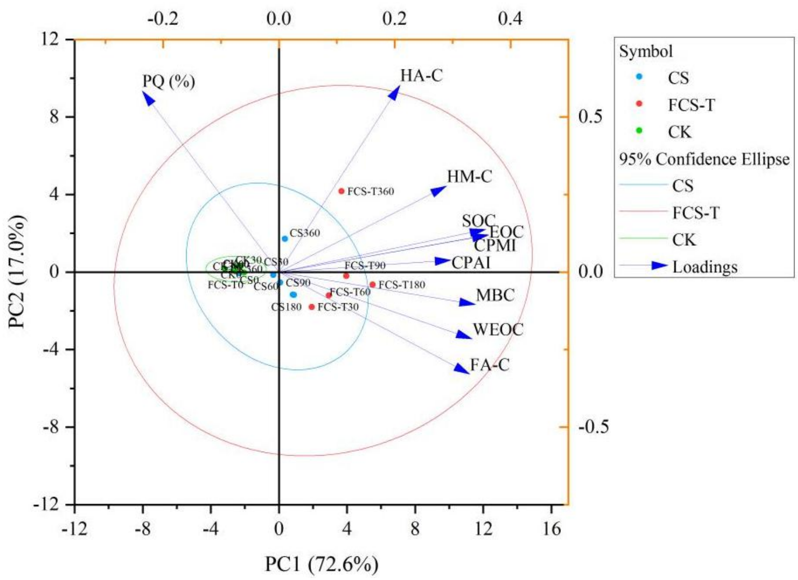
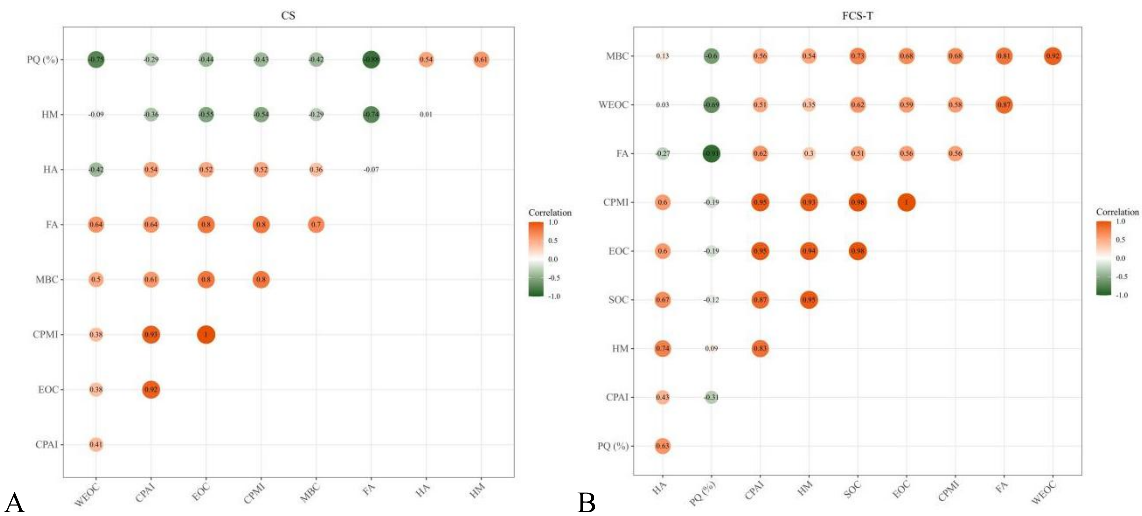


Figure 6: Principal component analysis (PCA) of soil organic carbon parameters and humus components affected by different treatments during 360 d of the experiment.



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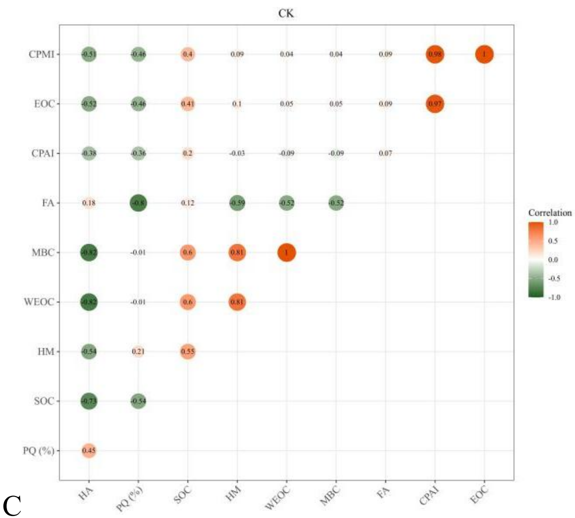

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Figure 7: The heatmap of Pearson's correlations among soil parameters from different treatments soil during the 360 d of the experiment. (A, CS; B, FCS-T; C, CK).

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.

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 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

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or FA among the treatments in a particular time (d) of the experiment. Different lowercase letters mean significant difference ($P < 0.05$) 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. (A: PCA bi-plot in 0-360 d; B: PCA bi-plot in the 360 d). Notes: PC, principal component; SOC, soil organic carbon; CPMI, carbon pool management index; CPAI, carbon pool activity index; WEOC, dissolved organic carbon; EOC, easily oxidizable organic carbon; MBC, microbial biomass carbon; HA, humic acid; FA, fulvic acid; HM, humin; PQ, humification degree. 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 1. Basic properties of the soil in field experiments

Soil	pH	Organic matter (g/kg)	Alkaline N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
Black soil	6.55	51.18	7.443	565.0	59.00

Table 2. Elemental composition of materials used in field experiments

Materials	C (g/kg)	H (g/kg)	N (g/kg)	O (g/kg)	C/N
CS	376.4	51.18	7.443	565.0	50.57
FCS-T	319.4	43.87	29.50	607.2	10.82

Note: CS, corn straw returned to the field; FCS-T, fermented corn straw treated with *T. reesei* returned to the field

Table 3. The carbon available ratio (CAR) of dissolved (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

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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. 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 experimental 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.

Table 5. Changes of relative content of each humus (HS) 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

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	CS	22.5±0.6a	12.6±1.6b	61.2±0.1a	64.2±3.6a
60	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
	CS	21.5±1.4a	14.1±2.3ab	58.0±1.6a	60.55±4.8b
90	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
	CS	22.2±0.7a	14.9±1.6b	57.1±2.5b	59.8± 2.4b
180	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
	CS	28.0±1.9a	11.5±1.3a	58.9±2.6b	71.0±1.4ab
360	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 treatment.