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Nitrogen Application Practices to Reduce Cd Concentration in Rice (*Oryza Sativa* L.) Grains

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Abstract

Cd pollution in paddy soils creates challenges in rice grain production, thereby threatening food security. The effectiveness of different base-tillering-panicle urea application ratio 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 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 for basal application of CMV at high levels, Cd concentrations in brown rice were significantly lower (40.7% and 34.1%, respectively) than that of control. Cd 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 urea and CMV were applied basally, whereas the Cd transport coefficient from straw to brown rice was relatively lower. Moreover, soil pH, or the concentrations of CEC and $CaCl_2$-Cd under different N fertilizer treatment was not significantly different. However, rice grain yield increased by 29.4% with basal application of a high amount of CMV 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 amounts of CMV can effectively reduce Cd concentration in brown rice.

Introduction

For most of the human populace, rice is the primary staple food, feeding more than 50% of the population worldwide (Ladha et al. 2005). Owing to the consequence of anthropogenic activities, paddy fields have been polluted with heavy metals, particularly in southern China (Chen et al. 2015). Cd is a harmful environmental pollutant, that can enter the human body via food chain and threaten human health (Rizwan et al. 2017; Chen et al. 2018). Cd pollution in soil-rice systems has become a topic of increasing interest in the field of environmental pollution research worldwide (Hu et al. 2016; Li et al. 2017; Li et al. 2021), and 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; Wu et al. 2021). N fertilizer is essential for rice production, and its 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 soil (Jia et al. 2010; Xu et al. 2015; Xie et al. 2016; Sun et al. 2018; Zhang et al. 2019). N fertilizer 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 treated with ammonium or urea are significantly lower than that treated with single application under similar total amount 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 and accumulation in rice have mostly been studied via pot experiments (Li et al. 2009; Wang et al. 2013), and with few being performed under field conditions.

Urea and CMV are two important N sources used in agricultural production. Currently, the effectiveness of urea and its combination with CMV on Cd absorption and accumulation in rice has not been explored. Therefore, the purpose of this research was 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 at the experimental site are described in detail by Wang et al. (2018). The basic physical and chemical characteristics of the tested soils are described in Table 1. The rice cultivar, Zhuliangyou189, considered for this experiment is a widely planted early rice cultivar used in Hunan Province, China.

Field experiment

A randomized block experiment was designed, and each treatment had three replicates. 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 at 40% N, topdressing at 30% N at tillering stage, and topdressing at 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 22500 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 37500 kg hm^{-2} CMV and urea (basal fertilizer N accounts for 60% of total N), and topdressing 40% N at tillering stage (N4). In all treatments, the application amounts of N, P_2O_5, and K_2O were 225, 112.5, and 225 kg hm^{-2}, respectively. The chemical N fertilizer, P fertilizer and K fertilizers considered 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 fertilizer, P fertilizer and K fertilizer were applied on April 20, 2016, one 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. Details regarding rice plant sampling and sample processing are provided by Wang et al. (2018). After harvesting rice, surface soil samples (0 – 20 cm) were collected from each experimental plot using a five-point method, and were 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), following which Cd, Zn, Ca, Mg, and Cu concentrations in the solution were determined via inductively coupled plasma-optical emission spectrometry (ICP-OES 720; Varian, USA) (Lu 2000). The concentrations of N in the plants and soils were determined using an elemental analyzer (Vario MAX CN, Elementar, Germany).

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). A one-way analysis of variance with multiple comparisons by Tukey's honest significant difference (HSD) test was adopted to analyze the statistical significance of differences among various N treatments at $p < 0.05$ level. In addition, correlation analysis was conducted by 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 the different base-tillering-panicle N fertilizer treatment ratios had an effect on the soil pH, CEC, or $CaCl_2$-Cd concentration (Table 2).

Grain dry weight

Rice grain yield was significantly influenced by various N treatments (Fig. 1). Under the same N dosage, the application of CMV increased rice grain yield. However, compared with the control, the grain yield was not significantly affected by different ratios of base-tillering-panicle N fertilizer treatment (Fig. 1). The grain yield treated with a combination of urea and a high amount of CMV was 29.4% higher than that of the control ($p < 0.05$) (Fig. 1).

Cd concentrations in rice tissues

The order of Cd concentrations in various parts of the rice plants were 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 compared with that in control were 36.7%, 43.0%, 22.8%, and 33.2% lower ($p < 0.05$), respectively. Meanwhile, in 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 N2 and N4 treatments compared with that in control were 51.5% and 40.6% lower ($p < 0.05$), respectively. In addition, the Cd concentrations in the brown rice in N2 and N4 treatments significantly decreased by 40.7% and 34.1% ($p < 0.05$), respectively. Thus, the application ratio of base-tillering-panicle inorganic N at 3:4:3 and a basal application of 37500 kg hm^{-2} CMV could significantly reduce the Cd concentrations in brown rice. The Cd concentration in brown rice in this study ranged from 0.73 mg kg^{-1} 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).

N 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 N2 treatment conditions. Compared with the control, the N concentration in brown rice in N2 treatment was 25.9% higher ($p < 0.05$). The N concentrations in the straw and brown rice in N3 treatment were 42.2% and 27.7% ($p < 0.05$) lower than those in N2 treatment, respectively. The N concentrations in the root, straw, and brown rice in the N4 treated were 26.1%, 36.1%, and 26.8% ($p < 0.05$) lower than those of the N2 treated, 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 effect (Table 3). Compared with the control, the concentrations of Zn and Cu in brown rice decreased with increasing basal CMV application rate. The Zn and Cu concentrations in the brown rice in the N4 treatment was 17.2% and 24.7%, respectively, which was lower than that of the control.

Cd transport coefficients

Cd 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 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 N1, N2, N3, and N4 treatments decreased by 42.2%, 46.3%, 50.4%, and 57.5%, respectively.

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 and root Cd concentrations ($p < 0.05$) (Table 4). The correlation between Cd concentration in brown rice and that in $TF_{S\ (brown\ rice/straw)}$ was significantly positive ($p < 0.05$) (Table 4), while that between in brown rice and $TFs_{(brown\ rice/root)}$ was significantly negative ($p < 0.05$) (Table 4).

Discussion

Effect of different N fertilizer management practices on rice grain yield

Our results showed that combined application of CMV as a basal fertilizer under similar total N quantity increased the 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), 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 the excess N accumulated in CMV is released into the soil after its return to the field, which is subsequently absorbed and utilized by rice. When CMV and urea were both applied, urea met the varying nutrient demand during the early growth stage of rice, since the decomposition of CMV was relatively slow. Thus, CMV can continuously release and provide the N needed 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

The results of this study showed that under simialr total N application amount, 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). 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 available soil Cd.

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. Meanwhile, Wu et al. (2017) found that the decreases in available soil Cd with CMV incorporation considerably varied with different test 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 fallowing stage, the DTPA-Cd (0.05 M diethylene-triamine-pentaacetic acid, 0.1 M triethanolamine, and 0.01 M $CaCl_2$) concentration in CMV-treated soil was 10.7% – 33.6% lower than that in the control (Zhang et al. 2020). The DTPA-Cd concentration in CMV-treated soil at 20, 60, and 100 days of cultivation was 10% – 37%, 7% – 14%, and 0.3% – 8% lower, respectively, than that in the control. With increased cultivation time, the difference in DTPA-Cd concentration between the CMV-treated and the control gradually decreased (Cui 2014). However, the effect of CMV on 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 Cd availability in paddy soils require further study.

The amount of urea significantly affected Cd availability in the soil. The effects of different amounts of urea on available Cd in neutral purple soil varied significantly with different periods of waterlogged incubation (Jia et al. 2009). At 15 days, 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. Meanwhile, at 30 days, the available Cd in soils treated with low and high urea concentrations were significantly higher than that in the control (28.0% and 45.5%, respectively). At 60 days, the available Cd during low urea treatment was significantly lower, which significantly increased during 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 soil available Cd concentration varied because of parent material and soil pH. Therefore, under similar N application rate, the dynamic effects of N application on soil Cd availability during 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 enter the grain through transport and redistribution, which is one of the major source of Cd in the grain. The transport capacity of phloem to Cd determines the Cd level in the grains (Uraguchi et al. 2009). In addition, the Cd sorbed by roots from the soil can be transported rapidly to grains from rice filling to the maturity stage (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 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 et al. 2018). Pig manure, humus soil, and sludge compost significantly increased Cd transfer from root to stem in moderately polluted soil, while humus soil and sludge compost significantly reduced the Cd transfer from 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). 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 rate, Cd transport coefficient from root 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). 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 (Zhang et al. 2021a), 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, reducing 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 rate, a reasonable ratio of N fertilizer base application, and, topdressing at the tillering and panicle stages could reduce Cd Cd in brown rice, reaching a minimum in 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, whereas 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 rate, 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 requires further study.

Cd 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). The reasons for this decrease are as follows: (1) The abundance of sulfate-reducing bacteria increased after applying CMV during the rice growth stage, and the Cd activity in the soil at the heading stage decreased 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), decreasing soil Cd bioavailability (Zhang et al. 2020b). (2) The rice grain yield increased, and its "dilution effect" reduced the Cd concentration in brown rice to a certain extent (Wang et al. 2013; Wu et al. 2017). (3) Cd uptake was 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 soil $CaCl_2$-Cd between the CMV treatments and the control. In addition, the Cd in brown rice significantly negatively correlated with $TFs_{(brown\ rice/root)}$ of Cd (Table 4). The results indicate that under the experimental conditions of this study, the decrease in Cd concentration in brown rice with 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.

Conclusions

This study validates that in Cd-contaminated paddy soil, applying a certain amount of urea at the panicle stage or the combined basal application of urea and CMV decreased the Cd absorbed by rice roots and its level in rice grains compared to that of conventional N fertilizer application. The 3:4:3 base-tillering-panicle application ratio of urea and the basal application of 37500 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 simialr 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.

Although an appropriate application mode for N fertilizer can significantly reduce the Cd concentration in brown rice, the concentration is more than 3 times the national standard limit (0.2 mg kg^{-1}, GB2762-2017). Thus, under what levels of pollution, can a suitable application method for N fertilizer reduce Cd concentrations in brown rice to levels lower than the national standard limit is worth studying. In addition, the effect of Cd reduction in brown rice combined with other agronomic measures also needs to be further studied.

Declarations

Author contribution

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethical approval Not applicable.

Consent to participate Not applicable.

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

Competing interests The authors declare no competing interests.

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Tables

Table 1

Basic physical and chemical properties of the experimental soil and Chinese milk vetch

	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	–

Table 2

Soil pH, cation exchange capacity (CEC), and $CaCl_2$-Cd treated with 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 is 6:4:0; N1, ratio of basal-tillering-panicle of N is 4:3:3; N2, ratio of basal-tillering-panicle of N is 3:4:3; N3, basal application of 22500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage; N4, basal application of 37500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage. Each value is presented as the mean ± standard error (n = 3), and different letters in a column indicate a significant difference at $p < 0.05$ level, according to Tukey's honest significant difference (HSD) multiple range test.

Table 3

Concentration of Zn, Cu, Ca, and Mg in brown rice treated with 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 is 6:4:0; N1, ratio of basal-tillering-panicle of N is 4:3:3; N2, ratio of basal-tillering-panicle of N is 3:4:3; N3, basal application of 22500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage; N4, basal application of 37500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage. Each value is presented as the mean ± standard error (n = 3), and different letters in a column indicate a significant difference at $p < 0.05$ level, according to Tukey's honest significant difference (HSD) multiple range test.

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

	Cd 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
$TFs_{(straw/root)}$ of Cd	-0.688**
$TFs_{(brown\ rice/root)}$ of Cd	0.355
$TFs_{(brown\ rice/straw)}$ of Cd	0.809**

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

Figures

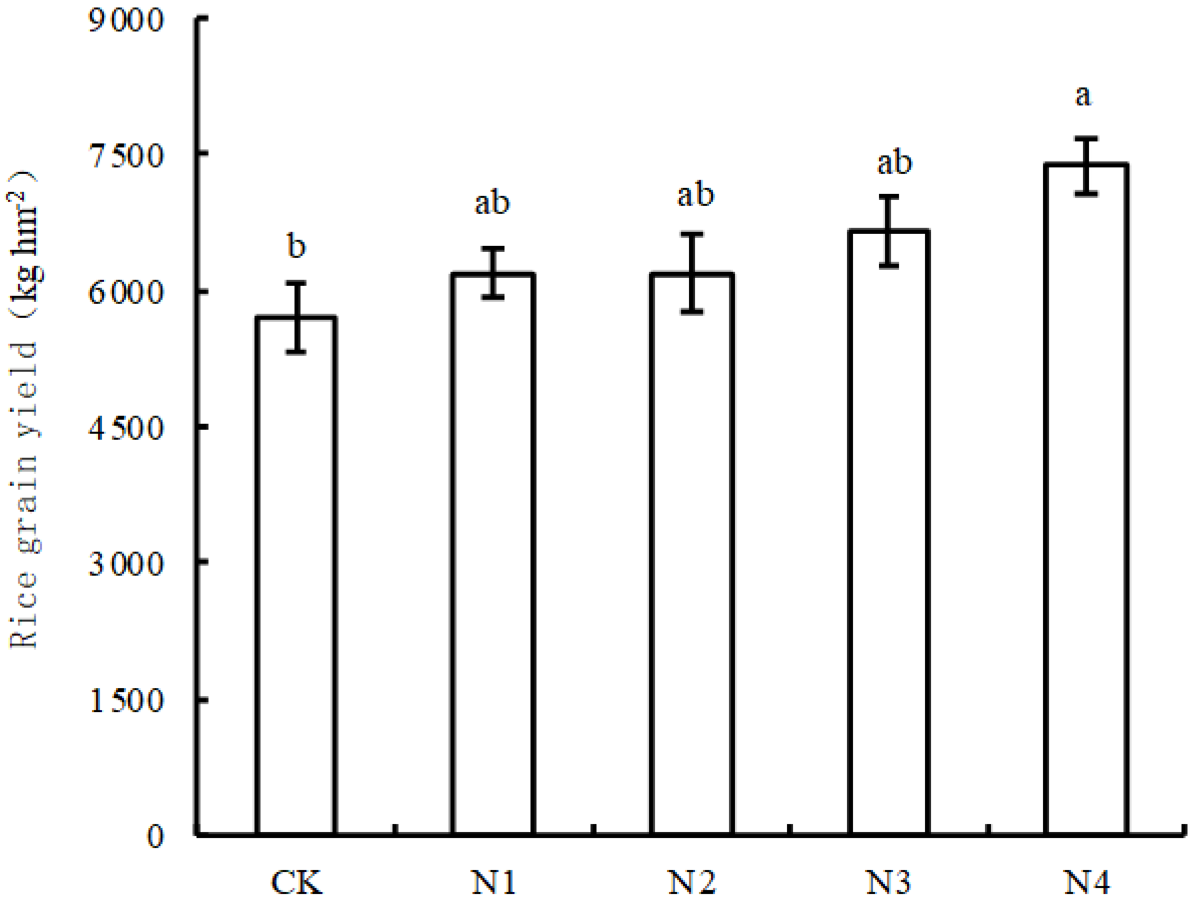

Figure 1

Effect of different N treatments on rice grain yield. CK, control, ratio of basal-tillering-panicle of N is 6:4:0; N1, ratio of basal-tillering-panicle of N is 4:3:3; N2, ratio of basal-tillering-panicle of N is 3:4:3; N3, basal application of 22500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage; N4, basal application of 37500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage. Data are presented as the mean ± standard error (n = 3). The different letters above the columns indicate significant differences between treatments (p < 0.05).

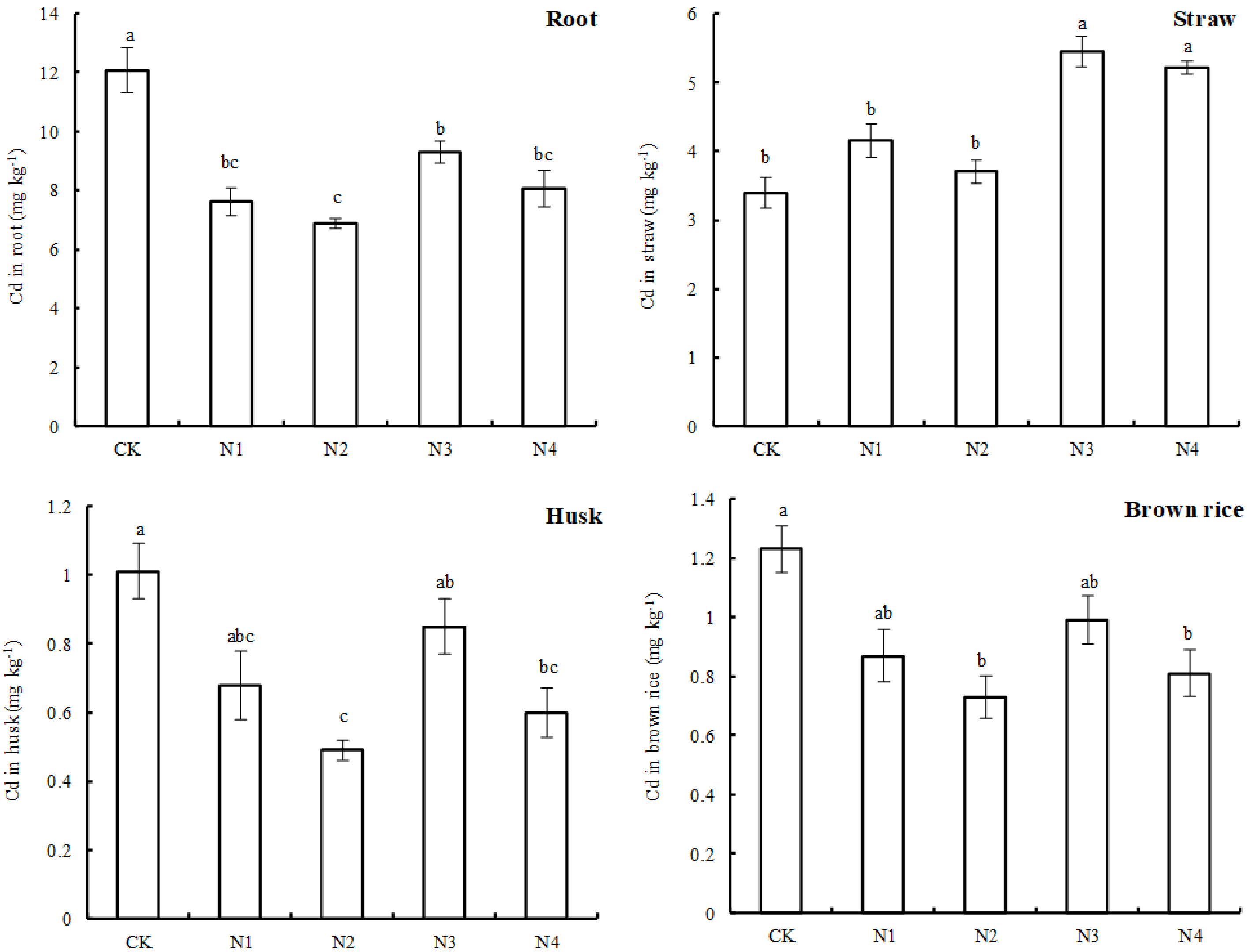

Figure 2

Effect of different N treatments on Cd concentrations in rice tissues. CK, control, ratio of basal-tillering-panicle of N is 6:4:0; N1, ratio of basal-tillering-panicle of N is 4:3:3; N2, ratio of basal-tillering-panicle of N is 3:4:3; N3, basal application of 22500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage; N4, basal application of 37500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage. Data are presented as the mean ± standard error (n = 3). The different letters above the columns indicate significant differences between treatments (p < 0.05).

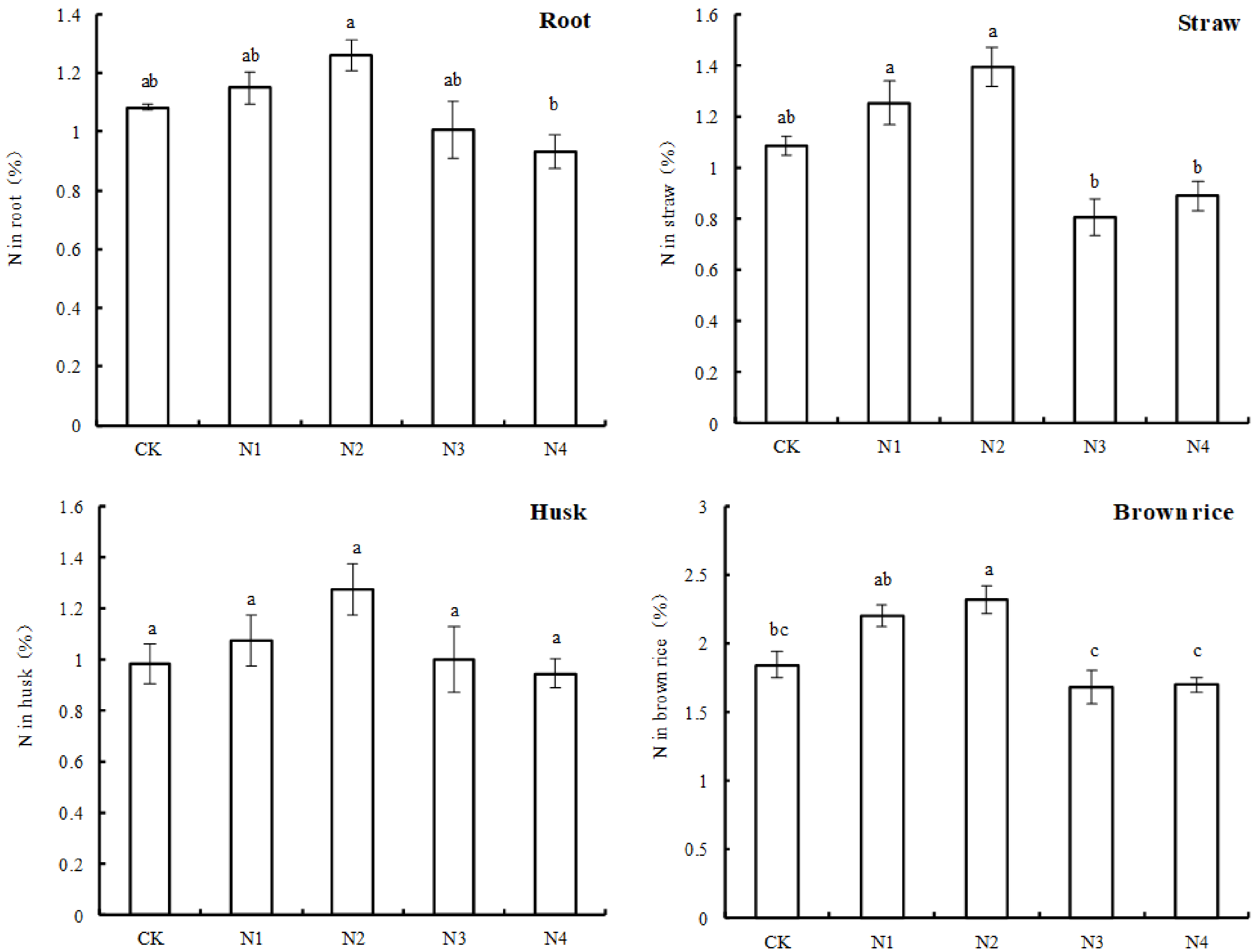

Figure 3

Effect of different N treatments on N concentrations in rice tissues. CK, control, ratio of basal-tillering-panicle of N is 6:4:0; N1, ratio of basal-tillering-panicle of N is 4:3:3; N2, ratio of basal-tillering-panicle of N is 3:4:3; N3, basal application of 22500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage; N4, basal application of 37500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage. Data are presented as the mean ± standard error (n = 3). The different letters above the columns indicate significant differences between treatments ($p < 0.05$).

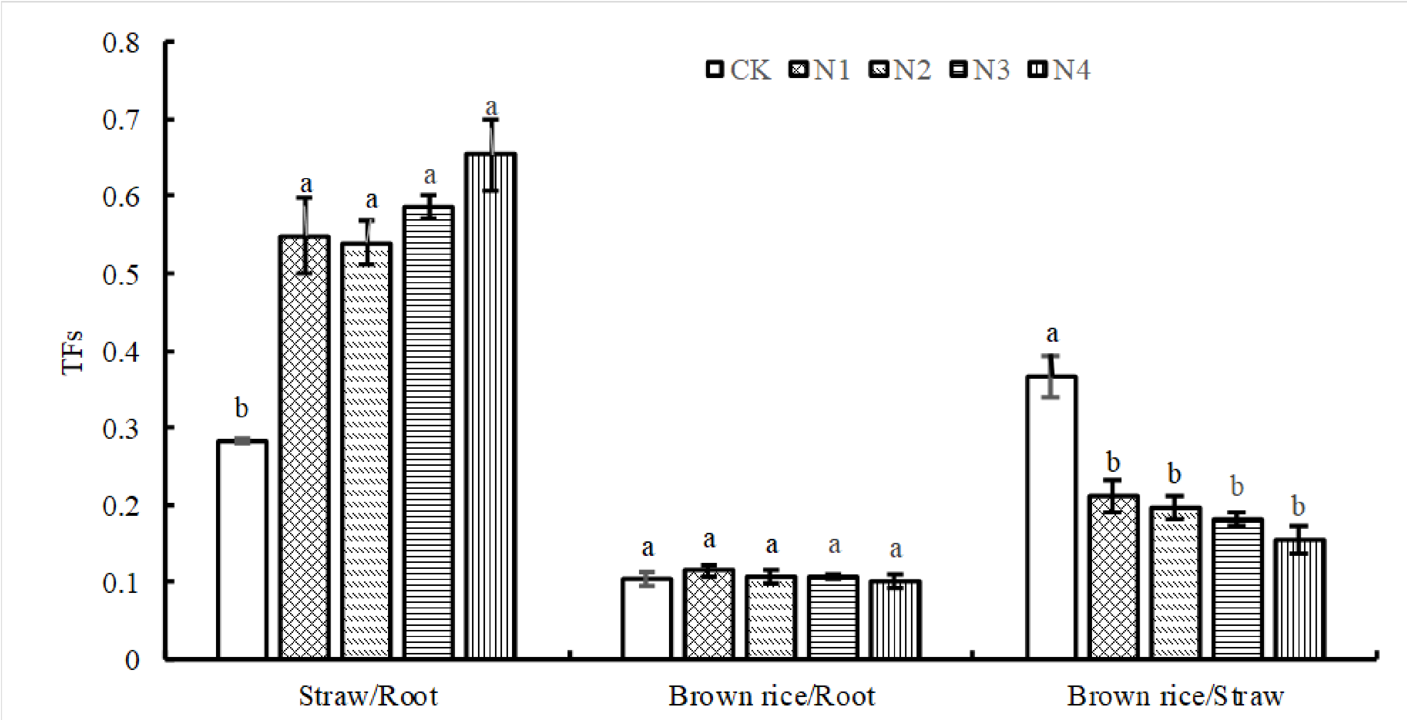

Figure 4

Effect of N treatment on translocation factors (TFs) of Cd between root, straw, and brown rice of rice plants. CK, control, ratio of basal-tillering-panicle of N is 6:4:0; N1, ratio of basal-tillering-panicle of N is 4:3:3; N2, ratio of basal-tillering-panicle of N is 3:4:3; N3, basal application of 22500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage; N4, basal application of 37500 kg hm^{-2} CMV and urea, and topdressing 40% N at tillering stage. Data are presented as the mean ± standard error (n = 3). The different letters above the columns indicate significant differences between treatments (p < 0.05).