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The influence of liming on cadmium accumulation in rice grains via iron-reducing bacteria

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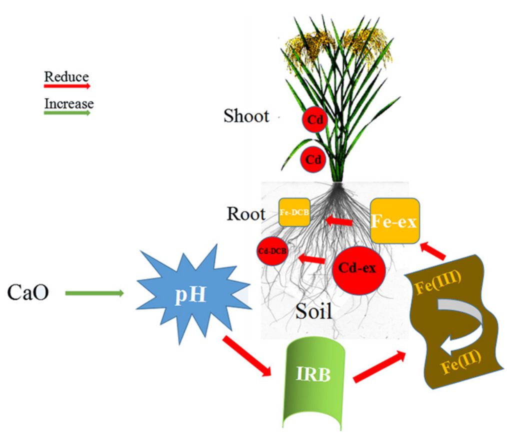
HIGHLIGHTS

- The application of liming alone (without Cd-contaminated rice straw) significantly reduced the rice gran Cd.
- The positive correlation was found between available Fe and Cd in soil and the Cd contents in plant.
- The soil pH influenced the IRB community structure and available Fe and Cd in soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium (Cd) in soil is attracting worldwide attention, and many valuable measures and suggestions of minimizing the rice grain Cd are available. Among these methods, liming can increase the soil pH and decrease the rice grain Cd content. Here, we report that soil pH was negatively and significantly correlated with the concentration of soil extractable Fe and Cd. In addition, the iron concentration on root surface was significantly and positively associated with the available metals in soil and the rice grain Cd. However, the return of contaminated rice straw significantly increased the Cd accumulation in the rice grain, although the returned straw did not significantly influence the concentration of extracted soil Cd. Furthermore, an analysis of the functional microbe community was performed, and the response of iron-reducing bacteria (IRB) under the six treatments provides valuable insights for reducing the available Cd concentration in soil. A LEfSe (LDA coupled with effect size measurement for significant differences) analysis showed that the application of liming reduced the abundance of IRB. The results of a redundancy analysis (RDA) indicated that soil pH was significantly and negatively associated with the abundance of *Proteobacteria* and *Geobacter* and the concentration of bioavailable Fe and Cd in the soil, which could explain the reduced accumulation of bioavailable Cd in rice grain. Collectively, our results demonstrated that liming in Cd-polluted paddy soil is a reasonable strategy for minimizing rice grain Cd by increasing the soil pH, which reduces the soil available iron and Cd concentration by shifting the diversity and composition of IRB, thus ultimately resulting in decreased Cd in rice.

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1. Introduction

The heavy metal cadmium (Cd) possesses high mobility and is toxic to living organisms (Song et al., 2015). Cd contamination of agricultural soils leads to considerable Cd accumulation in the edible parts of crops, including rice, which promotes Cd toxicity in humans via the food chain (Wolnik et al., 1983). Cd is easily transferred from soil to plants with a high bio-concentration factor, and it affects the soil physicochemical properties and physiological features of plants (Liu et al., 2015). Rice (*Oryza sativa*) is globally one of the most important staple cereal crops and feeds >50% of the world's population (FAO, 2014). It is especially highly consumed in Asian countries, including China, India, Japan and Korea (Zhang et al., 2005). Unfortunately, Cd contamination of soil occurs widely in the paddy fields of subtropical China (Zhang et al., 2015) and represents a serious problem that has gradually become more severe because of mine exploration, the metallurgy industry, solid waste disposal, paint pigments, and irrigation with wastewater from mines. Obviously, this contamination is not only a major threat to food safety but also represents a threat to the well-being of humans (Aziz et al., 2015). Thus, agronomic practices, which primarily include the use of soil amendments (Guo et al., 2006), fertilizers (Yan et al., 2015), water (Honma et al., 2016) and tillage management (Yu et al., 2014), can represent potential strategies for improving the physicochemical characteristics of soil and immobilizing Cd in polluted soils.

Inorganic (e.g., gypsum and lime) and organic materials (e.g., rice straw, *Astragalus sinicus*, and manure) applied as heavy metal soil amendments can effectively reduce the bioavailability of Cd in soil and prevent Cd uptake into plants, and these approaches have begun to attract attention in China (Rehman et al., 2015). The inorganic and organic materials minimize the bioavailability and transport of heavy metals in the soil environment by increasing the soil pH, adsorption, ionic exchange, complexation or precipitation reactions. Another study reported that extractable Cd increases by 0.35–0.37 fold with one pH unit decline in acidity (pH 4.7–6.7) in the paddy soils of subtropical China (Rao et al., 2013). Inorganic liming may increase the soil pH, which may lead to the increased immobilization of heavy metals via increased soil adsorption and enhanced transformation from soluble metals to residuals (Wang et al., 2015). Thus, limestone soil amendments are a popular practice to reduce the availability of soil heavy metals and their uptake by crops (Bolan et al., 2003; Zhou et al., 2014). The inexpensive and effective organic materials available red mud and compost could lower the ecological risk of heavy metals in soil (Zhou et al., 2017). Nevertheless, many natural amendments promote the release of heavy metals into the environment and do not improve soil productivity, microbial activity, or plant growth (Bolan et al., 2014). A thorough evaluation of the effectiveness of chemical materials for the immobilization of heavy metals via soil remediation practices involves not only the assessment of soil chemical characteristics by chemical and physical approaches (Cheng and Hseu, 2002) but also analyses of the restoration of soil habitat function by biological methods (Adriano et al., 2004). Thus, microbial communities as soil biological indices can directly and indirectly indicate contaminated soils for remediation.

Iron plaque can inhibit the uptake of heavy metals by plants (Batty et al., 2000). More importantly, metal(loid) uptake by plants increases/decreases depending on the amount of iron plaque (Zhang et al., 1998), and iron plaque increases the uptake of elements by plants during periods of diminished supply and acts as a reservoir (Ye et al., 2001). The formation of iron plaque in wetland and submersed aquatic plant species is caused by the oxidation of ferrous to ferric iron and the precipitation of iron oxide on the root surface, which involves the release of O_2 and oxidants in the rhizosphere (Taylor and Crowder, 1983; Taylor et al., 1984) and/or to biological oxidation by microorganisms (Emerson et al., 1999; Weiss et al., 2003). Additionally, because of the high capacity of functional groups on iron hydroxides to sequester metal(loid)s by adsorption and/or co-precipitation, the availability of the metal(loid)s in the rhizosphere can be affected by the iron plaque on root surfaces, which may lead to changes in the uptake and accumulation of metal elements by plants (Batty et al., 2003; Chen et al., 2006). However, in paddy soils, microorganism-mediated dissimilatory Fe(III)-reduction is recognized as the dominant mechanism for Fe(III)-reduction to Fe(II), and the role of microorganisms in contaminated soil remains unclear.

In this study, we evaluated the efficiency of liming and *Astragalus sinicus* applications alone or combination with Cd-containing rice straw in a Cd-contaminated field on reducing Cd availability in soil and accumulation in rice grain. Furthermore, we investigated the potential role of iron plaque in the uptake and accumulation of Cd by rice in contaminated paddy soil. Most importantly, we analyzed the community structure of soil microorganisms in six treatments using high-throughput sequencing. The results indicated that liming applications shifted the community structure of iron-reducing bacteria (IRB) and decreased the activity of heavy metals possibly via increases in the level of soil pH. Our results are expected to provide novel insights toward changing the process of iron reduction in contaminated paddy soil to minimize Cd accumulation in rice grains by stimulating the activity of IRB.

2. Materials and methods

2.1. Study area

This study was performed in a northern suburb of Changsha City, Hunan Province (28°22′–28°27′ N, 112°58′–113°05′ E; 47.3–597.8 m above sea level), which is in central subtropical China. The region covers 14.848 km^2, has a subtropical monsoon climate, an annual mean temperature of 17.2 °C, and an annual precipitation of 1200–1500 mm. The area soil type is classified as red soil and paddy soil according to the soil parent material, and it is primarily developed from granite. Paddy fields are the primary cropland, accounting for >75% of total cropland in the subtropical region, and they have been cultivated for double rice production for centuries. Generally, the early rice is transplanted in early March and harvested in late June, whereas the late rice is transplanted in early July and harvested in late October. The fields are primarily irrigated with water from adjacent reservoirs. The characteristics of the experimental soils are described in Table 1.

2.2. Field experimental designs

Six treatments were applied in the study region: T1, Cd-containing rice straw applied alone (approximately 7500 kg ha^{-1} rice straw); T2, the control; T3, Cd-containing rice straw and burnt lime combined (approximately 7500 kg ha^{-1} rice straw and 2250 kg ha^{-1} CaO); T4, burnt lime applied alone (approximately 2250 kg ha^{-1} CaO); T5, Cd-containing rice straw and *Astragalus sinicus* combined (approximately 7500 kg ha^{-1} rice straw and 2250 kg ha^{-1} *Astragalus sinicus*); and T6, *Astragalus sinicus* applied alone (approximately 2250 kg ha^{-1} *Astragalus sinicus*). Each of the treatments had four replicates. Rice straw and *Astragalus sinicus* were obtained from Cd-contaminated paddy fields and ground before use.

2.3. Soil and plant sampling

In each treatment, a soil sample was bulked synchronously from the top 20 cm soil layer. Each soil sample was air dried, homogenized

Table 1
Physicochemical properties of rice soil from the experiment field (mg kg^{-1}).

Soil type	pH	Cd	Fe	Zn	Mn	Pb	Cu	Ni	Cr
Rice soil	5.54	1.18	16,612.24	105.82	90.17	82.83	18.61	12.26	8.87