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Does the Biennial Straw Return Have an Identical Characteristic of Soil Organic Carbon Sequestration as the Annual? A Case Study of Cornfield in Northeast China

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Abstract: Straw return is a common cultivation to improve soil fertility and realize sustainable agricultural development. However, the effect of returning interval on the corn straw humification process in northeast China is little known. In this study, a four-year field trial was conducted to investigate the characteristics of soil carbon sequestration under the annual deep straw return (T1), the biennial deep straw return (T2), and the non-straw return (T3) in Jilin Province, China. In order to precisely evaluate the soil organic carbon density (SOCD), each soil horizon was divided differently according to the actual situation, rather than a fixed thickness. The results show that both the annual and the biennial deep straw return had a significantly positive influence on the content of soil organic carbon (SOC), humic acid, fulvic acid, and humin in the plough pan (straw-applied horizon), compared to the no-straw return. SOC of the cambic horizon and the C horizon in annual straw return was 28.78%, 47.44% higher than the biennial straw return, but it was 27.58% lower in the plough pan. The SOCD in the plough pan in the biennial straw return was higher than the annual straw return, but their difference in the entire soil profile was not significant. However, the conversion rate of straw carbon to SOC was 18.42% in the annual straw return and 21.05% in the biennial straw return. The straw return amount was not a key factor affecting the SOC sequestration in the cold area; it was restricted by the comprehensive effects of the cold weather, the intensity of soil disturbance, C/V and the initial SOC content. In conclusion, the biennial deep straw return was a better management tool, as it generally had an identical quality and quantity of soil organic carbon and a higher straw conversion rate relative to the annual deep straw return.

Keywords: corn straw; returning interval; soil organic carbon density; humification; conversion rate

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Citation: Liu, J.; Zhao, X.; Zhang, Z.; Zhao, C.; Huang, N.; Wang, H. Does the Biennial Straw Return Have an Identical Characteristic of Soil Organic Carbon Sequestration as the Annual? A Case Study of Cornfield in Northeast China. *Agronomy* **2024**, *14*, 1174. https://doi.org/10.3390/agronomy14061174

Received: 28 April 2024
Revised: 23 May 2024
Accepted: 28 May 2024
Published: 30 May 2024

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1. Introduction

Spring corn is a dominant crop in Jilin Province, Northeast China. The total yield of straw has risen sharply in recent years as planting area and yield increase. Straw return is recommended by the local government to improve soil fertility. And numerous studies have also proven that it is beneficial for increasing water holding capacity [1,2], soil carbon sink [3,4], microbial activity [5,6], and mineral nutrient availability [7,8]. At present, most farmers in the area adopt the immediate straw mulch after harvest in the autumn [9], while its negative impact is gradually emerging because the cold climate limits the straw decomposition rate [10,11]. Straw return once a year makes a large amount of undecomposed straw accumulate on the soil surface, which negatively influences sowing. And it also decreases germination, seedling growth, and root growth because of the low soil temperature caused by straw mulching return [12]. Based on the above considerations, a deep straw return has been suggested in recent years [13]. Tang et al. (2023) proposed a

ditch-burying return management in the cold region of Northeast China and pointed out that returning in spring was more beneficial for improving the soil physical properties than returning in autumn [14]. Wu et al. (2022) conducted a field experiment of deep-injected straw incorporation, and found that this return mode improved soil structure, water retention, and available nutrients in 20–40 cm [15]. However, a deep straw return once a year increases field operation times and economic investment. What's more important is that straw return once a year leads to all straw turning back into soil, which can't provide enough feed for livestock. Thus, an optimized straw return strategy in this corn planting area should be developed to obtain a more significant effect.

Prolonging the interval of straw return is an achievable and simple approach to solving the contradiction [16]. But there is little literature in this area. The characteristics of soil carbon sequestration in such situations are still unknown. Does the reduction in straw return amount caused by extending the return interval impact the process greatly? How much difference is there in SOC stock, compared to the annual straw return? All of these should be studied further.

When the carbon sequestration capacity of soil is evaluated, most focus on the labile C pool in the surface horizon [17]. Even though some soil profiles have been studied, they are, generally, only to a depth of less than 50 cm [18]. The vertical distribution of soil organic carbon (SOC), in particular variation in the recalcitrant C pool under deep return, is not well understood. Moreover, most studies frequently adopt the traditional fixed-depth (FD) method to calculate SOC stocks [19,20]. However, FD overestimates the SOC stock in the topsoil and underestimates it in the subsoil because the soil bulk density and SOC concentration are not uniform in the soil profile. Thus, a proper method of soil stratification should be developed to correctly evaluate the C sequestration capacity in the soil profile, especially in the deeper horizons.

In the present contribution, a 4-year field study of annual and biennial deep straw return was conducted to exactly evaluate their effect on SOC sequestration in the whole soil profile. The differences of SOC between the two return intervals were analyzed in each actual soil genetic horizon, as well as the composition of organic carbon. Soil organic carbon density (SOCD) and the conversion rate of straw carbon to SOC was calculated in the soil profile of 0–100 cm. We hypothesized that the biennial deep straw return had the same effect as the annual, because the amount of annual straw return was too large to decompose in the local climate with low temperature and low humidity, as well as the high background of the initial SOC content. The straw saved in the biennial return can be used for other purposes, such as livestock breeding and paper production, on the basis that the carbon sequestration capacity of the soil is not affected.

2. Materials and Methods

Study Site. The research was conducted in the village of Wanggou (43°37′12″ N, 124°54′00″ E), Gongzhuling, Jilin Province, in the northeast of China. The site is characterized by a continental monsoon climate with a mean annual temperature of 5.6 °C and an average annual precipitation of 594.8 mm (over 60% occurring from June to August). The soil is classified as Phaeozem according to the FAO soil taxonomy with a pH of 6.72, soil organic matter of 12.13 g kg^{-1}, available N of 135.4 mg kg^{-1}, available P of 26.8 mg kg^{-1}, and available K of 158.62 mg kg^{-1} in the 0–20 cm soil profile. Before the experiment, maize was continuously planted for at least 30 years without straw return. The geographical location of the field experiment is shown in Figure 1.

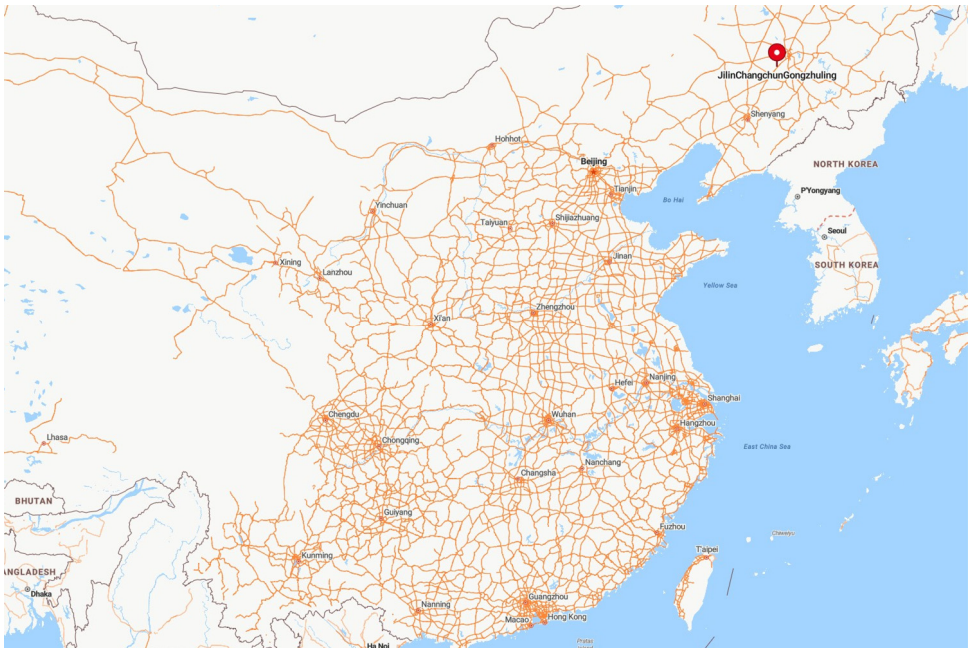


Figure 1. The geographical location of the field experiment.

Field Experiment. A field trial was conducted for 4 consecutive years from 2020 to 2023. And single-season spring corn was planted each year. Three treatments were set up in a completely randomized block design with three replicates after the harvest of the previous season in October, 2019. The treatments were (i) an annual straw return combined with deep burying (T1), in which corn straw was applied in 2019, 2020, 2021, and 2022 (4 times) during the experiment; (ii) a biennial straw return combined with deep burying (T2), in which corn straw was applied in 2019 and 2021 (2 times) during the experiment; (iii) conventional tillage without straw application (T3), in which all the previous straw residue was completely removed out of the field during the four years. The size of each plot was 70 m^2 with a length of 10 m and a width of 7 m. An uncultivated buffer of 3 m was established between plots. In the straw return treatments, the corn straw was returned in the autumn after the corn was harvested and the amount was 9500 kg/hm^2/year, which was close to the local average yield of corn straw. A deep soil cultivator displaced a soil mass with a 30 cm width and a 30 cm depth to the soil surface next to the working place in a single operation. Then the straw, which was crushed into a length of 5 cm by a crusher, was spread evenly at the bottom of the ditch. And the ditch was manually back filled with the original soil. The next one was immediately carried out closely next to the previous one. Therefore, the straw residual was returned to the level of 30 cm below the soil surface. Finally, the soil was compacted with a roller and kept fallow for six months until next spring.

After a tillage of 10 cm depth and a basal fertilizer of 1000 kg/hm^2 was conducted in all treatments at the end of April in every year, spring corn was planted traditionally. The corn hybrid variety XY335 was sown at a density of 6.5×10^4 plants/hm^2.The fertilizer was consisted of 28% N, 15% P_2O_5 and 12% K_2O and produced by DQL Chemical Fertilizer Co., Ltd. China (Shijiazhuang, China). No irrigation was applied during the whole plant growth. Weeds were controlled by herbicides (atrazine and acetochlor) according to traditional methods, and all farming practices were consistent in all treatments. The corn was harvested in early October, every year.

Soil Horizon Division. Sampling was conducted after harvest on 15 October 2023. Three points evenly distributed in each plot were randomly selected. The soil profile at each point was excavated to a 1 m depth and divided into 4 horizons of A, P, B, and C from the top to the bottom according to the actual soil characteristics. Horizon A was the cultivated horizon and horizon P was the plough pan, which jointly formed the topsoil.

Horizon B was the cambic horizon and horizon C was the C horizon. The thickness of each horizon at each point was measured and its average was calculated to represent the value of each treatment. The bulk density of each horizon is listed in Appendix A. Since T3 maintained the original farming management, its soil profile characteristics changed minimally compared with the initial soil before the test. The details are shown in Figure 2.

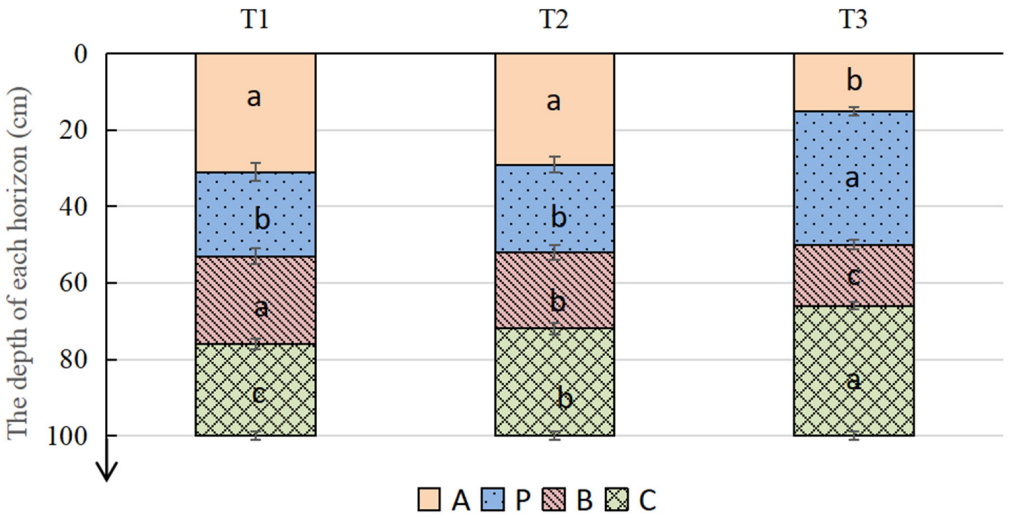


Figure 2. Soil stratification and corresponding depths in 2023 after 4 years of spring corn cultivation (2020, 2021, 2022, and 2023). A is the cultivated horizon, P is the plough pan, B is the cambic horizon and C is the C horizon. T1 is the annual straw return (2019, 2020, 2021, and 2022), T2 is the biennial straw return (2019 and 2021), and T3 is the no-straw return. Differing lowercase letters indicate a statistically significant difference between treatments in the same soil horizon (LSD, $p < 0.05$).

Sample Collection. After soil stratification was determined, a soil block of each horizon (an approximately 5 cm width × 15 cm length × the depth of the horizon) was extracted by spade from the top to the bottom. Three samples were taken from each horizon and mixed. Finally, 1 kg of soil was retained by the quartering method. All samples were packed into plastic containers and transported to the lab, where they were air-dried, sieved and stored in airtight containers at room temperature for analysis. The samples for soil bulk density were taken simultaneously using a soil cutting ring.

Soil Analyses. The soil bulk density was determined by the cutting ring method and the results are shown in Table A1. The SOC content was analyzed by the H_2SO_4-K_2CrO_4 external heating method [21]. The SOCD was calculated according to Equation (1) [22]. Fulvic acid (FA-C) and humic acid (HA-C) in the soil were separated by NaOH + $Na_4P_2O_7$ and measured by a TOC-VCPH Analyzer (Shimadzu) [23]. Extractable carbon in the humus (HE-C) was calculated as the sum of FA-C and HA-C, and humin (HM-C) was calculated as the difference between SOC and HE-C. SOCD was calculated as follows:

$$SOCD = SOC \times \rho \times H \times (1 - S) \times 10^{-2} \quad (1)$$

where SOCD is soil organic carbon density (kg/m^2), SOC is the content of soil organic carbon (g/kg), ρ is soil bulk density (g/cm^3), H is soil depth (cm), and S is the average content of gravel (%).

Statistical Analyses. Data processing and drawing were accomplished using Excel 2007. SPSS17.0 (IBM SPSS Statistics Inc., Chicago, IL, USA) was used to perform an analysis of variance. One-way ANOVA and least significant difference tests (LSD) were used to test the significance of differences in the SOC content, HA-C, FA-C, HM-C, HE-C, and HD among different treatments and soil horizons. The differences were declared statistically significant at the 0.05 level by the least significant difference test (LSD), and the values in the table are expressed as the mean ± standard deviation.

3. Results

3.1. The Distribution Characteristics of SOC

T1 and T2 generally increased the content of SOC in the entire profile compared to T3 (Figure 3). However, this influence greatly varied among soil depths. In horizon A, T1 and T2 increased the SOC by 0.71 and 1.06 g/kg, respectively, compared to T3. But this discrepancy was not statistically significant ($p > 0.05$). In horizon P, the SOC under T1 and T2 was significantly higher than that of T3 by 0.72 and 4.30 g/kg, respectively. In horizons B and C, it was significantly higher under T1 than that of T2 and T3 with an average increase of 2.89 and 2.68 g/kg ($p < 0.05$), respectively.

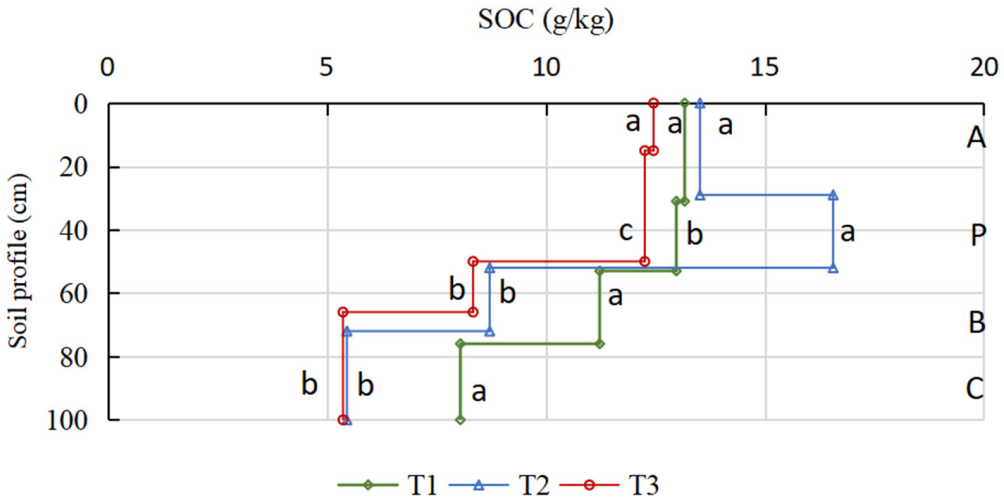


Figure 3. SOC concentration in the cultivated horizon (A), the plough pan (P), the cambic horizon (B), and the C horizon (C) in 2023, after 4 years of spring corn cultivation under annual deep straw return (T1), biennial deep straw return (T2), and no-straw return (T3) in Northeast China (g/kg). Different lowercase letters indicate a statistically significant difference between treatments in the same soil horizon (LSD, $p < 0.05$).

Across the entire soil profile, SOC decreased with soil depth except for T2, in which SOC in horizon P was significantly higher than that in horizon A (by 3.04 g/kg, $p < 0.05$). The top two horizons contained much more SOC than that in horizons B and C.

3.2. The Distribution Characteristics of SOCD

In horizon A, SOCD values were much greater under T1 and T2 than that of T3 by 2.20 and 2.42 kg/m^2, respectively ($p < 0.05$, Figure 4). In horizon B, SOCD under T1 was significantly higher than T2 and T3 by 1.34 and 1.47 kg/m^2, respectively ($p < 0.05$). In horizon P, SOCD under T3 was significantly higher than that ofT1 and T2 by 3.29 and 2.01 kg/m^2, respectively ($p < 0.05$), because this horizon was thicker. In addition, SOCD in horizon P under T2 was significantly higher than that under T1 by 1.28 kg/m^2 ($p < 0.05$). Total SOCD in the entire profile was higher under T1 and T2 than that of T3 with an increase of 0.28 and 0.16 kg/m^2, respectively. However, no obvious difference was observed between T1 and T2.

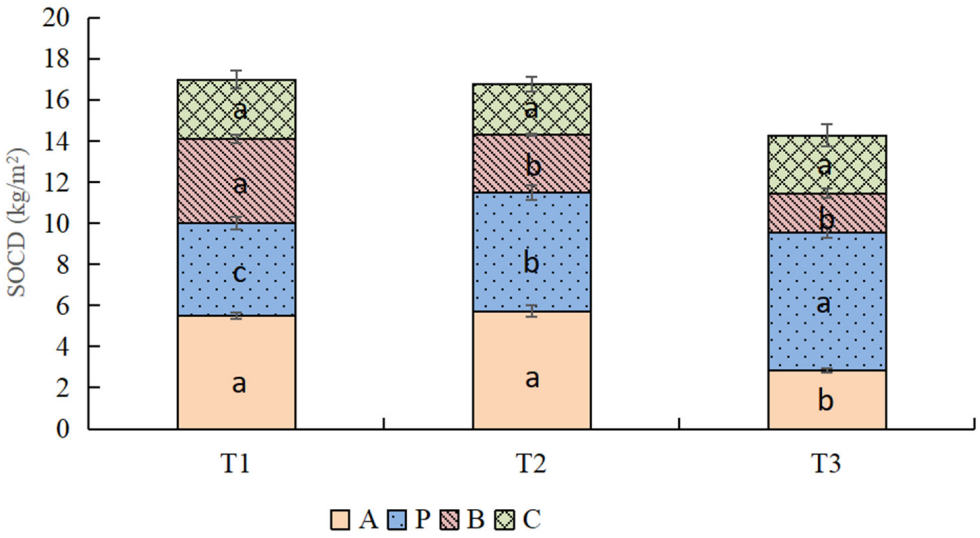


Figure 4. SOCD in the cultivated horizon (A), the plough pan (P), the cambic horizon (B), and the C horizon (C) in 2023, after 4 years of spring corn cultivation under annual straw return (T1), biennial straw return (T2), and non-straw return (T3) in Northeast China (g/kg). Different lowercase letters indicate a statistically significant difference between treatments in the same soil horizon (LSD, $p < 0.05$).

The distribution of SOCD in the soil profiles varied among treatments (Figure 4). In T3, SOCD first increased and then decreased with greater depth and reached the highest point of 7.80 kg/m^2 in horizon P. However, it showed a generally decreasing trend in T1 and T2. SOCD in horizons A and P, under T1 and T2, was significantly higher than other horizons. The "topsoil/full profile" ratio increased from 0.20 in no straw return treatment to 0.33–0.34 in deep straw return treatments. The ratios of SOCD in horizon B to the entire profile under T1 and T2 were 0.24 and 0.17, respectively, which were also higher than that of T3 (0.16). This indicates that even if straw is returned below the surface soil, its effect on the SOCD of the topsoil is also obvious.

3.3. The Composition and Characteristics of HE-C

Generally, the HA-C in T1 and T2 was higher than T3 in every soil horizon. T1 had higher values of 0.36, 1.52, and 0.41 g/kg in horizons A, P, and B than T3, and the difference was significant ($p < 0.05$, Figure 5). T2 also significantly increased HA-C by 0.23, 1.22, and 0.24 g/kg in horizons A, P, and C, respectively, compared with T3 ($p < 0.05$). No significant difference was observed between T1 and T2 ($p > 0.05$). Distribution of HA-C in the soil profile for all treatments followed the trend of increasing first and then decreasing, with the highest value appearing in horizon P. The difference between horizons A and P was significant for both T1 and T2 ($p < 0.05$).

Deep straw return significantly affected FA-C in the soil profile except horizon A ($p < 0.05$, Figure 4). In T1, FA-C was higher by 0.67 g/kg in horizon P and 0.45 g/kg in horizon B than that of T3. In T2, it was 0.82 and 0.24 g/kg higher than that of T3 in horizon P and C, respectively. Return intervals did not significantly affect FA-C except in horizon A, for which T2 showed a significant increase of 0.31 g/kg relative to T1 ($p < 0.05$). FA-C in horizon P was 0.61 and 0.45 g/kg higher than horizon A in T1 and T2, respectively, ($p < 0.05$), while it decreased with greater soil depth in T3.

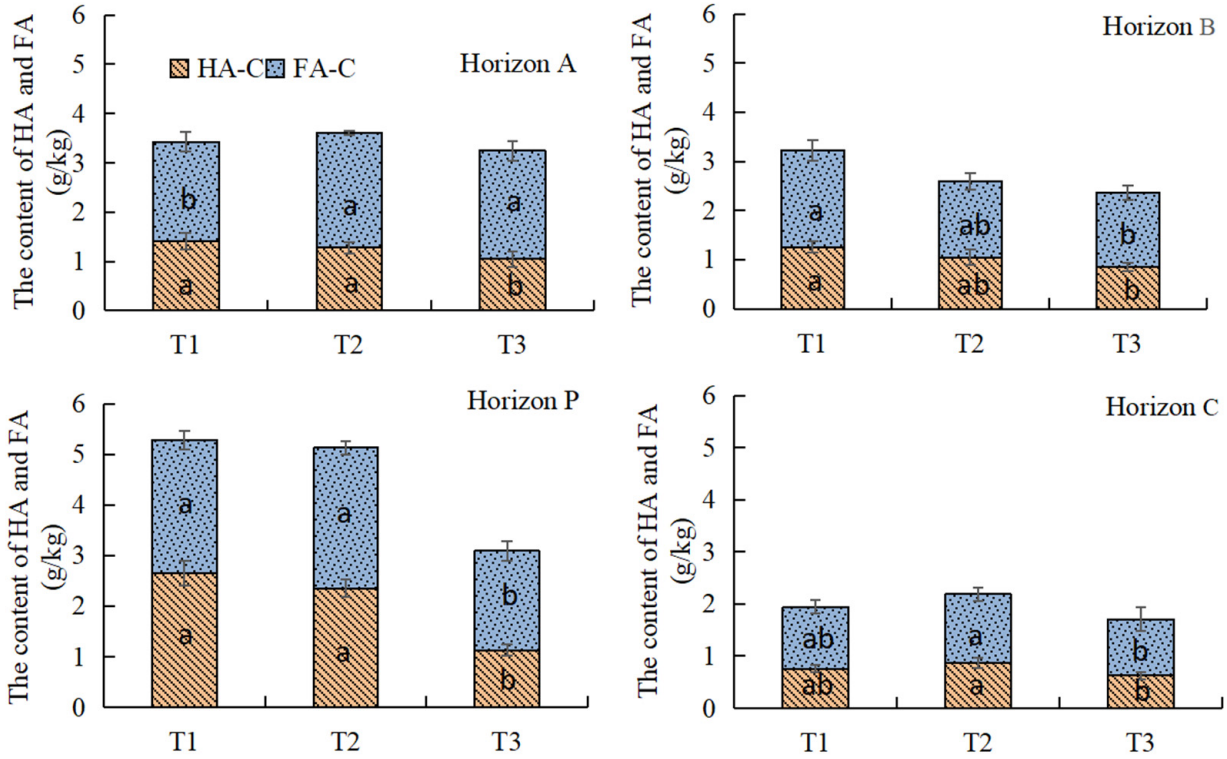


Figure 5. The content of humic acid (HA-C) and fulvic acid (FA-C) in the cultivated horizon (A), the plough pan (P), the cambic horizon (B), and the C horizon (C) in 2023, after 4 years of spring corn cultivation under annual straw return (T1), biennial straw return (T2), and non-straw return (T3) in Northeast China (g/kg). Different lowercase letters indicate a statistically significant difference for the same item between treatments in the same soil horizon (LSD, $p < 0.05$).

In all soil horizons, the content of HE-C was significantly increased by 0.18–2.19 mg/kg in T1 and 0.23–2.04 mg/kg in T2, compared to T3 ($p < 0.05$, Figure 5). The return intervals showed minimal difference in certain horizons. HE-C in T1 was significantly higher than T2 by 0.63 g/kg in horizon B, while HE-C in T2 was significantly higher than T1 by 0.18 and 0.25 mg/kg in horizons A and C, respectively ($p < 0.05$). Nevertheless, HE-C in horizon P was not significantly different among treatments. The distribution of HE-C in the soil profile declined with greater soil depth in T3, but the difference was not significant in the top two horizons. However, deep straw return promoted HE-C in horizon P by an increase of 1.85 and 1.52 g/kg, respectively, compared to horizon A.

Straw return increased HA-C/FA-C in horizons A and P (Figure 6). It was significantly higher by 0.23 and 0.36 in T1 than T3, respectively. T2 also had a significant higher of 0.26 than T3 in horizon P ($p < 0.05$). The different return intervals had the same influence in the soil horizons except for horizon A, in which the ratio of T1 was significantly higher by 0.16 than that of T2. In the whole soil profile, T1 and T2 had the highest level of HA-C/FA-C in horizon P ($p < 0.05$), while it changed minimally for T3.

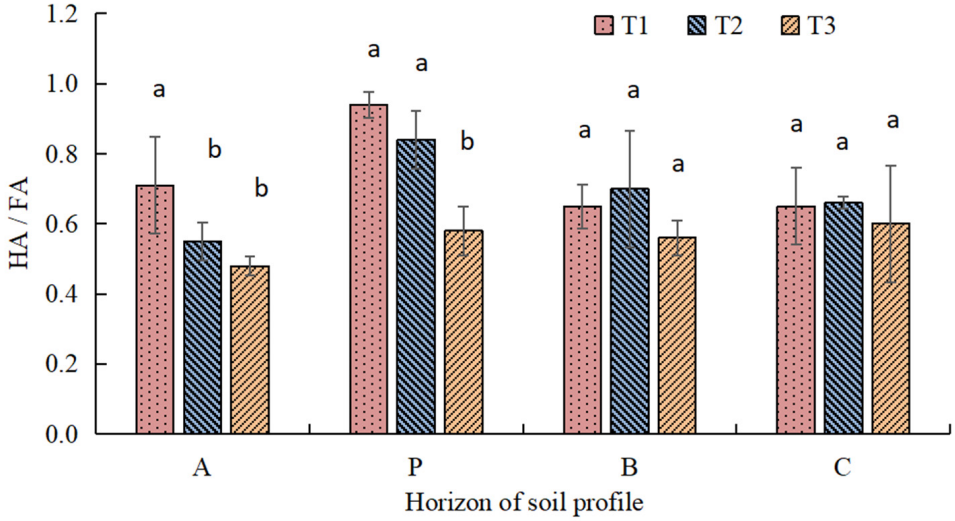


Figure 6. HA/FA in the cultivated horizon (A), the plough pan (P), the cambic horizon (B), and the C-horizon (C) in 2023, after 4 years of spring corn cultivation under annual straw return (T1), biennial straw return (T2), and non-straw return (T3) in Northeast China. Different lowercase letters indicate a statistically significant difference between treatments in the same soil horizon (LSD, $p < 0.05$).

3.4. The Distribution Characteristics of HM-C

Deep straw return significantly increased the content of HM-C ($p < 0.05$, Figure 7). Compared to T3, it was increased by 1.61 g/kg in horizon A, 3.01 g/kg in horizon P, 1.95 g/kg in horizon B, and 0.48 g/kg in horizon C in T1. For T2, it was increased by 0.79, 1.27, 1.20, and 0.50 g/kg, respectively. Significant increases of 0.82 g/kg in horizon A, 1.74 g/kg in horizon P, and 0.75 g/kg in horizon B were observed in T1, compared to T2. HM-C in the soil profile in T3 decreased with greater soil depth, while straw return treatments had higher HM-C in horizon P.

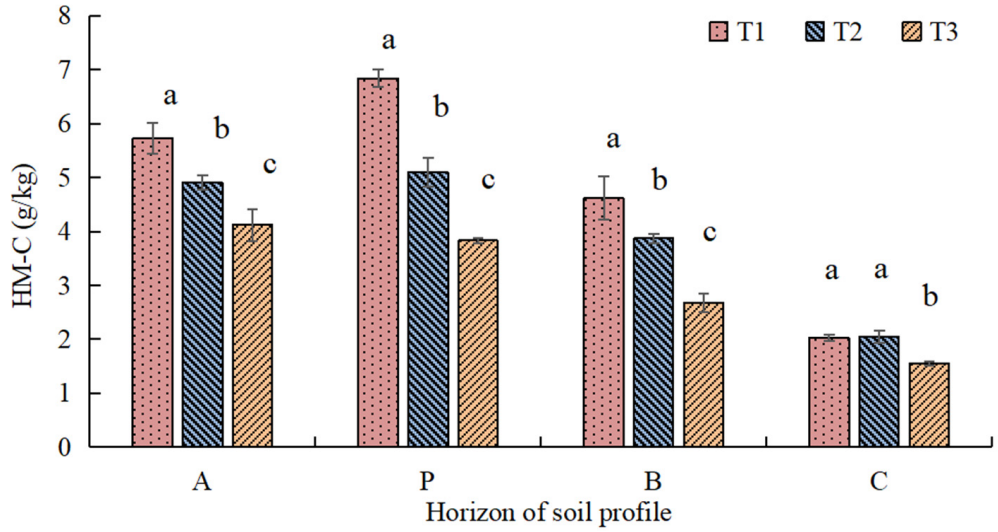


Figure 7. The content of HM-C in the cultivated horizon (A), the plough pan (P), the cambic horizon (B), and the C horizon (C) in 2023, after 4 years of spring corn cultivation under annual straw return (T1), biennial straw return (T2), and non-straw return (T3) in Northeast China (g/kg). Different lowercase letters indicate a statistically significant difference between treatments in the same soil horizon (LSD, $p < 0.05$).

3.5. Proportion of Straw Carbon Converted to SOC

The amount of SOCD in 0–100 cm soil profile was obtained by summing SOCD in each horizon and the results are shown in the Table 1. Compared to T3, T1 had the greatest increase of 0.28 kg/m^2, while T2 increased by 0.16 kg/m^2, which was 0.12 kg/m^2 lower than T1. However, each treatment had a different total straw returning amount, the proportion of straw carbon converted to SOC was different. It was 21.05% in T2 and 18.42% in T1, because T2 had a relatively small straw retuning amount.

Table 1. Carbon return by straw and accumulation in soil after 4 years of spring corn cultivation (2020, 2021, 2022, and 2023). T1 indicates the annual straw return (2019, 2020, 2021, and 2022), T2 is the biennial straw return (2019 and 2021), and T3 is the conventional tillage without straw return.

Treatments	Total Straw Returning (kg/hm^2)	Total Straw-C Returning (kg/hm^2) *	SOCD in Soil Profile of 0–100 cm (kg/m^2)	SOCD Increased (kg/m^2)	Conversion Rate from Straw Carbon to SOC (%)
T1	38,000	15,200	16.90	0.28	18.42
T2	19,000	7600	16.78	0.16	21.05
T3	0	0	16.62		

* Note: the carbon content of corn straw was calculated at 40%.

4. Discussion

The response of SOC and SOCD to deep straw return intervals: It is widely known that deep straw return has a more significant positive effect on SOC sequestration than mulching [24,25]. However, this kind of returning way was carried on in various regions and its impact on SOC and SOCD was evaluated in different soil depth. For example, Wang (2021) designed a field experiment with straw return at different soil depths and discovered that a 35 cm soil depth significantly benefited in straw C sequestration, compared to a 15 cm depth [7]. Yang et al. (2021) investigated the influence of ditch-buried straw return on soil microbial communities in soil depths of 0–30 cm and pointed out that 10–20 cm had an obviously positive effect relative to the above- and below-applied horizon [13]. Islam (2023) conducted a meta-analysis of 103 published studies to analyze the SOC stock in the topsoil of 0–20 cm and the subsoil of 20–40 cm under three tillage practices [18]. One conclusion drawn from the relative literature is that SOC or SOCD was mostly evaluated in the topsoil of 0–20 cm. Even when deeper soil is considered, soil horizons below 40 cm are seldom researched [25,26]. As the deep straw return was adopted by farmers and the government, the SOC in deeper soil horizons should be assessed in order to observe more realistic conditions. Therefore, 0–100 cm was surveyed in our study. And the results indicated that the influence of straw on SOC existed even in horizon C (Figure 3), and its effect on SOCD appeared down to horizon B (Figure 4). We attributed it to the following factors: (i) the content of SOC in subsoil is relatively lower than the topsoil, and there is a large gap to increase to the highest point of soil carbon saturation value [27]; (ii) the temperature and the water content in the subsoil are higher than in the topsoil, which is beneficial to microbial activity [28], and the new produced soluble organic carbon can migrate downward through the subsoil [29]; (iii) the subsoil has less disturbance, which is conducive to forming macroaggregates and decrease SOC mineralization by physical protection [30].

In this study, the biennial straw return had the strongest positive influence on SOC in horizon P, and the annual straw return seriously affected it in horizons B and C. However, these two treatments had no influence on horizon A (Figure 3). In other words, straw return had a positive effect on SOC below the applied horizon, and the annual straw return affected deeper soil horizon than the biennial. Our result was consistent with some previous studies. For instance, Wang (2015) pointed that ditch-buried straw return increased SOC content in the soil near the straw-applied horizon [31]. Huo et al. found that SOC in the 20–40 cm soil horizon was significantly enhanced by an adequate supply of straw at this depth [32]. Hobley et al. (2016) also pointed out that the vertical distribution of SOC was

most influenced by the place where straw was returned [33]. However, there are some contrary studies. Kan et al. (2022) came up with a result that no-tillage combined with straw mulching enhanced SOC in the topsoil, while its effect on the subsoil was minimal [34]. This difference was caused by the different returning ways. In our study, straw was cut into small pieces and buried in deep soil depth. This method increased the contact of straw with soil, which was a dominantly important factor to determine straw decomposition [35]. The straw decomposition rate was improved, and more labile organic carbon originated from straw was leached downward. Therefore, deep straw return influenced deeper soil than straw mulching.

In horizon A, although SOC content differed minimally among the three treatments, both the annual and the biennial straw return had a more significant positive influence on SOCD than the non-straw return. The same phenomenon, non-straw return had a higher SOCD even when its SOC content was less, occurred in horizon P (Figure 4). From the above change, it obviously reflected that the horizon depth, rather than the content of SOC, determined the SOC stock in the soil profile. But when we took SOCD from 0–100 cm into account, it was found that there was no significant difference between the annual and the biennial straw return. This meant that the straw return amount was not the key factor affecting the quantity of SOC sequestration in our study. It may be explained by the following reasons: (i) The tested field lies in Northeast China, where the annual mean temperature is 5.6 °C, which is lower than the optimum temperature of straw decomposition [36]. This climate is unconducive to forming SOC, even if other factors are more suitable. Therefore, more undecomposed straw was found in the annual straw return than the biennial. (ii) The lower disturbance under the biennial return treatment was beneficial to carbon sequestration and decreased SOC mineralization, which offset the gap caused by the straw return amount. Pang (2020) also pointed out that deep straw return induced greater SOC loss and could not be applied annually [37]. (iii) The higher ratio of fertilizer N to straw C under biennial straw return was beneficial to the reproduction and activity of microorganisms, which promoted microbial biomass carbon. Li (2021) had the same conclusion, as increasing N fertilizer would benefit the formation of SOC when straw was applied [38]. To illustrate the mechanism, the aggregation fraction and associated C and N stocks should be quantitatively investigated in the future.

The response of SOC composition todeep straw return intervals: HA-C, FA-C, and HM-C, which are products of humification, are the principal constituent of humic substances and represent the largest stable organic carbon pool [39]. Thus, they are the important indexes for evaluating SOC stock and play an essential role in improving soil fertility and carbon sequestration [40,41]. In our study, both the annual and the biennial straw return had a more significant effect on HA-C, FA-C, HE-C, and HA/FA in horizon P, where the straw was returned, than the non-straw return (Figure 5). This result was consistent with the distribution of SOC content in our study and further proves that the straw-applied horizon is affected sharply. Moreover, there was no significant difference between the two return intervals for HA-C, FA-C, HE-C, and HA/FA in horizon P. The decomposition process of straw is affected by many factors, such as straw input rate, agronomic practice, climate, and edaphic condition, and varied under certain circumstances [4,42]. Except for the different amount of straw, which did not have a linear relationship with SOCD in our study, it may be also related to C/N, the degree of soil disturbance, cold climate, and so on, just like what had been discussed in the SOCD section. The mechanism should be explored in greater detail in future experiments. Moreover, the interaction effect of carbon and nitrogen on straw humification should be extensively researched under different intervals of deep straw return, as nitrogen is a key factor determining the decomposition process.

HA-C/FA-C is an important indicator for evaluating the quality of soil humus, and a high value represents a high degree of humification and a complex molecular structure in soil [43]. Our results showed that HA-C/FA-C in annual and biennial straw return was significantly higher than non-straw return in horizons A and P (Figure 6), but its effect on horizons B and C was less. In horizon A, the annual straw return was significantly,

0.16, higher than the biennial straw return. This demonstrated that both straw return amount and its return interval affected the soil humification. Straw application increases HA/FA, promotes the formation of stable humus compounds, and increases the stability of SOC [44,45]. Zhang (2011) also found that the HA-C/FA-C ratio was significantly higher after seven years of applying maize straw combined with mineral fertilizers than with an application of mineral fertilizers alone [46]. Rivero (2004) attributed this increase in HA-C/FA-C to the stability of molecules and concluded that FA contained large amounts of labile components, which were easily converted into HA during humification [47].

The response of straw carbon converted to SOC to deep straw return intervals: In our study, the conversion rate from straw carbon to SOC was 21.05% in the biennial straw return and 18.42% in the annual straw return. This means that the straw return biennially is more conducive to carbon accumulation in the tested soil than the annual return. In other words, the factor that influenced SOC accumulation was the return interval rather than the amount of straw application. Nevertheless, a number of studies found a positive correlation between cumulative straw carbon input and SOC enhancement, which was contrary to our results [48]. As previously mentioned, the lower disturbance under the biennial return treatment was beneficial to carbon sequestration. It was also relative to the high background of SOC in the tested soil and the high return amount of the annual return. Stewart (2008) first divided the SOC accumulation into two types [49]. One was described by the linear first-order model with no saturation limit. The other was a C saturation model with an explicit soil C saturation limit. The tested soil belonged to the latter, which had a high level of SOC before the experiment and remained with a small gap to reach C saturation. Therefore, the relationship of the straw return amount and the newly accumulated SOC amount did not show a positively linear increase, especially under the condition of a large amount of straw input. In our study, more undecomposed straw was still found in the soil of the annual straw return than the biennial. It further testified that the proportion of straw carbon converted to soil carbon was low.

Nevertheless, our conversion rate was still higher than some studies carried out in northern China [50,51]. Apart from the quality of straw, soil property, and climate, it is mainly influenced by the returning depth. Zhao (2016) also indicated that straw mixing appeared to have better soil carbon sequestration performance than straw mulching [48]. In our study, straw was returned to a soil depth of 30 cm and buried rather than mulched. It increased the contact between straw and soil and stimulated the activity of soil microorganisms to form more stable organic matter. Moreover, deep straw return also decreased the damage caused by wind compared with straw mulching.

5. Conclusions

Both the annual and the biennial deep straw return had a significantly positive influence on SOC sequestration in the soil profile of 0–100 cm, and their effect was outstanding in the straw-applied horizon. Although the total straw return amount of the biennial straw return was less than the annual, its effect on the quality and quantity of SOC was generally the same as the annual, which was restricted by the comprehensive effects of the cold weather, the intensity of soil disturbance, C/N, and the initial SOC content. Considering the biennial deep straw return had a higher conversion rate of straw carbon to SOC, it should be extended to the cold area of northeast China. In this way, not only will the amount of carbon sequestration in the soil not be affected, but the saved straw may also be used for other purposes.

Author Contributions: Conceptualization, X.Z. and H.W.; methodology, H.W.; software, Z.Z.; validation, H.W., X.Z. and N.H.; formal analysis, C.Z.; investigation, Z.Z.; resources, H.W.; data curation, C.Z.; writing—original draft preparation, J.L.; writing—review and editing, J.L. and H.W.; visualization, X.Z.; supervision, X.Z.; project administration, H.W.; funding acquisition, H.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Department of Science and Technology of Jilin Province, grant number 20230303006SF.

Data Availability Statement: The data presented in this study are available on request.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

Table A1. Soil bulk density in the cultivated horizon (A), the plough pan (P), the cambic horizon (B) and the C-horizon (C) in 2023 after 4 years of spring corn cultivation (2020, 2021, 2022 and 2023). T1 is the annual straw return (2019, 2020, 2021 and 2022), T2 is the biennial straw return (2019 and 2021), and T3 is the conventional tillage without straw return. Unit: g/cm^3.

Soil Horizon	Treatments		
	T1	T2	T3
A	1.35 ± 0.06 b	1.46 ± 0.04 ab	1.59 ± 0.03 a
P	1.51 ± 0.05 b	1.52 ± 0.07 b	1.81 ± 0.03 a
B	1.59 ± 0.06 a	1.59 ± 0.05 a	1.68 ± 0.04 a
C	1.55 ± 0.08 b	1.63 ± 0.05 ab	1.79 ± 0.07 a

Note: The result shows mean ± standard deviations (n = 3). Differing lowercase letters indicate a statistically significant difference between treatments in the same soil horizon (LSD, $p < 0.05$).

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