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Manuscript Title: Effects of returning corn straw and fermented corn straw to fields on the soil organic carbon pools and humus composition
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Response to the second reviewer's comments

First of all, we would like to thank the reviewers for their valuable comments and suggestions which will help us improve our manuscript. We have taken all of the reviewers' comments and suggestions when we were greatly revising our manuscript. Please find changes and explanations point by point below in Blue.

RC3: 'Comment on soil-2021-105', Anonymous Referee #2, 08 May 2022

General comment

Comment: The paper of Zhang et al. deals with the effect of addition to soil of crop(corn) residues on the soil stable and labile organic C pools. The paper describes the results of a year-round field experiment where differently treated straw (FCS-T, CS) were added to so soil. I do not see great novelty in this work since the positive effect on SOM and SOM pools of the amendment with pre-treated crop residue is well known. The fermentation with *Tricoderma reesei* substantially mimed a composting effect, producing a material partly degraded and enriched of stable molecules. This fact, evidently favors both the SOM accumulation and production of labile substances from the portion not completely stabilized. This effect could be explored in future by testing different fermentation periods that should produce material with different stability.
The introduction is not very well informative about the study presented and, further, in some parts it has a textbook-style, which should be avoided in a research paper. The authors have to do a greater effort in displaying the state of the art in the recycling of crop residue as soil amendments and presenting their own hypotheses. I suggest to reduce the use of acronyms that make the paper very hard to follow. In my opinion the manuscript needs major revision before to be considered for publication.
Response: Thank you very much for your support of our manuscript. We have greatly revised our manuscript following the reviewers' comments and suggestion. We have deleted the textbook-style expressions, and reduced the use of acronyms for CPMI parameters, and keep the acronym of SOM to make the manuscript easier to understand and read.

Regarding the question of *novelty*, the information below tries to show and explain the relevance of the current research:

"Compared with direct application of crop residues and traditional composting methods, pretreatment of crop straws residues with microbial inoculants can effectively shorten the time for decomposition and humification of crop straws. This process fosters the increase in CPMI index, while also encouraging the accumulation of a more stable humus fraction of SOM. To-date, there are still conflicting reports and there is no general consensus on the effects of crop residue application on the formation process and composition of SOM.

The potential of using microbial-mediated fermented straw (especially *Trichoderma*-mediated straw fermentation) returned to the field as a crop residue management practice has gained renewed interest in recent years. This because when *Trichoderma*-mediated straw fermentation is used as a soil amendment and nutrient source (Gaind and Nain. 2006; Gaind and Nain. 2007; Siddiquee et al., 2017) has great potential to increase crop yield (Islam et al., 2014), enhance plant development and reduce biotic and abiotic stresses (Sarangi et al., 2021). In previous studies on the application of fermented corn straw treated with *Trichoderma reesei* (*T. reesei*) in the field, Gaind and Nain. (2006), Gaind and Nain. (2007), and Sarangi et al. (2021) reported an increase in SOC and humus content in a series of studies. However, these studies did not go into considerable detail on the specific SOM components, such as the dynamic changes of labile organic carbon components, humic substance components, and carbon pool management level. We also observed that *T. reesei* has the strongest ability to form humic acid-like (Zhang et al., 2020; Zhang et al., 2021) during the decomposition of corn straw when compared with other fungi (*Phanerochaete chrysosporium* and *Trichoderma harzianum*). Although there are many studies, we still lack a clear understanding about the potential applications of *T. reesei* fermented corn straw and how it can increase SOM when incorporated into the field. Our findings presented the dynamics of this process in more detail, and we formulated hypothesis that suggest returning corn straw treated with *T. reesei* would be beneficial to increase labile SOC fractions and the humus composition. Our research hypothesis was validated by our results and we concluded that, amending fields with corn straw treated with *T. reesei* would be beneficial for the accrual of SOC. This is because the application of fermented corn straw treated by *T. reesei* was more advantageous in increasing the contents of aromatic C compounds (HA-C, and HM-C) and WEOC, which resulted in the overall increase in SOC and EOC, as well as the carbon pool management index. This also provides better evidence for the application of fermented corn straw treated by *T. reesei* in the field."

Regarding the reviewers' comment about: "*The fermentation with Tricoderma reesei substantially mimed a composting effect, producing a material partly degraded and enriched of stable molecules. This fact, evidently favors both the SOM accumulation and production of labile substances from the portion not completely stabilized. This effect could be explored in future by testing different fermentation periods that should produce material with different stability*."

We completely agree with the reviewer that the effect of fermentation with *Tricoderma reesei* should be explored in future by testing different fermentation periods that should produce material with different stability.

Indeed, in our parallel studies we are currently undertaking, we are researching about fermented corn straw treated with different microorganisms and under different humid and heat conditions, and fermentation time. The end materials will be returned to the field based on the humification effects produced.

We have significantly revised the Introduction section, reformulate a clearer research hypothesis, and put more effort into showcasing the latest technology in crop residue recycling as soil amendments. The revised Introduction section reads as follows (page 1, line 23-page 2, line 84):

Text: "**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), as well as managing crop straw residues. However, the decomposition process of crop residues is slow when they 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 decomposition and stability of carbon (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).
Contrary to direct crop residue application and conventional compositing method, pre-treatment of crop residues with microbial inoculants is the most effective method for reusing crop residues as eco-friendly amendments to improve soil fertility and increase 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 compost to reach maturity when compared to conventional composting methods (Organo et al., 2022). When applied to the soil, the microbial inoculant product improves C sequestration (Ahmed et al., 2019) and increase humic substances in the soil (Vargas-Garcia et al., 2006; Huang et al., 2008). The microbial inoculant-based fermentation produces contain partially degraded materials and organic compounds enriched of stable molecules (Huang et al., 2008). This fact, favours the accumulation and production of SOM containing both labile organic substances from the partially degraded portion and stable humic fraction.
Soil organic carbon is 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. 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 provide information about the formation of SOC (Chen et al., 2009; Huang et al., 2018; Liu et al., 2019; Ma et al., 2021). Another important index to monitor the effects of agricultural management practices on soil C sequestration is the carbon pool management index (Tang et al., 2018; Ma et al., 2021). The carbon pool management index, an index that includes SOC pools (carbon pool index) and SOC lability (carbon pool activity index), is widely used as a sensitive tool to determine changes in soil C content (Blair et al. 1995; Duval et al. 2019). A high carbon pool management index indicates that soil management practices have a greater potential to promote soil C sequestration (Duval et al. 2019).

Humic substance (HS) is the most stable fraction of SOM and contributes to the largest proportion to the total SOC (Olk et al., 2019; Dou et al., 2020). As a result, studying changes in soil humus components together with labile organic C fractions after corn straw application, could inform about the formation and stabilization of SOC during litter 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). Yet there are still conflicting reports and there is no general 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 enrich soil humic acid structure with aromatic compounds (Fan et al., 2018; Zhang et al., 2019). While, other studies reported an increase in 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 in SOM composition is unclear and site specific, warranting the need for more research to focus on the transformation of corn straw residues into SOM.

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), and the role of *Trichoderma*-mediated straw fermentation in improving crop yield (Islam et al., 2014), promoting plant development, and alleviating biotic and abiotic stresses has been observed (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 decomposition when compared with other fungi (*Phanerochaete chrysosporium* and *Trichoderma harzianum*). The use of *T. reesei* based fermentation has been employed in incubation studies and these studies show an increase soil C and humus composition (Gaind and Nain. 2007; Yang et al., 2019; Zhang et al., 2021). However, there is limited knowledge on the potential application of *T. reesei* fermented corn straw and increase SOM when incorporated into the field. In particular, the dynamic change process of different soil organic carbon components has not been reported yet. 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 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 also increase carbon pool management index 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."

Specific comments

(page, line: comment)

Comment 1: 2, 37-39: This sentence is a partial repetition of that at lines 25-28.

Response: We revised the sentence to read as follows (page 2, line 46-47):

Text: "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."

References: "Chen, Z., Wang, H., Liu, X., Zhao, X., Lu, D., Zhou, J., Li, C.: Changes in soil microbial community and organic carbon fractions under short-term straw return in a rice-wheat cropping system, Soil. Till. Res., 165(1), 121–127, doi:10.1016/j.still.2016.07.018, 2017.

Ma, L. J., Lv, X. B., Cao, N., Wang, Z., Zhou, Z. G., Meng, Y. L.: Alterations of soil labile organic carbon fractions and biological properties under different residue-management methods with equivalent carbon input, Appl. Soil. Ecol., 161, 103821, doi:10.1016/j.apsoil.2020.103821, 2021."

Comment 2: 2, 40: Please, add how much time passed between the application of the straw and the determination of the C.

Response: Thank you for the suggestion. We added the time (which was five years) and we made revisions and updates in the text as follows (page 2, line 47-49):

Text: "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."

References: "Ndzelu, B. S., Dou, S., Zhang, X.: Corn straw return can increase labile soil organic carbon fractions and improve water-stable aggregates in Haplic Cambisol. J. Arid. Land., 12(6), 1018–1030, doi:10.1007/s40333-020-0024-7, 2020b."

Comment 3: 2, 41-42: this sentence need references.

Response: We thank the reviewer, and the suggestion of the reviewer was adopted. We added some references in the text and reference list as follows (page 2, line 49-51):

Text: "Therefore, assessing labile SOC fractions after crop straw applications may provide information about the formation of SOC (Chen et al., 2009; Huang et al., 2018; Liu et al., 2019; Ma et al., 2021)."

References: "Chen, H. Q., Hou, R. X., Gong, Y. S., Li, H. W., Fan, M. S., Kuzyakov, Y.: Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China, Soil. Till. Res., 106(1), 85 – 94, doi:10.1016/j.still.2009.09.009, 2009.

Huang, R., Tian, D., Liu, J., Lu, S., He, X., Gao, M.: Responses of soil carbon pool and soil aggregates associated organic carbon to straw and straw-derived biochar addition in a dryland cropping mesocosm system, Agric. Ecosyst. Environ., 265, 576–586, doi:10.1016/j.agee.2018.07.013, 2018.

Liu, Z., Gao, T., Liu, W., Sun, K., Xin, Y., Liu, H., Wang, S., Li, G., Han, H., Li, Z., Ning, T.: Effects of part and whole straw returning on soil carbon sequestration in C3-C4 rotation cropland. J. Plant. Nutr. Soil Sci. 50, 73–85, doi:10.1002/jpln.201800573, 2019.

Ma, L. J., Lv, X. B., Cao, N., Wang, Z., Zhou, Z. G., Meng, Y. L.: Alterations of soil labile organic carbon fractions and biological properties under different residue-management methods with equivalent carbon input, Appl. Soil. Ecol., 161, 103821, doi:10.1016/j.apsoil.2020.103821, 2021."

Comment 4: 2, 43: Please, explains what the CPMI index consists of.

Response: The suggestion of the reviewer was adopted. We have made revisions and updates in the text and references as below (page 2, line 52-56):

Text: "The carbon pool management index, an index that includes SOC pools (carbon pool index) and SOC lability (carbon pool activity index), is widely used as a sensitive tool to determine changes in soil C content (Blair et al. 1995; Duval et al. 2019). A high carbon pool management index indicates that soil management practices have a greater potential to promote soil C sequestration (Duval et al. 2019)."

References: "Blair, G., Lefroy, R., Lisle, L.: Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems, Aust. J. Agric. Res., 46, 1459–1466, doi:10.1071/AR9951459,1995.

Duval, M. E., Martinez, J. M., Galantini, J. A., Aitkenhead, M.: Assessing soil quality indices based on soil organic carbon fractions in different long-term wheat systems under semiarid conditions, Soil. Use. Manag., doi:10.1111/sum.12532 36:71 – 82, 2019."

Comment 5: 2, 45-49. This part should be deleted. This information is well known by the soil community.

Response: The suggestion of the reviewer was adopted. We have deleted the part as suggested by the reviewer, and revised the opening paragraph in the manuscript to read as below (page 2, line 57-59).

Text: "Humic substance (HS) is the most stable fraction of SOM and contributes to the largest proportion to the total SOC (Olk et al., 2019; Dou et al., 2020). As a result, studying changes in soil humus components together with labile organic C fractions after corn straw application, could inform about the formation and stabilization of SOC during crop residues decay."

References: "Olk, D., Perdue, E., McKnight, D., Chen, Y., Farenhorst, A., Senesi, N., Chin, Y. P., Schmitt, K. P., Hertkorn, N., Harir, M.: Environmental and agricultural relevance of humic fractions extracted by alkali from soils and natural waters, J. Environ. Qual., 48(2): 217–232, doi:10.2134/jeq2019.02.0041, 2019.
Dou, S., Shan, J., Song, X., Cao, R., Wu, M., Li, C., Guan, S.: 2020. Are humic substances soil microbial residues or unique synthesized compounds? A perspective on their distinctiveness, Pedosphere., 30(2), 159–167, doi:10.1016/s1002-0160(20)60001-7, 2020."

Comment 6: 3, 76 The coordinate are not the same from line 69. Please explain why if is the case.
Response: Yes, the coordinates are different from where soil and corn straw residues were collected. Corn straw residues were collected from a corn field (125.394028, 43.812083; N43°48′43.5″, E125°23′38.50″) which was planted with corn in the previous season (i.e., 2018). Then in 2019, a year-round experiment was was conducted in a corn monocropping experimental field (125.402222, 43.818056; N43°49′5″, E125°24′8″) also located at Jilin Agricultural University, which was adjacent to where corn straw residues were collected.
We added that corn straw was collected from the adjacent field to clarify. Please see below revised sentence (page 3, line 94-95):

"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″)."

Comment 7: 3, 86: please define the mineral salt solution fertilization and the C/N ratio adjustment.
Response: Thank you to the reviewer for the suggestion. We have detailed the mineral salt solution that was mixed with the corn straw as follows (page 4, line 106-110):

"The spore solution and a mineral salt solution (pH = 5) used were prepared similarly as described by Zhang et al (2020b), 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, $FeSO_4 \cdot 7H_2O$ 0.07 g, $CaCl_2$ 0.028 g, $MnSO_4$ 0.021 g, $ZnSO_4$ 0.019 g, and the pH = 5. The fermentation process lasted 90 days and was carried out at 30 °C, 60% humidity, and 6.0 rpm."

References: "Zhang, Y., Dou, S., Hamza, B., Ye, S., Zhang, D.: Mechanisms of three fungal types on humic-like substances formation during solid-state fermentation of corn straw, Intl. J. Agric. Biol., 24, 970–976, doi:10.17957/IJAB/15.1377, 2020."

Comment 8: 4, 114-116. how long lasted the shaking time with water?

Response: The shaking time with water lasted for 60 minutes. The sentence was revised to read as below.

"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)." (page 4, line 117-120).

References: "Changtingny, M. H., Curtin, D., Beare, M. H., Greenfield, L. G.: Influence of Temperature on Water-Extractable Organic Matter and Ammonium Production in Mineral Soils, Soil. Sci. Soc. Am. J., 74(2), 517－524, doi:10.2136/sssaj2008.0347, 2010."

Comment 9: 4, 117: No incubation and fumigation before the MBC extraction? How did you estimate the MBC without the difference between fumigated and not-fumigated samples? The simple extraction with K_2SO_4 gives you the OM soluble in a K_2SO_4 solution which has no connection with the microbial C!!

Response: Thank you to the reviewer for raising this issue. Indeed, in our previous version of the manuscript we did not detail the extraction procedure of MBC, we simply cited the reference we followed for the extraction procedure. But, upon the reviewers' comment, we added details of MBC estimation to provide some clarity. This was added as follows (page 5, line 142-149).

"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). The soil WEOC and MBC contents were determined by a TOC analyser (Shimadzu TOC-VCPH, Japan). MBC was calculated as below Eq. (1):

$$\mathrm{MBC} = \frac{\mathrm{F_c}}{\mathrm{k_c}} \qquad (1)$$

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. "

References: "Vance, E. D., Brookes, P. C., Jenkinson, D. S.: An extraction method for measuring soil microbial biomass C. Soil. Biol. Biochem., 19(6), 703 － 707, doi:10.1016/0038-0717(87)90052-6, 1987."

Comment 10: 5, 120-122: Here the authors refer to fumigation, but it is not clear. Please xplain better the methodology used. When and how did you fumigate soil during the cultivation period?

Response: Thank you to the reviewer for the suggestion. We collected fresh soil samples at 0 d, 30 d, 60 d, 90 d, 180 d, 360 d during the cultivation period. In each soil sampling day, the collected fresh soil was picked out all visible corn straw materials and passed through a 2 mm sieve. Then the soil sample was transported to the lab to analyze MBC in soil. The description of soil sampling in **2.3.2 Soil sampling and analysis**. During each sampling time, MBC was estimated according to Vance et al. (1987), as briefly described in the ***Reponse*** of **comment 9**.

Text: "**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."

"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). The soil WEOC and MBC contents were determined by a TOC analyser (Shimadzu TOC-VCPH, Japan). MBC was calculated as below Eq. (1):

$$\mathrm{MBC} = \frac{\mathrm{F_c}}{\mathrm{k_c}} \quad (1)$$

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. "

References: "Vance, E. D., Brookes, P. C., Jenkinson, D. S.: An extraction method for measuring soil microbial biomass C. Soil. Biol. Biochem., 19(6), 703 – 707, doi:10.1016/0038-0717(87)90052-6, 1987."

Comment 11: 5, 138: delete CPMI = CPI â x CPAI x 100

Response: Thank you for the suggestion. We deleted the repeated CPAI equation as advised by the reviewer (page 6, line 166).

Comment 12: 5, 146-147: The humification degree (PQ) was calculated as HA-C/HE-C ratio (Sugahara and Inoko, 1981).

Response: Thank you for the suggestion, we re-wrote the sentence following the suggestion of the reviewer (page 6, line 174-175).

Text: "The humification degree (PQ) was calculated as HA-C/HE-C ratio (Sugahara and Inoko, 1981)".

Comment 13: 8, 220, 221: Barley.

Response: We thank the reviewer for picking up this error. We change "barly" to "barley" throughout the discussion where the mistake appeared (page 9, line 258-260).

Text: "Ma et al. (2021) reported similar findings with barley treated with microbial inoculant, in which the WEOC content was significantly higher than that of barley residue without microbial inoculant, but the EOC content differed seldomly."

Comment 14: 8, 239-250. This part describe essentially the effect of addition to soil of pretreated material (e.g. compost). I think that this part does not add very much to the discussion and could be diluted along the section.

Response: Thank you to the reviewer for the suggestion. We greatly revised this part, by deleting most it and handful of lines were merged with section 4.1. Now the section reads as follows (Page 8 Line 232-Page 10 Line 286):

4 Discussion

4.1 Effects of different treatments on SOC, soil labile organic carbon fractions, and humus 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) 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 fermentation with *T. reesei* and had lower C/N ratio (**Table 2**). During the fermentation process, studies show that part of the organic matter input is converted into CO_2 and other substances, and the remaining residue is converted into stable organic matter similar to HS (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 (Hessenet al., 2004). Furthermore, according to Sprunger et al. (2019) low C/N ratio of organic residues promote the accumulation of soil organic matter. 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 soil had a negative connection (Dannehl et al., 2017) The aforesaid point of view was further supported by our research.

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 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., 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).

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 barley treated with microbial inoculant, in which the WEOC content 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 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., 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, 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.

Compared with other treatments, the FCS-T treatment significantly increased the contents of HA-C and FA-C, throughout the 360 d period (**Figure 5**). This is because the FCS-T material contain relatively higher alkyl, aromatic C contents, and humic-like substances (see Zhang et al., 2021); compounds that are resistant to microbial direct degradation. This promoted the formation of soil humic substances during the process of humification (Huang et al., 2008; Roldán et al., 2011). The 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 application of fermented corn straw was more conducive to the increase of HA-C, and CS was more conducive to the increase of FA-C. In other studies, Gaind and Mathur (2001) and Gaind and Nain (2007) reported statistically significant increasing humus content in soil treated with paddy/wheat straw compost with *T. reesei* under rice-wheat cropping system. In our study, by analyzing the changes of humic substances components in different time periods, we determined that administration of FCS-T can significantly and continuously increase FA relative content up to 180 days, while the increase in HA relative content can last up to 360 days (**Table 5**). The FA increased in the early stage may be converted into HA and stored in the soil in the later stage. Therefore, application of FCS-T materials is more conducive to increasing humus C content and HA relative content (include more aromatic C compounds), which is important for long-term storage of SOC.

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Thank you very much for your consideration.
Kind regards,
(Yifeng Zhang)