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Original Research

Ryegrass-Maize Intercropping for Cadmium Remediation: Efficacy and Mechanisms

Zhaoxin Zhang[1, 2, 3], Shenglan Ye[2, 3], Qiuli Zhao[1], Bei Zhang[4°*], Yan Niu[2, 3]

[1]Department of Ecological and Environmental Engineering, Shaanxi A&F Technology University,
Yangling 712100, China
[2]Key Laboratory of Degraded and Unused Land Consolidation Engineering, the Ministry of Natural Resources,
Xi'an 710075, China
[3]Technology Innovation Center for Land Engineering and Human Settlements, Shaanxi Land Engineering Construction
Group Co., Ltd, and Xi'an Jiaotong University, Xi'an 710049, China
[4]College of Landscape Architecture and Art, Northwest A&F University, Yangling 712100, China

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Abstract

Soil cadmium (Cd) pollution threatens agricultural sustainability and food security, necessitating eco-friendly remediation strategies. This study evaluated the efficacy of intercropping annual ryegrass with maize for the remediation of Cd-contaminated soil in a 120-day experiment with triplicate pots per treatment. Three treatments were applied: maize monoculture without Cd (CK), maize monoculture with Cd (1.8 mg/kg, C1), and ryegrass-maize intercropping (1:2 row ratio) with Cd (C2). Results showed that intercropping significantly reduced soil Cd content by 70%, surpassing monoculture maize (49.4%). Maize grain Cd levels decreased to 0.07±0.01 mg/kg, with a 39% lower bioconcentration factor (BCF) in C2 vs. C1. Ryegrass demonstrated a high cadmium translocation efficiency (translocation factor, TF: 0.6-0.8), effectively reducing Cd bioavailability in the rhizosphere. Intercropping maintained soil pH at 8.60 but decreased soil organic matter (SOM) by 19.5% and available phosphorus while increasing total potassium. Soil microbial communities showed increased Actinobacteriota abundance, though Cd stress reduced overall diversity. Enzyme activities (e.g., alkaline protease and urease) increased, indicating enhanced nitrogen cycling. Statistical analyses (SPSS 26.0; ANOVA, LSD tests, $p<0.05$) confirmed treatment effects. While nutrient competition requires management (e.g., tailored fertilization), ryegrass-maize intercropping balances Cd remediation, crop safety, and soil functionality, offering a practical framework for sustainable farmland management.

Keywords: agricultural soil, Cd contamination, phytoremediation, ryegrass-maize intercropping, soil microorganisms

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**e-mail: zhangbei94@nwafu.edu.cn
°ORCID iD: 0009-0000-0655-300X

Introduction

With the rapid growth of industry and cities, and the heavy use of fertilizers and pesticides in farming, heavy metal contamination in soil has become a serious global challenge. Heavy metals in soil build up gradually, are hard to detect, and difficult to manage. This not only hinders the nation's sustainable development but also threatens long-term soil use [1, 2]. Soil, the foundation of the ecosystem, is essential for agriculture and human survival [3]. The National Soil Contamination Survey Bulletin shows a soil pollutant exceedance rate of 16.1% nationwide, and 19.4% in arable areas. Cadmium (Cd) contamination is the most common, affecting nearly 13,300,000 m^2 of farmland [4, 5].

Cd-contaminated soil not only reduces crop yields and quality but also increases heavy metal accumulation in organisms through the food chain, which harms human health and can cause issues such as cancer and neurological disorders, posing a serious threat to food security [6]. Among major food crops, maize (*Zea mays* L.), used for food, feed, and industry, is increasingly important in agriculture; thus, controlling heavy metal levels in maize fields is crucial for human health [7, 8]. In China, maize is widely planted, and its yield and quality are directly linked to food security and animal husbandry. However, maize metabolism and yield are significantly inhibited in Cd-contaminated environments. Cd stress hinders the uptake of key nutrients (such as Zn, Fe) by maize roots, damages chloroplasts, reduces photosynthesis, and triggers oxidative stress, causing lipid peroxidation and cell death [9]. Additionally, Cd limits maize plant height, biomass, and kernel development, leading to lower yields, poorer quality, and heightened food security risks [10].

In the face of this serious situation, it is urgent to explore efficient, environmentally friendly, and economically feasible remediation technologies for heavy metal contamination. Heavy metal-contaminated soil remediation technology can be divided into two categories according to the purpose of remediation: one is to achieve changes in the chemical form of heavy metals in the soil and reduce their mobility and biological effectiveness in the environment [4]. Secondly, with the aim of reducing the total amount of heavy metals, the enrichment effect of plants, animals, and microorganisms is utilized to achieve the separation of soil and heavy metals [11]. Phytoremediation technology, as a green, in-situ method, has become a research hotspot due to its advantages of low cost and minimal environmental disturbance [12]. Numerous plants have been found to have heavy metal-enriching abilities, such as hyper-enriching plants like *Thlaspi caerulescens* and *Pteris vittata*, which absorb and accumulate heavy metals in large quantities, removing them from the soil [13, 14]. However, hyper-enriched plants generally have shortcomings such as small biomass, slow growth, and territoriality, which limit the development of large-scale restoration projects.

Ryegrass (*Lolium multiflorum*), as a common heavy metal super-enriched forage grass, shows great potential in the field of heavy metal-contaminated soil remediation. It has the advantages of rapid growth, large biomass, high tolerance to heavy metals, and outstanding absorption and enrichment ability, which can effectively extract heavy metals from the soil and reduce the heavy metal content in the soil [1]. In addition, the planting and harvesting of ryegrass is relatively easy and low-cost, which is suitable for large-scale popularization and application. Ryegrass has strong tolerance and enrichment ability to heavy metals such as Cd, Pb, Zn, etc. Its root secretion can change the inter-root soil environment, influence the bio-efficacy of heavy metals, and promote its own uptake of heavy metals; at the same time, under the mode of inter-cropping or intercropping with other plants, it can synergistically remediate the polluted soils and improve the efficiency of remediation through the mechanisms of root interactions, nutrient competition, and complementation [15, 16]. Ryegrass has shown some remediation ability in different areas and contaminated soils, and is relatively easy to grow and manage, and can adapt to a variety of climatic and soil conditions [17]. However, existing research on the specific mechanisms of ryegrass remediation of heavy metal contamination, especially in intercropping systems with crops, ryegrass and crop interaction mechanisms, the dynamics of inter-root microbial communities, and their fine regulation of heavy metal migration and transformation, remains limited. Through ryegrass-maize intercropping, it is expected to achieve effective remediation of heavy metal contamination while guaranteeing farmland fertility and providing a new approach to solving the problem of heavy metal contamination in maize farmland.

Based on this, this study aims to explore in depth the remediation mechanism of Cd contamination by ryegrass-maize intercropping in farmland, and the main research contents are as follows: 1) Quantify Cd remediation efficiency by measuring Cd reduction in rhizosphere soil under intercropping vs. monoculture; 2) Characterize soil health trade-offs through enzyme activity assays (urease, protease) and nutrient availability (SOM, AP, and AK); 3) Decipher microbial mechanisms via 16S rRNA sequencing to identify Cd-immobilizing taxa (e.g., Actinobacteriota) enriched in the intercropped rhizosphere. This will reveal the underlying mechanisms of Cd remediation by ryegrass-maize intercropping and provide scientific and technical support for the ecological remediation of Cd contamination in farmland.

Materials and Methods

Experimental Site and Devices

The experiment was conducted at the Qinling Field Monitoring Centre of Shaanxi Institute of Land Engineering Technology (107°54′E, 34°14′N), located in Meixian County, Shaanxi Province, China. This region has a warm-temperate, semi-humid monsoon climate (annual temperature: 12.8ºC; rainfall: 581.6 mm). These climatic conditions are conducive to the growth of both maize and ryegrass, ensuring optimal development for phytoremediation studies.

Agricultural topsoil (0-20 cm) was collected from Cd-contaminated farmlands in Meixian County, with initial Cd = 0.22±0.03 mg/kg (below China's risk threshold). To simulate moderate contamination common in affected regions, soil was artificially spiked with $CdCl_2 \cdot 2.5H_2O$ to 1.8 mg/kg (3 × GB 15618-2018 screening value). The limitation is that artificial spiking may not fully replicate field-aged Cd bioavailability. Soil was air-dried (25°C), sieved (≤2 mm), and homogenised. The basic soil properties are shown in Table 1. Pot design: Cubic pots (40×40×40 cm) were selected to accommodate mature root systems – maize root depth typically ≤35 cm. Each pot held 55 kg of soil (bulk density 1.15 g/cm³).

Crop Selection and Experimental Design

Plant materials: Maize (*Zea mays* L.) cv. "Zhengdan 958", a widely cultivated hybrid in Shaanxi Province, was chosen for its high yield potential and moderate Cd tolerance. Annual ryegrass (*Lolium multiflorum* Lam.), a recognized Cd hyperaccumulator with rapid growth, high biomass, and strong metal uptake capacity, was selected to maximize phytoremediation efficiency while minimizing competition with maize for resources.

Treatments and setup: The experiment comprised three treatments with three replicates each, as shown in Fig. 1. CK: Maize monoculture, no Cd; C1: Maize monoculture + 1.8 mg/kg Cd; C2: Ryegrass-maize intercropping (1:2 row ratio) + 1.8 mg/kg Cd. The 1:2 ratio optimizes Cd extraction by ryegrass while minimizing light/nutrient competition with maize. 8 maize plants/pot (20 cm spacing) + 20 ryegrass seeds/pot (inter-row distance: 15 cm).

Cd was added as $CdCl_2 \cdot 2.5H_2O$ dissolved in deionized water, mixed uniformly into the soil, and aged for 2 weeks to stabilize metal bioavailability. The pots were placed in a greenhouse with controlled conditions (temperature: 15-23ºC, humidity: 15%-20%) and watered weekly to 60% of field capacity to maintain optimal soil moisture for plant growth.

Sampling and Measurement of Agronomic Parameters

Plant Growth and Yield Traits

Throughout the 120-day growth period, plant height, leaf number, and biomass (root and shoot) were measured at key growth stages (tasseling and maturity). The duration (120 days) covers the full growth cycles of maize (tasseling to grain maturity) and ryegrass (peak Cd accumulation phase). At harvest, maize ears were collected to determine grain yield, 1000-grain weight, and Cd accumulation in grains. Ryegrass aboveground biomass was harvested separately to quantify Cd uptake and translocation efficiency.

Soil and Plant Analyses

Soil properties: Before and after the experiment, soil samples were collected from the rhizosphere (0-20 cm depth) to measure pH, organic matter, total nitrogen, total phosphorus, total potassium, available phosphorus, and available potassium, following the same methods as the baseline analysis.

Heavy metal determination: Soil and maize grain Cd concentrations were measured using graphite furnace atomic absorption spectrometry (GF-AAS, Hitachi Z-2000), with soil samples digested by HNO_3-$HClO_4$ (4:1 v/v) and plant samples by HNO_3-H_2O_2 (3:1 v/v) to ensure complete metal extraction.

Enzyme activities and microbial community: Soil enzyme activities (catalase, dehydrogenase, alkaline protease, urease) were assayed using colorimetric methods (Tabatabai, 1994), reflecting soil metabolic and nutrient cycling processes. Microbial DNA was extracted from soil using the MoBio PowerSoil Kit,

Table 1. Basic properties of soil used in the experiment (mean±standard deviation).

Index	Abbreviation	Determination method	Value
pH	-	Glass electrode in a 1:2.5 soil-water suspension	8.55±0.02
Soil organic matter	SOM	Walkley-Black titration method	13.75±0.31 g/kg
Total nitrogen	TN	Kjeldahl digestion	0.86±0.02 g/kg
Total phosphorus	TP	Molybdenum-antimony colorimetry (after H_2SO_4-$HClO_4$ digestion)	0.74±0.01 g/kg
Total potassium	TK	Flame photometry (after NaOH fusion)	21.30±0.22 g/kg
Available phosphorus	AP	Olsen method (molybdenum-antimony colorimetry)	31.67±1.25 mg/kg
Available potassium	AK	Flame photometry (ammonium acetate extraction)	202±3.16 mg/kg
Total cadmium	Total Cd	Graphite furnace atomic absorption spectrometry (GF-AAS)	0.22±0.03 mg/kg

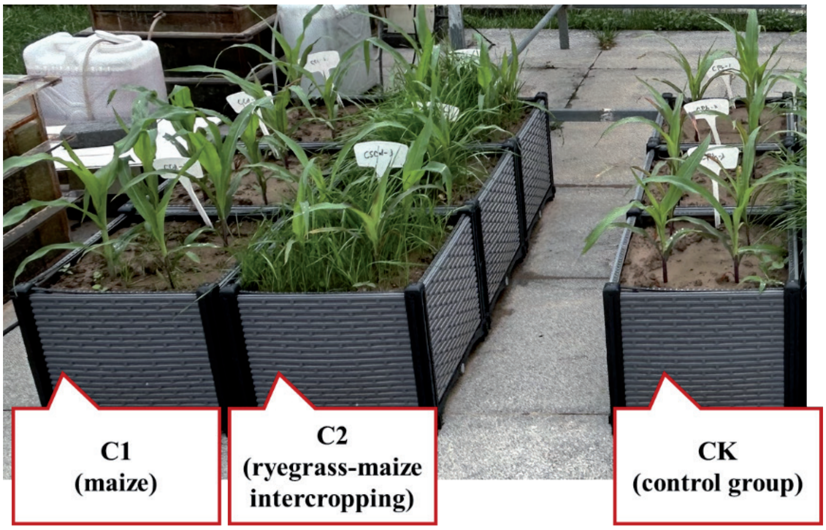


Fig. 1. Schematic diagram of experimental setup and grouping. CK, C1, and C2 represent the control group, maize group, and ryegrass-maize intercropping group, respectively.

and high-throughput sequencing of the 16S rRNA gene (V3-V4 region) was performed on the Illumina MiSeq platform to analyze microbial diversity and community structure at the phylum and genus levels.

Data Analysis

Agronomic data (plant growth, yield, biomass) and soil chemical properties were analyzed using one-way ANOVA with least significant difference (LSD) tests ($p<0.05$) in SPSS 26.0 to compare treatment effects. The bioconcentration factor (BCF) for maize grains was calculated as BCF = Cd concentration in grains (mg/kg)/Cd concentration in soil (mg/kg), indicating the potential for Cd accumulation in edible parts. The safety threshold (T) for soil Cd was derived from GB 2762-2022 (grain Cd limit = 0.10 mg/kg) using T = 0.10/BCF, providing a practical index for safe agricultural production under intercropping.

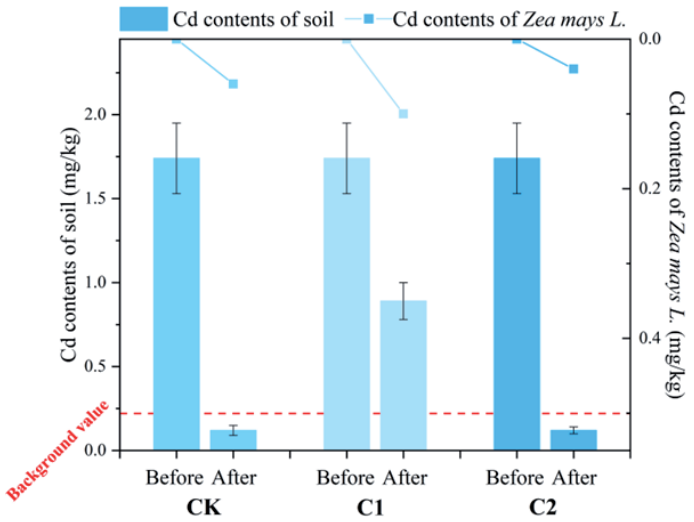
Microbial diversity indices (Chao1, Shannon, Simpson) were calculated in QIIME 2, and canonical correspondence analysis (CCA) was performed to explore correlations between soil properties and microbial community composition, highlighting the ecological mechanisms underlying Cd remediation in intercropping systems.

Results and Discussion

Remediation Effect of Ryegrass-Maize Intercropping on Cd Contamination in Farmland

In this study, the Cd content in soil before and after the experiment in different treatment groups was precisely determined, and the results showed significant differences (Fig. 2). Before the experiment, the mean value of Cd content in the soil of each treatment group was 1.74 mg/kg according to the experimental setup, which provided a uniform starting level of contamination for subsequent studies. After 120 days of the planting cycle, the content of Cd in the soil of the maize treatment group (C1) was reduced to a certain extent, but the overall decrease was limited, and the Cd content was reduced to 0.89 mg/kg, which was mainly attributed to the fact that maize itself has a certain degree of heavy metal tolerance. The enrichment capacity of maize for heavy metals is relatively low, and it is difficult to achieve deep removal of heavy metals from the soil. It can only achieve a small amount of uptake by the roots and part of the physiological process, so that the heavy metal content in the soil is reduced to a uniform starting level. Only through a small amount of root uptake and the influence of some physiological processes does the soil's heavy metal content change. In contrast, the ryegrass-maize intercropping group (C2) showed excellent remediation effects. The Cd content in the soil was significantly reduced by an average of >70%, and the Cd in some soils was even close to 80%.

Although the Cd content in C2 soil was still different from that of the control group, it was lower than the Cd risk screening value (0.6 mg/kg) stipulated in the Soil Environmental Quality – Soil Contamination Risk Control Standard for Agricultural Land GB 15618-2018. As a heavy metal hyper-enriched plant, the root system of ryegrass continuously secretes organic acids, phenols, and other substances to the surrounding soil during the growth process, and these secretions undergo complexation, chelation, and other chemical reactions with Cd and other heavy metal ions, desorb heavy metals from the surface of soil particles, and convert them into a form that can be easily absorbed by the root system [18]. The well-developed root system of ryegrass has a large surface area, which can efficiently adsorb and uptake these activated heavy metal ions, and then



Experimental group	Bioconcentration factor ($F_{BC\text{-}Grain}$)	Risk threshold (T)
CK	0.77±0.14	0.13 mg/kg
C1	0.12±0.02	0.92 mg/kg
C2	0.07±0.01	1.19 mg/kg

Fig. 2. Variation of Cd content in soil and maize in different experimental groups. CK (Before and After), C1 (Before and After), and C2 (Before and After) represent the Cd content in soil and maize before and after treatment for the control group, maize group, and ryegrass-maize intercropping group, respectively.

transport the heavy metals through the xylem to the aboveground part for storage, thus realizing the purpose of removing a large number of heavy metals from the soil and effectively reducing the total amount of heavy metals in the soil [19].

Among the two Cd treatment groups, the content of Cd in C1 maize kernels reached the limit value in cereals and their products (0.10 mg/kg) stipulated in GB2762-2022 National Standard for Food Safety Limits of Pollutants in Foods. This means that maize in this treatment group did not meet food safety standards. The Cd content in the kernels of C2 maize was lower than the above-mentioned standard and even lower than that of the untreated control group. The Cd content in maize kernels was effectively controlled after ryegrass-maize intercropping treatment. The BCF reflects the crop's ability to accumulate heavy metals in soil; the larger the BCF, the stronger its ability to accumulate heavy metals. The BCFs in different treatment groups are shown in Fig. 2. There were significant differences between the BCFs of Cd in corn kernels. Except for the CK group in which the BCF seed was >0.5, the BCF seed range of Cd in maize in the two treatment groups, C1 and C2, was between 0.103~0.145 and 0.062~0.081, respectively. The BCF of Cd in maize kernels in C2 was significantly lower than that in C1; the ryegrass-maize intercropping treatment effectively prevented the accumulation of Cd in maize kernels. CK had the lowest Cd safe utilization risk threshold of 0.13 mg/kg, which indicated that once the Cd content in soil exceeded this threshold under conventional cropping conditions, the risk of Cd contamination of maize significantly increased, which in turn threatened the quality and safety of agricultural products. The risk threshold for safe utilization of Cd in the maize treatment group (C1) was elevated to 0.92 mg/kg, which was a significant increase compared to CK. This indicates that the pattern of monocropping maize enhanced soil tolerance to Cd to a certain extent. The risk threshold for safe utilization of Cd in the ryegrass-maize intercropping treatment group (C2) was as high as 1.19 mg/kg, which exceeded the soil risk control value (0.6 mg/kg).

Ryegrass has a strong root system, and its root secretions may not only further improve the inter-root environment but also interact with corn root secretions to jointly promote the immobilization or transformation of Cd in the soil [20]. Ryegrass-maize intercropping showed a more significant synergistic effect, which greatly enhanced the safe utilization of soil Cd [21]. The contrasting Cd accumulation patterns between treatments highlight the importance of crop pairing in phytoremediation. Maize, with its low Cd translocation efficiency (translocation factor <0.3), acts as a "safe" food crop, while ryegrass serves as a "phytoremediator", creating a win-win scenario for food security and environmental cleanup. This synergy is supported by the 1.19 mg/kg safety threshold in C2, 9.2 times higher than the control (0.13 mg/kg), indicating that intercropping can expand the allowable Cd range for safe maize production. From a practical perspective, this technology enables concurrent crop production and pollution control, addressing the conflict between remediation and agricultural productivity – a key challenge in contaminated farmland management.

Effects of Changes in Soil Properties and Fertility in Ryegrass-Maize Intercropping

Soil properties changed before and after the experiment, showing significant differences (Table 2). pH and organic matter content increased in CK after the experiment. The soil properties were relatively stable under natural conditions, with no special amelioration measures. The small increase in SOM originated from the natural microbial decomposition in the soil and the slow accumulation of plant residues. The significantly higher pH in C1 was related to the release of alkaline substances from maize root secretions, which altered the acid-base balance of the soil at the inter-root level. The pH of the C2 was intermediate, indicating that ryegrass root secretions neutralized or moderated the effect of the maize root secretions on the soil pH to a certain degree. SOM was significantly reduced in both C1 and C2, which was attributed to the consumption of soil organic matter during the growth of the two plants, as well as the root secretions changing the structure of the microbial community and accelerating the decomposition and transformation of organic matter. The decrease in SOM content affected the soil's ability to retain fertilizer and water, as well as its ability to adsorb and fix heavy metals [22].

TN content in CK was relatively stable, probably due to the relative balance between nitrogen mineralization and fixation in natural ecosystems. The decrease in TN content in C1 and C2 was due to the competitive uptake of nitrogen by plants during growth and the more complex inter-root micro-ecological environment under the cropping pattern, which altered microbial nitrogen utilization pathways and led to a decrease in soil total nitrogen content [23]. TP content was generally lower in the different treatment groups, and the continuous uptake and utilization of phosphorus by crop growth was also an important reason for the decrease in total phosphorus content. TP content was similar in C1 and C2, and although maize root secretions may have a role in the activation of soil phosphorus, this role may be offset by the strong fixation of phosphorus by the soil in an alkaline soil environment [24]. The TK content of both C1 and C2 further increased to 22.44 g/kg and 21.73 g/kg. In the absence of external disturbances, the slow decomposition of soil mineral potassium led to an increase in the total potassium content, and the protons secreted by the root system could exchange with potassium ions on the surface of the soil minerals, which increased soil TK content [25]. However, the TK content of C2 was significantly lower than that of C1. Due to the competition for potassium between the two crops, ryegrass root secretions changed the existence pattern and migration and transformation pattern of potassium in the soil, resulting in lower soil TK content in C2.

AP in all soils decreased dramatically after the experiment, in which the AP content in CK, C1, and C2 decreased by 56.83%, 39.22%, and 70.35%, respectively. The AP content in CK decreased drastically, and microbial transformation of phosphorus and crop uptake of phosphorus in the soil were not effectively regulated under natural conditions, which led to the continuous depletion of AP. The lowest AP content in the intercropping group was due to the more intense competition between ryegrass and maize for AP uptake during the growth process. Changes in AP content directly affected physiological processes such as photosynthesis and energy metabolism of crops and may also indirectly affect the behavior of Cd in soil by influencing soil microbial activity and soil physicochemical properties [26]. Except for C1, the AK content in the rest of the treatment groups showed a decreasing trend. The increase in AK content in C1, on the one hand, was attributed to the strong selective absorption of potassium ions by the corn root system, which contributed to the transformation of soil mineral potassium to AK; on the other hand, the substances secreted by the corn root system might have changed the balance between adsorption and desorption of potassium ions in the soil colloid, so that more potassium ions were released to the soil solution, which increased the AK content. AK content in C2 decreased, and the competition between the two crops for potassium ion uptake intensified under the cropping pattern, and the ryegrass root secretions exerted a different effect on

Table 2. Changes in soil properties and fertility indicators in different experimental groups (mean ± standard deviation).

	pH	SOM (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)	AP (mg/kg)	AK (mg/kg)
Before	8.55±0.02	13.75±0.31	0.86±0.02	0.74±0.01	21.30±0.22	31.67±1.25	202±3.16
CK-After	8.58±0.03	14.22±0.28	0.86±0.01	0.72±0.02	21.96±0.18	13.67±0.82	181±2.54
C1-After	8.70±0.04	11.12±0.42	0.82±0.03	0.69±0.01	22.44±0.25	19.25±1.06	206±4.21
C2-After	8.60±0.03	11.06±0.35	0.71±0.02	0.69±0.02	21.73±0.16	9.39±0.68	170±3.07

Note: Before represents the soil prior to the experiment. CK-After, C1-After, and C2-After represent post-experiment soil from the control group, maize group, and ryegrass-maize intercropping group, respectively.

the morphological transformation of soil potassium from that of monocropping maize [27]. Ryegrass root secretion promotes the conversion of potassium ions in the soil to stationary potassium, which reduces the content of quick-acting potassium. Overall, changes in soil fertility affect plant growth and development, so in practical application, it is necessary to pay attention to the changes in soil fertility under the cropping system and take corresponding measures to regulate it, such as rational fertilization, to maintain the sustainable productivity of the soil.

The trade-off between Cd removal and nutrient depletion underscores the need for integrated soil fertility management in intercropping systems. Ryegrass, as a high-biomass crop, accelerates nutrient cycling but also increases nutrient export upon harvest. Farmers could adopt strategies like returning maize stover (low Cd content) to the field or applying organic amendments (e.g., compost) to replenish SOM and nitrogen, balancing remediation with soil health. The observed increase in total potassium (TK) in both Cd treatments (22.44 g/kg in C1, 21.73 g/kg in C2) suggests that root-induced mineral weathering may offset potassium uptake, a finding consistent with previous studies on intercropping-induced soil mineral transformation [25]. These results emphasize that while intercropping enhances Cd remediation, proactive nutrient management is essential for sustaining long-term agricultural productivity.

Changes in Microbiological Systems of Soil

Changes in Soil Enzyme Activities in Different Treatment Groups

The changes in rhizosphere soil enzyme activities in different treatment groups can effectively reveal the trend in the soil ecosystem before and after the experiment (Fig. 3). Soil catalase activity is mainly related to the redox state and the organic matter decomposition process. Comparing conditions before and after the experiment, the levels of catalase were basically the same in the different treatment groups before the experiment. After the experiment, catalase activity increased significantly, by 277.90% in CK (from 377.09 μmol/h before the experiment to 1425.04 μmol/h after the experiment), whereas in the C1 and C2 treatment groups, although catalase activity also increased (221.92% and 179.92%), the increase was lower than that in CK, resulting in a lower level of catalase activity than that in CK after the experiment. In the C1 and C2 treatment groups, although catalase activity also increased (221.92% and 179.92%), the increase was lower than that in CK, leading to reduced decomposition of hydrogen peroxide in C1 and C2 after the experiment [28]. Soil dehydrogenase activity was significantly and positively correlated with soil microbial metabolic activity, and high dehydrogenase activity indicated strong soil microbial activity and fast organic matter decomposition. The dehydrogenase activities in CK, C1, and C2 were 36.36 μg/d, 28.09 μg/d, and 20.36 μg/d, respectively, before the experiment, and the dehydrogenase activities in the Cd treatment groups (C1 and C2) were lower than those in the control group. Dehydrogenase activity was significantly reduced in all treatment groups after the experiment, and the differences were not significant at 6.31 μg/d (CK), 6.34 μg/d (C1), and 6.57 μg/d (C2), respectively.

The significant decrease in dehydrogenase activity after the experiment was attributed to the toxic effect of Cd on soil microorganisms that inhibited their metabolic activity, leading to a decrease in the decomposition capacity of organic matter, which in turn affected the nutrient cycling of the soil [29]. Alkaline protease activity reflects the intensity of nitrogen cycling in soil, especially the process of protein breakdown to amino

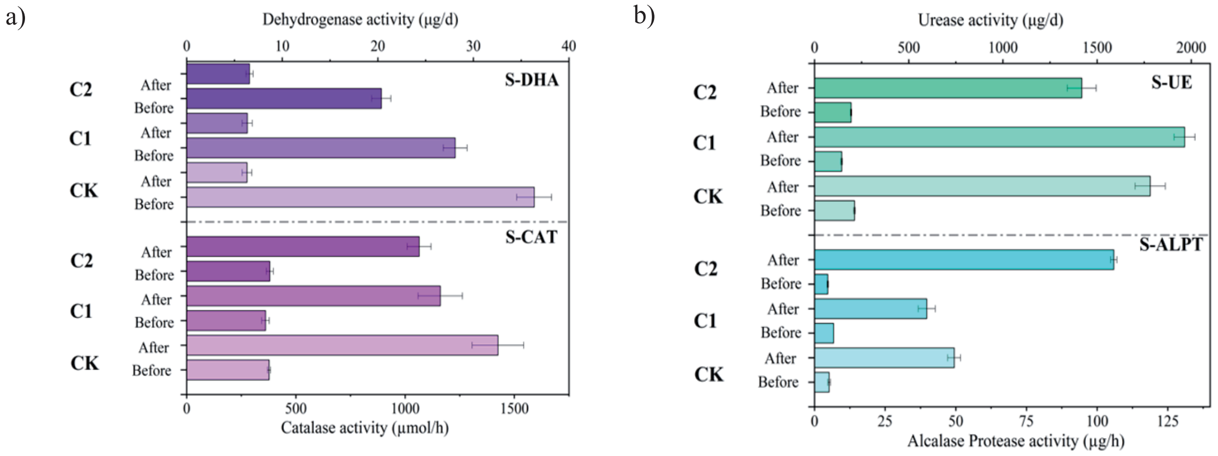


Fig. 3. Changes in soil enzyme activities in different experimental groups. a) Catalase (S-CAT) and dehydrogenase (S-DHA) activities; b) Protease (S-ALPT) and urease (S-UE) activities. CK (Before and After), C1 (Before and After), and C2 (Before and After) represent the soil enzyme activities before and after treatment for the control group, maize group, and ryegrass-maize intercropping group, respectively.

acids. Before the experiment, alkaline protease activity in CK, C1, and C2 was 5.15 μg/h, 6.74 μg/h, and 4.67 μg/h, respectively, and the activity of C1 was higher than that of CK, while the activity of C2 was lower than that of CK. In the post-test period, the alkaline protease activity of all three treatment groups was significantly increased. The alkaline protease activity in C2 increased to 105.87 μg/h, which was significantly higher than that of CK and C1. The significant increase in alkaline protease activity after the experiment, especially the greatest increase in C2, suggests that the interaction of zoysiagrass with Cd may stimulate the growth of specific microorganisms and enhance the protein decomposition capacity, thus increasing the effectiveness of soil nitrogen [30]. Urease activity is closely related to the process of urea decomposition to ammonia in soil, reflecting the efficiency of the soil nitrogen cycle. The pattern of change in urease activity and alkaline protease activity before and after the experiment was similar, and the urease activity was significantly increased in all treatment groups after the experiment, and the urease activities in CK, C1, and C2 were 1781.48 μg/d, 1964.37 μg/d, and 1418.39 μg/d after the experiment, respectively, and the urease activity of C2 was higher than that of CK, while the activity of C3 was lower than that of CK. The significant increase in the urease activity after the experiment, especially in C2, was due to the stimulation of the growth of some Cd-tolerant microorganisms by Cd. Overall, Cd treatment had a complex effect on soil organic matter decomposition, which may inhibit the activity of some microorganisms or stimulate the growth of other microorganisms; at the same time, Cd treatment may change the pathway of soil nitrogen cycling, enhance the decomposition capacity of proteins and urea, and thus improve the effectiveness of soil nitrogen [31, 32].

Evolution of Microbial Community Structure

A. Phylum level

The changes in microbial community structure in different treatment groups before and after the experiment are shown in Fig. 4a). The dominant species (at the phylum level) in the different treatment groups before the experiment were Proteobacteria, Actinobacteriota, Acidobacteriota, Gemmatimonadota, Chloroflexi, Bacteroidota, Myxococcota, Methylomirabilota, Crenarchaeota, and Verrucomicrobiota, and the sum of the relative abundance of these species was >90%. Before the experiment, the relative abundance of Proteobacteria in CK was 32.97%, while the abundance of C1 and C2 increased to 42.38% and 39.31%, respectively, indicating that the Cd treatment stimulated the growth of some Proteobacteria. The opposite trend to Proteobacteria was Actinobacteriota, whose relative abundance in CK was 21.51%, while the abundance of C1 and C2 decreased to 16.10% and 14.51%, respectively, indicating that Cd treatment inhibited the growth of some Actinobacteriota. The relative abundance of Proteobacteria decreased significantly after the test, and the abundance of CK, C1, and C2 decreased to 15.42%, 19.96%, and 20.33%, respectively, suggesting that the growth of Proteobacteria was inhibited after the test due to the long-term toxic effect of Cd, especially for sensitive strains [33, 34]. The relative abundance of Actinobacteriota increased significantly after the test, and the abundance of CK, C1, and C2 decreased to 47.16%, 36.17%, and 43.99%, respectively. Due to its strong tolerance and adaptation, Actinobacteriota became the dominant flora after the test. The effect of Cd treatment on Acidobacteriota varied depending on the treatment: the relative abundance of CK decreased to 10.62%, the abundance of C1 increased to 15.18%, and the abundance of C2 decreased to 11.38% after the experiment, depending on the treatment (due to the fact that cadmium stimulated the growth of some tolerant strains) [35]. The relative abundance of Gemmatimonadota, Chloroflexi, and Bacteroidota generally decreased after the experiment, suggesting that long-term exposure to Cd may have inhibited the growth of these phyla. Long-term exposure to Cd altered the structure of the microbial community, allowing the more tolerant Actinobacteriota to become the dominant group. The changes in microbial community structure were similar to C1, but the abundance of Actinobacteriota was higher, suggesting that the introduction of ryegrass may have further promoted the growth of Actinobacteriota.

B. Genus level

By analyzing the microbial community structure at the genus level, it can be observed that the treatment groups (CK, C1, and C2) before and after the experiment showed a significant trend of variation (Fig. 4b)). Before the experiment, the relative abundance of *Sphingomonas* and *Lysobacter* was higher than that of CK in both C1 and C2, where the abundance of *Lysobacter* in C2 (4.41%) was significantly higher than that of CK (2.98%), suggesting that the Cd stress promoted the growth of these tolerant genera. *Arenimonas* was significantly enriched in C2 (1.72%), which was 2% higher than that of CK (0.68%), which is 2.5 times more than CK, and this genus has Cd-tolerant characteristics. After the experiment, the microbial community of each treatment group was significantly altered, with the abundance of *Sphingomonas* decreasing from 5.21% to 1.19% in CK, and from 6.26% to 2.19% and 6.07% to 0.66% in C1 and C2, respectively, suggesting that the growth of this genus was inhibited by long-term Cd exposure. On the contrary, *Lysobacter* abundance in C2 increased dramatically from 4.41% to 6.23%, which was significantly higher than the other treatment groups, indicating that ryegrass cultivation specifically promoted the proliferation of this genus. The abundance of *Arenimonas* (3.13%) significantly increased in CK after the experiment, while the increase was smaller in C1 and C2, and the genus may be more adapted to non-contaminated environments in natural soils [36]. *Streptomyces* increased nearly fourfold after the abundance test in CK (from 0.42% to 1.66%), while the increase was even greater in C1 and C2 (from 0.23% to 2.05% in C1), suggesting that the genus has a dual character: the ability to survive in Cd-contaminated environments and the ability to multiply rapidly in non-contaminated conditions [37]. Together, these changes suggest that 1) Cd contamination significantly alters the structure of soil microbial communities, inhibiting the growth of sensitive genera and promoting the growth of tolerant genera [38]; 2) Ryegrass cultivation specifically enriches beneficial bacterial genera such as *Lysobacter*, possibly mitigating Cd toxicity through inter-root effects [39]; 3) There were significant differences in the response patterns of different genera to Cd stress, which provide important microbial resources for bioremediation of Cd-contaminated soils [40].

Correlation between Changes in Soil Properties and Changes in Microbial Ecosystems

Pearson's correlation analysis between soil property factors and microbial community phylum-level structure

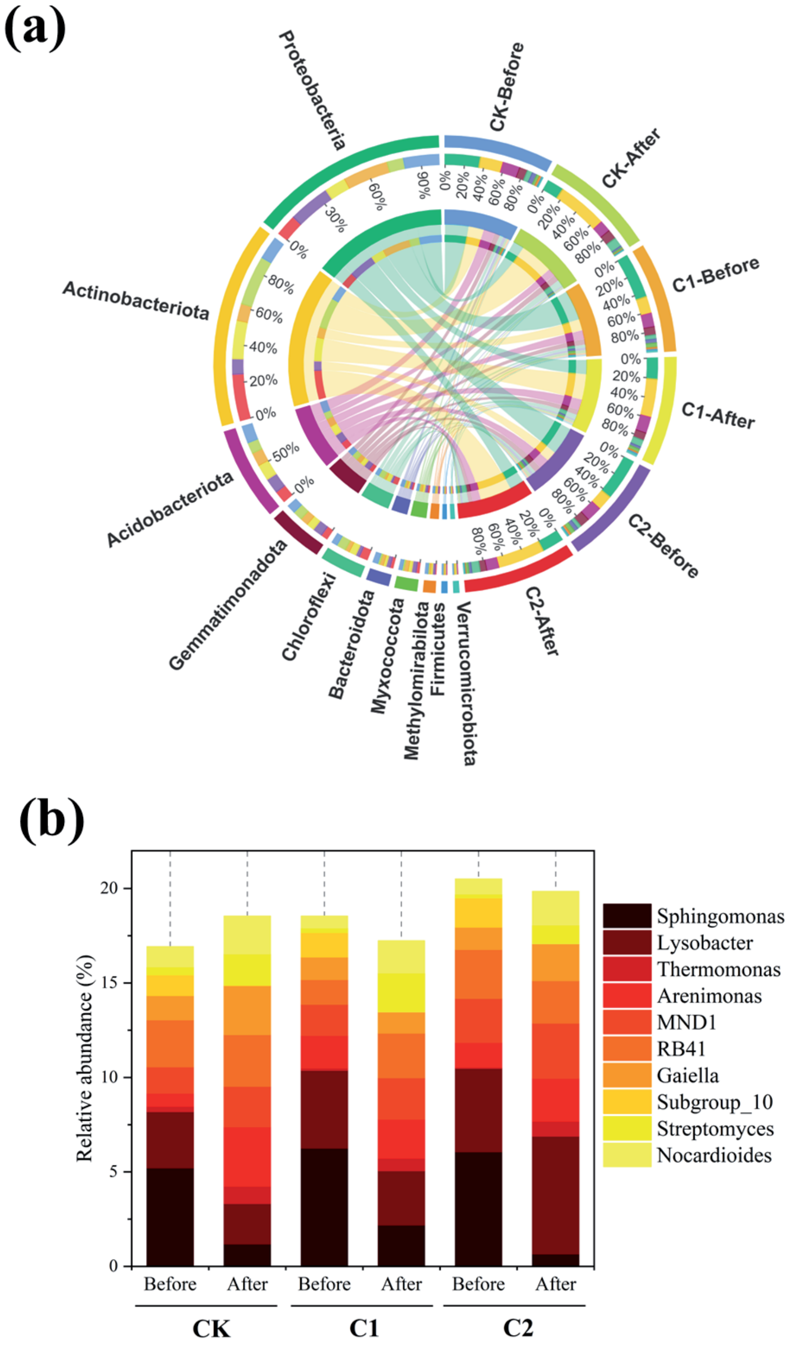
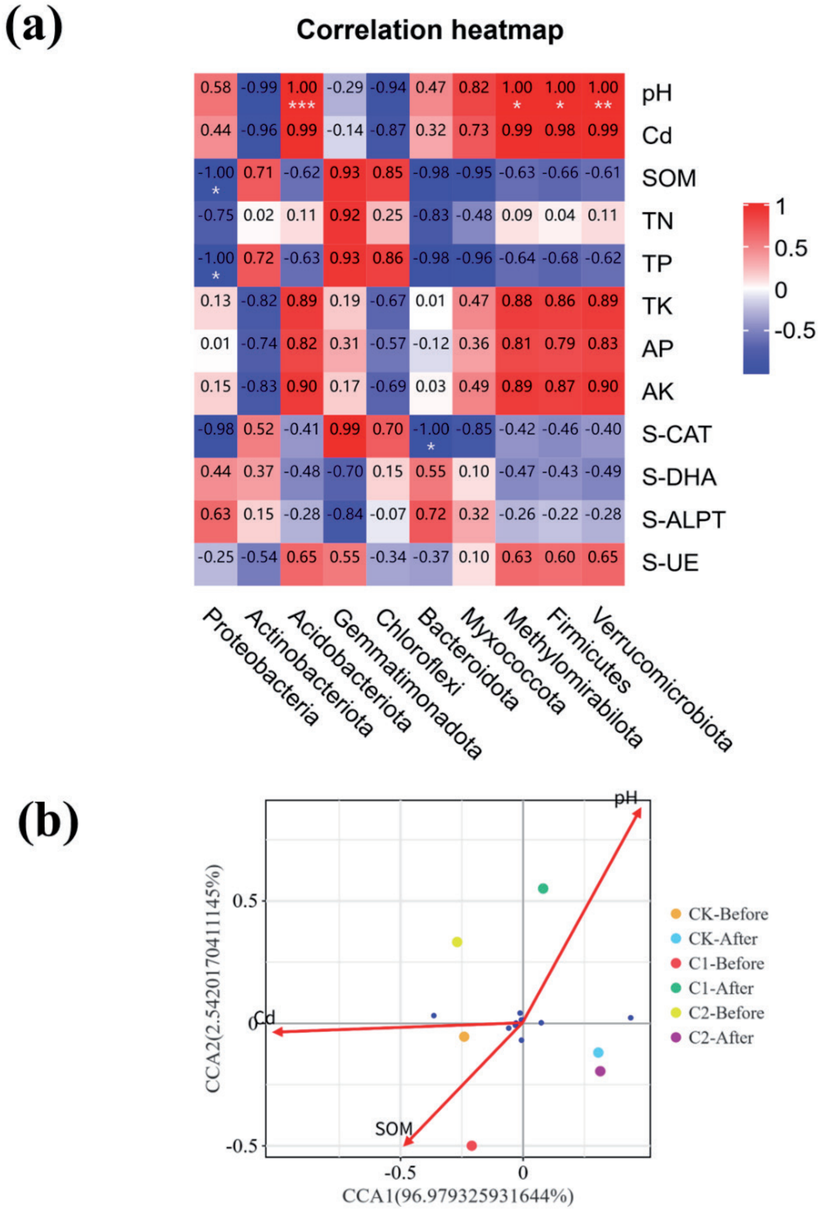


Fig. 4. Evolution of soil microbial community structure in different experimental groups. a) Phylum level; b) Genus level. CK (Before and After), C1 (Before and After), and C2 (Before and After) represent the soil microbial community structure before and after treatment for the control group, maize group, and ryegrass-maize intercropping group, respectively.

is shown in Fig. 5a). Proteobacteria showed a significant positive correlation with pH but a strong negative correlation with SOM and TN, indicating that it is more adapted to nutrient-poor alkaline environments, whereas Actinobacteriota showed a highly significant negative correlation with pH and Cd, but a positive correlation with SOM and TP, indicating a preference for organic matter-rich meso-acidic environments and a potential for Cd tolerance. It is worth noting that Acidobacteriota, Methylomirabilota, and Verrucomicrobiota showed a highly consistent pattern of association, with nearly perfect positive correlation with pH and Cd content, negative correlation with SOM and TP, and significant positive correlation with AP and AK. Gemmatimonadota showed unusually high positive correlations with SOM, TN, and TP, and almost completely positive correlations with S-CAT activity, which may play a central role in organic matter transformation and oxidative stress. On the contrary, Bacteroidota showed extremely strong negative correlations with SOM and TP, as well as a complete negative correlation with S-CAT activity, suggesting that its ecological niche is unfavorable to high organic matter environments.

The result of CCA is shown in Fig. 5b). It shows the associations between samples (before and after different treatments: CK, C1, C2) and environmental factors (pH, SOM, Cd). Axis CCA1 explains 96.98% of the variation, acting as the main dimension. pH is



	Proteobacteria	Actinobacteriota	Acidobacteriota	Gemmatimonadota	Chloroflexi	Bacteroidota	Myxococcota	Methylomirabilota	Firmicutes	Verrucomicrobiota
pH	0.58	-0.99	1.00 ***	-0.29	-0.94	0.47	0.82	1.00 *	1.00 *	1.00 **
Cd	0.44	-0.96	0.99	-0.14	-0.87	0.32	0.73	0.99	0.98	0.99
SOM	-1.00 *	0.71	-0.62	0.93	0.85	-0.98	-0.95	-0.63	-0.66	-0.61
TN	-0.75	0.02	0.11	0.92	0.25	-0.83	-0.48	0.09	0.04	0.11
TP	-1.00 *	0.72	-0.63	0.93	0.86	-0.98	-0.96	-0.64	-0.68	-0.62
TK	0.13	-0.82	0.89	0.19	-0.67	0.01	0.47	0.88	0.86	0.89
AP	0.01	-0.74	0.82	0.31	-0.57	-0.12	0.36	0.81	0.79	0.83
AK	0.15	-0.83	0.90	0.17	-0.69	0.03	0.49	0.89	0.87	0.90
S-CAT	-0.98	0.52	-0.41	0.99	0.70	-1.00 *	-0.85	-0.42	-0.46	-0.40
S-DHA	0.44	0.37	-0.48	-0.70	0.15	0.55	0.10	-0.47	-0.43	-0.49
S-ALPT	0.63	0.15	-0.28	-0.84	-0.07	0.72	0.32	-0.26	-0.22	-0.28
S-UE	-0.25	-0.54	0.65	0.55	-0.34	-0.37	0.10	0.63	0.60	0.65

Fig. 5. Correlation between changes in soil properties and changes in microbial ecosystems. a) The correlation heatmap of soil property factors and microbial community phylum-level structure (by Pearson's correlation analysis); b) Canonical correspondence analysis (CCA). CK (Before and After), C1 (Before and After), and C2 (Before and After) represent before and after treatment for the control group, maize group, and ryegrass-maize intercropping group, respectively.

positively correlated with CCA1, SOM is negatively correlated with it, and the correlation with Cd is weak; samples like C1-After lie in the high-pH area, while C1-Before is close to the low-SOM area, with a distinct difference in sample distribution before and after the treatment. In conclusion, pH and Cd content were the key factors driving microbial community differentiation; different phyla differentiated their ecological niches by developing specific resource utilization strategies (e.g., Actinobacteriota relied on organic phosphorus, Acidobacteriota preferred inorganic nutrients). The strong association between enzyme activities (e.g., S-CAT) and specific phyla (Gemmatimonadota) provides a microbiological basis for the regulation of soil biochemical processes. The enrichment of Cd-tolerant taxa like Lysobacter in C2 suggests that ryegrass root exudates selectively promote beneficial microbes, forming a "protective microbiome" that reduces Cd bioavailability. This aligns with the "rhizosphere engineering" concept, where plant-microbe interactions enhance metal immobilization and plant tolerance [41]. The positive correlation between Actinobacteriota and soil organic phosphorus implies these bacteria may facilitate phosphorus mobilization, compensating for the low available phosphorus in C2 (9.39 mg/kg) and supporting plant growth under stress. These microbial adjustments highlight the ecosystem's adaptive capacity to pollution, offering potential targets for developing microbial inoculants to enhance intercropping efficiency.

Mechanisms for Remediation of Cd Contamination by Ryegrass-Maize Intercropping

The translocation factor (TF) of ryegrass for Cd ranged from 0.6 to 0.8, which indicated that ryegrass has a strong ability to transport heavy metals from the root system to the aboveground part, and can effectively achieve aboveground enrichment of heavy metals and create conditions for the subsequent harvesting and removal [42]. Maize has a low translocation coefficient for Cd, a phenomenon that is of great significance in the study of Cd contamination remediation in agricultural fields. On the one hand, for people who consume maize kernels, the relatively small amount of Cd translocated to the kernels is beneficial and reduces the risk of Cd intake through the food chain. On the other hand, from the perspective of farmland contamination remediation, it is difficult to effectively reduce the Cd content in the soil by harvesting the aboveground part because a large amount of Cd is retained in the root system, making maize alone less efficient in remediating Cd-contaminated soil [43]. When maize is intercropped with ryegrass, the strong Cd transport capacity of ryegrass can compensate for the deficiencies of maize, and the two act synergistically to enhance the overall remediation effect, providing a more effective way to treat Cd-polluted farmland. Three key mechanisms drove the superior performance of C2: 1) Phytoremediation synergy: Ryegrass's high Cd uptake (shoot Cd concentration: 215 mg/kg) complemented maize's low grain translocation, creating a "barrier effect" that reduces Cd accumulation in edible parts; 2) Soil chemical transformation: Increased soil pH in Cd treatments promoted Cd precipitation as hydroxides, while ryegrass-secreted organic acids enhanced Cd desorption from soil colloids, facilitating its uptake; 3) Microbial functional adaptation: Tolerant microbial communities in C2 accelerated Cd immobilization via extracellular polymeric substances (EPS) and metal-binding proteins, reducing Cd mobility in the rhizosphere.

Under more alkaline conditions, Cd is more likely to form hydroxide precipitates or be adsorbed by soil colloids, thus reducing its bioavailability and allowing the soil to tolerate higher levels of Cd without affecting the safe production of maize [44]. Under this pH environment, the synergistic effect of ryegrass and maize root secretions further promoted the transformation of soil Cd to stable forms on the one hand, and enhanced the microbial fixation or transformation of Cd, possibly by regulating the structure of the inter-root microbial community, on the other hand. During the growth process, the corn root system will secrete some substances to change the physicochemical properties of the inter-root soil, such as pH, thus affecting the morphology and distribution of Cd in the soil [41]. Some of the Cd was converted from the more bioactive form to a relatively stable form, which reduced its toxicity to maize and allowed the soil to accommodate higher levels of Cd without significantly affecting the safe production of maize. In addition, the structure of the inter-root microbial community was changed under the cropping pattern, and the microbial utilization and transformation pathway of phosphorus became complicated, which may lead to part of the AP being immobilized by microorganisms or transformed into other forms that are difficult to be absorbed by the crop [45]. Ryegrass-maize intercropping as a phytoremediation technique may reduce the bioavailability of heavy metals in the soil by uptake, accumulation, and translocation of heavy metals through the ryegrass root system. This suggests that ryegrass-maize intercropping can enhance the biosorption and enrichment of Cd, providing a potential mechanism for the remediation of heavy metal contamination in agricultural fields.

It is important to note that these conclusions stem from controlled pot experiments with artificially added cadmium, which differ significantly from field environments with historical cadmium contamination. This discrepancy may lead to variability in the actual remediation effectiveness of the ryegrass-maize intercropping system. First, the artificially added cadmium in the experiment existed in relatively mobile forms with high bioavailability. In contrast, cadmium in historically contaminated soils undergoes prolonged aging processes (such as chelation with soil organic matter, coprecipitation with minerals, and immobilization within soil aggregates), forming stable components with low bioavailability. This implies that cadmium extraction efficiency by ryegrass in historically contaminated areas may be substantially lower than the over 70% reduction rate observed in the experiment. While ryegrass root exudates effectively activate readily mobile cadmium, their ability to mobilize aged cadmium is limited, thereby reducing the overall remediation efficacy of the intercropping system. Second, soil microbial communities in historically contaminated areas have undergone long-term adaptive evolution, forming functionally specialized cadmium-tolerant microbial communities. This contrasts with the relatively diverse initial microbial communities in control experiments. Under these conditions, ryegrass's capacity to further enrich beneficial microbial populations (e.g., *Bacillus lyticus*) may be constrained, diminishing the synergistic effects of plant-microbe interactions on cadmium immobilization. Third, historically contaminated soils often present compound issues, including degraded soil structure, long-term nutrient depletion, and the coexistence of multiple heavy metals. In nutrient-depleted historical contamination sites, nutrient competition between ryegrass and maize within the intercropping system intensifies. Additionally, reduced soil organic matter (decreased by 19.5% in the experiment) may become more severe, further limiting the growth of both crops and remediation plants and hindering the sustainability of the remediation process. Furthermore, although the maize variety used in the experiment exhibits moderate cadmium tolerance, prolonged cadmium stress in historically contaminated fields may diminish its "barrier effect" against cadmium translocation to grains, increasing the risk of cadmium contamination of edible parts.

Conclusions

Ryegrass-maize intercropping significantly reduced soil Cd content (restoration efficiency up to 97%), and ryegrass had outstanding Cd enrichment and translocation ability (translocation factor, TF: 0.6-0.8). However, intercropping implies competition for some soil nutrients, and the ratio of intercropping needs to be set reasonably. The changes in catalase activity reflected the changes in soil redox status, and the Cd treatment might have inhibited the activity of some microorganisms. The increase in alkaline protease and urease activities indicated that Cd treatment might have enhanced the effectiveness of soil nitrogen by changing the microbial community structure. A decrease in dehydrogenase activity indicated that Cd inhibited the metabolic activity of soil microorganisms, which may affect soil organic matter decomposition and nutrient cycling. These changes in enzyme activities collectively reflect the complex effects of Cd on soil organic matter decomposition, nitrogen cycling, and microbial activity. Cd contamination had a significant inhibitory effect on soil microbial diversity, whereas the effects of ryegrass-maize intercropping on microbial communities were more complex, and may either mitigate Cd toxicity or have an additional impact on microbial diversity as a result of altered soil conditions. Cd contamination significantly altered the structure of soil microbial communities, resulting in a decrease in the abundance of sensitive phyla such as Proteobacteria and a significant increase in the abundance of the more tolerant Actinobacteriota. Ryegrass-maize intercropping mitigated the negative effects of Cd on the microbial community to a certain extent, but its effect was limited and failed to completely restore the diversity of microbial communities. These changes reflected the long-term toxic effects of Cd on soil microbial communities and the adaptive advantages of tolerant flora in Cd-contaminated environments. From an ecological perspective, the ryegrass-maize intercropping system embodies a "nature-based solution", achieving sustainable remediation through plant-microbe interactions while maintaining soil functionality. Future research should explore genotype-specific interactions (e.g., cadmium-tolerant maize varieties) and climate adaptability to broaden this technology's application across diverse agricultural regions. Long-term field trials in historically cadmium-contaminated areas should quantify the actual remediation efficiency of intercropping systems while developing targeted optimization strategies (e.g., combining soil amendments to activate immobilized cadmium, breeding cadmium-tolerant high-efficiency remediation crop varieties) to enhance their adaptability to historically contaminated fields.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. SHI G., HU J., ZHANG S., NI G., SHI W., HU C., ZHAO X. The application of exogenous Se improved the remediation efficiency of *Lolium multiflorum* Lam grown in nonylphenol-Cd Co-contaminated soil. Journal of Environmental Chemical Engineering, **10** (6), 108962, **2024**.
2. WU Y., LI X., YU L., WANG T., WANG J., LIU T. Review of soil heavy metal contamination in China:

Spatial distribution, primary sources, and remediation alternatives. Resources, Conservation and Recycling, **181**, 106261, **2022**.

3. QIN G., NIU Z., YU J., LI Z., MA J., XIANG P. Soil heavy metal contamination and food safety in China: Effects, sources and removing technology. Chemosphere, **267**, 129205, **2021**.
4. MAI X., TANG J., TANG J., ZHU X., YANG Z., LIU X., ZHUANG X., FENG G., TANG L. Research progress on the environmental risk assessment and remediation technologies of heavy metal contamination in agricultural soil. Journal of Environmental Sciences, **149**, 1, **2025**.
5. ZHANG R.J., JI X.H., XIE Y.H., XUE T., LIU S.H., TIAN F.X., PAN S.F. A novel graph convolutional neural network model for predicting soil Cd and as contamination: Identification of influencing factors and interpretability. Ecotoxicology and Environmental Safety, **292**, 117926, **2025**.
6. YIN Z., XIE Y., WANG S., LI Q., WAN S., CHEN L., DAI X., WANG R., DESNEUX N., ZHI J., TANG B. Bioaccumulation and transferring for impacts on Cd and Pb by aphid consumption of the broad bean, *Vicia faba* L, in soil heavy metal contamination. Chemosphere, **360**, 142429, **2024**.
7. CHEN X., CHU S., CHI Y., WANG J., WANG R., YOU Y., HAYAT K., KHALID M., ZHANG D., ZHOU P., JIANG J. Unraveling the role of multi-walled carbon nanotubes in a corn-soil system: Plant growth, oxidative stress and heavy metal(loid)s behavior. Plant Physiology and Biochemistry, **200**, 107802, **2023**.
8. MENG F., LIU D., BU T., ZHANG M., PENG J., MA J. Assessment of contamination and health risks from exposure to heavy metals in soil, wheat grains, drinking water, and atmospheric particulate matter. Journal of Environmental Management, **376**, 124448, **2025**.
9. PEKEL A.Y., ÇALIK A., ALATAŞ M.S., KUTER E., CENGIZ Ö., OMURTAG G.Z., İNAN G. Evaluation of Correlations Between Nutrients, Fatty Acids, Heavy Metals, and Deoxynivalenol in Corn (*Zea mays* L.). Journal of Applied Poultry Research, **28** (1), 94, **2019**.
10. ZHANG J., HAO A., ZHAO B., MA F., ZHANG X., ZHANG Y., DUAN K., LI Y. Effects of microplastics and cadmium co-contamination on soil properties, maize (*Zea mays* L.) growth characteristics, and cadmium accumulation in maize in loessial soil-maize systems. Environmental Contamination, **356**, 124363, **2024**.
11. RAJENDRAN S., PRIYA T.A.K., KHOO K.S., HOANG T.K.A., NG H.S., MUNAWAROH H.S.H., KARAMAN C., OROOJI Y., SHOW P.L. A critical review on various remediation approaches for heavy metal contaminants removal from contaminated soils. Chemosphere, **287**, 132369, **2022**.
12. LIU N., ZHAO J., DU J., HOU C., ZHOU X., CHEN J., ZHANG Y. Non-phytoremediation and phytoremediation technologies of integrated remediation for water and soil heavy metal contamination: A comprehensive review. Science of The Total Environment, **948**, 174237, **2024**.
13. DENG S., ZHANG X., ZHU Y., ZHUO R. Recent advances in phyto-combined remediation of heavy metal contamination in soil. Biotechnology Advances, **72**, 108337, **2024**.
14. LIU Y.Q., CHEN Y., LI Y.Y., DING C.Y., LI B.L., HAN H., CHEN Z.J. Plant growth-promoting bacteria improve the Cd phytoremediation efficiency of soils contaminated with PE–Cd complex contamination by influencing the rhizosphere microbiome of sorghum. Journal of Hazardous Materials, **469**, 134085,**2024**.
15. SUN J., JIA Q., LI Y., DONG K., XU S., REN Y., ZHANG T., CHEN J., SHI N., FU S. Effect of arbuscular mycorrhiza fungus *Diversispora eburnea* inoculation on *Lolium perenne* and *Amorpha fruticosa* growth, cadmium uptake, and soil cadmium speciation in cadmium-contaminated soil. International Journal of Environmental Research and Public Health, **20** (1), 795, **2023**.
16. WANG W., XUE J., ZHANG L., HE M., YOU J. Extraction of heavy metals from copper tailings by ryegrass (*Lolium perenne* L.) with the assistance of degradable chelating agents. Scientific Reports, **14** (1), 7663, **2024**.
17. SHU Y., LI D., XIE T., ZHAO K., ZHOU L., LI F. Antibiotics-heavy metals combined contamination in agricultural soils: Sources, fate, risks, and countermeasures. Green Energy & Environment, **10** (5), 869, **2024**.
18. ZHAO Y., YAO J., LI H., SUNAHARA G., LI M., TANG C., DURAN R., MA B., LIU H., FENG L., ZHU J., WU Y. Effects of three plant growth-promoting bacterial symbiosis with ryegrass for remediation of Cd, Pb, and Zn soil in a mining area. Journal of Environmental Management, **353**, 120167, **2024**.
19. KOU B., YU T., TANG J., ZHU X., YUAN Y., TAN W. Kitchen compost-derived humic acid application promotes ryegrass growth and enhances the accumulation of Cd: An analysis of the soil microenvironment and rhizosphere functional microbes. Science of The Total Environment, **919**, 170879, **2024**.
20. KOU B., HUO L., CAO M., HUI K., TAN W., YUAN Y., JIANG Y. New insights into the stages of cadmium remediation in ryegrass enhanced by kitchen compost-derived dissolved organic matter: Activation, absorption, and storage. Science of The Total Environment, **955**, 177138, **2024**.
21. XIE H., MA Y., WANG Y., SUN F., LIU R., LIU X., XU Y. Biological response and phytoremediation of perennial ryegrass to halogenated flame retardants and Cd in contaminated soils. Journal of Environmental Chemical Engineering, **9** (6), 106526, **2021**.
22. LI J., LI Q., LIANG S., ZHANG B., WANG J., YU Y., XIA L., ZHANG Y., WANG Y. Aging mechanism of biochar based on fluorescence spectroscopy: Assessing soil dissolved organic matter (DOM) dynamics and Cd bioavailability. Chemical Engineering Journal, **505**, 159538, **2025**.
23. LI H., WANG G., HU F., FAN Z., YIN W., CAO W., CHAI Q., YAO T. Additive intercropping green manure enhances maize water productivity through water competition and compensation under reduced nitrogen fertilizer application. Agricultural Water Management, **311**, 109388, **2025**.
24. HE Y., HOU X., WU X., DUAN C., LIU C., YIN L., ZHANG M., FU D. Fertilization and intercropping reduce Pb accumulation in plants by influencing rhizosphere soil phosphorus forms in soil-plant systems. Ecotoxicology and Environmental Safety, **293**, 118011, **2025**.
25. BASSEGIO D., SECCO D., ANDRADE D. DE S., ZANÃO JÚNIOR L.A., MARINS A.C. DE, DE SOUZA S.N.M., CHANG P., MESSA V.R., SAVIOLI M.R., CASTRO M.B.S., SILVA É.L. Impact of sowing time of maize and ruzigrass intercropping systems on soil chemical, physical and microbiological properties in an Oxisol from southern Brazil. Geoderma Regional, **40**, e00937, **2025**.

26. LO PRESTI E., KAVAMURA V.N., ABADIE M., ROMEO M., REID T.E., HEUER S., MONTI M., MAUCHLINE T.H. Phosphorus availability drives the effect of legume-wheat intercropping on prokaryotic community interactions. Applied Soil Ecology, **199**, 105414, **2024**.
27. REICHMANN M., BLANC L., LAMPURLANÉS J., SIMON-MIQUEL G., PLAZA-BONILLA D. Does Intercropping improve soil aggregation and organic carbon protection? A case-study in the Semi-Arid Mediterranean. Agriculture, Ecosystems & Environment, **385**, 109563, **2025**.
28. AGHABABAEI F., RAIESI F. Mycorrhizal fungi and earthworms reduce antioxidant enzyme activities in maize and sunflower plants grown in Cd-polluted soils. Soil Biology and Biochemistry, **86**, 87, **2015**.
29. LI X., KONG H., HUANG J., YAN J., HE W., WANG H., PAN H., YANG Q., ZHUGE Y., LOU Y. Intercropping wheat and rapeseed in Cd-polluted weakly alkaline soil: Crop productivity, Cd enrichment capacity, and rhizosphere soil characteristics. Journal of Agriculture and Food Research, **19**, 101721, **2025**.
30. HANNAN F., IQBAL M., ISLAM F., FAROOQ M. A., SHAKOOR M. B., AYYAZ A., KHAN M.S.S., LI J., HUANG Q., ZHOU W. Remediation of Cd-polluted soil, improving Brassica napus L. growth and soil health with Hardystonite synthesized with zeolite, limestone, and green Zinc oxide nanoparticles. Journal of Cleaner Production, **437**, 140737, **2024**.
31. CHANG H., ZHANG C., ZANG S., ZHU Y., ZHAO W., QU Y., WANG H. Study on the influencing mechanism of biochar and ethylenediaminetetraacetic acid combination on the remediation of Cd polluted soil by Sedum alfredii Hance. Environmental Technology & Innovation, **36**, 103875, **2024**.
32. YANG J., GUO Z., AL-DHABI N.A., SHI J., PENG Y., MIAO B., LIU H., LIANG Y., YIN H., LIU X., TANG W., JIANG L. The succession of microbial community and distribution resistance gene in response to enrichment cultivation derived from a long-term toxic metal(loid) s polluted soil. Science of The Total Environment, **954**, 176385, **2024**.
33. GAI X., LI X., XING W., ZHANG X., CHEN G. Mechanism insights into amendments enhanced dendroremediation for Cd and Zn-polluted soil: Bacterial co-occurrence networks' complexity and stability. Geoderma, **451**, 117088, **2024**.
34. BAO Q., SHI J., LIU Z., KAN Y., BAO W. Application of crop straw with different C/N ratio affects CH4 emission and Cd accumulation in rice (*Oryza sativa* L.) in Cd polluted paddy soils. Climate Smart Agriculture, **2** (1), 100036, **2025**.
35. LI T., WANG S., ZHAO L., YUAN X., GAO Y., FU D., LIU C., DUAN C. Improvement of soil nutrient cycling by dominant plants in natural restoration of heavy metal polluted areas. Environmental Research, **263**, 120030, **2024**.
36. GUO J., LUO X., ZHANG Q., DUAN X., YUAN Y., ZHENG S. Contributions of selenium-oxidizing bacteria to selenium biofortification and cadmium bioremediation in a native seleniferous Cd-polluted sandy loam soil. Ecotoxicology and Environmental Safety, **272**, 116081, **2024**.
37. HUANG L., MENG Y., PAN B., PAN B., WEI J., DING J., DENG Y., SU X., YUAN Z., ZHANG M. Multidimensional effects of green waste vermicomposting on cadmium contaminated soil ecosystems: From physicochemical properties to microbial communities. Journal of Hazardous Materials, **480**, 136429, **2024**.
38. SHI G., HOU R., FU Q., LI T., CHEN Q. Effects of biochar and compost on microbial community assembly and metabolic processes in glyphosate, imidacloprid and pyraclostrobin polluted soil under freeze thaw cycles. Journal of Hazardous Materials, **471**, 134397, **2024**.
39. PENG H., DENG K., SHI Y., LIU S., JIAN Z., LI C., JI X., LI S. Alleviation of Cd-polluted paddy soils through Si fertilizer application and its effects on the soil microbial community. Science of The Total Environment, **855**, 158735, **2023**.
40. LAN W., ZHOU Q., LI J., LIU M., DENG Y., HUANG Y., ZHOU Y., YANG H., XIAO Y. Investigation of Cd and Pb enrichment capacities of Erigeron sumatrensis across three polluted regions: Insights into soil parameters and microbial communities. Environmental Research, **262**, 119868, **2024**.
41. YANG A., XIAO X., SHEN M., ZHOU C., WANG X., ZHANG C., WANG X. Enhancing arsenic and cadmium phytoextraction and soil microbial response with fertilizers or oxalic acid in *Pteris vittata* L. and *Sedum alfredii* Hance intercropping system. Journal of Environmental Chemical Engineering, **13** (1), 115250, **2025**.
42. SHI J., ZHAO D., REN F., HUANG L. Spatiotemporal variation of soil heavy metals in China: The contamination status and risk assessment. Science of The Total Environment, **871**, 161768, **2023**.
43. CHI G., FANG Y., ZHU B., GUO N., CHEN X. Intercropping with *Brassica juncea* L. enhances maize yield and promotes phytoremediation of cadmium-contaminated soil by changing rhizosphere properties. Journal of Hazardous Materials, **461**, 132727, **2024**.
44. LI Y., XING T., FU Z., PU T., DING P., WU Y., YANG F., WANG X., YONG T., YANG W. Rhizosphere bacterial communities mediate the effect of maize-soybean strip intercropping and nitrogen management on cadmium phytoextraction. Applied Soil Ecology, **207**, 105934, **2025**.
45. WANG X., FAN X., CHANG W., LI K., ZHANG M., PU G., KURAKOV A.V., PING Y., SONG F. Enhancing soil quality in soybean cultivation: Mycorrhizal technology combined with intercropping under high cadmium stress. Ecotoxicology and Environmental Safety, **290**, 117558, **2025**.