# Impacts of increasing maize stalk retention amount on soil respiration and temperature sensitivity

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**Abstract:** Conservation tillage with maize stalk retention is an effective method to replenish soil nutrients. Nutrient availability plays a major role in the control of soil respiration (SR). However, it is not known how different degrees of maize stalk retention control SR and its temperature sensitivity ($Q_{10}$). To investigate the effect of maize stalk retention amount on SR and $Q_{10}$, four maize (*Zea mays* L.) stalk retention treatments, including (i) control treatment (CT) without maize stalk retention, (ii) standing maize stalk retention (SCR), (iii) partial maize stalk retention with 'three-year cycle' (TYR) and (iv) chopped maize stalk retention (CCR) was set up. In order to investigate the differences in soil nutrient, soil organic carbon (SOC) quality and soil microbial biomass among four treatments, soil analysis with 6 replicates was conducted. The experimental results showed that SR rates were 1.07, 0.88, 0.59 and 0.37 g/kg of dry soil, and the average $Q_{10}$ was 1.535, 1.585, 1.62 and 1.725 for CT, SCR, TYR and CCR, respectively. Increasing maize stalk retention led to the reduction of soil microbial abundance and labile carbon compositions. Pearson correlation analysis showed that soil microbial abundance had a positive correlation with SR, while labile carbon fraction had a negative correlation with $Q_{10}$. In short, increasing the amount of maize stalk retention decreases SR while increasing $Q_{10}$ in northeast China. This research could provide a reference value for balancing carbon sequestration and carbon decomposition in farming practice.

**Keywords:** stalk management, soil nutrient, carbon composition, microbial biomass, laboratory incubation
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## 1 Introduction

Mollisols in Northeast China are suitable for maize (*Zea mays* L.) cultivation because of high fertility. There are about 6.86 million $hm^2$ of maize fields in northeast China[1]. In recent several decades, soil degradation has become a big concern, which may lower soil fertility and increase environmental pollution. In order to improve soil fertility and conserve environment, conservation agriculture has been promoted for several decades in China[2]. Returning maize stalks into fields is viewed as a key procedure of conservation agriculture[3]. Currently, prevalent management schemes include whole maize stalk retention, partial maize stalk retention, standing maize stalk retention, chopped maize stalk retention and mixed maize stalk retention[4]. Whole maize stalk retention (CCR) refers to using chopped maize stalks to cover soil surface year after year. In addition to replenishing soil nutrient, standing maize stalk retention (SCR) has a unique function of decreasing soil wind erosion[5]. SCR means leaving a certain height of bottom maize stalks standing in fields to improve stalk usage efficiency, of which the upper parts can be used for other purposes[6]. Another type of maize stalk retention named after 'three-year rotation (TYR)' has been applied for more than ten years in northeast China[7]. TYR means whole maize stalks should be returned to the field after harvesting for the first year, subsequently, whole maize stalks should be removed for the second year, at last, 1/3 of maize stalks need to be left standing in field for the third year.

Soil respiration (SR) is the second largest carbon flux between atmosphere and terrestrial ecosystem[8], which generates 75 to 100 Pg C efflux every year[9]. Soil microbe and soil organic carbon (SOC) are major factors affecting SR[10]. Soil microorganisms play an important role in decomposing SOC, and ~85% to 90% of SOC is decomposed by soil fungi and soil bacteria[11]. Temperature is another important control factor for SR. Exponential model of $Q_{10}$ is usually used for predicting SR based on temperature variation, which describes $CO_2$ releasing amplification for a 10℃ increase[12]. In some studies, $Q_{10}$ was considered ~2.0 and employed in the Century, TEM, Roth-C and PnET models[13]. However, some scholars believe that $Q_{10}$ is quite variable, and is affected by landscape, setting, climate, ecosystem types, and land use[14].

Maize stalk retention can sequester C in soil, which contributes to C storage, and soil C decomposition through SR is an essential nutrient resource for plant growth, while how to find an optimal balance between C sequestration and C decomposition is still an ongoing debate in managing soil health[15]. Meanwhile, long-term maize stalk management affects soil biochemical characters, and soil biochemical characters play a pivotal role in

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controlling SR. However, figuring out the influence of maize stalk retention on SR and $Q_{10}$ remains a longstanding challenge. To investigate the influence of different maize stalk retention amounts on SR and $Q_{10}$, this study was initiated in an experimental maize field, where CT, SCR, TYR and CCR have been applied for 17 years. We hypothesized that maize stalk retention amount would induce the variations of soil microbial structures and corresponding abundances, different retention amounts may also impact carbon qualities. Soil microbial abundance will determine SR rate at a certain level, and carbon quality changes will have an obvious influence on SOC decomposing by soil microbes, such changes should be expected to have close correlations with SR and $Q_{10}$.

## 2 Material and methods

### 2.1 Study site description

The study site is located in Fufeng County, Changchun City, Jilin Province (43.78 °N, 125.2 °E), where the annual mean temperature is 4.8 ℃, annual precipitation ranges from 522 to 615 mm, and annual accumulative temperature ranges from 2770 ℃ to 2910 ℃. According to the USDA soil taxonomy[16], the soil of such study site is Mollisols, which basically belongs to Black soil with soil order of Semi-Luvisols according to Chinese soil taxonomy. A soil test was conducted according to the description of Sumner[17] in the autumn of 2000. The concentrations of SOC, soil nitrogen (N), soil phosphorus (P) and soil potassium (K) within top 30 cm were 1.963%, 0.123%, 0.0590% and 1.6112%, respectively. Soil pH ranged from 6.5 to 7.1; the concentrations of sand, silt, and clay were 57%, 13% ,and 30%, respectively.

### 2.2 Experimental design

Monoculture maize was cultivated every year since 2000. The maize cultivar is *Jidan* 209, which was sowed between late April and early May, then harvested in early October. Plant distances of inter-row and intra-row were 65 and 24 cm, respectively. Manually hoeing was applied during the growing period, in other words, no chemical or biological herbicide was applied during the weeding control process. To limit experimental factors, no chemical fertilizers or manure was used. Since 2000, a randomized complete block design with six replicates was established at the study site. For each replicate, four zones with each area of 2 $hm^2$ were chosen randomly to apply different maize stalk retention managements. In one zone, SCR was used to cover the soil surface, which means 50 cm bottom maize stalk was retained in the field after harvesting. In two zones, TYR and CCR were applied respectively. The last zone acted as the control zone (CT), where the conventional agricultural practice was applied, i.e. all maize stalk was removed after harvesting. As for the specific implementation methods of four treatments, this study applied one maize harvester to realize partial bottom stalk retention, the maize harvester has a rotary cutting disk, whose height from the soil surface can be adjusted according to specific requirements. The work of whole maize stalk retention was finished by a commonly used maize harvester, the maize harvester has a stalk crushing device, which can crush the maize stalks into small pieces, then spread them on the soil surface. The work of whole maize stalk removal was realized according to the following steps, firstly, maize ears were picked by a maize harvester. Secondly, the stalks were cut by manually sickle, then the stalks were removed away manually. Likewise, partial maize stalk removal was realized manually. Four zones accepted the same treatments except for maize stalk retention management. In the spring of 2017, 25 soil sampling points were arranged randomly in each zone so as to acquire mean values. According to the methods proposed by Sumner[17], the concentrations of SOC, N, P and K, soil pH as well as soil texture were measured on April 5$^{th}$ , 2017.

SCR, TYR, and CCR led to different initial amounts of maize stalk retention. To quantify (i) initial concentrations of C and N, (ii) final concentrations of C and N and (iii) initial fresh stalk mass and final dry stalk mass within each treatment, 30 one-square-meter matrices were selected randomly in each zone. Half of them were used for C and N concentration analysis in fresh maize stalk. The other half was used for residue mass analysis of fresh stalk and dry stalk, as well as C and N concentration analysis in dry maize stalk. Specifically, on the maize harvesting day of October 15$^{th}$ , 2016, 15 one-square-meter matrices were selected randomly. Within each one-square-meter matrix, all the maize stalk residues were picked up manually and transported to the lab, then C and N concentration analysis in fresh maize stalk was conducted according to the methods by Yang et al.[18] and Heckman et al.[19] As for the other 15 one-square-meter matrices, all the maize stalk residues within each matrix were weighed every day since October 15$^{th}$ , 2016, the average mass of 15 matrices was regarded as the retention amount for each measuring day. The measuring results on October 15$^{th}$ , 2016 were regarded as the fresh mass. Until there was no significant difference (student's *t* test at a significance level of 0.05) between two measurements, the average mass obtained from last weighing was regarded as the final dry mass, and this measurement ended on November 7$^{th}$ , 2016. Subsequently, these dry maize residues were transported to lab for concentration analysis of C and N. The chemical testing procedure was conducted according to the methods proposed by Yang et al.[18] and Heckman et al.[19] As for SCR, the bottom standing maize residue would be removed manually for weighing and chemical testing, other procedures were the same as the other two treatments. As for TYR, whole maize stalks were returned to the field for the first year, whole maize stalks were removed for the second year, and leaving 1/3 mass of maize stalks standing in the field for the third year, thus 4/9 mass was regarded as its annual retention amount.

### 2.3 Soil sampling and analysis

On March 28$^{th}$ of 2017, soil samples from the tillage layer (0-10 cm) were collected. Twenty-five sampling points were arranged randomly in each zone, 25 soil cores were going to be obtained and then analyzed separately, so as to display an average value for each zone. After discarding about 1 cm of surface soil at each sampling point, about 1 kg of soil was vertically excavated by a spade and then contained in a cloth bag. Subsequently, impurities such as rocks, and roots were removed, and about 0.5 kg of soil sample was obtained by the quartering method at last. After the soil samples were naturally drying and then ground further to pass 1-mm sieve, the final soil was separated into three subsamples. The first subsample was air-dried at room temperature (25 ℃) and then ground with a mill to pass through a 0.25 mm sieve before physicochemical analysis. The second subsample was kept at 4 ℃ for soil microbial analysis. The third subsample was also kept at 4 ℃ for laboratory incubation.

Solid-state $^{13}C$ nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (CPMAS-NMR) technique was used to study the chemical composition of soil organic carbon. In order to increase the sensitivity of nuclear magnetic resonance (NMR), soil samples were treated with hydrofluoric acid (HF) to remove paramagnetic compounds and minerals[20]. Specifically, approximately 10 g of soil samples

were put into sealed polyethylene centrifugation vessels (100 mL) and treated with 40 mL of 10% (v/v) HF, then shaken for 2 h. After centrifugation (3000 r/min) for 10 min, the supernatant was discarded. After repeating the procedure five times, the remaining sediment was washed with distilled water until the pH was above 5 and then dried in an oven. NMR spectra were acquired on a 400 MHz spectrometer (Bruker Biospin-AVANCE II, Germany). Samples were placed into a zirconium oxide rotor with Kel-F caps. Spectra of all samples were acquired under the conditions of 12 kHz spinning speed, a ramp contact time of 1 ms, and a recycle delay of 1 s. About 20 K scans were applied to all samples. Spectra were calibrated by using the carboxyl signal of glycine as an external standard (176.03 mg/kg)[21]. The NMR spectrum was divided into four chemical shift regions to represent specific organic carbons[22,23]: i.e. (i) 0-45 mg/kg (Alkyl-C); (ii) 46-110 mg/kg (O-alkyl-C); (iii) 116-165 mg/kg (Aromatic-C); (iv) 166-210 mg/kg (Carboxylic-C). Specific concentration of each C was obtained by integrating corresponding chemical shift regions. Alkyl-C/O-alkyl-C ratio (Aliphaticity index), aromatic-C/ O-alkyl-C ratio (Aromaticity index) and (aromatic-C + alkyl-C)/O-alkyl-C ratio (Combined index) were also calculated as potential indicators to evaluate the recalcitrant level of soil carbon[22]. Microbial community structure and phenotypic diversity were assessed by using phospholipid-fatty acids (PLFA) analysis. Specifically, lipids were extracted from 5 g of dried soil by using a mixture of chloroform, methanol and citrate buffer (volume ratio: 1:2:0.8), and the phospholipids in the organic phase were separated using a solid-phase silica column (Agilent Technologies, Palo Alto, CA, USA). After mild alkaline methanolysis, the resulting fatty acid methyl esters were identified using an Agilent 6850 Gas Chromatograph. More specific operational procedures were described by Guo et al.[21]. Fatty acid nomenclature used in this study was described by Zak et al.[24] The abundance of each individual PLFA was expressed as nmol fatty acid per gram of dry soil.

In order to determine SR and $Q_{10}$, soil samples used for laboratory incubation were adjusted to have 60% water holding capacity. Briefly, 15 g of dry-equivalent soil was put in 0.5-L plastic jars with butyl-rubber stoppers. As the soil was taken from the field and undergone the pre-processes, the soil was disturbed. In order to recover the soil, the soil samples were pre-incubated at room temperature (25 ℃) for a week, then incubated at 5 ℃, 15 ℃, and 25 ℃ for 30 d. Respired $CO_2$ was trapped in NaOH solution. Specifically, 5.0 mL of 0.1 M NaOH was contained in a beaker suspended inside each plastic jar. The NaOH solution beaker was replaced with a new one no more than every 12 h, then the already used NaOH solution was removed and titrated with 0.05 M HCl to determine the evolved $CO_2$ amount. In other words, SR was measured at least twice a day throughout the incubation period, and the hourly SR was obtained by mathematical interpolation.

### 2.4 Data analysis

During the incubation period, NaOH solution beaker was replaced at 9:00 and 21:00 approximately every day, so $Q_{10}$ was calculated at the above two timepoints during the 30 d incubation, and the $Q_{10}$ was calculated according to Equation (1).

$$Q_{10} = \left( \frac{R_{T_2}}{R_{T_1}} \right)^{\frac{10}{(T_2 - T_1)}} \quad (1)$$

where, $R_{T2}$ and $R_{T1}$ are respiration rates for incubation temperature $T_1$ and $T_2$, respectively. In this work, $Q_{10}$ was calculated by using respiration rates under incubation temperatures of 5 ℃, 15 ℃ and 25 ℃ respectively. Hence, we have both $Q_{10}$ (15 ℃/5 ℃) and $Q_{10}$ (25 ℃/15 ℃).

Statistical analysis was carried out by using SPSS 11.5 for Windows (SPSS Inc., USA). The least significant difference (LSD) analysis was performed for significance comparison. Student's *t* test and *F* test were conducted according to sample numbers. Pearson correlation analysis was conducted to investigate the correlation among SR, $Q_{10}$, soil microbial abundance and carbon quality indices.

## 3 Results

### 3.1 Maize stalk and soil chemical characteristics

The determination of maize stalk retention amount lasted for 23 d. Study results showed that annual fresh retention amounts for SCR, TYR and CCR were 5750, 7590 and 17 250 kg/hm$^2$, respectively, annual dry retention amounts for SCR, TYR and CCR were 2500, 3300 and 7500 kg/hm$^2$, respectively. These data indicated that fresh maize stalk lost about 56% of mass from 15$^{th}$ October to 7$^{th}$ November despite of maize stalk retention regime.

Concentration testing results of C and N showed that in fresh maize stalk, C concentrations of SCR, TYR and CCR were 1000, 1320 and 3000 kg/hm$^2$, respectively, N concentrations of SCR, TYR and CCR were 14.3, 18.8 and 42.9 kg/hm$^2$, respectively. In dry maize stalk, C concentrations of SCR, TYR and CCR were 985, 1315 and 2990 kg/hm$^2$, respectively, N concentrations of SCR, TYR and CCR were 14.1, 18.4 and 42.7 kg/hm$^2$, respectively. Paired student's *t* test showed no significant ($p > 0.05$) difference between fresh and dry stalk in terms of C concentration or N concentration.

Seventeen years past since the initiation of this study. Different stalk managements did not show significant ($p > 0.05$) influence on soil pH or soil texture, because the measuring results indicated that soil pH values of four treatments still ranged from 6.5 to 7.1; concentrations of sand, silt and clay were 56%, 14% and 30%, respectively, which did not show significant ($p > 0.05$) changes. Compared with the original testing record conducted in 2000, CT lost 0.032, 0.0078, 0.1761 and 0.622% for N, P, K and SOC, respectively. Table 1 shows that compared with either CT or the original testing record, SCR, TYR and CCR showed an obvious increase in concentrations of N, P, K and SOC. Compared with CT, the maximum increasing amount of N, P, K and SOC occurred in CCR, which increased N, P, K and SOC by 0.040%, 0.0181%, 0.1959% and 0.764%, respectively. The minimum increasing amount occurred in SCR, which increased N, P, K and SOC by 0.029%, 0.0137%, 0.1459% and 0.6810%, respectively. *F* test showed in terms of the concentrations of N, P, K and SOC, there were also significant ($p < 0.05$) differences among the four treatments.

**Table 1 Concentrations of soil nutrients and SOC**

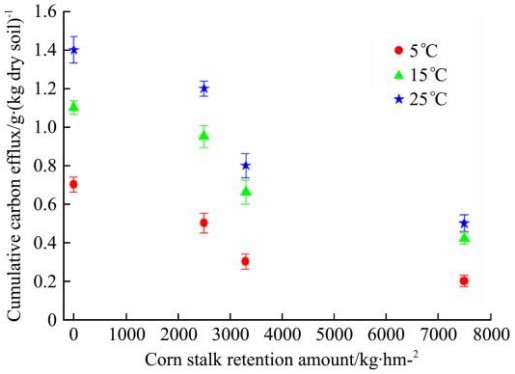
| | N/% | P/% | K/% | SOC/% |
|---|---|---|---|---|
| CT | 0.0910±0.0061 | 0.0512±0.0044 | 1.4351±0.0109 | 1.3410±0.0061 |
| SCR | 0.1200±0.0072 | 0.0649±0.0028 | 1.581±0.0039 | 2.0220±0.0201 |
| TYR | 0.1220±0.0042 | 0.0651±0.0101 | 1.589±0.0027 | 2.0290±0.0033 |
| CCR | 0.1310±0.0113 | 0.0693±0.0031 | 1.631±0.0016 | 2.1050±0.0017 |

Notes: CT means control management without maize stalk retention, SCR means standing maize stalk retention, TYR means 'three-year rotation' maize stalk retention, CCR means chopped maize stalk retention. N means soil total nitrogen concentration, P means soil total phosphorus concentration, K means soil total potassium concentration, SOC means soil organic carbon concentration. Each data was derived from six replicates, and each replicate contained 25 measurements from 25 soil samples. Each mean value is displayed with its standard errors.

### 3.2 SR and $Q_{10}$

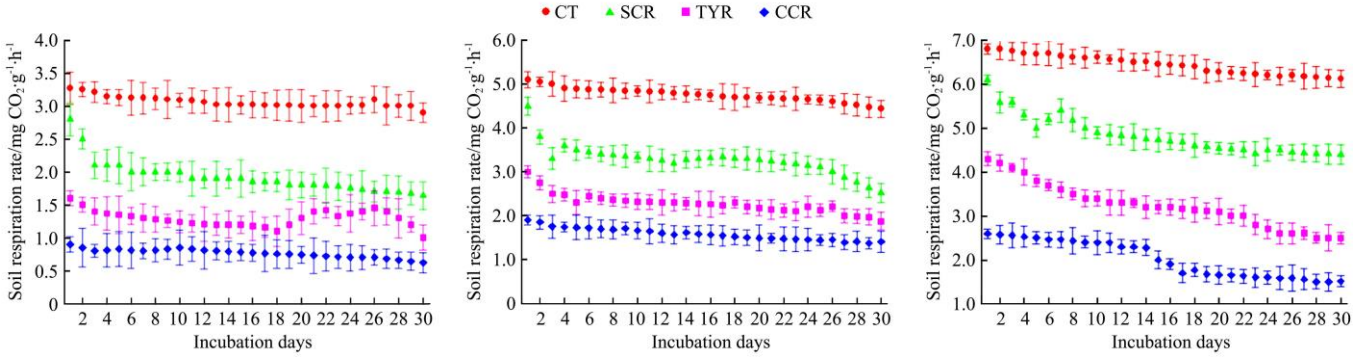
Cumulative C efflux reflected total SR of four treatments. Figure 1 shows that compared with CT, SR decreased along with the increase of stalk retention amount. Under three incubation temperatures, average SR values were 1.07, 0.88, 0.59 and 0.37 g/kg dry soil for CT, SCR, TYR and CCR, respectively, which showed significant differences ($p < 0.05$). When considering SR within any specific treatment, SR decreased along with the decrease of incubation temperature.

Hourly SR was obtained by mathematical interpolation. Under each individual incubation temperature, a curved line was fitted based on the relationship among hourly SR, maize stalk retention amount and incubation days, as displayed in Figure 2. It was obvious that hourly SR declined along with the increase of incubation days at all incubation temperatures. Statistical analysis showed that SR under SCR was significantly higher than TYR and CCR at all three incubation temperatures.




Note: Error bars designate the standard errors.

Figure 1 Cumulative carbon (C) efflux after 30-d incubation at 5 ℃, 15 ℃, 25 ℃, respectively

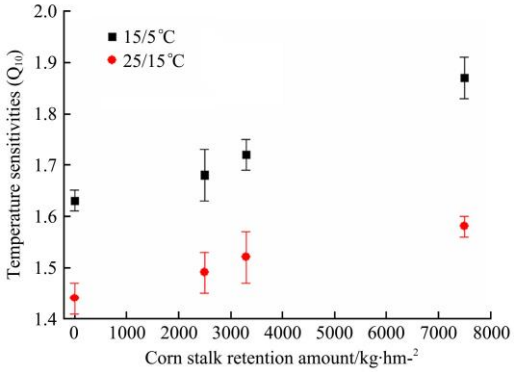



a. 5 ℃ b. 15 ℃ c. 25 ℃

Note: Error bars designate the standard errors.

Figure 2 Hourly soil respiration (SR) rate, maize stalk retention amount and incubation days at incubation temperatures of 5 ℃, 15 ℃ and 25 ℃

Figure 3 demonstrates the average $Q_{10}$. The lowest $Q_{10}$ (15 ℃/5 ℃) and $Q_{10}$ (25 ℃/15 ℃) belonged to CT, while the highest $Q_{10}$ (15 ℃/5 ℃) and $Q_{10}$ (25 ℃/15 ℃) occurred in CCR. Average $Q_{10}$ values were 1.535, 1.585, 1.62 and 1.725 for CT, SCR, TYR and CCR, respectively. Compared with average $Q_{10}$ under CT, SCR and TYR, average $Q_{10}$ (15 ℃/5 ℃) under CCR treatment was significantly higher ($p < 0.05$). The average $Q_{10}$ (25 ℃/15 ℃) under TYR and CCR was slightly higher than that under SCR, but there was no significant difference ($p > 0.05$) among the three stalk retention treatments.




Note: Error bars designate standard errors.

Figure 3 Temperature sensitivities ($Q_{10}$) for 4 treatments

### 3.3 Soil Carbon

Soil carbon compositions are presented in Table 2. Compared with the other three treatments, CT acquired the highest percentages of alkyl-C and O-alkyl-C, while the lowest percentages of Aromatic-C and Carboxylic-C. If considering soil carbon composition only in maize stalk retention fields, the highest fractions of alkyl-C, O-alkyl-C, Aromatic-C and Carboxylic-C were ascribed to TYR, SCR, TYR and CCR, respectively, while the lowest fractions were attributed to CCR, CCR, CCR and SCR. Carboxylic-C of CCR was higher than that of SCR and TYR by 10% and 9%, respectively.

**Table 2 Carbon compositions for 4 treatments**

| | Alkyl-C/% | O-alkyl-C/% | Aromatic-C/% | Carboxylic-C/% |
|---|---|---|---|---|
| CT | 27±0.73* | 53±0.22* | 10±1.66* | 10±0.65 |
| SCR | 24±0.49 | 50±0.85* | 14±0.42 | 12±0.62 |
| TYR | 25±1.17 | 47±3.12* | 15±0.73 | 13±0.43 |
| CCR | 20±0.53* | 45±1.23* | 13±0.91 | 22±0.07* |

Notes: Each mean value is displayed with its standard deviations. Numbers followed by '*' designate significant differences compared with the other three treatments at a significance level of 0.05.

Table 3 shows the calculated results of carbon quality indices. TYR had the highest indices for all three carbon qualities. The lowest aliphaticity index and combined index belonged to CT. Compared within maize stalk retention fields, the lowest aromaticity index, aliphaticity index and combined index were obtained by CCR, SCR and CCR, respectively.

**Table 3 Carbon quality indices of CT, SCR, TYR and CCR**

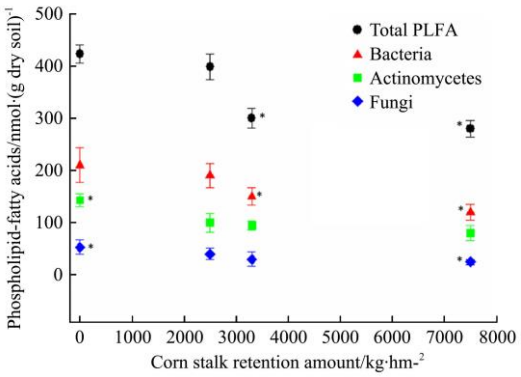
| | Aromaticity index/% | Aliphaticity index/% | Combined index/% |
|---|---|---|---|
| CT | 50.94 | 18.87 | 69.81 |
| SCR | 48.00 | 28.00 | 76.00 |
| TYR | 53.19 | 31.91 | 85.11 |
| CCR | 44.44 | 28.89 | 73.33 |

### 3.4 Soil microbial abundance

Figure 4 shows soil microbial abundances. CT occupied the highest total PLFA, bacteria, actinomycetes and fungi, and its actinomycetes and fungi were significantly higher ($p<0.05$) than the other three treatments. Total PLFA under SCR was higher than that under TYR and CCR by 33% and 42%, respectively. CCR led to significant decrease ($p<0.05$) in bacteria and fungi, while CCR did not have significant influence on actinomycetes ($p>0.05$). Figure 4 also indicates that SCR, TYR and CCR had nearly equivalent biomass of actinomycetes.

### 3.5 Pearson correlation analysis

Person correlation analysis was carried out based on the above experimental data in Table 4. Total PLFA, fungi and bacteria had positive correlations with SR. Alkyl-C and O-alkyl-C had positive correlations with SR, but negative correlations with $Q_{10}$ (15 ℃/5 ℃). Aliphaticity index and combined index had negative correlations with SR, but positive correlations with $Q_{10}$ (15 ℃/5 ℃). Aromaticity index had a negative correlation with SR.




Note: Error bars designate standard errors, '*' designates significant difference compared with the other three treatments at a significance level of 0.05.

Figure 4 Total phospholipid-fatty acids (PLFA), bacteria, actinomycetes and fungi for 4 treatments

**Table 4 Pearson correlation coefficients among SR, $Q_{10}$, soil microbial abundance and carbon quality indices**

| | Indices | Cumulative C efflux (5 ℃) | Cumulative C efflux (15 ℃) | Cumulative C efflux (25 ℃) | $Q_{10}$ (15 ℃/5 ℃) | $Q_{10}$ (25 ℃/15 ℃) |
|---|---|---|---|---|---|---|
| Soil microbial abundance | PLFA | **0.66*** | **0.75*** | **0.86*** | -0.69 | -0.67 |
| | Fungi | **0.79*** | **0.88*** | **0.87*** | -0.64 | 0.45 |
| | Actinomycetes | 0.29 | 0.47 | 0.31 | -0.44 | -0.35 |
| | Bacteria | **0.67*** | **0.78*** | **0.85*** | -0.34 | -0.45 |
| Carbon quality | Alkyl-C | **0.83*** | **0.82*** | **0.66*** | **-0.68**** | 0.64 |
| | O-alkyl-C | **0.92*** | **0.92*** | **0.77*** | **-0.58**** | -0.69 |
| | Aromatic-C | -0.21 | -0.22 | -0.38 | -0.89 | -0.36 |
| | Carboxylic-C | -0.32 | -0.42 | -0.48 | 0.68 | 0.53 |
| | Aromaticity | **-0.82*** | **-0.94*** | **-0.96*** | 0.78 | 0.45 |
| | Aliphaticity | **0.82*** | **0.78*** | **0.92*** | **0.71**** | 0.36 |
| | Combined index | **-0.94*** | **-0.91*** | **-0.87*** | **0.59**** | 0.39 |

Notes: Bold numbers with "*" means the correlation between two variables is significant at $p<0.05$; bold numbers with "**" means the correlation between two variables is significant at $p<0.1$.

## 4 Discussion

### 4.1 Soil quality changes over long term stalk management

Long-term stalk management changed soil nutrient concentration and soil carbon composition ratio. Maize stalk retention amount was in the order of SCR < TYR < CCR in this study. Along with the increase in retention amount, the concentrations of N, P, K and SOC increased. Soil organic carbon is acted as a robust indicator used for evaluating management options[25], and SCR, TYR and CCR increased SOC by 0.681%, 0.688% and 0.764% compared with CT, the N concentration of CCR increased by 9.167% compared with SCR, the concentration of TYR increased by 0.506% compared with SCR. This study is in line with the well-known academic point that maize stalk retention can replenish N, P, K and SOC[26]. In general, labile carbon fraction decreased and recalcitrant carbon fraction increased along with the increase in retention amount. The stalk retention amount of CCR increased by 200% compared with SCR, but CCR decreased Alkyl-C and O-alkyl-C by 16.667% and 10%, respectively. The retention amount of TYR increased by 32% compared with SCR, and TYR increased Aromatic-C and carboxylic-C by 7.143% and 8.333%, respectively. One seven-year study was conducted in Shandong Province (36°09′N, 117°09′E) China, the labile carbon variation was compared between stalk retention and stalk removal, and it was concluded that along with the increase in stalk retention amount, the labile carbon fraction declined significantly ($p<0.05$) within top 30 cm[27], which indicates their findings and our findings support each other. Furthermore, one study aimed to evaluate the effects of crop-residue retention amount on carbon compounds (labile/recalcitrant) in the semiarid region of Argentina, five retention amounts were adopted in the research, and their results hold the same viewpoint that along with the increase of stalk retention amount, the labile carbon fraction declined, the recalcitrant carbon fraction increased[28].

A larger amount of maize stalk retention favors higher N replenishment, SCR, TYR and CCR increased soil N concentrations by 0.029%, 0.031% and 0.040% compared with CT. One study concluded that N addition can significantly accelerate the decomposition of labile carbon fractions while further stablizing carbon compounds in heavier, mineral-associated fractions[29], which is interpreted in Table 2 in this study.

### 4.2 SR responses to N addition and soil microbial biomass

Figure 1 shows the SR (referred to as 'cumulative C efflux amount') should be in a descending order of CT, SCR, TYR, and CCR. There is an obvious trend that SR decreased with the increasing amount of maize stalk retention. SCR, TYR and CCR increased soil N by 0.029%, 0.031% and 0.040% compared with CT, while decreased average SR by 17.2%, 45.0% and 65.0%, respectively. Instead of adding N by stalk retention, one research added N directly, their research agrees that SR declined when N was added[30]. By using similar research methods as this study, one rather long-term (15-year) N and P addition experiment was conducted in the Tibetan Plateau, China, their major finding was that N addition significantly reduced SR[21].

Stalk retention has impacts on soil physical and biochemical

variations, which will cause microbial abundance changes[31], and microbial abundance has a positive correlation with SR[32]. Figure 4 shows that CCR was involved with the lowest PLFA and three individual microbes, which is contrary to the conventional impression. The reason why higher retention amount resulted in less soil microbial abundance was the formation of crop residue crusts. Owing to this study area having a long and cold winter which starts from early November to late March of next year, this area does not have enough accumulative temperature to decompose crop residue completely all year round. Moreover, as no-till planters have a good performance on crop-residue-covering fields, crop residue will not receive any additional management before spring sowing, therefore, crop-residue-crusts formed year after year. Previous studies conducted in the same locations as this study have shown that the thicker crop residue crust, the lower soil temperature[33], and the lower of soil temperature led to less soil microbial abundance[34]. As ~85% to 90% of SOC is decomposed by soil fungi and soil bacteria[11], but soil bacteria and soil fungi of CCR were only 100 and 13 nmol/g dry soil, which were significantly lower ($p$<0.05) than those of the other three treatments. The lower of soil bacteria and soil fungi was a major reason that led to lower SR. Therefore, cumulative C effluxes were in the descending order of CT, SCR, TYR, and CCR.

### 4.3 Effects of soil carbon on SR and $Q_{10}$

It is worth noticing that CCR resulted in lower labile carbon and higher recalcitrant carbon (Table 2). Pearson correlation analysis (Table 4) shows that labile carbon fraction and aliphaticity had positive correlations with SR, thus increasing recalcitrant carbon (i.e. Aromatic-C and Carboxylic-C) and aromaticity will hinder cumulative C efflux, above interpretation can partially explain why CCR acquired the lowest SR.

Average $Q_{10}$ (25 ℃/15 ℃) was far below average $Q_{10}$ (15 ℃/5 ℃). As shown in Figure 1, averaged across four treatments, mean cumulative C effluxes at 5 ℃, 15 ℃ and 25 ℃ were 0.425, 0.7825 and 0.975 g/kg dry soil, which indicates the mean SR amplification from 5 ℃ to 15 ℃ was much larger than that from 15 ℃ to 25 ℃. Figure 2 demonstrates that when the incubation temperature increased from 15 ℃ to 25 ℃, the hourly SR did not keep the same amplification as from 5 ℃ to 15 ℃. Since SR is controlled by thermal regimes[32,35,36], presumably SR was approaching its maximum when incubation temperature exceeded 15 ℃. Therefore, the average $Q_{10}$ (25 ℃/15 ℃) was far below the average $Q_{10}$ (15 ℃/5 ℃) in this study. The study results show that the average $Q_{10}$ (15 ℃/5 ℃) under CCR was significantly higher ($p$ < 0.05) than that under SCR and TYR, and the average $Q_{10}$ (25 ℃/15 ℃) under SCR was the lowest. Thus, our study asserts that average $Q_{10}$ increases with the increasing amount of stalk retention.

CCR acquired the highest fresh maize stalk mass (17 250 kg/hm$^2$), highest dry maize stalk mass (7500 kg/hm$^2$) as well as corresponding highest C concentrations of fresh and dry stalks (3000 and 2990 kg/hm$^2$). The SOC concentration of CCR was 2.105%, which was significantly higher ($p$ < 0.05) than the other three treatments. SOC is essential resource for SR[10,11]. Higher SOC concentration under CCR indicates that CCR has the potential to release more $CO_2$ when the incubation temperature increases. Stalk retention amount was the only difference among the four treatments, different retention amount led to the changing of soil carbon. Soil carbon compositions have paramount impacts on $Q_{10}$[35]. Since crop residue crusts make SOC more recalcitrance and more resistant to decomposers[28], soil samples obtained from CCR were characterized by a high concentration of recalcitrant carbon (Tables 2 and 3). Average $Q_{10}$ is positively correlated with recalcitrant carbon indices (i.e. Aliphaticity index, and Combined index), but negatively correlated with labile carbon (i.e. Alkyl-C and O-alkyl-C), which can be seen in Table 4. The above interpretation can explain why CCR acquired higher $Q_{10}$ compared with the other three treatments. In order to figure out the relationship between soil carbon and $Q_{10}$, one investigation with four soil carbon compositions was completed in Qinghai-Tibetan Plateau, China, the investigation showed $Q_{10}$ increases according to the ascending order of labile carbon, particulate organic carbon, hydrolysable carbon, and recalcitrant carbon[37], as the carbon quality varies according to the stalk managements in our study, their major finding is in consistence with ours.

## 5 Conclusions

After 17 years of unchanged agricultural practice, soil respiration (SR), temperature sensitivity ($Q_{10}$), soil nutrients, soil organic carbon (SOC), soil microbial abundance and carbon compositions were analyzed. The main conclusions are as follows:

(1) Stalk retention amounts for standing maize stalk retention (SCR), 'three-year cycle' (TYR) and chopped maize stalk retention (CCR) were 2500 kg/hm$^2$, 3300 kg/hm$^2$ and 7500 kg/hm$^2$, respectively. With the increase of maize stalk retention amount, SR gradually decreases while $Q_{10}$ gradually increases in northeast China.

(2) Larger maize stalk retention amount leads to the reduction of labile carbon fraction while increasing recalcitrant carbon fraction. Moreover, larger maize stalk retention induces a decrease of soil microbial biomass.

(3) Pearson correlation analysis illustrated that carbon compositions and soil microbes have significant impacts on SR and $Q_{10}$.

In real farming practice, conservation tillage with maize stalk retention is one effective method to realize soil nutrient replenishment, a larger amount of maize stalk retention favors C sequestration, thus CCR is more suitable for balancing C sequestration and C decomposition in northeast China.

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