Effects and Comprehensive Evaluation of Different Organic Fertilizer Substitution Ratios on Maize Yield, Soil Organic Carbon Fractions, Nutrients and Heavy Metals

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Research Article

Keywords: organic fertilizer substitution, maize yield, SOC fractions, nutrients, heavy metals

Posted Date: July 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-676927/v1>

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Abstract

Organic fertilizer substitution technology is an effective measure to solve the excessive application of chemical fertilizers in agricultural production. A pot experiment was set up with 5 treatments: no fertilizer (CK) and organic fertilizer substituting 0% (CF), 8% (OF8), 16% (OF16), and 24% (OF24) of chemical N fertilizer to analyze their effects on maize yield, soil organic carbon (SOC) and its fractions, carbon pool management index (CPMI), nutrients and heavy metals to provide a scientific basis for safe fertilizer application to maize. This study found that OF8, OF16, and OF24 all increased the content and proportion of SOC and labile organic C (LOC) fractions, CPMI, most of the middle and trace elements, and heavy metals content and their pollution indices in soil and grain compared to CF. Grain was more vulnerable to pollution compared to soil. There was a strong positive correlation between the content of middle and trace elements, and heavy metal, SOC and its fractions (except LLOC), and organic fertilizer substitution ratio, all with no significant correlation with yield. OF8 and OF16 promoted maize growth with a significant increase yield of 35.65% and 30.28% ($P < 0.05$), respectively. A comprehensive analysis determined the optimum substitution ratio of 8% (OF8), which can reduce chemical fertilizer and increase yield, improve soil fertility, low heavy metal pollution risk, is beneficial to promote sustainable agricultural development.

Keywords: organic fertilizer substitution; maize yield; SOC fractions; nutrients; heavy metals

Introduction

Maize (*Zea mays* L.) is the major grain and feed crop and is important to ensure food security and maintain social stability in China. However, excessive application of chemical fertilizer to ensure yield is common in agricultural production, resulting in reduced soil fertility, increased planting costs and environmental pollution, which is detrimental to the sustainable development of agriculture (Lu et al. 2020). Therefore, a fertilizer application measure that can ensure crop yields, reduce fertilizer application and environmental pollution is urgently needed to achieve sustainable agricultural development. In recent years, the average annual chemical fertilizer application to maize has been 12 Mt, accounting for 20.1% of total chemical fertilizer application (Xu et al. 2019). Therefore, the reduction in the amount of chemical fertilizers applied to maize production is conducive to the achievement of the "Zero Growth Strategy for Chemical Fertilizers".

Studies have shown that the technology of substituting chemical fertilizer with organic fertilizer is the main way to reduce the application of chemical fertilizer, which is of great significance in ensuring crop yield and reducing agricultural environmental pressure (Liu et al. 2015; Xin et al. 2017; Song et al. 2017). Organic fertilizer substitution technology is a fertilization method that combines the advantages and disadvantages of chemical fertilizer and organic fertilizer. It can timely supply the nutrients needed for crop growth in the early growth stage, and effectively coordinate the contradiction between vegetative growth and reproductive growth in the late growth stage (Redding et al. 2016; Ning et al. 2017), it also increases the content of SOC and further affects the soil physicochemical properties and crop yield (Li et al. 2018; Saikia et al. 2015; Manna et al. 2007).

SOC content cannot explain the change of soil quality and the stability of the organic C pool, whereas LOC is easy to transform in soil, which is not only the main energy used by microbes, but also the nutrient pool of plants. Although the proportion in the C pool is very small, it can reflect the small change of soil C pool in a short time, which is a sensitive index to reflect the change of soil C pool (Mandal et al. 2020). Therefore, this is a

more scientific method for using LOC and SOC to jointly evaluate the quality of the organic C pool (Benbi et al. 2015). Loginow et al. (1987) obtained 4 LOC fractions by measuring with 3 concentrations of KMnO_4 (33, 167, 333 mmol/L), and Blair et al. (1995) proposed a CPMI based on this, which can reflect the changes of SOC fractions and indicate the changes of soil fertility and soil quality. In recent years, soil LOC and CPMI have gradually become major indicators for evaluating soil quality (Zhang et al. 2021b; Saha et al. 2021; Duval et al. 2019). Studies have shown that a combined application of organic fertilizer and chemical fertilizer increases SOC content and LOC content, thereby improving soil C pool liability (CL) and CPMI (Zhang et al. 2020; Tang et al. 2020). However, there are few reports of the changes to SOC and its fractions, and CPMI under different organic fertilizer substitution ratios.

In recent years, with the development of China's "*Farmland Soil Quality Improvement Project*" and the implementation of "*Zero Growth Strategy for Chemical Fertilizer*", the demand and application amount of organic fertilizer showed an increasing trend. Organic fertilizer not only contains rich nutrient elements for plant growth and utilization, but also contains heavy metals elements such as Cu, Zn, As, etc. which pose a certain threat to the safety of agricultural products, soil, and the ecological environment (Ning et al. 2017; Zacccone et al. 2010). Therefore, the study on the effects of organic fertilizer substitution on agricultural products and soil heavy metals is of guiding significance for the rational application of organic fertilizer in production. Jia (2017) found that substituting 20% and 40% of chemical fertilizer with pig manure significantly reduced the contents of Cu, Zn, Cd, and Pb in different organs of wheat. While Xia et al. (2020) found that substituting part of chemical fertilizer with pig manure increased the contents of Cu, Zn, As, and Cd in soil, and increased significantly with the increase of the application rate. Ning et al. (2017) found that continued application of commercial organic fertilizer increased the content of Zn, Cd, and Cr in soil, while Xie et al. (2016) applied commercial organic fertilizer not only increased the accumulation of Cr, Cu, Zn, Cd, Pb and as in soil, but also increased the content

of heavy metals in agricultural products, and the degree of influence depended on the amount of commercial organic fertilizer applied. Zhang et al. (2021a) found that organic fertilizer substitution increased the content of Cu and Zn in wheat for two consecutive years, which was lower than the food standard limit in China. At present, research of organic fertilizer substitution on heavy metals mainly focuses on farm manure, and there is little research on commercial organic fertilizer and its different substitution ratios. Most of the above studies focused on the effects of organic fertilizer substitution on SOC and CPMI or heavy metals, while few attentions were paid to the effects of commercial organic fertilizer substituting part of chemical fertilizer on them.

Based on the previous (2017-2018) field experiment results (He et al. 2021), the pot experiment with different organic fertilizer substitution ratios under the condition of equal N, P, and K was carried out. To explore: (i) the effects on maize growth and yield; (ii) the effects of SOC and its fractions, and CPMI; (iii) the effects of nutrients and heavy metals in maize grain and soil; (iv) and through heavy metals risk assessment of soil and grain and comprehensive analysis, and determine the optimum organic fertilizer substitution ratio. It can provide a theoretical basis for reducing chemical fertilizer and increasing yield, improving soil fertility, low risk, and sustainable scientific organic fertilizer substitution in maize production, to promote the green and sustainable development of agriculture.

Materials and methods

Study site

The experiment is located in the Experimental Station of the Agricultural College of Shihezi University, Xinjiang, China. The soil type is calcareous desert soil (*Calcaric Fluvisol*), and the basic physicochemical properties of the soil are pH 7.96, SOC 5.52 g/kg, total nitrogen (N) 0.65 g/kg, available phosphorus (AP) 12.38 mg/kg, available potassium (AK) 149.86 mg/kg.

Materials

Chemical fertilizer uses urea (46.0% N), diammonium phosphate (18.0% N, 46.0% P_2O_5), potassium sulfate (51.0% K_2O), the commercial organic fertilizer (26.10% C, 1.77% N, 1.95% P_2O_5 , and 0.53% K_2O).

Experimental design

Before the start of the experiment (early April 2019), the pot experimental soil was selected from the 0-20 cm soil of the experimental station, and the fresh soil was air-dried and screened with a sieve of 5 mm to remove stones, rhizomes, and other sundries. The compactness of the soil was the same as that of the field soil when the weighed fertilizer was mixed with 10.0 kg soil and packed into a plastic pot. Five treatments were set up: no fertilizer (CK) and organic fertilizer substituting 0% (CF, conventional fertilization), 8% (OF8), 16% (OF16), and 24% (OF24) of chemical N fertilizer, respectively, where the same amount of N, P and K were applied to fertilization treatments. Each treatment was set up with 3 repetitions, 3 times of sampling, a total of 45 pots, random block arrangement. 6 maize seeds (KWS2030) were sown in each pot on 27 April 2019, and 1 plant was left in each pot when the seedlings reached the two-leaf and one-heart stage. The pot fertilization rates of 3.00 g N /pot, 1.80 g P_2O_5 /pot, and 0.60 g K_2O /pot. Before sowing, 40% N, 100%P, 100%K fertilizer, and organic fertilizer were applied as base fertilizer, the remaining 60% N fertilizer was applied as topdressing at the booting stage (Table 1). The weighing method was used to strictly control the soil moisture content throughout the experiment.

115

Table 1 Fertilization schemes for different treatments (g/pot)

Treatment	OFSR (%)	Base fertilizer						Topdressing	Total nutrient contents			
		Chemical fertilizer			Organic fertilizer				N	N	P ₂ O ₅	K ₂ O
		N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O					
CK	–	0	0	0	0	0	0	0	0	0	0	
CF	0	1.20	1.80	0.60	0	0	0	1.80	3.00	1.80	0.60	
8%OF	8	0.96	1.54	0.53	0.24	0.26	0.07	1.80	3.00	1.80	0.60	
16%OF	16	0.72	1.27	0.46	0.48	0.53	0.14	1.80	3.00	1.80	0.60	
24%OF	24	0.48	1.01	0.39	0.72	0.79	0.21	1.80	3.00	1.80	0.60	

Note: Organic fertilizer substitution ratio in different treatments was calculated as follows: OFSR (%) =

$$\frac{\text{Organic fertilizer N contact}}{\text{Total N contact}}$$

Sample collections and chemical determination

Before the start of the experiment, the mixed soil samples were air-dried and sieved to determine the basic physical and chemical properties of the soil. A destructive sampling at the jointing (V6), tasseling (VT), and maturity (R6) stages, collecting soil samples and different organs of maize. The dry matter of maize was determined by the drying method, and the content of total N, P, and K in the plant and the content of SOC, total N, P, and K in soil were determined as reference (Bao, 2000). The total amount of the middle elements (Ca, Mg, S, g/kg), trace elements (Mo, B, Fe, Mn, mg/kg), and heavy metals (Cu, Zn, Cd, Cr, Pb, As, and Ni, mg/kg) in maize grain and soil were extracted by the HCl-HNO₃-HF digestion method and the content of each element was determined by ICP-OES (Thermo ICAP 6300) (Tessier et al. 1979).

2.5. SOC fractions

KMnO₄ concentrations of 33.3 mmol/L, 167 mmol/L, and 333 mmol/L were used to determine the high labile organic C (HLOC, g/kg), high and medium labile organic C (HLOC + MLOC, g/kg), and labile organic C (LOC, g/kg) contents. SOC fractions were measured according to the method of Logninow et al. (1987). The concentrations of MLOC, low labile organic C (LLOC), and recalcitrant organic C (ROC) were calculated as

132 follows:

133
$$\text{MLOC (g/kg)} = (\text{HLOC} + \text{MLOC}) - \text{HLOC} \quad (1)$$

134
$$\text{LLOC (g/kg)} = \text{LOC} - (\text{HLOC} + \text{MLOC}) \quad (2)$$

135
$$\text{ROC (g/kg)} = \text{SOC} - \text{LOC} \quad (3)$$

136
$$\text{The proportion of SOC fractions (\%)} = \text{SOC fraction} / \text{SOC} \times 100 \quad (4)$$

137 2.6. C pool related indices

138 C pool related indices include CL, C pool liability index (CLI), C pool index (CPI), CPMI, which are
139 calculated according to Blair et al. (1995) using the following formula:

140
$$\text{CLI} = \text{CL}_{(\text{organic fertilizer substituting treatment})} / \text{CL}_{(\text{CK})} \quad (5)$$

141
$$\text{CL} = \text{LOC} / \text{ROC} \quad (6)$$

142
$$\text{CPI} = \text{SOC}_{(\text{organic fertilizer substituting treatment})} / \text{SOC}_{(\text{CK})} \quad (7)$$

143
$$\text{CPMI} = \text{CPI} \times \text{CLI} \times 100 \quad (8)$$

144 **Assessment methods of heavy metals pollution**

145 The total amount of heavy metals is a common index to evaluate the degree of heavy metals pollution (Tandy
146 et al. 2009). The single factor index method and Nemerow comprehensive pollution index methods are often
147 used to evaluate the heavy metals pollution of soil and agricultural products (Zhang et al. 2017). Single factor
148 index method calculation formula:

149
$$P_i = C_i / S_i \quad (9)$$

150 Where P_i is the heavy metals pollution index, C_i is heavy metals content; S_i is the limit standard of heavy
151 metals content in soil and grain, referring to China's latest limit standard for soil pollution (GB 15618-2018) and
152 food safety (GB 2762-2017) (Table 2). $P_i \leq 1$ is non-pollution level, $1 < P_i \leq 2$ is light pollution level, $2 < P_i \leq 3$
153 is medium pollution level, and $P_i > 3$ is heavy pollution level.

Table 2 Limit standards of China of heavy metals content in soil and grain

Type	Standard of China	Heavy metals content (mg/kg)						
		Zn	Cu	As	Cd	Cr	Pb	Ni
Soil (pH>7)	GB 15618-2018	300.0	100.0	25.0	0.6	250.0	170.0	190.0
Grain	GB 2762-2017	-	10.0	0.5	0.1	1.0	0.4	-

Nemerow pollution index method calculation formula:

$$P_N = \sqrt{\frac{(P_{i \text{ ave}})^2 + (P_{i \text{ max}})^2}{2}} \quad (10)$$

Where P_N is the Nemerow comprehensive pollution index; $P_{i \text{ ave}}$ and $P_{i \text{ max}}$ are the average and maximum values of the single factor pollution index, respectively. The standard of pollution index is divided into five levels: $P_N \leq 0.7$ is security; $0.7 < P_N \leq 1.0$ is alert; $1.0 < P_N \leq 2.0$ is the mild pollution; $2.0 < P_N \leq 3.0$ is the moderate pollution; $P_N > 3.0$ is the serial pollution (Wu et al. 2020).

Statistical analysis

Data analysed using SPSS 21.0 and then plotted with GraphPad Prism 7.0 and Origin 2018. The statistical methods were one-way analysis of variance and Duncan's test ($P < 0.05$). All figures were combined and processed with Adobe Illustrator CS6.

Results

Analysis of maize growth and yield

At V6, the accumulation of dry matter, N, P, K of maize in different treatments were OF8 > CF > OF16 > OF24 > CK. At the beginning of VT, OF16 was better than CF, and the accumulation of dry matter, N, P, K of maize in different treatments were OF8 > OF16 > CF > OF24 > CK (Fig. 1). At R6, with the increase of organic fertilizer substitution ratios, each index showed a quadratic function of one variable (Fig. 2). OF8 and OF16 are compared with CF, the accumulation of dry matter, N, P, K of maize increased by 25.78%-15.04%,

31.38%-7.11%, 25.25%-6.57%, 18.64%-9.83%, respectively; the yield, N, P, K accumulation of grain significantly increased ($P < 0.05$) by 35.65%-30.28%, 38.89%-31.67%, 29.17%-26.39%, 33.87%-22.48%, respectively (Fig. 1). Therefore, a suitable organic fertilizer substitution ratio (OF8 and OF16) can promote maize dry matter accumulation and nutrient absorption, thereby increasing yield.

Fig. 1 Effects of different organic fertilizer substitution ratios on growth and yield of maize. The different small letters in the picture show a significant difference between treatments ($P < 0.05$), the same below.

Fig. 2 Regression analysis of different organic fertilizer substitution ratios and maize indexes

Analysis of SOC and its fractions

During the whole growth stage (V6, VT and R6), compared with CF, OF8, OF16 and OF24 significantly ($P < 0.05$) increased the content of LOC by 25.92%-40.39%, 22.73%-29.96%, 15.37%-26.23%, respectively, by

increasing the content of different LOC fractions (HLOC, MLOC, and LLOC); the content of ROC increased by -2.90%-6.84%, 4.93%-16.47%, 8.10%-12.85%, respectively; the content of SOC increased by 4.08%-14.97%, 9.88%-20.22%, 12.06%-18.32%, respectively ($P < 0.05$), in which SOC and LOC were OF24 > OF16 > OF8 > CF > CK (Fig. 3-A). The increase of SOC and its fractions content will inevitably lead to the change of its fractions proportion, among them, OF8, OF16, and OF24 compared with CF, the LOC/SOC by 20.98%-22.11%, 8.10%-11.69%, 3.16%-8.58%, respectively; the ROC/SOC decreased by 6.71%-7.07%, 3.12%-4.50%, 1.20%-3.26%, respectively (Fig. 3-B). Therefore, organic fertilizer substitution treatment can increase the content and proportion of SOC and LOC fractions.

Fig.3 Effects of organic fertilizer substitution ratios on the content (A) and proportion (B) of SOC and its fractions. The accumulation sum of different blue columns is the LOC fractions content, and the accumulation sum of all columns is the SOC content.

Analysis of soil C pool related indices

Organic fertilizer substitution treatment increased the C pool related indices in maize growth stage (V6, VT and R6) (Fig. 4). OF8, OF16 and OF24 compared with CF, the CL increased by 25.00%-31.25%, 10.26%-17.95%, 5.26%-13.16%, respectively (Fig. 4-A); the CLI increased by 24.80%-30.40%, 11.11%-17.04%, 4.96%-11.57%, respectively (Fig. 4-B); the CPI increased by 4.67%-14.95%, 9.52%-20.00% ($P < 0.05$),

9.73%-15.93% ($P < 0.05$), respectively (Fig. 4-C); the CPMI increased by 34.25%-50.35%, 28.46%-34.25%, 17.45%-30.31%, respectively ($P < 0.05$), and showed the order of OF24 > OF16 > OF8 > CF > CK (Fig. 4-D).

Fig. 4 Effects of different organic fertilizer substitution ratios on soil C pool related indices

Correlation analyses of SOC and its fractions, C pool related indices, organic fertilizer substitution ratio, and yield

There was a significant positive correlation ($P < 0.05$) between all SOC fractions (except LLOC) and SOC. MLOC, ROC were not significantly positively correlated with LLOC ($P > 0.05$), and the other SOC fractions indicators have a significant positive correlation ($P < 0.05$) (Fig. 5-A). SOC and LOC fractions were significantly positively correlated with CL, CLI, CPI, and CPMI ($P < 0.05$). ROC was significantly positively correlated with CPI ($P < 0.05$), and ROC was significantly negatively correlated with CL ($P < 0.05$) (Fig. 5-C). SOC and its fractions, C pool related indices (CL, CLI, CPI, and CPMI) were significantly positively correlated ($P < 0.05$) with organic fertilizer substitution ratios, but there was no significant correlation with maize yield ($P > 0.05$) (Figs. 5-B, 5-D).

Fig.5 Correlation analysis of SOC fractions (A), organic fertilizer substitution ratios (B), C pool related indices (C), and yield (D). In this figure, *, $P < 0.05$, **, $P < 0.01$. OFSR is the organic fertilizer substitution ratios, in which 0, 8, 16 and 24 are CK, OF8, OF16 and OF24, respectively.

Analysis of nutrients and heavy metals in soil and maize grain

The organic fertilizer substitution treatment obviously improved the content of most of the trace elements and heavy metals in the grain and soil, and there was a strong positive correlation between their content (except Mg in grain) and the SOC and its fractions (LLOC correlation was weak), of which the content of heavy metals was lower than the corresponding standard limits in China (Fig. 6). For the grain, OF8, OF16, OF24 compared to

CF, the Ca, Fe, Zn, Cu, As, Pb and Ni content of the grain increased by 7.87%-13.98%, 13.04%-19.10%, 9.01%-20.46%, 4.46%-29.06%, 22.69%-62.06%, 30.00%-90.00%, 34.35%-89.66%, respectively, which reached a significant positive correlation ($P < 0.05$) with organic substitution ratio (Fig. 6-A). For the soil, OF8, OF16, OF24 compared to CF, the Ca, B, Fe, Mn, Mo, Zn, Cd, Cr, and Pb content of the soil increased by 1.93%-8.80%, 30.17%-68.33%, 0.99%-7.49%, 0.38%-4.59%, 1.37%-14.69%, 3.96%-7.96%, 10.70%-36.79%, 5.18%-18.41%, 5.58%-11.20%, respectively, which reached a significant positive correlation ($P < 0.05$) with organic substitution ratio (Fig. 6-B). In grain and soil, the nutrients and heavy metals with a significant positive correlation ($P < 0.05$) with organic fertilizer substitution ratios were also significantly positively correlated with SOC and its fractions (except LLOC) (Fig. 6).

Maize yield was positively correlated ($P < 0.05$) with grain N, P content, and soil N content, while there was no significant correlation ($P > 0.05$) with the content of middle and trace elements, and heavy metals. This indicates that the effect of N, P on yield was greater than that of middle and trace elements under the condition of organic fertilizer substitution, while some soil heavy metals would affect maize yield and grain heavy metals content. The clustering results showed that the nutrients and heavy metals contents of both soil and grain from the different treatments were divided into three groups: (i) CK, (ii) CF and OF8, (iii) OF16 and OF24 (Fig. 6).

Fig. 6 Effects of different treatments of nutrients and heavy metals in grain (A) and soil (B) and their correlation with organic fertilizer substitution ratios, yield, and SOC fractions. The units for macro (N, P, K) and middle elements (Ca, Mg, S) are g/kg, and for trace elements (Mo, B, Fe, Mn) and heavy metals (Cu, Zn, Cd, Cr, Pb, As, Ni) are mg/kg. *, $P < 0.05$, **, $P < 0.01$, OFSR is the organic fertilizer substitution ratio.

Evaluation of heavy metals pollution in maize grain and soil

The single factor index of heavy metals in soil and grain of each treatment ranged from 0.100 to 0.470, and 0.157 to 0.510, respectively, both of which were non-polluting levels ($P_i < 1$) (Fig. 7-A, 7-B). The comprehensive pollution index of soil and grain of each treatment ranged from 0.277 to 0.373, and 0.292 to 0.452, respectively, the pollution levels were all security ($P_N < 0.7$) (Fig. 7-C). The comprehensive pollution index of grain was higher than that of soil, and all showed $OF24 > OF16 > OF8 > CF > CK$, among them, OF8, OF16, OF24 compared with CF, increased by 5.57%-2.55%, 4.06%-16.56%, respectively.

The clustering results showed that the single factor index for grain and soil were grouped in the same way as the combined pollution indices, and both could be divided into 3 groups, with soil divided into (i) CK, (ii) CF

and OF8, (iii) OF16 and OF24; and grain divided into (i) CK and CF, (ii) OF8, and (iii) OF16 and OF24 (Fig. 7). In short, although the heavy metals pollution index of grain and soil after organic fertilizer substitution treatment did not reach the pollution level, the pollution risk increased with the increase of the organic fertilizer substitution ratio, and the grain was more vulnerable to pollution than soil.

Fig. 7 Evaluation of heavy metals pollution in grain and soil under different treatments

Discussion

SOC is an important component of the soil C pool, which promotes crop growth and improves soil quality. In this study, the organic fertilizer substitution treatments (OF8, OF16, OF24) obviously increased the content of SOC and its fractions during the growth stage (V6, VT, and R6), and its content was significantly ($P < 0.05$) positively correlated with the organic fertilizer substitution ratios. This is because the application of organic fertilizer directly inputs organic C into the soil, increasing microbial activity, accelerating the turnover of organic matter, and releasing more LOC, thereby increasing the content of LOC fraction ($P < 0.05$) and its proportion (Li et al. 2018a, 2018b). At the same time, the ROC content was also increased, which shows that the application of organic fertilizer not only increased the content of SOC and LOC fractions, but also promoted the fixation of organic C in the soil. Some studies found that organic fertilizer application significantly increased SOC and LOC content and was positively related to the amount of organic fertilizer applied (Gai et al. 2018; Li et al. 2017). Organic fertilizer application facilitated organic C fixation (Zhang et al. 2016), while chemical fertilizer

application reduced SOC and LOC content (Li et al. 2018b). CPMI can sensitively reflect the dynamics of the soil C pool and reveal changes in soil quality, and the defining formulas show that CL, CLI, CPI, and CPMI are a set of interrelated indicators (Mandal et al. 2020). In this study, there was a significant positive correlation between CPMI, SOC and LOC fractions content, and organic fertilizer substitution ratios ($P < 0.05$), and the increase of their contents would inevitably lead to the increase of CL, CLI, and CPI, leading to a significant increase of CPMI ($P < 0.05$), all of which showed $OF_{24} > OF_{16} > OF_8 > CF$. This is consistent with the law of SOC and its fractions, then it indicates that organic fertilizer substitution ratios are an important factor in its influence on SOC and its fractions, and CPMI.

In general, OF_8 , OF_{16} , OF_{24} effectively increased SOC and its fractions content, and CPMI, which was beneficial to improve soil fertility and quality, but had no significant correlation with maize yield. Maize yield has a significant positive correlation with grain N, P content, and soil N content ($P < 0.05$), but has no significant correlation with middle and trace elements, and heavy metals content of grain and soil (Fig. 6). This is because the increase in organic fertilizer substitution ratios in this study under equal N, P, and K conditions inevitably led to an increase in the amount of organic fertilizer and a decrease in the amount of chemical fertilizer, reducing the soil AN, AP, AK and increasing the heavy metals content of soil and grain, which was detrimental to maize growth and yield increase, thus each indicator of maize is related to organic fertilizer substitution ratios in a quadratic function (Fig. 2). However, suitable organic fertilizer substitution ratios (OF_8 and OF_{16}) can coordinate the balanced supply of organic and inorganic nutrients to meet the early crop growth on time, and effectively coordinate the relationship between nutrient and reproductive growth in the late reproductive stage, promoting maize dry matter accumulation and nutrient uptake, thus increasing maize yield, which is consistent with some studies (Gai et al. 2018; Li et al. 2017; Zhang et al. 2016; Geng et al. 2019). In addition, HLOC was significantly positively correlated with grain N, P content ($P < 0.05$) (Fig. 6-A), which indicated that organic

fertilizer substitution promoted N and P uptake in the grain by increasing HLOC content, thereby increasing maize yield, and that some studies have been limited to considering the effect of the LOC level on plant growth and development (Li et al. 2020).

Organic fertilizer is not only rich in nutrient elements, but also contains a certain amount of heavy metals. Organic fertilizer application and its amount are key factors affecting the content of heavy metals such as Cr, Cu, Zn, Cd, Pb and As in soil and agricultural products (Wu, et al. 2020), therefore, clarifying the effect of different organic fertilizer substitution ratios of heavy metals in soil and grain can provide a safe fertilizer application for maize provides a scientific basis. This study found that organic fertilizer substitution treatments obviously increased the content of middle and trace elements, and heavy metals in maize grain and soil, and were positively correlated with the organic fertilizer substitution ratios (Fig. 6). This indicates that their contents were affected by the amount of organic fertilizer applied, which is consistent with the results of some studies (Ning et al. 2017; Xie et al. 2021; Zhang et al. 2021a). Through correlation analysis, it was found that most of the middle and trace elements (except grain Mg, soil S), and heavy metals had a significant positive correlation with SOC and its fractions (except LLOC), with the correlation being stronger for the soil than for grain. This indicates that an increase in the content of SOC and its fractions in OF8, OF16, and OF24 inevitably leads to an increase in the content of middle and trace elements, and heavy metals in soil and grain. This may be because SOC contains a variety of functional groups, such as carboxyl, alcohol hydroxyl, enol hydroxyl, etc. which affect the migration and accumulation of heavy metals in the soil through sorption, chelation, and complexation, thus affecting the accumulation of heavy metals in maize grain (Zhao et al. 2014; Leszczynska and Kwiatkowska, 2015).

For this study, OF8, OF16, and OF24 increased the content of SOC and its fractions, CPMI, the middle and trace element content. However, it also increased the heavy metals content in soil and grain. Therefore, while actively promoting the application of organic fertilizer substitution technology, it is important to be alert to the

risk of heavy metals pollution that may be brought about by organic fertilizer application, and to carry out soil and grain heavy metals pollution risk evaluation, which will be of guidance for scientific fertilizer application to maize in agricultural production. This study found that OF8, OF16, and OF24 obviously increased the single factor index and comprehensive pollution index of heavy metals in soil and grain, and neither reached pollution levels. While grain was more susceptible to heavy metals pollution than soil. However, the risk of heavy metals pollution increased with organic fertilizer substitution ratios, indicating that organic fertilizer application was the main cause of heavy metals content in soil and grain. This is consistent with Wu et al. (2020) study, while Ilker Ugulu et al. (2021) found that the application of cattle and poultry manure treatment reduced the risk of heavy metals compared with chemical fertilizer treatment.

The plant and soil effects should be considered comprehensively to determine the suitable organic fertilizer substitution ratio. In this study, in terms of plants, OF8 and OF16 significantly increased the yield ($P < 0.05$), among them, grain nutrients and heavy metals content of OF8 is similar to CF, and its heavy metals pollution risk is also lower than OF16 and OF24 (Figs. 1, 6-7). In terms of soil, organic fertilizer substitution has increased the SOC fractions and CPMI, the soil nutrient and heavy metals content of OF8 are similar to that of CF, and its heavy metals pollution risk is also lower than OF16 and OF24 (Figs. 3-4, 6-7). After comprehensive soil and plant effects, 8% is determined as the optimum organic fertilizer substitution ratio (Fig. 8), which is consistent with our previous field research (He et al. 2021). Therefore, the optimum organic fertilizer substitution ratio is helpful to prevent the risk of heavy metals pollution in soil and agricultural products, and promote the sustainable and healthy development of agricultural production.

348 **Funding** This research was supported by the National Natural Science Foundation of China (31660598,
349 31360501) and the National Key R&D Program of China (2017YFD0201808, 2018YFD0200406).

350 **Authors' contributions** Hao He: Conceptualization, Data curation, Formal analysis, Writing – original draft,
351 Writing – review and editing. Zhenan Hou: Writing – review and editing, Supervision. Sibor Ru: Methodology,
352 Re-sources. Junhua Li: Conceptualization, Funding acquisition, Project administration, Validation, Writing –
353 review and editing. All authors read and approved the final manuscript.

354 **Acknowledgements** The authors are thankful to the support of the National Natural Science Foundation of
355 China (31660598, 31360501) and the National Key R&D Program of China (2017YFD0201808,
356 2018YFD0200406). We thank all the technicians for their valuable technical support.

357 **Declarations**

358 **Ethical approval and consent to participate** Not applicable.

359 **Consent for publication** All authors have read and approved the final version of the manuscript for
360 publication.

361 **Availability of data and materials** All data generated or analyzed during this study are included in this
362 published article.

363 **Competing interests** The authors declare no competing interests.

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