



Two-year and multi-site field trials to evaluate soil amendments for controlling cadmium accumulation in rice grain[☆]

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ABSTRACT

Representing the staple crop for half of the world population, rice can accumulate high levels of cadmium (Cd) in its grain, posing concerns on food safety. Different soil amendments have been proposed to decrease Cd accumulation in rice grain by either decreasing soil Cd availability, introducing competitive ions on Cd uptake, or down-regulating the expression of transporters for Cd uptake. However, the effectiveness of soil amendments applied alone or in combinations needs to be tested under field conditions. Here, we present results of field trials with two rice cultivars differing in Cd accumulation grown at three field sites in southern China in two years, to investigate the effects of two Mn-containing soil amendments (MnO_2 , Mn-loaded biochar (MB)), Si fertilizer (Si), limestone, and K_2SO_4 , as well as interactions among MnO_2 , Si, and limestone on decreasing Cd accumulation in rice grain. We found that single applications of MnO_2 or MB to acidic soils low in Mn decreased grain Cd concentrations by 44–53 % or 78–82 %, respectively, over two years without decrease in performance. These effects were comparable to or greater than those induced by limestone liming alone (45–62 %). Strong interactions between MnO_2 and limestone resulting from their influence on soil extractable Cd and Mn led to non-additive effects on lowering grain Cd. MB addition minimized grain Cd concentrations, primarily by increasing extractable and dissolved Mn concentrations, but also by decreasing Cd extractability in soil. In comparison, Si and K_2SO_4 amendments affected grain Cd levels only weakly. We conclude that the amendments that decrease labile Cd and increase labile Mn in soils are most effective at reducing Cd accumulation in rice grain, thus contributing to food safety.

1. Introduction

Cadmium (Cd) accumulation in rice grain poses a serious threat to food safety and human health in many rice-growing regions of the world. For example, special concern has been placed to southern China where Cd release from decades of mining and smelting, acidic soil conditions, and intensive rice cultivation overlap (Hu et al., 2016; Zhao et al., 2015), leading to wide-spread Cd contamination in rice (Chen et al., 2018a, 2018b, 2018c; Zhu et al., 2016). In some of the contaminated areas, intake of Cd from consumption of rice by the local populations has exceeded the tolerable intake limit recommended by the FAO/WHO (Chen et al., 2018a, 2018b, 2018c; Wang et al., 2019), posing serious threat to human health (Wang et al., 2019).

The transfer of Cd from soil to rice grain can be divided into (i) a soil-related part, including Cd mobilization and transport of Cd to the root surface, and (ii) a plant-related part comprising Cd uptake by roots and translocation of Cd from roots to shoot and into the grain. As an intermediate element in the hard-soft metal classification (Smith et al., 2002), Cd exhibits a medium affinity to oxygen groups and a high affinity to reduced sulfur groups (Karlsson et al., 2007). Consequently, Cd is mainly weakly bound to negatively charged surfaces or oxygen groups of minerals and organic matter in oxic soils (Loganathan et al., 2012), e.g., when the rice fields are drained. Under anoxic conditions, strong Cd binding to reduced inorganic and organic sulfur-containing groups can be established, e.g., during flooding of paddy soils (Fulda et al., 2013; Furuya et al., 2016). Thus, Cd mobility in oxic soils is controlled by adsorption to

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solids such as oxides, clay minerals and organic matter, which are strongly influenced by soil pH (Lee et al., 1996; Loganathan et al., 2012). Under oscillating redox conditions, the availability of reduced sulfur ligands additionally controls Cd mobilization in soil (Fulda et al., 2013). Paddy soils which are acidic under oxic conditions are widespread in southern China (Zhu et al., 2016). Soil flooding increases soil pH to circumneutral and induces microbial sulfate reduction (Kögel-Knabner et al., 2010), with both processes favoring Cd immobilization in soils. In contrast, soil drainage triggers reoxidation of Cd sulfides and other reduced chemical species by O₂. Thereby, Cd sulfide oxidatively dissociate and soil acidity is recovered during soil drainage, making Cd in soil highly available to plant uptake (Huang et al., 2021a, 2021b).

Being a non-essential element for plants, Cd²⁺ uptake occurs mainly via membrane transporters for the uptake of essential divalent cations, such as Mn²⁺, Zn²⁺, Fe²⁺, and Ca²⁺ (Zhao and Wang, 2020). Specifically, uptake of Cd into rice roots occurs through manganese (Mn) transporters, most importantly OsNramp5 (Ishikawa et al., 2012; Sasaki et al., 2012), and to a smaller degree OsNramp1 (Chang et al., 2020) and OsCd1 (Yan et al., 2019). After entering rice roots, a substantial fraction of Cd is sequestered into root vacuoles via OsHMA3, a tonoplast Cd transporter (Ueno et al., 2010; Yan et al., 2016), and possibly stored as Cd-phytochelatin (PCs) complexes. A portion of Cd is loaded into the xylem possibly through OsHMA2 and OsZIP7 (Satoh-Nagasaki et al., 2012; Tan et al., 2019). Finally, OsHMA2, OsZIP7, OsLCT1, and OsCCX2 are likely involved in the inter-vascular transfer of Cd in rice nodes (Hao et al., 2018; Tan et al., 2019; Uraguchi et al., 2011; Yamaji et al., 2013a, 2013b), a process that controls the distribution of Cd to the grain.

Two types of strategies can be employed to minimize Cd accumulation in rice grain, i.e., decreasing Cd lability in soil and decreasing Cd uptake and/or translocation in plants (Zhao and Wang, 2020). To lessen Cd lability in paddy soil, prolonged soil flooding during rice cultivation especially during the critical grain-filling stage before harvest has been shown to be effective for acidic soils by keeping soil pH in the neutral range (Chen et al., 2020; Honma et al., 2016) and also by precipitation of Cd sulfides (Fulda et al., 2013). However, the need for dry ground for machine-harvesting of rice and variations in the weather conditions before harvest make precise control on the timing of soil drainage often impractical. Soil amendments targeted to decrease soil Cd lability could prove beneficial. Amendments designed to decrease competitive Cd desorption by other cations (mainly protons) and/or to increase sorption sites such as liming and biochar applications have been shown to be effective to decrease grain Cd concentrations in field trials (Bian et al., 2014; Chen et al., 2018a, 2018b, 2018c; Cui et al., 2016, 2019; Zhu et al., 2016). Different to lime(stone), biochar could immobilize Cd in soil by introducing a large amount of sorption sites even without significantly increasing soil pH (El-Naggar et al., 2018; Qi et al., 2018). Sulfur amendments are also expected to introduce additional sulfides or thiols to promote Cd precipitation or sorption in flooded soils (Fulda et al., 2013). However, its actual performance under field conditions is inconsistent (G. Wang et al., 2020; Yuan et al., 2019).

Plant uptake and translocation of Cd may be decreased by increasing the supply of competitive ions. For example, it has been shown that increasing Mn supply in hydroponic culture decreased both Cd uptake and translocation by rice (Yang et al., 2014). Low levels of Mn in some paddy soils are thought to be a factor contributing to high Cd accumulation in rice (Wang et al., 2018). A Mn-loaded biochar has been shown to decrease grain Cd accumulation in a pot experiment (Zhou et al., 2018). Although these authors attributed its effect to enhanced Cd immobilization in soil and Fe/Mn plaque formation, it is possible that the competition by Mn on Cd uptake made a major contribution. Compared to Mn, the effects of other competitive ions are less consistent. Studies applying Zn (Cai et al., 2019; Green et al., 2017; Hassan et al., 2005), Fe (Nakanishi et al., 2006; Shao et al., 2007), and Ca (Ye et al., 2020) in hydroponic systems have shown a tendency of decreasing root Cd accumulation but increasing root-to-shoot translocation, resulting in inconsistent performance regarding Cd levels in rice shoot or grain.

Soil amendments may also influence rice grain Cd levels by regulating the expression of transporter genes or facilitating *in-planta* Cd sequestration by phytochelatins. Silicon was shown to decrease root and shoot Cd concentrations by down-regulating the expression of OsNramp5 and OsHMA2 in a hydroponic experiment (Shao et al., 2017). Wang et al. also showed that some Si fertilizers decreased Cd accumulation in rice straw and grain in a field trial (Wang et al., 2016). However, the effects of soil Si fertilization were inconsistent (Li et al., 2020; Wang et al., 2016). In addition to the potential effect on controlling Cd solubility in paddy soil, sulfur supply was suggested to decrease Cd uptake and translocation in rice plant by enhancing iron plaque formation as well as Cd chelation and vacuole sequestration in roots (Cao et al., 2018).

Except for liming, most of the aforementioned soil amendments to decrease Cd accumulation in rice have mainly been tested in hydroponic or pot experiments, with few or inconsistent results from field trials. However, it is important that any amendments are evaluated under field conditions, taking into account variations in soil properties, climatic conditions, and rice cultivars. The aim of this study was to evaluate the effects of six soil amendments including MnO₂, Mn-loaded biochar, limestone, Si fertilizer, K₂SO₄ and a slow-release nitrate fertilizer, as well as amendment interactions via selected combinations, on Cd accumulation in rice grain of two cultivars. The effects of these amendments on As accumulation will be reported separately.

2. Materials and methods

2.1. Selection of field sites

Field trials were carried with two rice cultivars grown at three field sites in Hunan province of China (Fig. 1). The three sites were selected because of the high risk of producing Cd and As contaminated rice based on preliminary soil data. In 2018, the experiment was carried out on two paddy fields in Changsha (CS) and Xiangtan (XT). In 2019, the same field in CS was used again in order to investigate possible second-year effects. Additionally, the experiment was carried out on a third paddy field in Hengnan (HN) in 2019. Selected properties of the soils without amendments are reported in Table S1 (supplementary material).

2.2. Soil amendments, rice cultivars, and experimental design

Twelve treatments were used with four replicates in a randomized block design consistently for all four field trials (Fig. 1). These include a control (C) without soil amendments, and six single amendments comprising addition of limestone (L), MnO₂ (Mn), a Si fertilizer (Si), a slow-release potassium nitrate fertilizer (N), K₂SO₄ (S), and Mn-loaded biochar (MB), respectively. In addition, four combined treatments of L, Mn, and Si amendments (L + Mn, L + Si, Mn + Si, L + Mn + Si), and one combined treatment of L and N amendments (L + N) were applied. In order to study the possible residual effects, L, Mn, and MB amendments applied in 2018 were not applied a second time in 2019 at the CS site. The timing of application of each amendment in each trial can be found in Table S3. Plots (7 m long and 3 m wide) were confined and separated by a ridge (20 cm high and 25 cm wide) surrounding the plot. Irrigation channels separated the rows and also provided access for sampling (Fig. 1).

Liming in the form of ground limestone (CaCO₃) was applied at 5.25 ton/ha and increased soil pH to 6.49, 6.53, and 6.55 at CS, XT, and HN, respectively (Fig. S2). Commercial MnO₂ (Table S2, Fig. S1), composed of ramsdellite (orthorhombic MnO₂) and akhtenskite (hexagonal MnO₂), was applied at 0.90 ton/ha to increase soil Mn concentrations by 200 mg/kg (assuming soil mixing depth to be 0–20 cm and soil bulk density to be 1.3 g/cm³, same below; Fig. S3). The purpose of using MnO₂ instead of a soluble Mn(II) salt as the Mn amendment was to simultaneously decrease the phytoavailability of arsenic in the soils by adding a solid oxidant (Ehlert et al., 2016; Xu et al., 2017). Mn-loaded biochar (Table S1, Fig. S1) designed as a Mn source and Cd adsorbent was produced by

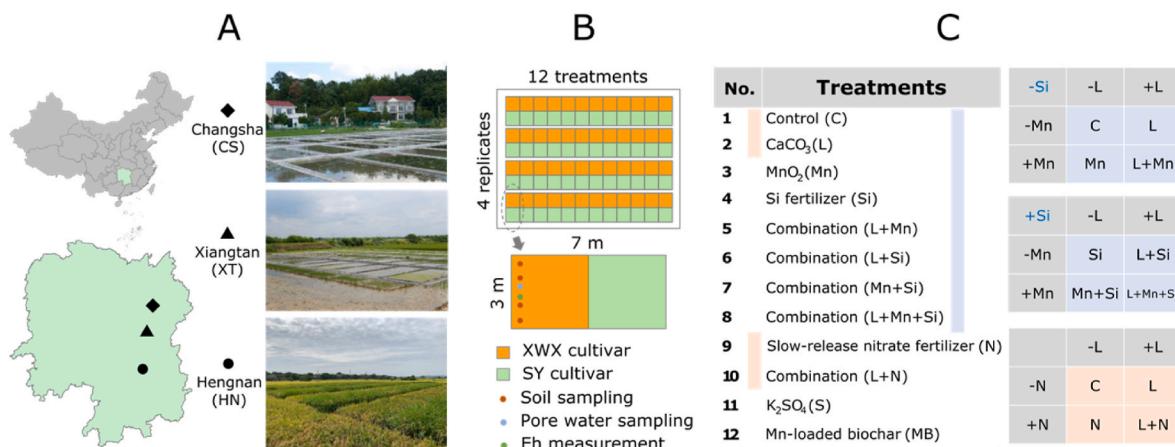


Fig. 1. Schematic of experimental design. (A): Locations and photos of the three field sites. (B): Design of field trials and sampling strategy. (C): Twelve treatments and two treatment sets with fully crossed factorial structure. Treatment set 1 (blue background) contains all possible combinations between limestone (L), MnO₂ (Mn) and Si fertilizer (Si). Treatment set 2 (orange background) contains all possible combinations between limestone (L) and slow-release nitrate fertilizer (N). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

pyrolysis of sawdust, which had been soaked in a MnSO₄ solution, at 600 °C for 2 h. Hausmannite (Mn₃O₄), szemikite (MnSO₄·H₂O), manganosite (MnO) and anhydrite (CaSO₄) were found as the major phases in the final MB material (Fig. S1). In contrast to many biochar materials reported, MB in this study was not highly alkaline (pH = 7.24, Fig. S2). MB was applied at 2.60 ton/ha which introduced 200 mg/kg Mn, 117 mg/kg S and 0.1 % biochar to the soil (Fig. S3, Fig. S4). Commercial silicon fertilizer (Table S2) was applied at 1.70 ton/ha, which increased soil Si and K contents by 170 mg/kg and 12 mg/kg, respectively. The slow-release nitrate was provided by Prof. Min Zhang (Shandong Agricultural University, China) as polyacrylate coated KNO₃ (99.7 % KNO₃) and applied to soil at 1.11 ton/ha which corresponds to total N and K additions of 58 mg/kg and 164 mg/kg, respectively (Fig. S4). All six soil amendments used were mixed into the soils (roughly 0–20 cm depth) using shovel and rake at the same time of basal fertilizer application before transplanting at both sites in 2018. In 2019, Si and N amendments were top-dressed to planted plots at rice late-tillering stage at both sites, while the other amendments were applied before transplanting as in 2018.

In each plot, two rice cultivars including one local rice cultivar, Xiangwanxian 13 (XWX), and one low Cd accumulating cultivar, Shenyou 957 (SY) (Duan et al., 2017), were used to study interactions between rice cultivar and soil treatment with respect to grain Cd. Both cultivars were transplanted at the same time after fertilizer application, but cultivar XWX was harvested 25–27 days later than SY except for the CS site in 2018, where both cultivars were harvested at the same time (Table S3). Local practices on basic fertilizer applications and water management were adopted in each field trial (supplementary material section 1.3).

2.3. Sampling and sample analyses

During the growing season, pore water and soil samplings, soil pH and redox potential measurements, and field-moist soil extraction were carried out for each plot at different growth stages (Table S3). To minimize disturbance of the growing crop, all pore water and soil samplings and in-situ measurements were carried out from the edge (~0.5 m inward) of XWX-planted half plots (Fig. 1).

Soil pore waters were taken at rooting depth (5–15 cm) using inserted Rhizon samplers (Rhizosphere Research Products; 10 cm porous

part with 0.6 µm pore size). Pore water pH was measured immediately using pH-glass electrodes. Acidified pore water samples were used for total elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer NexION 300X, USA and Agilent 8800, USA). Soil redox potential (Eh) was measured in the field plots at 7–10 cm below soil surface using platinum electrodes. Soil samples (0–20 cm below soil surface) representing a thoroughly mixed composite of four subsamples were collected from each plot at the grain-filling stage using a soil auger. For on-site CaCl₂ extractions of soils, 6 g of moist soil and 45 g 0.01 M CaCl₂ solution were weighed into a 50 mL plastic tube using a portable scale and manually shaken periodically for 15 min to extract moist-soil extractable elements. After settling for 10–15 min, the supernatants were filtered (0.45 µm) and acidified for elemental analysis by ICP-MS. Soil residues after extraction were oven-dried at 105 °C to constant weight to determine water contents of field moist soil so that soil extractable elemental concentration can be calculated on a dry-soil basis. The remaining composite soil samples were oven-dried (40 °C) and sieved (2 mm) prior to soil pH measurement (soil:water = 1:2.5; w/v), total elemental analysis using ICP-MS after aqua regia digestion (at 120 °C for 90 min) and X-ray fluorescence spectrometry (XRF; XEPOS, Spectro) using pressed pellets of milled soil, respectively.

Rice plants were harvested by cutting shoots above the ground. All grains from each cultivar in each plot were separated from panicles using a thresher and weighed after drying under sunlight at each field site to calculate yields. After dehusking, 0.2 g brown rice samples and a certified rice flour standard (GBW10045/GSB-23 or NIST 1568 b) were microwave-digested (MARS 6240/50, USA) in 5 mL concentrated nitric acid and analyzed for total element concentrations using ICP-MS.

2.4. Adjusted soil extractable Cd concentration

The effectiveness of soil extractable Cd for uptake is linked to the level of soil extractable Mn as Cd uptake is mainly via Mn transporters. Thus, it depends on both soil extractable Cd and the ratio of extractable Cd to extractable Mn. To account for this, extractable Cd was multiplied with the molar fraction of extractable Cd with respect to the sum of extractable Mn and Cd (i.e. the percentage of extractable Cd) as adjusted soil extractable Cd:

$$Cd_{soil, CaCl_2}^{adjusted} = Cd_{soil, CaCl_2} \cdot Cd_{soil, CaCl_2}^{mol/kg} / (Cd_{soil, CaCl_2}^{mol/kg} + Mn_{soil, CaCl_2}^{mol/kg})$$

when $Mn_{soil, CaCl_2}^{mol/kg} \gg Cd_{soil, CaCl_2}^{mol/kg}$:

$$Cd_{soil, CaCl_2}^{adjusted} \approx 0.489 \cdot Cd_{soil, CaCl_2}^2 / Mn_{soil, CaCl_2}$$

2.5. Statistical analyses and regression

All statistical analyses including one-way ANOVA, multi-way ANOVA, Dunnett's test and customized contrasts were done in R studio (Version 1.2.5033) using corresponding packages. Briefly, treatment effects compared to the control were assessed by Dunnett's test after one-way ANOVA. Within the factorial treatment sets (Fig. 1C), amendment interactions were identified using multi-way ANOVA. Based on the results of amendment interactions, amendment effects were assessed between proper treatment groups with and without the target amendment by customized contrast analyses after one-way ANOVA. Details on statistical analyses are provided in the supplementary material.

Multiple regression models were constructed for Cd concentrations (log-transformed) in brown rice of XWX and SY cultivars respectively from soil extractable Cd and Mn concentrations (log-transformed) after pooling data from all four trials. Variable weight in each regression model was calculated for each model variable as its relative contribution to the explained variation (R^2) after Shapley-based decomposition (Israeli, 2007; Simmler et al., 2017). For regression model with two variable A and B, weight of variable A could be calculated accordingly to the equation below:

$$\text{variable weight } A = \frac{(R_A^2 + R_{AB}^2 - R_B^2)/2}{R_{AB}^2} \times 100\%$$

where R_A^2 , R_B^2 and R_{AB}^2 are explained variation by models using only variable A, only variable B or both variable A and B respectively.

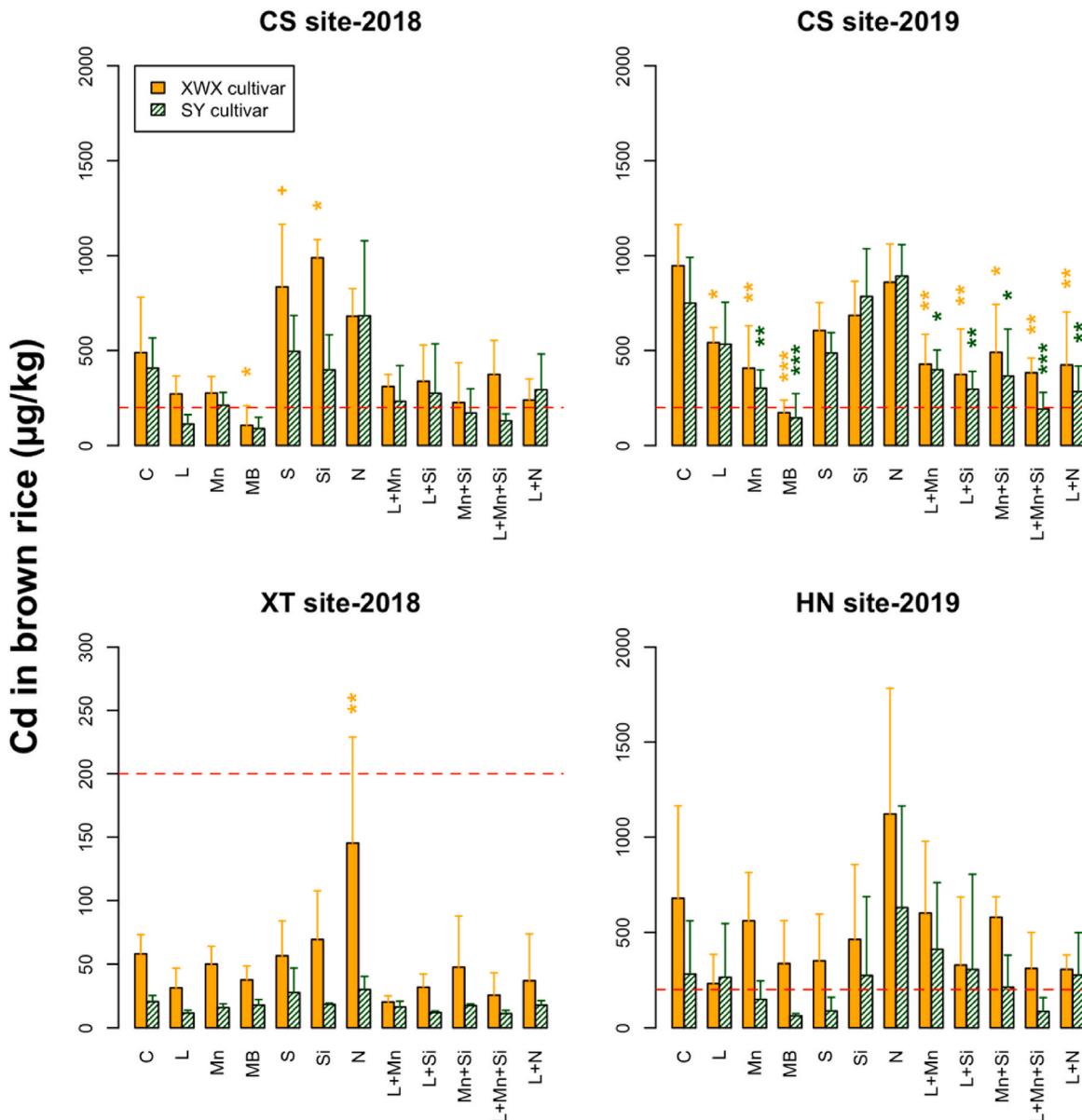


Fig. 2. Cd concentration in brown rice from 12 treatments in 4 trials. Error bars indicate standard deviations from four treatment replicates. Treatment abbreviations on the x-axis correspond to control (C), limestone (L), MnO₂ (Mn), Mn-loaded biochar (MB), K₂SO₄ (S), Si fertilizer (Si), slow-release nitrate fertilizer (N), and their combinations. Statistical significance from Dunnett's test (comparison to C) is shown in each plot with the following label code for adjusted p-values using a "single-step" method: '+' for $0.05 \leq p < 0.1$; '*' for $0.01 \leq p < 0.05$; '**' for $0.001 \leq p < 0.01$; '***' for $p < 0.001$. The threshold Cd concentration according to rice safety regulations in China is shown as red dashed line in each plot. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Data and R-scripts for all analyses and figures are provided as supplementary files.

3. Results

3.1. Grain Cd concentrations

Cadmium concentrations in brown rice of both XWX and SY cultivars are shown in Fig. 2 for all treatments in the four field trials. Except for a few treatments (N in trials CS2018 and CS2019 and Si in CS2019), the SY cultivar, known to be low in grain Cd accumulation (Duan et al., 2017), had lower mean grain Cd concentrations than the XWX cultivar. When pooled data from all treatments were compared (Fig. S5), SY cultivar had significantly lower Cd concentrations than XWX rice at XT and HN sites ($p \leq 0.01$), while the differences were less significant at the CS site in both years ($p > 0.05$). Grain Cd concentrations differed greatly

between trials, with the lowest grain Cd concentrations in trial XT2018 (mean of control plots for XWX and SY: 58 and 21 $\mu\text{g}/\text{kg}$; same below) compared to much higher values in HN2019 (679 and 282 $\mu\text{g}/\text{kg}$), CS2018 (489 and 407 $\mu\text{g}/\text{kg}$), and the highest values in CS2019 (947 and 750 $\mu\text{g}/\text{kg}$).

All limestone-containing and Mn-containing treatments decreased the mean Cd concentrations in brown rice by 23–78 %, 29–82 %, and 13–65 % compared to the corresponding control plots for both cultivars in trials CS2018, CS2019 and XT2018, respectively, and by 11–66 % for cultivar XWX in trial HN2019. Due to large variations among replicates, the decrease in grain Cd by those treatments was, however, not always statistically significant according to the Dunnett's tests, especially in HN2019 (Fig. 2). The high significance of all limestone-containing and Mn-containing treatments in CS2019 clearly indicated a pronounced second-year effect, as these treatments were applied only in 2018 at CS. The Si-containing treatments and S treatment overall displayed

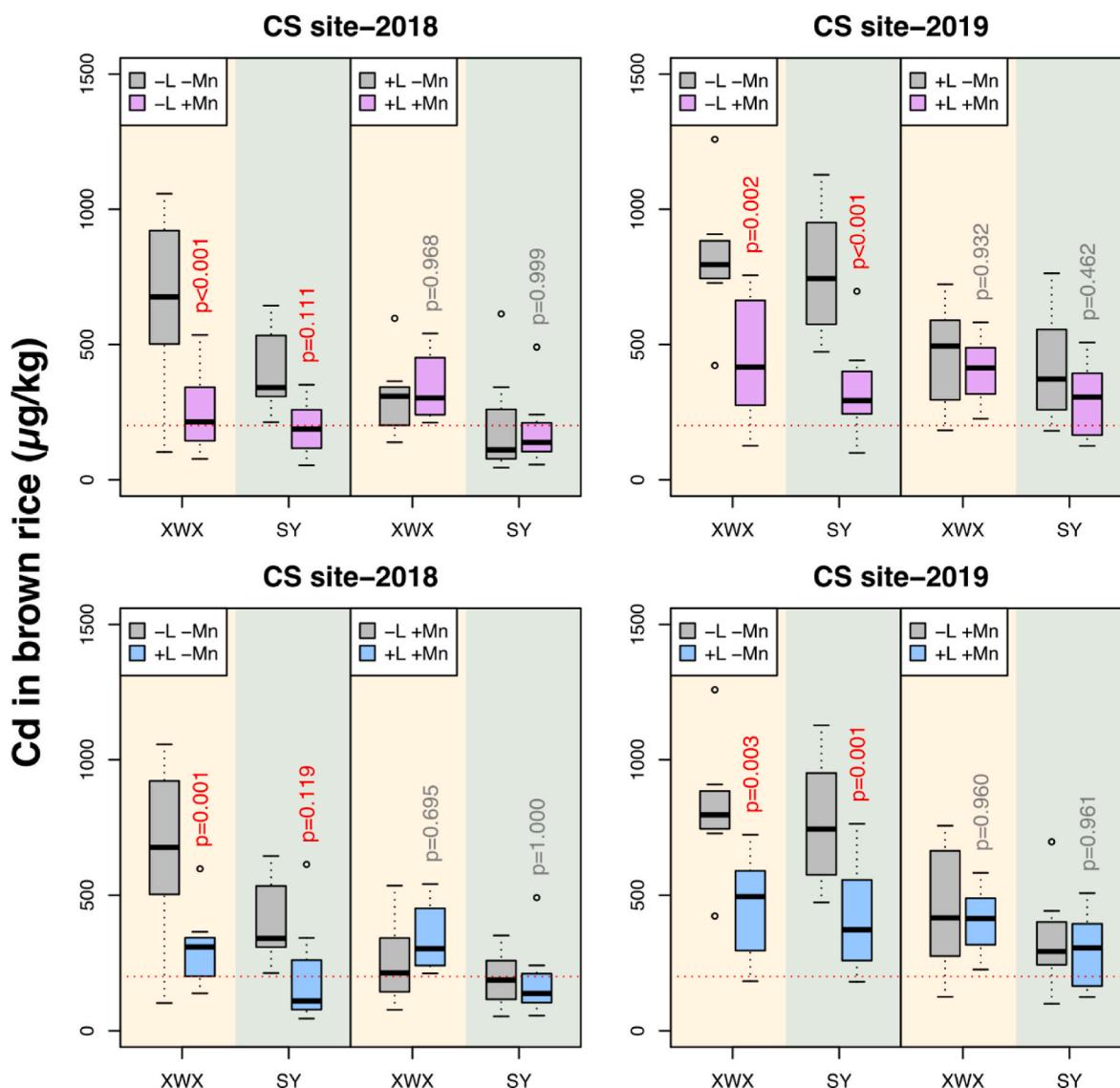


Fig. 3. Interaction between MnO_2 (Mn) and limestone (L) amendments on grain Cd concentration at the CS site in two years. “-L-Mn” contains C and Si treatments; “-L+Mn” contains Mn and Mn + Si treatments; “+L-Mn” contains L and L + Si treatments; “+L+Mn” contains L + Mn and L + Mn + Si treatments. The five horizontal lines in each boxplot from top to bottom indicate the highest value lower than the third quartile plus 1.5 times the interquartile range, third quartile, median, first quartile, the lowest value higher than the first quartile minus 1.5 times the interquartile range of corresponding data, respectively. Data values out of the range between the top and bottom lines are shown as open circles (outliers). p-values from contrast analyses are adjusted by the “single step” method to account for multiple comparisons. p-values below 0.2 are colored red. The threshold Cd concentration according to rice safety regulations in China is shown as red dashed line in each plot. Corresponding plots for the XT and HN sites are shown in Fig. S6. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

inconsistent effects on grain Cd, while the N treatment tended to even increase grain Cd. With respect to the Chinese maximum permissible value of 200 µg/kg Cd in rice, only the cultivar SY grown on MB amended soil met the food safety limit, in all trials consistently. For more detailed statistical analyses of amendment effects, a multi-way ANOVA and contrast analyses were performed, as presented in the following sections.

3.2. Amendment effects of limestone, MnO₂ and Si fertilizer on grain Cd

According to the results from multi-way ANOVA within a factorial treatment set containing limestone, MnO₂, and Si fertilizer (Table S5), no main effects from Si fertilizer and its weak interaction with limestone or MnO₂ suggested that no additional information would be obtained from contrast analysis on effects of Si fertilizer. However, strong main effects from both limestone and MnO₂ and their strong interaction justified testing the following contrasts on grain Cd concentration for each rice cultivar in each trial separately:

- Effect of limestone in the absence of MnO₂ (+L -Mn vs. -L -Mn): (L, L + Si) vs. (C, Si)
- Effect of limestone in the presence of MnO₂ (+L +Mn vs. -L +Mn): (L + Mn, L + Mn + Si) vs. (Mn, Mn + Si)
- Effect of MnO₂ in the absence of limestone (-L +Mn vs. -L -Mn): (Mn, Mn + Si) vs. (C, Si)
- Effect of MnO₂ in the presence of limestone (+L +Mn vs. +L -Mn): (L + Mn, L + Mn + Si) vs. (L, L + Si)

Analysis of these contrasts showed significant interactions between limestone and MnO₂ on grain Cd at the CS site in both years but not at XT and HN sites (Fig. 3, Fig. S6). Specifically at CS site (Fig. 3), limestone and MnO₂, in the absence of each other, significantly decreased the mean grain Cd concentration by 62 % and 53 % for XWX cultivar and by 53 % and 52 % for SY cultivar, respectively, in 2018. While in 2019, the corresponding decrease in grain Cd amounted to 45 % and 44 % for the XWX cultivar and to 56 % and 50 % for the SY cultivar, respectively. Whereas in the presence of limestone or MnO₂, additional application of MnO₂ or limestone showed no significant additional effect on grain Cd in all cases. Such information on treatment interactions obtained from multi-way ANOVA and customized contrasts was not revealed by the Dunnett's test. At XT and HN sites (Fig. S6), no significant effects of MnO₂ were found by contrast analysis, irrespective of limestone, while limestone showed only marginally significant effects in the absence of MnO₂.

3.3. Amendment effects of limestone, MnO₂, and MB on soil labile Cd and Mn

To further elucidate the amendment effects of limestone, MnO₂, and MB on grain Cd concentration, their effects on soil labile Cd and Mn (as 0.01 M CaCl₂ extractability in moist-soil and solubility in pore water) were examined. Following a procedure similar to the one applied for grain Cd (Table S6), the following contrasts were analyzed to reveal effects of limestone, MnO₂ and MB on both soil extractable Cd and Mn at the grain-filling stage:

- Effect of limestone (+L vs. -L): (L, L + Si, L + Mn, L + Mn + Si) vs. (C, Si, Mn, Mn + Si)
- Effect of MnO₂ (+Mn vs. -Mn): (Mn, L + Mn, Mn + Si, L + Mn + Si) vs. (C, L, Si, L + Si)
- Effect of MB (MB vs. C): MB vs. C

Limestone significantly decreased soil extractable Cd and Mn concentrations in all trials, with 46–90 % and 25–73 % decrease in the mean values for Cd and Mn, respectively (Fig. 5A). The data also indicated that limestone may decrease soil extractable Mn more strongly in soils also

receiving MnO₂ (Fig. S7). Although MnO₂ had no significant effects on soil extractable Cd in any trial when comparing all MnO₂-containing treatments to the rest within treatment set 1 (Fig. 5A), it tended to increase soil extractable Cd in the presence of limestone at CS site (Fig. S8). As expected, MnO₂ significantly increased soil extractable Mn concentrations at the CS site in both years ($p < 0.001$) and less significantly at XT in 2018 ($p < 0.01$). However, soil extractable Mn at the HN site, which had a high concentration of total Mn in the soil (Table S1), remained unaffected by the MnO₂ amendment. The MB amendment significantly increased soil extractable Mn in both years at CS ($p < 0.01$) and marginally at XT ($p = 0.15$), but also tended to decrease soil extractable Cd at marginal level ($p = 0.10$ – 0.15) except for CS in 2018. Pore water chemistry at grain-filling stage in general showed similar trends as observed for soil extractions. Details of pore water data from each sampling in each trial can be found in the supplementary material.

3.4. Amendment effects of Si fertilizer on grain yields, pore water Si, and soil labile Cd

Effects of Si treatments on grain yields, Si and Cd concentrations in pore water as well as soil extractable Cd levels are shown in the supplementary material. Briefly, Si amendment significantly increased grain yield except at the XT site (Fig. S9, Fig. S10). Medians of pore water Si concentrations from all plots dropped substantially from 7.9–10.0 mg/L before mid-season drainage to 1.2–1.8 mg/L after mid-season drainage in CS2018 and from 7.2–8.9 mg/L to 1.5–2.9 mg/L in XT2018. Corresponding medians after mid-season drainage in CS2019 (0.2–0.6 mg/L) and HN2019 (1.1 mg/L) also agreed with this trend. The Si amendment significantly increased pore water Si concentrations within 20 days after Si application in CS2018 and XT2018 but lost effects in the later samplings (Fig. S11). After changing Si application time to tillering stage in CS2019, Si treatment increased pore water Si concentrations by 279 % at the heading stage ($p = 0.027$) and by 49 % at the grain-filling stage ($p = 0.064$), suggesting a later Si application at tillering stage could be more effective than application before transplanting. Overall, the Si treatment showed very limited effects on soil Cd lability. The addition of Si had little significant effects on soil extractable Cd in all four trials and only significantly ($p < 0.05$) decreased pore water Cd in grain-filling sampling for CS2019 (Fig. S12), consistent with the ineffectiveness of Si amendment on grain Cd.

3.5. Regression for grain Cd from soil extractable Cd and Mn

Complementary to contrast analysis, where effects of a certain treatment or amendment could be masked by variations among replicate plots, regressions on individual plot-based grain Cd concentrations using corresponding soil extractable Cd and Mn concentrations in all four trials could provide insights into the role of Cd-Mn competition on grain Cd accumulation and elucidate how it was influenced by limestone, MnO₂, and MB amendments and site variations. As shown in Fig. 5C, regression models for grain Cd of both XWX and SY cultivars were successful with $p < 0.001$ for both soil extractable Cd and Mn as the explanatory variables and R^2_{adj} at 0.64 and 0.71, respectively. Soil extractable Cd exhibited positive coefficients (0.404 and 0.464) and dominant variable weights (92 % and 82 %) while soil extractable Mn had negative coefficients (−0.170 and −0.339) and minor variable weights (8 % and 18 %) in the constructed models for both XWX and SY (Fig. 5B and C).

4. Discussion

4.1. Soil amendments decreasing Cd lability and/or increasing Mn lability in soil could decrease grain Cd concentration

In this study, we showed that amendments decreasing Cd lability (limestone and MB) and/or increasing Mn lability (MnO₂ and MB) in soil

could be highly effective in decreasing grain Cd concentrations under field conditions; however, the treatment performance differed among sites (Figs. 2 and 5). The two-year field trial at the CS site also showed that a single application of limestone, MnO₂, or MB amendments in the first year showed effects over two years without decrease in performance. With respect to decreasing soil Cd lability, this finding agrees well with previous studies applying limestone or biochar to decrease grain Cd concentrations (Bian et al., 2014; Chen et al., 2018a, 2018b, 2018c; Cui et al., 2016, 2019; Wang et al., 2019). With respect to increasing soil Mn lability, our study provided clear evidence for the effectiveness of soil Mn application on decreasing grain Cd levels via Mn–Cd competition in rice under field conditions. It is known that Mn can compete with Cd for both sorption on the soil solid phase and absorption by rice roots via the OsNramp5 transporter (Loganathan et al., 2012; Yang et al., 2014). However, their expected impacts on Cd availability to rice are opposite. Our study showed that enhanced overall

Mn–Cd competition including both soil and plant processes after Mn amendment to soils was beneficial to decreasing Cd accumulation in rice grain without yield loss from Mn toxicity (Fig. S9). Rice is known to be highly tolerant to excess Mn (Yamaji et al., 2013a, 2013b), which can grow without Mn toxicity symptoms in media containing Mn from submicromolar to hundreds of micromolar levels (Sasaki et al., 2011).

Regression models on pooled data from four trials also revealed that soil extractable Cd and Mn were the two factors which explained the majority of variations in grain Cd concentrations in both cultivars among treatments and sites (Fig. 5C). It is worth noting in the models that differences in the extractable Cd and Mn of field-moist soil at grain-filling stage at CS site between 2018 and 2019, as affected by climatic differences between two years (Fig. S21), also explained their differences in grain Cd concentrations. The positive and negative coefficients of soil extractable Cd and Mn, respectively, indicated their competitive nature in regulating grain Cd levels. The ratios between

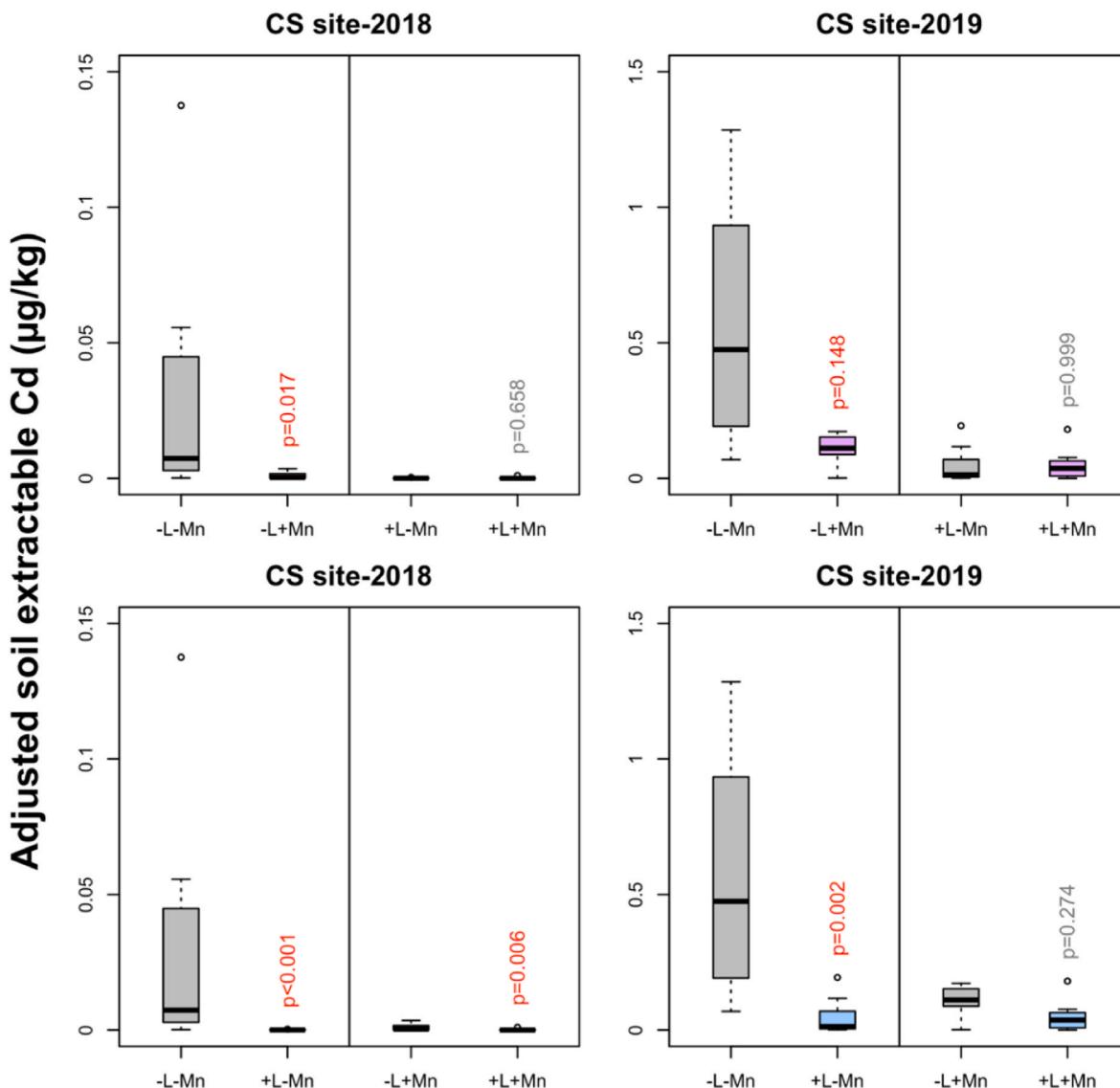


Fig. 4. Interaction between MnO₂ (Mn) and limestone (L) amendments on adjusted soil extractable Cd concentration in CS site in two years. Adjusted soil extractable Cd concentration is defined as soil extractable Cd adjusted by the molar ratio of soil extractable Cd to the sum of soil extractable Cd and Mn. “-L-Mn” contains C and Si treatments; “-L+Mn” contains Mn and Mn + Si treatments; “+L-Mn” contains L and L + Si treatments; “+L+Mn” contains L + Mn and L + Mn + Si treatments. The five horizontal lines in each boxplot from top to bottom indicate the highest value lower than the third quartile plus 1.5 times the interquartile range, third quartile, median, first quartile, the lowest value higher than the first quartile minus 1.5 times the interquartile range of corresponding data, respectively. Data values out of the range between the top and bottom lines are shown as open circles (outliers). p-values from contrast analyses are adjusted by the “single step” method to account for multiple comparisons. p-values below 0.2 are colored red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

coefficients of soil extractable Cd and Mn were 2.4 and 1.4 in regression models for XWX and SY respectively, which were also close to the theoretical value of 2 for the log-transformed term $0.489 \cdot Cd_{soil, CaCl_2}^2 / Mn_{soil, CaCl_2} \approx Cd_{soil, CaCl_2}^{adjusted}$, providing $Mn_{soil, CaCl_2} \gg Cd_{soil, CaCl_2}$ in our case. Thus, results here also supported the assumption under the adjusted soil extractable Cd concentration that competitive uptake and/or translocation between Cd and Mn existed in rice. Furthermore, the lower Cd to Mn coefficient ratio for SY cultivar compared to XWX cultivar agreed with SY being the lower Cd accumulating cultivar (Duan et al., 2017). Together with the fact that grain Mn to Cd ratio was significantly higher in SY cultivar than XWX cultivar in all trials (Fig. S13), this suggested the lower-Cd trait of SY rice could be due to its higher selectivity for Mn over Cd compared to XWX rice.

4.2. Interactions between limestone and MnO_2 amendments and their site-dependent effectiveness on decreasing grain Cd

The statistical analyses evidenced a strong interaction between MnO_2 and limestone amendments on grain Cd at CS site, showing no additional effect on decreasing grain Cd when both MnO_2 and limestone were applied. Different field sites also responded differently to soil limestone and MnO_2 amendments, with more significant decrease in grain Cd concentration observed at CS site than at the XT and HN sites (Figs. 2 and 3, Fig. S6). Those varying performances of limestone and MnO_2 amendments on grain Cd could be explained by the different responses of the adjusted soil extractable Cd concentration after amendment application in different cases (Fig. 4, Fig. S14).

For the acidic soil low in Mn at the CS site (110 mg/kg total soil Mn, Fig. 1), MnO_2 amendment increased soil total and extractable Mn concentrations by 0.6–1.5 fold and 3.9–8.7 fold respectively while it had no significant overall influence on soil extractable Cd (Table S1, Fig. 5A). Liming to CS soil decreased extractable Cd but also Mn concentrations (Fig. 5A). After calculation, both limestone and MnO_2 amendments, in the absence of each other, substantially decreased the adjusted soil extractable Cd at CS site in both years (Fig. 4). However, when MnO_2 and limestone were applied together at CS site, liming resulted in larger decrease of soil extractable Mn concentrations (Fig. S7) due to the extra Mn introduced. This limited the enhanced competition of soil labile Mn on Cd uptake by rice after Mn addition (Fig. 5). Alternatively, MnO_2 application limited the effect of Cd immobilization by liming as evidenced by slightly higher soil extractable Cd from L + Mn and L + Mn + Si treatments compared to L and L + Si treatments (Figs. S8B and D), likely through Mn competing for Cd occupied sorption sites in limed soils. Thus, the adjusted soil extractable Cd concentrations were comparably low among single and combined applications of MnO_2 and limestone at CS site, leading to their non-additive effects between limestone and MnO_2 on grain Cd.

For acidic soils high in Mn at the XT (321 mg/kg total soil Mn, Table S1) and HN sites (554 mg/kg total soil Mn, Table S1), MnO_2 amendment only increased soil total and extractable Mn concentrations by 36–54 % and 29–57 %, respectively. MnO_2 amendment was not able to significantly decrease the adjusted soil extractable Cd even in the absence of limestone (Fig. S14), resulting in no effects on grain Cd, regardless of whether limestone was applied or not (Fig. S6). Similarly, limestone also showed weaker effects on adjusted soil extractable Cd here than at CS site though its effects were stronger than MnO_2 (Fig. S6, Fig. S14).

The tendency of high grain Cd accumulation from combination of low soil total Mn and low pH had already been shown by correlation analysis using 64 paired soil-grain samples (Wang et al., 2018). A tradeoff between increasing soil pH and decreasing soil amorphous Mn upon liming had also been linked to the ineffectiveness of limestone in decreasing grain Cd at higher limestone dosage (Yang et al., 2020, 2021). Here, we demonstrated the interplay between soil pH and soil Mn level on grain Cd is by affecting the soil Cd phytoavailability taking into account soil Cd mobilization and competitive plant uptake and

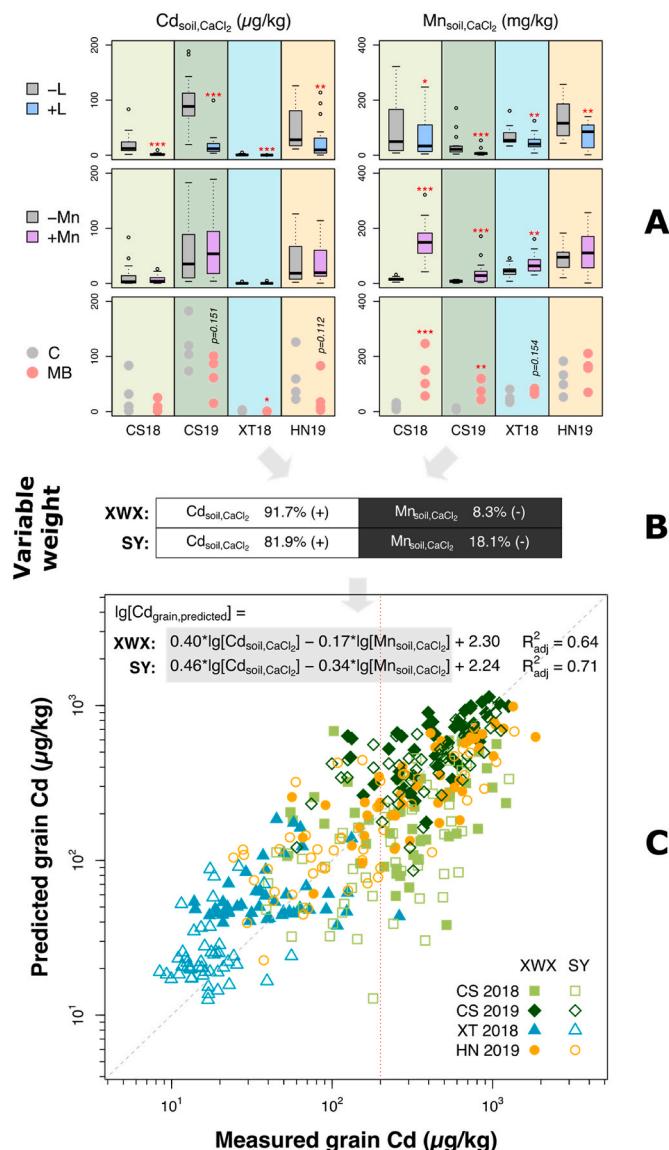


Fig. 5. Role of limestone (L), MnO_2 (Mn) and Mn-loaded biochar (MB) amendments on reducing grain Cd concentration. (A): Effect of MnO_2 (Mn), limestone (L) and Mn-loaded biochar (MB) on moist soil extractable Cd and Mn. “-L” contains C, Mn, Si and Mn + Si treatments; “+L” contains L, L + Mn, L + Si and L + Mn + Si treatments; “-Mn” contains C, L, Si and L + Si treatments; “+Mn” contains Mn, L + Mn, Mn + Si and L + Mn + Si treatments. The five horizontal lines in each boxplot from top to bottom indicate the highest value lower than the third quartile plus 1.5 times the interquartile range, third quartile, median, first quartile, lowest value higher than the first quartile minus 1.5 times the interquartile range of corresponding data, respectively. Data values out of the range between the top and bottom lines are shown as open circles (outliers). Statistical significance from contrast analyses is shown in each plot with following label code for the unadjusted p-values: ‘+’ for $0.05 \leq p < 0.1$; ‘**’ for $0.01 \leq p < 0.05$; ‘***’ for $0.001 \leq p < 0.01$; ‘****’ for $p < 0.001$. P values between 0.1 and 0.2 are also shown in corresponding plots. (B): Relative contribution (weight) of variables to the explained variation (R^2_{adj}) of each 2-variable regression model in panel C. (C): Regression model of grain Cd concentration of pooled rice samples based on $CaCl_2$ extractable Cd and Mn of moist soil. The vertical red dash line indicates the Chinese maximum permissible value of 200 $\mu\text{g}/\text{kg}$ Cd in rice. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

translocation against Mn. In summary, the results of our field study suggest that limestone and MnO_2 amendments are most effective at decreasing grain Cd for acidic soils with low Mn levels. For such soils,

one-time application may represent an option to lower grain Cd in rice for at least two cultivation periods.

4.3. Why MB was most effective in decreasing grain Cd accumulation

Although limestone, MnO₂ and Mn-loaded biochar (MB) were all found to be effective soil amendments in controlling Cd accumulation in rice grain at least for acidic soils low in Mn, MB showed the best performance consistently and decreased grain Cd concentrations to their corresponding minimums for SY cultivar at CS and HN sites and for XWX cultivar at CS site. In combination with the use of the low Cd accumulating SY cultivar, MB was the only amendment that kept grain Cd concentrations from all four trials below the regulation threshold value of 200 µg/kg. The outstanding performance of MB in decreasing grain Cd concentration could be mainly explained by its ability to simultaneously decrease soil extractable Cd concentrations and increase soil extractable Mn concentrations in all trials (Fig. 5A). Compared to limestone and MnO₂ amendments and their combination treatments, MB amendment maximized the effect of Mn competition on rice Cd uptake by avoiding changing soil Mn and Cd lability in the same direction. When MB was introduced to soils, Mn mixed with or adsorbed by the biochar could be released to soil matrix thus increasing soil Mn lability. Simultaneously, those originally Mn-rich sorption sites on biochar would be available for adsorbing other cations including Cd²⁺ and remain stable in soils for at least two years. It should be noted that the decrease of soil extractable Cd was not caused by pH increase in soil or pore water after MB amendment (Fig. S2, Fig. S15). Although MnO₂ could also be a sorbent for Cd in soil in addition to its role as a Mn source, it suffered from reductive dissolution in flooded soils which led to its small contribution to Cd immobilization. Instead, the released Mn from MnO₂ even showed the tendency to compete with Cd on sorption sites in limed CS soil thus increase soil extractable Cd (Fig. S8). Therefore, MB combined the benefits from both increasing soil sorption capacity and increasing the concentration of Mn²⁺ ions in soil competing for Cd uptake by rice, which was not achieved by applying limestone and MnO₂ together. In addition, MB increased pore water Mn concentration more strongly than MnO₂ amendment for CS and HN sites (Fig. S15), which was likely due to the higher Mn solubility of MB material compared to MnO₂ (Table S2). MB amendment also introduced similar amount of S to soils as S amendment (Fig. S4), which may have contributed to the decreased Cd accumulation in grain (section 4.4).

4.4. Si and S amendments cannot consistently lower grain Cd

Si has been shown to decrease Cd accumulation in rice root and shoot in hydroponic systems by several studies (Kim et al., 2014; Shao et al., 2017; Shi et al., 2005), probably through a down-regulation of the expression of *OsNramp5* and *OsHMA2* genes (Shao et al., 2017). However, field and pot experiments using soil-based Si fertilizers showed inconsistent effects on Cd accumulation in rice grain, with observed decreases in grain Cd always associated with increases of soil pH (Li et al., 2020; Wang et al., 2016), also inducing decreases in soil Cd extractability (Rehman et al., 2019). Si fertilization is also well known to potentially increase rice grain yield by counteracting abiotic and biotic stresses (Meharg and Meharg, 2015). In our field trials, Si amendment did not show evidence to decrease grain Cd concentrations consistently though it increased yields at CS and HN sites (Fig. 2, Fig. S10). On the one hand, pore water Si was always observed at low levels after mid-season drainage, especially at CS and HN sites (<5 mg/L), though Si amendment significantly increased pore water Si in CS2019 at heading and grain-filling samplings (Fig. S11). The concentrations of porewater Si in our field trials were at least 5 times lower than those used in hydroponic studies (1.0–1.8 mM) (Kim et al., 2014; Shao et al., 2017; Shi et al., 2005), even after Si amendment. On the other hand, although Si amendment increased dry soil pH slightly at CS site and strongly at XT and HN sites (Fig. S2), its effects on pore water pH and Cd lability were

less clear (Fig. S16, Fig. S12). Thus, it is possible that current Si applications were unable to significantly decrease grain Cd by neither influencing transporter gene expression nor decreasing soil Cd lability. The different responses of yield and grain Cd concentration to Si amendment may be due to their demand for Si at different time windows. The effects on grain yields were likely from an early boost of Si uptake during the vegetative growth phase before mid-season drainage while effects on grain Cd required higher Si or lower Cd lability in soil during grain filling stage when most of the grain accumulated Cd was taken up by the plants.

The S amendment in this study showed inconsistent effects on grain Cd. Possibly, effects of sulfate application may have been masked by high variations in total soil S and/or Zn among plots (Fig. S4, Fig. S22) (Huang et al., 2021a, 2021b; Weber et al., 2009). At XT site, where variations in soil S were the highest due to the strong spatial gradient (Fig. S17), total soil S concentrations were not significantly different between S amended soils and control soils with no effect on grain Cd from S amendment. While at HN site where total soil S concentration without S additions had the lowest variations, clear increase of soil S was found following S additions (S and MB) with >48 % decrease of grain Cd contents, though statistical significance was masked by large variations of grain Cd among replicates. Interestingly, at the CS site, the first S amendment in 2018 resulted in even higher grain Cd but the second S amendment in 2019 decreased grain Cd concentrations by >35 %. Also, in trials only receiving S amendment once (except CS site in 2019), the pore water S concentrations under S treatment were only higher than in the control shortly after sulfate application but not in the later samplings (Fig. S23), likely due to the rapid incorporation of applied sulfate into the organic fraction (Fitzgerald et al., 1982; Knights et al., 2001; Swank et al., 1984; Zhao et al., 2006). After S amendment was applied again at CS site in 2019, increase of pore water S under S treatment were noticeable in later samplings (Fig. S23). Overall, the effects of S amendment on grain Cd in our field trials appeared inconsistent. Other chalcophile metals (e.g., Zn, Cu, Pb) can compete with Cd for sulfide formation under reducing conditions (Weber et al., 2009). They can also strongly influence the reoxidation kinetics of mixed-metal sulfides upon soil drainage (Huang et al., 2021a, 2021b). Additionally, the CdS oxidation kinetics upon drainage of paddy soils can also vary among field sites, e.g. due to differences in soil texture. Further studies on S speciation and dynamics in different paddy soils under field conditions are needed to understand the role of soil S amendment in the formation and oxidation of Cd sulfides at the presence of other chalcophile metals (Huang et al., 2021a, 2021b; Weber et al., 2009), thus its role in Cd accumulation in rice grain.

5. Conclusions

Through a series of field trials in two years combining three field sites, two rice cultivars and 12 soil treatments, we found that soil extractable Mn (by 0.01 M CaCl₂ using moist-soil at rice grain-filling stage; same below) as a key negative contributor, in combination with the positive contribution from soil extractable Cd, which controls Cd accumulation in rice grain under field conditions (Fig. 5B and C). We showed that soil amendments either decreasing soil labile Cd (limestone and MB) or increasing soil labile Mn (MnO₂ and MB) to rice plants were effective in decreasing grain Cd accumulation (Figs. 2 and 5A), without causing Mn toxicity. Strong interactions and non-additive effects between limestone and MnO₂ on grain Cd were reported for the first time on an acidic soil low in Mn (CS), which can be explained by the comparably low adjusted soil extractable Cd after simultaneous decrease (by limestone) or increase (by MnO₂) of soil extractable Cd and Mn in the presence of the other amendment (Figs. 3 and 4). For acidic soils high in Mn (XT and HN), the weaker effect of liming and the ineffectiveness of MnO₂ addition on grain Cd also agreed well with such interactions (Fig. S6, Fig. S14). By simultaneously increasing pore water and soil extractable Mn and decreasing soil extractable Cd, MB showed

the best performance in keeping grain Cd at safe levels. Therefore, our study suggests that competition between Cd and Mn in all processes from soil to plant should be considered when designing strategies to increase Mn lability or decrease Cd lability in soils in order to safeguard rice from Cd contamination.

Contrary to the expected role of Si fertilization in controlling grain Cd accumulation as suggested by some studies, the performance of Si fertilizer tested in this study was, however, not convincing though it increased yields. Future studies should focus on strategies to sustain high Si phytoavailability throughout rice growing season after application to soil or use foliar Si applications instead (Li et al., 2020; Liu et al., 2009; C. Wang et al., 2020).

The use of a low Cd accumulating rice cultivar (SY957) in this study did not always produce rice grain Cd below the regulation threshold, unless in combination with soil amendments such as MB. In this sense, agronomic measures to decrease rice Cd uptake and translocation to grain such as soil amendment seem indispensable in the near future.

Author contributions

Xu Fang: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – Original Draft, Writing – Review& Editing, Visualization. Jing Wang: Methodology, Validation, Investigation, Resources. Hongping Chen: Methodology, Validation, Investigation. Iso Christl: Conceptualization, Validation, Supervision, Writing – Review & Editing. Peng Wang: Supervision, Writing – Review & Editing. Ruben Kretzschmar: Conceptualization, Validation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. Fang-Jie Zhao: Conceptualization, Validation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

Main finding

Mn amendments, especially Mn-loaded biochar, can effectively decrease Cd accumulation in rice grain grown on acidic soils low in Mn under field conditions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.117918>.

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