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Straw burial depth and manure application affect the straw-C and N sequestration: Evidence from ^{13}C & ^{15}N-tracing

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ABSTRACT

Straw return to farmlands has great potential to increase C sequestration and decrease its losses. Combined influences of straw burial depth and manure application are important factors which determines the decomposition or retention of straw C and N in the soil profile. The influence of straw amendment on soil-derived C and N is not well- known in the presence of manure application with at different straw burial depths. In a 360-day field study an in situ ^{13}C & ^{15}N-tracing technique was used to elucidate the effects of straw burial depth (15 and 35 cm), manure (presence or absence) and their interaction on C and N sequestration. Soil organic carbon (SOC) and total nitrogen (TN) contents were significantly ($P < 0.05$) higher in treatments including manure plus straw (SMS) compared to the soil (S), soil plus straw (SS) and soil plus manure (SM) treatments at both soil burial depths. After 360 days, straw N sequestration was unaffected by burial depth, but significantly more straw C was sequestrated at the 35 cm soil depth compared with the 15 cm soil depth: 39.9 % vs. 35.8 %, respectively. The straw-derived C was 37.9 % for the SS treatments and it was lower, 2.2–10.0 % and 5.7–13.7 % for the SMS treatments at depths of 15 and 35 cm, respectively. The mean residence time of the C and N was longer, 16 and 27 days, respectively at the 15 cm soil depth and 31 and 37 days, respectively at the 35 cm depth in the SMS treatments, compared to the SS treatments. Hence, the SMS treatments improved stability of carbon and nitrogen in deep soil layers. We concluded that returning straw at subsurface soil increased straw C sequestration in soils; combining the application of manure with returning straw to deep soil layers improves the stability of organic C and N in soil without compromising the potential for carbon and nitrogen sequestration.

1. Introduction

Soil carbon sequestration at farmland has the greatest potential for increasing carbon storage in the global ecosystem and mitigating carbon emissions (Lal, 2004; Wiesmeier et al., 2015, 2014). However, the soil organic carbon (SOC) pool, particularly agricultural SOC, has decreased dramatically in some regions (Huang, 2005). This has been especially serious in northeast China, one of the world's third most recognized black soil terrains. Unsustainable agricultural management practices have caused a loss of 31.1 % of SOC to the atmosphere over the past few decades, causing serious soil degradation and nutrients depletion (Zhang et al., 2019). Increasing carbon sequestration in agricultural soils has significant implications for alleviating soil degradation, and has induced much interest from scientists and farmers worldwide (Kirkby et al., 2011; Yu et al., 2006; Blanco and Lal, 2004; Wang et al., 2018).

In the agroecosystems, plant residues incorporation in soils acts as the primary source of SOC. About 34.4 Tg yr^{-1} of straw is produced worldwide, which when used properly could sequester a considerable amount of carbon (200 Tg C yr^{-1}) (Wang et al., 2012; Sun et al., 2013). Previous work has reported that straw return is a promising method of increasing the carbon sequestration potential of cultivated soils (Cai et al., 2018; Pei et al., 2015; Liu et al., 2014). However, continuous straw

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return would not result in increased SOC content in certain regions (Wang et al., 2018, 2013). The sequestration of straw-C in soils is affected by many factors, such as straw buried depth, nutrition amendment, and manure application (Sun et al., 2013; Wang et al., 2013, 2015; Zhang et al., 2015; Zou et al., 2017). Traditionally, soil scientists have focused on the topsoil when examining soil C and N turnover following straw amendment (Pei et al., 2015; An et al., 2015a, 2015b; Xie et al., 2016). Although the highest C and N contents are found in the topsoil as compared to greater depths, still a high proportion of SOC is present in deep soil horizons, sequestering some exogenous carbon in deeper soils (Sanaullah et al., 2011). Several studies have reported that straw C and N sequestration in surface soils were associated with the use of appropriate fertilization practices, straw burial depths and soil conditions (Pei et al., 2015; An et al., 2015a, 2015b; Xie et al., 2016). These factors have the potential to affect soil C and N sequestration, but there is a lack of information on the distribution of C and N in soils treated with combined application of straw and manure at various soil depths.

It is generally accepted that the residence time of deep soil carbon is long (Sanaullah et al., 2011). Some studies reported more straw degradation in the subsoil compared to the topsoil (Sanaullah et al., 2011; Gill and Burke, 2002). In addition, numerous studies have shown that application of manure can improve SOC stability (Rudrapa et al., 2006; Gong et al., 2009). Liang et al. (2011) reported that the mean residence time of SOC in the manure treatment was longer compared to the no fertilization treatment. Huang et al. (2010) also found that the combined application of manure and NPK fertilizers enhanced C storage in the micro-aggregates by 39.8 % compared to the control. To date, although some research has been carried out on carbon stabilization in topsoil under straw return or manure application (Pei et al., 2015; An et al., 2015a, 2015b; Zhao et al., 2018). However, the impacts of straw burial at different depths, combined with manure application on straw-C and N stabilization in soils are still unclear.

Phaeozem and Luvisol mainly located in Northeast China, and are the country's most important agricultural region, which is suffering from serious SOC depletion following long-term conventional cultivation (Pei et al., 2015). Previous research in this region has focused on the contribution of straw to SOC sequestration in a long-term fertilizer experiment (Wang et al., 2018, 2013), but these studies focused solely on topsoil. In recent years, a method for returning straw to deep soil layers has been widely used in this area. A stable isotope labeling technique has been used to accurately trace the distribution and fixation of added straw-C in soils (Bird et al., 2008).

The aims of this study were to determine, the effects of manure amendment and burial of maize straw over a 360-day period in different soil layers on: (1) SOC and total N contents; (2) the relative contributions of straw-derived C and N as well as soil-derived C and N; (3) the stability of organic C and N in soils. We hypothesized that the combination of manure application and returning straw to deep soil layers would increase the distribution of straw C and N in soil and the stabilization of organic C and N. This study allows us to identify the best straw return approach for soil C and N sequestration.

2. Materials and methods

2.1. Site description

The experiments were initiated on May 8, 2016 and simultaneously carried out at two different sites in the northeastern region of China. The first site was in Keshan county (48°02′N, 125°47′), Heilongjiang province, in the north of the region. The second site was in Gongzhuling city (43°30′N, 124°48′E), Jilin province, in the southern part of the region. Phaeozem of the studied sites, which originated from quaternary loess-like sediments, is typical of the region. Soil textures are clay loam and loamy clay at Keshan and Gongzhuling, respectively. The clay contents are 33.4 % (Keshan), and 31.1 % (Gongzhuling). The climate is temperate sub-humid with an average annual temperature of 2.4 °C (Keshan site) and 4.5 °C (Gongzhuling site), and annual precipitation of 500 mm (Keshan) and 730 mm (Gongzhuling). Over 60 % of the annual precipitation occurs between July and September. The annual frost-free period is 125 days at Keshan and 140 days at Gongzhuling. Soil properties at different depths and climatic conditions at the two sites are given in Table 1. Total organic carbon (TOC) contents in the surface soil (0−15 cm) were 12.9 g kg^{-1} at Gongzhuling, and 20.2 g kg^{-1} at Keshan. TOC for the 15−35 cm soil layer were 11.7 g kg^{-1} at Gongzhuling and 15.4 g kg^{-1} at Keshan.

2.2. Experimental materials

Soil samples were collected before planting from both sites in three replicates from both depths for analysis of physico-chemical properties. After air-drying, a portion of the soil samples was passed through a 0.15 mm sieve for chemical analyses, including the total organic carbon content and $\delta^{13}C$ signature. Another portion was passed through a 2 mm sieve and was prepared for the in-situ experiment.

Maize straw used in this study was obtained from a ^{13}C & ^{15}N pulse-labeling experiment. Maize was grown in a chamber with an atmosphere including $^{13}C-CO_2$ (98 % ^{15}C atom excess) and a nutrient solution containing $(^{15}NH_4)_2SO_4$ (90 % ^{15}N atom excess) was supplied three times throughout the growth season (An et al., 2015a, 2015b). The mature maize plants were harvested in October 2015. The labeled straw was cut after 70℃ for 12 h and passed through a 0.5 mm sieve. The ^{13}C-enriched and ^{15}N-labeled straw contained 436.9 g kg^{-1} total carbon, 14.9 g kg^{-1} total nitrogen, 435.9‰ $\delta^{13}C$ signature, and 138.412‰ $\delta^{15}N$ signature. The composted cattle manure used in the study was obtained from local farmer, and had 154.9 g C kg^{-1} and 13.2 g N kg^{-1}.

A nylon mesh litter bag containing ^{13}C and ^{15}N labelled straw was used to monitor the transformation of straw-C and N at each site. The bags were 20 cm long and 15 cm wide (mesh size: 0.074 mm) and only water, gas, and microorganisms could freely penetrate through these bags but not the maize roots.

The experimental setup is shown in Fig. S1. The straw-amendment treatments with and without manure for both soils were prepared by mixing 3 g of straw with 100 g air-dried soil collected from surface (0−15 cm) and subsurface (15−35 cm) soil. For the manure treatment, dry cattle manure was added to the soil samples. The non-amended treatment was prepared with 100 g soil (or 100 g soil plus 2 g manure) as the control (no straw added). Each sample mixture was put into a nylon mesh bag. Four treatments were therefore included in this study: soil (S), soil plus straw (SS), soil plus manure (SM), soil and manure plus straw (SMS).

In May 2016, these bags were separately buried in eight vertical pits at 15 and 35 cm depth (35 cm wide ×200 cm long), and each unit corresponds to a wall (35 cm in thickness). Each treatment was replicated 9 times, and bags were taken out at 70, 160 and 360 days after burial, leading to a total of 144 bags (8 treatments, 9 replications and two experimental sites). Soil temperature sensors (Fig S2) (MicroLite USB, Zealquest Scientific Technology Co., Ltd) were placed at each depth.

2.3. Analytical methods

Samples were analyzed to determine key physical and chemical properties. Organic C, total nitrogen content, $\delta^{13}C$ and $\delta^{15}N$ values in the samples were determined by an elemental analyzer (Elementar vario PYRO cube, Germany) coupled to a PDZ Europa isotope ratio mass spectrometer (IsoPrime 100 Isotope Ratio Mass Spectrometer, UK). ^{13}C and ^{15}N abundance (‰) in this study were determined relative to a standard calibrated against Pee Dee Belemnite (Werner and Brand, 2001). Soil pH was determined with a PHS-3C meter (REX Instrument Factory, Shanghai, China) by mixing 10 of soil with 35 ml of distilled water. Soil clay content was obtained by the pipette method (Gee and

Table 1
Key properties of tested soils at the start of the experiment (mean ± SD, n = 3).

sites	Soil depth (cm)	TOC (g kg^{-1})	TN (g kg^{-1})	C/N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	pH (H_2O)	Clay (%)
GZL	15	12.9 ± 0.3	1.27 ± 0.08	10.2	−15.1 ± 0.9	31.7 ± 3.4	5.4 ± 0.1	31.4%
	35	11.7 ± 0.2	1.01 ± 0.10	11.6	−17.7 ± 0.8	32.0 ± 2.6	5.9 ± 0.0	32.2%
KSH	15	20.2 ± 0.2	1.75 ± 0.05	11.5	−22.9 ± 0.2	25.2 ± 2.8	6.0 ± 0.0	33.3%
	35	15.4 ± 0.3	1.38 ± 0.04	11.2	−23.4 ± 0.2	27.8 ± 4.3	6.2 ± 0.1	34.1%

GZL, Gongzhuling site; KSH, Keshan site; C/N ratio, ratio of SOC to TN; TOC, total organic carbon, TN, total nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

Bauder, 1986).

2.4. Isotopic C, N analysis and calculations

The proportions of straw-derived C, N (FMc, FM_N, %) and soil-derived C, N (FSc, FS_N) were calculated using the following equation (Conrad et al., 2012):

$$FMc = (\delta^{13}C_{sample} - \delta^{13}C_{control}) / (\delta^{13}C_{straw} - \delta^{13}C_{control}) \times 100 \quad (1)$$

$$FM_N = (\delta^{15}N_{sample} - \delta^{15}N_{control}) / (\delta^{15}N_{straw} - \delta^{15}N_{control}) \times 100 \quad (2)$$

$$FSc = 1\text{-}FMc;\ FS_N = 1\text{-}\ FM_N \quad (3)$$

where $\delta^{13}C_{sample}$ or $\delta^{15}N_{sample}$ represents the $\delta^{13}C$ or $\delta^{15}N$ value of SOC or TN in the treatment with straw; $\delta^{13}C_{control}$ or $\delta^{15}N_{control}$ represents the $\delta^{13}C$ or $\delta^{15}N$ value of the initial soil in the corresponding soil treatment without straw; $\delta^{13}C_{straw}$ or $\delta^{15}N_{straw}$ represents the $\delta^{13}C$ or $\delta^{15}N$ value of the straw applied.

The proportion (%) of total (C or N), straw -derived (C or N) and soil -derived (C or N) remaining at each sampling date can be expressed as follows:

$$RSMc = C_{SM} / C_{SMo} \times 100 \quad (4)$$

$$RSM_N = N_{SM} / N_{SMo} \times 100 \quad (5)$$

$$RMc = FMc \times C_{SM} / C_{SMo} \times 100 \quad (6)$$

$$RM_N = FM_N \times N_{SM} / N_{SMo} \times 100 \quad (7)$$

$$RSc = FSc \times C_{SM} / C_{SMo} \times 100 \quad (8)$$

$$RS_N = FS_N \times N_{SM} / N_{SMo} \times 100 \quad (9)$$

where RSMc (%) is the amount of organic C (g) in the remaining straw in the treatment with straw on each sampling date; C_{SM0} is the initial total amount of organic C (g) in the treatment with straw prior to burial; C_{SM} is the total organic C amount (g) in the treatment with straw on a given sampling date; RSM_N is the remaining amount of total nitrogen in the treatment with straw; N_{SM0} is the initial total nitrogen amount (g) in the treatment with straw prior to burial; N_{SM} is the total nitrogen amount (g) in the treatment with straw on a given sampling date; RMc (%) and RM_N (%) are respectively, the remaining straw-derived C and N in the treatment with straw on a particular sampling date; RSc (%) and RS_N (%) are respectively, the remaining soil-derived C and N in the treatment with straw on a particular sampling date.

The mean residence time of C (MRTc, days) and N (MRT_N, days) in the soil was calculated as a reciprocal of the corresponding turnover rate using the following equations (Dorodnikov et al., 2011; Gregorich et al., 1995):

$$MRTc = -t / \ln (1\text{-}FMc / 100) \quad (10)$$

$$MRT_N = -t / \ln (1\text{-}FM_N / 100) \quad (11)$$

2.5. Statistical analyses

As described for the experimental layout, the means of three replicates and standard deviations are shown. Statistically significant differences at a significance level of $P < 0.05$ were determined via a two-way analysis of ANOVA and LSD test using SAS 8.0 for Windows. Student's *t*-test was applied to compare the soil C and N variables in the different straw amendment treatments using JMP 10 statistical software (SAS Institute Inc., 2012).

3. Results

3.1. Changes in TOC and TN content under straw and manure application

Straw and manure application enhanced TOC and TN sequestration in soil over the 360-day incubation period at both sites, especially for the SMS treatments (1.3- and 1.5-fold) at the Gongzhuling site (Fig. 1). Specifically, the TOC and TN were maximum in SMS treatment varying between 15.1–23.6 g kg^{-1} and 1.6–2.2 g kg^{-1} respectively, and the lowest in SM treatment ranged from 12.2 to 21.2 g kg^{-1} and 1.1 to 1.9 g kg^{-1} respectively. By the end of incubation, compared to the initial TOC, the average TOC content of the two sites increased by 2.2 g kg^{-1} (15.5 %) for the SS treatment, and by 0.9 g kg^{-1} (5.8 %) for the SM treatment, and by 3.4 g kg^{-1} (23.9 %) for the SMS treatment. At the end of the incubation period (360 days), the TOC and TN contents increased by 16.3 % and 20.8 % at surface layer, and by 19.4 % and 30.0 % at subsurface layer compared to soils without straw amendment. Compared to the initial TN, the average TN content increased by 0.3 g kg^{-1} (26.0 %) for the SS treatment, by 0.2 g kg^{-1} (13.9 %) for the SM treatment, and by 0.5 g kg^{-1} (39.9 %) for the SMS treatment, whereas TN for the CK treatments (soil alone) remained relatively constant.

Interaction effects among straw burial depths, straw and manure application were also investigated. Straw burial depth significantly affected the SOC and TN content in the treatment with straw at both sites ($P < 0.01$, Tables 2 and 3). There were significant interaction effects between soil depths and manure application on the TOC content for the treatment with straw. However, this effect was not apparent for the TOC and TN contents in the absence of straw and manure application: the CK treatments were not significantly different for the entire experiment ($P <$ 0.05). In contrast, the TOC and TN contents were greater in the SS treatments than in the SM treatments.

3.2. Contribution of straw-(C or N) and soil-(C or N) to C and N pools

After 360 days of incubation, 26.4–46.3 % of SOC originated from straw C (Fig. 2 and Table 2). The FMc values at subsurface layer were significantly higher than those at surface layer. The FMc values were 2.2–10.0 % and 5.7–13.7 % lower in the straw-amended treatments than in the straw and manure-amended treatments, at both depths respectively. The contribution of the native soil carbon to TOC (FSc) were opposite among the treatments with straw. Manure application significantly increased the FSc ($P < 0.05$) compared to straw. On average, the FSc values at surface layer were 16.8 % higher at Gongzhuling and 3.2 % higher at Keshan for the manure plus straw treatment compared to straw only. At subsurface layer, the FSc value at both sites was 31.1 % and 8.8 % higher in manure plus straw treatment when compared to straw only.

The contribution of straw-N to TN (FM_N) accounted for 9.3–18.0 % and 10.7–17.7 % of the total soil organic N at surface and subsurface

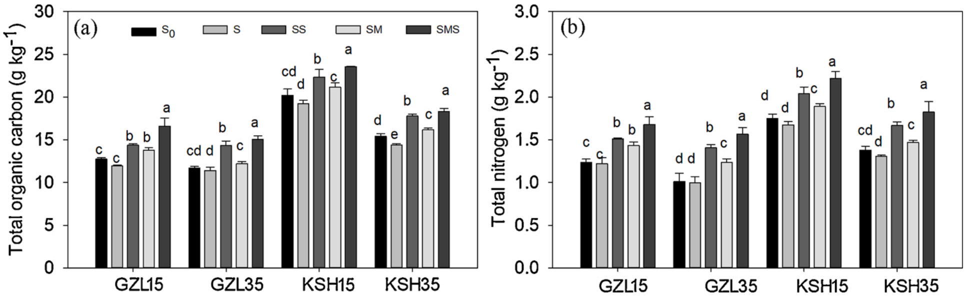

Fig. 1. Total organic carbon (TOC; a) and total nitrogen (TN, b) content in soil from litter bags buried at depths of 15 and 35 cm. GZL15, topsoil (15 cm) at Gongzhuling site; GZL35, subsoil (35 cm) at Gongzhuling site; KSH15, topsoil (15 cm) at Keshan site, and KSH35, subsoil (35 cm) at Keshan site. S_0: initial soil; S: soil only; SS: soil plus straw; SM: soil plus manure; SMS: soil plus manure and straw. Data are expressed as the mean ± standard deviation ($n = 3$). Different lowercase letters represent significant differences between straw addition treatments ($P < 0.05$).

Table 2
Analysis of variance table for FMc, FSc, RMc, RMSc, RSc with four main factors (days of incubation: 70, 160, 360 days; soil: topsoil and subsoil at Gongzhuling site, topsoil and subsoil at Keshan site; manure: with manure and straw, without manure; depth: 15 and 35 cm depths).

Source	TOC	FMc	FSc	RMc	RMSc	RSc
Day (A)	40.0 (0.0002***)	12.1 (0.0084**)	45.1 (0.0002***)	69.5(< 0.0001***)	94.0 (< 0.0001***)	7.1 (0.0285*)
Soil (B)	487.2 (< 0.0001***)	18.6 (0.0026*)	32.4 0.0005*)	6.3 (0.0363*)	7.4 (0.0260*)	8.1 (0.0216*)
Manure (C)	45.5 (<0.0001***)	5.2 (0.0518)	14.5 (0.0052**)	0.2 (0.6329)	0.2 (0.6388)	13.0 (0.0070**)
Depth (D)	403.4 (< 0.0001***)	25.5 (0.0010***)	53.3 (< 0.0001***)	3.4 (0.1018)	6.5 (0.0346*)	0.1 (0.7876)
A*B	0.7 (0.4397)	3.4 (0.1020)	0.0 (0.8411)	0.0 (0.9799)	0.0 (0.8740)	10.9 (0.0108*)
A*C	6.4 (0.0356*)	1.1 (0.3343)	1.3 (0.2847)	3.2 (0.1138)	2.3 (0.1690)	0.5 (0.5121)
A*D	0.4 (0.5582)	7.6 (0.0247*)	29.9 (0.0006***)	1.8 (0.2202)	3.4 (0.1021)	7.3 (0.0269*)
B*C	2.4 (0.1563)	2.3 (0.1692)	9.4 (0.0154*)	5.4 (0.0478*)	5.3 (0.0504)	2.5 (0.1525)
B*D	133.4 (< 0.0001***)	0.4 (0.5684)	0.7 (0.4382)	1.2 (0.3026)	2.0 (0.1977)	0.2 (0.6417)
C*D	9.3 (0.0158*)	1.3 (0.2804)	3.4 (0.1036)	3.7 (0.0917)	4.4 (0.0694)	1.2 (0.3103)
A*B*C	3.3 (0.1086)	0.2 (0.6923)	4.7 (0.0614)	2.1 (0.1843)	3.2 (0.1100)	2.5 (0.1533)
A*B*D	5.2 (0.0527)	0.3 (0.6091)	4.4 (0.0697)	2.7 (0.1411)	5.4 (0.0482*)	1.3 (0.2816)
A*C*D	1.4 (0.2746)	1.1 (0.3204)	1.4 (0.2636)	0.2 (0.6614)	0.3 (0.5782)	3.5 (0.0992)
B*C*D	2.4 (0.1563)	0.1 (0.7154)	1.2 (0.2103)	0.3 (0.6009)	0.1 (0.7614)	1.1 (0.3204)
A*B*C*D	2.3 (0.1657)	0.2 (0.6860)	0.5 (0.4908)	2.0 (0.1933)	2.2 (0.1723)	0.1 (0.9373)

Data expressed as the F (*P*) value in the ANOVA results. FMc, FSc, RSMc, RMc, RSc used 72 samples in total. FM, proportion of straw-derived C; FSc, proportion of soil-derived C; RSMc, proportion of total C remaining; RMc, proportion of straw C remaining in those treatments with straw (straw + manure); RSc, proportion of soil C remaining in those treatments with straw (straw + manure). *, **, *** indicate significant differences at $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively.

Table 3
Analysis of variance table for FM_N, FS_N, RM_N, RMS_N, RS_N with four main factors (days of incubation: 70, 160, 360 days; soil: topsoil and subsoil at Gongzhuling site, topsoil and subsoil at Keshan site; manure: with manure and straw, without manure; depth: 15 and 35 cm depths).

Source	TN	FM_N	FS_N	RM_N	RMS_N	RS_N
Day (A)	1.6 (0.2473)	13.1(0.0068**)	6.8 (0.0309*)	4.8290(0.0492*)	14.4(0.0053**)	1.6882(0.2300)
Soil (B)	22.3(0.0015**)	3.7 (0.0911)	6.6 (0.0329*)	14.3317(0.0053**)	0.3 (0.5905)	0.7284(0.4182)
Manure (C)	3.8 (0.0865)	1.8 (0.2181)	2.9 (0.1293)	2.6801(0.1402)	0.1 (0.7414)	5.1664(0.0496*)
Depth (D	14.9(0.0048**)	4.6 (0.0484*)	4.8 (0.0609)	2.5734(0.1473)	0.5 (0.5145)	13.6689(0.0061**)
A*B	0.3 (0.5812)	0.3 (0.6200)	0.5 (0.5188)	0.1557(0.7034)	2.2 (0.1732)	0.0772(0.7882)
A*C	0.0 (0.8607)	0.0 (0.8755)	0.0 (0.9561)	0.0334(0.8596)	0.0 (0.9322)	0.0373(0.8516)
A*D	0.2 (0.6406)	0.5 (0.4968)	0.2 (0.6471)	1.9009(0.2053)	4.3 (0.0716)	1.5991(0.2416)
B*C	0.0 (0.8348)	1.9 (0.1961)	1.9 (0.2091)	0.0504(0.8281)	0.9 (0.3618)	0.3584(0.5659)
B*D	3.0 (0.1194)	0.0 (0.8615)	4.3 (0.0706)	0.0572(0.8170)	0.1 (0.7301)	0.1988(0.6675)
C*D	0.0 (0.898)7	1.1 (0.3159)	0.1 (0.7702)	0.0244(0.8797)	0.1 (0.7190)	0.2345(0.6412)
A*B*C	0.1 (0.7638)	0.0 (0.9156)	0.1 (0.7900)	0.0388(0.8488)	0.5 (0.4865)	0.1032(0.7562)
A*B*D	0.0 (0.8453)	0.0 (0.8491)	1.7 (0.2235)	3.3342(0.1053)	0.2 (0.7080)	2.1027(0.1851)
A*C*D	0.0 (0.9983)	0.1 (0.7829)	0.4 (0.5576)	0.0055(0.9429)	0.5 (0.4848)	0.0014(0.9715)
B*C*D	0.0 (0.8348)	0.0 (0.9472)	0.1 (0.7916)	0.0007(0.9801)	0.0 (0.9529)	0.0041(0.9508)
A*B*C*D	0.1 (0.8258)	0.0 (0.9688)	0.1 (0.8120)	0.0115(0.9172)	0.1 (0.7991)	0.1860(0.6777)

Data expressed as the F (*P*) value in the ANOVA results. FM_N, FS_N, RSM_N, RM_N, RS_N used 72 samples in total. FM_N, proportion of straw-derived N; FS_N, proportion of soil-derived N; RSM_N, proportion of total N remaining; RM_N, proportion of straw N remaining in those treatments with straw (straw + manure); RS_N, proportion of soil N remaining in those treatments with straw (straw + manure). *, **, *** indicate significant differences at $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively.

layer respectively (Fig. 2). The FM_N value was 2.6–25.2 % higher in straw-amended treatments, compared to the SMS. The differences in FS_N values were not significant for both soil depths. With manure addition, a higher proportion of native soil-C contributed (FS_N) at surface and subsurface layer compared to the treatment with straw only. No significant difference was found among these treatments. At the end of the decomposition period (360 days), the FS_N values were 84.4 % (SS at the Gongzhuling site), 87.9 % (SMS at Gongzhuling), 85.4 % (SS at Keshan), and 88.9 % (SMS at Keshan site) at surface layer and 84.4 %, 88.4 %, 88.1 %, and 88.5 % respectively at subsurface layer.

3.3. Mean residence time of SOC and TN in straw and manure amendment treatments

After straw addition, soil carbon was significantly affected by fertility level, incubation time, manure addition and soil depth (Fig. 3a and b). Straw buried at surface layer resulted in a higher MRTc than that buried at subsurface layer. Manure application (SMS) also increased

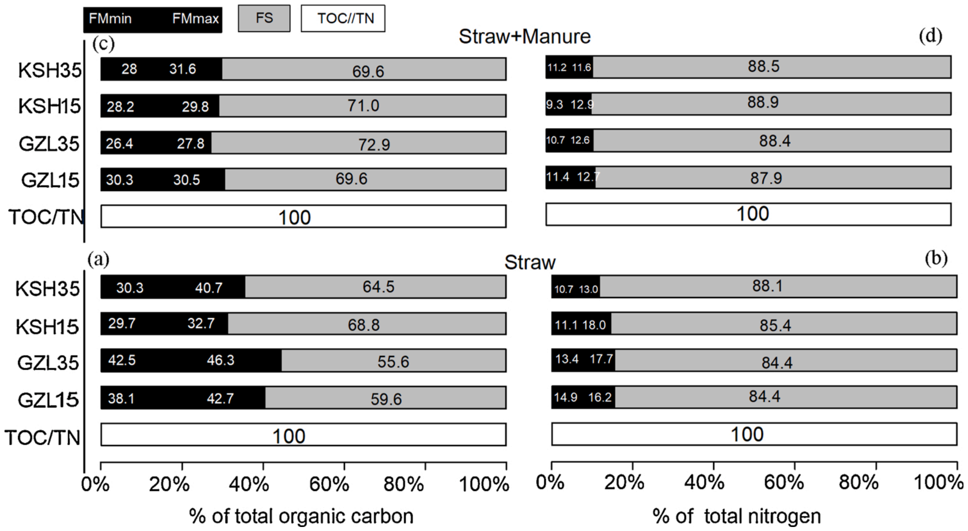

Fig. 2. Straw-derived carbon (a) and (c), straw-derived nitrogen (b) and (d) in soil from litter bags buried at depths of 15 and 35 cm over a period of 360 days of incubation. FMmin, the minimum proportion of straw-derived C (N); FMmax, the maximum proportion of straw-derived C (N); FS, the proportion of soil-derived C (N); TOC, total organic carbon; TN, total nitrogen; GZL15, topsoil (15 cm) at Gongzhuling site; GZL35, subsoil (35 cm) at Gongzhuling site; KSH15, topsoil (15 cm) at Keshan site, and KSH35, subsoil (35 cm) at Keshan site.

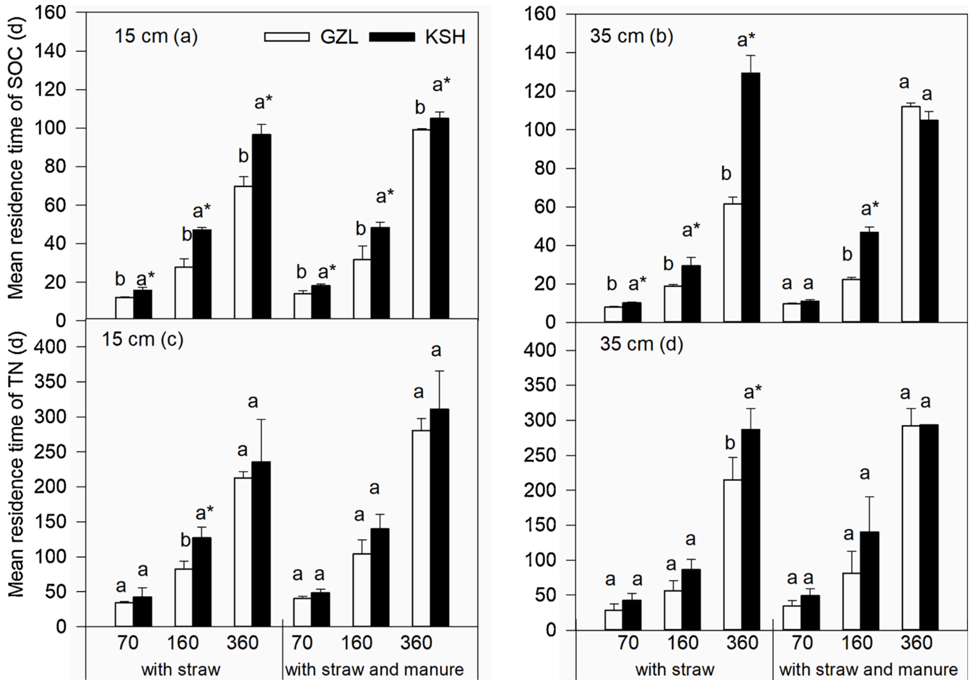

Fig. 3. Mean residence time of C (MRTc; a, b) and N (MRT_N, c, d) in soil from litter bags buried at 15 and 35 cm. GZL15, topsoil (15 cm) at Gongzhuling site; GZL35, subsoil (35 cm) at Gongzhuling site; KSH15, topsoil (15 cm) at Keshan site, and KSH35, subsoil (35 cm) at Keshan site. Data are expressed as the mean ± standard deviation (n = 3). Different lowercase letters represent significant differences between straw addition treatments ($P < 0.05$).

MRTc throughout the entire experiment (70, 160 and 360 days) compared with no manure treatment.

There was noticeable variation in soil nitrogen following straw addition (Fig. 4). The addition of manure (SMS) increased the mean residence time of soil nitrogen (MRT_N) by 22.0 % and 30.3 % for surface and subsurface layer, compared to the straw treatment only (SS). Compared with the low SOC soil (Gongzhuling), the MRT_N for the soil with high SOC (Keshan site) varied between 17.6–25.4 % and 18.3–38.6 % for surface and subsurface layer, respectively. The average value of MRT_N at surface layer was 3.1 % higher than that at subsurface layer after 360 days.

4. Discussion

4.1. *Effect of straw burial depth on straw C and N sequestration in soils*

In this study, FMc and FM_N values were positively correlated with straw burial depth ($R = 403.4$, $P < 0.0001$; $R = 17.9$, $P = 0.0048$), suggesting a role for straw layer burial in determining straw C and N distribution (Tables 2 and 3). The subsoil had higher average soil temperature and water content than the topsoil, especially in the cold season (Fig. S2). We therefore think that the better abiotic conditions for straw decomposition in the deep soil layer (Cai et al., 2015). Several studies have shown that the proportion of dissolved organic C with ^{13}C-enrichment increases with soil depth (Kaiser et al., 2001; Sanaullah et al., 2011; Cai et al., 2015; Luo et al., 2015). However, the differences that we observed did not result in significantly higher straw C and N

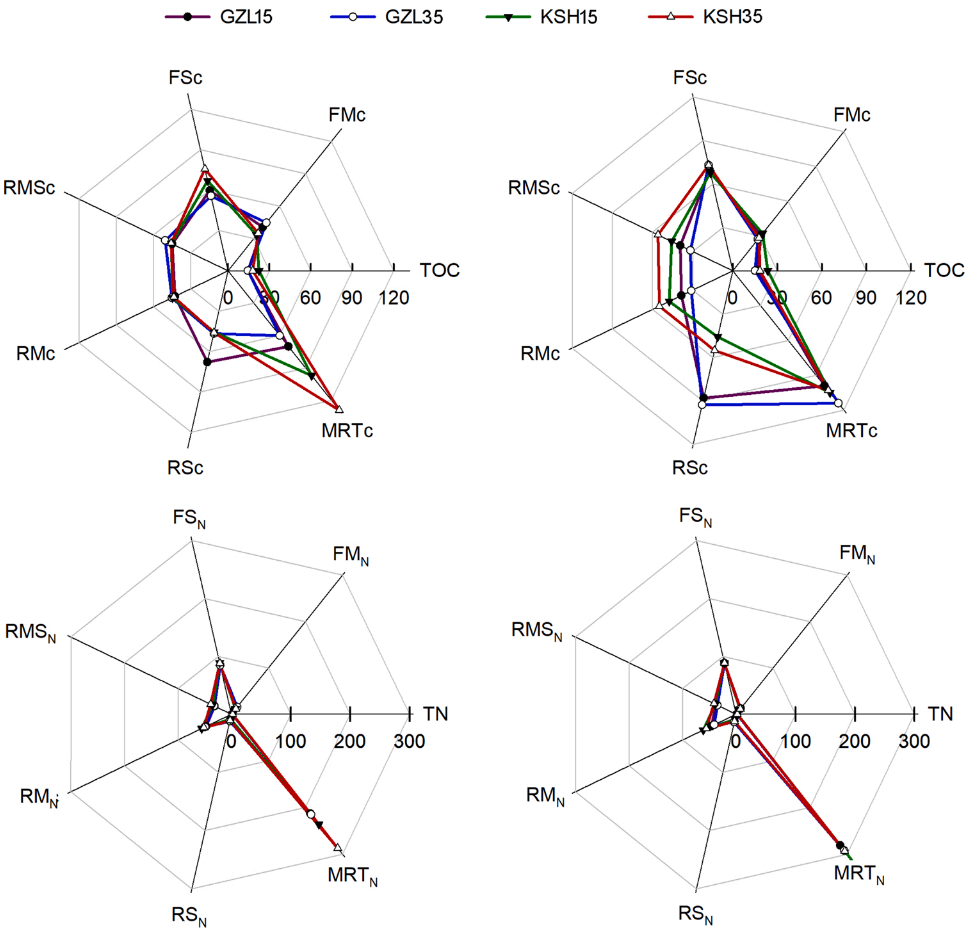

Fig. 4. Straw-derived C (FMc), soil-derived C (FSc), remaining rate of total organic carbon (RSMc), remaining rate of straw-derived C (RMc), remaining rate of soil-derived C (RSc), mean residence time of C (MRTc), total organic carbon (TOC); maize-derived N (FM_N), soil-derived N (FS_N), remaining rate of total organic carbon (RSM_N), remaining rate of straw-derived N (RM_N), remaining rate of soil -derived N (RS_N), mean residence time of N (MRT_N), total organic carbon (TN). Note that the unit of the nutrition content in Fig. 4 is %.

retention levels (Tables 4 and 5). Sanaullah et al. (2011) also found that similar amounts of root C and N remained at depths of 30, 60 and 90 cm during a 3-year field experiment.

The fact that this change in the buried straw layers is significant for C but absent for N in the SS and SMS treatments after 360 days of incubation (Fig. 2) suggests that straw N is already present in an available form before it is introduced into the subsoil and can be rapidly transferred to soil microbial biomass (Sanaullah et al., 2011). Maize residues are generally considered to be low-N plant materials (Table 1, Cai et al., 2015). Soil nutrient and microbial community composition in deep soil also decrease straw decomposition (Goberna et al., 2005; Brabcová et al., 2018; Fontaine et al., 2007), causing a decoupling of straw C and N decomposition coinciding with a substantial increase in gross nitrogen mineralization.

Table 4
Percentage of straw-derived SOC in total input straw C and the proportion of straw C remaining (%).

Depth (cm)	Treatment	Gongzhuling		Keshan	
		Amount of straw-derived SOC/ Total straw C	Straw C remaining after one year decomposition	Amount of straw-derived SOC/ Total straw C	Straw C remaining after one year decomposition
15	SS	43.4 ± 2.6b	42.7 ± 5.2ab	39.8 ± 1.4a	44.7 ± 6.1a
	SMS	37.7 ± 2.0c	38.4 ± 0.3ab	38.1 ± 1.1a	47.4 ± 9.1a
35	SS	48.6 ± 1.0a	45.2 ± 4.5a	43.2 ± 5.3a	43.6 ± 7.1a
	SMS	32.0 ± 1.5d	36.2 ± 1.0b	38.4 ± 2.6a	54.7 ± 1.6a

SS, soil with straw; SMS, soil with straw and manure. Different letters represent significantly different means ($P < 0.05$) among treatments for day 360.

Table 5
Percent of the straw-derived SON in total input straw N and the proportion of straw N remaining (%).

Depth (cm)	Treatment	Gongzhuling		Keshan	
		Amount of straw-derived N/Total straw N	Straw N remaining after one year decomposition	Amount of straw-derived N/Total straw N	Straw N remaining after one year decomposition
15	SS	4.8 ± 0.1a	47.2 ± 0.7a	4.2 ± 0.4a	51.3 ± 13.0a
	SMS	4.6 ± 6.5a	46.2 ± 5.4a	4.1 ± 0.6a	41.6 ± 10.1a
35	SS	5.5 ± 0.9a	49.3 ± 8.7a	4.8 ± 0.5a	54.9 ± 5.3a
	SMS	4.4 ± 0.3a	40.7 ± 2.8a	4.9 ± 0.1a	55.6 ± 1.6a

SS, soil with straw; SMS, soil with straw and manure. Different letters represent significantly different means ($P < 0.05$) among treatments for day 360.

4.2. The distribution of straw C and N in soils amended by manure application

Although all treatments receiving manure (+ straw) had higher SOC and TN content than those without, these differences did not result in significantly higher straw-derived C and N sequestration (Fig. 4, Tables 4 and 5). The increase in SOC and TN contents resulting from manure treatment in the present study could possibly be attributed to a larger proportion of recalcitrant organic compounds in manure compared to straw (Yan et al., 2007). This result was not fully supportive of our second hypothesis that the combination of manure application and straw to deep soil layers would improve the distribution of maize straw C and N in soil. Hence, the distribution of straw C and N in soils may be related not only to straw burial depth, but also to the chemical properties of manure (Kuzyakov et al., 2000). Compared to the straw treatments, manure application decreased straw-derived C and N, possibly due to a high level of N immobilization from manure, which had a low C/N ratio (> 10) (Kuzyakov et al., 2000). Another possible reason for the decrease in straw-derived C and N sequestration after manure application could be the low level of manure input. Other researchers have shown that manure promotes C and N turnover because of the available nutrients (Blagodatskaya et al., 2007; Blagodatskaya and Kuzyakov, 2008). According to Mando et al. (2005), manure amendment was more beneficial than straw for the formation of particulate organic matter (POM), which was a sensitive indicator of changes in SOC (Mando et al., 2005). It has been proven that the application of manure, in comparison with that of straw, contributes to greater carbon sequestration (Pei et al., 2015; An et al., 2015a, 2015b). Additionally, the effect of soil chemical properties (such as soil nutrient status, soil C-to-N ratio) could decrease straw decomposition (Cai et al., 2018; Mahmoodabadi and Heydarpour, 2014). Our results also showed that soil C and N sequestration is a complex response to straw and manure amendment, as well as burial depth (Fig. 2). The consequences of applying different kinds and amounts of manure therefore also warrant further study in order to estimate their role in sequestrating straw C and N in soils.

4.3. *Mean residence time of incorporated residue carbon and nitrogen*

The combination of returning straw to deep soil layers and manure application enhanced soil C and N stabilization (Fig. 3). This is in agreement with the results of many studies (Pei et al., 2015; An et al., 2015a, 2015b). Both MRTc and MRT_N increased continuously throughout the experiment (Fig. 3). The turnover time for C and N was shortest in the early part of the experimental period (up to 70 days) and turnover subsequently slowed down due to the incorporation of new straw-derived C into the soil within a relatively short time interval (Pei et al., 2015). A substantial decrease in the amount of straw resulted in 8- and 7-fold increases in MRTc and MRT_N respectively during the subsequent 360 days (Fig. 3). Manure amendments slightly increased the MRTc and MRT_N compared to the soils with straw only. This was mainly because the proportion of recalcitrant organic compounds in manure was larger than that in straw (Liang et al., 2011). Thus, the application of manure could improve soil carbon and nitrogen stabilization.

Our study showed that combined application of manure and returning straw to deep soil layers can improve the stabilization of organic C and N (Fig. 4). These findings are in agreement with our hypothesis. Soil tillage can affect the bioavailability of aggregate-associated SOC and nutrient mineralization (Six et al., 2002; Zhang et al., 2014). Furthermore, the estimated MRT of recalcitrant SOC can be affected by different tillage and straw treatments (Zhang et al., 2014). In the present study, the mean residence time of the C and N was longer by, respectively, 16 and 27 days at a soil depth of 15 cm and by 31 and 37 days at a depth of 35 cm for the SMS treatments compared with the SS treatments (Fig. 3). Similarly, it was observed that manure combined with straw can increase TOC and TN content (Fig. 1). These findings suggest that strategic use of straw combined with management practices such as “deep” tillage for straw incorporation can enhance straw-derived C sequestration.

5. Conclusions

This study demonstrated that during 1 year of field incubation with stable isotope labeling, the burial of the straw layer in deep horizons significantly increased the flow of fixed straw-C into the SOC. The inconsistent differences in straw carbon and nitrogen distribution between the soils with and without manure addition demonstrated that manure addition likely promoted the decomposition of straw carbon & nitrogen and suppressed the fixation of new carbon and nitrogen. Accordingly, the combined application of manure and a buried straw layer represent good field-management practices for soil carbon and nitrogen sequestration. Future studies are needed to ensure that the beneficial effects of manure application, straw return and optimal straw burial depths are maximized in order to increase carbon and nitrogen sequestration.

Significant statement

Black soil region in Northeast China is one of three black soil regions in world and this area is also one of the most important agricultural production regions in China. However, the land is intensively cultivated by shallow tillage for 1 or 2 decades which leads to the sharp decrease of soil organic carbon (SOC) and soil fertility. Manure and straw play important roles to maintain SOC, but impacted by tillage depth. In an attempt of solving the problems that long-term shallow tillage has brought about the lack of SOC in the subsurface soil, soil carbon and nitrogen sequestration were studied after application of straw combined with manure in different depth. The results will not only expand the theory of carbon and nitrogen turnover after adding exogenous materials, but also provide scientific basis for improving SOC with the straw returning techniques.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.still.2020.104884.

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