Effects of Corn Stalks Returning on Soil Microbial Carbon Use Efficiency and Corn Yield in Semi-Arid Cropland

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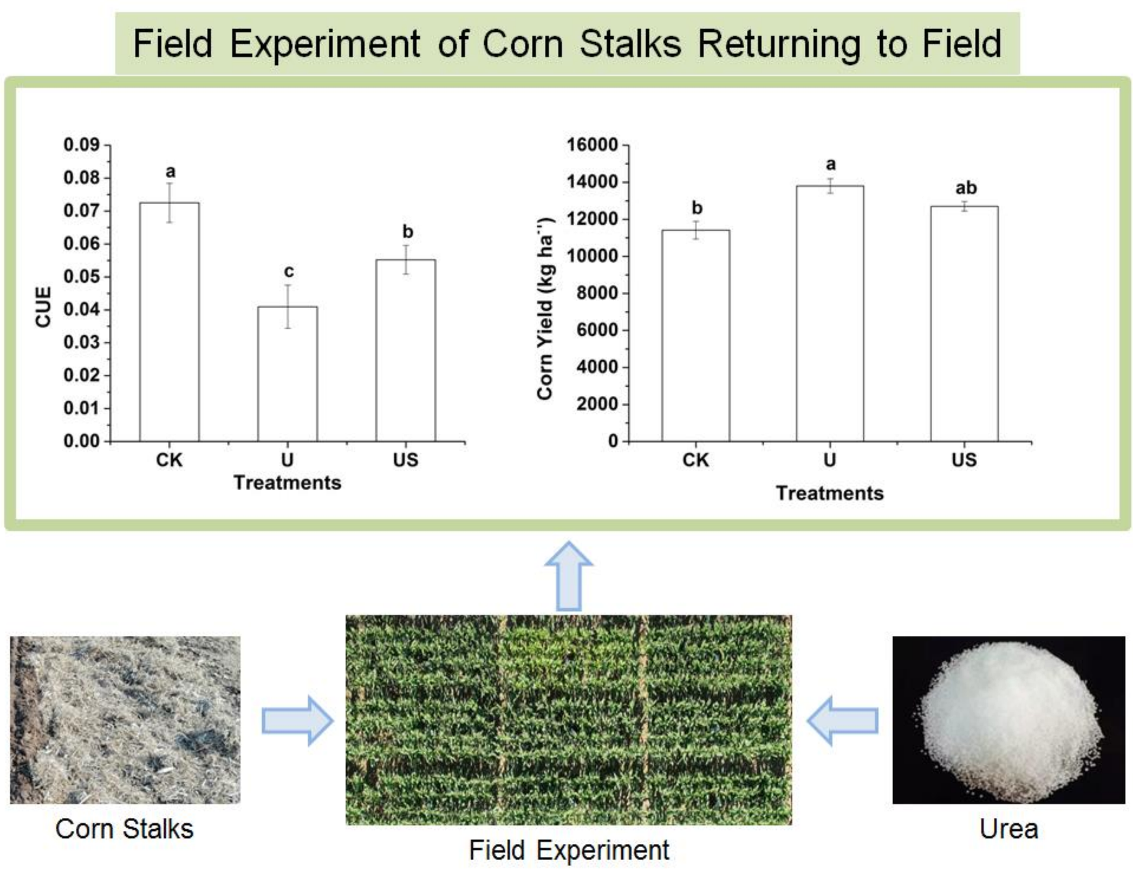
GRAPHICAL ABSTRACT



Effects of Corn Stalks Returning on Soil Microbial Carbon Use Efficiency and Corn Yield in Semi-Arid Cropland

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Soil microbial carbon use efficiency (CUE) is a key parameter controlling the short-term carbon (C) cycle in terrestrial ecosystems. The effect of urea application (156 kg N ha^{-1}) and corn stalks returning (9.0 tons ha^{-1}) on soil microbial CUE and corn yield in semi-arid cropland was studied using the ^{18}O-labeled water approach during a one-year experiment. In semi-arid cropland, applying urea reduced soil microbial CUE by 44%, while the soil microbial CUE was increased significantly by 34% after returning corn stalks to the field. The application of urea increased the total nitrogen content of soil by 23%, and corn stalks returning further increased nitrate nitrogen (NO_3^--N) by 45%, dissolved organic carbon (DOC) by 53%, and dissolved organic nitrogen (DON) by 122%. Compared with no fertilization, urea application increased the corn height by 4% and the corn yield by 21%. Corn stalks returning combined with urea reduced the corn stalks by 37% compared with no fertilizer. There was no significant difference in corn yield between corn stalks returning combined with urea and single urea application. Therefore, corn stalks returning combined with urea may be an effective agronomic measure to increase soil carbon sequestration, improve soil fertility, maintain corn yield, restore soil fertility, and improve production capacity.

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Keywords: Microbial carbon use efficiency; Corn stalks resources; O-labeled water approach; Corn field; Corn biological indicators

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INTRODUCTION

Global warming is one of the most important environmental issues in the 21st century, and the extreme climate and natural disasters it brings threaten the survival and development of humans (Wheeler and Von Braun 2013; Badewa *et al*. 2022). The increase of greenhouse gases (CO_2, CH_4, N_2O, *etc*.) in the atmosphere is the main cause of global warming (Davidson 2009). Soil organic matter (SOM) is a vast carbon (C) pool in terrestrial ecosystems, with an organic C storage of up to 2344 GT (Stockmann *et al*. 2013). Small changes in soil C pools may also strongly impact the concentration of the largest greenhouse gas, carbon dioxide (CO_2) (Xia *et al*. 2020). Cropland soil is one of the soil types most severely affected by human activities. Frequent tillage changes the redox state, converting a large amount of SOM into CO_2 and releasing it into the atmosphere, which is one reason for the increase in atmospheric CO_2 (Ye and Horwath 2016). Therefore, taking steps to sequester more C in cropland soils is critical to slow global warming.

China is one of the largest agricultural countries, and its corn output accounts for 21% of the world (Xia *et al*. 2020). Liaoning Province is one of the 13 major grain-producing regions in China. The semi-arid region in the northwest of Liaoning Province accounts for more than two-thirds of the corn planting area in the province (Zhang *et al*. 2020). The corn production in this region is crucial to ensuring national food security. However, the soil degradation in this area severely restricts the corn production potential (Jiang *et al*. 2022; Kumar *et al*. 2023). A large corn planting area may also bring large soil C emissions (Ye and Horwath 2016). While exploring agronomic measures that can maintain more carbon in the soil, it is important to consider whether corn yield is unaffected or can be improved, which is a major challenge for agricultural development.

Synthetic nitrogen (N) fertilizer plays a key role in increasing food production and ensuring that half of the world's population has enough food (Zhang *et al*. 2013; Denk *et al*. 2017). However, the long-term application of N fertilizer without organic material input will lead to the decline of farmland fertility, which is not conducive to guaranteed grain yield and sustainable agricultural development (Triplett and Dick 2008; Zhang *et al*. 2012). Straw returning is a widely used agronomic measure with many advantages, such as improving soil physicochemical properties, increasing soil aggregates, promoting soil moisture and fertilizer conservation, and ensuring high crop yields (Velthof *et al*. 2002; Wu *et al*. 2021) and reducing the risk of heavy metal pollution in semi-arid areas by improving the pH (Jiang *et al*. 2022). However, the input of exogenous organic matter into the soil may exacerbate or delay the decomposition of SOM, resulting in positive or negative priming effects, that is, the effect of fresh organics on the microbial decomposition of SOM (Chen *et al*. 2014). The acceleration of the C cycle was primed by exogenous organic C and was controlled by N (Chen *et al*. 2014). Therefore, it is necessary to explore the impact of C and N dual addition on SOM changes and corn yield.

Soil microbial CUE is the proportion of substrate C that microorganisms assimilate for growth out of the total uptake C. It is a key parameter controlling the short-term C cycle in terrestrial ecosystems and is significantly correlated with changes in soil organic carbon (SOC) (Wang *et al*. 2021; Hu *et al*. 2022). A high microbial CUE reflects highly efficient conversion of plant litter or root sediments to microbial biomass, which may subsequently sequester C for a long time, while a low microbial CUE implies a relatively large loss of C through respiration (Manzoni *et al*. 2012; Hu *et al*. 2022). Empirical relationships among plant, soil, microbial physiological traits, and SOC formation are still lacking today (Wang *et al*. 2021). Therefore, exploring the effect of corn stalks returning on soil microbial CUE and corn yield in semi-arid cropland and the relationship between soil microbial CUE and soil physicochemical properties can provide data support for formulating a reasonable strategy to improve SOM in semi-arid farmland. The main objectives of the present work are to explore the impact of corn stalks returning to the field on soil microbial CUE and to investigate the impact of corn stalks returning to the field on corn yield.

EXPERIMENTAL

Field Site and Experimental Design

The experiment was conducted at the field experimental station of the Liaoning Key Laboratory of Water-Saving Agriculture in Fuxin County, northeast China. The annual average temperature is 7.2 °C, the annual average precipitation is about 480 mm, and the average sunshine time is about 2865.5 h. Before the experiment, a 5 point sampling method was used to take 0 to 20 cm of soil in the area and mix it thoroughly. After passing through a 2 mm sieve, it was used to determine the basic soil physicochemical properties. The test soil was cinnamon soil with an organic matter content of 13.4 g kg^{-1}, a total N of 1.0 g/kg, a total phosphate (P) of 0.4 g/kg, a total potassium (K) of 22.8 g/kg, an available P of 53.6 mg kg^{-1}, and an exchangeable K of 86.7 mg kg^{-1}. Soil bulk density (0 to 20 cm) was 1.51 g cm^{-3}, and the pH (H_2O) was 5.5. The field was set up in spring 2021. The farming system is continuous corn with one season per year. Three treatments were established: 1) control check (CK); 2) urea (U); 3) urea + corn stalks (US). No fertilizer was applied in the CK treatment, while N, P, K fertilizer was applied in the U and US treatments. Fertilizers were urea, superphosphate, and potassium chloride (KCl), and the application levels were N 156 kg ha^{-1}, P 26 kg ha^{-1}, and K 60 kg ha^{-1}, respectively. All fertilizers were applied once during planting on 10 May 2021. Corn stalks were crushed (5 to 10 cm) and returned to the field (9.0 tons ha^{-1}). A randomized block design with three replicates was arranged. The area of each plot was 40 m^2 (5 m × 8 m). The planting density of the corn was 55,000 plants ha^{-1}. The corn variety was "H188" spring maize (*Zea mays* L., a late maturing maize variety based on Food and Agriculture Organization of the United Nations (FAO) standards, with a 127 day growing period). Fertilizer and corn stalks were spread, and rotary plowed to a depth of 15 to 20 cm before sowing. Plant protection and irrigation were not used, weeds were pulled manually, and the corn was harvested manually.

Sample Collection and Analysis

Three soil cores were randomly collected from the plow layer (0 to 20 cm) of CK, U, and US plots after corn harvest. The soil samples were composited, sieved (2 mm), and stored at 4 °C until used for analysis. Soil microbial CUE was the proportion of substrate C absorbed by microbial growth to the total C absorbed, and the process of calculating CUE using the ^{18}O-labeled water approach was referred to (Qu *et al*. 2020; Li *et al*. 2021a). Soil organic matter and total N contents were determined by dry combustion of the samples using an Elemental Analyzer (Vario EL III, Hanau, Germany) (Yang *et al*. 2017). Soil ammonium N (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were extracted with 2 M KCl solution (Wu *et al*. 2021), filtered, and analyzed with a continuous flow analyzer (AA3, Bran + Luebbe, Norderstedt, Germany). Soil pH was measured in a 1:2.5 soil/water suspension with a combination electrode (Yang *et al*. 2017). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were determined using a procedure described by Jones and Willett (2006). Soil microbial biomass carbon (MBC) was fumigated with the chloroform ($CHCl_3$) fumigation–extraction method, extracted with 0.5 M K_2SO_4 (soil: solution = 5 g : 20 mL), and determined by a total organic carbon (TOC) analyzer (Elementar vario TOC Analyzer, Germany), and the unfumigated samples were subtracted (Yang *et al*. 2017).

Statistical Analysis

All analyses were performed using Statistical Package for the Social Sciences (SPSS) Statistics 16.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for testing the treatment effects with Duncan. Significance was $P < 0.05$. Pearson correlation analysis was used for the correlation between soil microbial CUE and soil physicochemical properties. Figures were prepared with Origin 8 (Origin Lab Corp., USA). The data in the figures and tables are the average value ± standard error.

RESULTS AND DISCUSSION

Soil Microbial Carbon Use Efficiency

As shown in Fig. 1A, the soil microbial CUE of CK, U, and US treatments were 0.073, 0.041, and 0.055, respectively, and the differences were significant.

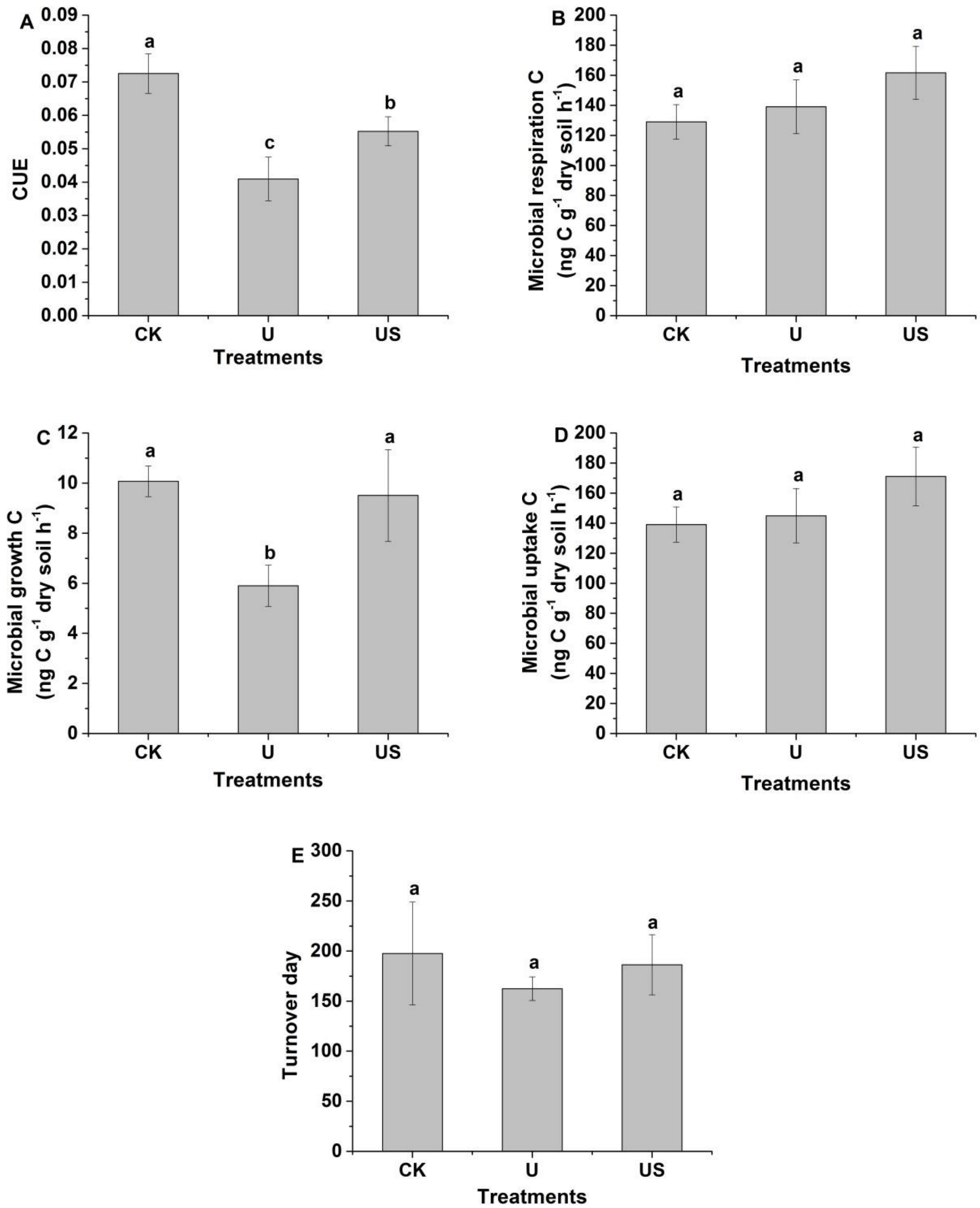

Fig. 1. Effects of fertilization and corn stalks returning on microbial carbon use efficiency (CUE). CUE (A), microbial respiration C (B), microbial growth C (C), microbial uptake C (D), and turnover day (E). CK: control check with no fertilization, U: urea application, US: urea application + corn stalks returning. Bars are ± one standard error of means (n=3). Different letters indicate significant difference at $p < 0.05$.($P < 0.05$). The same considerations apply to subsequent tables and figures.

Compared with the single application of urea, the soil microbial CUE of corn stalks combined with urea increased by 34%. Compared with the CK treatment, the soil microbial CUE of the U and US treatments significantly decreased by 44% and 25%, respectively. Although there was no significant difference in microbial respiration C between the CK, U, and US treatments, the microbial respiration C increased with urea addition and corn stalks returning (Fig. 1B). There was no significant difference in microbial growth C between the CK and US treatments, but both were significantly greater than that of the U treatment, increasing by 71% and 61%, respectively (Fig. 1C). Figure 1D showed that there was no significant difference in microbial uptake C between the CK, U, and US treatments. Turnover day represented the turnover time of microbial biomass. Figure 1E showed that there was no significant difference between the CK, U, and US treatments.

Soil Physicochemical Properties

The U treatment significantly increased soil total N by 23%. Compared with the CK treatment, the US treatment significantly increased soil total N, NO_3^--N, DOC, and DON by 21%, 70%, 41%, and 159%, respectively. Compared with the single application of urea, the combined application of urea with corn stalks returning significantly increased NO_3^--N, DOC, and DON by 45%, 53%, and 122%, respectively.

Table 1. Total N, NH_4^+-N, NO_3^--N, pH, DOC, DON and MBC in the Topsoil (0 to 20 cm)

Treatments	Organic matter (g kg^{-1})	Total N (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	pH	DOC (mg kg^{-1})	DON (mg kg^{-1})	MBC (mg kg^{-1})
CK	13.4 ± 0.8 a	1.0 ± 0.1 b	2.3 ± 0.3 a	2.7 ± 0.2 b	5.5 ± 0.1 a	13.3 ± 2.5 b	4.2 ± 0.8 b	37.9 ± 8.6 a
U	13.0 ± 0.7 a	1.3 ± 0.1 a	2.4 ± 0.7 a	3.2 ± 0.6 b	5.7 ± 0.3 a	12.3 ± 0.1 b	4.9 ± 1.5 b	33.8 ± 5.7 a
US	13.0 ± 0.8 a	1.3 ± 0.1 a	2.4 ± 1.2 a	4.6 ± 0.3 a	6.1 ± 0.7 a	18.8 ± 0.2 a	11.0 ± 2.4 a	34.4 ± 0.4 a
CK: control check with no fertilization, U: urea application, US: urea application + *corn* stalks returning								
Letters in columns indicate significant difference at $p < 0.05$ according to the Duncan's test (n=3), the same below.								

Pearson Correlation Analysis of Soil Microbial Carbon Use Efficiency and Soil Physicochemical Properties

Soil microbial CUE was mainly affected by microbial growth C and total N and may be related to soil NO_3^--N, pH and DON. Soil microbial CUE was significantly positively correlated with microbial growth C and significantly negatively correlated with total N (Table 2). Microbial respiration C was significantly positively correlated with NO_3^--N, PH, and DON. Microbial growth C was significantly positively correlated with soil microbial CUE. Microbial biomass carbon was significantly positively correlated with SOM. Total N was significantly negatively correlated with soil microbial CUE. Nitrate nitrogen was significantly positively correlated with microbial respiration C, pH, DOC, and DON. The pH was significantly positively correlated with microbial respiration C, NO_3^--N, and DON. Dissolved organic carbon was significantly positively correlated with

NO_3^--N. Dissolved organic nitrogen was significantly positively correlated with microbial respiration C, NO_3^--N, pH, and DOC.

Table 2. Pearson Correlation Analysis of Microbial Growth Attributes and Soil Physicochemical Properties

	R	G	T	MBC	Organic matter	Total N	NH_4^+-N	NO_3^--N	pH	DOC	DON
CUE	-0.281	0.842**	0.653	0.292	0.315	-0.742*	-0.117	-0.288	-0.172	0.146	-0.114
R		0.274	0.012	-0.185	-0.24	0.61	-0.122	0.779*	0.882**	0.513	0.781*
G			0.629	0.252	0.211	-0.41	-0.209	0.141	0.319	0.416	0.324
T				-0.153	0.126	-0.261	0.023	0.083	0.274	0.37	0.132
MBC					0.689*	-0.333	-0.183	-0.2	-0.224	-0.352	-0.221
Organic matter						-0.444	-0.546	-0.11	-0.336	-0.18	0.049
Total N							0.44	0.478	0.494	0.183	0.307
NH_4^+-N								-0.071	-0.08	0.024	-0.327
NO_3^--N									0.712*	0.8**	0.917**
pH										0.483	0.697*
DOC											0.852**

The values in the table are Pearson correlation coefficients
**The correlation is significant at P < 0.01
*The correlation is significant at P < 0.05
R: microbial respiration C, G: microbial growth C, T: turnover day.

Table 3. Means (±SE) of 100 Grain Weight, Corn Unit Weight, Corn Height, Corn Stalks Weight and Corn Yield

Treatments	100 grain weight (g)	Corn unit weight (g L^{-1})	Corn height (cm)	Corn stalks weight (kg ha^{-1})	Corn Yield (kg ha^{-1})
CK	40.2 ± 0.3a	810.2 ± 3.7a	302 ± 2b	19260 ± 2167a	11418 ± 477b
U	41.8 ± 0.9a	802.9 ± 1.8a	314 ± 4a	18343 ± 1610ab	13803 ± 392a
US	39.6 ± 1.0a	795.8 ± 10.1a	306 ± 4ab	12075 ± 1723b	12702 ± 255ab

Corn Biological Indicators

There was no significant change in the 100 grain weight of corn regardless of urea application alone or combined application of corn stalks and urea, which was between 39.6 and 41.8 g. The unit weight of corn was not affected by fertilization and corn stalks returning and remained between 796 and 810 g L^{-1}. The highest corn height was 314 cm under urea application alone, which was significantly higher than 302 cm under the CK treatment and increased by 4%. The corn height under the US treatment was 306 cm, which was not significantly different from that under the CK and U treatments. The weight of corn stalks under the CK treatment was the highest, reaching 19,300 kg ha^{-1}, which was significantly higher than that under the US treatment (12,100 kg ha^{-1}). Compared with the CK treatment, the weight of corn stalks in the US treatment was reduced by 37%. There was no significant difference in the weight of corn stalks between the U treatment (18,300 kg ha^{-1}) and the CK and US treatments. Urea application was an important means to increase corn yield. The highest corn yield was 13,800 kg ha^{-1} with urea application, followed by the US treatment and the CK treatment, which were respectively 12,700 and 11,400 kg ha^{-1}. The corn yield of the U treatment was 21% higher than that of the CK treatment, and there was no significant difference between the U treatment and the US treatment.

Effects of Corn Stalks Returning on Soil Microbial Carbon Use Efficiency

N application reduced soil microbial CUE by 44% (Fig. 1A), which is consistent with previous studies (Silva-Sánchez *et al*. 2019; Miao *et al*. 2021). The mineral N addition reduced bacterial growth, while higher soil microbial CUE levels were associated with higher bacterial dominance (Aldén *et al*. 2001; Kristensen *et al*. 2018; Rosinger *et al*. 2019; Silva-Sánchez *et al*. 2019; Miao *et al*. 2021). Soil microbial CUE was significantly negatively correlated with total N. The low soil microbial CUE may be due to the depletion of soil nutrients during corn harvest (Fang *et al*. 2018). However, some studies show that short-term N addition increases the content of soil available N, resulting in a decrease in the demand for microbial N acquisition and an increase in soil microbial CUE (Thiet *et al*. 2006; Spohn *et al*. 2016). Therefore, it is necessary to explore the changes of microbial CUE in different periods after fertilization in the future to accurately grasp the soil C sequestration potential. Corn stalks returning combined with urea increased soil microbial CUE by 34% (Fig. 1A). This may be the fact that corn stalks brought abundant amino acids, cellulose, and other unstable C to the soil (Hadas *et al*. 2004). The simultaneous availability of unstable C and mineral N significantly promotes the availability of nutrients in the soil, leading to rapid growth and reproduction of the fast-growing r-strategy microbial community (Lopez-Urrutia and Moran 2007; Manzoni *et al*. 2012; Chen *et al*. 2014), as well as an increase in bacterial dominance, promoting soil microbial CUE (Silva-Sánchez *et al*. 2019). There was no significant difference in microbial uptake C among all treatments (Fig. 1D). When the total uptake of C remained constant, a higher CUE usually implied more production of microbial biomass and other microbial products, which may be beneficial for the storage of microbially derived C in soils (Wang *et al*. 2021). Soil microbial CUE was mainly affected by microbial growth C, and high microbial growth C was beneficial for long-term C sequestration (Table 2). The combination of corn stalks and urea increased microbial growth C by 61% (Fig. 1C), which may be due to the sensitivity of bacteria to C input (Lee and Schmidt 2014), and the increase in bacterial abundance was conducive to the increase in microbial growth C (Silva-Sánchez *et al*. 2019; Wei *et al*. 2022). Microbial respiration C was significantly positively correlated with NO_3^--N, pH, and DON (Table 2). Li *et al.* (2021a) found that high N addition reduced soil microbial CUE, and the main reason for the decrease in CUE was the increase in microbial respiration rate under the condition of N addition, which was consistent with research results that NO_3^--N and DON were significantly positively correlated with microbial respiration C (Table 2). The soil microbial CUE level was between 0.034 and 0.079, which may be related to the lower soil pH (Table 1). The effect of pH on microbial respiration C may be because, when pH increased, the growth rate of fungi decreased and the growth rate of bacteria increased, and the growth ratio of fungi to bacteria decreases significantly, which in turn increases soil respiration (Silva-Sánchez *et al*. 2019).

Effects of Corn Stalks Returning on Soil Physicochemical Properties

The total N of a single application of urea and a combined application of urea with corn stalks returning was significantly increased by 23% and 21%, respectively, over that of no fertilization. This is because the consumption of N by corn growth and the soil acidification induced by N addition improves the activity of lignin-degrading enzymes and stimulates litter decomposition (Table 1; Hou *et al*. 2021). Soil organic matter, NH_4^+-N, pH, and MBC were not affected by fertilization and corn stalks returning (Table 1), which may be due to the short time of the field experiment. Long-term monitoring is needed to determine the effects of fertilization and corn stalks returning. Compared with urea application, corn stalks returning combined with urea application further increased NO_3^--N by 45%, DOC by 53%, and DON by 122%, similar to Li *et al*. (2021b). Corn stalks returning was considered to improve the physicochemical properties of soil (Chan *et al*. 2002; Wu *et al*. 2021). Wu *et al*. (2022) also showed that straw returning could increase soil NO_3^--N. The increase in NO_3^--N, DOC, and DON may be due to the stimulation of extracellular enzyme activity by the addition of corn stalks (Chen *et al*. 2014), and the decomposition of corn stalks releases macronutrients and changes nutrient availability (Devevre and Horwath 2000). Therefore, corn stalks returning may be beneficial to improving soil fertility in semi-arid cropland, but the effect of improving SOM needs long-term monitoring.

Effects of Corn Stalks Returning on Corn Biological Indicators

The 100 grain weight and unit weight of corn have been shown to be important biological indicators of corn yield and quality (Wang *et al*. 2020; Zhao *et al*. 2023). The application of urea and returning corn stalks to the field had no effect on the 100 grain weight and unit weight of corn (Table 3). The application of nitrification inhibitors and long-term corn stalks returning have a significant impact on 100 grain weight (Wang *et al*. 2023; Zhao *et al*. 2023), while corn varieties have a significant impact on corn unit weight (Li *et al*. 2023). Therefore, the restoration of soil fertility and the improvement of corn productivity in semi-arid cropland may require the cooperation of breeding, improved N fertilizer application and long-term corn stalks returning to the field. No matter whether fertilizing or adding corn stalks, the height of corn was more than 300 cm (Table 3). However, compared with no fertilization, applying urea would increase the height of corn by 4%, which may be because urea provided sufficient N for corn growth (Venterea *et al*. 2011). N application also significantly increased soil total N.

This study found that the no fertilization treatment resulting in harvesting of the most corn stalks, but the corn yield was the lowest. This may be due to the presence of fertilizer residues from the previous season in the cropland, and soybean being a crop from the previous season. Its N fixation effect also promoted more available N in the cropland. In addition, this year's rainfall (655 mm) increased by 36% compared to the average annual rainfall (480 mm), alleviating drought, and promoting corn growth. However, owing to N deficiency, corn yield declined in the later period (Venterea *et al*. 2011). Corn stalks returning and urea combination application not only can ensure corn yield, but it also can reduce corn stalks. This may be because corn stalks addition provides sufficient C source (more DOC), so that more N was immobilized by microorganisms at the early stage of corn growth, reducing the growth of corn stalks, while the later release (more NO_3^--N and DON) promoted the formation of corn yield (Wu *et al*. 2021).

The combined application of corn stalks and urea not only can fertilize the soil, but also it can reduce the consumption of soil fertility by reducing corn stalks production. However, the favorable experimental results of corn stalks returning combined with urea may be disturbed by increased rainfall, and more experimental data are needed in the future to determine the feasibility of corn stalks returning in semi-arid areas (especially the impact on the improvement of organic matter).

CONCLUSIONS

1. Soil microbial carbon use efficiency (CUE) decreased with urea application, while soil microbial CUE increased by adding corn stalks after urea application. The increase in microbial growth C was the main reason for the increase in soil microbial CUE.
2. Corn stalks returning increased soluble organic matter (DOC and DON) and available nutrients (NO_3^--N) in soil.
3. Urea application affected the corn biological indicators, significantly improving corn height and increasing corn yield. In semi-arid cropland, corn stalks returning combined with urea ensure corn yield and reduce corn stalks production.

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