RESEARCH ARTICLE

Nitrogen application practices to reduce cadmium concentration in rice (*Oryza sativa* L.) grains

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Abstract

Cadmium (Cd) pollution in paddy soils creates challenges in rice grain production, thereby threatening food security. The effectiveness of different base-tillering-panicle urea application ratios and the combined basal application of urea and Chinese milk vetch (CMV, *Astragalus sinicus* L.) in minimizing Cd accumulation in rice grains was explored in a Cd-contaminated acidic soil via a field experiment. The results indicated that under similar nitrogen (N) application rates, an appropriate amount of urea applied at the panicle stage or the combined basal application of urea and CMV decreased Cd absorption by rice roots and its accumulation in rice grains, as compared with that of conventional N application (control). Furthermore, under a 3:4:3 base-tillering-panicle urea application ratio or under a high basal application of CMV (37,500 kg hm^{-2}), Cd concentrations in brown rice were significantly lower (40.7% and 34.1%, respectively) than that of control. Cadmium transport coefficient from root to straw was significantly higher than that of control when an appropriate amount of urea was applied at the panicle stage or when urea and CMV were applied basally, whereas the Cd transport coefficient from straw to brown rice was relatively lower. Moreover, soil pH, or the CEC and CaCl$_2$-Cd concentrations under different N fertilizer treatment was not significantly different. However, the rice grain yield increased by 29.4% with basal application of a high CMV amount compared with that of control. An appropriate amount of urea applied at the panicle stage or the combined basal application of urea and CMV decreased Cd absorption by rice roots and inhibited its transport from straw to brown rice, thus reducing Cd concentration in brown rice. Therefore, combined with the key phase of Cd accumulation in rice, a reasonable urea application ratio or a basal application of high CMV amounts could effectively reduce Cd concentration in brown rice.

Keywords Cadmium · Urea · Chinese milk vetch · Transport coefficient · Rice

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Introduction

For majority of the human populace, rice is the primary staple food, feeding more than 50% of the population worldwide (Ladha et al. 2005). Severe anthropogenic activities have polluted paddy fields with heavy metals, particularly in southern China (Chen et al. 2015). Cadmium is a harmful environmental pollutant that can enter the human body via food chains, thereby threatening human health (Rizwan et al. 2017; Chen et al. 2018; Yang et al. 2022). Therefore, Cd pollution in soil-rice systems has become a topic of increasing interest in environmental pollution research worldwide (Hu et al. 2016; Li et al. 2017, 2021).

Effective measures must be adopted to reduce Cd accumulation in rice grains. The application of fertilizers is a promising, low-cost, environmentally friendly, and an effective approach that can mitigate Cd concentrations in the

edible parts of crops grown in contaminated soils (Sarwar et al. 2010; Xu et al. 2015; Wu et al. 2018, 2021). Nitrogen fertilizers are essential for rice production, and their application changes the physicochemical characteristics of paddy soil, including the pH, Eh, and cation exchange capacity (CEC), thereby affecting the soil Cd availability (Yang et al. 2006; Jia et al. 2009; Zhao et al. 2010; Liang et al. 2013; Han et al. 2018; Xu et al. 2020). The Cd uptake by rice is significantly affected by the type, form, application rate, and application period of N fertilizer in contaminated paddy soils (Jia et al. 2010; Xu et al. 2015; Xie et al. 2016; Sun et al. 2018; Zhang et al. 2019). Nitrogen fertilizers can be applied to soil as a base fertilizer or a tillering fertilizer (7–15 days after transplanting). In actual rice cultivation, N fertilizer is typically used as a panicle fertilizer to improve rice yield and N use efficiency (Zhang et al. 2012; Sun et al. 2014). Recent research has revealed that Cd concentrations in Chinese cabbage (*Brassica chinensis* L.) split-treated with ammonium or urea are significantly lower than that treated with single application under similar amounts of N fertilizer (Fan et al. 2017). However, information regarding Cd uptake by rice under different basal and topdressing ratios of N fertilizer is limited.

Some reports have indicated that applying Chinese milk vetch (CMV), a farmland green manure widely cultivated in southern China, can significantly affect Cd uptake and accumulation in rice (Li et al. 2009; Tang et al. 2011; Wang et al. 2013; Wu et al. 2017; Zhang et al. 2020b). Nevertheless, the effects of CMV on Cd uptake by rice have mostly been studied via pot experiments (Li et al. 2009; Wang et al. 2013). Few studies have been conducted under field conditions.

Urea and CMV are two important N sources used in agricultural production (Xu et al. 2020; Jiang et al. 2022; Wang et al. 2022). Currently, the effectiveness of urea and its combination with CMV on Cd absorption and accumulation in rice has not been explored. Therefore, this study aimed to study the effect of different basal and topdressing ratios of urea and its combination with CMV on rice grain yield, and assess Cd and N uptake by rice through a field experiment in Cd-contaminated acidic paddy soil.

Materials and methods

Soil and plants

The location, climate characteristics, and pollution sources of the experimental site are described comprehensively by Wang et al. (2018). The basic physicochemical characteristics of the tested soils are listed in Table 1. The rice cultivar, Zhuliangyou 819, which was used in this study, is a widely planted early rice cultivar in Hunan Province, China.

Table 1 Basic physicochemical properties of the experimental soil and Chinese milk vetch

Parameters	Soil	Milk vetch
pH (H_2O)	4.93	–
Organic carbon (g kg^{-1})	30.83	–
Total N (g kg^{-1})	2.65	29.70
Cation exchange capacity (CEC) (cmol kg^{-1})	12.4	–
Total Cd (mg kg^{-1})	0.94	0.08
CaCl$_2$-extractable Cd (mg kg^{-1})	0.60	–

Field experiment

A randomized block experiment was designed. The area of each experimental plot was 18 m^2 (6 m × 3 m). Five N treatments were considered: (1) ratio of basal-tillering-panicle of N at 6:4:0 (CK), indicating basal application with 60% N and topdressing with 40% N at tillering stage; (2) ratio of basal-tillering-panicle of N at 4:3:3 (N1), wherein basal application was with 40% N, topdressing with 30% N at tillering stage, and topdressing with 30% N at panicle stage; (3) ratio of basal-tillering-panicle of N at 3:4:3 (N2), representing basal application of 30% N, topdressing 40% N at tillering stage, and topdressing 30% N at panicle stage; (4) basal application of 22,500 kg hm^{-2} CMV and urea (basal fertilizer N accounts for 60% of total N), and topdressing 40% N at tillering stage (N3); and (5) basal application of 37,500 kg hm^{-2} CMV and urea (basal fertilizer N accounts for 60% of total N), and topdressing 40% N at tillering stage (N4). Each treatment had three replicates. In all treatments, the application amounts of N, P$_2$O$_5$, and K$_2$O were 225, 112.5, and 225 kg hm^{-2}, respectively. The chemical N, P, and K fertilizers for application were urea, calcium superphosphate, and potassium chloride, respectively. CMV, applied to the plots, was turned over 7 days before transplanting rice. Basal chemical N, P, and K fertilizers were applied on April 20, 2016, 1 day before transplanting rice. The topdressing of urea at the tillering and panicle stages was performed on May 6 and May 30, respectively. Rice was harvested on July 19, 2016.

Sampling and processing

Rice grains in each plot were harvested manually after maturation, and subsequently air-dried and measured. Five rice plants were collected from each plot after reaching maturity. Rice plant sampling and sample processing were according to Wang et al. (2018). After harvesting rice, surface soil samples (0–20 cm) were collected from

each experimental plot using a 5-point method, air-dried indoors, ground with a wood grinder, sieved (< 1 mm), homogenized, and stored.

Sample analysis

Soil pH, organic carbon, CEC, and total Cd were determined based on the methods described by Wang et al. (2018). The concentration of 0.01 M calcium chloride ($CaCl_2$)-extractable Cd in the soil was measured using the method described by Hu et al. (2008).

The plant materials were digested in a mixture of HNO_3 (10 mL) and $HClO_4$ (2 mL) (open system), and the Cd, Zn, Ca, Mg, and Cu concentrations in the solution were determined via inductively coupled plasma-optical emission spectrometry (720; Varian, USA) (Lu 2000). The N concentrations N in the plants and soils were determined using an elemental analyzer (Vario MAX CN, Elementar, Germany).

Quality assurance and control for Cd, Zn, Ca, Mg, and Cu in the plant materials were determined using reagent blanks, triplicate samples, and certified reference materials GBW07602 (GSV-1) obtained from China Standard Materials Research Center, Beijing, China.

Data analysis

Translocation factors (TFs) were estimated using the ratios of Cd concentrations in different organs of the rice plants.

$$TF_{straw/root} = Cd_{straw}/Cd_{root}$$
$$TF_{brown\ rice/root} = Cd_{brown\ rice}/Cd_{root}$$
$$TF_{brown\ rice/straw} = Cd_{brown\ rice}/Cd_{straw}$$

The data were statistically analyzed using SPSS software (SPSS 19.0, SPSS Inc., Chicago, USA). One-way analysis of variance with multiple comparisons using Tukey's honest significant difference (HSD) test was adopted to analyze the statistical significance of differences among various N treatments at $p < 0.05$. In addition, correlation analysis was conducted using bivariate correlations, and Pearson's correlation coefficients at $p < 0.05$ and $p < 0.01$ were determined concurrently.

Results

Soil pH, CEC, and concentration of available Cd

Under similar N dosage, neither CMV application nor different base-tillering-panicle N fertilizer treatment ratios had an effect on the soil pH, CEC, or $CaCl_2$-Cd concentration (Table 2). The soil pH, CEC, and $CaCl_2$-Cd concentration of the five N treatments were 4.68–4.98, 11.47–14.17 cmol

Table 2 Soil pH, cation exchange capacity (CEC), and $CaCl_2$-Cd for different N treatments

Treatments	pH	CEC (cmol kg^{-1})	$CaCl_2$-Cd (mg kg^{-1})
CK	4.75 ± 0.12 a	12.33 ± 0.37 a	0.48 ± 0.04 a
N1	4.91 ± 0.14 a	12.67 ± 0.67 a	0.45 ± 0.03 a
N2	4.68 ± 0.07 a	11.68 ± 0.99 a	0.43 ± 0.01 a
N3	4.98 ± 0.13 a	11.47 ± 0.44 a	0.46 ± 0.02 a
N4	4.93 ± 0.09 a	14.17 ± 0.78 a	0.44 ± 0.02 a

Note: *CK*, control, ratio of basal-tillering-panicle of N=6:4:0; *N1*, ratio of basal-tillering-panicle of N=4:3:3; *N2*, ratio of basal-tillering-panicle of N=3:4:3; *N3*, basal application of 22,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage; *N4*, basal application of 37,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage. Each value is presented as mean ± standard error ($n = 3$), and different letters in a column indicate significant differences at $p < 0.05$, according to Tukey's honest significant difference (HSD) multiple range test
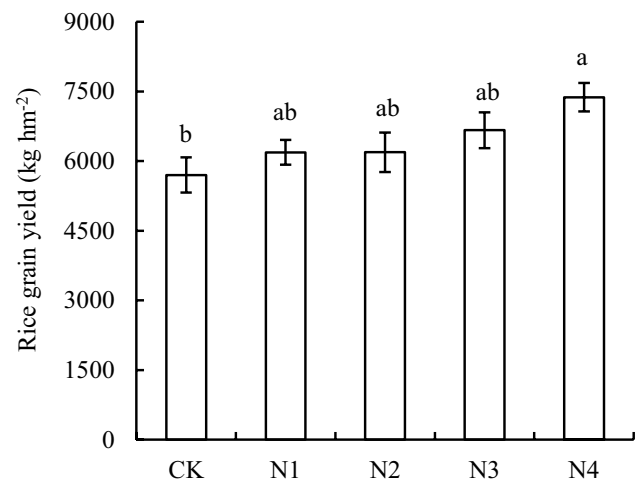

Fig. 1 Effect of different N treatments on rice grain yield. CK, control, ratio of basal-tillering-panicle of N=6:4:0; N1, ratio of basal-tillering-panicle of N=4:3:3; N2, ratio of basal-tillering-panicle of N=3:4:3; N3, basal application of 22,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage; N4, basal application of 37,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage. Data are presented as mean ± standard error ($n = 3$). Different letters above the columns indicate significant differences between the treatments ($p < 0.05$)

kg^{-1}, and 0.43–0.48 mg kg^{-1}, respectively. No significant differences were observed in soil pH, CEC, and $CaCl_2$-Cd among the five N treatments.

Grain dry weight

Rice grain yield was significantly influenced by the different N treatments (Fig. 1). Under the same N dosage, CMV application increased the rice grain yield. However, compared with the control, the grain yield was not significantly

affected by the different ratios of base-tillering-panicle N fertilizer treatment (Fig. 1). The grain yield treated with a combination of urea and a high CMV amount was 29.4% higher than that of the control ($p < 0.05$) (Fig. 1).

Cadmium concentrations in rice tissues

The order of Cd concentrations in various parts of the rice plants was as follows: root > straw > brown rice > husk. The Cd concentrations in the rice tissues were significantly affected by the application of base-tillering-panicle urea, and the combined application of urea and CMV (Fig. 2). The Cd concentrations in the roots of N1, N2, N3, and N4 treatments were reduced by 36.7%, 43.0%, 22.8%, and 33.2% ($p < 0.05$) compared with that in the roots of the control, respectively. Moreover, in the N3 and N4 treatments, the Cd concentrations in the straw significantly increased by 60.3% and 53.5% ($p < 0.05$), respectively (Fig. 2). The Cd concentrations in the husk of the N2 and N4 treatments were 51.5% and 40.6% lower ($p < 0.05$), respectively, than that in the husk of the control. In addition, the Cd concentrations in the brown rice in the N2 and N4 treatments significantly decreased by 40.7% and 34.1% ($p < 0.05$), respectively. Thus, the application ratio of the base-tillering-panicle inorganic N at 3:4:3 and a basal application of 37,500 kg hm^{-2} CMV significantly reduced the Cd concentrations

in brown rice. The Cd concentration in brown rice in this study ranged from 0.73 to 1.23 mg kg^{-1}, which was 3.65 to 6.15 times higher than the national limit of Cd in rice grains (0.2 mg kg^{-1}, GB2762-2017).

Nitrogen concentrations in rice tissues

The application ratio of base-tillering-panicle urea, and the combined application of urea and CMV significantly affected the N concentrations in rice tissues (Fig. 3). The N concentrations in the root, straw, husk, and brown rice were the highest under the N2 treatment conditions. Compared with the control, the N concentration in brown rice in the N2 treatment was 25.9% higher ($p < 0.05$). The N concentrations in the straw and brown rice in the N3 treatment were 42.2% and 27.7% ($p < 0.05$) lower than those in the N2 treatment, respectively. The N concentrations in the root, straw, and brown rice in the N4 treatment were 26.1%, 36.1%, and 26.8% ($p < 0.05$) lower than those of the N2 treatment, respectively.

Concentrations of Zn, Cu, Ca, and Mg in brown rice

The combined application of urea and CMV significantly affected the Zn and Cu concentrations in brown rice, whereas Ca and Mg concentrations displayed no significant

Fig. 2 Effect of different N treatments on Cd concentrations in rice tissues. CK, control, ratio of basal-tillering-panicle of N = 6:4:0; N1, ratio of basal-tillering-panicle of N = 4:3:3; N2, ratio of basal-tillering-panicle of N = 3:4:3; N3, basal application of 22,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage; N4, basal application of 37,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage. Data are presented as mean ± standard error ($n = 3$). Different letters above the columns indicate significant differences between the treatments ($p < 0.05$)
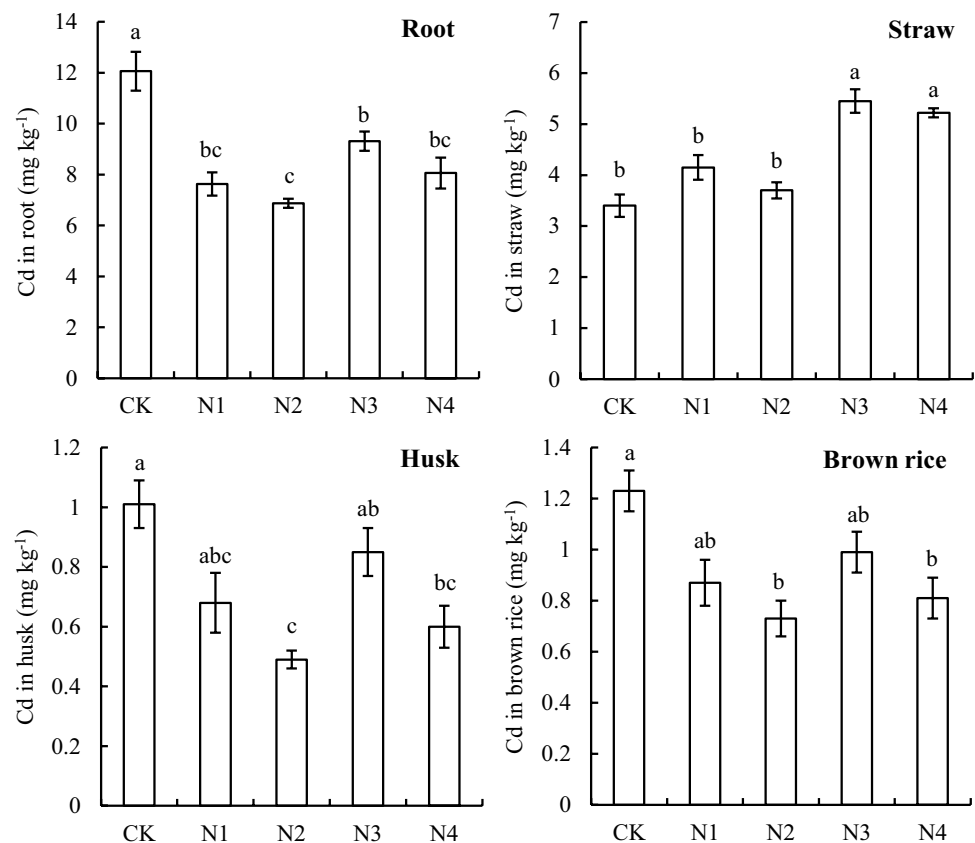
Fig. 3 Effect of different N treatments on N concentrations in rice tissues. CK, control, ratio of basal-tillering-panicle of N=6:4:0; N1, ratio of basal-tillering-panicle of N=4:3:3; N2, ratio of basal-tillering-panicle of N=3:4:3; N3, basal application of 22,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage; N4, basal application of 37,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage. Data are presented as mean±standard error (n=3). Different letters above the columns indicate significant differences between the treatments ($p < 0.05$)
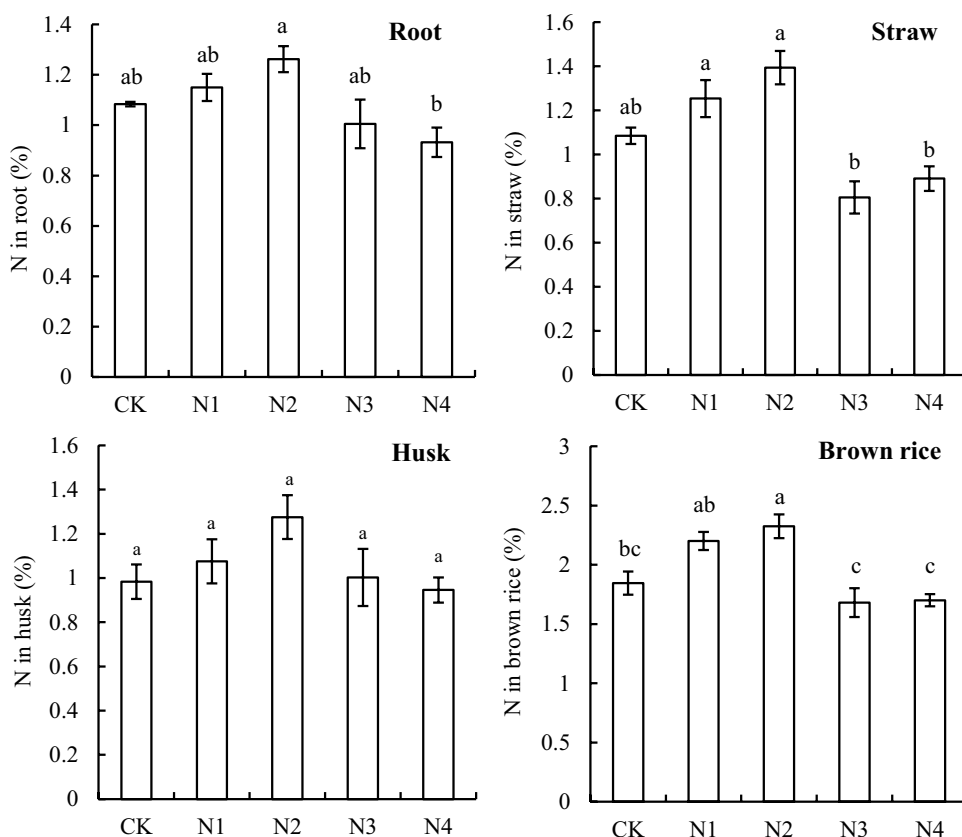

Table 3 Concentration of Zn, Cu, Ca, and Mg in brown rice for different N treatments

Treatment	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
CK	23.80 ± 0.72 ab	4.01 ± 0.03 ab	70.33 ± 2.25 a	939.8 ± 22.6 a
N1	23.83 ± 0.52 ab	4.43 ± 0.33 a	74.87 ± 7.65 a	941.8 ± 33.4 a
N2	24.43 ± 1.03 a	4.11 ± 0.48 ab	72.13 ± 1.84 a	954.4 ± 22.6 a
N3	21.13 ± 1.03 ab	3.79 ± 0.15 ab	78.63 ± 6.42 a	887.7 ± 27.2 a
N4	19.70 ± 1.06 b	3.02 ± 0.03 b	74.05 ± 6.68 a	862.1 ± 26.3 a

Note: *CK*, control, ratio of basal-tillering-panicle of N=6:4:0; *N1*, ratio of basal-tillering-panicle of N=4:3:3; *N2*, ratio of basal-tillering-panicle of N=3:4:3; *N3*, basal application of 22,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage; *N4*, basal application of 37,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage. Each value is presented as mean±standard error (n=3), and different letters in a column indicate significant differences at $p < 0.05$, according to Tukey's honest significant difference (HSD) multiple range test

effect (Table 3). Compared with the control, the Zn and Cu concentrations in brown rice decreased with increase in the basal CMV application rate. The Zn and Cu concentrations in the brown rice of the N4 treatment were 17.2% and 24.7%, respectively, which were lower than that of the control.

Cadmium transport coefficients

Cadmium transport coefficients from root to straw (TF$_{straw/root}$) and from straw to brown rice (TF$_{brown\ rice/straw}$) were significantly affected by the application ratio of base-tillering-panicle

urea and the combined application of urea and CMV (Fig. 4). Compared with the control, the TF$_{straw/root}$ of the N1, N2, N3, and N4 treatments increased by 94.7%, 91.5%, 107.8%, and 131.9%, respectively, while the TF$_{brown\ rice/straw}$ values of the N1, N2, N3, and N4 treatments decreased by 42.2%, 46.3%, 50.4%, and 57.5%, respectively.
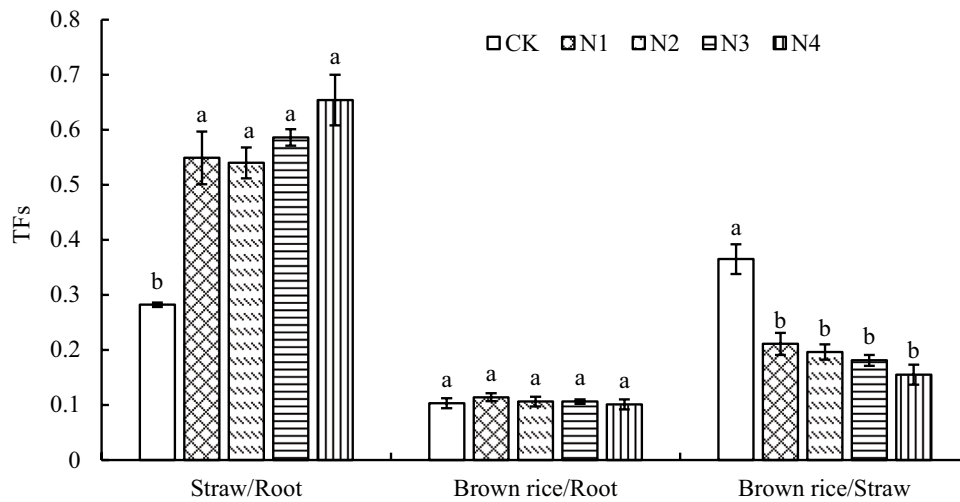
Fig. 4 Effect of N treatment on the translocation factors (TFs) of Cd between root, straw, and brown rice of rice plants. CK, control, ratio of basal-tillering-panicle of N=6:4:0; N1, ratio of basal-tillering-panicle of N=4:3:3; N2, ratio of basal-tillering-panicle of N=3:4:3; N3, basal application of 22,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage; N4, basal application of 37,500 kg hm^{-2} CMV and urea, and topdressing 40% N at the tillering stage. Data are presented as mean ± standard error ($n=3$). Different letters above the columns indicate significant differences between the treatments ($p < 0.05$)

Table 4 Pearson's correlation coefficients (r) between Cd concentration in brown rice and CaCl$_2$-Cd, Cd concentration in root and straw, grain yield, and the translocation factors (TFs) of Cd in rice plants

	R values for Cd concentration in brown rice
CaCl$_2$-Cd	0.600*
Cd concentration in root	0.861**
Cd concentration in straw	−0.134
Rice grain yield	−0.391
N concentration in brown rice	−0.403
Zn concentration in brown rice	0.174
Cu concentration in brown rice	0.098
Ca concentration in brown rice	0.172
Mg concentration in brown rice	−0.052
TFs$_{(straw/root)}$ of Cd	−0.688**
TFs$_{(brown rice/root)}$ of Cd	0.355
TFs$_{(brown rice/straw)}$ of Cd	0.809**

* Correlation is significant at $p < 0.05$

** Correlation is significant at $p < 0.01$

Correlation between Cd in brown rice and Cd in root and straw, soil CaCl$_2$-Cd, and TFs of Cd in rice plants

The Cd concentrations in the brown rice were significantly positively correlated with the soil CaCl$_2$-Cd ($r=0.600$, $p < 0.05$) and root Cd concentrations ($r=0.861$, $p < 0.05$) (Table 4). The correlation between Cd concentration in brown rice and that in TF$_{S(brown rice/straw)}$ was significantly positive ($r=0.809$, $p < 0.05$) (Table 4), while that between

in brown rice and TFs$_{(straw/root)}$ was significantly negative ($r = −0.688$, $p < 0.05$) (Table 4). However, no significant correlation was observed between the Cd concentration and the N, Zn, Cu, Ca, and Mg concentrations in brown rice.

Discussion

Effect of different N fertilizer management practices on rice grain yield

Our results showed that the combined application of CMV as a basal fertilizer under similar total N quantity increased the rice grain yield (Fig. 1). Previous studies have shown that a balanced application of CMV and urea could effectively increase rice grain yield (Gao et al. 2013; Lu et al. 2017; Zhang et al. 2018; Qiao et al. 2021), which is consistent with the results of this study. In addition, the rice grain yield increased with increasing CMV dosage (Fig. 1). This is because excess N accumulated in CMV was possibly released into the soil after its return to the field, which was subsequently absorbed and utilized by rice. When both CMV and urea were applied, urea met the varying nutrient demand during the early growth stage of rice, because the decomposition of CMV was relatively slow. Thus, CMV could continuously release and provide the N required by rice to promote its growth and development (Zhang et al. 2020a). In addition, the low C/N ratio of CMV promotes soil microbial growth and the release of nutrients during organic matter decomposition, which improves the available nutrient

content in soil, and promotes rice growth and development (Cherr et al. 2006).

Effect of different N fertilizer management practices on soil Cd availability

Under similar total N application amounts, the soil $CaCl_2$-Cd concentration in rice at its mature stage was neither significantly affected by the different base-tillering-panicle urea treatments nor the combined application of urea and CMV (Table 2); moreover, the correlation between available Cd and pH was not significant (r = −0.424, p > 0.05). This may be because of the limited effect of N treatment on the soil pH value during maturity (Table 2), which reduced the effect on the available soil Cd. Changes in the soil pH value considerably affect the available Cd in soil, with a negative correlation observed between the two (Zhu et al. 2016; Siddique et al. 2022). Nitrogen fertilizer decreased the soil pH and increased the concentration of available Cd in paddy soil (Zhao et al. 2010). Under 60–100% field water capacity, the soil pH of the urea treatment increased initially and then decreased, while the available Cd concentration decreased initially and then increased during the cultivation process; moreover, pH and available Cd are significantly negatively correlated (Yang et al. 2006; Zhao et al. 2010). During waterlogged cultivation, soil pH in the low and high urea treatment increased initially and then decreased, available soil Cd concentration in the low urea treatment decreased initially, later increased, and finally decreased, whereas the available soil Cd concentration in the high urea treatment decreased initially and then increased. There is no significant negative correlation between soil pH and available Cd during waterlogged cultivation (Jia et al. 2009). Liang et al. (2013) also found that there was no significant relationship between the concentration of available soil Cd and pH in multi-metal contaminated red soil in different urea levels under flooding conditions, and other factors considerably influenced the available Cd. Although pH is a basic factor affecting the available Cd in soil, it is not a limiting factor (Yang et al. 2018). Urea application in the soil can change the soil pH, and then affect the Cd availability. The relationship between available Cd and pH is related to factors, such as urea application level and soil moisture content.

Wang et al. (2013) found that the application of similar amounts of CMV did not significantly affect the available soil Cd in rice in its mature stage. Moreover, Wu et al. (2017) found that the decrease in the available soil Cd with CMV application considerably varied among different areas. In particular, the available Cd in the Fulin and Liling test fields decreased by 1.8–12.1% and 1.5–9.0%, respectively, and the decrease rate was the lowest at the rice maturity stage. Except the following stage, the available Cd concentration in the CMV-treated soil was 10.7–33.6% lower than

that in the control (Zhang et al. 2020a, b). The available Cd concentrations in the CMV-treated soil at 20, 60, and 100 days of cultivation were 10–37%, 7–14%, and 0.3–8% lower, respectively, than that in the control. With increased cultivation time, the difference in the available Cd concentration between the CMV-treated and the control gradually decreased (Cui 2014). However, the effect of CMV on the available Cd in the paddy soil differed, which may be related to the soil properties and sampling period. The dynamic effects of different N application modes on the Cd availability in paddy soils require further research.

The amount of urea significantly affected Cd availability in the soil. The effects of different amounts of urea on the available Cd in neutral purple soil varied significantly with different periods of waterlogged incubation (Jia et al. 2009). On day 15, the available Cd content in the low urea-treated soil was lower than that in the control, and the available Cd in the high urea-treated soil was 32.8% higher. Moreover, on day 30, the available Cd contents in soils treated with low and high urea concentrations were significantly higher than that in the control (28.0% and 45.5%, respectively). On day 60, the available Cd during the low urea-treatment was significantly lower, which significantly increased during the high urea-treatment (Jia et al. 2009). During the 60 days of waterlogged incubation, the available Cd concentration in the acidic red soil first increased and then decreased with increasing urea application rate (Liang et al. 2013). Thus, under waterlogged conditions, the effects of urea dosage on the soil available Cd concentration varied because of differences in the parent material and soil pH. Therefore, under similar N application rate, the dynamic effects of N application on soil Cd availability during the different stages of rice growth must be further studied.

Effect of different N fertilizer management practices on Cd transfer in rice plants

After heading, the Cd in the straws enters the grain through transport and redistribution, which is one of the major sources of Cd in the grains. The transport capacity of phloem for Cd determines the Cd level in the grains (Uraguchi et al. 2009). In addition, the Cd absorbed by the roots from the soil can be transported rapidly to the grains from the rice filling to the maturity stages (Fujimaki et al. 2010). The transport coefficient reflects the absorption, transport, and distribution of Cd in rice plants. The higher the coefficient value, the stronger the migration ability of Cd in rice plants.

Under identical total N application rate, the transport coefficient of Cd from root to straw significantly increased by combining the basal application of urea and CMV, while the transport coefficient of Cd from straw to brown rice decreased (Fig. 4). Some studies have shown that CMV application increases the Cd transport from roots to stems

and from leaves to grains (Wang et al. 2013; Fan et al. 2020). The transfer of Cd from root to straw and from straw to grain was promoted by the application of particulate organic matter (Guo and Zhao 2018). Pig manure, humus soil, and sludge compost significantly increased the Cd transfer from root to stem in moderately polluted soil, while humus soil and sludge compost significantly reduced the Cd transfer from the roots to stems in heavily polluted soil (Liu et al. 2019). The application of rapeseed cake significantly reduced Cd transport from straw to brown rice in Shanyou 63, but increased the transfer from straw to brown rice in Zhongzheyou 1 (Zhou et al. 2013). Thus, the effects of organic materials on Cd transport in rice plants vary, which is related to the organic materials, rice cultivars, and the degree of soil pollution.

Under the same total N application rates, the Cd transport coefficient from roots to straw significantly increased by applying a certain amount of urea at the panicle stage, while that from straw to brown rice decreased (Fig. 4). Zhang et al. (2021a) reported that N application at the initial heading and full heading stages promoted the transport of Cd from roots to straw in Zhuliangyou 819, and decreased its transport from straw to brown rice, which is consistent with our results. Applying a certain amount of N fertilizer at the panicle stage of rice can delay the senescence of leaves and stems and reduce the proportion of assimilates present in leaves and stems from being transported to rice, thereby reducing the Cd content in rice by "hitchhiking".

Effect of different N fertilizer management practices on grain Cd concentration

Our results showed that under identical N application rates, a reasonable ratio of N fertilizer base application, and topdressing at the tillering and panicle stages could reduce Cd content in brown rice, with a minimum value observed in the N2 treatment (Fig. 2). Compared with no N treatment, under similar N application rates (180 kg hm^{-2}), Cd concentrations in grains of low-Cd varieties (Zhuliangyou 819 and Xiangwanxian 12) were not significantly affected by different N fertilizer ratios. Conversely, the Cd concentration in grains of high-Cd varieties (Luliangyou 996 and Yuzhenxiang) decreased significantly by 52.72% and 74.13%, respectively, at a ratio of 6:0:2:2 (Zhang et al. 2021b). In conclusion, under similar total N application rates, the effects of N application ratio at different growth stages on Cd concentration in grains varied for different rice varieties. Applying a certain amount of N fertilizer at the panicle stage of rice decreased the Cd concentration in brown rice (Fig. 2), which was related to the reduction in Cd uptake by roots (Fig. 2), and the Cd in brown rice was significantly positively correlated with root Cd ($p < 0.05$) (Table 4). Thus, the mechanism of

N application at the panicle stage to regulate Cd uptake and transport in rice should be studied further.

Cadmium content in the brown rice and roots decreased when CMV and urea were applied simultaneously (Fig. 2). Some studies observed that the application of CMV reduces Cd concentration in brown rice (Wang et al. 2013; Wu et al. 2017; Zhang et al. 2020b). This is because: (1) The abundance of sulfate-reducing bacteria increases after applying CMV during the rice growth stage, and the Cd activity in the soil at the heading stage decreases with the formation of CdS (Zhang et al. 2020b). Furthermore, CMV could transform exchangeable Cd to oxidizable and residual Cd over time (Li et al. 2012; Wang et al. 2013; Wu et al. 2017), thus, decreasing the soil Cd bioavailability (Zhang et al. 2020b). (2) The rice grain yield increases, and its "dilution effect" reduces the Cd concentration in brown rice to a certain extent (Wang et al. 2013; Wu et al. 2017). (3) Cadmium uptake is inhibited by rice roots (Wang et al. 2013; Wu et al. 2017). In this study, Cd in the brown rice was significantly positively correlated with soil CaCl$_2$-Cd and the root Cd ($p < 0.05$) (Table 4), while no significant difference was observed in the soil CaCl$_2$-Cd between the CMV treatments and the control. In addition, the Cd in brown rice was significantly positively correlated with TFs $_{(brown rice/straw)}$ of Cd (Table 4). The results indicated that under the experimental conditions of this study, the decrease in the Cd concentration in brown rice under the application of CMV was mainly achieved by inhibiting the Cd uptake in rice roots and its transfer from straw to brown rice. However, the mechanism by which CMV regulates Cd uptake and transfer in rice requires further investigation.

Although an appropriate application mode for N fertilizer can significantly reduce the Cd concentration in brown rice, the concentration is more than three times the national standard limit (0.2 mg kg^{-1}, GB2762-2017). Thus, understanding a suitable application method for N fertilizer to reduce Cd concentrations in brown rice to levels lower than the national standard limit under different pollution levels is worth studying. In addition, studying the effect of Cd reduction in brown rice combined with other agronomic measures is also required.

Conclusions

This study verified that in Cd-contaminated paddy soil, urea application at the panicle stage or combined basal application of urea and CMV decreased the Cd absorbed by rice roots and its level in rice grains compared with the conventional N fertilizer application. The 3:4:3 base-tillering-panicle application ratio of urea and the basal application of 37,500 kg hm^{-2} CMV significantly reduced Cd levels in rice grains. Compared with conventional N application,

applying an appropriate amount of urea at the panicle stage or the combined basal application of CMV and urea did not significantly affect soil pH, CEC, and CaCl$_2$-Cd concentration under similar total N application. However, applying a certain amount of urea at the panicle stage or the combined basal application of urea and CMV promoted Cd transport from root to straw, but reduced its transport from straw to brown rice. Furthermore, applying urea at the panicle stage or the combined basal application of urea and CMV reduced the Cd content in brown rice by reducing Cd uptake by rice roots and inhibiting its transport from straw to brown rice. Thus, in Cd-contaminated paddy soil, under the same total N quantity, a suitable application mode of N fertilizer could effectively reduce the Cd concentration in rice grains.

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All authors read and approved the finial manuscript.

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

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors have given their consent to publish this research article.

Competing interests The authors declare no competing interests.

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