

**Mechanism of carbon sequestration under different maize straw return practices in Northeast China**

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**MECHANISM OF CARBON SEQUESTRATION UNDER DIFFERENT MAIZE STRAW RETURN PRACTICES IN NORTHEAST CHINA**

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Promoteurs: Prof. Gilles Colinet & Prof. Minggang Xu

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# Résumé

**Enjun Kuang (2022) Mechanism of carbon sequestration under different maize straw return practices in Northeast China.** **(Thèse de doctorat en anglais).** Gembloux, Belgique, Gembloux Agro-Bio Tech, University of Liège, 131 pages, 19 tables, 18 figures.

**Résumé:**

Le retour des pailles sur les sols cultivés est redevenu un moyen important de gestion de la fertilité du sol par ses effets sur les stocks de carbone organique du sol (COS) et le cycle des éléments nutritifs. Dans un contexte de faibles températures moyennes annuelles, la décomposition de la paille peut ne pas être complète et affecter de la sorte la qualité des semis et l'émergence et la croissance des plantules. Par ailleurs, la décomposition de la matière organique du sol libère une grande partie du carbone dans l'atmosphère. Les recherches actuelles visent à stabiliser le carbone du sol tout en maintenant une certaine dynamique de décomposition des pailles. Il est de la plus haute importance de trouver des méthodes appropriées de retour de la paille sur le champ compatibles avec des objectifs de production. Il est donc nécessaire d'améliorer la compréhension des mécanismes de stockage du carbone organique sous différentes modalités de retour de paille.

Nos recherches ont dans un premier temps porté sur l’étude de la décomposition de pailles à différentes profondeurs dans un sol noir en Chine et les mécanismes de séquestration du COS. Deuxièmement, quatre modalités de retour de paille ont été suivies pendant plusieurs années. Les teneurs en COS, en éléments nutritifs et l'activité enzymatique ont été analysées quantitativement. Enfin, l’évolution des caractéristiques d’humification a été suivie en fonction des modalités de retour de paille de maïs. Les principaux résultats sont présentés ci-après.

Des différences significatives de résidus de paille de maïs sont observées en fonction de la profondeur d’enfouissement : 68,7% de perte de carbone (p < 0,01) après 3 ans de décomposition en surface. L’enfouissement entre 15 et 30 cm semble la meilleure méthode pour ce qui concerne les teneurs en lignine, le taux de décomposition et le carbone microbien (SMBC). Le retour de paille en profondeur peut ainsi favoriser le stockage de carbone stable dans le sol. La teneur en carbone organique du sol apparaît positivement corrélée avec le rapport C/N et la température du sol, tandis qu’une corrélation négative significative entre la sucrase et l’humidité du sol a été observée. Les résultats soulignent l'importance de l'analyse des fractions du COS et du SMBC dans les sols profonds.

La composition du carbone organique dissous (COD) à différentes profondeurs a été analysée par spectroscopie de fluorescence tridimensionnelle. Le traitement CK - T4 engendre une dominance de substances semblables à l'humus et au tryptophane, tandis que le traitement T5 montre des substances similaires à l'humus et à la tyrosine. Il y a une petite quantité d'ingrédients autogènes à une profondeur de 31 - 40 cm et le coefficient d'humification est le plus élevé. Avec l’augmentation de la profondeur d’enfouissement de la paille, l'intensité de fluorescence du composant C1 augmente, tandis que celle du composant C2 fluctue. Le COD dans le sol est affecté par des sources endogènes et exogènes (fi > 1,4, 0,6 < Bix < 0,8) et présente un état d'humification faible (hix < 1,0). L'analyse de corrélation a montré que la profondeur du sol, la quantité de paille retournée sur le terrain et leur interaction avaient une influence significative sur la teneur en COD et sa composition.

Les expériences sur le terrain ont montré que la restitution de la paille avait également un effet significatif sur l'augmentation de la teneur en carbone organique du sol tant sur la couche 0 ~ 20 cm que celle 20 ~ 40 cm, mais qu’un retour tous les trois ans ne montrait pas de différences par rapport au témoin sans apports. Une corrélation positive significative a été observée entre le COS, le COD, l'EOC, la sucrase et l'uréase dans le sol entre 0 et 40 cm, mais il n'y avait pas de corrélation significative entre les fractions du carbone organique labile. L’analyse des structures indique que le COD provient à la fois des plantes et des micro-organismes, avec une grande contribution autobiogène, une biodisponibilité élevée et un faible degré d'humification après le retour de la paille (FI 1,59 ~ 1,69, Bix 0,90 ~ 0,95, hix 0,64 ~ 0,74). La transformation des acides fulviques est favorisée par un retour annuel de la paille, tandis qu’un retour un an sur deux conduit à la formation de macromolécules et d’un humus plus stable.

En résumé, des pratiques appropriées de retour de pailles sont nécessaires dans le nord - est de la Chine. L'objectif de cette étude était d'évaluer la faisabilité d’utiliser la paille pour modifier l'aromaticité du COD dans un sol noir et accélérer sa décomposition. Le COD du sol accélère la transformation de l'humus du sol par les micro-organismes indigènes, mais l’apport d’humus du traitement de retour continu de paille était faible. En bref, les résultats montrent l’importance du COD dérivé de la paille et permettent d’orienter les conseils techniques de restitution de pailles dans les sols étudiés. La fréquence de retour de la paille, le degré de broyage, la quantité, l'application combinée d'engrais chimiques et les méthodes de restitution doivent être adaptés.

**Mots-clés**: Incorporation de paille; profondeur; utilisation de la paille; fractions de carbone organique du sol; activité enzymatique; fluorescence tridimensionnelle; analyse PARAFAC

# Abstract

**Enjun Kuang (2022). “Mechanism of carbon sequestration under different maize straw return practices in Northeast China.” (Ph.D. Dissertation in English).** Gembloux, Belgique, Gembloux Agro-Bio Tech, University of Liège, 131 pages, 19 tables, 18 figures.

**Abstract:** Straw return has become an important method in Northeast China for the improvement of soil fertility via increasing soil organic carbon (SOC) and complementing the nutrient contents as China has a relatively abundant straw resource. Because of low annual average temperature, the decomposition of straw could be slowed down, and agriculture therefore face lots of problems such as poor quality of sowing, emergence, and growth of seedlings. On the other side, in the process of decomposition, a considerable portion of carbon can be released into the atmosphere. The present research dealt with experiments to improve straw carbon stabilization in the soil and still maintain the decomposition of crop straw. Taking into consideration specific regional characteristics, it is our primary task to find suitable practices for the straw return to the soil and to solve the problems in production practices. Therefore, it is essential to improve the understanding of SOC sequestration mechanisms under different straw return practices.

In this thesis, firstly, we simulated the field tillage practice with mesh bags in black soil to evaluate the straw decay tendency according to different depths and the underlying mechanism of SOC sequestration. Secondly, four different straw return frequencies were surveyed to quantity the effects of the practices on SOC, soil nutrient, and enzyme activity as well as their relationship. Finally, dynamics of humification after maize straw return was assessed for different practices. The main results are as follows:

The simulation experiment in a micro-area plot showed that incorporation of maize straw residues to the sub-surface (D1-D3) layers lead to significant difference of decomposition compared with D0 (68.7% C lost, *P*<0.01) after 3 years. Incorporating the straw at 15-30 cm soil depth , was the best method regarding evolution of straw lignin, decomposition rate, and soil microbial content (SMBC). Straw returning to the deeper horizonsalso can produce more stable carbon in the soil and increase the accumulation of organic matter. The SOC content showed a significant positive correlation with C/N ratio and soil temperature. On the other hand, sucrase and moisture showed a significant negative correlation. The present study reinforces the importance of analyzing SOC fractions and SMBC for the subsoil.

Two fluorescence components of the dissolved organic carbon (DOC) were analyzed by three-dimensional fluorescence spectra according to different soil depths. CK-T4 treatment (bury of straw between 21 and 30 cm) showed dominance of humus-like and tryptophan-like substances, while the T5 treatment (bury of straw between 31 and 40cm) showed humus-like substances and tyrosine-like substances, with small authigenic components at the depth of 31-40 cm, and the humification coefficient was the highest. The fluorescence intensity of the component C1 increased with the deepening of straw returning depth, while the C2 component showed a fluctuating state. The DOC in soil was affected by both endogenous and exogenous sources (FI>1.4, 0.6<BIX<0.8), showing a state of weak humification (HIX<1.0). Correlation analysis showed that the soil depth, straw return, and their interaction had significant effects on DOC and its components.

The field experiment with difeferent frequencies of straw return indicated that returning straw every year to the field had a significant effect to improve SOC content both at the 0-20 cm and 20-40 cm, while straw return every three-year nor NS treatment could not increase SOC. There were significant and positive correlations among the soil SOC, DOC, easily oxidizable carbon (EOC), sucrase, and urease at 0-40 cm soil depth, but there were no significant correlations between the labile organic C fractions. The structure analysis showed the DOC came from mixture of plants and microorganisms, with a strong contribution of authigenic source, high bioavailability, and weak degree of humification after a straw return (FI was 1.59~1.69, BIX was 0.90-0.95, HIX was 0.64-0.74). Continuous straw return promoted the transformation of fulvic acids, while straw return every two years induced accumulation of macromolecular substances which form a more stable humus.

In conclusion, identification of proper straw return practices is crucial in Northeast China. Research was conducted to evaluate the if application of straw could enhance the aromaticity and acceleratethe decomposition of DOC released from the black soil. With the contribution of indigenous microorganisms in the soil, soil DOC improved and speeded up the transformation of soil humus, but continuous straw return treatment showed a weak degree of humification. Briefly, our findings reinforced by statistical analysis could provide some valuable and unique spectroscopic information on DOC derived from straw and offer technical guidance when straw return to black soil. China's land straddles many dimensions, and the straw returning time, crushing degree, straw returning amount, combined application of chemical fertilizer and suitable farming methods for straw return are different. Appropriate straw-returning technologies should be selected according to the regional conditions, a regional straw-returning technology system should be established, and straw returning should be carried out reasonably.

**Keywords:** Straw return; depth; frequence; soil organic carbon fractions; enzyme activity; three-dimensional fluorescence; PARAFAC analysis

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List of Abbreviations

|  |  |
| --- | --- |
| AVN | Alkali hydrolyzed nitrogen |
| AVP | Available phosphorus |
| AVK | Avalabile potassium |
| LF | free light fraction |
| O-LF | occluded light fraction |
| HF | heavy fraction |
| DOC | Dissolved organic carbon |
| EOC | Easily oxidizable carbon |
| LOC | Labile organic carbon |
| EEM | Excitation-emission matrix |
| Ex | excitation wavelength |
| Em | emission wavelength |
| PARAFAC | Fluorescence combined with a parallel factor analysis |
| FI | Fluorescence index |
| HIX | Humification index |
| BIX | Biological index |
| g | Gram |
| SMBC | Soil M biology carbon |
| SOC | Soil organic carbon |
| TN | Total nitrogen |
| TP | Total phosphorus |
| TK | Total potassium |
| D0 | Mesh straw bag was put in 0-5 cm |
| D1 | Mesh straw bag was put in 5-15 cm |
| D2 | Mesh straw bag was put in 15-30 cm |
| D3 | Mesh straw bag was put in 30-45 cm |
| T1 | 0-2 cm soil layer was excavated, straw and soil were fully mixed and backfilled into the original soil; |
| T2 | the 0-2 cm soil layer was excavated and placed separately, the 3-10 cm soil layer was fully mixed with straw, and then the original soil layer was backfilled, and then the 0-2 cm soil was backfilled to the surface; |
| T3 | treatment: the 0-10 cm soil layer was excavated and placed separately, the 11-20 cm soil layer was fully mixed with straw, and then the original soil layer was backfilled, and the 0~10 cm soil was backfilled in situ; |
| T4 | treatment: 0-10 cm and 11-20 cm were dug out according to layers and placed separately. 21~30 cm soil layer was fully mixed with straw and then backfilled with the original soil layer, and each layer of soil was backfilled according to the original position; |
| T5 | treatment: excavate 0-10 cm, 11-20 cm and 20-30 cm soil layer by layer and place them separately. After 31-40 cm soil layer is fully mixed with straw, the original soil layer is backfilled, and each layer of soil is backfilled according to the original position. |
| S-1 | maize straw residue returned to soil every year |
| S-2 | maize straw residue returned to soil every two years |
| S-3 | maize straw residue returned to soil every three years |
| S-4 | CK, without straw returned to soil every year |

**Chapter I**

**General Introduction**

As we all know, black soil is among the most fertile soils. However, with overuse and nutrient problems in recent years, black soil faced degradation and organic matter decline. Straw return is an effective measure to improve soil fertility and soil structure. Appropriate straw return to the soil could reduce soil bulk density, increase soil porosity, and increase soil nutrients. Researches show that straw return improves soil carbon sequestration capacity and increases soil organic carbon storage. With the continuous expansion of the corn planting area, big quantities of straw are produced.

In addition, in a context of prohibition of burning straw and of promotion of circular agriculture, the reuse of straw is very important. The commonly used agricultural harvest equipment can disperse and crush all straws to less than 15 cm when harvesting maize. The temperature in Heilongjiang Province is lower than in other provinces, the annual average temperature is less than 8 ℃, and the effective decomposition time of the straw is only from May to September per year. Most straws can be fully decomposed in the south because of the high temperature, but not in the north. It brings some problems such as affecting the quality of sowing, emergence, and growth of seedlings. Straw return to the deep soil effectively alleviated the situation and became the most effective method to return corn straw to the field in Northeast China.

## 1. Background

Straw is a relatively abundant resource in Northeast China, and the increase in the production of grain has led to enormous production of straw (Xia et al., 2014). However, nearly 50% of the straw is burned which results in serious environmental pollution (L. Zhou et al., 2012). Straw return to soil is a common and effective field management practice with a strong effect on the soil physical, chemical, and biological properties and on crop yield (Mu et al., 2016; Smitha et al., 2019). The straw, root, and other C inputs contributed about 40%, 30%, and 30% of total soil organic carbon (SOC) storage, respectively, following the large-scale implementation of the crop straw return policy in China (Zhao et al., 2018).

Although straw incorporation is extensively practiced across the world, most studies have been focusing on the effect of straw mulching or mixing with topsoil (Kahlon et al., 2013; Tao et al., 2015). The decomposition rate of straw varies with the depth of burial. Li (2001) found that straw incorporated into the top 5 cm of soil decomposed faster than that used as surface mulching only. However, due to the low temperature in Northeast China all year round - the annual average temperature is less than 8°C,- an effective decomposition period of straws occurs only from May to September. On the other hand, all types of corn straw completely get decomposed in the south of China because of the high temperature, while it was more difficult in Northeast. As a result, more than half of the straw residue is still left over the soil in Northeast China, affecting the quality of sowing, emergence, and growth of seedlings. Previous studies showed that the application rate of corn straw should be 30%~50% of the annual yield (Wu et al., 2002). The period of complete maize straw decomposition in the soil is long and takes about 2~3 years. Excessive amounts of straw return hinder the complete degradation of the straw over a short period, and the addition of insufficient amounts of straw does not meet the carbon pool maintenance requirement (Andruschkewitsch et al., 2012; H. Liu et al., 2021). Furthermore, many scholars have focused on the deep return of straw to the field (Wang et al., 2015; Jiao et al., 2014).

The black soil region of Northeast China, , performs a significant role in crop production and food security in China (Y. Zhang et al., 2007). It includes Heilongjiang, Jilin, and Liaoning provinces with a total area of 790,000 km2. However, serious soil and water losses have caused the degradation of the ecological system due to cultivation practices inappropriate to the physical enviroment of the local region (Fang et al., 2006). Therefore, it is of great significance to protect the black soil resources and improve the comprehensive grain production capacity (Wei et al, 2006). Meanwhile, due to long-term intensive agricultural production, this area has been suffering from considerable soil carbon losses, while it is also characterized by a large surplus of maize straw (Jiang et al., 2017). With the continuous development of modern agriculture, the maize planting areas has been expanded four times in the past 40 years, the yield and straw amount had increased 15 times (Q. Wang et al., 2014). The utilization rate of straw in black soil area is 75.2% in Jilin Province and 83% in Liaoning Province, while only 35% in Heilongjiang Province (figure 1.1). Thus, the lowest relative straw utilization compared with other province was noted in the Northeast of China could be due to the large amount of straw generation and low temperature here.

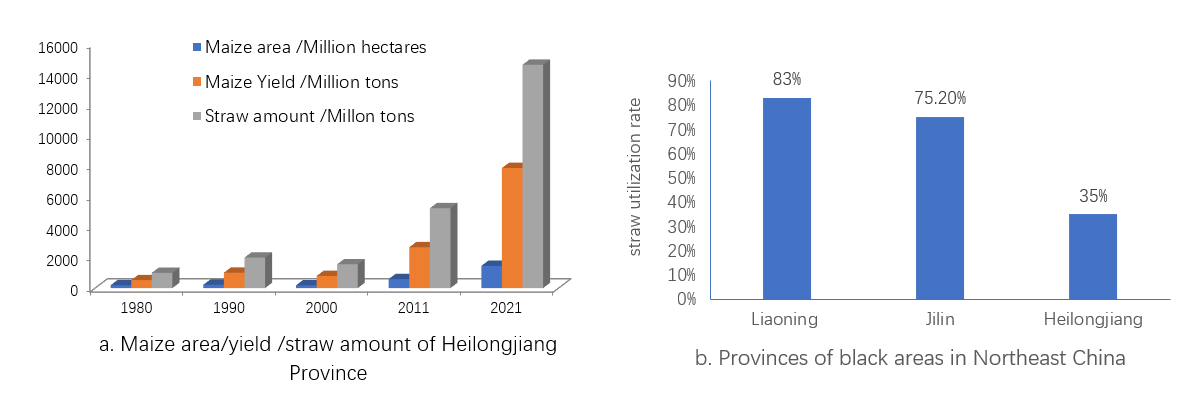


Figure 1-1 (a) the maize areas, yields, and straw amounts in recent decades in Heilogjiang province; (b) straw utilization of different black areas in NE China

We believe that the agro-ecosystems in Northeast China have greater opportunities for soil carbon sequestration by straw incorporation than other regions in China (Qin et al., 2013; Zou et al., 2016, Wang et al., 2015). However, the black soil areas also present diverse climate and soil types which requires different planting systems and complicates the situation.

* 1. ***Study area***

Heilongjiang Province is located in Northeast China, facing Russia across the river in the north and east, bordering the Inner Mongolia in the west, and Jilin Province in the south. It is the northernmost and easternmost provincial administrative region in China, between 121°11′~135°05′ E longitude and 43°26′~53°33′N latitude. The total area of its juridiction is 473 000 square kilometers, of which 15.9 million hectares are occupied by agricultural land, 23.2 million hectares of forest land, 2 millions hectares of pasture land, and other agricultural lands 1.8 million hectares.

* A cold temperate and temperate continental monsoon climate prevails in the study area.
* From south to north, the province can be divided into the middle temperate zone and the cold temperate zone according to temperature indicators.
* From east to west, it can be divided into a humid area, semi-humid area, and semi-arid area according to the dryness index.
* The climate is characterized by low temperature and drought in spring, warm and rainy in summer, easy waterlogging and early frost in autumn, long cold winter, and large regional differences in climate.
* The precipitation is abundant in summer, and the winter is dry and cold.
* Main soil type are dark brown soil, meadow soil, black soil, brown coniferous forest soil, albic soil, chernozem, aeolian sand soil, and meadow soil (figure 1.2).

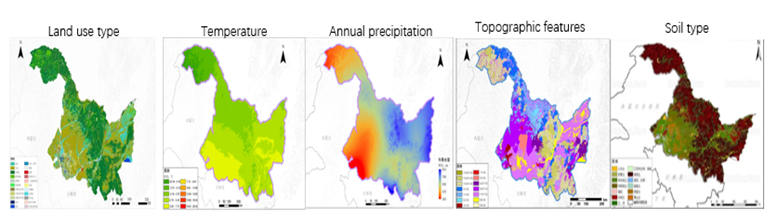


Figure 1-2 Thematic maps of Heilongjiang province bio-physical environment

Different soil types and land use lead to different farming systems (practices, planting types…). This study mainly focused on the different straw return practices in the black soil area.

* 1. ***Location of study sites***

Two sites were used for our study, both belonging to the black soil area (often called as the Songnen Plain). Both sites showed similar precipitation, different temperature regime, and one crop per-year planting system (Figure 1-3). The first site of the study was a micro-area test conducted at Harbin (Hrb, 126.62 °E, 45.68°N) in Heilongjiang province. The second site was at Gong Zhuling (GZL, 124.82 °E, 43.50°N) with typical black soil in Jinlin Province. The chemical properties of the soil are presented in Table 1-1.

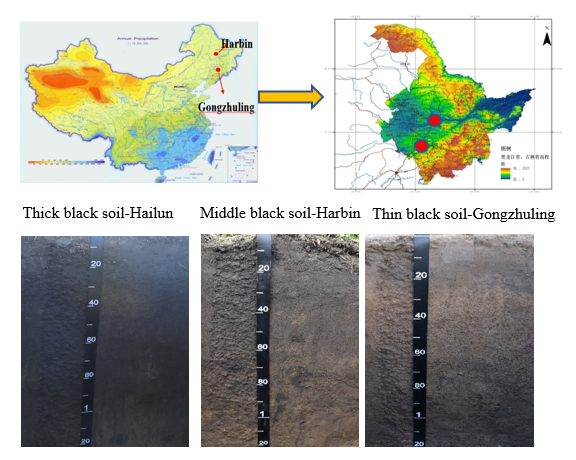


Figure 1-3 Location of study sites and differences of of black soil thickness observed

Table 1-1 Chemical properties of the soil at two sites

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site | P. (mm) | T. (°C) | EAT (°C) | thickness of black soil layer(cm) | SOC  g·kg-1 | Total N  g·kg-1 | A-N  mg·kg-1 | Olsen-P  mg·kg-1 | A-K  mg·kg-1 | pH |
| Hrb | 553.5 | 3.6 | 2 600~3 000 | 30~60 | 18.5 | 2.40 | 103.1 | 70.8 | 167.7 | 6.62 |
| GZL | 595.2 | 5.2 | 2 500~2 800 | 0~30 | 22.4 | 2.11 | 79.4 | 24.1 | 85.3 | 6.18 |

Abbreviations: P- Precipitation, T- Temperature, EAT- Effective accumulative temperature, A-N - Available nitrogen, and A-K - Available potassium.

## 2. Literature review

***2.1 Decomposition characteristics of the crop straw***

***2.1.1 Straw decomposition in soil***

Crop straw return to soil has become an effective way to supplement the soil nutrients and increase the crop yield in modern agriculture because it is rich in various nutrients and physiologically active substances (Zhang et al., 2008; Han, et al., 2009; Mu P., et al., 2011; Song et al., 2020). Crop return is a reasonable farmland management measure in the agricultural ecosystem. As a nutrient recycling strategy (Cayuela ML et al., 2009), it reduces the dependence of agricultural production to inorganic fertilizers, and the nutrients in the straw are directly released after decomposition (Blanco Canqui et al., 2009; Lal, 2009), thereby leading to an indirect increase in the soil nutrient availability (Lal, 2004). In soils with high fixing capacity of phosphorus, crop straws may act as slow release fertilizers.

Regarding the composition of the crop straw in the study area, the main soil components are divided into three categories: 1) water-soluble small molecular organics, such as amino acids and sugars that are easily decomposed and used by rapidly growing microorganisms (Fioretto et al. 2005; Adair et al., 2008); 2) macromolecular organics such as cellulose and hemicellulose, accounting to 28~ 44% of the dry weight of plant straw, which make the cell wall hard (Rehman et al., 2013); 3) lignin and other aromatic compounds. Lignin is the most difficult polymer to degradade in the straw and a complex aromatic matrix. During the first phase of the initial decomposition of straw, due to leaching and the activity of soil microorganisms and animals, the straw quickly loses the soluble compounds (starch, amino acids and sugars) and releases mineral nutrients. The weight of straw decreases rapidly, during the decomposition period. In a second decomposition stage, cellulose and lignin become the main compounds of straw and are degraded by specific microbial communities. Compared with the former stage, this stage has a lower decomposition rate, and is thus a slow decomposition stage (Purahong et al., 2015).

At present, the biggest obstacle in the straw return in Northeast China is the low decomposition rate, mainly because of the high carbon-nitrogen ratio of corn straw, the common spring drought, and the cold in late spring, thereby leading to poor crop emergence and low yield (Shi et al., 2018). Under the appropriate tillage mode, the straw can reach a higher decomposition rate. The main factors affecting decomposition in farmland production include the composition of organic materials (Talbot et al., 2012), soil type (Vesterdal, 1999), composition of biological communities (Cleveland et al., 2014), temperature and rainfall (Aerts, 1997), enzyme activity (Flores et al., 2005), and decomposition time (Tian P et al., 2019). The main method for studying straw decomposition is the mesh bag method (Varelaet al., 2014; Xu et al., 2017). Kamota et al. (2014) studied the decomposition process of the bioengineered maize leaves in Africa, turning transgenic maize and nontransgenic maize leaves into the soil or covering the ground. The results showed that the decomposition rate of straw returned to the soil was higher than that of mulched maize, and there was no correlation between the decomposition rate of straw and the genetically modified maize varieties. Anahí et al. (2010) showed that no-tillage significantly reduced the decomposition rate of litter, mainly because it increased the surface soil bulk density, decreased the organic matter content and pH compared to the original grassland. The decomposition rate of straw increased with an increase of the returning and nitrogen application, and the rapid decomposition of straw in the first two weeks had a strong incentive effect on the carbon contained in the soil itself (Guo TF, 2019).

Soil tillage can determine the depth of straw return and change the distribution of nitrogen in different soil layers (Chen et al., 2017; Latifmanesh et al., 2018). The nutrient supply during straw decomposition depends on different straw return depths (Kisselle et al., 2001). In the rotary tillage returning system, the straw and soil are mixed in 5-10 cm soil layers, and the straw is buried in 20-30 cm soil layers under the condition of straw returning (Stockfifisch et al., 1999). Latifmanesh (2020) demonstrated the corn straw with 0-10 cm return depth had a higher straw decomposition rate than those with 10-20 and 20-30 cm return depth.

Zhou et al. (2016) studied soil respiration and microbial community composition during the decomposition of corn straw under different temperatures and water content. The proportion of gram-positive bacteria and gram-negative bacteria showed a significant negative correlation with the amount of straw decomposition. Due to the input of fresh organic materials, the process of promoting or inhibiting the decomposition of farmland organic matter is called the priming effect (PE). It has been confirmed that the excitation effect plays a crucial role in the soil carbon and nitrogen cycle (Finzi et al., 2015; Keiluwei et al., 2015), and regulates the response of a series of ecological processes to global change (Sulman et al., 2014). The growth of crops has an impact on the decomposition and mineralization of returned straw, since the crops compete with soil microorganisms for water and nutrients (Jannoura et al., 2012), or crop roots can secrete substances that are absorbed more easily than straw carbon/nitrogen, thereby changing the growth environment of the microorganisms (Wichern et al., 2007). The interaction between crops and microorganisms in cultivated land can change the decomposition and utilization of straw carbon/nitrogen by microorganisms. The litterbags method was used to study the decomposition rate of different crop stalks in the field by Xu et al (2017). The mass of maize residues decreased to 52%, and the decomposition rate of residue decreasing from 0.223-0.379 month-1 in the first month to 0.054-0.076 month-1 after 12 months (Xu et al., 2017).

***2.1.2 Nutrients release***

Tillage treatment changes the straw decomposition rate and affects the nutrient release at the same time (Lu, 2015, Lin et al., 2017; Muhammad et al., 2011; Zhao et al., 2019). Straw decomposition and nutrient release occur simultaneously. To maximize the crop productivity, it is important to clarify the impact of decomposition on carbon and nitrogen cycling in the soil environment (Chen et al., 2017).

For different crops, in the traditional tillage straw return and no-tillage mulching returning treatments, the phosphorus release rate of green manure straw was found higher than that of no-tillage mulching within 2-10 weeks after returning, and its phosphorus release rate was found to be significantly positively correlated with the straw phosphorus content and negatively correlated with the straw C/P (Lupwayi et al., 2007). The law of nitrogen release and phosphorus is the same, and the nitrogen release rate of traditional tillage is higher than that of no-tillage mulching treatment. In addition, the nitrogen in corn straw after harvest is not released much in the first year of returning to the field, but gets released in the following years of returning to the field (Lupwayi et al., 2006a). Within 10 weeks after the start of the experiment, the potassium release rate of wheat straw under traditional tillage treatment was found to be significantly higher than that under mulching returning to the field (Lupwayi et al., 2006b). In a rice straw decomposition experiment, the increase in mineralization amount was mainly due to the input of straw (Devêvre and Horwáth, 2000). When fresh organic materials were put into poor soil, the decomposition process promoted the activity of microorganisms (Blagodatskaya and Kuzyakov, 2008). In soil rich in organic materials, the decomposition of organic matter is more likely to be affected by environmental factors, such as soil water content, nutrient content, etc. (Chowdhury et al., 2014; Nottingham et al., 2015). After 112 days of incubation experiment, Luce et al. (2014) found that 13-20% of soil mineral nitrogen and 4-8% of microbial biomass nitrogen came from straw nitrogen release. Quantitative analysis of the transport of phosphorus in straw and soil and the contribution rate of phosphorus from crop straw was studied. The results showed that only when a large amount of crop straw with high phosphorus content was applied to the soil, the effectiveness of straw on soil phosphorus reached a significant level. When the phosphorus content in straw or soil was low, the release of total phosphorus from straw was significantly reduced (Damon et al., 2014).

***2.2 The effect of different straw return practices on soil physical, chemical, and biological properties***

At present, the main straw return practices consist of the straw direct return and straw indirect return. The direct return includes the surface mulching and straw crushing and turn back, while the indirect returning includes feed livestock with straw and then returning livestock manure to the field, and retting return. It is difficult to promote the technology of indirect return, so the straw direct return still remains the main way of straw return in China (Ji et al., 2012). The traditional direct returning of farmland includes mostly surface covering and shallow returning of the cultivated layer. The surface covering improves the soil water storage and moisture conservation capacity (Li et al., 2020). The shallow return of the cultivated layer can improve the soil aggregation structure and increase the organic matter content in the cultivated layer (Wang et al., 2019).

However, there are some problems with the current surface mulch and shallow return in topsoils, such as affecting the growth of next season's crop, causing soil C/N imbalance, reduced seed viability, germination, seedling growth, and the greenhouse effect (Ji et al., 2012). It could also lead to an easy air leakage and moisture missing, which was not conducive to seed germination (Zou et al., 2013), and had no obvious effect on the accumulation of organic matter. Additionally it led to problems such as shallow tillage and loss of organic matter (Zhu et al., 2016). The concentration of straw buried in the ditch to 40 cm reduces the CO2 emission compared with that of straw buried in the ditch to 20 cm (Wu et al, 2014). Therefore, the method of directly burying straw into deep soil layer of 40 cm was gradually popularized and widely used. Straw deep return refers to the help of mechanical crushing and deep reclamation of straw into the soil subsurface (20-40 cm), extending the straw surface fertilization to the subsurface fertilization. This method does not only improve the soil mechanical properties of the subsurface, but also promote the accumulation of organic matter, and solve the problem of shallowing the arable layer (Dou et al., 2019). The concentrated depth of straw can also improve the water storage capacity of soil and promote the growth of crop roots (Wang et al., 2015). Many scholars developed technical systems such as straw enrichment and deep reclamation (Dou, 2019), and fully mechanized straw and full deep reclamation (Wang et al. 2017) that provided a strong support in the promotion of straw deep reclamation.

Ma et al. (2003) showed that the overall planting of straw effectively improved the physical and chemical properties of soil and reduced the bulk density of 0~10 cm soil by 0.1-0.25 G. Zhang et al. (2016) showed that the bulk density decreased after straw returning, which resulted in a downward trend in the bulk density of the 0-20 cm topsoil and 20-40 cm subsoil. Among them, due to the tillage depth, the surface soil bulk density at 0-20 cm decreased significantly, resulting in obvious differences among the treatments, and the subsoil bulk density at 20-40 cm decreased slightly. It had no significant effect on the soil porosity below 20 cm, and serves to be a major indicator of soil porosity. The great measure of returning straw to the field helps in an effective and significant improvement in the saturated hydraulic conductivity of the topsoil of 0-20 cm soil.

It also had a significant impact on the improvement of soil organic matter, soil nitrogen, phosphorus, and potassium, especially the content of soil available potassium, which reached 2.6 times. Liu et al. (2018) showed that the combined application of nitrogen fertilizer could significantly improve the content of soil organic carbon and available nutrients of wheat and maize. Tan et al. (2017) showed that after the straw was returned to the field, the organic carbon content of soil and humic acid (HA) increased with increasing ΔlgK value, and the application effect of deep straw application was more significant than that of shallow straw application. The deep application of straw had little effect on the contents of available nitrogen, phosphorus, and potassium in the soil. However, Zou et al (2013) also showed that straw return could not increase the total amount and quality of the soil carbon pool but increased the soil microbial carbon.

Soil microbial carbon is the most active part of soil organic carbon. Some researchers believe that the soil microbial parameters such as soil microbial diversity, microbial carbon and nitrogen can be used to estimate the soil health and quality. The vast majority of soil materials were in a stable or semistable state. Although the microbial biomass carbon accounts for only a small part of soil carbon, microorganisms play a key role in the soil material cycle through mineralization, decomposition of organic matter and self-assimilation of inorganic matter. It is well-known that the application of organic matter could increase the number of soil microorganisms, which has been confirmed by most studies. However, the effect of straw return on the microbial diversity and activity is still controversial. Many factors affect the soil microbial biomass, such as the soil type, human activities, fertilization measures, land use mode, the impact of different temperature and humidity environments, etc. (Zhao et al., 2005). The alternation of dry and wet soil can cause a large amount of death and renewal of soil microorganisms. Also, the soil pH value also significantly affects the soil microbial biomass. The microbial biomass of strong acid, strong alkali and saline alkali soils is significantly low. Low temperature (less than 6 ℃) or high temperature (more than 35 ℃) has a great impact on soil microbial biomass. Generally, the changes in the soil environment produce two results on the microorganisms: first, the number of unsuitable microorganisms could be reduced or even get killed; microorganisms adapted to the environment multiply and accumulate in large numbers. Straw return changes the original soil environment, and the input of straw provided energy and nutrients for soil microorganisms. However, others believed that soil original C was still the main source of microbial nutrients and energy through 14C tracer technology. Straw return to the field accelerated the turnover rate of the soil microbial carbon, and the acceleration of the microbial biomass carbon turnover rate accelerated the separation of soil original carbon and straw carbon (Wang et al., 2003). Zhang et al. (2006) proposed that the types and advantages of soil microbial communities with straw returning were higher than those without straw returning. Straw returning increased the diversity and activity of the soil microorganisms to a certain extent. However, a long-term positioning test of red soil showed that the microbial carbon source utilization capacity of straw returning to red soil was low (Rosie et al., 2009). Other studies have pointed out that rotten straw was conducive to maintain the diversity and activity of soil microorganisms (Zhu et al., 2003).

There is no doubt that straw can provide a carbon source for the soil microbial activities, but whether it can improve the species richness and dominance of the microbial community is still controversial. It is possible that the initial stage of straw returning can reduce the ability of microorganisms to use carbon sources and the uniformity of community species, resulting in the decline of soil carbon and nitrogen utilization, but the long-term effect would increase the diversity and activity of soil microorganisms (Zhu et al., 2014). Returning straw to the field significantly increases the number of soil phosphorus dissolving bacteria and promotes the transformation of insoluble phosphorus to soluble phosphorus(Ji et al. 2014). The activities enzymes like dehydrogenase, urease, sucrase and neutral phosphatase in soil had a good correlation with soil nutrients, reflecting the fertility of soil in the current season, and served to exhibit a good index for evaluating soil management (Deng et al., 2013). Soil enzyme activity could represent the direction and degree of the corresponding biochemical processes in soil. Straw return improves the activities of urease, alkaline phosphatase and catalase in soil and invertase (Ji et al., 2014). However, the effects of straw return on the soil enzyme activities in different soil layers were inconsistent. The field positioning experiment in the dry farming area of the Western Loess Plateau showed that straw return improved the activities of soil urease, alkaline phosphatase and sucrase in the 0~10 cm soil layer, but had no significant effect on the activities of soil urease, alkaline phosphatase and sucrase in the 10~30 cm soil layer. Also, it had no significant effect on the activity of soil catalase in the 0~30 cm soil layer (Luo et al., 2009).

***2.3 Mechanism of soil organic carbon sequestration and stabilization according to straw return practices***

***2.3.1 Soil organic carbon sequestration***

Straw return is a vitally important practice for improving the soil structure and complementing the favorable nutrient contents (Chatterjee, 2013), which is conducive to the crop growth (Wang et al., 2014) and accelerates the SOC sequestration (Liu et al., 2014). Dynamic changes in SOC in agricultural soils are mainly determined by the balance between the organic material inputs and degradation rates of existing SOC (Laird and Chang, 2013). Adding organic inputs is the most direct practice to accelerate the SOC mineralization in a positive priming effect, or adversely slow down its mineralization causing a negative the priming effect (Kuzyakov et al., 2000). However, the magnitude and direction of priming effect are not only impacted by the quantity and quality of exogenous organic carbon, but also influenced by the SOC stability (Kuzyakov et al., 2000).

The primary factor influencing the effects of straw return on the SOC sequestration is the amount and type of C-containing straw (Lou et al., 2011; Wang et al., 2012; Shadrack et al., 2014). Many studies have shown that increasing the amount of the straw residue increased the SOC levels untill the soil was C-saturated (Kunlanit et al., 2014; Poeplau et al., 2015). Moreover, there were large differences in the composition of plant leaves, stems, and roots, which had different effects on SOC sequestration (Gong et al., 2009; Clemente et al., 2013; Fan et al., 2014; Menichetti et al., 2015). Furthermore, other factors such as experimental period, test site, planting system, tillage, application of fertilizer, and the practice of straw return (Shen et al., 2007; Triberti et al., 2008; Liu et al., 2014; Villamil et al., 2015, Yang et al., 2015; Zheng et al., 2015; Zhang et al., 2015) were vital to exhibit significant differences in the magnitude and direction of SOC changes after straw return. Studies showed that long-term straw return was an effective agricultural management practice for increasing the SOC sequestration (Wang et al., 2014; Yang et al., 2015), while maize straw return in combination with sub-soiling was the more suitable practices. This increased the yield and soil C sequestration in short-term straw return than high amount, ultimately achieving a sustained agricultural development in this cropping system (Li et al., 2016). Therefore, to achieve the maximum environmental benefits, it is necessary to obtain a quantitative and qualitative understanding of the effects of straw return on SOC sequestration.

***2.3.2 Soil organic carbon molecular structure***

Spectral analysis plays a vital role to characterize the complexity of straw decomposition products and the molecular structure of soil organic matter. Spectral analysis methods mainly include the infrared spectroscopy, 13C nuclear magnetic resonance, three-dimensional fluorescence spectroscopy and ultraviolet visible absorption spectroscopy.

Infrared spectroscopy analyzes the characteristic functional groups on the basis of the absorption peaks of organic materials. It is an easy to operate and fast technique (Younis&Iqbal, 2015). In the long-term fertilization process, different organic functional groups of soil organic matter (-CH3, =CH2, C=0-COOH, -NH2, etc.) have specific absorption peaks in the infrared spectrum, so the structural composition of unknown samples can be accurately analyzed quantitatively (Plaza et al. 2007). Cao FY et al. (2016) used nylon mesh bag method and FTIR spectroscopy showed that maize and soybean straw mainly formed humic acid after decomposition. There were no obvious differences in organic structures among different land uses and between fresh and dry treatments (P>0.05), but the crop residue type and residue N content impacted structural changes. Pei et al. (2021) examined the regularity of distribution and chemical structure characteristics of organic carbon in soda alkaline fluvo-aquic soil aggregates after straw returning, and showed that the straw return significantly increased the content of light organic caron in 53-250 um aggregates and promoted the fixation of organic carbon by saline soil aggregates in short time, but did not change their structural characteristics. Zhu et al. (2021) showed that the total straw return increased the polysaccharide content of the top layer and mid-layer soils, and maintained a soil carbon stability of the whole soil layers in Northeast China by using mid-infrared spectroscopy.

Three-dimensional excitation-emission matrix fluorescence spectroscopy (3D-EEM) continuously scans the emission spectrum at different excitation wavelength positions by using the pop scanning method to analyze the structure of soil organic matter and more intuitively reflects the fluorescence spectrum information of each organic matter component. Compared with the traditional fluorescence spectrum, 3D-EEM can also analyze multicomponent complex systems (such as humus) and the medium fluorescence spectrum (excitation/emission, Ex/Em). The identification and characterization of overlapping objects are very important to study the source and composition of dissolved organic matter in the soil (Shao et al. 2009). This fluorescence analysis technology has been widely used in many research fields to conduct qualitative or quantitative analyses of the water-soluble organic matter from different sources. When using three-dimensional fluorescence spectroscopy to study dissolved organic matter from different sources, the program is usually set to excitation wavelength (HX), the range is 200~600 nm, the scanning interval is 10 nm; the emission wavelength (AEM) range is 200~600 nm, the scanning interval is 2 nm; and the excitation and emission slit width is 10 nm. The DOM sample of sewage has three obvious fluorescence peaks: peak A (Ex/Em=330/426nm) belongs to fulvic acid-like fluorescence in the visible region, peak B (Ex/Em=245/436 nm) belongs to fulvic acid-like fluorescence in the ultraviolet region, and peak C (Ex/Em=275/346 nm) belongs to protein-like fluorescence (Ruscalleda et al. 2014). In an earlier study, the three-dimensional fluorescence spectrum characteristics of soil under different fertilization treatments was analyzed, and it was found that the control group (no fertilization) had strong protein-like, humic acid-like, and fulvic acid-like peaks in the soil. However, there were only humic acid-like and fulvic acid-like substances in the two treatments of applying organic fertilizer and organic-inorganic compound fertilizer, indicating that the application of organic fertilizer and organic-inorganic compound fertilizer promoted the transformation of protein-like substances in the soil into humic acid-like substances (Ruscalleda et al. 2014).

***2.4 Comprehensive straw utilization***

At present, the output of crop straw in China is large. If it is not dealt properly or intervened in a proper practice, it may cause serious environmental pollution, which not only affects the development of rural economy, but also hinders the farmers' income. With the introduction of a series of national policies on straw utilization, the efficient measures and technical specifications for straw utilization are proposed. The "five modernizations" of crop straw utilization refer to the fertilizer, feed, base material, raw material and fuel of straw (figure 1-4). Comprehensive utilization of straw can turn waste into treasure, turn harm into benefit, increase farmers' income, cultivate new economic growth points, and reduce the environmental pollution. It is the main way to solve the problem of straw burning, and is of great significance.

In accordance with the principle of "multiple utilization and priority for agriculture", the key technologies for the comprehensive utilization of corn straw were studied, with the direct return of corn to the field and the return of over rotten corn to the field as the main technology. This technology was supplemented by the industrialization of straw energy, base material and raw material, to achieve the direct return of corn straw to the field rate of more than 60%, and the comprehensive utilization rate of resources of more than 90%.

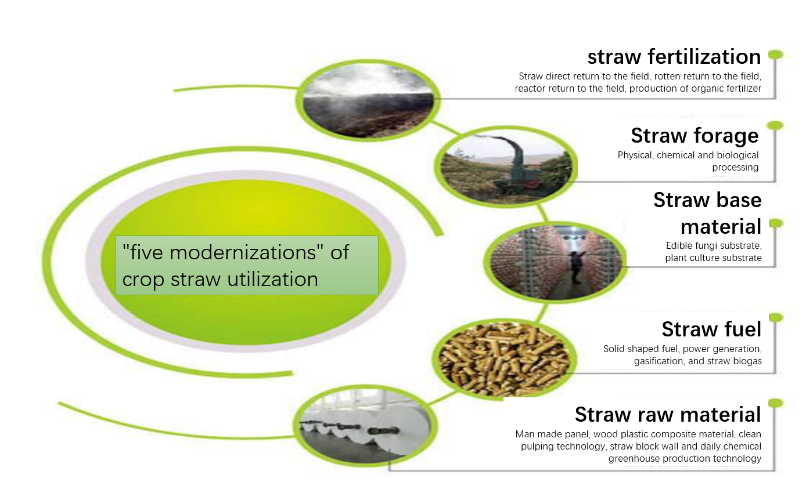


Figure 1-4 Five modernizations of straw utilization

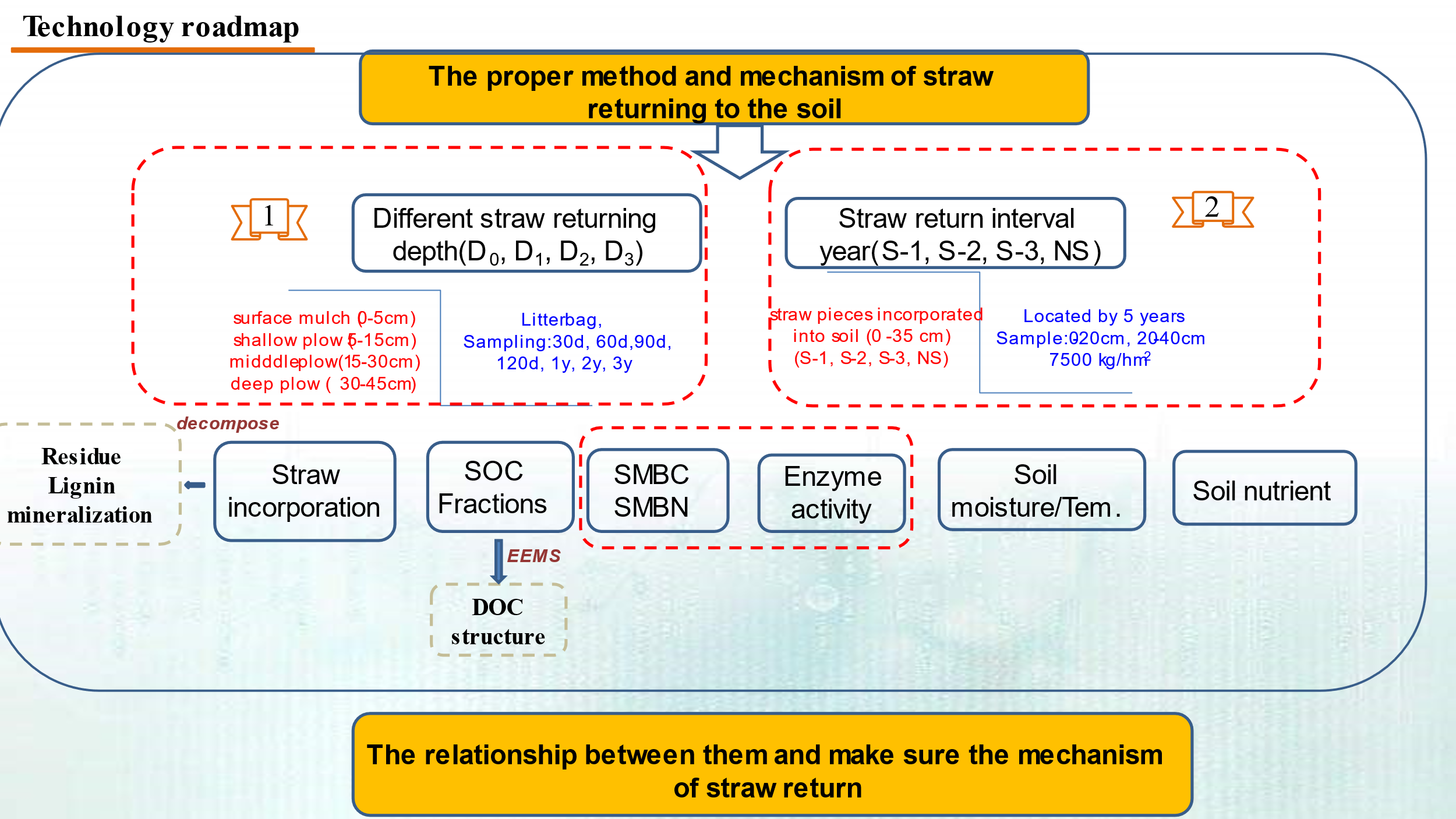
## 3. Objectives

The general objectives of this work were to decipher the underlying mechanisms of soil carbon sequestration in different modalities of straw return, and to understand the effects of the soil carbon pool, soil nutrients and enzyme activities, so as to provide a theoretical basis for the straw returning mode and comprehensive utilization in Heilongjiang Province. To expound on the above main objectives, the specific aims of the present research are as follows:

a: To understand the effects of organic carbon content, soil moisture, temperature, MBC, and enzyme activity on soil nutrient cycling and C sequestration in the specific context of maize straw return to black soils.

b: To evaluate the importance of bury depth and frequency of straw return as driving factors of C sequestration and nutrient cycling.

c: To study the effects of the different treatments on the structure and composition of DOC, as a key component of SOM transformations.

Figure 1-5 Technology roadmap of the thesis

## 4. Overview of the chapters

This thesis was structured into the following 6 chapters:

**Chapter Ⅰ** General introduction.

In this chapter, the general information of research progress and objectives was described. We presented the comprehensive evaluation of straw incorporation, it contains the effect on soil physical, chemical and biological characteristics, and SOC fractions and structure in different straw incorporation methods. Finally, the objectives and structure of this thesis were summarized in this chapter.

**Chapter II** Degradation characteristics of maize straw under different buried depths in northeast black soil and their effects on soil carbon and nitrogen

The objective of this chapter was to make sure the effects on labile organic carbon fractions under different buried depths. Whether depth could make straw carbon to stabilize and to maintain the decomposition of crop straw. Based on this concept, the effects of maize straw incorporation at different depths on the nutrient status of soil were observed via three experiments conducted for 3 years on black soil in Northeast China. Four soil depths were tested, these are D0 (0-5 cm), D1 (5-15 cm), D2 (15-30 cm) and D3 (30-45 cm).

**Chapter III** Analysis of DOC component structure of black soil profile under straw returning condition based on florescence spectrum

Taking black soil as the research object, the difference of three-dimensional fluorescence spectrum of soil dissolved organic carbon (DOC) after returning maize straw to different soil depths was analyzed, and the change characteristics of humification degree of maize straw return to deep soil were discussed.

**Chapter IV** Dynamic changes of straw return with different years on soil labile organic carbon fractions and enzyme activities in black soil areas.

The objective of this chapter was to monitor the effects on soil labile organic carbon fractions, enzyme activities, and three-dimensional fluorescence spectrum soil dissolved organic carbon (DOC) with straw return through different years. After maize harvested in October 2011, the straw was crushed into pieces and incorporated into deep soil by tillage (about 30-35 cm). The average maize straw production was about 10000 kg·hm-2. Four treatments were designed and shown as following: S-1 (maize straw residue returned to soil every year), S-2 (maize straw residue returned to soil every two years), S-3 (maize straw residue returned to soil every three years), NS (CK, without straw return to soil every year).

**Chapter V** Fluorescence spectral characteristics of straw return with different years on the DOC components in black soil areas.

This chapter was main focus on the DOC structure using Excitation-emission matrix spectroscopy (EMMs) combined with parallel factor analysis (PARAFAC). It indicated that DOC came from the mixture characteristics of plants and microorganisms, with strong contribution of authigenic sources and weak degree of humification. Tryptophan like substances were dominant in three fluorescent spectral components which identified by PARAFAC analysis. The treatment, similar to the return of straw every year, reduced fulvic acid like substances and promoted the transformation of fulvic acid. While straw return every two-year was easy to facilitate the accumulation of macromolecular substances and the phenomenon helped to form a more stable humus.

**Chapter Ⅵ** General discussion, conclusions, and perspective

In this chapter, the meaning, importance, and relevance of general results in the three main chapters were introduced. We stated the answers to the main questions of this study, made future prospects for this topic and demonstrated what new knowledge we have contributed.

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**Chapter Ⅱ**

# Degradation Characteristics of Maize Straw under Different Buried Depths in Northeast Black Soil and Their Effects on Soil Carbon and Nitrogen

From Kuang E. J., Xu, M. G., Colinet, G., Chi, F. Q., Su, Q. R., Zhu, B. G., Zhang J.M. (2020). Degradation characteristics of maize straw under different buried depths in northeast black soil and their effects on soil carbon and nitrogen. *International Journal of Agriculture & Biology,* 24:77‒84.

## Abstract

The incorporation of straw in the improvement of soil fertility via increasing soil organic carbon has become an important method. But in this process of decomposition, a considerable portion of carbon will be released into the atmosphere. The present research dealt with experiments to make more straw carbon to stabilize the soil and to maintain the decomposition of crop straw. Based on this concept, the effects of maize straw incorporation at different depths on the nutrient status of soil were observed via three experiments conducted for 3 years on a black soil in northeast of China. Four soil depths were tested. These are D0 (0-5 cm), D1 (5-15 cm), D2 (15-30 cm) and D3 (30-45 cm). The results showed that maize straw residues incorporation to the sub-surface (D1-D3) layer had a significant difference compared with D0 (68.7% C lost, P<0.01) after 3 years of decomposition. The three treatments with buried residues into the soil had almost similar average diminution of C content (10.37-14.01%). Meanwhile, D0 had a lower decomposition constant, straw lignin, and cellulose decomposition than D1-D3 treatments. The content of urease and sucrose declined with the deep soil, and straw return increased the enzyme activity in this study. The D1 treatment also had higher soil microbial biomass carbon (SMBC) and labile soil organic carbon (SOC) fractions. These components also increased significantly with the seasonal change in the D2 treatment. The content of SOC showed a significant positive correlation with C/N and soil temperature. While sucrose and moisture showed a significant negative correlation between them. The present simulation study reinforces the importance of analyzing SOC fractions and SMBC in deep soil. It indicated that maize straw incorporation in deep soil was very important for the maintenance of soil fertility. At the same time, it suggested a solution to the problem of a large quantity of straw production in the maize cultivation zones. The results bear significant importance for agriculture.

**Keywords:** Straw incorporation; Soil depth; Soil organic carbon fractions; Microbial biomass carbon; Enzyme activity

## Introduction

In the People’s Republic of China, the North East area is one of the most important maize (Zea mays L., Fam.: Poaceae) growing areas. It produces annually more than 35% of country’s total maize production and occupies 31% of maize growing areas of China (Fan et al., 2018). Residues produced after harvesting and processing of maize grains are important renewable resources. But managing this huge amount of maize residues is a big challenge. The annual production of maize residue has been estimated 239 mio MT/y. From this huge stock, only 23% of the residues are used for forage, 4% for industry materials and 0.5% for biogas generation. The rests of the production are then discarded and even directly burnt in the field (Liu et al., 2008).

After harvesting, straws return to the soil is beneficial and can be considered as an important management practice (Zhang et al., 2014, 2016b; Wang et al., 2015a; Yin et al., 2018). It increases the input of nutrients and carbon storage in the top soil (Choudhury et al., 2014; Zhang et al., 2016a). Thereby, opens a great deal of potential in enhancing soil fertility, soil organic matter (SOM) content and microbial population (Lal, 2004; Powlson et al., 2008). All these activities help improving the soil structure (Zhang et al., 2008), especially the soil porosity (Wuest, 2007). Unfortunately, in northeast China, leaving residues onto the soil surface would not be efficient for soil quality improvement. Because the left out straw in the field could not be decomposed completely under the low temperature (Wang et al., 2012). Moreover, maize straw returning to the field would lead to an exhaustion of soil moisture, and be harmful to the seed germination of the next crop (Liu, 2014). Incorporating the straw into the subsurface soil may decrease the adverse effect in crop seeding and enhance the soil organic carbon (SOC) stabilization (Choudhury et al., 2014). This may be considered as a beneficial practice for the improvement of environment in the northeastern region of China (Kuang et al., 2014; Chen et al., 2017; Wang et al., 2015b; Yang et al., 2016).

For the cultivated lands in the northeastern China, the soil organic status can be maintained at a relatively stable level after being returned the crop residues to the field. However, there are some strong physical constraints such as existence of hard pan below the plough layer at 20 cm depth. It limits the development of the root system. On the other hand, it was observed that because of low temperature the straw applied into the plough layer, decomposes slowly over a long winter. So, it hinders the seedling activity for the next planting season. However, putting the straw residues into the deeper part of the soil is a widespread practice in this region (Kuang et al., 2014). The process helps in improving fertility of the deep soil. This very concept, actually helped to develop the present research plan. In order to understand the effects of burying residues in the cultivated fields of northeastern China, a field experiment was needed to be carried out. The basis of this experiment would be to put straw residues into the soil at different layers, and to measure the evolution of indicators of SOM dynamics. We hypothesized that, (i) the localization in deep horizons can accelerate the speed of maize straw decomposition due to temperature effect, (ii) the soil properties and microbial characteristics respond differently after straw return to different soil layers. In order to test the components of this hypothesis, the specific objectives for the present research undertaken, were: to return maize straw to different soil depths, to make sure that the straw biomass decomposition is accelerated into deep than the surface of soil and to make sure that the process enhances the storing of straw carbon in a deep soil and improves the soil nutrient content.

## 1 Materials and methods

***1.1 Experiment Design***

All the experiments for the present research were carried out in the micro-area test of the Academy of Agricultural Sciences of Heilongjiang, Northeast China. Mesh bags were used for the decomposition experiment. Maize straw (MS) was collected during harvesting time of September. Specifically, 50 g of dried maize straw were chopped into about 2-5 cm lengths in each bag (300 mesh). The amount of straw in the bags were selected according to the total maize straw biomass by the year which was about 7500 kg hm-2. Urea was used to adjust the C/N ratio to 25:1 and field capacity was adjusted to 60%. Bags were placed in four different soil horizons. The depths of the horizons for burying the MS were: D0, D1, D2 and D3. Triplicate samples of bags were collected after 30, 45, 60, 90, 120 d and after 1, 2 and 3 y from the beginning of the experiment. At the same time, the soil of the upper and lower 5 cm of the mesh bags were also sampled. Immediately after sampling, part of the soil was sieved (1 mm mesh) and used for the analysis of enzyme activities and soil microbial biomass. The other part of the soil was air-dried and sieved (2 mm and 0.15 mm mesh) to test its chemical properties. Before the chemical analysis, the maize straw samples were oven dried at 60°C without washing. After this a definite volume of it weighed, and the residual rate of straw was calculated. The samples were crushed to determine the straw organic carbon, lignin and cellulose contents. The Residue percentage of the straw was calculated using the formula St/50×100 (where, S is the residual mass of straw (g) and 50 is the original straw mass (g), t is the different sampling time).

By putting a thermometer at soil layers of 5, 10, 15, 20 and 25 cm, the temperature was recorded on the sampling dates.

The moisture and temperature showed in Figure 2-1.

Figure 2-1. The soil moisture (H2O %) and temperature in the 5, 10, 15, 20 and 25 cm soil layers at sampling days.

***1.2 Methods Sample and analysis***

***1.2.1 Straw organic carbon***

The above-mentioned oven dried straw sample (unwashed, 60°C) was smashed through a 100-mesh sieve and used for the determination of soil organic carbon (SOC) (Multi N/C 2100 TOC total organic carbon/total nitrogen analyzer).

***1.2.2 Soil organic carbon fractions analysis***

The density fraction of soil organic carbon (SOC) refers to Golchin (Golchin *et al.*, 1998). In it, SOC was divided into free light fraction (LF), occluded light fraction (O-LF) and heavy fraction (HF). The methodology in brief follows: 5 g of air-dried soil was homogenized with 25 ml NaI solution (gravity 1.8 g·cm-3) in a 50 ml centrifuge tube. The sample was gently shaken and let stand overnight at room temperature. Next day, it was centrifuged at 3500 rpm for 15 min. The supernatant was poured out, 50 ml of NaI was added to it and centrifuged again. This process was repeated twice. The residue was finally washed by 25 ml 0.01 mol L-1 CaCl2 and 50 ml of distilled water, then dried on a water bath below 60℃ and weighed. This dried part was LF. The extraction process was continued by adding 25 mL NaI solution to the residue material in the centrifuge tube, shaken and centrifuged for twice. This part was O-LF. Thirdly, 25 ml distilled water was added, shaking done for 20 min and then centrifuged at 4000 rpm for 20 min. The precipitation in the tube was repeatedly washed with 95% ethanol to colorless and was put into an oven below 40℃ and dried to a constant weight. This part was HF. All dried parts passed through 0.25 mm sieve and analyzed for organic carbon by wet oxidation method with K2CrO7 at 170~180℃.

***1.2.3 Soil microbial carbon and nitrogen analysis***

Soil microbial biomass was determined by chloroform fumigation method. However, for the determination of soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) potassium dichromate oxidation method and Kjeldahl method were used, respectively.

***1.2.4 Soil enzyme activity***

Urease determination was carried out by indophenol blue colorimetry method. And 3, 5-dinitrosalicylic acid colorimetry was used for the determination of sucrase enzyme (Guan, 1987).

The soil urease activity was determined by sodium phenolate-sodium hypochlorite colorimetric method, and the data was expressed as milligrams of NH3-N produced per gram of soil at 24 h. On the other hand, the soil sucrase activity was determined by 3,5-dinitrosalicylic acid colorimetric method. The data were expressed as milligrams of glucose produced per gram of soil at 24 h (Guan, 1987).

***1.3 Statistical analyses***

All the statistical analysis of the data was subjected to ANOVA using the Statistical Package for Social Science (SPSS 17.00). Significant difference among means was identified using Duncan (D) test at P<0.05.

## 2 Results

***2.1 The decomposition of maize straw biomass***

Figure 2-2 shows the effect of straw decomposition on straw residue over time and soil depth. When different soil depths are compared, accelerated straw decomposition was evident in the deeper part of the tested soil. D0 treatment, which is a surface soil showed a different response. At this level (D0) 68.7% of the mass was still left at the end of the experiment. While the other treatments at deeper soil layers (D1-D3) had almost similar average straw residue (10.4%-14.0%). Compared with the whole stage of decomposition, there was a fast stage which just began before 90 days (Figure 2-2). The evolution of straw residue can be represented by decreasing power functions (table 2-1).



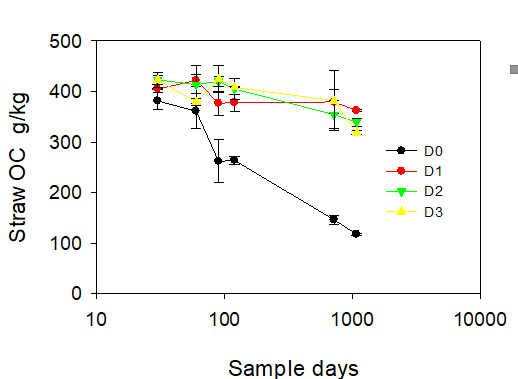
**Figure 2-2. Maize straw residues with time [D0 (0-5 cm), D1 (5-15 cm), D2 (15-30 cm) and D3 (30-45 cm)]**

**Table 2-1 Equations of straw residue evolution according to treatments**

|  |  |  |
| --- | --- | --- |
| Soil depth | Equation | R2 |
| D0 | y=983.29x-0.875 | R2=0.9926 |
| D1 | y=3844.3x-0.857 | R2=0.9791 |
| D2 | y=2636.0x-0.8 | R2=0.9821 |
| D3 | y=2897.9x-0.811 | R2=0.9833 |

***2.2 The organic carbon content of straw residue incorporation in different soil depths***

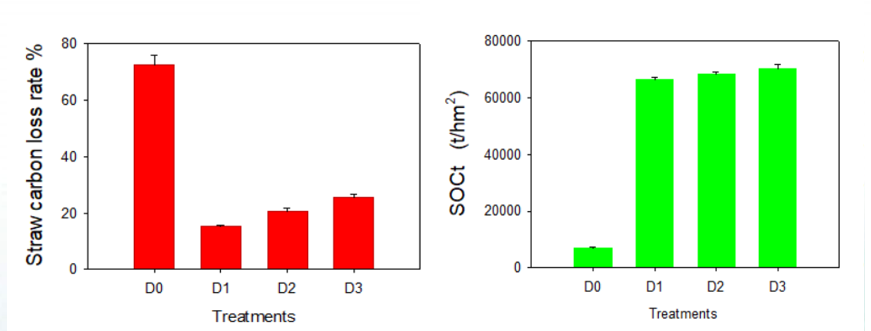
Figure 2-3 shows the mineralization pattern of maize straw organic carbon at different soil depths over time. The effects of depth and time on the mineralization process are very clear. The organic carbon content of the straw put into deep soil is higher. It means at those depths the straw keeps more carbon. On the other hand, straw left on the top of the soil (D0) keeps less organic carbon. D3 treatment had more organic carbon content than D2 and D1. After 1 year of decomposition, D3, D2 and D1 were higher than D0 by 50.5%, 58.3% and 65.1%, respectively. It indicated a significant difference between D0 and other deep straw returning treatments.

**Figure 2-3. The straw organic carbon contents with time [D0 (0-5 cm), D1(5-15 cm), D2 (15-30 cm), and D3 (30-45 cm)].**

Evolution of straw with time in days is best modelled by polynomial functions (table 2-2).

|  |  |  |
| --- | --- | --- |
| Soil depth | Equation | R2 |
| D0 | y=380.5569-0.6569x+0.0004x2 | R2=0.8779 |
| D1 | y=490.2679-0.1004x+0.000057x2 | R2=0.5155 |
| D2 | y=426.5623-0.1440x+0.000059x2 | R2=0.9890 |
| D3 | y=412.8137+0.0188x-0.000096x2 | R2=0.8214 |

**Table 2-2 Equations of Straw OC according to treatments**



**Figure 2-4.** **The straw organic carbon loss rate and SOC storage under different soil depths [D0 (0-5 cm), D1(5-15 cm), D2 (15-30 cm), and D3 (30-45 cm)].**

The results of straw decomposition after three years showed the straw carbon loss rate was 75% in the D0 treatment, while the D1-D3 treatments were less 20% in Figure 2-4. The SOC storage in D1-D3 treatments were approx. 200 times than (215%, 224%, 234%) the D0 treatment. It indicated that straw return to deep soil could storage more straw carbon.

All the carbon fractions had a declined trend from the topsoil to the deep soil layers. On the day 1 year after the straw returned, the content of the LF group in the D3 treatment was stable, but it declined with sampling time. This trend indicated that the LF group was faster than others in the process of decomposition. It can also be seen from these data that the existence of light organic carbon is unstable. The O-LF was the physical protection component of soil organic carbon because it is existing as randomly distributed between soil aggregates. From Table 2-3, in 30 days of straw return, O-LF content of D0, D1, and D2 were significantly increased than in D3 treatments. While the latter did not change too much. The soil's heavy organic carbon humidification degree was higher. Because soil organic carbon combines with different graded mineral particles to form organic-inorganic compounds. It reflected the ability to hold soil organic carbon and ascertained the stability of soil carbon and soil quality. All these play significant roles in the mobilization of soil organic carbon. It showed that the HF content did not vary among all the soil depths after decomposing for 1 year.

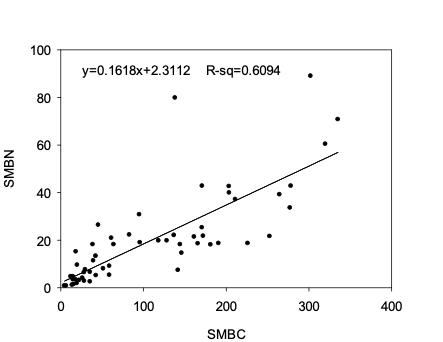
Table 2-3. The soil carbon fractions of different soil layers with the decomposing days, which D0 (0-5 cm), D1(5-15 cm), D2 (15-30 cm), and D3 (30-45 cm)/ mg·kg-1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | 30d | 60d | 90d | 120d | 360 d |
| LF | D0 | 89.07±19.43a | 127.95±6.00a | 51.84±0.76c | 106.83±8.89b | 117.44±12.12a |
|  | D1 | 110.55±16.75a | 51.83±1.74c | 68.06±3.22b | 164.91±33.67a | 83.11±16.49b |
|  | D2 | 99.21±9.34a | 77.69±2.88b | 69.41±8.08b | 104.77±12.16b | 91.47±10.49ab |
|  | D3 | 42.83±1.64b | 58.28±7.87c | 91.94±5.29a | 58.78±7.15c | 97.31±20.24ab |
| O-LF | D0 | 115.17±19.92a | 102.79±14.94ab | 52.40±0.63b | 88.23±10.99b | 95.80±6.31a |
|  | D1 | 115.66±3.32a | 67.29±21.61c | 84.17±10.97a | 84.76±10.27b | 53.56±1.01bc |
|  | D2 | 118.43±9.65a | 130.88±17.98a | 58.42±2.19b | 117.43±4.57a | 34.94±25.40c |
|  | D3 | 51.28±4.34b | 89.87±14.17bc | 78.82±10.24a | 72.37±17.26b | 78.38±3.62ab |
| HF | D0 | 13.71±0.73b | 13.00±0.23c | 14.12±0.39a | 15.17±0.50a | 13.27±0.54a |
|  | D1 | 13.95±0.47ab | 14.66±0.08a | 13.50±0.25b | 15.23±0.89a | 12.35±0.84a |
|  | D2 | 14.77±0.14a | 13.77±0.50b | 14.22±0.19a | 14.50±0.45a | 13.36±0.19a |
|  | D3 | 9.06±0.35c | 14.33±0.22ab | 14.00±0.35ab | 14.20±0.42a | 13.02±0.61a |
| SOC | D0 | 16.14±1.33 | 15.90±0.32 | 16.63±1.36 | 18.83±1.94 | 16.99±0.07 |
| g·kg-1 | D1 | 15.18±1.16 | 15.70±0.26 | 16.27±1.18 | 18.42±0.36 | 16.53±0.21 |
|  | D2 | 14.97±0.31 | 15.65±0.34 | 14.92±0.64 | 18.27±1.16 | 16.21±0.13 |
|  | D3 | 13.85±0.65 | 13.12±0.26 | 13.66±0.34 | 15.86±0.61 | 13.67±0.09 |

***2.3 The SMBC/N in different soil depths***

The SMBC and SMBN content have been plotted in Figure 2-5. From the figure, it is seen that the straw lignin and cellulose decreased with sampling days. The straw lignin of the D0 treatment was lowest than all other treatments.

We could find the change of SMBC not obvious except D2 treatment which had a high SMBC value and occurred from 90~120 d and also had a peak in the whole sampling period. The content was higher than D0, D1 and D3 by 43.7%, 24.3% and 23.8%, respectively (Figure 2-5). D0 had the lowest content in all the soil horizons and there was no significant difference between D1 and D3 throughout the whole period of the experiment. In the D2 treatment and at 120 d of the experimental period, the SMBN value was also higher. There was no significant difference with D0, D1 and D3 treatments. There was a positive, linear, and significant relationship between SMBC and SMBN (y=-2.087-0.1636x, R-sq=0.88%, P<0.01). Regression analysis showed that the retention rate increased significantly with time.



(b)

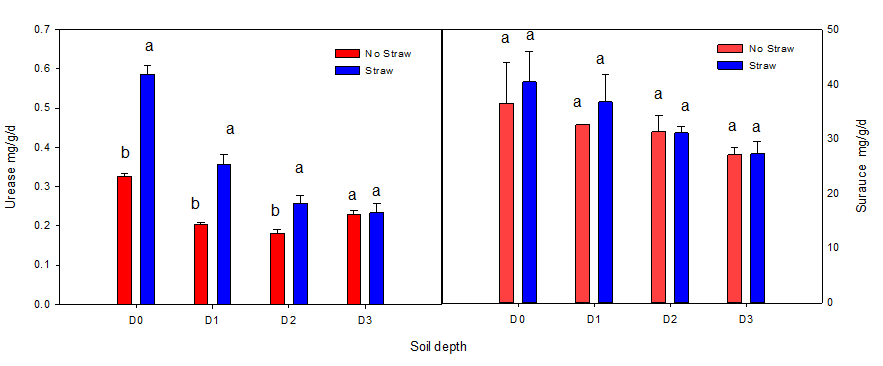
(a)



Figure 2-5. Soil microbial carbon and nitrogen contents (a) and their relationship (b)

***2.4 The urease and sucrase of straw residue incorporation in different soil depths***

Straw incorporation into the soil could increase the urease and sucrase content in the different soil depths (Figure 2-6). There was a significant difference between straw incorporation and no incorporation in D0, D1 and D2 treatments (P<0.05). But this difference was not significant in D3 experiment. Sucrase did not show a significant difference in different treatments but showed a downward trend with soil depth.



**Figure 2-6. The different content of urease and sucrase after straw return to different soil depths, which D0 (0-5 cm), D1(5-15 cm), D2 (15-30 cm) and D3 (30-45 cm).**

***2.5 Relationship between the factors***

After standardizing the results of the correlation analyses for all the soil indicators and as presented in Table 2-4, it had been seen that SOC significantly and positively correlated with C/N (0.819) and temperature (0.508). On the other hand, sucrase correlated negatively and significantly with moister (-0.555).

**Table 2-4: The correlation analysis between soil organic carbon and other factors**

|  | SOC | SMBC | C/N | SMBN | Urease | Sucrase | Temperature | Moisture |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SOC | 1 | -0.270 | 0.819\*\* | -0.202 | 0.352 | 0.353 | 0.508\* | -0.060 |
| SMBC |  | 1 | -0.282 | -0.166 | 0.068 | 0-.444 | 0.066 | 0.220 |
| C/N |  |  | 1 | -0.090 | 0.390 | 0.433 | 0.319 | -0.336 |
| SMBN |  |  |  | 1 | 0-.151 | 0.212 | -0.161 | -0.155 |
| Urease |  |  |  |  | 1 | 0.200 | 0.119 | -0.130 |
| Sucrase |  |  |  |  |  | 1 | 0.276 | -0.555\* |
| Temperature |  |  |  |  |  |  | 1 | 0.129 |
| Moisture |  |  |  |  |  |  |  | 1 |

## 3 Discussions

Three years after maize straw disposal to the experimental fields, the residues applied at 5-45 cm were completely decomposed. But the straws on the topsoil layer were only partially decomposed. The residues of D1, D2, and D3 treatments reached less than 20% and declined dramatically during D0 treatment. But correlation analysis showed no significant differences among the D1, D2, and D3 treatments. Straws returned into deep soil have been recommended as an effective method to reduce the straw biomass (Zou et al., 2016; Yang et al., 2016).

Crop straw is a source of organic carbon that can influence the balance of SOC accumulation and decomposition (Bakht et al., 2009), especially the LOCF (Malhi et al., 2011). There had been some other reports about straw mulch that showed positive (Whitbread et al., 2003), or no obvious (Xu et al., 2011) or negative effects (Ma et al., 2013) in 1-2-year experiments. Generally, maize straw returning to deep soil had benefited from decomposition and carbon storage in Northeast China (Lal, 2004; Wu et al., 2016). Kuang et al. (2014) showed a regularity in the decomposition of straw which showed a fast rate in the early stage but went slow in the later stage. The decomposition of straw under buried conditions showed 9~20% higher than those mulched on the soil. But the straws were buried only at 20 cm soil layer without considering the effect of seeding for the next year. In the present research, similar results were shown. The straw returning to the deep soil (D1-D3) treatments showed beneficial effects for straw decomposition (70%~80%). The reasons were that the soil layers had good conditions for moisture, temperature, and more microorganisms for straw decomposition (Zou et al., 2016). At the stage of 30 d of straw incorporation into the experimental soil, the decomposition rate reached in peak.

The C/N ratio is an important factor that affects the decomposition of maize straw (Billings, 2006). A C/N ratio of 25:1 facilitates the maize straw decomposition and the release of N (Chan et al., 2002). On the other hand, a suitable C/N ratio could increase crop production (Li et al., 2016). Therefore, it was necessary to apply appropriate amounts of nitrogen fertilizer to adjust the C/N ratio.

SOC played an important role in mediating soil available nutrients, soil structure, and carbon balance (Shafi et al., 2007). The phenomenon has a certain lag in response to climate change, land cultivation and farmland management measures could be considered as an optimal way of sustainable crop production (Chen et al., 2008). However, most of the researches focus on the returning of straw to deep soil layers because of having an effective increase in the soil organic carbon content. And this could be done by using a deep-ditching-ridge-ploughing method (Soon and Lupwayi, 2012) and DB-SR method (Wang et al., 2015b). The methodology is different from the methods used in the present investigation. But there is a similarity and the result provides a good conclusion about returning of the straw to 20 cm soil depth.

Soil organic carbon pool is one of the most important dynamic carbon pools in the earth's terrestrial ecosystem. Most important to it is that its small change can lead to a large fluctuation in the global atmospheric CO2 content (Kumar et al., 2010). Different land use patterns and management measures have a great impact on the soil organic carbon storage (Han et al., 2017). From the perspective of carbon sequestration in farmland, it is hoped that the higher the stability of organic carbon, lower will be the carbon emission. Straw returning increases the content of active organic carbon and the proportion of active organic carbon in the total organic carbon pool (Navarro-Noya, et al., 2013).

Marschner et al. (2011) showed no significant differences of SOC during the growth stages. This result was similar to those obtained in some previous studies, where the SOC was insensitive to recent agricultural management activities (Laird and Chang, 2013; Cusack et al., 2011). There may be more influence in physical protection of straw returning. So, we choose the physical method to analyse the effect of the straw returning which was referred to Golchin (Golchin et al., 1998). Chen et al. (2008) opined that straw returning could increase the content of LF and had a significant effect on improving soil organic carbon quality. From the perspective of the grouping of organic carbon, the content of LF and O-LF would have been changed easily in all the soil depths, in those HF was relatively stable. Straw incorporation could stimulate microorganisms and might produce more active organic carbon. So the net effect could consequently be predicted on a short term basis (Soon and Lupwayi, 2012). The arable degree of culturing in the cultivated soil layer was relatively higher, and the soil recombined organic carbon content does not fluctuate significantly in the short term. However, our study showed that the straw OC of D3 treatments had a highest content than other depths, except for D0 treatment which had a lower straw OC (58.0%) than D1 and D3. In other words, there was more than 58% of straw carbon flowing into the air when the straws were put on top soil. It indicated that the straw carbon could be saved in the soil when straw returned into deep soil while reducing the volatilization of straw carbon and lower CO2 emission (Kumar et al., 2010). According to Han et al. (2017), a straw application could increase CO2–C emission because they change the soil's total porosity and organic carbon content.

Bolinder et al. (1999) indicated that soil microbial biomass, specifically soil enzymes, is more sensitive to changes in soil quality. It showed that the long-term incorporation of crop residues caused significant increases in urease and invertase activity levels over five years (Wei et al., 2015). The trends in the enzyme activity levels were also similar in the present study. Compared with no straw incorporation (CK), the treatments of straw return greatly increased the activity levels of soil urease. The function was evident, especially in D0 treatment which had the highest content, but there was no significant difference in soil sucrase. As described in the previous studies (Jin et al., 2009), the activity levels were higher in the topsoil which may have been caused due to the “surface activation effect” (Bandick et al., 1999). These increases may have been attributable to both microbial growth and the stimulation of microbial activity due to enhanced resource availability (Zhao et al., 2009).

Crop residues return significantly affected bacterial community structure and increased their population (Navarro-Noya et al., 2013). Different microbial communities are responsible for specific functions in the decomposition of crop residues. For example, bacteria dominate in the initial phases, while fungi dominate in the later stages of crop residue decomposition (Marschner et al., 2011). Although the SMBC only has a 5-8% of SOC, it has higher activity and dynamics in soil carbon which plays a key role in nutrient cycling (Cusack et al., 2011) and acts as a driving force for microbial activity (Li et al., 2012). It is considered a sensitive indicator of changes in soil quality and soil health caused by cultivation (Powlson et al., 1987). In this study, SMBC was decreased with the deepening of soil layers and showed a significant difference between soil layers. The D2 treatment had the highest content of SMBC which is consistent with the result of Zou et al. (2016). For this, conditions fulfilled should be to put the straw into deep soil and that a phenomenon of surface microbial aggregation in the soil does exist (Lal, 2004).

## 4 Conclusions

In a 3 years trial, the maize straw residue returning to deep soil could decompose more quickly than putting the maize straw on top of the soil (P<0.01). To incorporate the straw, especially for the straw lignin, decomposing rate, and SMBC content, 15~30 cm soil depth was the best method. Straw returning to the deep soil also can store more stable carbon in the soil and can increase the accumulation of organic matter. The effects of farming practices and straw returning to the field and activating carbon, not only stir up the soil layer but also distribute crop residues, long term application will also affect the physical, chemical, and biological properties of soil. However, our current experiment has only conducted short-term research, and long-term monitoring is needed for prediction. Therefore, the impact of more than 10 years on the composition of soil-activated carbon needs to be further explored.

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**Chapter Ⅲ**

**Analysis of DOC component structure of black soil profile under straw returning condition based on fluorescence spectrum**

From Kuang, E. J., Chi, F. Q., Zhang, J. M., Xu, M. G., Colinet, G., Su, Q. R., Hao, X. Y., Zhu, B. G., 2022. Analysis of DOC component structure of black soil profile with straw deeply buried and based on fluorescence spectrum. *Spectroscopy and Spectral Analysis,* 42(10): 3243-3248.

## Abstract

Taking black soil as the research object, this study aimed to analyze the difference in the three-dimensional fluorescence spectrum of dissolved organic carbon (DOC) in soil after returning maize straw to the field at different depths (0-2 cm, 3-10 cm, 11-20 cm, 21-30 cm, and 31-40 cm), and discuss the change characteristics of humification degree of maize straw return to deep soil. The results showed that straw return could increase the content of DOC in soil. The characteristics of three-dimensional fluorescence spectra showed that two kinds of fluorescence components of DOC in soil. The control (CK) toT4 treatments were humus-like component C1 [excitation wavelength (Ex)/emission wavelength (Em)= 250-275 nm/ 420-450 nm] and tryptophan-like component C2 (Ex/Em = 270-300, 200-250 nm/ 340-360 nm) and tyrosine-like component (Ex/Em = 225 nm/ 304 nm). Small authigenic components appeared at a depth of 31-40 cm, and the humification coefficient was the highest. The fluorescence intensity of the DOC component C1 in soil increased with the increase in straw returning depth. However, the component C2 showed a fluctuating state, and the fluorescence intensity increased first and then decreased. The DOC in soil was affected by both endogenous and exogenous sources (FI > 1.4, and 0.6 < BIX < 0.8), showing a state of weak humification (HIX < 1.0), and the FI values of each treatment were between 1.4 and 1.6, indicating that the main source of DOC in soil was the microbial decomposition of straw after returning to the field. The FI value after each treatment was higher at a depth of 21-30 cm. The linear regression analysis showed that the effects of soil depth, straw returning, and their interaction with DOC and its components were significant. The DOC improved and sped up the transformation of soil humus with the help of indigenous microorganisms in soil. Straw return could store more carbon, improve the quality of soil available in the carbon pool, and maintain the balance of soil organic carbon.

**Keywords:** Dissolved organic carbon; PARAFAC analysis; straw return; three-dimensional fluorescence.

## Introduction

Dissolved organic carbon (DOC) is an active component in the soil nutrient pool, accounting for only 0.04%-0.22% of the total soil organic carbon (Li et al., 2016). It is also an important indicator of the environmental health and quality change of soil. Therefore, further investigation on DOC is of great significance for understanding the process of the soil carbon cycle. The DOC content is affected by land use, applied organic materials (Wei et al., 2020), soil environment, and other conditions (Liu et al., 2006). Straw return can significantly improve the DOC content of the topsoil (Li et al., 2019). According to the classification of the fluorescence group, the content of fulvic acid with a relatively simple structure in humus increases, and the structure is simplified. The hydrophobic components of DOC and aromatic molecules have strong adsorption capacity (Sun et al., 2018). Straw combined with tillage measures such as deep plowing also has an impact on the deep organic carbon pool below 40 cm (Sheng, et al., 2014), and improve the nutrient content of deep soil (Kuang et al., 2019).

In recent years, the rapid transformation of vegetation and the difference in soil carbon input caused by high-intensity land use have significantly affected the accumulation of deep organic carbon pools (Zheng et al., 2020). In the effective analysis of DOC characterization in soil, the three-dimensional fluorescence spectroscopy technology showed good reproducibility and high sensitivity. It was found that the three-dimensional fluorescence spectra were different for low and high-carbon soils. The low–carbon soil showed mainly three fluorescence peaks, including protein–like fluorescence peaks, fulvic acid–like fluorescence peaks, and humic acid-like fluorescence peaks, while the high–carbon soil had only two fluorescence peaks (Shi et al., 2016). From the perspective of DOC, this study explored the mechanism of soil carbon sequestration after straw returning to the field at different depths, analyzed the role of DOC and soil carbon sequestration, and further invesigated the change characteristics of DOC material composition and structure in the process of straw decomposition using three-dimensional fluorescence spectroscopy combined with parallel factor analysis, aiming to provide a scientific theoretical basis for farmland soil carbon sequestration mechanism in the black soil area.

## 1 Materials and Methods

***1.1*** ***Experimental Design***

This experiment began in 2016 and was carried out at the frame planting test site of the Heilongjiang Academy of Agricultural Sciences, with a frame planting area of 2 m2. The maize straw was crushed to 2 cm in length, and applied to different depths of the soil according to the total amount of straw returned to the field (approx. 9000 kg·hm-2). The Carbon/Nitrogen ratio was adjusted to 25, and the field water capacity to 60%. The depth of straw returned to the field was 0–2 cm, 3–10 cm, 11–20 cm, 21–30 cm, and 31–40 cm, represented by T1, T2, T3, T4, and T5, respectively. At the same time, the non-straw returned to the field was set as the control (CK). The specific operations were as follows:

T1 treatment: A soil layer of 0–2 cm was excavated, and the straw and soil were fully mixed and backfilled into its original layer.

T2 treatment: A soil layer of 0–2 cm was excavated, and placed separately; the soil layer of 3–10 cm was fully mixed with straw and then backfilled into its original layer; and finally, the 0–2 cm soil was backfilled to the surface.

T3 treatment: A soil layer of 0–10 cm was excavated and placed separately; the soil layer of 11–20 cm was fully mixed with straw and backfilled into its original layer; and finally, the soil layer of 0–10 cm was backfilled in situ.

T4 treatment: Soil layers of 0–10 cm and 11–20 cm were dug out and placed separately; then, the soil layer of 21–30 cm was fully mixed with straw and backfilled into its original layer; and finally, each layer of soil was backfilled into its original position.

T5 treatment: Soil layers of 0–10 cm, 11–20 cm, and 20–30 cm were excavated and placed them separately; then, the soil layer of 31–40 cm was fully mixed with straw and backfilled into its original layer; and finally, each layer of soil was backfilled into its original position.

***1.2 Sampling and Analyses***

The soil samples (0–2 cm, 3–10 cm, 11–20 cm, 21–30 cm, 31–40 cm, 41–50 cm) were collected for each treatment according to the soil profile level in 2019. The roots and surplus straws were removed, dried, and screened using a 2-mm sieve to determine the DOC in soil and its fluorescence structure characteristics.

The air-dried soil sample (weighing 0.01 g), was mixed with 2mol/L hydrochloric acid solution for acidolysis, and the SOC content of the soil (Multi N/C 2100, Germany) was determined with a total organic carbon analyzer. Then, 5 g of air-dried soil sample was taken, water was added to it (the volume ratio of dry soil weight to water was 1:10), oscillated horizontally at 200 rpm at room temperature for 24 h, and then centrifuged at 12, 000 rpm at 4℃ for 20 min. The supernatant was passes through a 0.45-μm filter, and the concentration (Multi N/C 2100 TOC instrument) was measured using the TOC instrument, which was DOC; The DOC concentration of all samples was adjusted to 15 mg·L-1, and the three-dimensional fluorescence spectrum was measured with a fluorescence spectrometer (Hitachi F-7000, Japan). The scanning range of the excitation wavelength (Ex) and emission wavelength (Em) was 200–500 nm, the bandwidth was 10 nm, and the scanning speed was 1200 nm·min-1, The influence of Raman scattering on the fluorescence data was eliminated when PARAFAC analysis was performed.

***1.3 Statistical Analyses***

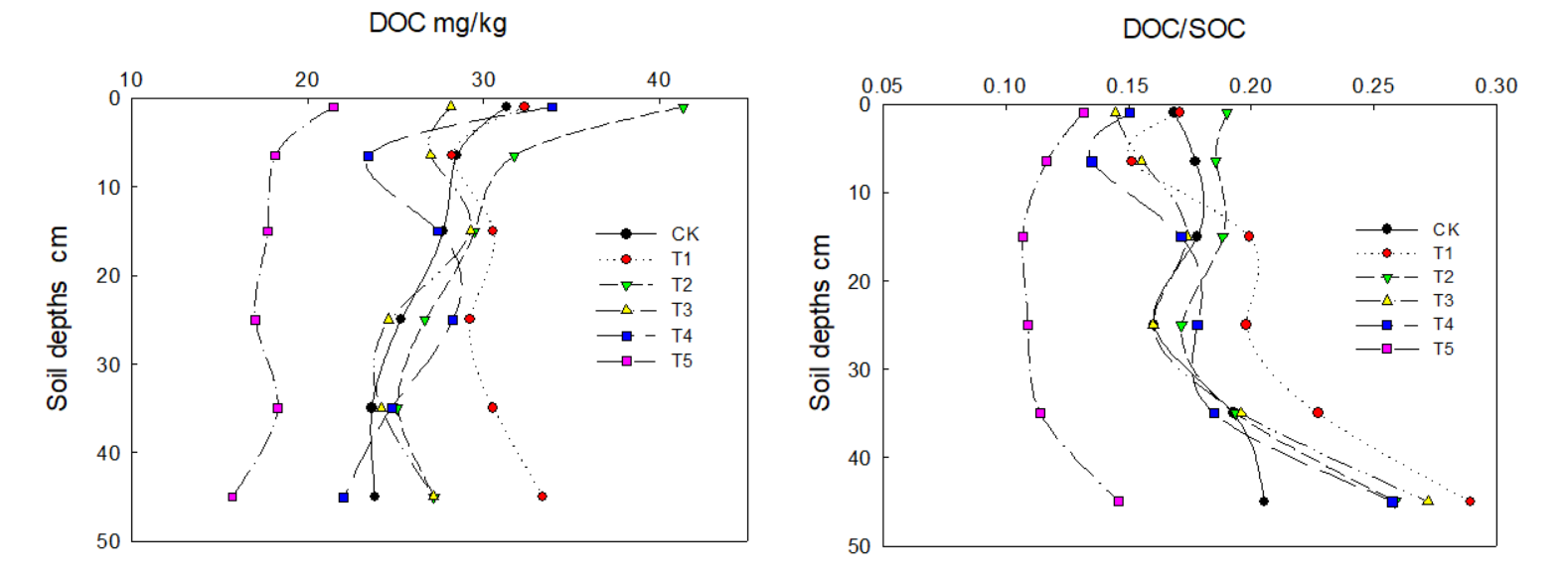
The data were processed and analyzed using Excel 2010 and SPSS 17.0. The Matlab 2013 software was used for three-dimensional fluorescence map drawing and parallel factor analysis, and Origin 2019 was used for the regional integration of fluorescence spectrum index.

## 2 Results

***2.1 The content of DOC after different treatments***

The DOC activity in soil was strong, but it was also easy to loss. The input of straw affected its content (Wei et al., 2020). Figure 3-1 shows that after returning straw to the field, the DOC content at different soil depths increased, and the vertical distribution in the soil showed a gradually decreasing trend, which was consistent with the previous research results (Xiong et al., 2015). The DOC content of the 0–2 cm soil layer was higher than that of the other soil layers. This was because the DOC concentration of the surface soil was higher due to the influence of straw returning. The DOC content gradually decreased with the increase in depth. This was because the clay of deep soil increased, the adsorption of DOC also increased, and the decomposition of organic matter by soil microorganisms decreased (B. Li et al., 2019). The DOC content after the T1 and T2 treatments was the highest, with an average of more than 30 mg·kg-1, and the DOC content after the T5 treatment was the lowest. From the depth of straw returning, the DOC concentration in the 11- to 20-cm after T3 treatment was 8.4% higher than that in the 3- to 10-cm soil layer; the DOC content in the 21- to 30-cm soil layer after T4 treatment was 3.0% higher than that in the 11- to 20-cm soil layer; and the DOC content in the 31- to 40-cm soil layer after the T5 treatment was 7.6% higher than that in the 21- to 30-cm layer. Although the difference did not reach a significant level, the DOC content in the soil layer applied with straw increased. Straw returning provided good material and energy for microbial growth and further stimulated the decomposition of easily decomposed organic matter in the soil itself. In addition, straw carbon mineralization also increased the content of DOC in soil.

The greater the ratio of DOC/SOC, the easier its decomposition by microorganisms. Also, it was sensitive to the response of management measures (Li J, et al., 2013). In the soil layer below 10 cm, the proportion of DOC and SOC after the T1 treatment was the highest, and all the treatments increased with the deepening of the soil profile.



**Figure 3-1 The Content of DOC and the DOC/SOC ratio after different treatments.**

***2.2 The fluorescence index of DOC components in soil***

Fluorescence spectral indexes FI, BIX, and HIX were often used to characterize the structural characteristics of soil humus (Jiang, et al., 2014). Among these, FI could reflect the source of humus. Table 3-2 showed that the value of FI after each treatment was between 1.4 and 1.6 whether straw was returned to the field or not, indicating that DOC in soil was derived from the mixture of plants and microorganisms. The average value of FI after each treatment was slightly higher than that after T3, which was 1.54 ± 0.03. When the straw was returned to the field to a depth of 11–20 cm, the DOC in soil improved with the help of indigenous microorganisms in the soil, and the transformation rate of soil humus was accelerated. In the deeper soil layer, the influence of foreign substances on the soil gradually decreases due to the influence of soil moisture and temperature, and the process also slowed down, but the difference between different depths was not obvious.

BIX is usually used to measure the contribution of authigenic organic matter. When BIX was in the range 0.6–0.7, 0.7–0.8, and 0.8–1.0, DOC had less, medium, and strong authigenic characteristics, respectively. When BIX was greater than 1, it was produced by the activity of biological bacteria. In this study, except for the T5 treatment, the BIX after each treatment was between 0.8 and 1.0, indicating a strong characteristic of authigenic source. The T5 treatment had smaller autogenous components with BIX<0.7.

The humification degree of soil organic matter was expressed by HIX (Jiang et al., 2014). The higher the HIX value, the higher the humification degree of DOC in soil, and the better the stability. It still implied existence for a long time. When HIX was less than 1.5, it belonged to biological or bacterial sources, and when HIX was greater than 3.0, it belonged to strong humus characteristics. As shown in Table 3-2, the HIX value after each treatment was less than 1.5, the minimum value was 0.72 after the CK and T1 treatments, and the maximum value was 0.83 after the T5 treatment. The straw surface covering or shallow returning to the field was not conducive to the process of humification, and the degree of deep humification was high. This might be because when the straw was returned to the soil at a depth of 40 cm, the aeration and permeability of the soil were poor, and the microbial activity was weak. After decomposing the simple and easily degradable substances in the soil, the microorganisms could only use the more difficult-to-decompose substances in the soil to synthesize more complex and stable humic acid substances, so that the humification degree of DOC in soil increased. The difference in soil humification degree was significant with the increase in returning years (B. Li et al., 2017).

Table 3-1 Average fluorescence spectral index of DOC in soil after different treatments

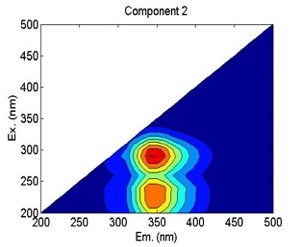
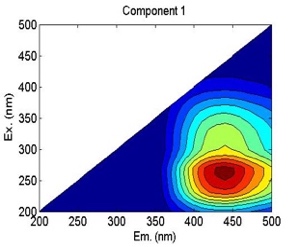
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| --- | --- | --- | --- |
| Treatment | FI | BIX | HIX |
| CK | 1.49±0.02a | 0.89±0.20a | 0.72±0.16a |
| T1 | 1.49±0.04a | 0.97±0.06a | 0.72±0.04a |
| T2 | 1.50±0.06a | 0.83±0.15b | 0.76±0.09a |
| T3 | 1.54±0.03a | 0.88±0.17a | 0.76±0.09a |
| T4 | 1.51±0.05a | 0.91±0.17a | 0.75±0.06a |
| T5 | 1.52±0.05a | 0.68±0.03b | 0.83±0.04a |

Note: The values are mean±standard deviation. Different lowercase alphabets represent significant difference (P < 0.05).

***2.3 Fluorescence spectrum characteristics of DOC components after different soil treatments***

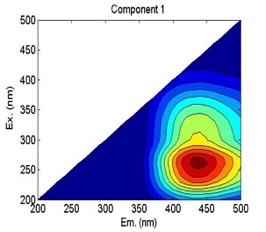
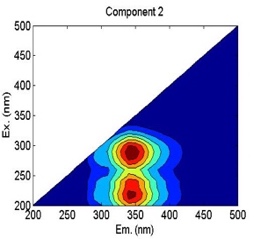
The three-dimensional fluorescence spectrum data of DOC in soil after different treatments were analyzed using PARAFAC. As shown in Figure 3-2, two fluorescence components were analyzed from six groups of samples. The CK–T4 comprised humus-like component C1 (Ex/Em = 250–275 nm/ 420–450 nm) and tryptophan-like component C2 (Ex/Em = 270–300, 200–250 nm/ 340–360 nm). The component C1 contained one excitation peak and one emission peak, corresponding to peak A. It was a kind of fulvic acid in the ultraviolet region, which represented the organic matter with less degradable substances with high molecular weight, and reflected the supply and buffer capacity of soil fertility. The component C2 contained two excitation peaks and one emission peak, corresponding to the main peak, T peak (tryptophan-like substance). The T peak mainly corresponded to the metabolites degraded by bacteria and microorganisms, which were bound or free in proteins. The T5 treatment comprised humus-like component C1 (250–275 nm/ 420–450 nm) and tyrosine-like component C2 (Ex/Em=225 nm/ 304 nm). The component C1 contained an excitation peak and an emission peak, corresponding to the main peak A. The component C2 contained one excitation peak and one emission peak, corresponding to peak B, which was a tyrosine-like substance with a lower molecular weight that was more easily degraded than tryptophan-like acid (Zheng et al., 2020).

The fluorescence components of straw returning to 0–30 cm were related to carboxyl and hydroxyl groups, which were commonly used to represent the exogenous organic matter input to the soil. When the straw was returned to the field to 30–40 cm, more easily degradable tyrosine-like substances appeared. The research results of Kuang (2020) showed that deep burial helped improve the decomposition rate of straw biomass in the soil and reduce the residue rate. At the same time, the fluorescence intensity promoted the accumulation of humic acid–like and fulvic acid–like substances, and also promoted the degradation of soluble microbial metabolites. Studies showed that when the DOC content did not change significantly, the effect on its structure was obvious (Shi et al. 2016).



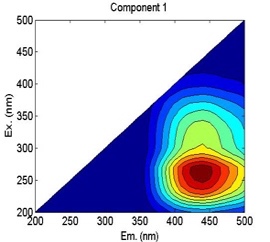
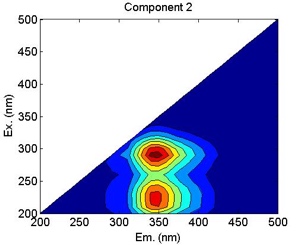
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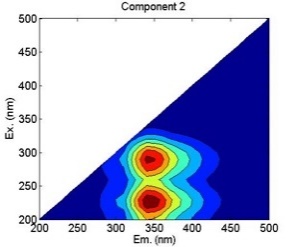
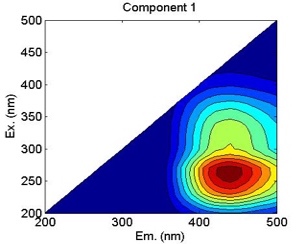
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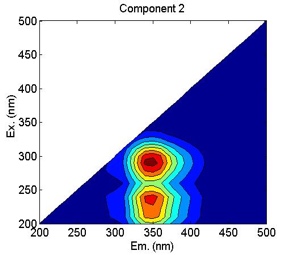
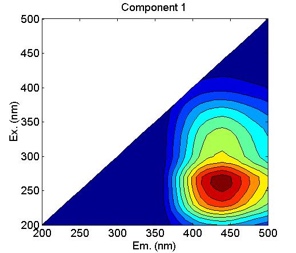
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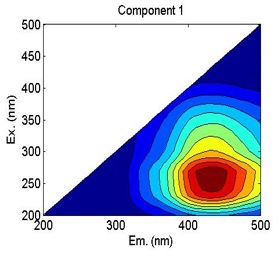
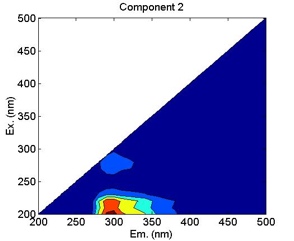
T2

T2



T4

T4



T5

T5

**Figure 3-2 Three-dimensional fluorescence components of DOC in soil after different treatments.** Peak A: Fulvic-acid-like substances in the UV region; Peak T: short-wave tryptophan-like substances (protein-like); Peak B: tyrosine-like substances (protein-like).

***2.4 Fluorescence intensity analysis of DOC in soil after straw returning***

The fluorescence intensity of different components of DOC in soil was different at different depths. The fluorescence intensity value of each component reflected its relative content, which could be used to characterize the structural change of DOC in soil. After the straw return, the fluorescence intensity of fulvic acid–like acid increased with the increase in straw returning depth. The decomposition of plant residues produced high-molecular-weight organic matter. The component C1 increased with the increase in soil depth, and the fluorescence intensity of straw returning to the field increased. The fluorescence intensity of the component C2 increased first and then decreased, showing a continuous fluctuation state. The total fluorescence intensity (C1 + C2) was the highest after the T4 treatment, 26.5% higher than that after CK treatment, followed by that after the T1 treatment, which was 9.0% higher than that after CK treatment. From straw returning to different soil depths, the proportions of components C1 and C2 were different (Table 3-2). The percentage of C1 after each treatment was higher than that of C2. Only the fluorescence intensity of the component C2 after the T1 treatment was enhanced. The fluorescence intensity of the component C1 treated with T5 exceeded by 67%, humus-like substances increased and protein-like substances decreased. The fluorescence component of DOC in soil was mainly fulvic acid with the increase in the straw returning depth, and the relative percentage of tryptophan was low.

Table 3-2 Fluorescence intensity and relative percentage of DOC fluorescence components in different soil depths

| Treatment | C1 (R.U.) | C2 (R.U.) | C1+C2(R.U.) | Relative percentage % | |
| --- | --- | --- | --- | --- | --- |
| C1 | C2 |
| CK | 1191.21±571.61a | 827.41±416.34ab | 2018.62±860.58a | 55.79 | 44.21 |
| T1 | 1129.28±529.87a | 1143.47±176.79a | 2272.75±567.54a | 46.84 | 53.16 |
| T2 | 1253.68±562.98a | 794.99±273.44ab | 2048.67±768.88a | 57.77 | 42.23 |
| T3 | 1269.22±418.39a | 932.04±127.29ab | 2201.26±407.54a | 56.24 | 43.76 |
| T4 | 1396.80±145.95a | 1156.04±418.43a | 2552.84±498.05a | 55.87 | 44.13 |
| T5 | 1404.37±144.76a | 697.19±209.51b | 2101.56±276.95a | 67.19 | 32.81 |

Note: The fluorescence component values are mean ± standard deviation. Different lowercase alphabets represent significant differences (*P*<0.05).

***2.5 The correlation of DOC in soil after straw returning***

The significance of DOC, its fluorescent components, and the DOC/SOC value after each treatment are analyzed in Table 3-3. The effects of soil depth, treatment, and their interaction with DOC and its fluorescent components were significant (*P*< 0.01). Small changes in the soil environment could affect the DOC content. Soil microbial activity was an important factor affecting the DOC in soil. In addition, soil nutrients and pH conditions also affected the DOC content, which promoted the transformation of proteins into humic acid and fulvic acid (Shi et al., 2016).

Table 3-3 Significance analysis of effects of different treatments, depths, and their interactions on *Fmax* of the DOC structure (*P* value)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | SOC | DOC | C1 | C2 |  |
| Treatment(T) | 0.072 | 0.000 | 0.000 | 0.000 |  |
| Soil layer(D) | 0.000 | 0.000 | 0.000 | 0.000 |  |
| T×D | 0.000 | 0.000 | 0.000 | 0.000 |  |
| R-Sq | 98.14% | 94.60% | 99.68% | 99.05% |  |

## 3. Conclusions

3.1 The content of DOC in soil increased after straw returning to the field, and its source was affected by the joint action of endogenous and exogenous sources; Straw returning to 31–40 cm had smaller authigenic components, and the humification coefficient was the highest.

3.2 When the straw was returned to the field to a depth of 0–30 cm, the fluorescence components of DOC in soil contained fulvic acid-like and tryptophan-like substances. The fluorescence intensity of fulvic acid-like substances increased with the deepening of soil depth, while tryptophan-like substances showed a fluctuating state; The fluorescence components of straw returning to a depth of 31–40 cm included fulvic acid and tyrosine. The DOC content and its fluorescent components were significantly affected by straw returning, soil depth, and their interaction.

In conclusion, straw returning to the field provided more stored carbon for deep soil and played a role in carbon sequestration and emission reduction in farmland production.

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**Chapter Ⅳ**

**Dynamic changes of straw return with different years on soil labile organic carbon fractions and enzyme activities in the black soil areas**

## Abstract

Incorporation of straw in the improvement of soil fertility via increasing soil organic carbon has become an important method. In the present investigation, taking black soil as the research object, the difference of labile organic carbon and enzyme activities after returning maize straw to the field in different years were tested. The experimental design was based on taking into account of four treatments namely, S-1 (maize straw residue returned to soil every year), S-2 (maize straw residue returned to soil every two years), S-3 (maize straw residue returned to the soil every three years), NS (CK, without straw every year). Data on the change characteristics of humification degree of maize straw return were collected and discussed. The results showed that it had significant effects on soil organic carbon (SOC), microbial biomass carbon (MBC), dissolved organic carbon (DOC), and easily oxidizable carbon (EOC) contents among different treatments except LOC which did not show significant differences. The SOC content had a significant difference between topsoil (0-20 cm) and deep soil (20-40 cm) compared with the NS. The soil microbial biomass carbon (SMBC) and DOC concentrations in the two soil layers by all the straw return treatments were significantly higher than those of NS treatment (p<0.05). The result indicated that return of straw every year to the field had a significant effect to improve SOC content both at the 0-20 cm and 20-40 cm, and S-3 and NS had similar content after three years of treatment. Significant positive correlations were obtained among the soil SOC, DOC, EOC, sucrase and urease at 0-40 cm soil depth. But there was no significant correlation between the labile organic C fractions. In brief, the research findings obtained in the present investigation supported by statistical analysis, could provide some valuable and distinctly visible information of DOC derived from straw and offer technical guidance when incorporating straw into black soil.

**Keywords:** Straw return; labile organic carbon; enzyme activities; soil dissolved organic carbon

## Introduction

Crop residue incorporation into the cultivated soils directly or indirectly can promote the production of a favorable soil environment (Edward et al., 2009) It plays a crucial role in soil organic carbon (SOC) sequestration, soil nutrient turnover, and greenhouse effect regulation (X. Chen et al. 2017; Gregorich et al. 2017). Studies have suggested that cropland soils have great carbon sequestration potentials if crop residue incorporation (Lal and Bruce, 1999;Triberti et al. 2008) through increasing C input and/or decreasing C output (Brar et al., 2013; Lal, 2004), soil structure (Mulumba and Lal 2008), and enzyme activities (Sá and Lal 2009).

SOC, happens to be the main source of energy for soil microorganisms (Guo et al. 2016) and plays a vital role in increasing crop productivity and improving soil quality (Y. T. He et al. 2015). However, the responses of SOC content to crop residue vary widely in magnitude and even method (Fontaine et al., 2004; Wang et al., 2018). Reduced SOC concentration, after residue incorporation has also been frequently reported (Poeplau et al., 2015; J. B. Wang et al., 2011). With respect to SOC concentrations after straw mulch, they showed positive (S. Li et al. 2016), or no obvious (M. Xu et al. 2011) or negative effect (Ma et al., 2013) in experiments of 1-2-year duration. Soil carbon, under short and medium terms of returning to the field, cause changes in soil total organic C but were difficult to detect due to the high background C and temporal and spatial variabilities (Gong et al. 2009). Labile organic C fractions i.e., water-soluble organic carbon (WSOC), dissolved organic carbon (DOC), microbial biomass carbon (MBC), and easily oxidizable carbon (EOC) were much more sensitive than SOC to bring changes in fertilization practices (Ghani, Dexter, and Perrott 2003; Haynes 2000; Plaza-Bonilla, Cantero-Martínez, and Álvaro-Fuentes 2014).

Soil enzyme activity was observed closer to the shifts in rates of SOC decomposition and patterns of turnover in organic carbon pools (Bending, Turner, and Jones 2002; Veres et al. 2015). It showed a positive relationship with SOC but soil phenol oxidase activity showed a negative correlation with SOC increase and the degree of soil humidification (J. Li et al. 2015). It can provide useful insights into the mechanisms of microbial sensitivity to the added N and C. These two elements can support primary metabolism, and the synthesis of oxidative enzymes, which degrade recalcitrant compounds like lignin in the metabolic acquisition of nutrients (Tiemann and Billings 2011). Soil with higher SOC content, especially composed of higher labile organic carbon fractions was reported to have higher soil enzyme activities (Bowles et al. 2014; J. Li et al. 2015).

In the black soil area, due to the development of intensive agriculture, the average output of straw has reached to its highest production state. To solve the problem, a huge amount of accumulated straw are burnt down directly causing serious environmental pollution in the last decade (S. Li et al. 2016). However, an effective practice of disposal can be the return of straw to the deep soil of the agricultural fields, thus minimizing air pollution and adding SOC to the soil of this region (Chen et al. 2017; Kuang et al. 2014; X. Wang et al. 2015; Yang et al. 2016).

China produces about 800 million MT of straw every year, of which corn straw accounts 32.5%. The large production quantity and wide distribution of maize straw could be an important biological resource. The comprehensive treatment and utilization of maize straw have been becoming an important practice in agriculture in China (Liu et al. 2019). But there is also a risk that a considerable portion of gas released into the atmosphere via straw decomposition can be a threat to the environment (Ghimire, Bista, and Machado 2019). So, proposals containing environmentally sound and comprehensive utilization methods of straw processing can get government support and subsidy. Therefore, the integration of maize straw may be a better strategy for increasing the soil quality and other processes relevant to soil amendments. Consequently, comprehensive understanding of the effects of deep straw return to improve soil nutrient and soil biological properties has been critical for the scientific management of maize straw in the black soil area of China. Therefore, the present study was conducted targeting the objectives like: (i) to test that the soil carbon content and soil nutrient will not have significant influence in S-3 treatment, where straw return to the soil will be followed every 3 years (ii) to confirm that the increased microbial biomass carbon and enzyme activity after straw return to the soil for every 1 and 2 year interval, provides a scientific basis for high efficiency and reasonable utilization of straw return to deep soil in the northeast of China. It is also a fact that only by clarifying various effective methods of utilization, we will be able to recognize the value of straw as a resource, based on actual production, and active measures can therefore be undertaken to reduce its misuse as waste.

## 1. Materials and Methods

***1.1 Experimental Design***

The field experiment for the present study was carried out from 2011-2015 and after the maize harvest in October 2011, the accumulated straw biomass was crushed into pieces and incorporated into deep soil by tillage (about 30-35 cm) at Gongzhuling in Jinlin province. The average maize straw production per year was about 7500 kg·hm-2 using a random design with three replications, and each plot covered an area of 500 m2. One time fertilization was adopted by N 225 kg·ha-1, P2O5 100 kg·ha-1, K2O 100 kg·ha-1.

Table 4-1 The layout of straw amount for five years

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Treatment | 2011 | 2012 | 2013 | 2014 | 2015 | The total amount of straw |
| S-1 | Yes | Yes | Yes | Yes | Yes | 5×7500 kg·ha-1 |
| S-2 | Yes | No | Yes | No | Yes | 3×7500 kg·ha-1 |
| S-3 | Yes | No | No | Yes | No | 2×7500 kg·ha-1 |
| NS | No | No | No | No | No | 0 |

***1.2 Sampling and Analyses***

Soil samples were collected from the experimental fields using 5-point-S-shape method. After collection, all the samples were mixed together to obtain a single composite one from two soil depths (0-20 and 20-40 cm). The samples were allowed air-dried and then screened by picking out roots and stones. Finally, the samples were passed through 0.01 and 0.25 mm sieves and the sieved portion of the soil was used for laboratory analysis. Soil organic carbon determination was done by KMnO4 oxidation method and urease by indophenol blue colorimetry method, and for sucrase enzyme 3, 5-dinitrosalicylic acid colorimetry method was used (Guan, 1986).

Soil MBC was analyzed by the fumigation–extraction method (Vance ED 1987). Each sample was weighed into to equivalent portions, one of which was fumigated for 24 h with ethanol-free chloroform, while the other was an unfumigated control. Both of them were shaken 1 h with a 0.5M potassium sulfate, centrifuged and filtered. The MBC was measured by TOC analyzer (N/Y2100, Analytik Jena AG, Germany).

DOC was extracted from moist soil (5.0 g) with a 1:5 ratio of soil to deionized water at 25℃ (Jones and Willett 2006), and shaken for 30 min at a speed of 250 rpm. The samples were subsequently centrifuged for 10 min at 5000 rpm, the supernatant was filtered with a 0.45 μm membrane filter. Oxidation was carried out to the filtrates with potassium dichromate and the final value was measured via titration with ferrous ammonium sulfate. The easily oxidizable carbon (EOC) was determined as described by Blair et al. (1995). A portion of the finely ground air-dried soil sample was reacted with 333 mmol·L−1 KMnO4 by shaking at 60 r min−1 for 1 h. The suspension was then centrifuged at 2000 r min−1 for 5 min. The supernatant was diluted and measured spectrophotometrically at 565 nm.

LOC concentration was determined by using heavy liquid flotation separation (Spedding et al. 2004). In a test tube, 10 g of air-dried soil (<2 mm) was weighed and to it 50 mL NaI liquid solution was added. The test tube was then shaken for 30 min and the floating, light fraction of the sample was immediately removed and poured into a plastic bottle. This process was repeated three times. The light fraction was collected and washed to remove excess NaI by drying at 60°C for 24 h. Weighed the dried part and then passed the 0.01 mm sieves to test the organic carbon content.

***1.2 Statistical analyses***

The SPSS 17.0 (SPSS, Inc., Chicage, IL, USA) analytical software package was used for all the statistical analysis. The data were checked for normal distribution. Pearson correlation coefficients of soil activated organic carbon, and soil activated organic carbon components with soil enzyme activities.

## 2. Results

***2.1 Response of soil organic carbon and soil labile organic carbon***

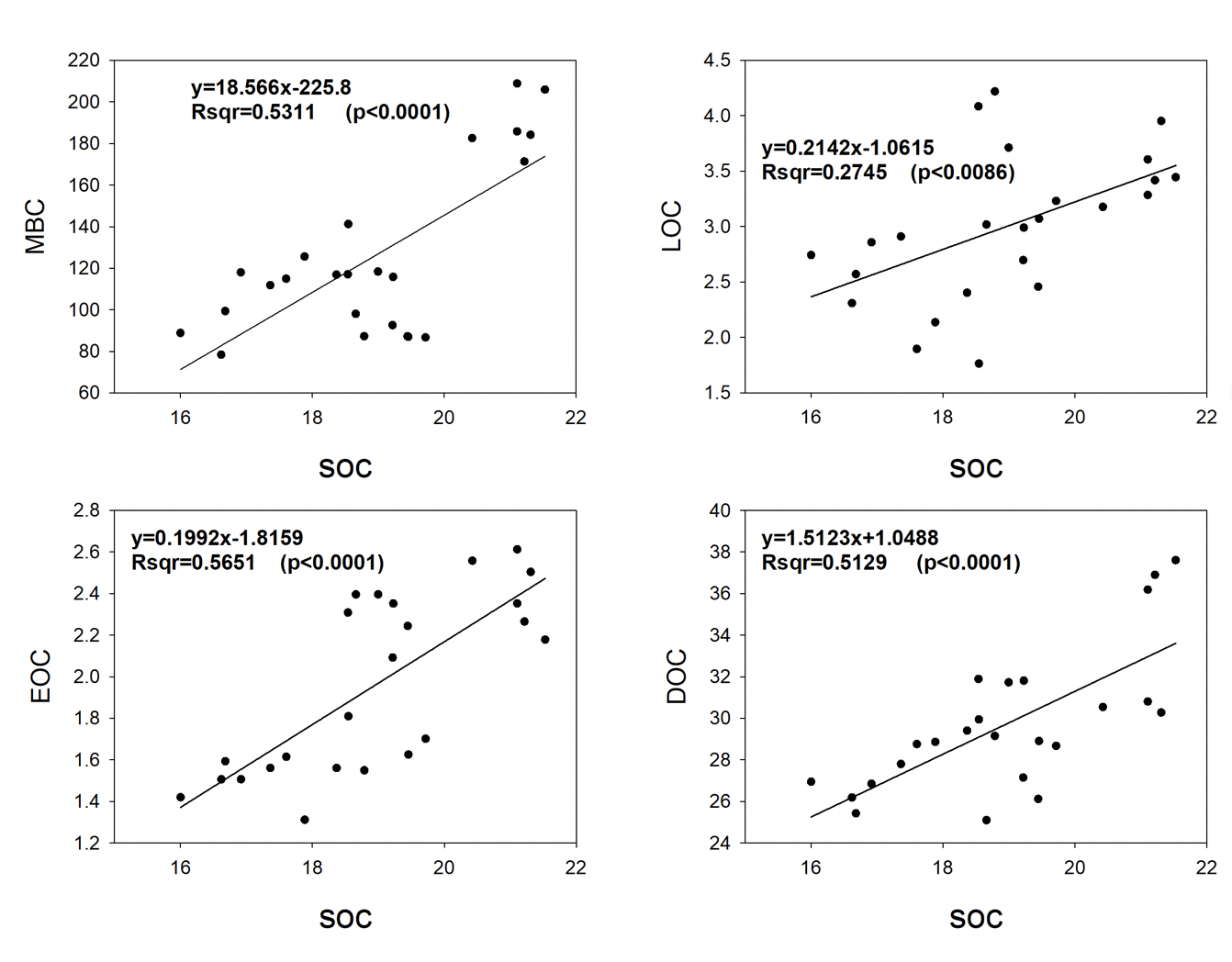
The treatments of straw return in different years showed significant effects on SOC and soil labile organic carbon fractions such as SMBC, EOC, DOC, except LOC (Table 4-2). Compared with the CK (NS treatment), the SOC content had a significant difference both in the top soil (0-20 cm) and the deep soil (20-40 cm) strata. However, S-3 and NS had a similar concentration in the topsoil, both of two treatments were lower than S-1 and S-2 treatments. Similar trend was also found with C/N ratio, in which case S-1 and S-2 was higher than other treatments, and the S-3 treatment was the lowest. The SMBC and DOC concentrations of straw return treatments in the two soil layers were significantly higher than NS treatment (p<0.05).

The result indicated that continuous return of straw at every year rate had a significant effect to improve SOC content in the 0-40 cm, and straw return at every three-year (S-3) had similar content with NS treatment after five years later. There was also a good effect on increasing SOC content of subsoil layer after maize straw return.

In addition, straw return treatments and soil layer had significant impact on SOC, SMBC, DOC, and LOC contents except EOC (Table 4-2). Their interaction had no significant impacts on SOC and EOC. However, SMBC, EOC, LOC, and DOC showed significant correlations with SOC separately (Figure 4-1).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table 4-2 Variation of the fractions of soil activated organic carbon components with treatments | | | | | | | |
| T | SL cm | SOC g·kg-1 | SMBC mg·kg-1 | EOC mg·kg-1 | DOC mg·kg-1 | LOC mg·kg-1 | C/N |
| S-1 | 0-20 | 21.28±0.22a | 195.33±20.87a | 2.26±0.12b | 36.89±1.00a | 123.22±6.75a | 8.00±0.08a |
| S-2 | 0-20 | 20.95±0.46a | 184.14±2.32a | 2.56±0.08a | 30.54±0.37b | 115.08±22.59a | 8.25±0.18a |
| S-3 | 0-20 | 18.92±0.35b | 117.04±1.79b | 2.35±0.06b | 31.80±0.11b | 126.11±9.30a | 6.73±0.12b |
| NS | 0-20 | 19.11±0.40b | 92.56±7.68c | 2.24±0.21b | 26.12±1.45c | 125.05±3.18a | 6.33±0.13c |
| S-1 | 20-40 | 19.32±0.48a | 86.96±0.42b | 1.62±0.11a | 28.90±0.34ab | 112.42±18.87a | 8.29±0.20a |
| S-2 | 20-40 | 18.27±0.34b | 127.84±12.33a | 1.56±0.35a | 29.40±0.77a | 123.54±12.98a | 7.22±0.14c |
| S-3 | 20-40 | 17.30±0.35c | 114.87±4.35a | 1.56±0.08a | 27.80±1.35ab | 134.07±5.35a | 7.72±0.16b |
| NS | 20-40 | 16.43±0.38d | 88.80±14.83b | 1.51±0.12ab | 26.18±1.07b | 135.25±7.21a | 5.23±0.12d |
|  |  |  |  |  |  |  |  |
| Two-way ANOVA | T | 0.000 | 0.000 | -- | 0.000 | 0.024 | 0.000 |
| SL | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.003 |
| T×SL | -- | 0.000 | -- | 0.000 | 0.015 | 0.000 |
| R-sq | 96.12% | 96.17% | 94.57% | 97.39% | 71.74% | 98.59% |

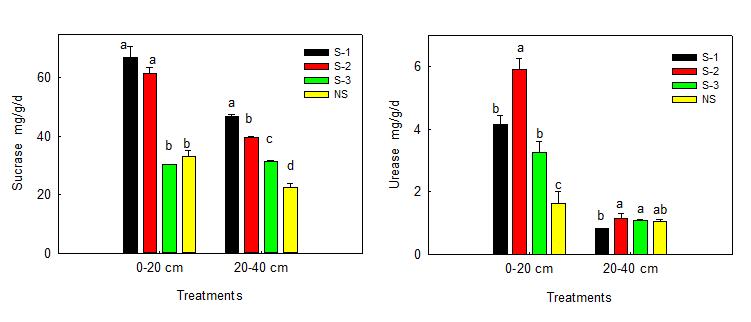
Changes in soil organic carbon (SOC), microbial biomass carbon (MBC), dissolved organic carbon (DOC), and easily oxidizable carbon (EOC) contents in soils under straw return by different year. T was treatments, SL was soil layer. Two-way analysis of variance analysis of the interaction between treatments and soil layer on SOC, MBC, DOC, EOC and LOC contents, the averages (mean±SD) followed by the same letter in the same soil layer were not significantly different (Duncan’s test, *p*<0.05) in different treatment. “--”indicates that SOC, MBC, DOC, EOC and LOC contents were not significantly affected by treatments, soil layer, or their interaction at the p<0.05 level.

**Figure 4-1 Relationship between soil organic carbon fractions**

***2.2 Response of soil enzyme activity***

Straw return in different years had significant effects on soil enzyme activities both in the 0-20 cm and 20-40 cm, while the urease activity had no significant impact on 20-40 cm.

Sucrase activity of all the straw return treatments in two soil layers were higher than those of NS (Figure 4-2). Sucrase activities of S-1 and S-2 treatments had higher activities than NS treatment which showed and increase of 102.1 and 85.6% in 0-20 cm soil layer. However, there was no significant difference in S-3 and NS. In the 20-40 cm, the sucrase activities of S-1, S-2, and S-3 treatments had significant differences compared with the NS treatment (P<0.05), i.e., 107.2, 75.9, and 38.9% higher, respectively.

The change of urease activity in all straw treatments had significant differences in topsoil compared with NS treatment, while no obvious effect in deep soil layer was observed (Figure 4-2). Urease activity values of S-1 and S-2 treatments were 1.6-5.9 g·kg-1 in the 0-20 cm soil layer, 0.8-1.1 g·kg-1 in the 20-40 cm soil, respectively. Compared with NS of 0-20 cm soil layer, the urease activity of the S-1, S-2, and S-3 increased by 156.3, 265.5, and 100.3%, respectively. The enzyme content in surface soil was higher than that in the deep soil, because with the deepening of soil depth, the content of organic matter and inorganic matter in soil decreases obviously, so the enzyme activity decreases gradually. Secondly, the air circulation in deeper soil layer was blocked, and the species and quantity of bacteria and fungi in soil were reduced, which leads to the decrease of soil enzyme activity.

**Figure 4-2 Activity of soil Sucrase and Urease in different soil depths under the different years of straw incorporation**

***2.3 Response of soil nutrient***

Soil chemical properties of different treatments were shown in Table 4-3. Maize straw application significantly increased the SOC content in 0~40 cm compared with NS and the highest vale was found in S-1 treatment in both the soil layers. With the application of straw, the content of AN was changed greatly in two soil layers. The variation range at 0-40 cm was 35.8-11.7%, while at 20-40 cm layer, it was relatively stable than the 0-20 cm layer. The AP and AK content of all treatments decreased with the deepening of soil depth, while the highest content was recorded in S-1 treatment, followed by S-2 treatment in two soil layers after straw return.

Maize straw return increased soil pH value in 0-20 and 20-40 cm soil layers showing highest soil pH in S-1 and S-2 treatments compared with NS.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table 4-3 Soil available nutrient in different treatments | | | | | | | |
| Treatment |  | SOC g·kg-1 | AN mg·kg-1 | AP mg·kg-1 | AK mg·kg-1 | pH |
| S-1 | 0-20cm | 21.28±0.22a | 60.84±0.46a | 60.78±0.13a | 185.02±1.97a | 5.53±0.01b |
| S-2 | 0-20cm | 20.95±0.46a | 54.95±1.85b | 51.61±2.54b | 179.69±0.91b | 5.56±0.04b |
| S-3 | 0-20cm | 18.92±0.35bc | 59.21±0.46a | 32.63±3.56cd | 165.06±1.10c | 5.15±0.01d |
| NS | 0-20cm | 19.11±0.40bc | 62.15±1.85a | 27.88±1.05d | 157.96±2.72d | 5.14±0.01d |
| S-1 | 20-40cm | 19.32±0.48b | 53.97±1.39b | 35.78±1.64c | 162.52±2.88c | 6.18±0.06a |
| S-2 | 20-40cm | 18.27±0.34c | 47.76±0.93c | 27.70±3.15d | 151.67±2.25e | 6.18±0.02a |
| S-3 | 20-40cm | 17.30±0.35d | 39.91±0.93d | 19.75±0.67e | 153.99±0.10de | 5.52±0.02b |
| NS | 20-40cm | 16.43±0.38e | 52.99±1.85b | 15.61±2.37e | 145.32±1.46f | 5.22±0.01c |

Note: Different letters indicate significant differences between samples(*p*<0.05). Values were mean±standard errors(n=3)

***2.4 Yield***

Application of straw to the field at interval years might be reflecting the difference of straw amount in the total five years. There was no significant difference between the rate of straw application in successive years and every other year in yield of the latest two years (2014 and 2015). The yield of NS treatment did not increase significantly for several consecutive years, but there was still an increasing trend. The yield of S-1 treatment increased first and then decreased, the same was S-2 and S-3 treatments. The yield of each treatment with different return years showed instability.

The input amounts of straw and straw carbon were different according to the amount of straw return. More was the input straw amount, soil organic carbon content was higher. It was found that the carbon sequestration rate and efficiency of S-2 treatment was higher than other treatments, S-3 treatment was the lowest.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 4-4 The yield of different treatments and straw carbon amount input | | | | | | | | |
| Treatments | 2012 Yield  kg·hm-2 | 2013 Yield  kg·hm-2 | 2014 Yield  kg·hm-2 | 2015 Yield  kg·hm-2 | Amount of straw input for five years/kg | Amount of straw carbon input for five/kg | Carbon sequestration rate | Carbon sequestration efficiency | | |
| S-1 | 11569±278ab | 11820±697b | 12378±338a | 11915±236a | 71102±1083 | 1513.2±23.1 | 89.35 | 0.30 | | |
| S-2 | 11717±390ab | 12571±447a | 12311±288a | 12493±399a | 43960±816 | 920.8±17.1 | 178.35 | 0.58 | | |
| S-3 | 11327±349b | 12705±593a | 12510±327a | 12485±384a | 28896±392 | 546.7±7.4 | 12.73 | 0.05 | | |
| NS | 12147±306a | 12109±396ab | 12460±537a | 12491±440a | ---- | ---- | ---- | ---- | | |
| Note: Different letters indicate significant differences between samples(*p*<0.05). Values were mean±standard errors(n=3) | | | | | | | | | |

***2.5 Correlations***

As shown in Table 4-5, significant positive correlations were found among the soil SOC, DOC, EOC, sucrase and urease at 0-40 cm soil depth, but there were no significant correlations with the labile organic C fractions. The SOC was significantly correlated with the soil MBC, EOC, DOC, sucrase and urease. But there were no significant correlation between the LOC and MBC, SOC, and EOC. MBC and enzyme had the highest significant correlations.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Table 4-5 Pearson correlation coefficients of soil activated organic carbon components with soil enzyme activities | | | | | | | |
|  | SOC | MBC | LOC | EOC | DOC | Sucrase | Urease |
| SOC | 1.000 |  |  |  |  |  |  |
| MBC | 0.728\*\* | 1.000 |  |  |  |  |  |
| LOC | 0.523\*\* | 0.307 | 1.000 |  |  |  |  |
| EOC | 0.751\*\* | 0.586\*\* | 0.518\*\* | 1.000 |  |  |  |
| DOC | 0.716\*\* | 0.773\*\* | 0.433\* | 0.466\* | 1.000 |  |  |
| Sucrase | 0.893\*\* | 0.826\*\* | 0.456\* | 0.498\* | 0.732\*\* | 1.000 |  |
| Urease | 0.764\*\* | 0.832\*\* | 0.549\*\* | 0.835\*\* | 0.627\*\* | 0.702\*\* | 1 |
| \* Significant at *P*<0.05. \*\* Significant at *P*<0.01. | | | | | | | |

## 3. Discussion

***3.1 The change of soil available nutrient after straw returning in different years***

The effects of farming practices and straw return on soil labile carbon include not only the disturbance of soil and the distribution of crop residues, but also the effects of soil physical, chemical and biological changes brought about by long-term effects. Since, we have studied only the short-term farming (1 year the lack of understanding of long-term farming (>10 years) on the impact of soil activated carbon components requires further exploration), long term effects were not evident.

A large number of studies have shown that conventional and appropriate crop residues have a significant impact on soil physical and chemical properties (Dolan et al. 2006; Roldán et al. 2003), which plays an important role in improving soil organic matter dynamics and nutrient cycling (Naresh, Gupta, and Panwar 2018). Therefore, the mineralization after returning crops to the field also provided necessary nutrients (N, P, K) and even reduced the amount of potassium fertilizer in the field (Shan and Buresh 2008). The change of effective nutrient content in soil was more complex, and its content was related to soil nutrient supply level and crop absorption and utilization. However, various studies have reached different conclusions on the impact of straw return on soil available nutrients. According to the long-term analysis of low fertility moist soil, the content of soil hydro-N increases significantly with the increase of straw incorporation in the soil. This might be attributed to the nitrogen supply of crop residues and/or the improvement of soil physical and chemical conditions(Utomo, Frye, and Blevins 1990). Due to the low nitrogen content of straw, nitrogen resources could be re-utilized after straw was returned to the field. In addition, some studies showed that when straw was transferred to deep soil, the risk of nitrate leaching was increased due to the interference of the soil layer (X. Xu et al. 2018). On the other hand, it improves the aeration of deep soil and promotes the utilization of nitrate-nitrogen by crops.

The combined application of chemical fertilizer and crop residue could increase the AP compared with the application of chemical fertilizer alone (Garg and Bahl 2008). But Mu et al. (2011) had showed that with the continuous return of straw, soil organic matter, AN, and AK increased significantly, while AP nutrients change a little. It was possible that in the early stage of corn straw decomposition in soil, more than 90% of potassium was released, and the potassium content in wheat straw accounts for about 80% of the whole plant, thus increasing the potassium content (De-shui et al. 2007).

In this study, the content of soil alkaline nitrogen (AN) content fluctuates greatly in all the sampling layers. There was no obvious regularity between straw application and soil layer, which may be due to the heterogeneity caused by straw income and soil disturbance. Both AP and AK increased mostly in the S-1 treatment, followed by S-2, and S-3 treatment was the lowest. It could be seen that the amount of straw intake has a significant impact on the content of soil AP and K, the content of AK in 20-40 cm was higher than that of topsoil. Although the straw applied in the S-3 treatment was very low, it was still higher than that in the no straw treatment (NS). This had a greater relationship with soil P fixation and crop absorption maybe due to the release of organic acids in the decomposition process. So as to support the release of P by dissolving natural P sources, which should be specifically discussed in the future.

At present, the problem of soil acidification in the black soil area of Northeast China was mainly caused by improper land use (Pietri and Brookes 2008), farmers always adopt predatory planning, which was the main aspect of soil acidification caused by the carbon and nitrogen cycle (Helyar and Porter 1989). In the study of long-term effects with no fertilizer, the soil pH remained stable (Qiao et al., 2007). A large amount of nitrogen fertilizer applied to the farmland for planting corn every year, and 2/3rd of these fertilizers should be input in the form of topdressing in July with the highest temperature and rainy season (J. Zhang et al. 2021). The nitrification of ammonium nitrogen in the soil and the subsequent leaching of nitrate nitrogen moved downward with the movement of water, and may leach out of the root zone, leading to acidification of foundation soil (Fan et al., 1992) which makes farmland soil acidified seriously. However, returning straw or organic fertilizer to the field could effectively alleviate the above problems (Qiao et al., 2007). The results of this study showed that the straw return increased deep soil pH higher than the surface and had a significant influence in S-1 and S-2 treatments. But the S-3 treatment was similar with NS which means straw return for every three years was too long/less to affect soil. Because a large number of organic anions would be accumulated during the growth of crops, when plants were removed out of the field, these alkaline substances would also be removed. Corn straw and organic fertilizer application to the soil could improve soil acidification. This was one of the main ways to alleviate soil acidification.

***3.2 Response of soil organic carbon fractions***

DOC, EOC, and MBC were used to indicate the labile organic carbon fractions under different management practices(Haynes 2005). In the present study, SOC, DOC, EOC, and MBC content were significantly enhanced under S-1 and S-2 treatments, indicating that straw incorporation played an important role in rising SOC and labile organic carbon fractions(Gong et al. 2009). Because the straw application not only increased overall inputs of carbon into the soil but also changed the rate of microbial decomposition. Soil labile organic caron fractions showed significant correlations with SOC, and all fractions significantly increased with the amount of straw, supporting the findings of many previous studies(Z. Chen et al. 2017; H. Zou et al. 2016).

Many studies indicated that long-term straw incorporation increased SOC concentration compared to no straw incorporation(Tian et al. 2015; D. Yan et al., 2007; S. Zhao et al. 2016), especially in top soil layer (Zhu et al. 2015). However, a short-term study conducted, showed that crop residue return did not significantly change the TOC concentration in central China (Gangwar et al. 2006; Guo et al. 2014). This study also indicated that straw return every three years did not have obvious effects on the SOC content in 0-20 cm, but it had a significant difference among them in the 20-40 cm soil layer.

To the findings of no differences in S-3 and NS treatment at 0-20 cm, a possible explanation for this was that NS and S-3 treatments receiving no/less straw return contained the least amount of fresh organic biomass, therefore, the lowest amount of readily available organic carbon. This suggested that at higher amounts of straw application, the decomposition degree, and the composition of the straw were also potentially important factors influencing SOC sequestration (X.Wang et al. 2012), even increased subsoil SOC content (Zou et al. 2016).

Although the SMBC only have a 5-8% of SOC, it has higher activity and dynamics in soil carbon which plays an important role in the nutrient cycling and maintaining ecosystem function (Moore et al., 2000; Vance ED 1987), and it was the driving force for microbial activity (C. Li et al., 2012). DOC and MBC were the most sensitive indicators for assessing change of SOC under short-term straw return in the rice-wheat cropping system, and soil properties in response to straw return were different (Z. Chen et al. 2017). SMBC was decreased with the deepening of soil layers. There was a significant difference in the SMBC content between different straw return years, and a phenomenon of surface microbial aggregation in the soil. The S-1 and S-2 treatments had higher amounts of straw, which provided larger amounts of substrates for soil microorganisms and converted to labile fractions (MBC and DOC)(Tiemann and Billings 2011). It was cleared that straw return interval for many years was not a best choice for the soil nutrient supply. The free light fraction was mostly derived from fresh, undecomposed organic material (Mueller et al. 1998). One previous research has reported that straw return significantly increases soil-free light fraction content (Yan et al., 2007). However, in the present study, straw incorporation in different year did not increase LOC content, which was consistent with the results of Zhao (H. Zhao et al. 2019).

***3.3 The change of enzyme activity***

Soil enzyme happens to be one of the important active components which participates in various biochemical processes in soil. These are: decomposition and synthesis of humus and animal and plant residues, microbial residues, synthesis, hydrolysis and transformation of organic matter, and soil nutrient cycling (Allison and Vitousek 2004; Zheng et al. 2015). Tillage and straw returning had major effects on soil enzyme activities. The different years of straw return had significant effects on soil enzyme activities, which was consistent with other findings (Mandal et al. 2007; H. Zhao et al. 2019). It could be found that the activities of urease and sucrase in S-1 and S-2 treatments were higher than S-3 and NS treatments in the 0-40 cm soil layers. This suggested that straw return increased most soil enzyme activities and the low quantity and quality of maize straw could not support higher microbial substrate. The enzyme activities on topsoil were more effective than those of subsoil, because with the deepening of soil depth, the content of organic and inorganic matter in the soil decreased obviously, so the enzyme activity decreased gradually. The air circulation in the deeper soil layer was blocked, and the species and quantity of bacteria and fungi in soil were reduced, which leads to the decrease of soil enzyme activity. Ji et al. (2014) showed tillage management greatly affected the distribution of soil microorganism in different soil depths. Deep tillage loosened the soil and added the organic matter into the soil, which increased the abundance of soil microorganism. The more the soil microorganisms, the higher the soil enzyme activities would be. Jin et al. (2009) also showed the subsoil with mulch consistently had higher enzyme activities compared with no till with mulch. Activities of enzyme species may be related to organic matter mineralization and humification in the soil.

***3.4 The yield***

Many researchers focus on the effect of different amounts of straw return (Xu et al., 2018; Cong et al., 2019; Gao et al., 2020) showed the increase of yield was not in direct proportion to the increase of straw return amounts. Xu (X. Xu et al. 2018) showed that 75% straw return to the field had a significant effect on increasing soil nutrient content, but the yield was lower than less amount of straw. In the study, different treatments of straw return were similar with the different amounts of return during a certain period. S-1 treatment was a full straw return, S-2 was equivalent to full return every 2 years. But in essence, compared with returning farmland every year and half the amount, it was different in terms of labor, material, and financial resources. In this study, there was no significant difference among treatments, because the site was most fertile soil which riched nutrients. A long-term of straw return research in Cinnamon soil showed straw mulch and straw incorporation after crashing could increase maize yield (H. Xu et al., 2021), while was consistent with the research in red soil (Kong et al., 2021), however, the long-term of straw return research in black soil showed no significant differences in average yields among the manure, straw with chemical fertilizer treatments, in which the same N amounts were applied. (Gao, et al., 2015). On the one hand, it came down to the different soil texture and climate; on the other hand, the straw contained lots of activated carbon and nitrogen, which helped to fix soil nitrogen while the application of chemical fertilizer promotes the soil nitrogen mineralization (Malhi et al., 2007). Yang et al. (2020) showed straw return could increase rice, maize and wheat yield by meta-analysis from 274 literatures, but the yield significantly affected by temperature, rainfall, texture, pH, tillage practices and fertilization. As an input of organic nutrient resources, straw return with the cooperation of optimized chemical fertilizer could improve SOC content and enable soil fertility to continuously develop its potential for yield increase. To sum up, it was necessary to find appropriate cultivation, fertilization and straw return practices to maintain the continuous increase in crop production.

## 4. Conclusions

Our results demonstrated that SOC and labile fraction SOC contents increased significantly with the different year of straw return, which inputs residue-derived C. The frequency of year of straw incorporation also led to significantly higher activities of enzyme. The result indicated that straw return in every three years had the similar effect on no straw return treatment. There were significant and positive correlations with the soil SOC, DOC, EOC, sucrase and urease in 0-40 cm soil depth in different treatments. Straw returning every two years had more carbon sequestration and carbon sequestration efficiency.

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**Chapter Ⅴ**

**Fluorescence spectral characteristics of straw return with different years on soil DOC components in black soil areas**

From Kuang E. J., Chi, F. Q., Zhu, B. G., Zhang J.M., Xu, M. G., Colinet, G., Zhu P., Hao X. Y. & Sun L. (2022). Effects of maize straw return on soil dissolved organic carbon characteristics in crop fields. (Pending approval, Journal of Sensors)

## Abstract

In the present study, the effects of maize (Zea mays L.) straw decomposition on soil carbon pool under different return methods were studied for five years. Soil dissolved organic carbon (DOC) is an easily decomposed and highly active C component, which is affected by external organic material input. Excitation-emission matrix spectroscopy (EMMs) combined with parallel factor analysis (PARAFAC) was applied to analyze the soil dissolved organic carbon (DOC) content. In addition, its fluorescence characteristics and the variation characteristics of humification degree after maize straw return in the field were also studied. The results showed that the content of DOC decreased gradually with the increase in soil depth, but the same after the application of straw increased within 0-20 and 20-40 cm soil layers. The increase of DOC in continuous straw return, i.e., in S-1 treatment, was higher than that in others. The DOC/SOC value of straw return to the field every other year, i.e., S-2 treatment was the lowest. It has, therefore, indicated that the stability of the soil organic carbon pool became better. From the analysis of the spectral indices (FI, BIX, and HIX were 1.59~1.69, 0.90~0.95, and 0.64~0.74, respectively) it is indicated that DOC input into the soil resulted from the mixture characteristics of plant and microorganisms. There occurred a strong contribution of authigenic source, high bioavailability, and weak degree of humification after straw incorporation. Three fluorescent spectral components were identified by PARAFAC analysis. Component C1 was fulvic acid in the ultraviolet region which was 26.8%~35.7% of the total, while components C2 and C3 were both tryptophan substances (64.3%~73.2% of the total) and these two compounds were also dominant in all substances. Continuous straw incorporation promoted the transformation of fulvic acid, while straw incorporation every other year (S-2) helped easy accumulation of macromolecular substances and did form more stable humus. Correlation analysis showed that the DOC/SOC was closely related. And the interaction between straw incorporation years and soil depth had a significant effect on C1. The interaction between soil depth, incorporation year, and soil depth itself had a significant effect on the C2 component.

**Keywords:** Straw return; soil dissolved organic carbon; fluorescence spectrum; PARAFAC analysis

## Introduction

Crop straw returning to the agricultural field as a waste resource can improve the soil structure and soil nutrients (K. Kumar and K. M. Goh, 2003; L. M. Zibilske and L. A. Materon 2005). Corn straw has a slow decomposition rate, but its return to the deep field increases the fixation of straw carbon into the soil (Kuang, et al., 2020). It is conducive to the accumulation of humus in the crop field (Tian P., 2020) and can also solve the negative impact caused by the low temperature, particularly in Northeast China. Due to the gradual promotion of diversified utilization of straw, the ways of returning straw practice to the field vary in different regions (Wang, et al., 2008; Liu et al., 2007; Pan D. and Li R., 2021). However, either way, it can improve soil nutrients, fertility, and energy reuse. Dissolved organic carbon (DOC) is an active component in the soil nutrient pool and an important part of the material cycle (Qin, et al., 2014). DOC can be transformed into other components of soil organic carbon as well. Straw return can significantly improve the content of soil DOC (Li, et al., 2019). It has been reported at home and abroad that the main source, distribution, and composition characteristic of DOC was characterized by three-dimensional fluorescence spectroscopy (3DEEM) combined with multivariate statistical analysis (Praise S. and Ito H., 2016; Niu, et al., 2016; Wang, et al., 2008; Zhu, et al., 2021). It has an obvious effect on the fluorescence peaks of soil DOC components followed by different soil organic carbon content. Additionally, they also affect different tillage combined with straw return practices, and the interval time of straw return, while the humification of DOC was also different (Shi, et al., 2016; Li, et al., 2020; Fan, et al., 2013). Research results showed that the content of fulvic acid in the organic carbon fluorescence component was increased and the structure was simplified after straw return (Dong and Dou, 2017). Li et al. (2017a) and other researchers used UV Vis and 3DEEM technology to study the structural characteristics and sources of DOC in the soil of the Dianchi Lake area. He found that the soil in that area was dominated by fulvic acid-like substances with no obvious characteristics of exogenous and endogenous sources. To improve the utilization of corn straw in the fermentation of organic materials, the reference value of the actual composting ratio is proposed according to the fluorescent components of DOM in the substrates with different ratios of straw and cow dung (Li, et al., 2021). In addition, according to the characteristics of DOC composition, and the structure of different soil types, the adsorption behavior of soil on the DOC was also analyzed. The result has guided the rational application of compost to different types of soil (Xiong, et al., 2015). Therefore, 3DEEM technology was used with the PARAFAC method to analyze the composition, characteristics, and distribution of DOC under the condition of returning straw to the field. This could more accurately show the characteristics of various fluorescent components of DOC and provide theoretical support for the diversified utilization of straw in Northeast China.

In this study, we hypothesize the application of maize straw obtained at three different return years varied soil DOC fractions of fluorescent materials. Herein, the main objects of this study were to (1) investigate the dynamics of DOC concentrations in different treatments; (2) characterize the composition and structure of soil DOC using spectroscopic techniques, and (3) gain the fluorescence components using PARAFAC modeling and analyze the variations of relevant optical indices.

## 1. Materials and methods

***1.1 Experimental Design***

See the 1.1 of chapter Ⅳ.

***1.2 Sampling and Analyses***

Soil samples were collected by the "S" shape method at five points and from the two soil depths (0-20 and 20-40 cm) in April 2016. In each plot, five cores were excavated randomly and collected soils were mixed to give a representative composite sample for further processing. After collection, the integrated mixed soil sample was cleaned by hand-picking of straw residues and small stones and then allowed for air-drying. The dried samples were passed through though 0.01 mm and 2 mm soil sieves for laboratory analysis.

Determination of SOC: For determining the SOC, 0.01 g of air-dried soil sample was weighed and to that 2 mol/L hydrochloric acid solution was added for acidolysis. SOC was determined with a total organic carbon analyzer (Multi N/C 2100, Germany).

Determination of DOC content: From the air-dried sieved sample, 5 g was weighted and to it distilled water was added (the ratio of dry soil weight to water volume was 1:10). The slurry was shaken horizontally with the help of a shaker at room temperature for 1 h at 200 r·min-1. The sample was then centrifuged at 4 ℃ for 20 min at 12000 r·min-1. The supernatant was filtered with the help of 0.45 μm filter paper, and thereafter a clear solution was obtained. This solution was used to determine the DOC concentration with a carbon analyzer (Multi N/C 2100 TOC, Germany).

Spectral analysis: The DOC solution was adjusted to a concentration of 10 mg·L-1, and the three-dimensional fluorescence spectrum was measured with a fluorescence spectrometer (Hitachi F-7000, Japan). The scanning range of excitation wavelength (Ex) and emission wavelength (Em) was 200–600 nm, and the bandwidth and the scanning speed were 10 nm, and 1200 nm·min-1, respectively. When PARAFAC analysis was applied, the influence of Raman scattering on the fluorescence data was eliminated.

***1.3 Statistical analyses***

The data were processed and analyzed by Excel 2010 and SPSS 17.0. Matlab 2013 software was used for three-dimensional fluorescence map drawing and parallel factor analysis, and origin 2019 was used for regional integration of fluorescence spectrum index.

## 2. Results

***2.1 DOC and DOC/SOC variations***

From Figure 5-1, it is seen that the DOC content gradually decreased with the increase of soil depth. The straw application could increase the DOC content both in 0-20 and 20-40 cm soil layers, but the S-1 treatment was always the highest. Compared with NS treatment, the DOC content of S-1, S-2, and S-3 treatments were 41.3, 16.9, and 21.7% higher, respectively, at 0-20 cm. and 10.4, 12.3, and 6.2% higher at 20-40 cm, respectively (*P*<0.05). A higher value of DOC/SOC in S-1 treatment at 0-20 cm was obtained. However, S-1, S-2, and S-3 were 26.8, 6.7, and 23.0% higher than NS, respectively. The difference between them was significant (*P* <0.05). At the 20-40 cm soil layer, the performance of the S-1 treatment was lower than that of other treatments, and there was no significant difference among treatments.



Figure 5-1 The content of DOC and DOC/SOC under different treatments (Lowercase letters indicated the significance analysis at the level of 0.05 between different treatments at the same level)

***2.2 The fluorescence region integral***

3DEEM research includes common peak picking, PARAFAC, and FRI. Based on the different substance types represented by the five regions divided by excitation and emission wavelengths (Table 5-1), the ordinary peak-seeking method can track the source of active organic matter and compare the degree of humification(Marhuenda-Egea et al. 2007; Y. Zhao et al. 2017). PARAFAC and FRI technology can overcome the difficulty of heterogeneity of single fluorescent components in the measured samples (Stedmon, Markager, and Bro 2003), and their analysis was simple and suitable for multivariable data analysis. PARAFAC decomposes the fluorescence spectrum (EEM) into individual fluorescent components and provides relatively complete data analysis of organic matter components (such as FA) (X. S. He et al. 2013); FRI can analyze the volume integral under each excitation-emission region and analyze the spectral data of relevant fluorescence intensity at all wavelengths obtained from EEM (Wu et al. 2012).

The fluorescence region integral(FRI) method has been successfully used for the analysis of three-dimensional fluorescence spectra of water (Chai et al., 2012; Yu et al. 2011) FRI method divides the two-dimensional fluorescence region formed by excitation and emission wavelengths into five parts, representing five different types of organic substances, including aromatic protein substances I, aromatic protein substances II, fulvic acids, soluble microbial metabolites, and humic acids, as shown in Table 1. Then, the integral volume of a specific fluorescent region was calculated by integration, that was, the cumulative fluorescence intensity of organic compounds with similar properties. Finally, it was standardized to obtain the integral standard volume of a specific fluorescent region, which reflected the relative content of specific structural organic compounds in this region.

Table 5-1 Location and descriptions of EEM peaks in ranges of excitation and emission wavelengths

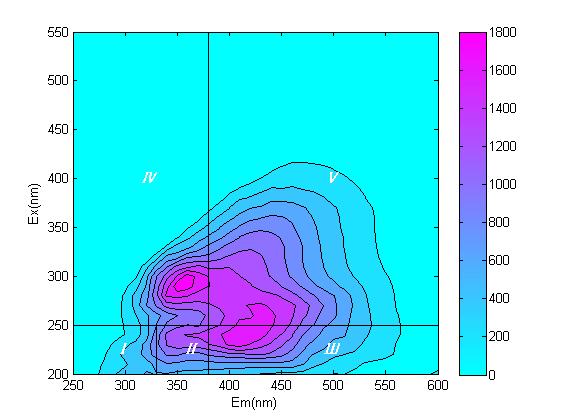
|  |  |  |  |
| --- | --- | --- | --- |
| Fluorescence region | Types of substances | Ex (nm) | Em (nm) |
| Ⅰ | Tyrosine-like protein | 200-250 | 250-330 |
| Ⅱ | Tryptophan-like protein | 200-250 | 330-380 |
| Ⅲ | Fulvic acid-like | 200-250 | 380-550 |
| Ⅳ | Soluble microbial metabolites | 250-490 | 250-380 |
| Ⅴ | Humic-like | 250-490 | 380-550 |

***2.2 Fluorescence spectrum***

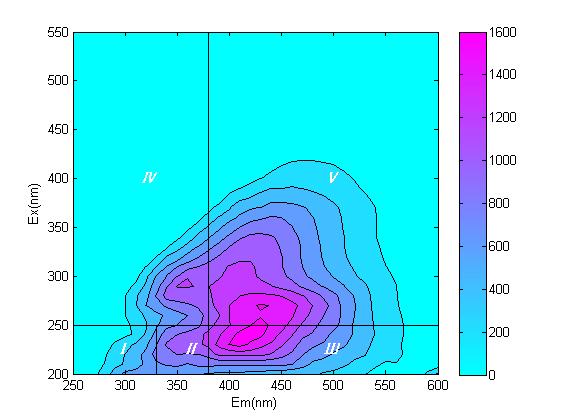
After returning straw to the field, the protein-like substances had a decreasing trend, and the decrease was more and more with the continuous years of returning straw to the field. After continuous straw returning (S-1 treatment), tryptophan-like protein substances and soluble microbial metabolites increased, tyrosine-like protein substances and humus-like substances decreased, and fulvic acid-like substances had no obvious change trend in these treatments. Studies had shown that protein-like components were an important factor to control the characteristic fluctuation of dissolved organic matter. There was a significant difference in the proportion of protein-like/humic-like fluorescent components in soil dissolved organic matter after land tillage change. In this experiment, the humification degree of dissolved organic carbon among all treatments was the lowest with the continuous straw return, showing a weak humification degree. There was no significant difference among other treatments. The sources of dissolved organic matter were the dual contributions of internal (microorganisms, algae) and external (humus)(Bardgett, Hobbs, and Frostegård 1996).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Table 5-2 Percentage of various substances in total substances % | | | | | | |
| Treatments | Tyrosine-like protein | Tryptophan-like protein | Fulvic acid-like | Soluble microbial metabolites | Humic-like |
| S-1 | 2.88±0.12bc | 7.30±1.64a | 19.13±1.38a | 17.49±4.80a | 53.19±5.15a |
| S-2 | 2.80±0.05c | 6.38±0.64a | 20.21±0.80a | 14.40±1.71a | 56.20±1.67a |
| S-3 | 3.08±0.21ab | 6.82±0.20a | 20.30±0.78a | 14.62±1.42a | 55.18±0.66a |
| CK | 3.17±0.07a | 6.44±0.43a | 19.03±0.24a | 15.75±1.32a | 55.62±1.55a |
| Note: The values are mean ± standard deviation. Different lowercase represents a very significant difference(*p*<0.05). | | | | | | |

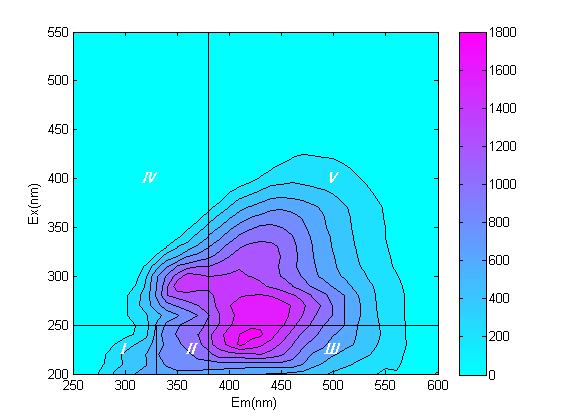
**Figure 5-2 Fluorescence spectrum of different treatments**



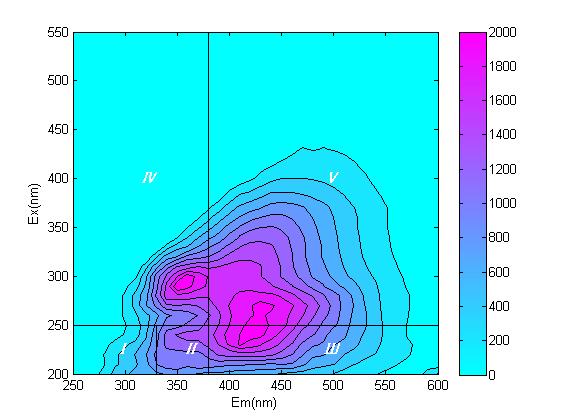
CK



S-3



S-2



S-1

***2.3 PARAFAC***

As shown in Figure 5-2, Three components were obtained from the three-dimensional fluorescence spectrum of soil DOC analyzed by PARAFAC. The maximum values of excitation wavelength and emission wavelength of each fluorescent component were listed in Table 5-3, and the types of fluorescence peaks of each component were determined at the same time.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table 5-3 Spectral characteristics of three fluorescent components | | | | |
| Component | Type | Peak | Excitation maxima(nm) | Emission maxima(nm) |
| C1 | Fulvic acid-like compounds in the ultraviolet region | A | 250 | 440 |
| C2 | Tryptophan like substance | T | 280-300, 230 | 350-370 |
| C3 | Tryptophan like substance | T | 220 | 340 |
|  | | | | |

Component C1 (excitation wavelength: 250 nm, emission wavelength: 440 nm) contained one excitation peak and an emission peak, indicating fulvic acid like in the ultraviolet region, corresponding to a traditional peak (in Figure 5-2). Peak A was mainly produced by relatively stable organic substances with large relative molecular weight, which was related to hydroxyl and carbonyl in DOC and generally indicated exogenous input.

Component C2 (excitation wavelength 280-300 nm, 230 nm; emission wavelength 350-370 nm) indicated the fluorescence of tryptophan-like substances related to carboxyl functional groups. It contained two excitation peaks, and one emission peak, corresponding to a traditional T peak (Guo, 2010). Tryptophan-like acids were considered to be soluble microbial metabolites produced by the degradation and metabolism of microorganisms and bacteria and were easy to bind to macromolecular proteins, they could easily be transferred energy with tyrosine bound in the same protein, which had a complex impact on the fluorescence peak (Zhang B, et al, 2018). Nonprotein fluorescent substances such as lignin and gallic acid in some humus also have similar fluorescence peaks, which might be related to the structure of phenol and live aniline. Component C3 (excitation wavelength: 220 nm, emission wavelength: 340 nm) and C2 belonged to tryptophan fluorescence.

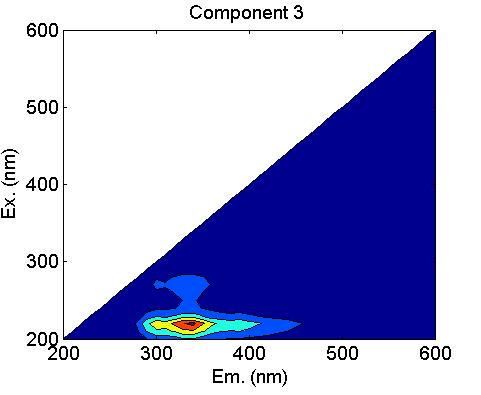
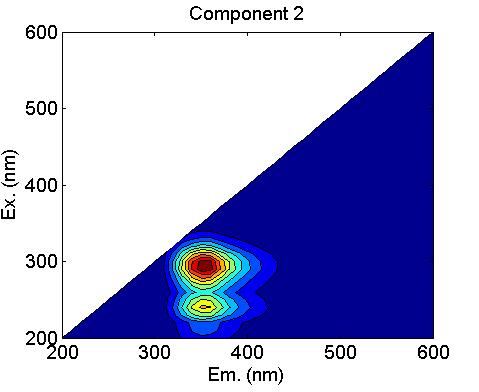
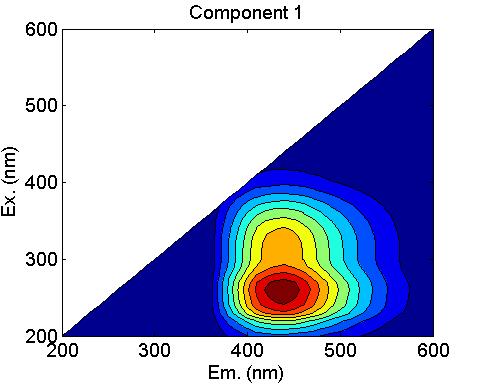


Figure 5-3 Fluorescence spectrum of different treatments in PARAFAC

The fluorescence intensity was closely related to the structure of the DOC. The fluorescence intensity of each fluorescence peak can indirectly explain the active functional groups and DOC properties contained in DOC. The higher fluorescence intensity may be related to the accumulation of components with simple and low relative molecular weight, such as hydroxyl, methoxy, amino, and other electron donor groups with low aromaticity and low degree of polymerization.

Table 5-4 Fluorescence intensity and relative percentage of soil DOC fluorescence components

| **Treatment** | **C1** (R.U.) | **C2** (R.U.) | **C3** (R.U.) | **C1+C2+C3** (R.U.) | **Relative percentage%** | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **C1** | **C2** | **C3** |
| S-1 | 1620.6b | 2520.2a | 1818.7a | 5959.5 | 29.58 | 42.90 | 27.52 |
| S-2 | 2038.8a | 2441.1a | 1080.7a | 5560.6 | 37.76 | 42.73 | 19.51 |
| S-3 | 1683.9a | 2673.2a | 1049.0a | 5406.1 | 33.27 | 46.55 | 20.17 |
| NS | 1740.6b | 3129.3a | 1373.1a | 6242.9 | 31.03 | 47.06 | 21.91 |

Note: The fluorescence component values are mean ± standard deviation. Different lowercase represents a very significant difference (*P*<0.05).

The lower fluorescence intensity may be related to the component accumulation of DOC containing electron absorbing groups such as carboxyl and carbonyl with high relative molecular weight (D’Orazio, Traversa, and Senesi 2014). Through PARAFAC analysis, it was identified that there were three components of soil SOC in each treatment of straw return. C1 was fulvic acid in the ultraviolet light region, and C2 and C3 were tryptophan-like substances. It can be seen from Table 5-3 that tryptophan-like substances dominate, followed by fulvic acid-like substances. The molecular structure of the components of continuous straw return treatment was simpler and the relative molecular weight was lower.

***2.4 Fluorescence index of soil DOC components***

The indices of FI, BIX, and HIX are currently used to quantify the compositional variation and source of DOC. FI can reflect the source of humus. When the FI value was less than 1.4, it indicated that the main source of dissolved organic matter was planted. When FI>1.9, the components of dissolved organic matter came from the metabolic activity of soil microorganisms. When the FI value was between 1.4~1.9, it was produced by the mixed action of plants and microorganisms. Among them, FI could reflect the source of humus. The FI values of all treatments were between 1.59 and 1.69 and the data have been presented in Table 5-5. Which the NS treatment was the highest and the S-2 treatment was the lowest. The difference between NS and straw return treatments was significant.

whether straw was returned to the field or not, indicated that soil DOC came from the mixture of plants and microorganisms. The average value of FI of each treatment was slightly higher than that of other treatments, which was 1.69±0.02, which had a significant difference.

BIX was usually used to measure the contribution of authigenic organic matter. When BIX was between 0.6~0.7, 0.7~0.8, and 0.8~1.0, it represented that dissolved organic matter had less, medium, and strong authigenic characteristics respectively. When BIX was greater than 1, the substance was produced by the activity of biological bacteria. In this study, the value of BIX in each treatment was 0.8~1.0, indicating that there was a strong characteristic of authigenic source, but no significant difference among the treatments.

Table 5-5 Average fluorescence spectral index of soil DOC in different treatments

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **FI** | **BIX** | **HIX** |
| S-1 | 1.65±0.01b | 0.90±0.10a | 0.64±0.05b |
| S-2 | 1.59±0.02c | 0.93±0.10a | 0.74±0.03a |
| S-3 | 1.61±0.01c | 0.95±0.07a | 0.72±0.02ab |
| CK | 1.69±0.02a | 0.95±0.07a | 0.71±0.02ab |

Note: The values are mean±standard deviation. Different lowercase represents very significant difference (*P*<0.05). The unmarked part was not significant, the same below.

The humification degree of soil organic matter was expressed by HIX. The higher the HIX value, the higher the humification degree of soil DOC, and the better its stability, and it also indicated that it existed for a long time. When HIX was less than 1.5, it belonged to biological or bacterial sources, and when HIX was greater than 3.0, it belonged to strong humus characteristics. It could be seen from Table 5-5 that the HIX of each treatment was less than 1.5, belonging to biological or bacterial sources. S-1 treatment had a lower humification degree of straw return to the field year after year, which was significantly different from other treatments. Because the addition of fresh straw every year would stimulate the mineralization of original soil carbon, which was not conducive to the stability of soil humus; When straw was then returned to the field every two or three years, the excitation effect and mineralization of straw were weaker, which was conducive to the accumulation of humus. The difference in soil humification degree was more and more significant with the increase of returning years.

***2.5. Correlation analysis of soil DOC after straw return***

The relationship of DOC, its fluorescent components, and DOC/SOC values of each treatment was analyzed and presented in Table 4. The effect of soil depth, treatment, and their interaction on DOC was extremely significant (p<0.01). The effects of treatment and soil depth on DOC/SOC and C3 were significant or extremely significant. Treatment and their interaction had a significant impact on the C1 component. Soil depth and their interaction had a significant impact on the C2 component. In the analysis, a significant negative correlation was found with the C2 component.

Table 5-6 Significance analysis of different treatments, depths, and their interactions on Fmax of DOC structure (P value)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | DOC | DOC/SOC | C1 | C2 | C3 |
| Treatment (T) | 0.000 | 0.000 | 0.000 | 0.149 | 0.035 |
| Soil depth (D) | 0.000 | 0.000 | 0.551 | 0.000 | 0.008 |
| T×D | 0.000 | 0.068 | 0.000 | 0.001 | 0.062 |
| R-Sq | 97.39% | 96.12% | 89.94% | 83.59% | 64.63% |

## 3. Discussion

Soil DOC is an easily decomposed and highly active component of organic carbon, which is significantly affected by soil matrix and microbial degradation. In this trial, the DOC content gradually decreased with the deepening of the soil profile. The application of exogenous straw could increase the DOC content of the soil profile in the test area, which was consistent with the previous research results (Li, et al., 2019). The DOC content of topsoil increased due to tillage and repeated addition of exogenous substances. In addition, the increase of clay content in deep soil also accelerated the adsorption of DOC, and the decomposition of organic matter by soil microorganisms was weakened (Xiong, et al., 2015). The straw return provided a good material and energy basis for microbial growth and further stimulated the decomposition of easily decomposed organic matter in the soil itself. Straw carbon mineralization also increased the content of soil DOC, and the application of straw promoted the accumulation of soil DOC (Shi, et al., 2016).

The ratio of DOC/SOC could be used to characterize the activity of SOC and its sensitivity to management measures (Li, et al., 2013). For topsoil, the straw return had the trend of increasing DOC/SOC ratio, and straw return year after year (S-1) treatment was more obvious. With the extension of returning to the field years, the change of DOC/SOC first decreased and then increased. The value of DOC/SOC of straw return to the field every other year (S-2) was the lowest, which indicated that the continuous application of new carbon sources made the stability of the soil organic carbon pool worse (Tian, et al., 2011). In the 20-40 cm soil layer, the DOC/SOC of S-1 treatment was the lowest, that’s, because the content of DOC and SOC increased after straw return, and the straw inputs increased significantly for deep soil nutrients (Kuang, et al., 2020).

FI, BIX, and HIX could be used to characterize the structural composition of soil humus (Niu, et al., 2016). FI represented the source of soil DOC, and BIX represented an indicator of the characteristics and bioavailability of DOC autogenous sources (Li, et al., 2014). The FI value of each treatment was between 1.59 and 1.69, which indicated that the soil DOC came from a mixture of plants and microorganisms. The average value of FI was the highest in the NS treatment, which was significantly different from the straw return treatment. S-2 treatment was the lowest, and the source of DOC was more inclined to the characteristics of plant sources, had the straw was not returned to the field, DOC tended to move in the direction of microbial sources. Then the composition and source of DOC in the soil profile would also move in the direction of microbial sources with the increase in soil depth (Peng, et al., 2008). The humification coefficient HIX was usually used to indicate the degree of DOC humification in soil. The results showed that the BIX value of each treatment was > 0.9. Regardless of whether straw was returned to the field or not, soil DOC had strong characteristics of autogenetic sources. The humification coefficient of HIX was usually used to indicate the degree of DOC humification of soil. The higher the HIX value, the better its stability, and indicated that it existed for a long time (Huguet, et al., 2009). The HIX value of each treatment was between 0.64 and 0.74, and the degree of humification was weak. It was mainly from autotrophic sources due to the production of short-term organic matter decomposed by organisms and bacteria (Peng, et al., 2008). Among all treatments, S-1 was the lowest and S-2 was the highest. These indicated that continuous straw return reduced the humification degree of soil DOC and S-2 treatment was conducive to the accumulation of organic matter. Studies had shown that the difference in the degree of soil humification became increasingly significant with the increase in the number of years returned to the field (Ohno, et al., 2015). To sum up, the content of DOC after returning straw to the field for different years, was a mixed metabolite of straw and microorganisms, and the degree of humification was weak. DOC was unstable and could not exist in the soil for a long time. The instability of fresh DOC would be easy to be explained by carrying out the activities of soil microorganisms in CO2, water, etc., and the transformation of organic to inorganic matter.

DOC structure was closely related to fluorescence intensity, and many active functional groups contained in DOC could be explained by fluorescence intensity (Zhang, et al., 2018). The higher fluorescence intensity was related to the accumulation of DOC containing simple hydroxyl, amino, and other components with low relative molecular weight. The lower fluorescence intensity was related to the accumulation of carboxyl, carbonyl, and other components with complex and high relative molecular weight in DOC (Reader, et al., 2015). Through PARAFAC analysis, three components of soil DOC were identified, which were named C1 (a fulvic-acid-like substance in the UV light region), C2, and C3 (both tryptophan-like substances). Fulvic acid-like substance in UV light region was mainly produced by relatively stable organic substances with large relative molecular weight, which was related to hydroxyl and carbonyl groups in DOC, and generally indicated exogenous input (Li, et al., 2017). The fluorescence of tryptophan-like substances was related to carboxyl functional groups (S. Li, et al., 2017), which were generally considered easy to combine with macromolecular proteins and had complex effects on fluorescence peaks (Guo, et al., 2010). Studies had shown that straw application could promote the conversion of proteins to humic acid and fulvic acid (Shi, et al., 2016). In this trial, the total fluorescence intensity of S-2 and S-3 treatments were the lowest, and the C1 substance was higher than that of other treatments. All these indicated that the field returning every other year was conducive to the formation of macromolecular substances and tended to form more stable humus than other treatments. With the increase in the ratio of straw to organic fertilizer, the proportion of protein-like components gradually increased, which was consistent with the results of this paper (Li, et al., 2021). The decrease of fulvic acid-like substances treated with S-1 was because the energy-rich in exogenous organic substances provides the energy required for microorganisms to decompose complex macromolecular organic substances, resulting in the decrease of the content of fulvic acid-like substances (D’orazio, et al., 2015).

## 4. Conclusions

The DOC content decreased with the deepening of soil depth, the straw application could increase the DOC content both in 0~20 cm and 20~40 cm, while DOC/SOC value in 0-20 cm of S-1 treatment was the highest. It indicated that DOC came from the mixture characteristics of plants and microorganisms, with strong contribution of authigenic sources and weak degree of humification. Tryptophan like substances were dominant in three fluorescent spectral components which identified by PARAFAC analysis. The treatment, similar to the return of straw every year, reduced fulvic acid like substances and promoted the transformation of fulvic acid. While straw return every two-year was easy to facilitate the accumulation of macromolecular substances and the phenomenon helped to form a more stable humus. To sum up, straw return every two years was more conducive to the stability of soil organic carbon. The surplus straw can also be used in a diversified way to create more economic value.

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**Chapter Ⅵ**

**General Discussion, conclusions, and perspective**

The research in the thesis demonstrated that appropriate practices of straw return were crucial in Northeast China where the abundant crop straw plays a major role in soil fertility and organic carbon sequestration. The labile organic carbon fractions as the main part of SOC accumulation and exogenous OC sequestration played a central role in the soil carbon sequestration mechanism. The results clarify the relationship between changes of organic carbon fractions, microbial biomass carbon, enzymes, and soil temperature and moisture. The impacts and mechanism of straw incorporation at different soil depths and years on these parameters should be discussed to indicate the relevance of practices of straw incorporation in Heilongjiang province. Results should also be evalauated in the broader context of comprehensive use. Is the return to the field the most valuable use os the straw residues. Therefore, these aspects of the problems will be addressed.

## 1 General discussion

***1.1 Effect of different straw return practices on straw decomposition regularity, soil nutrients, and gas emission***

Straw is an important renewable resource, it is also the main by-product of crops which accounts for about 50% of the total biomass of crops. In the 1960s and 1970s, maize and wheat straw were mainly used as fuel. In the 1990s, with the continuous increase of crop yield and straw resources, chemical fertilizer replaced organic fertilizer, agricultural machinery power replaced animal power (straw used as feed decreased), and commercial energy replaced straw fuel. Most straws were used to directly return to the field to enrich the soil (Kuang et al., 2014). Straw is also rich in nutrients such as carbon, nitrogen, phosphorus, and potassium. As one of the most widely protective measures, the straw return was beneficial for optimizing soil fertility and reducing air pollution (Chen H., 2010).

Generally, maize straw return to deep soil benefited from decomposition and carbon storage in Northeast China (Lal, 2004; Wu et al., 2016). After three-year of the maize straw returned to the field, it was found that only part of the straw mulch was decomposed, while the residue of straw buried in the deep soil decreased rapidly to 10.4%~14.0% (Figure2-2). Because deep layers had good conditions in moisture, temperature, and more microorganisms for straw decomposition (Zou et al., 2016). Furthermore, straw return to deep soil could store more straw carbon (Figure 2-3), but with no significant differences among the D1, D2, and D3 treatments. Straw return to deep soil has been recommended as an effective practice to reduce straw biomass (Zou et al., 2016; Yang et al., 2016). Other researchers showed straw mulch had positive (Whitbread et al., 2003), no obvious (Xu et al., 2011), or negative effects (Ma et al., 2013) in 1-2-year experiments. However, in this study, the practices of straw mulch presented a negative effect on straw decomposition and gas emission. As we all known, the content of CO2, N2O, CH4, and other greenhouse gases in the atmosphere has continued to rise since 2011. Agricultural production activities are an important source of greenhouse gas emissions, accounting for 12% of total human production activities (Walling and Vaneeckhaute 2020). The research showed that long-term straw returning can completely compensate for the direct greenhouse gas emissions caused by fertilizer application, and the nutrients produced by straw returning can also reduce indirect greenhouse gas emissions by 20%~24% (Zhang et al., 2020). When straw is applied to soil, one part enters the soil and becomes the source of soil organic matter, and the other part is released into the atmosphere in form of CO2, leading to an increase in CO2 emissions (Heintze et al. 2017; Cheng et al. 2017). At the same time, the decomposition of straw carbon stimulated the mineralization of soil organic carbon then increasing CO2 emissions (Li et al., 2016; Wang et al. 2018; Yang et al. 2017). Studies showed the adjusted hydrothermal factors promoted CO2 emissions after straw mulch (Ding 2017). Some scholars believed that straw mulch hindered the release of CO2 because of less contact with the soil and the lower straw decomposition (Al‐Kaisi and Yin 2005).

Research at home and abroad still lacks consistent conclusions on the impact of straw return on N2O emissions. Studies showed that the increase of soil carbon from straw return, directly participated in soil nitrification process, increasing N2O emissions (Li et al., 2018; Tang et al., 2021; Liu et al., 2011). After the straw mixed with nitrogen fertilizer in black soil, N2O emissions increased greatly by two orders of magnitude ( Li et al., 2018). However, the C/N ratio of straw was large, it stimulated the soil microorganisms to absorb other nitrogen sources to meet metabolic activities, reducing the substrate for nitrification and denitrification, then reducing N2O emissions (Garcia et al., 2007; Zhang et al., 2015; Gao et al., 2017). In Songnen Plain, straw deep application and straw mulch with no tillage did not affect the soil N2O and CH4 emissions under soybean-corn-corn rotation mode (Zhang et al., 2015; Hao et al., 2022). The impact on CH4 emission after straw return was mostly focus in paddy field, and increased CH4 emission because the more carbon came from the straw could improve sufficient substrate for methanogens (Yao et al. 2013; Wang et al. 2020). However, ammonium produced by hydrolysis after applications of nitrogen fertilizer could promote the oxidation of CH4 and reduce CH4 emissions (Ma Jing et al. 2010; Yang Shuyun et al. 2010). The low water content in the dryland made it difficult to form a strict anaerobic environment, reducing the absorption of CH4 by the soil, then increasing the CH4 emission (Adviento-Borbe and Linquist, 2016).

The practice of straw return, straw amount, C/N ratio, temperature, and moisture content were the main factors affecting straw decomposition (Cao et al., 2016; Cai et al., 2019). The more straw biomass was decomposed, the more straw nutrients were released. The released straw nitrogen, phosphorus, and potassium could supplement the nutrients in the soil, increase the content of soil organic matter (Li et al., 2009), and reduce the amount of potassium fertilizer application (Shan and Buresh 2008, Kuang et al, 2014). The content of AN content fluctuates greatly in all the sampling layers in this study, in which AP and AK increased in the S-1 treatment (Table 4-2), followed by the S-2 treatment. It was no obvious regularity between straw application and soil layer, which might be due to the heterogeneity caused by straw input and soil disturbance. Maize straw retention increased soil pH both in the 0-20 cm and 20-40 cm soil layers which increased with the amount of straw return. The change of effective nutrient content in soil was more complex, and its content was related to soil nutrient supply level, crop absorption, and utilization. However, various studies showed different conclusions on the soil's available nutrients by straw return. According to the long-term analysis of low fertility moist soil, the content of soil hydro-N increases significantly with the increase of straw incorporation, which might be attributed to the nitrogen supply of crop residues (Utomo et al., 1990). When straw was transferred to deep soil, the risk of nitrate leaching was increased due to the interference of the soil layer (Xu et al. 2018), on the other hand, it improved the aeration of deep soil and promoting the utilization of nitrate-nitrogen by crops.

***1.2 Impact of different straw return practices on SOC and SOC fractions***

The application of straw could increase SOC content which increased with the amount of straw biomass. Many studies indicated that long-term straw incorporation increased SOC concentration compared to no straw incorporation (Tian et al. 2015; Yan et al., 2007; Zhao et al. 2016), especially on the top soil layer (Zhu et al. 2015). However, a short-term study conducted that crop residue return did not significantly change the SOC concentration (Table 2-2), consistent with some results (Gangwar et al. 2006; Guo et al. 2014). After five years of straw return, the SOC content of S-1 and S-2 treatments had a significant difference, compared with no straw return treatment (Table 4-1). This may be related to the input of straw (Table 4-3). It also indicated that straw return to the soil in three years did not have obvious effects on the SOC content in 0-20 cm, but it had a significant difference among them at the 20-40 cm soil layer. The correlation of all the soil indicators was presented in Table 2-3, it had been seen that SOC significantly and positively correlated with C/N (0.819) and temperature (0.508). On the other hand, sucrase correlated negatively and significantly with moister (-0.555). Tian (2007) indicated the decomposition rate of straw was higher at higher temperatures (25℃) and the content of microbial biomass carbon was higher when the soil humidity was lower.

Soil organic matter was not sensitive to the response of the soil organic carbon pool at the initial stage of straw return (Haynes, 2005). Therefore, many scholars researched the changes in organic carbon with different activities and different forms of existence from the stability of organic carbon, according to the sensitivity and turnover speed of soil organic carbon pool to external factors, DOC EOC, MBC as active organic carbon pool, which was easily affected by farmland management measures such as fertilization, tillage and straw return (Kalbitz, 2000; Mrabeta, 2001). Table 2-2 explored the reserve change and distribution proportion of soil organic carbon components under physical protection (Golchin et al., 1998) after corn straw was applied to different soil depths, it showed the LF group was faster in the process of decomposition than others. It was consistent with S. Chen et al. (2008)’s results which had a significant effect on improving soil organic carbon quality. From the perspective of the grouping of organic carbon, the content of LF and O-LF would have been changed easily in all the soil depths, HF had a relatively stable. The arable degree of culturing in the cultivated soil layer was higher, and the soil recombined organic carbon content did not fluctuate significantly in the short term.

In the present study, SOC, DOC, EOC, and MBC content were significantly enhanced under S-1 and S-2 treatments(Table 4-1), indicating that straw return played an important role in rising SOC and labile organic carbon fractions(Gong et al. 2009). It also found that SMBC and DOC concentrations in the two soil layers of straw return treatments were significantly higher than those of NS treatment (*P*<0.05). There were significant and positive correlations among the SOC, DOC, and EOC in 0-40 cm soil depths, but there were no significant correlations between the labile organic C fractions.

***1.3 Impact on soil enzyme activity after different straw return practices***

Soil enzymes are one of the important active components of soil. They participate in various biochemical processes in soil, such as the decomposition and synthesis of humus, decomposition of animal and plant residues and microbial residues, synthesis, hydrolysis and transformation of organic matter, and soil nutrient cycling (Allison and Vitousek 2004; Zheng et al. 2015). Both urease and sucrase showed a downward trend with soil depth, and increased obviously after straw return (Figure 2-5), except sucrase which had no significant difference between straw return and no straw return. The enzyme activities on topsoil were more effective than those of subsoil, because, with the deepening of soil horizon, the content of organic matter and inorganic matter decreased obviously, so the enzyme activity decreased gradually.

On the other hand, repeated straw returns had significant effects on soil enzyme activities, which was consistent with other findings (Mandal et al. 2007; H. Zhao et al. 2019). It significantly increased the activity of soil urease and surcease in the 0~40 cm soil layers by application of maize straw every year and every two years (Figure 4-2). It indicated the low quantity and quality of maize straw could not support higher microbial substrate use efficiency. A possible explanation was that the S-3 treatment receiving no straw contained the least amount of fresh organic biomass and, therefore, the lowest amount of readily available organic carbon which was not a valid measurement for increasing soil activity.

***1.4 Impact of Fluorescence spectral characteristics on different components of soil DOC***

DOC was an active component in the soil nutrient pool, accounting for only 0.04%~0.22% of the total soil organic carbon (Li et al., 2016). With the improvement of testing technology and data analysis technology, the understanding of the characteristics and concentration of fluorophores had been broadened. PARAFAC could analyze EEMs into a single fluorophore and estimate the concentration of each component, it provided a great help of fluorescent chromophores in dissolved organic matter (Jaffé et al., 2014). The DOC content in soil was increased no matter which kind of straw return practices, while continuous straw return treatment (S-1) had a higher proportion of DOC/SOC (Figure 3-1 and 5-1).

Two fluorescence components were obtained by PARAFAC in straw returning to different soil depths, which were fulvic-acid-like and tryptophan-like substances after straw returned to 0-30 cm soil layers and fulvic-acid-like and tyrosine-like substances after straw returned to 30-40 cm soil layers (Figure 3-2). The deeper soil layers environment changed the fluorescence components and the fluorescence intensity of fulvic-acid-like substances was higher than that one. Moreover, two fluorescence components were also obtained after the straw return of different years, which were fulvic-acid-like and tryptophan-like substances, but the fluorescence intensity of tryptophan-like substances was dominant (Table 5-4). A higher humification coefficient increased in deep soil return which got lots of macromolecular substances difficult to decompose. Meanwhile, Straw return every year reduced the amount of fulvic acid and promoted the transformation of fulvic acid, and straw return every two years was easy to promote the accumulation of macromolecular substances, which helped to form a more stable humus(Table 3-1, 5-5).

It was conducted to evaluate the feasible application of straw return and farming practices on soil organic carbon sequestration. Briefly, reinforced by statistical analysis could provide some valuable and distinct optical information on DOC derived from straw and offer technical guidance when straw return to black soil.

***1.5 straw utilization***

In modern agricultural production, straw return mainly includes composting, decomposition, burning, covering, and mechanical rotary tillage. Straw burning would destroy the surface soil structure, make a large amount of surface water evaporate, and destroy the drought resistance and moisture conservation capacity of the soil (Montgomery, et al., 2007; Srinivasan et al; 2012). Composting decomposition and return to the field would produce a large number of greenhouse temperatures, which is not driving the development of agriculture in an environment-friendly direction (Cui et al., 2006). Straw mulching and mechanical rotary tillage are two widely used methods of straw direct return, and a large number of research results have been reported (Zhao J. et al., 1996; Bu et al., 2006; Sharma et al., 2011).

Straw mulching could significantly increase the content of 1-5 mm aggregates and soil bulk density decreased by 1.86%~3.73% (Liang et al., 2021), which further promoted the infiltration of precipitation and improved the water storage capacity (Sonnleitner et al., 2003; Liang et al., 2021). At the same time, straw mulch was conducive to reflect sunlight and hindering heat conduction on the soil surface, inhibiting soil evaporation, to increase the ability of soil to retain precipitation (Zhang et al., 2020). However, the study in the black soil area of Northeast China found that straw mulch increased the soil bulk density of 0-5 cm and 10-15 cm, and reduced the soil saturated water content and stable infiltration rate, and reduced the soil storage capacity of atmospheric precipitation.

Another way of straw return was mechanical rotary tillage and plow, the depth, amount, and length of straw return were the main factors affecting the effect of straw return (Angers et al., 1995, Liang et al., 2021). The depth of straw mechanical rotary tillage returning to the field was generally 0-15 cm. Studies have proved that the mixed application of straw into the soil has a better effect on the improvement of soil structure. Spaccini (2001) found that compared with straw mulching, mixed application of corn straw into the soil effectively improved the stability of soil aggregates. At the same time, the mixing of straw and soil could accelerate the decomposition rate of straw, increase the number of soil microorganisms and improve the distribution of microorganisms in the soil (Nicolardota et al., 2007). However, mixing straw and topsoil in the albic soil reduced the topsoil bulk density, and the topsoil was too loose to increase the evaporation of topsoil soil water (Liang et al., 2021). Although the oxidation conditions of the soil surface were good and the rate of straw mineralization and humification was high, the increasing or decreasing of soil organic matter depended on the ecological conditions of the soil layer, especially the low temperature in Northeast China. Straw was difficult to rot, which seriously affected the sowing and emergence in spring (Kuang et al, 2014). The improvement of soil fertility by straw return to the field in topsoil was limited, it was also one of the reasons for the low efficiency of straw return. In addition, the soil space required for crop growth was very large, especially the root system of corn crop could extent one meter to ensure the absorption space of water and nutrients. Therefore, the improvement of soil fertility in the deep layer (bottom layer of plow or below) would be a important way to improve soil production capacity and an effective disposal that could decrease air pollution and provide SOC in this region( Kuang et al., 2014;J. Chen et al., 2017; X. Wang et al., 2015; Yang et al., 2016).

Wu et al. (2008) showed that when the tillage depth of wheat straw was greater than 20 cm, the decay degree of wheat straw was more than 90%, and the yield was significantly increased. At present, the depth of straw application did not exceed 25 cm below the ground surface, and the whole straw or crushed straw was generally laid at a certain depth in the soil (kuang et al., 2014). Han et al. (2009) found mixed crushing straw application to 20-35 cm can reduce the soil bulk density, increase the total soil porosity and saturated hydraulic conductivity, and deepen the thickness of the black soil plow layer. Deep trenching (40 cm) can improve the utilization efficiency of soil water and the retention of organic carbon in soil (Wang S, 2015). Zhang et al. (2011) found that the soil carbon mineralization rate and cumulative mineralization increased with the increase of straw return in each soil layer of 0~60 cm. As mentioned above, deep straw return had a significant effect on improving soil organic carbon content and mineralization.

China is a multi-dimensional country with complex and diverse temperature differences and planting structures, and there are also a variety of straw return and utilization methods in different regions. Because of the specificity of terrain and climate conditions of Heilongjiang Province, the most suitable way of straw return to the field was full crushing and deep returning of straw, matched with a series of machinery and equipment. In recent years, the state has issued many policies to support the diversified utilization of straw, more and more straws could be effectively used. Continuous straw return (S-1 treatment) could increase the content of SOC and soil nutrients, but through calculating the economic benefits from input of straw and organic carbon, S-2 treatment had the highest comprehensive benefit (Table 4-3). It could not only make full use of a straw to improve soil nutrient content, but also make full use of a straw to improve the diversified utilization of straw.

## 2. General conclusions

1. In a 3 years trial, the maize straw residue returned to deep soil could decompose more quickly (straw residue 10.4%~14.0%) than the maize straw left on top of the soil (straw residue 68.7%) (*P*<0.01). To incorporate the straw, especially for the straw lignin, decomposing rate and SMBC content, the soil depth of 15-30 cm was the best depth to return which showed that the deep soil environment stored more stable carbon in the soil and increased the accumulation of organic matter.

2. The DOC content and its fluorescent components were significantly affected by different straw return depths. From the analysis of the spectral index of DOC in soil (FI was 1.49-1.52, BIX was 0.68-0.97, HIX was 0.72-0.83), results showed that its source was affected by the joint action of endogenous and exogenous sources. The autogenous components of straw returning to 31-40cm are smaller, and the humification coefficient is the highest. Two fluorescence components were obtained by PARAFAC which were fulvic-acid-like and tryptophan-like substances after straw returned to 0-30 cm soil layers and fulvic-acid-like and tyrosine-like substances after straw returned to 30-40 cm soil layers. Deep straw return provided more stored carbon and it played an important role in carbon sequestration and emission reduction in farmland production.

3. Our results demonstrated that SOC and the labile fractions increased significantly with the year of increasing after straw return, which input more residue-derived C, a similar trend of enzyme activities, too. It indicated that straw return every three years had similar effects with no straw return. Significant and positive correlations were shown among the SOC, DOC, EOC, sucrase, and urease at 0-40 cm soil depth, but there were no significant correlations between the labile organic C fractions.

4. The DOC content and its fluorescent components were significantly affected by different straw return practices. The continuous straw return treatment (S-1) had a higher proportion of DOC/SOC. From the analysis of the spectral index of DOC in soil (FI was 1.59-1.69, BIX was 0.90-0.95, HIX was 0.64-0.74), it came from the mixture characteristics of plants and microorganisms, with a strong contribution of authigenic source, high bioavailability and weak degree of humification after a straw return. Two fluorescence components were obtained which were fulvic-acid-like (26.8-35.7%) and tryptophan-like substances (64.3%-73.2%). Straw return every year reduced the amount of fulvic acid and promoted the transformation of fulvic acid, and straw return every two years was easy to promote the accumulation of macromolecular substances, which helped to form more stable humus. Correlation analysis showed that the interaction between straw incorporation years and soil depth had a positive effect on component C1, and the interaction of soil depth, incorporation years, and soil depth had a positive effect on component C2.

5. At present, straw utilization was an important problem to be solved urgently in China. From the perspective of soil fertility, it was suggested to implement the method of continuous straw returning to the field. From the analysis of the comprehensive utilization of straw, the effect of returning to the field every two years has a higher soil carbon sequestration. The supporting mechanized facilities for straw returning are not perfect, straw returning takes time and labor, and the effects were complicated. In the future, we should publicize the advantages of straw returning to the field and enhance farmers' awareness of straw returning to the field. China's land straddles many dimensions, and the straw returning time, crushing degree, straw returning amount, combined application of chemical fertilizer, and suitable farming methods for straw returning are different. Appropriate straw-returning technologies should be selected according to the regional conditions, a regional straw-returning technology system should be established, and straw returning should be carried out reasonably.

## 3. Innovation

This thesis revealed the underlying mechanism of SOC sequestration under different straw return methods in Northeast China. Moreover, the effects of different straw returns on soil nutrients, soil-labile organic carbon, enzyme activities, and their relationship were investigated. Finally, through the method of parallel factor analysis (PARAFAC) analyzed the relatively independent fluorescence components, to evaluate the characterize the composition and structure of soil DOC.

## 4. Perspectives

Based on the results of this thesis, the following recommendations are proposed to better understand the mechanism of SOC sequestration and the better method to straw return in these areas:

(1) As for organic components, in addition to DOC structure, there is also the structural analysis of FA and HA. This part should be improved in the following-up to make the research complete.

(2) We only got part of the data from the experiment of labeling straws with 13C, further experiments should be carried out to quantify the carbon components decomposed from straws and get more clear about the soil organic carbon sequestration.

(3) Through the revision stage of the manuscript, I found many shortcomings in the study and the work, providing lots of ideas and methods for future research and high efficiency in work. That is vitally important for my further research.

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Appendix – Publications

1. **Kuang E.J**., Xu M.G., Gilles Colinet, Chi F., Su Q. R., Zhou B. K. Zhang J. M., 2020. Degradation Characteristics of Maize Straw under Different Buried Depths in Northeast Black Soil and Their Effects on Soil Carbon and Nitrogen. International journal of agriculture & biology, Intl J Agric Biol, 24:77-84.

2. **Kuang E.J.**, Chi F., Zhang J. M., Xu M.G., Gilles Colinet, Su Q. R., Hao X. Y. Zhou B. K., 2022). Analysis of DOC component structure of black soil profile with straw deeply buried and based on fluorescence spectrum. *Spectroscopy and Spectral Analysis,* 42(10): 3243-3248.

3. **Kuang E. J**., Chi, F. Q., Zhu, B. G., Zhang J.M., Xu, M. G., Colinet, G., Zhu P., Hao X. Y., Sun L., 2022. Effects of maize straw return on soil dissolved organic carbon characteristics in crop fields. (**Pending approval,** Journal of Sensors)

4. **Kuang E.J**., Chi F.Q., Zhang J. M., Zhu B. G., Su Q. R., 2021. Effects of soil tillage and straw return on soybean roots and soil nutrients, Heilongjiang Agricultural Sciences, (8): 27-33. (In Chinese)

5. **Kuang E.J.,** Li Z. X., Chi F.Q., Zhang J. M., Su Q. R., Zhu B. G., 2020. Effect of different plough and organic fertilizer on characteristics of soybean yield and soil nutrients, Soybean Science, 39(1):108-115. (In Chinese)

6. **Kuang E.J**., Chi F.Q., Zhang J. M., Su Q. R. Gao Z. C., Zhu B. G., 2019. Effects of different tillage methods with organic materials application on main soil properties, Soils and Crops, 8(4): 395-404. (In Chinese)

7. **Kuang E.J.**, Chi F.Q., Zhang J. M., Su Q. R. Zhou B. K., Gao Z. C., Zhu B. G., 2018 . Assessment on soil fusion effects of segmented and removed frozen black soil under long-term located experiment, Soils, 50(1): 148-154. (In Chinese)

8. **Kuang E.J.,** Chi F.Q., Zhang J. M., Su Q. R., 2018. The effect on crop selenium content and yield by foliar application of selenium, Chinese Soils and Fertilizers, (4): 133-136. (In Chinese)

9. **Kuang E.J**., Chi F., Zhang J. M., Su Q. R., Zhang Y W, Liu Y D, Li Y S, Zhu B G, Chen L., 2022. Effects of selenium concentrations and combined application of selenium and zinc on selenium content and quality of rice. (in Chinese)

**Rewards:**

1. Chi F Q, **Kuang E J,** Zhang J M, et al. (2017). Research and demonstration of key technologies of crop selenium enrichment in Heilongjiang Province, **the 2nd prize** of scientific and technological progress award of the provincial government. (In Chinese)

2. Hao X Y, Ma X Z, Zhou B K, Kuang E J, et al. (2019). Research and demonstration of carbon sequestration and emission reduction technology in farmland soil in black soil area, t**he 3rd prize** of scientific and technological progress award of the provincial government. (In Chinese)

**Local standards:**

1. **Kuang E J**, Chi F Q, Zhang J M, et al. (2017). Supporting technologies and procedures for different returning amounts of maize straw, DB23/T 2010-2017, Local standards of Heilongjiang Province. (In Chinese)

2. Chi F Q, **Kuang E J**, Zhang J M, et al. (2017). Technical specification for the production of selenium-enriched rice, DB23/T 1998-2017, Local standards of Heilongjiang Province. (In Chinese)

3. Chi F Q, **Kuang E J**, Zhang J M, et al. (2019). Technical specification for concentrated return of maize straw to the field, fallow, and fertilization, DB23/T 2521-2019, Local standards of Heilongjiang Province. (In Chinese)

**Chief Editors:**

Chi F. Q., Zhang J.M., **Kuang E.J.** & Wan S. M. (2021) Editor in chief “Supply capacity and application effect of sulfur and selenium in soil in Heilongjiang Province”

**Utility model patent:**

1. Kuang Enjun, a straw fertilizer crushing device, utility model, patent number ZL 2020 2 0504888.3, application date 2020.4.9, authorization 2020.11.10

2. Kuang Enjun, a straw fertilizer mixing device, utility model, patent No. ZL 2020 2 0538619.9, application date: April 14, 2020, authorization: November 13, 2020

**Software copyright:**

1. Enjun Kuang, Dynamic monitoring system of the straw fermentation process, Registration number,2020SR0536503 Continuous monitoring system of soil temperature change, Registration number, 2020SR0536510

2. Enjun Kuang, Intelligent analysis system of soil nutrients, Registration number, 2020SR0537705

3. Enjun Kuang, Dynamic monitoring system of straw decomposition in soil, Registration number, 2020SR0538307