Straw incorporation in deep soil layer promotes net photosynthetic carbon assimilation and maize growth in Northeast China

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Abstract

Background and aims

Returning straw into soil could increase soil organic carbon (SOC) and promote crop growth. However, little has been reported on the source of C for increased SOC (straw C or crop photosynthetic C).

Methods

To investigate the assimilation of photosynthetic C and its distribution in soil in the maize growth season, we set up a one-year $^{13}$C pulse-labelling experiment in a consecutive maize straw returning long-term trial. Four treatments were included: no straw return (control), straw mulching on the soil surface (cover), return in 0–20 cm layer (shallow) and 20–40 cm layer (deep).

Results

We found that the deep straw incorporation significantly ($P<0.05$) increased maize grain yield (by 2.9%) and SOC (by 13.4%). During the growing season, the deep straw incorporation increased photosynthetic $^{13}$C assimilation in shoots by 17.4% and the partitioning of photosynthetic $^{13}$C to soil by 7.9% at early jointing, and by 11.5% at maturity. The contribution of photosynthetic C to microbial biomass C (MBC) and dissolved organic C (DOC) was highest at jointing, and at harvest amounted to 39.1 % of MBC and 28.8% of DOC.

Conclusion

The results highlighted the importance of regulating the soil carbon dynamics via the deep straw return strategy. In conclusion, deep straw incorporation significantly increased photosynthetic efficiency and facilitated partitioning of photosynthetic C to roots and soil, thus promoting maize growth.

Introduction

Agriculture is an important source of global greenhouse gas (GHG) emissions (Poore and Nemecek 2018). The global potential for reducing GHG emissions exceeds 5500 Mt CO$_2$Eq. per annum, 90% of which comes from soil carbon (C) sequestration (Smith et al. 2007). Crop straw is an important component of the C cycle. Improving C fixation and stability of C in agricultural soil systems may reduce GHG emissions, thereby mitigating the negative impact of climate changes (Pan 2008). Alterations in soil C pools influence plant growth and development, soil fertility and nutrient cycling. However, the partitioning of photosynthetic C to roots, as well as to soil, remains poorly understood due to the complexity of the soil organic C pools.
China produces more than 800 million tons of crop straw annually, accounting for about 30% of the world's total straw production (Bi et al. 2009), and the amount is still growing at a net rate of 12.5 million tons per year (Xia et al. 2014). The straw contains considerable amounts of nitrogen (N), phosphorus (P), potassium (K) and other nutrient resources, which are equal to 40% of the national fertilizer consumption (Jia et al. 2018; Xu et al. 2016).

Straw return has been an important tillage practice in China. Straw return increases the content of soil organic C (SOC), thereby improving soil quality (Cong et al. 2019). It is estimated that around 0.6–1.2 billion tons of C is sequestered into soil each year through straw return (Lal 2009). Therefore, it is of great significance to explore the optimal use of straw for improving soil structure and quality as well as crop yield. Traditionally, straw is mostly used as soil surface mulching (cover) to increase soil moisture and crop yield (Li et al. 2020; Qin et al. 2015; Yan et al. 2007). However, straw surface-mulching may have some negative effects on the crops in the subsequent seasons, e.g., increased incidence of pests and diseases (Dinardo-Miranda and Fracasso 2013) and increased GHG emissions (Knoblauch et al. 2011).

Deep straw return denotes incorporation of straw into subsoil layer (20–40 cm) (Zou et al. 2014). Compared with surface straw-mulching, return of crushed straw in deep soil is more effective in improving soil physical and chemical properties (Li et al. 2021; Zou et al. 2014). However, little has been reported on the effects of various depths of straw return on crop growth and yield, at least partly due to a lack of appropriate measurement methods.

Soil organic C is an important C pool in the global C balance. Photosynthetic C assimilated by plants enters the soil in the form of plant residues and root secretions, contributing to various organic C pools, including dissolved organic C (DOC) and microbial biomass C (MBC). Thus, photosynthetic C, as the hub of the C cycle in the atmosphere-plant-soil-microbe system, is closely related to the circulation of C between the soil organic C pool and the atmospheric environment (Yevdokimov et al. 2006). MBC accounts for only 1–3% of soil organic C, and a much smaller proportion of the total soil C (Nie et al. 2012). Decomposition of SOC is closely linked to the dynamics of soil MBC as an indicator of soil activity. However, there are only a few studies on the turnover and dynamics of rhizosphere secretions as influenced by straw return (An et al. 2015b), especially regarding different depths of straw addition. The dynamics of soil organic C is influenced by the interaction among plants, soil and microorganisms, and is the main research topic in soil C sequestration. Thus, studying the effects of straw return to soil on the distribution of photosynthetic C and soil C pools is of great significance to the global C cycle and soil C sequestration. However, little has been reported on the effects of straw return on C partitioning in the soil-maize system. The C source (straw C or crop photosynthetic C) that contributes to increased SOC remains unclear.

Thus, the objectives of this study were: 1) to characterize the effects of straw return to various soil depths on maize growth and grain yield; 2) to determine changes in the photosynthetic C partitioning in maize shoots, roots, grains, SOC, DOC and MBC; and 3) to elucidate temporal dynamics of $^{13}$C partitioning in the maize-soil system. In this study, we used in-situ $^{13}$C pulse-labelling to trace the fate of photosynthesized...
C in the plant-soil-microbe system and quantify the contribution of the newly fixed C to soil organic C pools. We hypothesized that deep straw return: 1) would result in increased C sequestration in soil via improved root and shoot growth; and 2) would increase soil organic C and microbial activity, thereby enhancing maize growth and grain yield.

**Materials And Methods**

**Study site**

This study was conducted at the research station of Shenyang Agricultural University (41°31' N-123°24' E), Liaoning province, China, from May to September 2018. The soil type at the site is typical Brown Earth (Chinese Soil Taxonomy). The site has a temperate semi-humid continental climate. The annual temperature ranged between 6.2 and 9.7 °C, and the annual rainfall was between 584 and 692 mm. Air temperature and humidity in 2018 were shown in Fig. S1. The basic physical and chemical properties of the tested soil are shown in Table 1; they were determined by the methods specified by Bao (2001).

**Experimental design**

Air-dried and chopped maize straw (average length 3 cm; C:N = 75:1) from the preceding maize plants on the same research station was returned to field at 28,000 kg/ha in the autumn (year before the experiment took place) at the soil surface (cover), 0-20 cm (shallow) or 20-40 cm (deep), with the control treatment having no straw returned. The experiment was carried out in the field microplots (2.4 m × 1.1 m), with eight treatments (labelled and non-labelled sets of four treatments specified above) in three replicates. The labelled and non-labelled treatments were set apart by more than 10 m to avoid the interference. The straw was manually mixed with soil at 0-20 cm for the shallow treatment. In the deep-return treatment, 0-20 cm surface soil was removed, the straw was manually mixed with 20-40 cm soil, and then 0-20 cm surface soil was returned.

The amount of N, P and K fertilizers applied was based on the standard farming practice for growing maize in the area (N: 240 kg/ha, P: 33 kg/ha and K$_2$O, 87 kg/ha; as urea, superphosphate and potassium sulfate, respectively). The K and P fertilizers were applied as basal fertilizer at sowing, and N fertilizer was applied in three splits (as basal fertilizer and at jointing and tasseling) in the 3:4:3 proportion.

Maize (hybrid Jingke 968) was sown by hand planters and was thinned at the seedling stage to stand density of 57,000 plants/ha. Plant distance within rows was 30 cm, and the distance between rows was 50 cm. Border plots were included on the sides of the experimental field. Weed growth was controlled manually during the experiment.

**Photosynthetic C ($^{13}$C) labelling method**

In the maize early jointing stage (on 11th July), the $^{13}$C pulse labelling was done simultaneously on all four treatments within one replicate block. The pulse labelling method (shown in Fig. S2) followed the
published description (An et al. 2015a; Zhang et al. 2020) with modifications. A sealed and transparent labeling chamber measured 2.2 m length, 0.5 m width and 3 m height. This portable labeling chamber covered nine plants in each treatment and consisted of a transparent vinyl sheet on a steel frame. In order to provide a seal around the edges of the chamber, excess vinyl covered the contours of soil surface (Kong and Six 2010) and was sealed with wet soil (McMahon et al. 2005). Before the start of labelling, the black plastic film mulch was used to cover the soil surface of the micro-plot to prevent the labelled CO$_2$ from diffusing into the soil. The plastic black film was laid only during labelling and was removed immediately afterwards. To avoid any impact of plastic film cover, the non-labelled areas were also covered with black plastic film for the duration of the labelling period.

Labelling took place from 8:00 to 13:00 on a sunny day. An infrared gas analyzer was connected to the top of the labeling chamber to monitor the total CO$_2$ concentration (Wu et al. 2009). NaOH was used to absorb CO$_2$ in the chamber. After the CO$_2$ concentration fell below 80 μL/L, the sodium hydroxide trap was removed and H$_2$SO$_4$ (50 mL, 1 mol/L) was added to the first beaker containing labelled Na$_2$^{13}$CO$_3$ (99 atom% $^{13}$C, Sigma-Aldrich) to obtain $^{13}$CO$_2$ concentration of approximately 400 μL/L. When the CO$_2$ concentration in chamber fell below 80 μL/L again, H$_2$SO$_4$ (50 mL, 1 mol/L) was added to the second beaker containing labelled Na$_2$^{13}$CO$_3$. This process was repeated five times, and each labeling chamber required 9.12 g Na$_2$^{13}$CO$_3$. Finally, we added sulfuric acid to the No. 6 beaker filled with non-labelled sodium carbonate (1.81 g Na$_2$^{12}$CO$_3$) to enhance the $^{13}$C assimilation efficiency and minimize the loss of $^{13}$CO$_2$ (Butler et al. 2004). The entire labelling process ended, and the labelling chamber was removed, after the CO$_2$ concentration dropped below 80 μL/L after the final adjustment.

**Sample collection and processing**

Destructive sampling of maize plants in each treatment was conducted three times. Maize plants and soil samples were taken on 13rd July (the early jointing stage; two days after labelling), 26th July (the late jointing stage; 15 days after labelling) and 27th September (the grain maturity stage; 80 days after labelling). In each straw treatment, three labelled and three non-labelled plants were randomly selected from the respective plots. Shoots were cut at the base, and then roots and soil cores were dug out as a monolith (50 cm long × 50 cm wide and 40 cm deep). The aboveground material included shoots (stems and leaves) and grains (at maturity). All the visible small roots in the soil sample were picked out. Shoots (stems and leaves) and roots were washed in deionized water, oven-dried at 70 °C for 3 days and weighed. Dried root and shoot samples were ground in a mill (RetschMM200, Dusseldorf, Germany) for determining organic C.

The soil samples (0-40 cm) represented the mixture of rhizosphere and non-rhizosphere soil. The residual straw was carefully picked out (about 90% of straw was decomposed at grain harvest). The soil samples were stored in plastic bags at 4 °C and processed within 5 days. A portion of each soil sample was used for determining DOC and MBC. The remaining portion of each soil sample was air-dried, ground and passed through 0.25 mm sieve for the determination of total soil organic C. An elemental analyzer –
stable isotope ratio mass spectrometer (Elementar vario PYRO-isoprime100, Manchester, UK) was used to
determine total organic C content and $\delta^{13}$C value in soil and plant samples.

**Determination of soil DOC and MBC contents and $\delta^{13}$C values**

Microbial biomass C (MBC) was determined by the chloroform-fumigation extraction method (Vance et
al. 1987). Fresh soil equivalent to 10 g oven-dried soil was fumigated for 24 h and then extracted with 0.5
mol L$^{-1}$ K$_2$SO$_4$. The same amount of soil was also extracted without fumigation. The non-fumigated
extract was used to determine dissolved organic C (DOC). The soil extracts were measured to determine
the dissolved organic C content using a Total Organic Carbon Analyzer (Multi N/C UV HS, Analytik Jena
AG, Jena, Eisfeld, Germany). The MBC was calculated as the difference in dissolved organic C content
between fumigated and non-fumigated soil extracts, with the conversion coefficient $k_{EC}$ of 0.45 (Wu et al.
1990). All K$_2$SO$_4$ extracts were freeze-dried (EYELA Freeze Dryer FD-1, Tokyo, Japan) to analyze $^{13}$C
abundance (253Plus, Thermo Fisher, California, USA).

**Calculations**

1. $\delta^{13}$C value and $\delta^{13}$C abundance ($F_C$)

\[
\delta^{13}C (\text{‰}) = \frac{R_C - R_{\text{PDB}}}{R_{\text{PDB}}} \times 1000
\]

\[
F_C (\%) = \frac{(\delta^{13}C + 1000) \times R_{\text{PDB}}}{(\delta^{13}C+1000) \times R_{\text{PDB}}+1000} \times 100
\]

where $R_C$ is the $^{13}$C/$^{12}$C atomic ratio of the sample, and $R_{\text{PDB}}$ is 0.0112372 (Lu et al. 2002a).

2. The amount of $^{13}$C (mg) fixed in photosynthesis partitioned to maize shoots, roots, grains and soil
(without considering a loss due to respiration)

\[
^{13}C_i = C_i \times \frac{(F_{IC} - F_{\text{nlC}})}{100} \times 1000
\]

where $C_i$ is the C content (mg) of shoots, grains, roots or soil in the labelling treatment; $F_{IC}$ is the
abundance (%) of $^{13}$C in shoots, grains, roots or soil in the labelling treatment; and $F_{\text{nlC}}$ is the abundance
(%) of $^{13}$C in shoots, grains, roots or soil in the non-labelled treatment (Leake et al. 2006).

3. Partitioning of $^{13}$C (%)

\[
\text{Partitioning of }^{13}C_i = \frac{^{13}C_i}{^{13}C_{\text{fixed}}} \times 100
\]

where $^{13}C_{\text{fixed}}$ is the sum (mg) of $^{13}$C partitioned to shoots, grains, roots and soil in the labelling
treatment, and $^{13}C_i$ is the $^{13}$C content of individual plant parts or soil (Yu 2017).
(4) Soil microbial biomass C (C$_{MBC}$ mg/kg), dissolved organic C (C$_{DOC}$ mg/kg), and the content of $^{13}$C ($^{13}$C-C$_{MBC}$ µg/kg; $^{13}$C-C$_{DOC}$ µg/kg)

\[
MBC = (C_{fumC} - C_{nfumC})/k_{EC}
\]

\[
C_{DOC} = C_{nfumC}
\]

\[
^{13}$C-C$_{MBC} = ((F_{fumC,l} - F_{fumC,nl}) \times C_{fumC} - (F_{nfumC,l} - F_{nfumC,nl}) \times C_{nfumC})/(k_{EC} \times 100)
\]

\[
^{13}$C-C$_{DOC} = ((F_{nfumC,l} - F_{nfumC,nl}) \times C_{nfumC})/100
\]

where C$_{fumC}$ and C$_{nfumC}$ are the DOC content (mg/kg) in the K$_2$SO$_4$ extracts from fumigated and non-fumigated soils, respectively, in the same treatment; F$_{fumC,l}$ and F$_{fumC,nl}$ are the $^{13}$C abundances (%) in DOC in the K$_2$SO$_4$ extracts from fumigated and non-fumigated soils, respectively, from the labelled treatment; F$_{fumC,nl}$ and F$_{nfumC,nl}$ are the $^{13}$C abundances (%) in DOC in the K$_2$SO$_4$ extracts from fumigated and non-fumigated soils, respectively, from the non-labelled treatment. $k_{EC}$ is the conversion coefficient, and its value is 0.45 (Wu et al. 1990).

**Data analysis**

Two-way ANOVA was done on shoot biomass, root biomass, organic C in shoots, roots and soil, amount of assimilated C, and C partitioning to maize roots and soil, using sampling dates and treatments as independent variables. One-way ANOVA was conducted on parameters relative to four different treatments on each sampling date, or on twelve treatments (3 sampling dates × 4 treatments), depending on significance of the interaction between treatments and sampling dates. Means were compared with the Tukey’s honestly significant differences test at the 5% level of probability. All statistical analyses were done using the SPSS statistical software version 20.0 (IBM Corp., Armonk, NY, USA).

**Results**

The effects of straw treatments on plant growth and yield

The treatments and sampling dates significantly influenced root biomass and shoot biomass, but the interaction was non-significant (Table 2), indicating that the effects of straw return on maize plants growth increased uniformly over time. Root biomass and shoot biomass tended to have relatively high values in the shallow and deep treatments compared with those in the control and the cover treatment (Fig. 1a and 1b). At harvest, deep straw incorporation significantly increased shoot biomass (by 16.8%, averaged across the three sampling dates) compared to the control.

At harvest, there was no significant difference in grain yield across the treatments (Fig. 1c). Deep straw incorporation showed significantly higher hundred-grain weight compared with the control (Fig. 1d).
The effects of straw treatments on dynamics of organic C in maize plants and soil

Organic C in roots did not significantly differ among the three sampling dates (Table 2). Across sampling dates, average organic C concentration in roots in the shallow and deep treatments (406 and 413 g/kg, respectively) was significantly higher than that in the control and the cover treatment (391-392 g/kg) (Fig. 2a).

Treatments and sampling dates, but not the interaction between them, significantly influenced organic C in shoots (Table 2). Across sampling dates, deep straw incorporation significantly increased organic C in shoots; averaged across treatments, organic C in shoots significantly decreased from jointing stage to grain maturity. Both shallow and deep straw incorporation slightly but significantly increased organic C in grain (by 2.1% and 1.2%, respectively) compared with the control (Fig. 2b).

The interaction between sampling dates and treatments significantly influenced organic C in soil (Table 2). Both shallow and deep straw incorporation had the highest soil organic C (12.7 and 13.7 g/kg, respectively) on 13 July (sowing), and the control without straw had the lowest soil organic C on all three sampling dates (Fig. 2c).

The assimilation and partitioning of photosynthetic C

The effects of treatments and sampling dates, and their interaction significantly influenced the amount of assimilated C in shoots (Table 2). Deep straw incorporation significantly increased C in shoots compared with the control on all three sampling dates (Fig. 3a).

The interaction between treatments and sampling dates significantly altered C partitioning to roots and soil (Table 2). In roots, C partitioning rate in the treatments with shallow and deep straw incorporation was the highest (18.2% and 18.6%, respectively) on 25 July (late jointing), but the two treatments had the lowest C partitioning (11.4% and 11.6%, respectively) on 13 July (early jointing) (Fig. 3b). The C partitioning to soil tended to be lower on 13 July (early jointing) than 27 September (grain harvest) (Fig. 3c). Treatments had no significant influence on C partitioning to grain (4.2% on average; Fig. 3d).

Dynamics of DOC and MBC in soils

The treatments and sampling dates significantly influenced DOC and MBC in soils, but the interaction was non-significant (Table 2). The control had lower DOC (Fig. 4a) and MBC (Fig. 4b) than the three straw treatments regardless of the sampling date.

The $^{13}$C-DOC content as well as the ratio $^{13}$C-DOC/DOC were significantly influenced by the interaction (Table 2). On 25 July (late jointing), the $^{13}$C-DOC content in the straw treatments of cover, shallow and deep was 6.9, 7.3 and 7.7 μg/kg, respectively, all of which were significantly higher than the control. However, from late jointing to grain harvest, the $^{13}$C-DOC content in soil decreased significantly to around 0.45 μg/kg, with no difference among the four treatments (Fig. 4b).
Both main effects significantly influenced the MBC as well as $^{13}$C-MBC contents, but the interaction was non-significant (Table 2). At all three sampling dates, the control had significantly lower MBC than the other treatments. Regarding the temporal dynamics, $^{13}$C-MBC was relatively high in early jointing (about 36.8 µg/kg on average) and at grain harvest (about 29.1 µg/kg on average), but dropped at late jointing (25 July) (about 26.3 µg/kg on average) (Fig. 4d). The $^{13}$C-MBC content (Fig. 4d) followed exactly the same trends as MBC (Fig. 4b).

**Discussion**

**Effects of straw incorporation on maize growth**

We showed that straw incorporation in deep soil tended to increase root and shoot biomass compared with the control (Fig. 1a and 1b), which could be associated with a higher hundred-grain weight (Fig. 1d). This may be the nutrients released from straw filled SOC and accelerated microbial activities in the deep layer (Chen et al. 2020a; Li et al. 2021; Ma et al. 2019; Zou et al. 2016). Our results are in agreement with other studies, whereby maize plants in the treatments with straw tended to have a bigger root or shoot biomass or even higher grain yield compared with plants grown without straw added (Chen et al. 2020b; Han et al. 2020b; Xian et al. 2020).

Higher photosynthetic C allocation to the root at late jointing in the treatment with incorporated straw than the straw cover treatment (Fig. 3b) indicated that straw incorporation into soils was beneficial to the growth of maize roots (Fig. 1a). The bigger biomass of roots after deep straw incorporation would enhance uptake of water and nutrients from the deep soil (Huang et al. 2013).

Straw mulching on soil surface or shallow incorporation requires the optimal amount of straw because excessive straw or uneven distribution may directly reduce the germination of seeds, and cause adverse phenomena such as chlorotic seedlings and reduced growth (Zou et al. 2016). Decomposition of maize straw in northern China generally takes about 2 years (Han et al. 2020a). The straw trapped at shallow soil depth or on the soil surface can disturb sowing in spring, and the release of organic acids during straw decomposition may not be conducive to root growth (Ma et al. 2016). Thus, to avoid these problems, we tested straw incorporation into deeper soil. Deep straw incorporation could improve not only the capacity of soil to store water and conserve fertilizers (Zou et al. 2014), but also increase soil carbon content, improve soil fertility (Wang et al. 2015), promote soil microbial growth, and improve soil biological functions (Zhao et al. 2015).

**Effects of the depth of straw incorporation into soils on soil organic C and assimilation and partitioning of photosynthetic C in the maize-soil system**

Straw incorporation at 20–40 cm soil depth increased $^{13}$C assimilation in shoots compared with the control across the whole maize growth period (Fig. 3). This might have been because straw incorporation at 20–40 cm soil depth increased root and shoot biomass (Fig. 1), and also increased microbial biomass.
(MBC; Fig. 4b), thus enhancing crop and microbial respiration (Baptist et al. 2015). Rhizosphere deposition at the early stage of crop growth can be influenced by tillage methods (Munoz-Romero et al. 2013), soil fertility (Sun et al. 2019) and other factors, e.g., plant species (Baptist et al. 2015) and nutrient availability (Merckx et al. 1987). In our study, the photosynthetic $^{13}$C products were distributed mainly in the maize parts (Fig. 3), whereas a relatively small proportion entering soil increased during maize growth (Fig. 3c). These findings were in agreement with the early studies showing photosynthetic C had a fast conversion rate in plants: the photosynthetic C content in shoots reached a peak 6 hours after labelling, and photosynthetic C partitioned to roots was detected 4 hours after labelling (Johnson et al. 2002; Ostle et al. 2000).

In our study, the photosynthetic $^{13}$C products were distributed mainly in the maize parts (Fig. 3), whereas a relatively small proportion entering soil increased during maize growth (Fig. 3c). These findings were in agreement with the early studies showing photosynthetic C had a fast conversion rate in plants: the photosynthetic C content in shoots reached a peak 6 hours after labelling, and photosynthetic C partitioned to roots was detected 4 hours after labelling (Johnson et al. 2002; Ostle et al. 2000).

In the present study, straw incorporation at 20–40 cm soil depth was associated with the relatively high soil organic C content compared with the other treatments (Fig. 2c). This finding could be a consequence of enhanced above- and below-ground plant productivity in the treatment with deep straw incorporation (Fig. 1). Plants in the deep straw treatment invested relatively more assimilates into root growth than plants in the control (Fig. 3a), resulting in the higher root biomass (Fig. 1a) and root length in the deep treatment at harvest (data not shown). This can be explained by straw incorporation into deep soil promoting the formation of dominant aggregates and increasing organic C accumulation in them (Zhu et al. 2015).

**Effects of the depth of straw incorporation into soils on dynamics of photosynthetic C allocation in the maize-soil system**

In this study, we found that the proportions of photosynthetic C allocated were: 69–80% to shoots, 12–17% to roots, and about 7.9–12% to soil (Fig. 3). The observed values were similar to results reported by Tian et al. (2013) for a rice system. $^{13}$C partitioning to soil was significantly influenced by the depth of straw incorporation. The larger relative exudation of organic compounds from roots is often associated with enhanced microbial growth and enzymatic activities connected with nutrient mining from soil organic matter, which then facilitates plant nutrient uptake (Kaštovská et al. 2018).

The amount of soil photosynthetic C partitioning in each treatment was greater at early jointing than grain maturity, which might have been due to the maize roots growing vigorously at jointing, leading to a large amount of root exudates entering the soil. However, approaching grain maturity, maize root system gradually lost its activity, resulting in less root exudation (Barber 1995).

The distribution of $^{13}$C to roots was proportional to the development of root system over time. Root growth may also lead to a temporal increase in soil $^{13}$C allocation (Fig. 3c) due to an increase in root exudation. The observed differences in temporal rhizodeposition dynamics indicated treatment-related differences in the quantity of the released organic compounds. The partitioning rate of photosynthetic $^{13}$C to soil was not significantly different among treatments (Table 2 and Fig. 3c), indicating that straw addition regardless of soil depth did not change partitioning of photosynthetic C to soil in a short term.
Effects of the depth of straw incorporation into soils and maize growth stage on soil DOC and MBC

In our study, rate of photosynthetic C partitioning to DOC and MBC was influenced by growth stages of maize (Fig. 4). The contents of DOC and $^{13}$C-DOC increased from early to late jointing and decreased at grain maturity (Fig. 4a and 4c). It was probably due to relatively strong root exudation at jointing, with a decline toward maturity. Similarly, soil MBC in each treatment decreased from early to late jointing and increased at the maturity stage, which might have been associated with decomposition of dead roots (Fig. 4b and 4d). This is consistent with the previous study showing that the proportions of $^{14}$C in DOC and MBC varied with rice progressing from jointing to grain filling (Lu et al. 2002b).

MBC was the main component of active soil organic C because microorganisms could preferentially utilize dissolved C in the rhizosphere (Grantina-levina et al. 2014). In our study, three treatments with straw addition (especially deep and shallow incorporation treatments) increased the partitioning of photosynthetic C in MBC (Fig. 4d). This might have been due to straw addition promoting the growth of maize roots, improving exudation into the rhizosphere, and thus enhancing microorganism growth. Straw addition could also increase soil microbial activity via microbial decomposition of straw. Compared with the cover treatment, the higher content of soil organic C was found in the deep and shallow incorporation treatments (Fig. 2). In addition, straw incorporated in the deeper layer lowered soil bulk density and improved soil aeration (Zou et al. 2014), both of which would accelerate decomposition of straw.

Different natural conditions in various soil layers would be associated with varied composition and abundance of microbial populations, leading to differential straw degradation rates (Coppens et al. 2006; Frey et al. 1999). Soil moisture and nutrient availability interact in influencing plant C acquisition and partitioning in the plant-microbe-soil systems (Atere et al. 2017). In our study, we indeed found significant differences in soil water content (Fig. S3). In addition, temperature has an important effect on soil organic C and MBC (Ghosh et al. 2020; Yanni et al. 2020); however, in our study there was no significant difference in soil temperature among the four treatments (Fig. S4).

Straw incorporation at the 20–40 cm soil depth had the positive effects not only on maize plants at harvest such as a non-significant grain yield increase and significantly higher hundred-grain weight (Fig. 1c-d), but also on soil such as higher SOC compared with the surface and 0–20 cm depth straw addition (Fig. 2c, Fig. 5). However, based on the current research, the mechanisms underlying an increase in soil organic C can be predicted only to some extent, and the contribution of different factors cannot be determined qualitatively and/or quantitatively, like C emission and energy-consumption. There is still a scope for research on the soil mechanisms at the microscopic scale regarding the effect of straw incorporation at various soil depths.

Conclusions

The results have supported our hypothesis that deep straw incorporation can promote net photosynthetic C assimilation and maize growth via increased soil organic C and an increase in microbial activity (MBC).
The mechanisms were likely due to deep straw incorporation improving root growth, microbial activity and nutrient release from straw. The results also showed that plants grown with straw added in differential ways varied in C fixation and partitioning, resulting in the unique patterns of $^{13}$C dynamics that might have implications for C exudation into the rhizosphere soil, the DOC and MBC contents and nutrient availability. Deep straw incorporation increased the fixation of photosynthetic C by maize and partitioning to active soil organic C fractions such as DOC and MBC. Hence, deep straw incorporation could be recommended in the maize production in Northeast China.

Declarations

Acknowledgments

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References

1. An T, Schaeffer S, Li S, Fu S, Pei J, Li H, Zhuang J, Radosevich M, Wang J (2015a) Carbon fluxes from plants to soil and dynamics of microbial immobilization under plastic film mulching and fertilizer application using $^{13}$C pulse-labeling. Soil Biol Biochem 80:53–61
2. An TT, Schaeffer S, Zhuang J, Radosevich M, Li SY, Li H, Pei JB, Wang JK (2015b) Dynamics and distribution of $^{13}$C-labeled straw carbon by microorganisms as affected by soil fertility levels in the black soil region of northeast China. Biol Fertil Soils 51:605–613
3. Atere CT, Ge T, Zhu Z, Tong C, Jones DL, Shibistova O, Guggenberger G, Wu J (2017) Rice rhizodeposition and carbon stabilisation in paddy soil are regulated via drying-rewetting cycles and nitrogen fertilisation. Biol Fertil Soils 53:407–417
4. Bao S (2001) Analysis of soil agro-chemistry. Agricultural Press Chinese, Beijing
5. Baptist F, Aranjuelo I, Legay N, Lopez-Sangil L, Molero G, Rovira P, Nogués S (2015) Rhizodeposition of organic carbon by plants with contrasting traits for resource acquisition: responses to different fertility regimes. Plant Soil 394:391–406
6. Barber SA (1995) Soil nutrient bioavailability: a mechanistic approach. John Wiley & Sons, New York
7. Bi Y, Gao C, Wang Y, Li B (2009) Estimation of straw resources in China. Transactions of the Chinese Society of Agricultural Engineering 25:211–217 (in Chinese with English abstract)
8. Butler JL, Bottomley PJ, Griffith SM, Myrold DD (2004) Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. Soil Biol Biochem 36:371–382
9. Chen SY, Zhang XY, Shao LW, Sun HY, Niu JF, Liu XW (2020a) Effects of straw and manure management on soil and crop performance in North China Plain. Catena 187:104359
10. Chen YY, Fan PS, Li L, Tian H, Ashraf U, Mo ZW, Duan MY, Wu QT, Zhang Z, Tang XR, Pan SG (2020b) Straw incorporation coupled with deep placement of nitrogen fertilizer improved grain yield and nitrogen use efficiency in direct-seeded rice. Journal of Soil Science Plant Nutrition 20:2338–2347

11. Cong P, Li Y, Gao Z, Wang J, Zhang L, Pang H (2019) High dosage of pelletized straw returning rapidly improving soil organic carbon content and wheat-maize yield. Transactions of the Chinese Society of Agricultural Engineering 35:148–156 (in Chinese with English abstract)

12. Coppens F, Garnier P, De Gryze S, Merckx R, Recous S (2006) Soil moisture, carbon and nitrogen dynamics following incorporation and surface application of labelled crop residues in soil columns. Eur J Soil Sci 57:894–905

13. Dinardo-Miranda LL, Fracasso JV (2013) Sugarcane straw and the populations of pests and nematodes. Scientia Agricola 70:369–374

14. Frey SD, Elliott ET, Paustian K (1999) Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biol Biochem 31:573–585

15. Ghosh A, Das A, Das D, Ray P, Bhattacharyyya R, Biswas DR, Biswas SS (2020) Contrasting land use systems and soil organic matter quality and temperature sensitivity in North Eastern India. Soil Tillage Res 199:104573

16. Grantina-levina L, Karlsons A, Andersone-Ozola U, Levinsh G (2014) Effect of freshwater sapropel on plants in respect to its growth-affecting activity and cultivable microorganism content. Zemdirbyste 101:355–366

17. Han Y, Yao SH, Jiang H, Ge XL, Zhang YL, Mao JD, Dou S, Zhang B (2020a) Effects of mixing maize straw with soil and placement depths on decomposition rates and products at two cold sites in the mollisol region of China. Soil Tillage Res 197: https://doi.org/10.1016/j.still.2019.104519

18. Han YL, Ma W, Zhou BY, Yang XL, Salah A, Li CF, Cao CG, Zhan M, Zhao M (2020b) Effects of straw-return method for the maize-rice rotation system on soil properties and crop yields. Agronomy 10:461

19. Huang Y, Bi SY, Zou HT, Dou S (2013) Effect of straw deep returning on corn root system and yield. Journal of Maize Science 21(5):109–112 (in Chinese with English abstract)

20. Jia W, Qin W, Zhang Q, Wang X, Ma Y, Chen Q (2018) Evaluation of crop residues and manure production and their geographical distribution in China. J Clean Prod 188:954–965

21. Johnson D, Leake JR, Ostle N, Ineson P, Read DJ (2002) In situ $^{13}$CO$_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. New Phytol 153:327–334

22. Kaštovská E, Straková P, Edwards K, Urbanová Z, Bártá J, Mastný J, Šantrůčková H, Picek T (2018) Cotton-grass and blueberry have opposite effect on peat characteristics and nutrient transformation in peatland. Ecosystems 21:443–458

23. Knoblauch C, Maarifat AA, Pfieffer EM, Haefele SM (2011) Degradability of black carbon and its impact on trace gas fluxes and carbon turnover in paddy soils. Soil Biol Biochem 43:1768–1778
24. Kong AYY, Six J (2010) Tracing root vs. Residue carbon into soils from conventional and alternative cropping systems. Soil Sci Soc Am J 74:1201–1210
25. Lal R (2009) Soil quality impacts of residue removal for bioethanol production. Soil Tillage Res 102:233–241
26. Leake J, Ostle N, Rangel-Castro J, Johnson D (2006) Carbon fluxes from plants through soil organisms determined by field $^{13}$CO$_2$ pulse-labelling in an upland grassland. Appl Soil Ecol 33:152–175
27. Li F, Han X, Ma XL, Wang YJ, Song TY, Wang YY (2020) Straw mulch controls runoff and nitrogen and phosphorus loss from slope farmland in black soil region of Northeast China. J Soil Water Conserv 34:37–42 (in Chinese with English abstract)
28. Li JQ, Ye XH, Zhang YL, Chen J, Yu N, Zou HT (2021) Maize straw deep-burying promotes soil bacteria community abundance and improves soil fertility. Journal of Soil Science Plant Nutrition. https://10.1007/s42729-021-00448-6
29. Lu Y, Watanabe A, Kimura M (2002a) Contribution of plant-derived carbon to soil microbial biomass dynamics in a paddy rice microcosm. Biol Fertil Soils 36:136–142
30. Lu Y, Watanabe A, Kimura M (2002b) Input and distribution of photosynthesized carbon in a flooded rice soil. Global Biogeochem Cycles 16:32–31
31. Ma LJ, Kong FX, Wang Z, Luo Y, Lv XB, Zhou ZG, Meng YL (2019) Growth and yield of cotton as affected by different straw returning modes with an equivalent carbon input. Field Crops Research 243:107616
32. Ma Z, Marsolais F, Bernards MA, Sumarah MW, Bykova NV, Igamberdiev AU (2016) Glyoxylate cycle and metabolism of organic acids in the scutellum of barley seeds during germination. Plant Sci 248:37–44
33. McMahon SK, Williams MA, Bottomley PJ, Myrold DD (2005) Dynamics of microbial communities during decomposition of carbon-13 labeled ryegrass fractions in soil. Soil Sci Soc Am J 69:1238–1247
34. Merckx R, Dijkstra A, den Hartog A, van Veen JA (1987) Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biol Fertil Soils 5:126–132
35. Munoz-Romero V, Lopez-Bellido RJ, Redondo R, Lopez-Bellido L (2013) Nitrogen rhizodeposition by wheat under different tillage systems in a rainfed Vertisol. Field Crops Research 144:148–153
36. Nie SA, Zhou P, Ge TD, Tong CL, Xiao HA, Wu JS, Zhang YZ (2012) Quantifying rice (Oryza sativa L.) photo-assimilated carbon input into soil organic carbon pools following continuous $^{14}$C labeling. Environmental Science (in Chinese) 33:1346–1351
37. Ostle N, Ineson P, Benham D, Sleep D (2000) Carbon assimilation and turnover in grassland vegetation using an in situ $^{13}$CO$_2$ pulse labelling system. Rapid Commun Mass Spectrom 14:1345–1350
38. Pan GX (2008) Soil organic carbon stock, dynamics and climate change mitigation of China. Advances in Climate Change Research 4:282–289 (in Chinese with English abstract)

39. Poore J, Nemecek T (2018) Reducing food’s environmental impacts through producers and consumers. Science 360:987–992

40. Qin W, Hu C, Oenema O (2015) Soil mulching significantly enhances yields and water and nitrogen use efficiencies of maize and wheat: a meta-analysis. Sci Rep 5:16210

41. Smith P, Martin D, Cai Z, Gwary D, Janzen H, Kumar P, McCarl B, Ogle S, O’Mara F, Rice C, Scholes B, Sirotenko O, Howden S, McAllister T, Pan G, Romanenkov V, Schneider U, Towprayoon S (2007) Policy and technological constraints to implementation of greenhouse gas mitigation options in agriculture. Agriculture, Ecosystems and Environment 11: 6–28

42. Sun ZA, Wu SX, Zhang YW, Meng FQ, Zhu B, Chen Q (2019) Effects of nitrogen fertilization on pot-grown wheat photosynthate partitioning within intensively farmed soil determined by $^{13}$C pulse-labeling. J Plant Nutr Soil Sci 182:896–907

43. Tian J, Pausch J, Fan M, Li X, Tang Q, Kuzyakov Y (2013) Allocation and dynamics of assimilated carbon in rice-soil system depending on water management. Plant Soil 363:273–285

44. Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

45. Wang SN, Zou HT, Zhang YL, Yu N, Zhang YL, Fan QF, Huang Y (2015) Effect of straw deep returning on the soil water features and soil organic carbon components. J Soil Water Conserv 1:154–158 (in Chinese with English abstract)

46. Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. Soil Biol Biochem 22:1167–1169

47. Wu WX, Liu W, Lu HH, Chen YX, Devare M, Thies J (2009) Use of $^{13}$C labeling to assess carbon partitioning in transgenic and nontransgenic (parental) rice and their rhizosphere soil microbial communities. FEMS Microbiol Ecol 67:93–102

48. Xia L, Wang S, Yan X (2014) Effects of long-term straw incorporation on the net global warming potential and the net economic benefit in a rice–wheat cropping system in China. Agriculture Ecosystems Environment 197:118–127

49. Xian YR, Chen Y, Chen C, He RR, Chen XW, Chen Y, Wang XL (2020) Does extending recycling chain of using rice straw contribute to improving yield and reducing GHGs emissions in paddy field? An integrated analysis based on field research and system assessment. J Clean Prod 264:121508

50. Xu J, Hu N, Zhu L (2016) Effect of amount of annual straw returning on soil nutrients and yield in winter wheat field. Journal of Triticeae Crops 36:215–222 (in Chinese with English abstract)

51. Yan DZ, Wang DJ, Yang LZ (2007) Long-term effect of chemical fertilizer, straw, and manure on labile organic matter fractions in a paddy soil. Biol Fertil Soils 44:93–101
52. Yanni SF, Helgason BL, Janzen HH, Ellert BH, Gregorich EG (2020) Warming effects on carbon dynamics and microbial communities in soils of diverse texture. Soil Biol Biochem 140:107631

53. Yevdokimov I, Ruser R, Buegger F, Marx M, Munch J (2006) Microbial immobilisation of $^{13}$C rhizodeposits in rhizosphere and root-free soil under continuous $^{13}$C labelling of oats. Soil Biol Biochem 38:1202–1211

54. Yu P (2017) Distribution of photosynthetic carbon and fertilizer nitrogen in rice-soil system relative to rice growth stages. Master degree. Shenyang Agricultural University

55. Zhang Y, Hou W, Chi M, Sun Y, An J, Yu N, Zou H (2020) Simulating the effects of soil temperature and soil moisture on CO$_2$ and CH$_4$ emissions in rice straw-enriched paddy soil. Catena 194:104677

56. Zhao YL, Guo HB, Xue ZW, Mu XY, Li CH (2015) Effects of tillage and straw returning on microorganism quantity, enzyme activities in soils and grain yield. Chin J Appl Ecol 26:1785–1792 (in Chinese with English abstract)

57. Zhu S, Dou S, Chen LZ (2015) Effect of deep application of straw on composition of humic acid in soil aggregates. Acta Pedol Sin 52:747–758 (in Chinese with English abstract)

58. Zou H, Ye X, Li J, Lu J, Fan Q (2016) Effects of straw return in deep soils with urea addition on the soil organic carbon fractions in a semi-arid temperate cornfield. Plos One 11:e0153214

59. Zou HT, Wang SN, Yan HL, Ma YB, Fan QF, Huang Y, Zhang YL (2014) Effects of straw deep returning on soil structure moisture in semiarid of northeast China. Agric Res Arid Areas 32:52–60 (in Chinese with English abstract)

### Tables

**Table 1** Physical and chemical properties of soil (0-40 cm depth)

| Bulk density (g/cm$^3$) | pH$_{\text{water}}$ | Total carbon (g/kg) | Total nitrogen (g/kg) | Hydrolysable nitrogen (mg/kg) | Available phosphorus (mg/kg) | Available potassium (mg/kg) | $\delta^{13}$C value (%) |
|-------------------------|---------------------|---------------------|----------------------|-----------------------------|-----------------------------|-----------------------------|--------------------------|
| 1.32                    | 6.61                | 10                  | 1.4                  | 57                          | 22                          | 167                         | -18.7                    |

**Table 2** Results of analysis of variance (P values) for various parameters in the maize-soil system as influenced by different treatments at three sampling dates.
| Parts | Dependent variables                        | Treatment (T) | Sampling Date (D) | T * D |
|-------|-------------------------------------------|---------------|-------------------|-------|
| Roots | Biomass (g/plant)                         | 0.089         | <0.001            | 0.49  |
|       | Organic C (g/kg)                          | <0.001        | 0.78              | 0.24  |
|       | Carbon partitioning (%)                   | <0.001        | <0.001            | <0.001|
| Shoots| Biomass (g/plant)                         | 0.06          | <0.001            | 0.59  |
|       | Organic C (g/kg)                          | 0.004         | <0.001            | 0.23  |
|       | Carbon assimilation amount (mg/m$^2$)     | <0.001        | <0.001            | <0.001|
| Soil  | Organic C (g/kg)                          | <0.001        | <0.001            | <0.001|
|       | Carbon partitioning (%)                   | 0.89          | <0.001            | 0.02  |
|       | Dissolved organic C (DOC) (mg/kg)         | <0.001        | <0.001            | 0.37  |
|       | Microbial biomass C (MBC) (μg/kg)         | <0.001        | <0.001            | 0.10  |
|       | $^{13}$C-DOC (μg/kg)                      | <0.001        | <0.001            | <0.001|
|       | $^{13}$C-MBC (μg/kg)                      | <0.001        | <0.001            | 0.12  |

**Figures**

(a) Root biomass (g/plant) for different treatments and sampling dates.
(b) Shoot biomass (g/plant) for different treatments and sampling dates.
(c) Grain yield (g/plant) for different treatments.
(d) Hundred grain weight (g) for different treatments.
Figure 1

Biomass of maize roots (a) and shoots (b) on the three sampling dates (13 Jul: early jointing, 62 days after sowing (DAS); 25 Jul: late jointing, 74 DAS; 27 Sep: grain maturity, 138 DAS) and grain yield (c) and hundred-grain weight (d) at maturity. Means ± SE (n=3). Different letters denote significant differences among treatments on a specific sampling date (a, b) or among the treatments (c, d) (P<0.05). Control, no straw added; Cover, straw added to the soil surface; Shallow, straw incorporated at the 0-20 cm soil depth; Deep, straw incorporated at the 20-40 cm soil depth.

Figure 2

Organic carbon (C) content of maize roots (a), shoots (b), grain (the insert in b) and 0-40 cm soil layer (c) on the three sampling dates (13 Jul: early jointing, 62 days after sowing (DAS); 25 Jul: late jointing, 74 DAS; 27 Sep: grain maturity, 138 DAS). Means ± SE (n=3). Organic C in roots (a) was averaged across the sampling dates because the interaction (treatments × sampling dates) was not significant. Depending on significance of the interaction, different letters denote significant differences among treatments (a, the insert in b), among the treatments on a given sampling date (b) or among sampling dates × treatments.
(c) (P<0.05). Control, no straw added; Cover, straw added to the soil surface; Shallow, straw incorporated at the 0-20 cm soil depth; Deep, straw incorporated at the 20-40 cm soil depth.

**Figure 3**

The amount of photosynthetic carbon (13C) accumulated in maize shoots (a) and partitioning of photosynthetic carbon (13C) to maize roots (b), 0-40 cm soil (c) and grain (d) on the three sampling dates (13 Jul: early jointing, 62 days after sowing (DAS); 25 Jul: late jointing, 74 DAS; 27 Sep: grain maturity, 138 DAS). Carbon partitioning: 13C content of individual plant parts or soil divided by the sum of 13Cfixed (13C found in the shoots, roots, soils and grains) (expressed as %). Means ± SE (n=3). Different letters denote significant differences among sampling dates × treatments (a, b, c) or among treatments (d) (P<0.05). Control, no straw added; Cover, straw added to the soil surface; Shallow, straw incorporated at the 0-20 cm soil depth; Deep, straw incorporated at the 20-40 cm soil depth.
Dissolved organic carbon (DOC) (a), microbial biomass carbon (MBC) (b), 13C in DOC (c) and 13C in MBC (d) in 0-40 cm soil layer on the three sampling dates (13 Jul: early jointing, 62 days after sowing (DAS); 25 Jul: late jointing, 74 DAS; 27 Sep: grain maturity, 138 DAS). Means ± SE (n=3). Depending on significance of the interaction, different letters denote significant differences among treatments on a specific sampling date (a, b, d) or among sampling dates × treatments (c) (P<0.05). Control, no straw added; Cover, straw added to the soil surface; Shallow, straw incorporated at the 0-20 cm soil depth; Deep, straw incorporated at the 20-40 cm soil depth.
Figure 5

Relative increase in various parameters at maize harvest in the three treatments with straw addition compared with the treatment without straw. Control, no straw added; Cover, straw added to the soil surface; Shallow, straw incorporated at the 0-20 cm soil depth; Deep, straw incorporated at the 20-40 cm soil depth. HGW, hundred-grain weight; SB, shoot biomass; RB, root biomass; SCA, shoot carbon assimilation; RCP, root carbon partitioning; SOC, soil organic carbon; DOC, dissolved organic carbon; MBC, microbial biomass carbon.

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