

## Article

# The Impact of Combining *Robinia pseudoacacia* Leaves and Corn Straw on Soil Carbon Content and Corn Yield in Loess Plateau

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**Abstract:** In the agroforestry system, the organic matter in the farmland and natural ecosystem enters the farmland soil in a mixed form to improve soil fertility and carbon pool quality. However, it is unclear how soil microbial carbon-degrading enzyme activity responds to carbon dynamics in this process. Therefore, we took farmland in the Loess Plateau as the research object, combining the application of corn straw and *Robinia pseudoacacia* leaves in a mass ratio of 4:0, 3:1, 2:2, 1:3, and 0:4 for returning to the field. We measured corn grain yield, carbon emission, organic carbon pool component content, and carbon-degrading enzyme activity of the farmland. The results showed that combining corn straw and *Robinia pseudoacacia* leaves had a significant impact on soil organic carbon components (readily oxidizable organic carbon and recalcitrant organic carbon), carbon-degrading enzymes (polyphenol oxidase, peroxidase, and cellobiohydrolase), and cumulative carbon emissions. The trend of different indicators in different treatments during the corn growth period was similar. We found that soil carbon emissions were closely related to ROC and soil oxidase activity, while soil carbon content was closely related to soil hydrolase activity. Compared to not returning straw to the field, the corn straw and *Robinia pseudoacacia* leaves returned to the field in a mass ratio of 1:3(Y1C3) can increase corn grain yield by 32.04%. The Y1C3 treatment has the highest soil carbon content and the lowest crop carbon emission efficiency. Soil water content plays a crucial role in the process of carbon pool transformation driven by soil carbon-degrading enzymes. In conclusion, soil carbon dynamics are closely related to the activity of soil carbon-degrading enzymes. Combining the application of corn straw and *Robinia pseudoacacia* leaves may be a more suitable farming measure for fragile habitats in the Loess Plateau than other solutions.

**Keywords:** soil carbon dynamics; soil carbon-degrading enzyme; soil carbon emission; soil carbon content; agroforestry system



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## 1. Introduction

Soil organic carbon (SOC) content has been regarded as an essential indicator of soil fertility and crop yield, and its dynamic balance is of great significance to the sustainable development of agricultural production [1–3]. One of the factors that affect soil carbon in agroecosystems is the surrounding landscape mosaic, where forest fragments may represent sources of litter in agricultural landscapes. Since the implementation of the “returning farmland to forests” restoration project in the ecologically fragile area of the Loess Plateau, a composite structure of farmland and forest land has been formed [4,5]. In the agroforestry system, exogenous organic matter from farmland and natural ecosystems enters the soil as a mixture, causing dynamic changes in the soil carbon pool of the farmland [6]. However, the impact of this “mixed decomposition effect” on farmland soil carbon fixation and emission

is still unclear. Researching the effects of carbon source application on soil carbon pool dynamics in agroforestry systems is expected to provide an essential theoretical basis for agricultural production practice.

The return of exogenous carbon is considered one of the main agricultural management measures to increase soil organic carbon [7]. In recent years, the impact of combining different types of materials for returning to the field in soil carbon pools has attracted widespread attention from scholars. To increase the effectiveness of exogenous carbon decomposition, diversified methods of return have gradually emerged [8,9]. For instance, the incorporation of a mixture of soybean and corn straw into the soil, returning composted straw to the field, deep-burying of ground-up straw, and so on [8,10]. In agroforestry systems, mixed litter in soil ecosystems largely determines the dynamic pattern of soil carbon and nitrogen, and different proportions of mixtures may lead to differences in the selection and supply of soil carbon and nitrogen. For example, returning green manure and straw to the field significantly improves soil fertility and increases rice yield [11]. The NO emissions in dryland agricultural ecosystems are significantly reduced by returning straw to the field and incorporating earthworms [12]. The scale of straw return to the field has been affected by the returning method and mixed species [13]. In addition, the return of organic matter with different carbon availability (straw or litter) affects the decomposition of straw and the stability of the carbon pool by alleviating carbon constraints and benefiting the activities of specific taxa [14]. Compared with returning single-type straw to the field, combining different types of straw regulates soil microbial community diversity and promotes the increase of soil organic carbon [15,16]. However, the response of soil carbon pool and organic carbon accumulation dynamics to mixtures of litter and straw in agroforestry systems is unclear.

The decomposition of straw is affected by biological and abiotic factors. Soil enzymes are widely recognized as crucial for mediating soil organic matter decomposition, transformation, and mineralization [17,18]. Based on their function, soil carbon-degrading enzymes can be broadly divided into two categories, i.e., oxidases and hydrolases [19]. In detail, soil oxidases such as polyphenol oxidase (PPO) and peroxidase (PX) can depolymerize poor-quality and chemically complex recalcitrant carbon. Soil hydrolases such as  $\beta$ -1,4-glucosidase (BG) and cellobiohydrolase (CBH) mainly acquire labile carbon [20]. Research shows that the combined application of different crop straw or biochar can improve the activity of soil carbon-degrading enzymes and increase soil organic carbon content [21,22] while increasing soil carbon emissions [23,24]. Therefore, clarifying the dynamic changes of soil enzymes is key to understanding the changes in the carbon pool caused by different straws being returned to the field.

Therefore, we conducted an in situ experiment in the Loess Plateau agroforestry area and returned corn straw and *Robinia pseudoacacia* leaves to the farmland. The soil carbon emissions, soil organic carbon and its component content, and soil oxidase and hydrolase activities were measured at different growth stages of corn. We aimed to clarify the impact characteristics of the combined application of corn straw and *Robinia pseudoacacia* leaves on soil carbon-degrading enzyme activity and soil carbon dynamics (including carbon content and carbon emissions) and to reveal their response relationships. We hypothesized that (1) the incorporation of *Robinia pseudoacacia* leaves mixed with corn stalks into the field can increase soil organic carbon content and corn grain yield and that (2) the application ratio of *Robinia pseudoacacia* leaves to corn stalks significantly influences the activity of soil carbon-degrading enzymes and drives soil carbon dynamics.

## 2. Materials and Methods

### 2.1. Site Description

This study was carried out in the Wuliwan watershed of Ansai District, Shaanxi Province (36°46'18"~36°46'42" N, 109°13'56"~109°16'03" E), which is a typical area of returning farmland to forests on the Loess Plateau, with an average altitude of 1371.9 m (Figure S1). It belongs to the warm temperate semiarid continental monsoon climate, with

an average annual temperature of 9.5 °C and an average yearly rainfall of 525 mm. Rain is mainly concentrated from July to September, accounting for about 70% of the annual rainfall (Figure S6). This area is a rain-fed agricultural area. The study was conducted on sloping farmland where corn is the primary crop. The soil is derived from loess parent material and has a loose texture with low organic matter content. Specifically, the soil organic carbon content is 4.41 g·kg<sup>-1</sup> and the total nitrogen content is 0.41 g·kg<sup>-1</sup>, as detailed in Table S1.

## 2.2. Experimental Design

This study started in 2019. *Robinia pseudoacacia* leaves were collected by arranging a litter net around the farmland in the *Robinia pseudoacacia* forest. Corn straw was harvested from the corresponding corn field. Corn stalks and *Robinia pseudoacacia* leaves were cut into 2–3 cm pieces and then manually mixed and buried across a 0–40 cm soil profile after corn harvest. The research shows that the optimal return of crop straw to farmland is about 6000 kg·ha<sup>-2</sup> in the Loess Plateau [25]. In this experiment, each small quadrat was 20 m<sup>2</sup>. Our experiment involved six treatments, namely 12 kg of corn straw (Y4C0), 9 kg of corn straw and 3 kg of *Robinia pseudoacacia* leaves (Y3C1), 6 kg of corn straw and 6 kg of *Robinia pseudoacacia* leaves (Y2C2), 3 kg of corn straw and 9 kg of *Robinia pseudoacacia* leaves (Y3C1), 12 kg of *Robinia pseudoacacia* leaves (Y0C4), and a control treatment without returning materials (CK). The primary chemical properties of the returning materials are shown in Table 1.

**Table 1.** Basic chemical properties of returned materials.

Item	CK	Y4C0	Y3C1	Y2C2	Y1C3	Y0C4
SO (g·kg <sup>-1</sup> )	—	415.39 ± 5.22 ns	420.91 ± 3.37	423.08 ± 5.82	424.39 ± 3.49	415.47 ± 3.39
TN (g·kg <sup>-1</sup> )	—	7.71 ± 0.16 e	9.65 ± 0.17 d	11.57 ± 0.18 c	15.95 ± 0.23 b	21.94 ± 0.14 a
C: N	—	53.85 ± 1.45 a	43.63 ± 1.12 b	36.57 ± 1.15 c	26.61 ± 1.22 d	18.94 ± 1.13 e

Note: The data are mean ± standard error. Data that do not share a letter significantly differ between treatments ( $p < 0.05$ ). The ns indicates no significant difference between different treatments. SOC: soil organic carbon; TN: total soil nitrogen; C: N: soil carbon-nitrogen ratio. CK: without returning materials; Y4C0: corn straw only; Y3C1: combined application of three-quarters corn straw and one-quarter *Robinia pseudoacacia* leaves; Y2C2: half corn straw and half *Robinia pseudoacacia* leaves; Y1C3: combined application of three-quarters *Robinia pseudoacacia* leaves and one-quarter corn straw; Y0C4: *Robinia pseudoacacia* leaves only.

A randomized block design was employed, with each treatment replicated three times. The corridor width between the quadrats was set at 1 m, and a protective row was established. Our field planting management measures are consistent with those of local farmers. The corn variety planted in the study was Xianyu 335. The planting density of corn was 60,000 plants per hectare. A recommended full dose of fertilization was given before sowing at rates of N = 225 kg·ha<sup>-1</sup>, P = 125 kg·ha<sup>-1</sup>; nitrogen and phosphate fertilizers were urea and diammonium phosphate.

## 2.3. Sample Collection

The soil samples were taken from 0–20 cm soil layers on 14 May 2022 (seedling stage, SS), 25 June 2022 (jointing stage, JS), 25 July 2022 (heading stage, HS), 30 August 2022 (grain filling stage, GFS), and 30 September 2022 (ripening stage, RS). The soil sample was mixed and passed through a 2 mm sieve, using the five-point sampling method. Then, the soil sample was divided into two parts. One part of the fresh soil sample was stored at 4 °C to determine soil microbial biomass carbon and soil carbon-degrading enzyme activity. The other part was used to determine the soil's physical and chemical properties after air-drying.

## 2.4. Measurements and Metrics

### 2.4.1. Soil Physical Properties

Soil water content (SWC) was determined by drying the soil at 105 °C to constant weight. To determine soil bulk density (BD), the ring knife method was adopted. Soil pH was determined via the potential approach [26].

### 2.4.2. Chemical Properties and Soil Respiration

The OC content in plants and soil was determined according to the Walkley–Black method [27]. The TN content was measured following the Kjeldahl method [28]. Soil readily oxidized organic carbon (ROOC) was determined through KMnO<sub>4</sub> oxidation colorimetry [29]. Soil microbial biomass carbon (MBC) was determined with a Shimadzu TOC-TN analyzer (Shimadzu Corp, Kyoto, Japan). Acid hydrolysis was used to determine soil recalcitrant organic carbon (ROC) [30].

When taking soil samples at different growth stages of corn, the LI-8100A portable soil carbon flux automatic measurement system (LI-COR Inc., Lincoln, NE, USA) was used to measure soil respiration between 9:30 and 11:00 a.m. Before the measurement, weeds in the PVC ring were removed and measured for three consecutive days. The average value was taken as the soil respiration data of the current corn growth period.

### 2.4.3. Soil Carbon-Degrading Enzyme Activity and Soil Respiration

The activity of soil hydrolases was measured using an improved standard fluorescence technique, repeated three times for each sample [31,32]. The fluorometric substrates of BG and CBH were 4-MUB-β-D- glucoside and 4-MUB-β-D-cellobioside. After the microplate was incubated for 4 h at 25 °C in the dark, the fluorescence value was detected with a multifunctional microplate reader (Tecan Infinite M200, Vienna, Austria). The excitation and detection wavelengths were 365 nm and 450 nm, respectively. The 1:2 mixture of L-dihydroxyphenylalanine and EDTA-3Na was used for the soil oxidase PPO and PX substrates. The absorbance value at 460 nm wavelength was measured with the microplate reader after the culture [33].

## 2.5. Statistical Analysis

### 2.5.1. Soil Carbon Emission

In this experiment, soil CO<sub>2</sub> flux, cumulative emission (CE), and carbon emission efficiency (CEE) at different growth stages of corn were used to reflect the change in soil carbon emission characteristics [34]. The calculation formula is as follows:

$$CE = \sum \left[ (t_{i+1} - t_i) \times \frac{R_{i+1} + R_i}{2} \times 0.1584 \times 24 \right] \times 0.2727 \times 10$$

where  $R$  is the carbon dioxide emission rate,  $t$  represents the number of days after sowing, and  $i + 1$  and  $i$  denote two consecutive instances of monitoring. The coefficients are used for unit conversion, and the soil CO<sub>2</sub> emission rate unit is kg C · ha<sup>-2</sup>.

$$CEE = \frac{CE}{Y}$$

$CEE$  is the carbon content released per unit output [35], reflecting the emission intensity of CO<sub>2</sub>, and the unit is kg C · kg<sup>-1</sup>;  $Y$  is the yield of corn crop (kg · ha<sup>-2</sup>).

### 2.5.2. Data Analysis

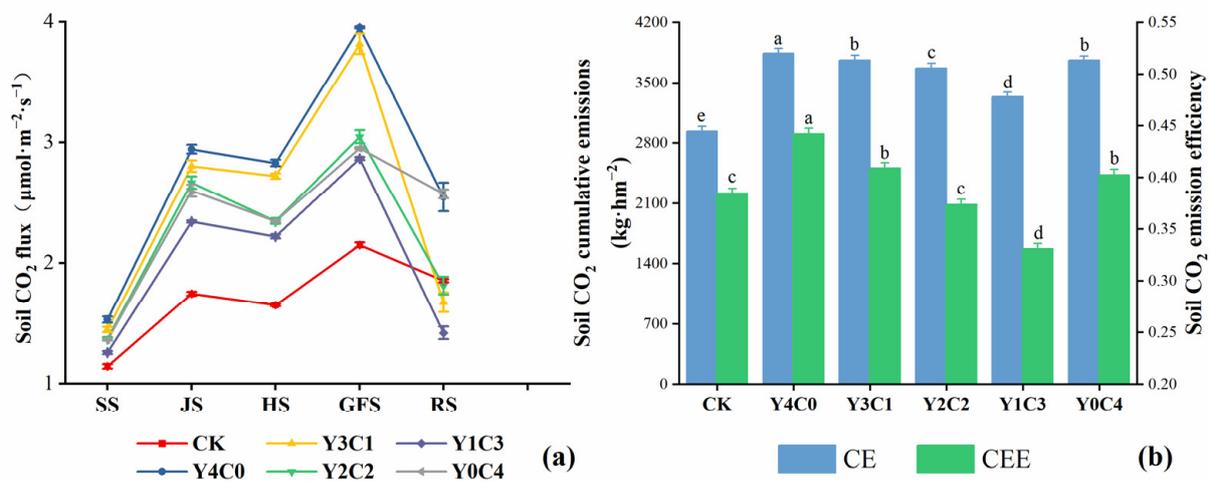
In this study, Excel 2021 was used to organize the experimental data. The ANOVA analysis was monitored after confirming the normality of data, and error heterogeneity assumptions were satisfied. The ANOVA, LSD, and correlation analysis were all conducted using R.4.2.1. The mean values in each group were compared using LSD at the

0.05 probability level. Finally, a drawing was completed with Adobe Illustrator 2022 and Photoshop 2020.

### 3. Results

#### 3.1. Characteristics of Carbon Emissions

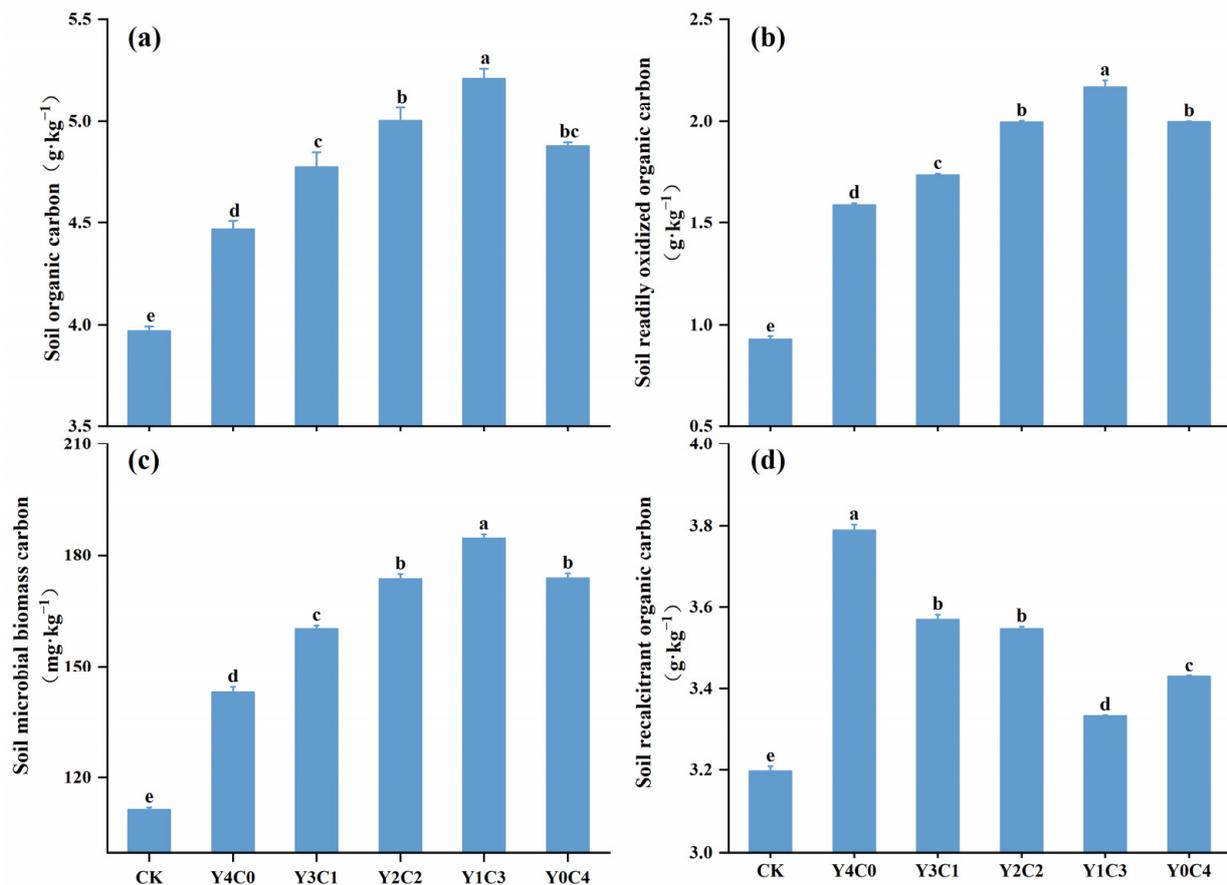
Combining corn straw and *Robinia pseudoacacia* leaves significantly impacts soil CO<sub>2</sub> emissions ( $p < 0.05$ ). In this study, compared with the CK, the combination treatment considerably increased soil CO<sub>2</sub> cumulative emissions. Specifically, the Y4C0 treatment increased the most, while the Y1C3 treatment increased the least, by 30.98% and 13.68%, respectively. This difference mainly came from soil CO<sub>2</sub> flux during the jointing and grouting stages (Figure 1a). In addition, the combined application treatment significantly increased corn grain yield, compared to the CK. Especially where Y1C3 was used, the product increased by 32.04% compared to the control (Table S2). Therefore, the response of CEE to the combination treatment was different. Compared with the CK, the Y4C0, Y3C1, and Y0C4 treatments increased CEE significantly, while the Y1C3 significantly decreased it by 16%.



**Figure 1.** Changes of soil CO<sub>2</sub> emissions under combined application of different carbon sources. (a) The variation of soil CO<sub>2</sub> flux during the growth of maize. (b) Different treatments of CE and CEE. Data that do not share a letter significantly differ between treatments ( $p < 0.05$ ). Note: Data that do not share a letter significantly differ between treatments ( $p < 0.05$ ). SS: seedling stage; JS: jointing stage; HS: heading stage; GFS: grain filling stage; RS: ripening stage. Y4C0: corn straw only; Y3C1: combined application of three-quarters corn straw and one-quarter *Robinia pseudoacacia* leaves; Y2C2: half corn straw and half *Robinia pseudoacacia* leaves; Y1C3: combined application of three-quarters *Robinia pseudoacacia* leaves and one-quarter corn straw; Y0C4: *Robinia pseudoacacia* leaves only; CK: without returning materials; CE: soil CO<sub>2</sub> cumulative emission; CEE: soil CO<sub>2</sub> emission efficiency.

#### 3.2. Characteristics of the Soil Carbon Pool

Compared to the CK, the combined application of corn straw and *Robinia pseudoacacia* leaves significantly increased SOC content. However, the effects of different components were not the same (Figure 2). Specifically, the Y1C3 treatment showed the highest increase in SOC, ROOC, and MBC content, with 31.23%, 134.42%, and 65.97%, respectively. The lowest rise in ROC content was observed in the Y1C3 treatment, at 4.24%.

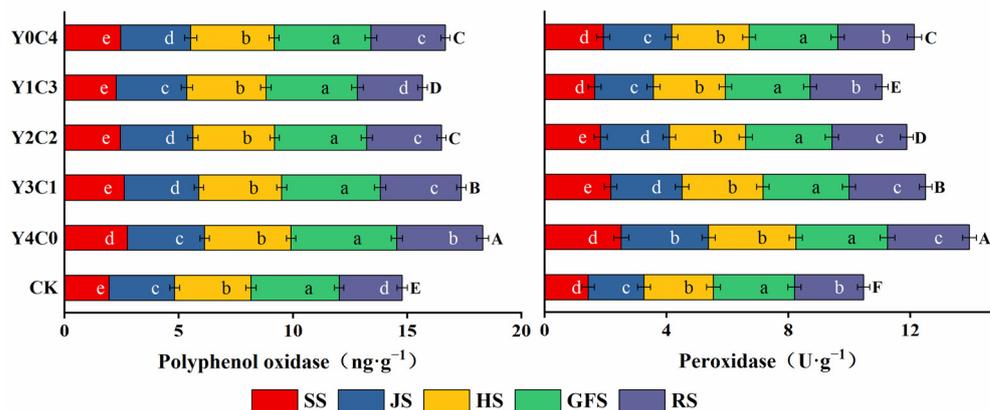


**Figure 2.** Changes in the mean content of soil organic carbon and its components under different carbon sources combined application. (a) Soil organic carbon. (b) Soil readily oxidized organic carbon. (c) Soil microbial biomass carbon. (d) Soil recalcitrant organic carbon. Data that do not share a letter significantly differ between treatments ( $p < 0.05$ ). Note: Data that do not share a letter significantly differ between treatments ( $p < 0.05$ ). Y4C0: corn straw only; Y3C1: combined application of three-quarters corn straw and one-quarter *Robinia pseudoacacia* leaves; Y2C2: half corn straw and half *Robinia pseudoacacia* leaves; Y1C3: combined application of three-quarters *Robinia pseudoacacia* leaves and one-quarter corn straw; Y0C4: *Robinia pseudoacacia* leaves only; CK: without returning materials.

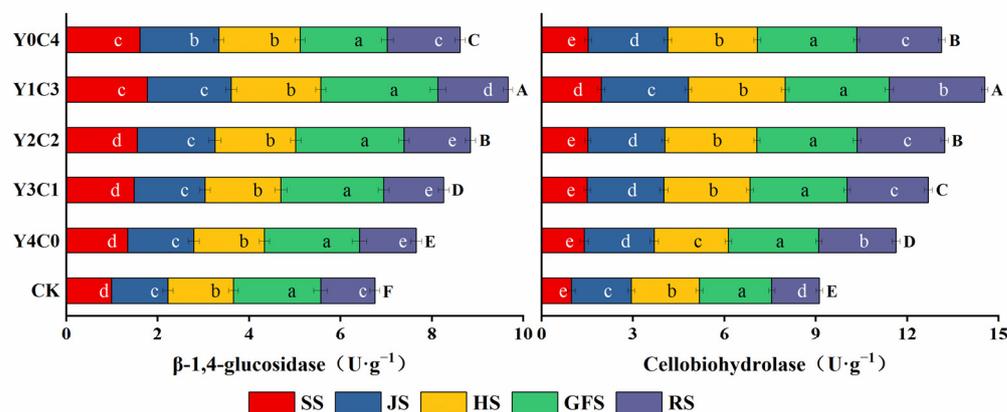
At the same time, we found that the soil organic carbon and its components for each treatment showed similar performance at different growth stages of corn (Figures S2–S5).

### 3.3. Characteristics of Soil Carbon-Degrading Enzyme Activity

The combined application of corn straw and *Robinia pseudoacacia* leaves significantly increased the activity of soil carbon-degrading enzymes (Figures 3 and 4). However, the response of hydrolytic enzymes and oxidase to the combination ratio was different. Compared with CK, polyphenol oxidase (PPO) and peroxidase (PX) activities in each treatment increased by 6.03–23.87% and 5.72–33.17%, respectively. The  $\beta$ -1, 4-glucosidase (BG) and cellobiohydrolase (CBH) increased by 13.41–43.17% and 27.64–59.60%, respectively. The difference is that, in each combination treatment, the Y1C3 treatment had a minimal increase in oxidase, while hydrolase had the highest increase. During the growth period of corn, all soil carbon-degrading enzyme activities peaked at the grain-filling stage.



**Figure 3.** Changes of soil oxidase activity in the different growth stages of corn under combined application of different carbon sources. Uppercase letters indicate significant differences between different treatments, while lowercase letters denote significant differences across various maize growth stages within the same treatment. Note: Data that do not share a letter significantly differ between treatments ( $p < 0.05$ ). SS: seedling stage; JS: jointing stage; HS: heading stage; GFS: grain filling stage; RS: ripening stage. Y4C0: corn straw only; Y3C1: combined application of three-quarters corn straw and one-quarter *Robinia pseudoacacia* leaves; Y2C2: half corn straw and half *Robinia pseudoacacia* leaves; Y1C3: combined application of three-quarters *Robinia pseudoacacia* leaves and one-quarter corn straw; Y0C4: *Robinia pseudoacacia* leaves only; CK: without returning materials.



**Figure 4.** Changes of soil hydrolase activity in the different growth stages of corn under combined application of different carbon sources. Uppercase letters indicate significant differences between different treatments, while lowercase letters denote significant differences across various maize growth stages within the same treatment. Note: Data that do not share a letter significantly differ between treatments ( $p < 0.05$ ). SS: seedling stage; JS: jointing stage; HS: heading stage; GFS: grain filling stage; RS: ripening stage. Y4C0: corn straw only; Y3C1: combined application of three-quarters corn straw and one-quarter *Robinia pseudoacacia* leaves; Y2C2: half corn straw and half *Robinia pseudoacacia* leaves; Y1C3: combined application of three-quarters *Robinia pseudoacacia* leaves and one-quarter corn straw; Y0C4: *Robinia pseudoacacia* leaves only; CK: without returning materials.

### 3.4. The Relationship between Soil Carbon-Degrading Enzymes and Soil Carbon Pool

The combined application of *Robinia pseudoacacia* leaves and corn straw had a significant impact on soil organic carbon components (ROOC and ROC), carbon degrading enzymes (PPO, PX, and CBH), and cumulative carbon emissions (CE). Soil hydrolases were significantly correlated with SOC, ROOC, and MBC, while oxidase was correlated considerably with ROC ( $p < 0.05$ ). The soil cumulative carbon emissions (CE) were significantly associated with soil carbon-degrading enzymes, especially oxidase (PPO and PX). On the

whole, soil carbon content was significantly associated with soil hydrolases. In contrast, soil carbon emissions were significantly correlated with soil oxidase.

#### 4. Discussion

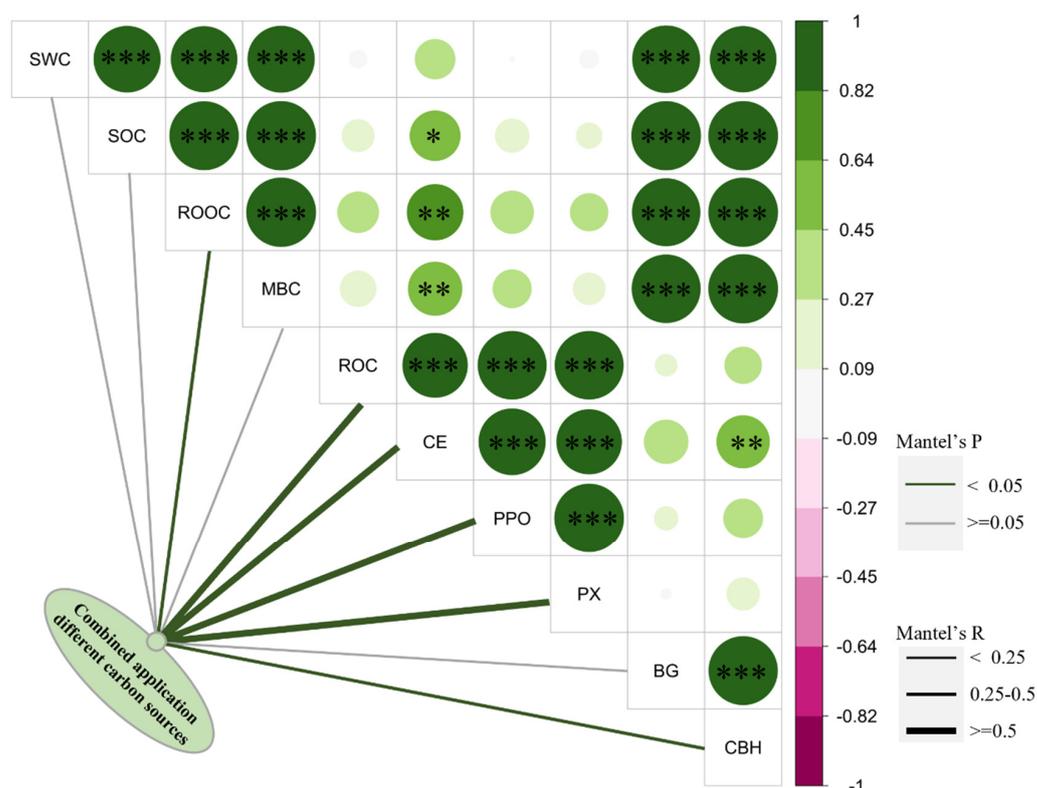
##### 4.1. Response of Soil Carbon-Degrading Enzymes to Combined Application of Different Carbon Sources

Soil carbon-degrading enzymes participate in the decomposition and transformation of soil carbon pools, playing an essential role in soil carbon pool transformation and energy flow [36,37]. In this experiment, compared with the CK, the combination of corn straw and *Robinia pseudoacacia* leaves significantly increased the activity of soil carbon-degrading enzymes. The main reason is that exogenous substances provide sufficient nutrients for crops and soil microorganisms, increasing soil microbial activity [38]. However, the application ratio significantly impacts the activity of soil carbon-degrading enzymes. Specifically, compared to returning only straw to the field (Y4C0), with an increase in the proportion of *Robinia pseudoacacia* leaves, the activity of hydrolytic enzymes significantly increases, and the activity of oxidase significantly decreases. These significant changes in soil enzymes are caused by different types of litter, such as carbon-to-nitrogen ratio, lignin-to-cellulose ratio, and other factors [39]. Oxidase is considered to be one of the main enzymes promoting the degradation of lignin and cellulose, and fertilization can promote the increase of peroxidase activity in farmland soil, accelerate the degradation of lignin, and promote the accumulation of soil organic carbon [11]. Soil hydrolase is an extracellular enzyme secreted by soil microorganisms and plants, and its activity is closely related to soil carbon, nitrogen, phosphorus, and other nutrients, and is affected by environmental factors such as underground roots, surface litter, soil microbial biomass, and soil nutrients [40]. In addition, soil C: N ratios greatly impact soil enzyme activities because extracellular enzyme activity is also determined by the elemental stoichiometry of microbial biomass [32,33]. Specifically, the microbial carbon utilization efficiency increases with the decrease in substrate C: N ratio [41]. Compared to corn straw (C: N = 53.85), *Robinia pseudoacacia* leaves (C: N = 18.94) have a lower C: N ratio. The application of *Robinia pseudoacacia* leaves reduces the soil C: N ratio and ROC content, thereby reducing the content of soil oxidase. The positive relationship between oxidase activities and ROC (Figure 5) further supports this point.

Meanwhile, different growth stages of corn also impact enzyme activity. In this experiment, the enzyme activity during the grain-filling stage was the highest compared to other growth stages. On the one hand, the grain-filling stage is the peak period of corn growth activity, and vigorous root exudates increase the activity of soil carbon degrading enzymes [42]. On the other hand, the grain-filling stage of spring maize usually coincides with the rainfall on the Loess Plateau, so the increase in soil water improves the turnover of nutrients in the soil, which is impacted by the utilization of nutrients by microorganisms, and thus increases soil enzyme activity [10].

##### 4.2. Relationship between Soil Carbon-Degrading Enzymes and Soil Carbon Dynamics

Compared to the CK, the combination of corn straw and *Robinia pseudoacacia* leaves significantly increases carbon content, despite increasing carbon emissions. The input of soil carbon sources provides sufficient substrates for microbial growth and metabolism, enhances microbial activity, increases the content of soil organic carbon and its components, and leads to an increase in soil carbon emissions [39,43]. In addition, compared with the corn straw returning treatment (Y4C0) only, as the proportion of *Robinia pseudoacacia* leaves application increased, soil carbon emissions significantly decreased, and soil carbon content significantly increased, except for the treatment of only *Robinia pseudoacacia* leaves returning to the field. The activity of soil carbon-degrading enzymes exhibited a similar change trend, possibly because stubborn substrates favor the diversity of microorganisms and their metabolic pathways [44,45]. The C: N ratio treated with Y1C3 is more suitable for soil microbial utilization [46].



**Figure 5.** Correlation analysis between soil carbon dynamics and soil carbon-degrading enzyme activity. Note: Mantel test on the relationship between soil carbon dynamics and soil carbon-degrading enzyme activity under combined application of different carbon sources; line width corresponds to Mantel's R; line color represents the significance; \* significant difference at 0.05 level, \*\* significant difference at 0.01 level, and \*\*\* significant difference at 0.001 level. SWC: soil water content; SOC: soil organic carbon; ROOC: soil readily oxidized organic carbon; MBC: soil microbial biomass carbon; ROC: soil recalcitrant organic carbon; CE: soil CO<sub>2</sub> cumulative emission. PPO: polyphenol oxidase enzyme; PX: peroxidase; BG:  $\beta$ -1,4-glucosidase; CBH: cellobiohydrolase.

This experiment's soil carbon emissions were closely related to ROC and soil oxidase activity. In contrast, soil carbon content was closely related to soil hydrolase activity (Figure 5). The reasons are as follows: (1) Low-quality carbon sources (high C: N ratio) are mainly driven by soil oxidase decomposition, which requires more energy consumption and results in higher carbon emissions [47]. (2) High-quality carbon sources (low C: N ratio) are more easily directly utilized by microorganisms to form soil organic carbon, which means that microbial residues and metabolites are critical contributors to soil organic carbon accumulation [48,49]. Compared to oxidase, MBC has a closer relationship with hydrolases, which supports this viewpoint (Figure 5). This study found that soil moisture content has a significant impact on soil organic carbon content and soil hydrolase activity, consistent with other research findings [24,50,51]. This is mainly due to the seasonal variation of soil moisture content affecting the biochemical properties of the soil [52]. Suitable soil moisture content can promote the growth of microorganisms, which in turn secrete more extracellular enzymes to break down nutrients and promote community growth [53]. This indicates that soil moisture may be one of the key environmental factors affecting the farmland ecosystem of the Loess Plateau.

## 5. Conclusions

In the dryland farmland of the Loess Plateau, the combined application of corn straw and *Robinia pseudoacacia* significantly impacts soil carbon dynamics. Response of soil

carbon-degrading enzymes to this process, where hydrolases are closely related to carbon content, and oxidase is closely associated with carbon emissions.

In different combination application ratios, corn straw and *Robinia pseudoacacia* leaves returned to the field in a mass ratio of 1:3 can significantly increase soil carbon content and carbon emission efficiency. Combination application may be a more suitable farming measure for fragile habitats in the Loess Plateau.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14040689/s1>, Figure S1: Sample site location and experimental design; Figure S2: Changes in soil organic carbon(SOC) of each treatment at different growth stages of corn; Figure S3: Changes in soil readily oxidized organic carbon(ROOC) of each treatment at different growth stages of corn; Figure S4: Changes in soil microbial biomass carbon(MBC) of each treatment at different growth stages of corn; Figure S5: Changes in soil recalcitrant organic carbon(ROC) of each treatment at different growth stages of corn; Figure S6: Daily rainfall and temperature during the experimental period; Table S1: Basic physical-chemical properties of soil in the experimental field; Table S2: Corn grain yield.

**Author Contributions:** Methodology analysis, H.L.; writing—original draft and revision process, J.L.; investigation, Z.Z.; visualization, W.L., Q.Z. and X.W.; writing—review and editing, C.R.; supervision, G.Y.; conceptualization and writing—review & revision, X.H. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Data are contained within the article and supplementary materials.

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**Conflicts of Interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

1. Cotrufo, M.F.; Soong, J.L.; Horton, A.J.; Campbell, E.E.; Haddix, M.L.; Wall, D.H.; Parton, W.J. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nat. Geosci.* **2015**, *8*, 776–779. [\[CrossRef\]](#)
2. Li, X.S.; Liang, Z.Y.; Li, Y.N.; Zhu, Y.H.; Tian, X.H.; Shi, J.L.; Wei, G.H. Short-term effects of combined organic amendments on soil organic carbon sequestration in a rain-fed winter wheat system. *Agron. J.* **2021**, *113*, 2150–2164. [\[CrossRef\]](#)
3. Lu, J.S.; Zhang, W.; Li, Y.; Liu, S.T.; Khan, A.; Yan, S.C.; Hu, T.T.; Xiong, Y.C. Effects of reduced tillage with stubble remaining and nitrogen application on soil aggregation, soil organic carbon and grain yield in maize-wheat rotation system. *Eur. J. Agron.* **2023**, *149*, 126920. [\[CrossRef\]](#)
4. Yu, Y.; Zhao, W.W.; Martinez-Murillo, J.F.; Pereira, P. Loess Plateau: From degradation to restoration. *Sci. Total. Environ.* **2020**, *738*, 140206. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Yi, Y.; Xin, Z.B.; Qin, Y.B.; Xiao, Y.L. Impact of ecological vegetation construction on the landscape pattern of a Loess Plateau Watershed. *Acta Ecol. Sin.* **2013**, *33*, 6277–6286.
6. Rouifed, S.; Handa, I.T.; David, J.F.; Hattenschwiler, S. The importance of biotic factors in predicting global change effects on decomposition of temperate forest leaf litter. *Oecologia* **2010**, *163*, 247–256. [\[CrossRef\]](#)
7. Caruso, T.; De Vries, F.T.; Bardgett, R.D.; Lehmann, J. Soil organic carbon dynamics matching ecological equilibrium theory. *Ecol. Evol.* **2018**, *8*, 11169–11178. [\[CrossRef\]](#)
8. Duan, F.Y.; Peng, P.; Yang, K.P.; Shu, Y.H.; Wang, J.W. Straw return of maize and soybean enhance soil biological nitrogen fixation by altering the N-cycling microbial community. *Appl. Soil Ecol.* **2023**, *192*, 105094. [\[CrossRef\]](#)
9. Guinet, M.; Adeux, G.; Cordeau, S.; Courson, E.; Nandillon, R.; Zhang, Y.Y.; Munier-Jolain, N. Fostering temporal crop diversification to reduce pesticide use. *Nat. Commun.* **2023**, *14*, 7416. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Li, H.Y.; Zhang, Y.H.; Sun, Y.G.; Zhang, Q.; Liu, P.Z.; Wang, X.L.; Li, J.; Wang, R. No-tillage with straw mulching improved grain yield by reducing soil water evaporation in the fallow period: A 12-year study on the Loess Plateau. *Soil Tillage Res.* **2022**, *224*, 105504. [\[CrossRef\]](#)
11. Zhou, Y.; Zhang, J.W.; Xu, L.; Nadeem, M.Y.; Li, W.W.; Jiang, Y.; Ding, Y.F.; Liu, Z.H.; Li, G.H. Long-term fertilizer postponing promotes soil organic carbon sequestration in paddy soils by accelerating lignin degradation and increasing microbial necromass. *Soil Biol. Biochem.* **2022**, *175*, 108839. [\[CrossRef\]](#)

12. Kan, Z.R.; Zhou, J.J.; Li, F.M.; Sheteiwy, M.S.; Qi, J.Y.; Chen, C.Q.; Yang, H.S. Straw incorporation interacting with earthworms mitigate N<sub>2</sub>O emissions from upland soil in a rice-wheat rotation system. *Sci. Total Environ.* **2023**, *859*, 160338. [[CrossRef](#)]
13. Erenstein, O. Crop residue mulching in tropical and semi-tropical countries: An evaluation of residue availability and other technological implications. *Soil Tillage Res.* **2002**, *67*, 115–133. [[CrossRef](#)]
14. Shang, W.H.; Razavi, B.S.; Yao, S.H.; Hao, C.K.; Kuzyakov, Y.; Blagodatskaya, E.; Tian, J. Contrasting mechanisms of nutrient mobilization in rhizosphere hotspots driven by straw and biochar amendment. *Soil Biol. Biochem.* **2023**, *187*, 109212. [[CrossRef](#)]
15. Su, Y.; Yu, M.; Xi, H.; Lv, J.L.; Ma, Z.H.; Kou, C.L.; Shen, A.L. Soil microbial community shifts with long-term of different straw return in a wheat-corn rotation system. *Sci. Rep.* **2020**, *10*, 6360. [[CrossRef](#)]
16. Kozjek, K.; Manoharan, L.; Ulrich, T.; Ahrén, D.; Hedlund, K. Microbial gene activity in straw residue amendments reveals carbon sequestration mechanisms in agricultural soils. *Soil Biol. Biochem.* **2023**, *179*, 108994. [[CrossRef](#)]
17. Lee, J. Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production. *Sci. Hortic.* **2010**, *124*, 299–305. [[CrossRef](#)]
18. Chen, J.; Luo, Y.Q.; Li, J.W.; Zhou, X.H.; Cao, J.J.; Wang, R.W.; Wang, Y.Q.; Shelton, S.; Jin, Z.; Walker, L.M.; et al. Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Glob. Chang. Biol.* **2017**, *23*, 1328–1337. [[CrossRef](#)]
19. Burns, R.G.; DeForest, J.L.; Marxsen, J.; Sinsabaugh, R.L.; Stromberger, M.E.; Wallenstein, M.D.; Weintraub, M.N.; Zoppini, A. Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biol. Biochem.* **2013**, *58*, 216–234. [[CrossRef](#)]
20. German, D.P.; Weintraub, M.N.; Grandy, A.S.; Lauber, C.L.; Rinkes, Z.L.; Allison, S.D. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biol. Biochem.* **2011**, *43*, 1387–1397. [[CrossRef](#)]
21. O'Brien, P.L.; Sauer, T.J.; Archontoulis, S.; Karlen, D.L.; Laird, D. Corn stover harvest reduces soil CO<sub>2</sub> fluxes but increases overall C losses. *GCB Bioenergy* **2020**, *12*, 894–909. [[CrossRef](#)]
22. Wei, T.; Zhang, P.; Wang, K.; Ding, R.X.; Yang, B.P.; Nie, J.F.; Jia, Z.K.; Han, Q.F. Effects of Wheat Straw Incorporation on the Availability of Soil Nutrients and Enzyme Activities in Semiarid Areas. *PLoS ONE* **2015**, *10*, 120994. [[CrossRef](#)]
23. Eberwein, J.R.; Oikawa, P.Y.; Allsman, L.A.; Jenerette, G.D. Carbon availability regulates soil respiration response to nitrogen and temperature. *Soil Biol. Biochem.* **2015**, *88*, 158–164. [[CrossRef](#)]
24. Zhou, L.Y.; Zhou, X.H.; Zhang, B.C.; Lu, M.; Luo, Y.Q.; Liu, L.L.; Li, B. Different responses of soil respiration and its components to nitrogen addition among biomes: A meta-analysis. *Glob. Chang. Biol.* **2014**, *20*, 2332–2343. [[CrossRef](#)]
25. Diao, S.P.; Gao, R.P.; Gao, Y.; Ren, Y.F.; Zhao, P.Y.; Yuan, W.; Gao, X.F. Effects of Straw Returning on Soil Hydrothermal and Yield of Maize in Loess Plateau of Inner Mongolia. *Crops* **2019**, *6*, 83–89.
26. Ren, C.J.; Chen, J.; Lu, X.J.; Doughty, R.; Zhao, F.Z.; Zhong, Z.K.; Han, X.H.; Yang, G.H.; Feng, Y.Z.; Ren, G.X. Responses of soil total microbial biomass and community compositions to rainfall reductions. *Soil Biol. Biochem.* **2018**, *116*, 4–10. [[CrossRef](#)]
27. Nelson, D.W.; Sommers, L.E. Total carbon, organic carbon, and organic matter. In *Methods of Soil Analysis; Part 2. Agronomy Monographs 9*; Page, A.L., Ed.; ASA and SSSA: Madison, WI, USA, 1982; pp. 539–579.
28. Bremner, J.M.; Mulvaney, C.S. Nitrogen-Total. In *Methods of Soil Analysis; Part 2. Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; American Society of Agronomy, Soil Science Society of America: Madison, WI, USA, 1982; pp. 595–624.
29. 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.* **1995**, *46*, 1459–1466. [[CrossRef](#)]
30. Rovira, P.; Vallejo, V.R. Examination of thermal and acid hydrolysis procedures in the characterization of soil organic matter. *Commun. Soil Sci. Plant Anal.* **2000**, *31*, 81–100. [[CrossRef](#)]
31. Saiya-Cork, K.R.; Sinsabaugh, R.L.; Zak, D.R. The effects of long-term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biol. Biochem.* **2002**, *34*, 1309–1315. [[CrossRef](#)]
32. Xu, Z.W.; Yu, G.R.; Zhang, X.Y.; He, N.P.; Wang, Q.F.; Wang, S.Z.; Wang, R.L.; Zhao, N.; Jia, Y.L.; Wang, C.Y. Soil enzyme activity and stoichiometry in forest ecosystems along the North-South Transect in eastern China (NSTEC). *Soil Biol. Biochem.* **2017**, *104*, 152–163. [[CrossRef](#)]
33. Deforest, J.L.; Moorhead, D.L. Effects of elevated pH and phosphorus fertilizer on soil C, N and P enzyme stoichiometry in an acidic mixed mesophytic deciduous forest. *Soil Biol. Biochem.* **2020**, *150*, 107993. [[CrossRef](#)]
34. Chi, Y.B.; Yang, P.L.; Ren, S.M.; Ma, N.; Yang, J.; Xu, Y. Effects of fertilizer types and water quality on carbon dioxide emissions from soil in wheat-maize rotations. *Sci. Total Environ.* **2020**, *698*, 134010. [[CrossRef](#)] [[PubMed](#)]
35. Zhang, J.T.; Tian, H.Q.; Shi, H.; Zhang, J.F.; Wang, X.K.; Pan, S.F.; Yang, J. Increased greenhouse gas emission intensity of major croplands in China: Implications for food security and climate change mitigation. *Glob. Chang. Biol.* **2020**, *26*, 6116–6133. [[CrossRef](#)] [[PubMed](#)]
36. Chen, Y.L.; Zhang, Y.H.; Li, C.; Xu, R.S.; Pei, Z.R.; Li, F.C.; Wu, Y.H.; Chen, F.; Liang, Y.R.; Li, Z.H.; et al. Linking soil organic carbon dynamics to microbial community and enzyme activities in degraded soil remediation by reductive soil disinfection. *Appl. Soil Ecol.* **2023**, *189*, 104931. [[CrossRef](#)]
37. Xu, H.W.; Qu, Q.; Li, G.W.; Liu, G.B.; Geissen, V.; Ritsema, C.J.; Xue, S. Impact of nitrogen addition on plant-soil-enzyme C–N–P stoichiometry and microbial nutrient limitation. *Soil Biol. Biochem.* **2022**, *170*, 108714. [[CrossRef](#)]
38. Maarastawi, S.A.; Frindte, K.; Bodelier, P.L.E.; Knief, C. Rice straw serves as the additional carbon source for rhizosphere microorganisms and reduces root exudate consumption. *Soil Biol. Biochem.* **2019**, *135*, 235–238. [[CrossRef](#)]

39. Curtright, A.J.; Tiemann, L.K. Chemical identity of carbon substrates drives differences in denitrification and NO reduction within agricultural soils. *Soil Biol. Biochem.* **2023**, *184*, 109078. [[CrossRef](#)]
40. Peng, X.Q.; Wang, W. Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate grasslands of northern China. *Soil Biol. Biochem.* **2016**, *98*, 74–84. [[CrossRef](#)]
41. Manzoni, S.; Jackson, R.B.; Trofymow, J.A.; Porporato, A. The global stoichiometry of litter nitrogen mineralization. *Science* **2008**, *321*, 684–686. [[CrossRef](#)]
42. Singh, S.; Kumar, S. Seasonal changes of soil carbon fractions and enzyme activities in response to winter cover crops under long-term rotation and tillage systems. *Eur. J. Soil Sci.* **2020**, *72*, 886–899. [[CrossRef](#)]
43. Brown, R.W.; Jones, D.L. Plasticity of microbial substrate carbon use efficiency in response to changes in plant carbon input and soil organic matter status. *Soil Biol. Biochem.* **2024**, *188*, 109230. [[CrossRef](#)]
44. Ding, X.L.; He, H.B.; Zhang, B.; Zhang, X.D. Plant-N incorporation into microbial amino sugars as affected by inorganic N addition: A microcosm study of 15 N-labeled maize residue decomposition. *Soil Biol. Biochem.* **2011**, *43*, 1968–1974. [[CrossRef](#)]
45. Bai, Z.; Bodé, S.; Huygens, D.; Zhang, X.D.; Boeckx, P. Kinetics of amino sugar formation from organic residues of different quality. *Soil Biol. Biochem.* **2013**, *57*, 814–821. [[CrossRef](#)]
46. Yeboah, S.; Zhang, R.Z.; Cai, L.Q.; Jun, W. Different carbon sources enhance system productivity and reduce greenhouse gas intensity. *Plant Soil Environ.* **2018**, *64*, 463–469. [[CrossRef](#)]
47. Kominoski, J.S.; Rosemond, A.D.; Benstead, J.P.; Ladislavgulis, V.; Maerz, J.C.; Manning, D.W.P. Low-to-moderate nitrogen and phosphorus concentrations accelerate microbially driven litter breakdown rates. *Ecol. Appl.* **2015**, *25*, 856–865. [[CrossRef](#)] [[PubMed](#)]
48. Liang, C.; Schimel, J.P.; Jastrow, J.D. The importance of anabolism in microbial control over soil carbon storage. *Nat. Microbiol.* **2017**, *2*, 17105. [[CrossRef](#)] [[PubMed](#)]
49. Miltner, A.; Bombach, P.; Schmidt-Brücken, B.; Kästner, M. SOM genesis: Microbial biomass as a significant source. *Biogeochemistry* **2012**, *111*, 41–55. [[CrossRef](#)]
50. Liu, Z.H.; Huang, F.Y.; Wang, B.F.; Li, Z.Y.; Zhao, C.X.; Ding, R.X.; Yang, B.P.; Zhang, P.; Jia, Z.K. Soil respiration in response to biotic and abiotic factors under different mulching measures on rain-fed farmland. *Soil Tillage Res.* **2023**, *232*, 105749. [[CrossRef](#)]
51. Zhang, J.L.; Zhang, J.L.; Zhao, X.J.; Liao, Y.C.; Jing, X.L.; Xue, J. Effects of different tillages on soil CO<sub>2</sub> flux, and its relation to soil moisture and soil temperature in dry-land maize field. *Agric. Res. Arid. Areas* **2018**, *36*, 88–93.
52. Potts, J.; Brown, R.W.; Jones, D.L.; Cross, P. Seasonal variation is a bigger driver of soil faunal and microbial community composition than exposure to the neonicotinoid acetamiprid within Brassica napus production systems. *Soil Biol. Biochem.* **2023**, *184*, 109088. [[CrossRef](#)]
53. Su, F.; Wei, Y.; Wang, F.; Guo, J.; Zhang, J.; Wang, Y.; Guo, H.; Hu, S. Sensitivity of plant species to warming and altered precipitation dominates the community productivity in a semiarid grassland on the Loess Plateau. *Ecol. Evol.* **2019**, *9*, 7628–7638. [[CrossRef](#)] [[PubMed](#)]

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