EFFECTS OF STRAW ENRICHMENT AND DEEP INCORPORATION ON HUMUS COMPOSITION AND HUMIC ACID STRUCTURE OF BLACK SOIL PROFILE IN NORTHEAST CHINA

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Abstract. Straw enrichment and deep incorporation (SEDI) is a new concept for soil restoration and conservation. It has been shown to improve soil organic carbon (SOC), humic C fractions, and enhance the structure of humic acid (HA). However, few studies have focused on the changes of humus components and structure of HA after SEDI at different depths of soil profile, especially under the subsurface soil. The purpose of study is to evaluate the effects of different soil profile (0-80 cm) on soil humus composition and structural characteristics of HA after SEDI in northeast China. Two different treatments, including CK (no straw) and SEDI (returning straw at a time with a total amount of 15385 kg.hm^{-2}, incorporating straw into 20-40 cm depth of black soil). Our results showed that SEDI significantly increased SOC contents, humus substances (HEC), humic acid carbon (HAC) and fulvic acid carbon (FAC). Among all the profiles, the increase most significant at the depth of 20-30 cm, the content of SOC, HAC, FAC and HMC was increased by 11.6%, 38.89%, 13.37% and 33.40%. The FTIR spectra and elemental results revealed that SEDI increased the aliphaticity of the HA structure. It can, therefore, be inferred that straw application will improve humus compositions, SOC content and enhance the stability of HA structure.
Keywords: *corn straw, deep incorporation, HA structure, soil profile, soil organic carbon*

Introduction

Over the past few years, the organic carbon storage of the black soil in Northeast China has significantly decreased (Han et al., 2018), due to excessive reclamation and long-term heavy application of chemical fertilizer, which resulted in shallow tillage layers and the degradation of black soil (Yi et al., 2020). According to Yin's study (Yin et al., 2018), crop straw is a good soil organic fertilizer. Consequently, straw return has become a routine practice in China, owing to its beneficial effects on crop productivity. Several studies (Wei et al., 2019; Chatterjee et al., 2013;Ingelmo et al., 2012; Hu et al., 2013; Li et al., 2018) have shown that the straw return is not only reflected in its effect on the change of soil organic carbon content, but can also improve the quality of SOM and crop productivity.

Scholars (Dou et al., 2011) usually divided the 0-40 cm soil layer into surface soil (0-20 cm) and subsurface soil (20-40 cm). However, most studies on straw return mainly focused on the surface soil. Shallow straw application significantly increases the content of organic carbon and humus components in soil surface according to a micro area experiment (Dong et al., 2017). Straw mulching improves the structure of surface soil and reduces soil compaction (Fan et al., 2018), but have little effect on the content of SOC in the subsurface soil, it affects seeding emergence and makes tillage difficult.

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However, Batjes showed that more than 50% of soil organic carbon is stored in the soil layer with a depth of 30-100 cm (Batjes et al., 1996). Thus, a new returning method is, hereby, invented, that is straw enrichment and deep incorporation (SEDI). Cong found that straw deep return improves the SOC content and humus C fractions in the 20-40 cm soil depth (Cong et al., 2019). Soil humic substances are important constituents of SOC (Brunetti et al., 2007). Zhang showed that straw deep return can also improve the quality and activity of soil humic substances (Zhang et al., 2012). However, Dou et al demonstrated that incorporating corn straw into 20-40 cm soil depth is more conducive to the accumulation of soil organic matter and humic C fractions (Dou et al., 2019), which enhances the aromatic C and aliphatic C groups, making HA structure more complicated (Cui et al., 2017; Zhang et al., 2016).

Previous studies mainly focus on the changing of soil organic matter after returning straw in the surface soil, but less talked about this kind of change in the subsurface soil. In this study, we conducted a year field trial, to (i) determine SOC and humic C fractions contents and (ii) to compare changes in HA characteristics under different depths.

Materials and methods

Site description

The soil was collected from the Enyu town of Changchun City, Jilin Province (N44°50′10.61″, E126°33′25.14″) at 0-80 cm soil depth. The region belongs to a temperate continental monsoon climate and receives an annual rainfall of 536.4 mm. According to the American soil classification system, the field is classified by black soil, belonging to Argiudolls. It belongs to the monsoon temperate semi humid climate in Northeast China. The temperature is mostly affected by the monsoon. It is dry and windy in spring, wet and rainy in summer, mild and cool in autumn and long and cold in winter. The basic properties of the tested soil are presented in *Table 1*. And the basic elemental composition of the straw is shown in *Table 2*.

Table 1. *Basic properties of the tested soil*

Depth/cm	Organic matter (g.kg^{-1})	2000-20 µm	20-2 µm	< 2 µm	pH
0-20	19.90	39.08	29.87	31.05	6.43
20-40	19.19	33.46	32.21	34.33	6.71

Table 2. *Basic properties of corn straw*

C (g.kg^{-1})	N (g.kg^{-1})	H (g.kg^{-1})	O + S (g.kg^{-1})	C/N
338.6	6.87	58.77	457.65	49.29

Experiment design

The experiment was initiated in the three week of June 2016. The straw was returned to the field under equal straw mass of 15385 kg/ha (equivalent to 100% straw return). Two treatments were randomly with three replications. The area of each plot is 18.2 m^2. The treatments included (i) CK (no straw applied), (ii) SEDI (rake the corn straw in the

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field together into rows at a ratio of 4:1 with a finger-plate rake, then crush the corn straw and bury it into subsoil, 20-40 cm deep along designated strips with a wind-driven input cylindrical plough (*Fig. 1*). Finally sow seeds into the strips with no corn straw buried in between the strips with corn straw buried in a normal way with a non-tillage seeder, to realize separation of the seeded strips (narrow rows) from the strips (wide rows) with straw buried in a wide and narrow row alternating cultivation mode). CK treatment was not disturbed.

Figure 1. *The wind-driven input cylinder plough*

Soil sampling and analysis

The soil was collected at the end of the growing season (October 2017), employing a 3-point sampling method, to make one composite mixture. Three random undisturbed soil samples were collected from each treatment at different profiles (0 to 10 cm, 10 to 20 cm, 20 to 30 cm, 30 to 40 cm, 40 to 50 cm, 50 to 60 cm, 60 to 70 cm, and 70 to 80 cm). After visible organic debris and stones were removed, the soil sample was air-dried and passed through a 2-mm sieve (*Fig. 2*).

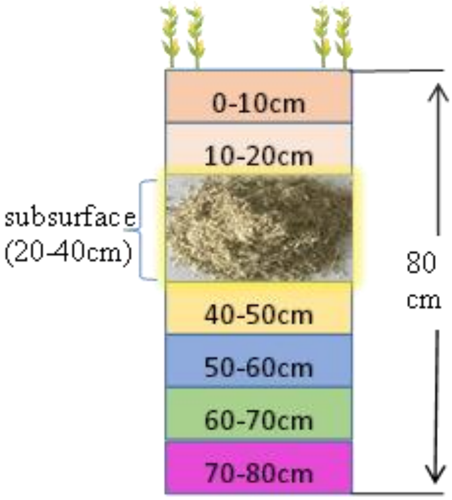

Figure 2. *Different soil layers in the same profile*

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Laboratory analysis

SOC was determined by potassium dichromate external heating method (Cambardella and Elliott, 1993), by treating 0.2 g soil sample into a 50 ml volumetric flask with 10 ml 0.4 N $K_2Cr_2O_7$-H_2SO_4 solution, and heating it at 180 ℃ until the first drop from the funnel. Then it was titrated with 0.2N $FeSO_4$.

Soil humus composition was analysed following the method by (Dou, 2010). Briefly, 5 g of soil sample was extracted with 30 ml of distilled water. After continuous shaking at 70 ℃ for 1 h in the water bath. The residue was extracted with 30 ml of a mixture of 0.1 N alkali solution (NaOH + $Na_4P_2O_7$). The C contents of HA (HA-C), FA (FA-C) and HM (HM-C) were determined.

HA isolation and purification were processed using the procedure described by International Humic Substances Society (Kuwatsuka, 1992). Briefly, 50 g of soil sample was treated with 1 N HCL in succession and shaking for 1 h. Then samples were extracted with 0.1 N NaOH solution to clean the precipitate and adjust pH to 13-14. The resulting liquid was low-speed centrifugation, high-speed centrifugation, electro-dialysed, rotary evaporation, freeze-drying and other processes.

HA element composition such as C, H, N and O were determined by a Vario EL III elemental analyzer (German).

The Fourier transform infrared spectrometer (Brucker TENSOR 27, German) was used to analyze the HAs, and the wave number was ranged from 4000 to 400 cm^{-1}, following the KBr tablet method.

Thermogravimetry (TG) and DTG analysis of solid HA samples were performed using STA 2500 Regulus thermogravimetric analyser (Netzsch, Germany). About 4–6 mg of solid HA sample was heated to 750 °C at a constant heating rate of 158 °C min^{-1}.

Data analysis

SPSS software was used for variance analysis to evaluate the effects of different depth on all measured soil parameters. Significance differences among treatment means were evaluated using the significant difference test with T test analysis at $p < 0.05$. All graphs and spectra were compiled using Origin 7.5 software. The DTA and TG charts were analyzed by Universal Analysis software. The humification index (PQ) was calculated as the formula:

$$PQ = \text{HA-C}/(\text{HA-C} + \text{FA-C}) \qquad \text{(Eq.1)}$$

Results

Organic C contents and humus composition under different soil profile

After 16 months of field trial (*Fig. 3*), the contents of SOC ranged from 7.09 to 20.31 $g.kg^{-1}$, the SOC content decreased as the soil layer was deeper from the surface. Compared to CK, the SOC in SEDI treatment in 0-10 cm and 10-20 cm soil layers increased by 3.8% and 1.9%, respectively. The increase was most significant at the depth of 20-30 cm (11.59% higher than CK). The C content of humus components significantly increased after corn straw was returned, increasing in the order of SEDI > CK in the 0–40 cm depth (*Table 3*), but the soil layer changes in 40-80 cm are not significant. With the increasing of the depth of soil, the content of humus components decreased. Among all the profiles, HA-C, FA-C and HM-C contents had

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the most significant change in the 20-30 cm soil layer increased by 38.89%, 13.37% and 33.40%.

PQ value (*Eq. 1*) is the proportion of HA in humic substances, which can reflect the degree of humification of SOC. Compared with CK, the treatment of straw returning to the field increased the PQ value of the soil to varying degrees. And the SEDI treatment increased the PQ value of in the 20-30 cm soil the most, which increased from 62.07% to 66.72% compared with CK.

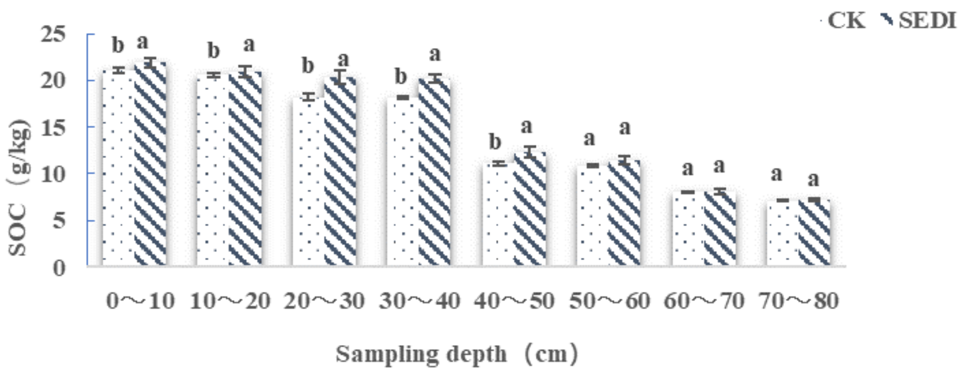

***Figure 3.** Effects of straw enrichment and deep incorporation on soil organic carbon content. CK: no straw is applied in normal tillage; SEDI: straw enrichment and deep incorporation. Error bars represent the standard deviations of the mean (n = 3). Different lowercase letters indicate significant differences among treatments (p < 0.05)*

***Table 3.** Effect of SEDI on the content of organic carbon in soil humus components*

Depth (cm)	Treatment	HA (g/kg)	FA (g/kg)	HM (g/kg)	PQ (%)
0~10	CK	3.51 ± 0.11^b	2.06 ± 0.26^a	5.26 ± 0.21^b	63.10
	SEDI	4.31 ± 0.14^a	2.14 ± 0.06^a	6.44 ± 0.21^a	66.82
10~20	CK	3.45 ± 0.11^b	2.04 ± 0.13^a	5.18 ± 0.24^b	62.82
	SEDI	4.26 ± 0.12^a	2.13 ± 0.17^a	6.42 ± 0.25^a	66.67
20~30	CK	3.06 ± 0.07^b	1.87 ± 0.05^b	4.67 ± 0.10^b	62.15
	SEDI	4.25 ± 0.07^a	2.12 ± 0.21^a	6.23 ± 0.17^a	66.72
30~40	CK	2.96 ± 0.09^b	1.83 ± 0.13^b	4.61 ± 0.23^b	61.80
	SEDI	4.03 ± 0.19^a	2.04 ± 0.26^a	6.04 ± 0.15^a	66.42
40~50	CK	1.76 ± 0.20^b	1.12 ± 0.26^a	3.17 ± 0.10^a	61.15
	SEDI	1.98 ± 0.06^a	1.17 ± 0.14^a	3.24 ± 0.17^a	62.79
50~60	CK	1.53 ± 0.21^a	0.96 ± 0.27^a	3.07 ± 0.17^a	61.50
	SEDI	1.57 ± 0.18^a	0.97 ± 0.21^a	3.13 ± 0.25^a	61.66
60~70	CK	1.31 ± 0.08^b	0.98 ± 0.24^a	2.05 ± 0.25^a	59.82
	SEDI	1.37 ± 0.16^a	0.91 ± 0.14^a	2.02 ± 0.20^a	60.05
70~80	CK	1.16 ± 0.13^a	0.79 ± 0.22^a	1.93 ± 0.26^a	59.63
	SEDI	1.23 ± 0.13^a	0.85 ± 0.24^a	1.97 ± 0.27^a	59.13

CK: no straw is applied in normal tillage; SEDI: straw enrichment and deep incorporation. The values represent the means ± standard error (SE). Means that do not share the same letter superscript within a column are significantly different (p<0.05). PQ is the humification index computed as PQ = HA-C/(HA-C + FA-C)

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Element composition of HA

Table 4 shows the elemental composition of soil HA across all treatments under different treatments, and the range of each attribute was as follows: C content was 478.5 to 525.8 g.kg^{-1}, N content was 20.00 to 29.85 g.kg^{-1}, H content was 35.70 to 41.90 g.kg^{-1}, and O content was 398.3 to 450.5 g.kg^{-1} (*Table 4*). Elemental C, H and N contents in HA were higher in SEDI treatment than in CK treatments. The O content showed a significant decrease in SEDI. These differences indicate that SEDI promotes the consumption of O element and the increases accumulation of C, N and H in soil HA. Among the all profiles, the H/C and O/C ratio decreased from the upper layer to lower layer of the soil. After SEDI, the H/C ratio of HA in surface layer and subsurface layer decreased. The O/C ratio decreased 9.75% compared with the CK group at the depth of 20-30 cm, which was the most significant decline in all profiles.

Table 4. *Effect of SEDI on HA element composition of black soil profile*

Depth (cm)	Treatment	C (g.kg^{-1})	H (g.kg^{-1})	N (g.kg^{-1})	O (g.kg^{-1})	The molar ratio	
						O/C	H/C
0~10	CK	510.4±0.4^{b}	40.40±0.30^{b}	25.18±0.19^{b}	420.1±0.6^{a}	0.617±0.001^{a}	0.950±0.007^{a}
	SEDI	525.8±0.4^{a}	41.90±0.35^{a}	29.85±0.08^{a}	415.2±0.5^{b}	0.592±0.001^{b}	0.956±0.007^{a}
10~20	CK	509.5±0.5^{b}	40.10±0.36^{b}	25.10±0.32^{b}	425.7±0.6^{a}	0.627±0.001^{a}	0.944±0.009^{b}
	SEDI	521.9±0.4^{a}	41.50±0.40^{a}	29.50±0.2^{a}	412.5±0.3^{b}	0.593±0.001^{b}	0.954±0.009^{a}
20~30	CK	498.2±0.6^{b}	38.70±0.46^{b}	22.70±0.26^{b}	436.2±0.6^{a}	0.657±0.001^{a}	0.932±0.011^{b}
	SEDI	520.3±0.6^{a}	40.90±0.44^{a}	29.00±0.47^{a}	411.1±0.5^{b}	0.593±0.002^{b}	0.943±0.009^{a}
30~40	CK	492.9±0.6^{a}	38.00±0.35^{a}	22.16±0.17^{b}	440.7±0.5^{a}	0.670±0.001^{a}	0.925±0.008^{a}
	SEDI	491.7±0.4^{a}	38.00±0.26^{a}	27.80±0.36^{a}	398.3±0.5^{b}	0.608±0.001^{b}	0.927±0.006a
40~50	CK	490.4±0.4^{a}	37.10±0.61^{a}	21.43±0.13^{b}	442.9±0.4^{a}	0.677±0.001a	0.908±0.015^{b}
	SEDI	491.0±0.3^{a}	37.80±0.36^{a}	22.79±0.17^{a}	438.9±0.6^{b}	0.670±0.002^{b}	0.924±0.008^{a}
50~60	CK	489.1±0.3^{a}	36.80±0.26^{b}	21.06±0.13^{a}	443.8±0.6^{a}	0.681±0.001^{a}	0.903±0.006^{a}
	SEDI	488.8±0.5^{a}	37.00±0.53^{b}	21.71±0.55^{a}	440.9±0.5^{b}	0.677±0.002^{b}	0.908±0.015^{a}
60~70	CK	486.3±0.5^{b}	36.30±0.44^{b}	20.81±0.12^{b}	445.7±0.3^{a}	0.687±0.001^{a}	0.896±0.011^{b}
	SEDI	488.7±0.4^{b}	36.70±0.36^{b}	21.10±0.23^{a}	441.0±0.3^{b}	0.676±0.001^{b}	0.901±0.008^{a}
70~80	CK	480.2±0.4^{a}	35.70±0.30^{a}	20.00±0.18^{b}	450.5±0.4^{a}	0.704±0.001^{a}	0.892±0.007^{b}
	SEDI	478.5±0.5^{b}	35.90±0.52^{a}	20.62±0.24^{b}	445.4±0.4^{b}	0.695±0.001^{b}	0.900±0.013^{a}

The values represent the means ± standard error (SE). Means that do not share the same letter superscript within a column are significantly different ($p<0.05$). CK: no straw is applied in normal tillage; SEDI: straw enrichment and deep incorporation

FTIR spectra of HA

The FTIR spectrum of soil HA for different treatments are shown in *Figure 4*, and the corresponding relative intensity of the main absorption peaks are listed in *Table 5*. The spectra bands across all treatments exhibited similar characteristics (*Fig.2*), differing only in their relative intensities of absorption (*Table 5*). Absorption peaks were assigned to organic functional groups following (Zhang et al.,2017). The SEDI treatment showed a relative abundance of aromatic peaks at 1620 cm^{-1} (*Table 5*).

The ratio of I_{2920}/I_{1720} and I_{2920}/I_{1620} which reflects the strength of the ratio of aliphatic C to carboxyl C and aromatic C in the HA structure, respectively, revealed that SEDI treatment increased the aliphatic/carboxyl C and aliphatic/aromatic C (*Table 5*). Compared with CK, the ratio of I_{2920}/I_{1720} and I_{2920}/I_{1620} of HA in 20-30 cm soil layer increased significantly than that of CK, with increase by 144.06% and 104.15%, respectively.

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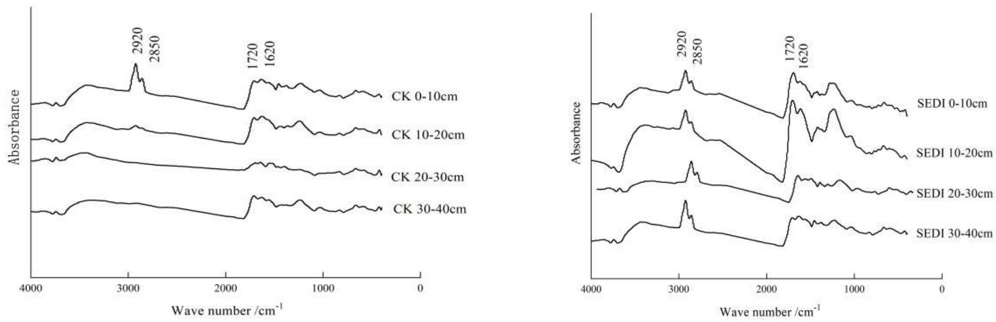

Figure 4. *FTIR spectrum of soil HA under different treatments. FTIR spectra of HA structure in soil layers 0~20 cm and 20~40 cm from left to right. CK: no straw is applied in normal tillage; SEDI: straw enrichment and deep incorporation*

Table 5. *Effect of SEDI on the relative absorption intensity of soil HA*

Depth (cm)	Treatment	Relative intensity (%)				Ratio	
		2920 cm^{-1}	2850 cm^{-1}	1720 cm^{-1}	1620 cm^{-1}	2920/1720	2920/1620
0~10	CK	3.78 ± 0.009^{b}	1.497 ± 0.007^{b}	7.563 ± 0.013^{a}	7.712 ± 0.010^{b}	0.473 ± 0.001^{b}	0.464 ± 0.001^{b}
	SEDI	5.547 ± 0.007^{a}	1.933 ± 0.013^{a}	7.315 ± 0.007^{b}	9.373 ± 0.014^{a}	0.758 ± 0.001^{a}	0.592 ± 0.001^{a}
10~20	CK	2.532 ± 0.015^{b}	1.217 ± 0.014^{b}	7.472 ± 0.006^{a}	7.531 ± 0.003^{b}	0.339 ± 0.002^{b}	0.336 ± 0.001^{b}
	SEDI	5.032 ± 0.019^{a}	1.872 ± 0.006^{a}	7.237 ± 0.091^{b}	9.019 ± 0.008^{a}	0.695 ± 0.007^{a}	0.558 ± 0.002^{a}
20~30	CK	2.019 ± 0.019^{b}	1.203 ± 0.007^{b}	7.381 ± 0.006^{a}	7.435 ± 0.007^{b}	0.274 ± 0.003^{b}	0.272 ± 0.002^{b}
	SEDI	4.752 ± 0.007^{a}	1.856 ± 0.010^{a}	7.118 ± 0.007^{b}	8.572 ± 0.007^{a}	0.668 ± 0.001^{a}	0.554 ± 0.001^{a}
30~40	CK	1.934 ± 0.010^{b}	1.119 ± 0.120^{b}	7.201 ± 0.107^{a}	7.353 ± 0.018^{b}	0.269 ± 0.002^{b}	0.263 ± 0.001^{b}
	SEDI	4.246 ± 0.010^{a}	1.801 ± 0.010^{a}	7.019 ± 0.005^{b}	8.351 ± 0.007^{a}	0.605 ± 0.001^{a}	0.508 ± 0.001^{a}
40~50	CK	1.851 ± 0.010^{b}	1.017 ± 0.056^{b}	6.985 ± 0.004^{a}	7.112 ± 0.005^{b}	0.265 ± 0.001^{b}	0.26 ± 0.002^{a}
	SEDI	2.038 ± 0.017^{a}	1.386 ± 0.066^{a}	6.873 ± 0.010^{b}	8.098 ± 0.015^{a}	0.297 ± 0.002^{a}	0.252 ± 0.002^{a}
50~60	CK	1.726 ± 0.010^{a}	0.996 ± 0.009^{b}	6.533 ± 0.007^{a}	6.688 ± 0.009^{b}	0.264 ± 0.002^{a}	0.258 ± 0.002^{a}
	SEDI	1.73 ± 0.012^{a}	1.217 ± 0.020^{a}	6.541 ± 0.008^{a}	7.579 ± 0.008^{a}	0.264 ± 0.002^{a}	0.228 ± 0.001^{b}
60~70	CK	1.558 ± 0.009^{b}	0.873 ± 0.008^{b}	5.897 ± 0.007^{b}	6.535 ± 0.007^{b}	0.264 ± 0.002^{a}	0.238 ± 0.001^{a}
	SEDI	1.603 ± 0.01^{a}	1.053 ± 0.013^{a}	6.003 ± 0.007^{a}	7.462 ± 0.011^{a}	0.267 ± 0.002^{a}	0.215 ± 0.001^{b}
70~0	CK	1.472 ± 0.010^{b}	0.862 ± 0.056^{b}	5.658 ± 0.007^{b}	6.432 ± 0.006^{b}	0.26 ± 0.002^{a}	0.229 ± 0.001^{a}
	SEDI	1.526 ± 0.006^{a}	1.012 ± 0.006^{a}	5.992 ± 0.092^{a}	7.361 ± 0.009^{a}	0.255 ± 0.003^{a}	0.207 ± 0.001^{b}

The values represent the means ± standard error (SE). Means that do not share the same letter superscript within a column are significantly different ($p<0.05$). $I_{2920}/I_{1720,}$ absorption intensity ratio of 2920 cm^{-1} and 2850 cm^{-1} calculated as (2920 + 2850)/1720; I_{2920}/I_{1620}, absorption intensity ratio of 2920 cm^{-1} and 1620 cm^{-1} calculated as (2920 + 2850)/1620. CK: no straw is applied in normal tillage; SEDI: straw enrichment and deep incorporation

Thermal analysis of HA

TG (Thermogravimetric) and DTA (Differential Thermal Analysis) curves of soil HA under different treatments are shown in *Figure 5*. During the pyrolysis process, HA samples mainly showed moderate temperature exotherm (299–340°C) and high-temperature exotherm (467–501°C) (*Fig. 5*). The TG curves of all studied HA samples showed gradual weight loss with increasing temperatures, and then steep weight loss at temperatures greater than 400°C was observed. The semi-quantitative fundamental results presented in *Table 6*, revealed that SEDI treatment had a significant increase in exothermic heat and weight loss at moderate temperature, compared with CK, but decreased at the high/moderate heat ratio temperature and the high/moderate mass loss.

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It shows that SEDI can also increase the aliphatic compounds and aromatic compounds, and reduce the thermal stability.

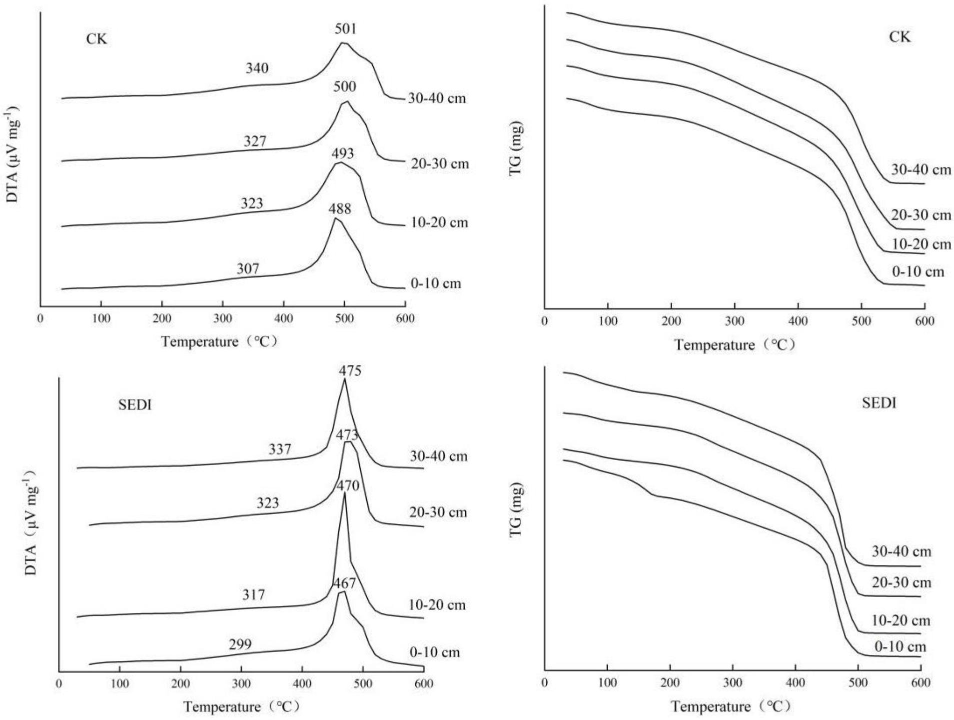

Figure 5. *Effect of different treatments on differential thermogravimetric analysis (DTA) curve of soil HA. From left to right are the heat DTA diagram of HA and the TG diagram of HA. CK: no straw is applied in normal tillage; SEDI: straw enrichment and deep incorporation*

Table 6. *Effects of SEDI on heat release and weight loss of HA*

Depth	Treatment	Exothermic heat ($kJ.g^{-1}$)		Exothermic heat ratio of moderate and high temperature	Mass loss ($mg.g^{-1}$)		Ratio of high and moderate mass loss
		Moderate temperature	High temperature		Moderate temperature	High temperature	
0~10	CK	0.108 ± 0.002^b	7.100 ± 0.122^a	65.946 ± 0.216^a	138.5 ± 0.458^b	576.8 ± 0.436^b	4.16 ± 0.110^a
	SEDI	0.125 ± 0.002^a	7.119 ± 0.045^a	57.107 ± 0.742^b	140.6 ± 0.520^a	583.1 ± 0.529^a	4.15 ± 0.017^a
10~20	CK	0.0902 ± 0.001^b	7.085 ± 0.007^b	78.550 ± 0.628^a	130.6 ± 0.520^b	558.2 ± 0.557^b	4.27 ± 0.013^a
	SEDI	0.141 ± 0.002^a	7.441 ± 0.027^a	52.903 ± 0.687^b	144.8 ± 0.520^a	599.5 ± 0.458^a	4.14 ± 0.017^b
20~30	CK	0.088 ± 0.001^b	7.077 ± 0.019^b	80.422 ± 0.707^a	128.4 ± 0.458^b	555.6 ± 0.436^b	4.33 ± 0.013^a
	SEDI	0.149 ± 0.002^a	7.715 ± 0.007^a	51.669 ± 0.555^b	170.1 ± 0.557^a	610.5 ± 0.400^a	3.59 ± 0.009^b
30~40	CK	0.084 ± 0.001^b	6.823 ± 0.068^b	81.229 ± 0.600^a	125.1 ± 0.458^b	541.3 ± 0.458^b	4.33 ± 0.013^a
	SEDI	0.165 ± 0.003^a	7.937 ± 0.007^a	48.208 ± 0.706^b	175.6 ± 0.794^a	630.3 ± 0.436^a	3.59 ± 0.015^b
40~50	CK	0.070 ± 0.002^b	5.788 ± 0.109^b	82.302 ± 0.253^a	115.2 ± 0.600^b	524.5 ± 0.360^b	4.55 ± 0.021^a
	SEDI	0.112 ± 0.002^a	7.079 ± 0.012^a	63.398 ± 0.780^b	138.6 ± 0.458^a	589.2 ± 0.721^a	4.25 ± 0.010^b
50~60	CK	0.063 ± 0.001^b	5.096 ± 0.046^b	83.713 ± 0.722^a	109.8 ± 0.624^b	513.7 ± 0.624^a	4.68 ± 0.024^a
	SEDI	0.083 ± 0.001^a	6.589 ± 0.013^a	79.396 ± 0.800^b	119.3 ± 0.458^a	503.7 ± 0.529^b	4.22 ± 0.013^b
60~70	CK	0.048 ± 0.001^b	4.463 ± 0.020^b	88.817 ± 0.679^a	95.6 ± 0.520^a	500.6 ± 0.557^b	5.24 ± 0.034^b
	SEDI	0.062 ± 0.002^a	5.349 ± 0.106^a	86.744 ± 0.787^a	96.7 ± 0.458^a	514.7 ± 0.458^a	5.32 ± 0.021^a
70~80	CK	0.053 ± 0.006^a	4.203 ± 0.012^b	89.006 ± 0.588^b	90.3 ± 0.557^a	500.1 ± 0.600^b	5.54 ± 0.028^a
	SEDI	0.054 ± 0.001^a	4.968 ± 0.011^a	92.571 ± 0.798^a	90.8 ± 0.557^a	504.7 ± 0.458^a	5.59 ± 0.030^a

The values represent the means ± standard error (SE). Means that do not share the same letter superscript within a column are significantly different ($p<0.05$). CK: no straw is applied in normal tillage; SEDI: straw enrichment and deep incorporation

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Discussion

Effects of SEDI on SOC and humus composition at different depths of the black soil profile

Our results showed that from the soil profile, the component of soil organic matter and soil humus decreased gradually from upper to lower layers in all the treatments (*Table 3*). This may be due to the fact that activated carbon tends to accumulate in the surface layer of soil (Liao et al., 2015). As the profile goes down, the source of organic carbon decreases, and the decomposition rate of straw decreases, so that the organic carbon content continues to decrease (Hao et al., 2017). In addition, the mineralization of microorganisms continuously consumes the original soluble organic matter, so that the increase of SOC is continuously reduced, showing a downward trend (Na et al., 2015). As the SOC content gradually decreases, the content of each component of humus also shows a downward trend. After straw enrichment and deep incorporation, as the depth of the profile increases, the content of soil organic matter and humic substances also decrease layer by layer, while they are all higher than CK on the same profile. Among them, the SEDI has the most significant effect on the accumulation of organic matter in the 20-30 cm soil layer, and its influence range is 0-50 cm. It may be because the straw is crushed and returned to the field, which reduces the volume of the straw and increases the contact area between the microorganisms and the straw. This leads to the decomposition rate of straw was accelerated, so that the organic matter and the nutrient can be fully released and enhance the organic matter content (Li et al., 2006; Kubar et al., 2018). And after SEDI, the soil was loosened, the soil microbial biomass and its activity were improved, which was conducive to the development of roots to the lower soil layer, and the content of organic matter in subsurface layer was higher than that of the surface layer.

Studies have shown that straw deep incorporation can significantly increase the organic matter content of subsurface layers (Cong, 2019). It also increases significantly the soil humus components and the PQ value (Cui et al., 2017). Our current study further confirmed that SEDI increased the carbon contents of humus components (HA, FA, and HM), with the greatest improvement in the 20-30 cm soil layer. The reason for a more significant increase of HA content may be that the stem, root or root exudates of crop straw provide a large amount of carbon source input to the soil (Ma et al., 2020), increase the content of soil organic carbon, improve the composition of humus, and then enhance the soil Hu Fu ratio (HA/FA), that is, increase the degree of soil humification (Cong, 2019). On the other hand, the enriched straw was buried in the subsurface of the soil, which disturbs the subsurface of the soil, increases the oxidizable carbon in the soil, and then increases the soil carbon source, stimulates the microbial activity (Lobe et al., 2011), and leads to the increase of microbial metabolic rate (Peng et al., 2013).

SEDI improves the structural characteristics of HA

Studies have shown that SEDI can enhance the HA aliphatic structure and make the HA molecular structure simpler and youthful (Cui et al., 2017). Our results show that H/C ratio, I_{2920}/I_{1720} and I_{2920}/I_{1720} ratio decrease from top to bottom with the increase of soil depth. This indicates that the degree of oxidation and aromaticity of HA in humus decreases as the soil layer increases. After straw enrichment and deep incorporation, the content of aliphatic chain hydrocarbon and aromatic carbon of HA increased, the

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content of Carboxylic Carbon decreased, the hydrophobicity of HA increased, and the structure of HA became simpler.

Research have shown that the hydrophobicity of aliphatic carbon was significant for maintaining the stability of soil organic carbon (Bai, 2017). Straw returning could increase the aliphatic carbon content of HA molecule (Zhang, 2020), and make the aliphatic carbon in HA molecule hydrophobic (Chen, 2020). As shown in *Table 3,* the (O + S)/C ratio of HA after straw enrichment and deep incorporation was lower than that of CK, indicating that straw application could increase the content of aliphatic compounds in soil and increase the hydrophobic C content of HA (Li et al., 2016). Among the different depth, the (O + S)/C ratio of 20-30 cm treatment in SEDI is the lowest, which indicates that HA molecules have less oxygen-containing functional groups, lower oxidation degree, and tend to simplify the structure (Vance, 1987). The H/C ratio of 20~30 cm treatment in SEDI was the highest, which indicated that the reduction trend of HA molecular condensation degree was the most obvious.

SEDI treatment increased the number of aliphatic chain hydrocarbon and aromatic hydrocarbon functional groups in FITR spectrum of HA and decreased the content of carboxyl group (*Table 4*). The results showed that straw returning could increase the content of aliphatic chain hydrocarbons and aromatic hydrocarbons in soil, which was consistent with Dong (Dong, 2017). For different treatments, the content of aliphatic chain hydrocarbon and aromatic hydrocarbon functional groups increased most obviously in 20-30 cm treatment of SEDI. This may be because that straw buried in the subsurface layer increases the carbon source of the soil, enhances the activity of microorganisms, and promotes the decomposition of HA (Kwiatkowska Malina, 2017). On the other hand, after the straw is enrichment and deep incorporation, the alkyl carbon and alkoxy carbon of HA will increase, which will increase the aliphaticity of HA and the carboxyl carbon will decrease, resulting in the reduction of the oxidation degree of soil HA (Wu, 2005). In elemental and differential thermal analysis, the (O + S)/C of the surface and sub-surface layers after deep incorporation are both higher than CK, and H/C ratio is lower than CK, the exothermic high/medium ratio and the mass loss high/medium ratio increased, indicating that the straw enrichment and deep incorporation reduces the degree of oxidation of HA molecules, the degree of condensation decrease and the thermal stability is reduced. This result is consistent with FTIR spectral analysis.

Conclusion

We used quantitative analysis of SOC and humus composition as well-qualitative analysis of humic acid (HA) and found that:

i. Addition of corn straw amendments in the soil significantly increased SOC content and humus composition. As the depth of the profile increases, the content of SOC and humic substances decreases layer by layer. Compared with CK, the SOC, HA-C, FA-C and HM-C of different depth soil layers in SEDI treatment increased in varying degrees, with the most increase in 20-30 cm soil layer.
ii. SEDI treatments increased the aliphaticity of the HA molecular structure, making it simpler.

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Therefore, in conventional planting, application of corn straw will improve SOC content, reduced the oxidation degree, condensation degree and thermal stability, and has the most significant effect on the 20-30 cm soil layer in the black soil of Northeast China. However, further research is needed to confirm the present conclusions by determining the long-term responses of humic substances and SOC to SEDI treatments.

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