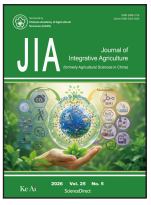
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RESEARCH ARTICLE

Legume–cereal intercropping with AMF reduces cadmium bioavailability and enhances land productivity

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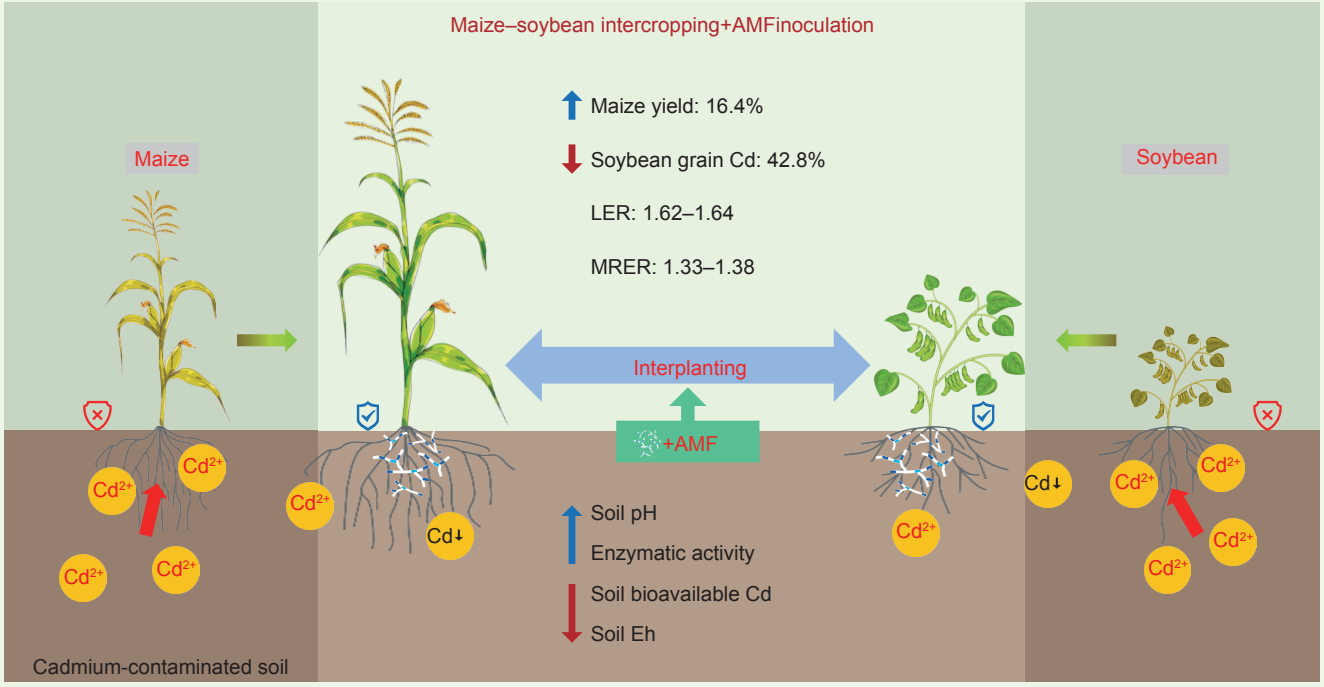
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Highlights

- Maize–soybean intercropping reduces grain Cd to safe levels (<0.20 mg kg^{-1}), ensuring compliance with China's food safety standard (GB2762-2022) while maintaining yield advantages (LER>1).
- AMF inoculation synergistically enhances intercropping benefits, improving Cd removal efficiency (MRER>1) and crop resilience through enhanced nutrient uptake and improved rhizosphere soil health.
- A "repair while producing" model demonstrates that strategic crop pairing combined with AMF integration can simultaneously remediate Cd-polluted soils, ensure food safety, and optimize land productivity.

Graphical abstract



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Abstract

The combined implementation of intercropping systems and arbuscular mycorrhizal fungi (AMF) inoculation represents a promising phytoremediation strategy for heavy metal-contaminated farmland, providing both ecological and economic benefits. However, additional research is necessary to understand the influence of AMF and intercropping on Cd bioavailability. This study examines the synergistic effects of maize–soybean intercropping and AMF inoculation on crop growth, cadmium (Cd) allocation patterns, and rhizosphere soil dynamics through comprehensive field and pot experiments. Field trials revealed significant yield advantages in maize–soybean intercropping systems, with land equivalent ratios (LERs) of 1.62 (common maize) and 1.64 (sweet maize). Intercropping decreased soybean Cd accumulation across all tissues, notably in grains (42.8% reduction), while maintaining maize grain Cd concentrations below China's food safety threshold (0.20 mg kg^{-1}). The metal removal equivalent ratio (MRER) achieved 1.33–1.38 in field conditions, validating intercropping's dual advantage in productivity and Cd phytoextraction. Pot experiments indicated the AMF-inoculated intercropping system (IN+A) increased maize yield by 16.4% while reducing Cd accumulation in both crops, with grain concentrations meeting safety standards. Rhizosphere analysis demonstrated IN+A treatment substantially improved soil health indicators: 34.5% reduction in bioavailable Cd, elevated pH, decreased redox potential (Eh), and enhanced catalase activity. AMF colonization rates were 2.2–4.3 times higher in inoculated treatments (11.5–14.0%) *vs.* controls (3.2–5.3%). These results establish that AMF-enhanced legume–cereal intercropping reduces Cd bioavailability through soil alkalinization (pH increase) coupled with redox potential reduction, and metal allocation plasticity redirecting Cd to root tissues. This interaction between microbial symbiosis and plant community design stabilizes Cd in soils while maintaining crop safety (grain Cd<0.20 mg kg^{-1}), establishing an ecoengineering approach for contaminated farmland remediation.

Keywords: phytoremediation, cadmium, interplanting, maize, soybean

1. Introduction

Heavy metal contamination of agricultural soils, particularly cadmium (Cd), represents a significant impediment to sustainable food production, with over 12% of global arable land currently exceeding safe Cd thresholds (Antoniadis *et al.* 2019). Unlike other heavy metals, Cd demonstrates exceptional soil mobility and plant bioavailability, facilitating its rapid entry into food chains at concentrations as low as 0.2 mg kg^{-1} (Rai *et al.* 2023). Modern chemical agricultural practices, including intensive phosphate fertilizer use and wastewater irrigation, have intensified Cd accumulation in soils by 22–45% across major grain-producing regions since 2010 (Zou *et al.* 2021). This contamination pattern threatens both ecosystem health and human food security, requiring remediation strategies that balance soil detoxification with agricultural productivity infiltrate the human food chain (Rai *et al.* 2023).

Phytoremediation, an effective and environmentally sustainable approach, employs plants to extract, sequester, or degrade contaminants from soil (Wang and Que 2013; Ashraf *et al.* 2019; Awa and Hadibarata 2020). However, hyperaccumulator-based monocultures frequently compromise agricultural productivity and lack scalability (Brooker *et al.* 2024). Intercropping systems, particularly legume–cereal combinations, present a rhizosphere engineering solution by combining contaminant stabilization with ecosystem services. The spatial complementarity of deep-rooted cereals (e.g., *Zea mays* L.) and nitrogen-fixing legumes (e.g., *Glycine max* L.) generates alkaline rhizosphere microsites, increasing pH by 0.3–0.8 units, promoting Cd carbonate precipitation and reducing dissolved Cd^{2+} by 38–54% (Li *et al.* 2023). Recent studies indicate that plant–plant interactions significantly influence soil organic carbon (SOC) turnover in rhizosphere soils (Wang *et al.* 2024) and enhances microbial diversity essential for heavy metal immobilization. These systems also reduce Cd bioavailability through root exudate-mediated chemodynamic shifts — soybean-derived citrate enhances Cd adsorption on Fe/Mn oxides, while maize phenolic acids inhibit Cd translocation *via* Casparian strip modification (Zhang *et al.* 2023).

To enhance these benefits, arbuscular mycorrhizal fungi (AMF) symbiosis can be strategically incorporated. AMF enhance plant resilience by modifying rhizosphere Cd speciation through hyphae-mediated pH modulation and glomalin secretion (Hao *et al.* 2022). Field trials demonstrate the synergistic potential of AMF inoculation and soybean–*Solanum nigrum* L. intercropping as a viable approach to mitigate Cd contamination in soybean while improving soil health in Cd-contaminated soils (Yan *et al.* 2024). This synergy likely stems from AMF-driven nutrient exchange networks that simultaneously upregulate metal chelators and downregulate Cd transporters in roots (Meena *et al.* 2018; Zhang *et al.* 2023).

This study examines the synergistic effects of maize–soybean intercropping and AMF (*Glomus mosseae*) inoculation on Cd bioavailability reduction and agricultural productivity. The research hypothesizes that AMF-enhanced nutrient exchange and metal chelation will amplify intercropping's rhizosphere engineering effects. The objectives are to: (i) quantify the impacts of cropping patterns and AMF on Cd allocation and yield; (ii) evaluate rhizosphere mechanisms driving Cd immobilization (pH, redox potential (Eh), enzyme activity); and (iii) assess the system's dual functionality in safe crop production and soil remediation. This research provides a theoretical foundation for understanding the combined effects of intercropping

and AMF inoculation in improving phytoremediation of Cd-contaminated soils.

2. Materials and methods

2.1. Plant cultivation

Field trials employed three cultivars: common maize (*Z. mays* L. cv. Huayu 1502) (H), sweet maize (*Z. mays* L. cv. Yuetian 8) (Y), and soybean (*G. max* L. cv. Huachun 12) (B). Pot experiments utilized cultivars Huayu 1502 (maize) and Huachun 12 (soybean). All seeds were procured from College of Agronomy, South China Agricultural University (Guangzhou, China).

2.2. Experimental soils and AMF inoculum

Field soil The field experiment employed cement pools with soil obtained from a cadmium-contaminated site (23°09′39′′N, 113°21′32′′E) maintained by South China Agricultural University. The soil exhibited the following properties: pH 5.21, organic matter 31.72 g kg^{-1}, available P 21.98 mg kg^{-1}, available K 112.6 mg kg^{-1}, total Cd 1.83 mg kg^{-1}.

Potting soil Soil for the pot experiment was collected from the Qujiang remediation base (24°29′31′′N, 113°48′39′′E) downstream of Dabaoshan mining area. The soil underwent pre-treatment including air-drying, sieving (<2 mm), and steam sterilization (121°C, 2 h). The soil characteristics were: pH 6.70, organic matter 17.76 g kg^{-1}, available P 5.85 mg kg^{-1}, available K 58.70 mg kg^{-1}, total Cd 0.73 mg kg^{-1}.

The AMF *G. mosseae* BEG167 supplied by College of Agriculture, Guangxi University (Nanning, China) underwent propagation on maize roots for 12 weeks. The inoculum (2% v/v) comprised spores (≥50 spores g^{-1}) and colonized root fragments (Wang *et al.* 2008).

2.3. Experimental setup

The field experiment utilized cement pools measuring 2 m^2 (1 m×2 m) arranged in a completely randomized design with three replicates. Five experimental treatments were established: (1) common maize monoculture (M-H); (2) sweet maize monoculture (M-Y); (3) soybean monoculture (M-B); 4) common maize–soybean intercropping (IN-HB), with the common maize and soybean components in this treatment denoted as IN-H and IN-HB-B, respectively; and (5) sweet maize–soybean intercropping (IN-YB), with the sweet maize and soybean components in this treatment denoted as IN-Y and IN-YB-B, respectively. All maize cultivars maintained a uniform row spacing of 0.3 m, while soybean rows were spaced at 0.22 m. In intercropping systems, the row spacing between maize and soybean was fixed at 0.22 m (Fig. 1-A). Standard agricultural practices for irrigation, fertilization, and field management were uniformly applied across all

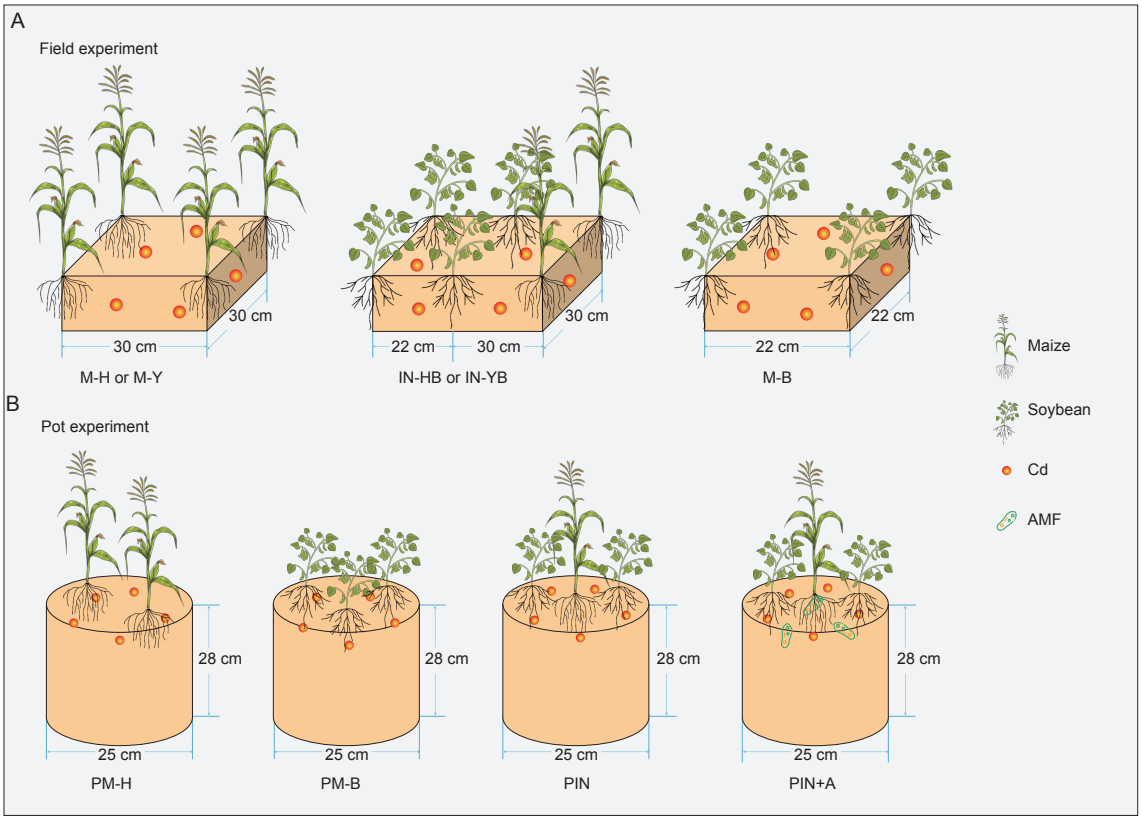


Fig. 1 Schematic representation of field experiment (A) and pot experiment (B). M-H, maize cultivar Huayu 1502 in monoculture; M-Y, maize cultivar Yuetian 8 in monoculture; IN-HB, maize–soybean intercropping with maize cultivar Huayu 1502; IN-YB, maize–soybean intercropping with maize cultivar Yuetian 8; M-B, soybean cultivar Huachun 12 in monoculture; PM-H, maize cultivar Huayu 1502 in monoculture; PM-B, soybean cultivar Huachun 12 in monoculture; PIN, maize–soybean intercropping; PIN+A, maize–soybean intercropping with AMF inoculation.

plots. The experimental period extended from 31 March to 18 June 2021. A complementary pot experiment was conducted from 22 March to 4 July 2021 using pots (25 cm diameter×28 cm height) containing 5.0 kg of soil. The experimental design comprised four treatments with three replicates: (1) maize monoculture (PM-H); (2) soybean monoculture (PM-B); (3) maize-soybean intercropping without AMF inoculation (PIN), in which PIN-H and PIN-B denote intercropped maize and soybean, respectively; and (4) AMF-inoculated maize–soybean intercropping (PIN+A), in which PIN+A-H and PIN+A-B denote intercropped maize and soybean under AMF inoculation, respectively. This yielded 24 experimental units (Fig. 1-B). Prior to planting, soil underwent sterilization by autoclaving at 121°C for 30 min. For AMF inoculation, 100 g of inoculant was thoroughly incorporated into the middle soil layer (1/3–2/3 depth) (Luo *et al.* 2017; Li *et al.* 2025). Non-inoculated treatments received sterilized AMF inoculum packs to maintain experimental consistency. Soil moisture was maintained at 60% water-holding capacity throughout the experiment. Compound fertilizer solution was applied weekly during the growing season to ensure adequate nutrient availability (Li *et al.* 2016).

2.4. Analytical procedures

Sampling Root and shoot samples were collected during the seedling, heading (maize-specific), and maturity growth stages. Rhizosphere soil was obtained through vigorous shaking, air-dried, and sieved (<150 μm). Root architecture measurements were performed using a root scanner (Epson Expression 1600 pro, Model EU-35, Seiko Epson Corporation, Japan) and analyzed by WinRHIZO Reg2009 to determine the root parameters.

Cadmium analysis For cadmium determination in plants, dried samples (0.10 g) underwent digestion with 10 mL of ultrapure nitric acid using a microwave digestion system (MARS6, CEM Corporation, USA). The digestion process occurred at 120°C until the solution volume decreased to 2–3 mL, followed by dilution to 25 mL with deionized water. Cadmium concentrations were measured by graphite furnace atomic absorption spectroscopy (GFAAS, TAS-990, Persee General Instrument Co., Ltd., Beijing, China)(Subramanian *et al.* 1983; Khairy *et al.* 2014). Quality control was performed using a certified reference material for rice flour (GBW(E) 100349, NCS Testing Technology Co., Ltd., Beijing, China) and procedural blanks (Kang *et al*. 2021).

Bioavailable cadmium in rhizosphere soil was extracted using 0.05 mol L^{-1} EDTA solution according to Sun *et al.* (2001). In brief, 5.00 g soil samples were combined with 25 mL extractant and oscillated reciprocally (300 r min^{-1}, 2 h). The suspensions underwent centrifugation (4,000×g, 20 min) and filtration through 0.45 μm membranes before GFAAS analysis.

For different forms of Cd, the methodology proposed by Tessier *et al.* (1979) was adopted. The operationally defined Cd fractions included exchangeable Cd (F1), carbonate-bound Cd (F2), Fe-Mn oxide-bound Cd (F3), organic matter- and sulfide-bound Cd (F4), and residual Cd (F5). The operation method follows Lam *et al.* (1997) Scheme.

Rhizosphere soil characterization and AMF colonization Soil Eh and pH measurements utilized a portable ORP meter (SX-630, Sanxin, Shanghai, China) and pH meter (S210, Mettler Toledo, Zurich, Switzerland), respectively. Enzyme activities were assessed through established methods: acid phosphatase *via* disodium phenyl phosphate colorimetry (Banerjee *et al.* 2012), invertase *via* 3,5-dinitrosalicylic acid assay (Frankeberger and Johanson 1983), and catalase activity through potassium permanganate titration (Johnson and Temple 1964).

For AMF colonization analysis, ethanol-preserved roots were processed following Luo *et al.* (2017) with modifications. Root segments (1 cm) underwent sequential treatment with 10% KOH (90°C, 1 h), 5% lactic acid (5 min), and 0.05% (w/v) Trypan Blue staining (90°C, 30 min). Colonization rates were determined microscopically using the grid-line intersect method (Giovannetti and Mosse 1980), with triplicate measurements per biological replicate.

2.5. Statistics and data analysis

Bioconcentration amount (BCA) BCA was used to express the total concentration of Cd accumulated by plants (Kang *et al.* 2020; Yang *et al.* 2021).

$$BCA=\sum B_p \times C_p$$

where C_p represents the cadmium concentration (mg kg^{-1}) in plant tissues, and B_p denotes the biomass (kg) of respective organs (roots, stems, leaves, and grains) for both maize and soybean.

Land equivalent ratio (LER) LER was employed to assess intercropping productivity advantages (Yu *et al.* 2015; Chen *et al.* 2023).

$$LER=\left(\frac{Y_{im}}{Y_{mm}}\right)+\left(\frac{Y_{is}}{Y_{ms}}\right)$$

where Y_{im} and Y_{is} refer the yields of maize and soybean in the intercropping mode, respectively, and Y_{mm} and Y_{ms} refer the yields of maize and soybean in the monocrop mode, respectively. A LER>1 indicates superior land utilization efficiency in the intercropping system.

Metal removal equivalent ratio (MRER) MRER was evaluated through a novel index adapted from Kang *et al.* (2021) and Chen *et al.* (2022):

$$MRER=\left(\frac{BCA_{im}}{BCA_{mm}}\right)+\left(\frac{BCA_{is}}{BCA_{ms}}\right)$$

where BCA_{im} and BCA_{is} represent the BCA of maize and soybean in the intercropping mode, respectively, while BCA_{mm} and BCA_{ms} represent the BCA of maize and soybean in the monoculture mode, respectively.

Statistical analyses were conducted using SPSS 20.0 (IBM Corporation) with significance level set at $\alpha=0.05$. Mean comparisons were performed using one-way ANOVA followed by Duncan's post-hoc test. Data visualization was executed in Origin 2021 (OriginLab Corporation), and data organization was conducted using WPS Office 2019 (Kingsoft, China).

3 Results

3.1. Field experiment

Plant growth parameters The cropping systems significantly affected maize and soybean biomass allocation (Table 1).

Table 1 Biomass (g/plant) of maize and soybean and land equivalent ratio (LER) under different treatments

Treatment[1)]	Maize			Treatment[2)]	Soybean			Treatment[3)]	LER
	Root	Shoot	Grains		Root	Shoot	Grains		
Seedling stage									
M-H	4.43±0.59 a	43.99±2.49 a		M-B	0.90±0.16 a	4.52±0.68 ab			
IN-H	2.66±0.54 a	17.17±3.71 c		IN-HB-B	0.79±0.08 a	6.87±0.06 a		IN-HB	1.62
M-Y	3.06±0.15 a	39.16±3.97 ab		IN-YB-B	0.72±0.02 a	3.83±0.70 b		IN-YB	1.64
IN-Y	2.68±0.68 a	27.27±8.27 bc							
Heading stage									
M-H	27.39±7.08 a	248.86±61.64 a		M-B	2.14±0.33 a	50.66±3.86 a			
IN-H	27.71±5.51 a	257.24±47.90 a		IN-HB-B	2.18±0.42 a	50.98±7.93 a			
M-Y	26.28±7.89 a	362.11±30.18 a		IN-YB-B	1.79±0.22 a	44.14±8.07 a			
IN-Y	15.08±2.80 a	334.53±1.44 a							
Maturity stage									
M-H	48.05±2.86 a	861.81±49.82 a	64.05±7.38 a	M-B	3.38±0.22 a	89.40±1.29 a	11.04±0.54 a		
IN-H	41.96±0.18 ab	728.60±25.02 a	65.33±4.13 a	IN-HB-B	3.71±0.59 a	67.58±5.61 ab	6.63±0.99 b		
M-Y	25.30±0.50 b	769.51±45.81 a	37.46±5.03 b	IN-YB-B	2.79±0.36 a	58.88±9.69 b	5.49±1.49 b		
IN-Y	57.15±12.60 a	404.72±202.53 b	42.80±5.32 b						

[1)] M-H, maize cultivar Huayu 1502 in monoculture; IN-H, maize cultivar Huayu 1502 intercropped with soybeans; M-Y, maize cultivar Yuetian 8 in monoculture; IN-Y, maize cultivar Yuetian 8 intercropped with soybeans.
[2)] M-B, soybean cultivar Huachun 12 in monoculture; IN-HB-B, soybeans intercropped with maize cultivar Huayu 1502; IN-YB-B, soybeans intercropped with maize cultivar Yuetian 8.
[3)] IN-HB, maize-soybean intercropping with maize cultivar Huayu 1502; IN-YB, maize-soybean intercropping with maize cultivar Yuetian 8.
Different letters indicate significant differences among treatments at $P<0.05$ (mean±SE, $n=3$).

During the seedling stage, IN-H and IN-Y demonstrated reduced root biomass compared to their monoculture counterparts (M-H: 2.66 g *vs.* M-Y: 2.68 g), although the differences were not significant ($P>0.05$). Shoot biomass decreased significantly by 61.0% in IN-H and 30.4% in IN-Y compared to monoculture ($P<0.05$). Soybean exhibited contrasting responses: IN-HB increased biomass by 51.99%, while IN-YB showed a 15.27% reduction.

At maturity, intercropped common maize roots decreased by 12.68% *vs.* monoculture, while sweet maize roots increased substantially by 125.89%. Shoot biomass decreased by 47.41% across intercropped maize varieties. Kernel biomass showed no significant variation between intercropping treatments, although common maize demonstrated superior grain production compared to sweet maize. Soybean shoot biomass decreased by 34.14% when intercropped with sweet maize, with grain biomass reductions of 39.98% (IN-HB) and 50.28% (IN-YB). The LER of 1.62 (IN-HB) and 1.64 (IN-YB) indicated significant yield advantages in both intercropping systems.

Root architecture analysis revealed substantial reductions in soybean total root length under intercropping: 64.01% with common maize and 57.79% with sweet maize (Fig. 2-A). In contrast, intercropped sweet maize developed the largest root surface area (360.89 cm²) at maturity, surpassing monoculture values for both maize varieties (Fig. 2-A). Soybean root surface area decreased by 48.70% (IN-HB) and 52.49% (IN-YB) compared to monoculture (Fig. 2-B).

Cd content and BCA in plants Intercropping significantly affected cadmium distribution in plant tissues (Fig. 3-E and F). At maturity, root Cd concentrations in intercropped common maize (IN-H: 2.37 mg kg^{-1}) and sweet maize (IN-Y: 2.57 mg kg^{-1}) decreased by 20.20 and 49.74%, respectively, relative to

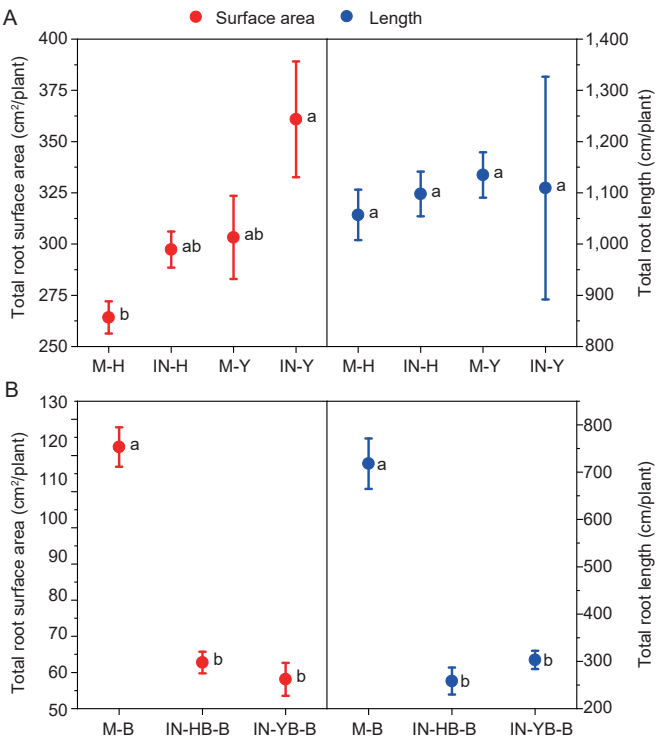


Fig. 2 Total root surface area and root length of maize (A) and soybean (B) under different treatments. M-H, maize cultivar Huayu 1502 in monoculture; IN-H, maize cultivar Huayu 1502 intercropped with soybeans; M-Y, maize cultivar Yuetian 8 in monoculture; IN-Y, maize cultivar Yuetian 8 intercropped with soybeans. M-B, soybean cultivar Huachun 12 in monoculture; IN-HB-B, soybeans intercropped with maize cultivar Huayu 1502; IN-YB-B, soybeans intercropped with maize cultivar Yuetian 8. Different letters indicate significant differences between treatments at $P<0.05$ (mean±SE, $n=3$).

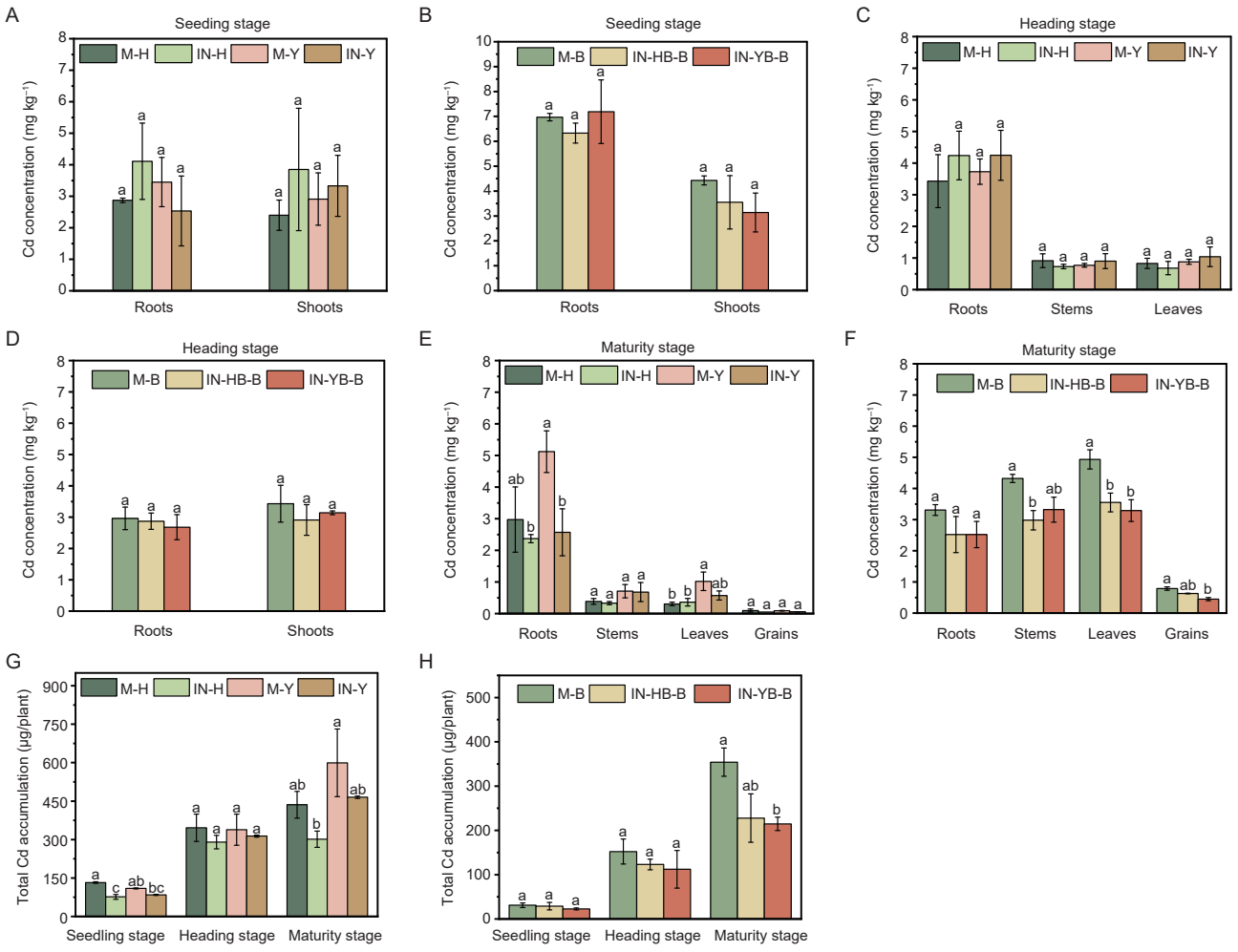


Fig. 3 Cadmium (Cd) concentrations and total Cd accumulation in maize (A, C, E and G) and soybean (B, D, F and H) under different treatments. M-H, maize cultivar Huayu 1502 in monoculture; IN-H, maize cultivar Huayu 1502 intercropped with soybeans; M-Y, maize cultivar Yuetian 8 in monoculture; IN-Y, maize cultivar Yuetian 8 intercropped with soybeans. M-B, soybean cultivar Huachun 12 in monoculture; IN-HB-B, soybeans intercropped with maize cultivar Huayu 1502; IN-YB-B, soybeans intercropped with maize cultivar Yuetian 8. Different letters indicate significant differences between treatments at $P<0.05$ (mean±SE, $n=3$).

monoculture (M-H and M-Y). Grain Cd levels remained below the Chinese food safety threshold (GB 2762–2017; 0.20 mg kg^{-1}) in all treatments: 0.10 mg kg^{-1} (M-H), 0.05 mg kg^{-1} (IN-H), 0.09 mg kg^{-1} (M-Y), and 0.06 mg kg^{-1} (IN-Y). Soybean demonstrated systemic Cd reduction under intercropping. Compared to M-B, IN-HB-B and IN-YB-B reduced Cd in roots by 23.69 and 23.89%, Cd in stems by 31.09 and 23.22%, Cd in leaves by 28.00 and 33.27%, and Cd in grains by 20.32 and 42.84% of soybean plants, respectively.

BCA progression followed the order of maturity> heading>seedling stages (Table 2). Intercropping decreased total Cd accumulation by 30.88% (IN-H) and 22.40% (IN-Y) in maize, and 39.36% (IN-YB-B) in soybean compared to monoculture. All MRER values exceeded 1 (MRER>1), indicating enhanced Cd phytoextraction efficiency in maize–soybean intercropping systems.

Rhizosphere soil characteristics Intercropping significantly modified cadmium bioavailability and soil physicochemical properties in the rhizosphere (Fig. 4). IN-H demonstrated maximum EDTA-extractable Cd concentrations of 48.59 μg kg^{-1} (heading stage) and 48.78 μg kg^{-1} (maturity), representing 164.74 and 88.45% increases over M-H, respectively (Fig. 4-A). In contrast, IN-Y displayed non-significant decreases in bioavailable Cd at maturity compared to M-Y. Soybean rhizospheres exhibited opposing patterns, with EDTA-Cd declining by 34.86% (IN-HB-B: 124.24 μg kg^{-1}) and 61.75% (IN-YB-B: 72.96 μg kg^{-1}) relative to M-B at maturity (Fig. 4-B).

Soil Eh dynamics indicated treatment-specific responses (Fig. 5). IN-H reduced rhizosphere pH during heading *vs.* M-H followed by significant alkalinization at maturity. No significant Eh variations were observed across maize treatments ($P>0.05$). Soybean intercropping systems elevated rhizosphere pH by 0.15–0.28 units compared to monoculture, while decreasing Eh by 2.89% (IN-HB-B) and 5.53% (IN-YB-B) during heading stage.

Enzyme activity profiles revealed distinct intercropping effects (Fig. 6). IN-H increased catalase (+20.90%) and invertase (+48.16%) activities at maturity *vs.* M-H (Fig. 6-A and E). IN-Y enhanced phosphatase activity by 83.21% at

Table 2 Bioconcentration amount (BCA; μg/plant) of maize and soybean and metal removal equivalent ratio (MRER) under different treatments

Treatment[1)]	Maize	Treatment[2)]	Soybean	MRER
Seedling stage				
M-H	132.25±3.00 a	M-B	31.02±5.21 a	
IN-H	76.65±9.44 c	IN-HB-B	28.87±8.32 a	1.51
M-Y	109.52±1.91 ab	IN-YB-B	22.81±2.18 a	1.50
IN-Y	84.20±2.20 bc			
Heading stage				
M-H	346.28±53.19 a	M-B	152.25±28.11 a	
IN-H	290.09±26.21 a	IN-HB-B	123.13±12.00 a	1.65
M-Y	338.68±60.82 a	IN-YB-B	112.21±42.44 a	1.66
IN-Y	313.67±4.00 a			
Maturity stage				
M-H	435.80±52.05 ab	M-B	354.10±31.93 a	
IN-H	301.22±31.68 b	IN-HB-B	227.82±54.61 ab	1.33
M-Y	599.29±131.95 a	IN-YB-B	214.74±15.15 b	1.38
IN-Y	465.07±4.73 ab			

[1)] M-H, maize cultivar Huayu 1502 in monoculture; IN-H, maize cultivar Huayu 1502 intercropped with soybeans; M-Y, maize cultivar Yuetian 8 in monoculture; IN-Y, maize cultivar Yuetian 8 intercropped with soybeans.
[2)] M-B, soybean cultivar Huachun 12 in monoculture; IN-HB-B, soybeans intercropped with maize cultivar Huayu 1502; IN-YB-B, soybeans intercropped with maize cultivar Yuetian 8.
Different letters indicate significant differences among treatments at $P<0.05$ (mean±SE, $n=3$).

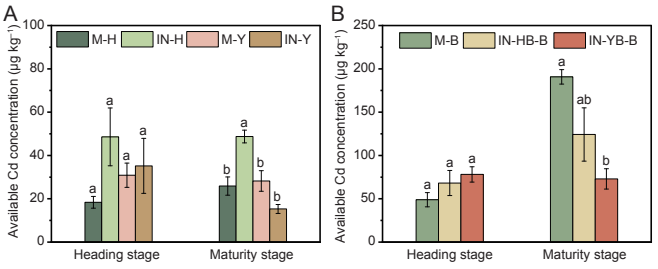


Fig. 4 Available cadmium (Cd) concentrations in maize (A) and in soybean (B) under different treatments. M-H, maize cultivar Huayu 1502 in monoculture; IN-H, maize cultivar Huayu 1502 intercropped with soybeans; M-Y, maize cultivar Yuetian 8 in monoculture; IN-Y, maize cultivar Yuetian 8 intercropped with soybeans. M-B, soybean cultivar Huachun 12 in monoculture; IN-HB-B, soybeans intercropped with maize cultivar Huayu 1502; IN-YB-B, soybeans intercropped with maize cultivar Yuetian 8. Different letters indicate significant differences between treatments at $P<0.05$ (mean±SE, $n=3$).

maturity (Fig. 6-C). Soybean rhizospheres demonstrated contrasting enzymatic responses: IN-YB-B reduced phosphatase activity by 27.82% while enhancing invertase activity by 211.66% during heading phase (Fig. 6-D and F).

3.2. Pot experiment

AMF colonization rate The AMF colonization rates for inoculated treatments ranged from 11.45 to 14.00%, while for treatments without AMF, the colonization rate ranged from 3.23 to 5.31% (Table 3). Additionally, a significantly higher colonization rate was observed in plants inoculated with AMF, compared to those without AMF colonization. However, the

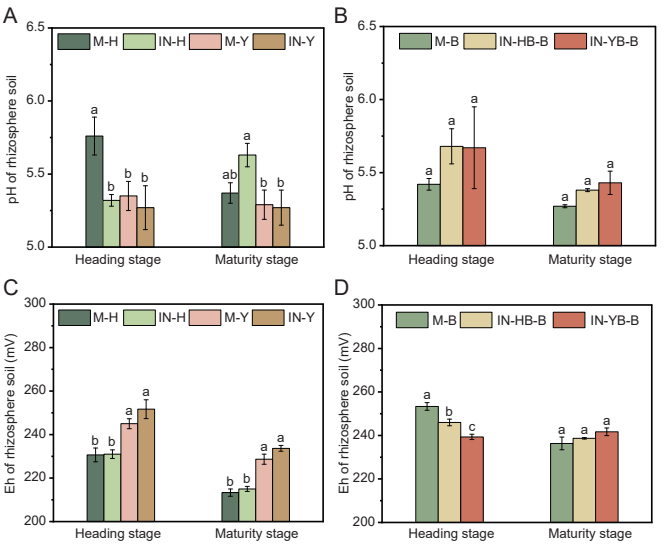


Fig. 5 pH and redox potential (Eh) of rhizosphere soil of maize (A and C) and soybean (B and D) under different treatments. M-H, maize cultivar Huayu 1502 in monoculture; IN-H, maize cultivar Huayu 1502 intercropped with soybeans; M-Y, maize cultivar Yuetian 8 in monoculture; IN-Y, maize cultivar Yuetian 8 intercropped with soybeans. M-B, soybean cultivar Huachun 12 in monoculture; IN-HB-B, soybeans intercropped with maize cultivar Huayu 1502; IN-YB-B, soybeans intercropped with maize cultivar Yuetian 8. Different letters indicate significant differences between treatments at $P<0.05$ (mean±SE, $n=3$).

colonization rate of maize and soybean showed no significant differences among the various treatments.

Plant growth parameters No significant biomass differences were detected between treatments at the seedling stage (Table 4). At maturity, PIN-H and PIN+A-H decreased maize shoot biomass by 24.93% and 20.09%, respectively, with corresponding grain biomass reductions of 23.73 and 20.13% *vs.* PM-H. Despite these reductions in maize yield, both intercropping systems exhibited clear land-use advantages, as reflected by LER values of 3.26 for PIN and 3.07 for PIN+A. AMF inoculation improved maize yield by 16.42% under intercropping, indicating synergistic benefits.

Cd content and BCA in plants Intercropping and AMF-inoculation significantly decreased Cd concentrations in maize stems (PIN-H: 0.14 μg kg^{-1}, 35.83% decrease; PIN+A-H: 0.13 μg kg^{-1}, 41.78% decrease) *vs.* PM-H (Fig. 7-C). Soybean tissues demonstrated progressive Cd reduction: PIN+A<PIN<PM. Total plant Cd accumulation followed maturity>seedling progression (Fig. 7-E and F; Table 5). At maturity, both maize and soybean total Cd contents showed a pattern of PIN+A<PIN<PM with significant differences. The MRER of PIN and PIN+A were 1.80 and 1.26 at the seedling stage and 1.43 and 0.98 at the maturity stage, suggesting AMF inoculation reduced Cd removal efficiency during later growth phases.

Rhizosphere soil characteristics The combined intercropping-AMF system significantly altered cadmium bioavailability and speciation (Fig. 8-A and B). Bioavailable Cd concentrations in maize rhizospheres exhibited a descending order: PIN+A<PIN<PM, with maturity-stage

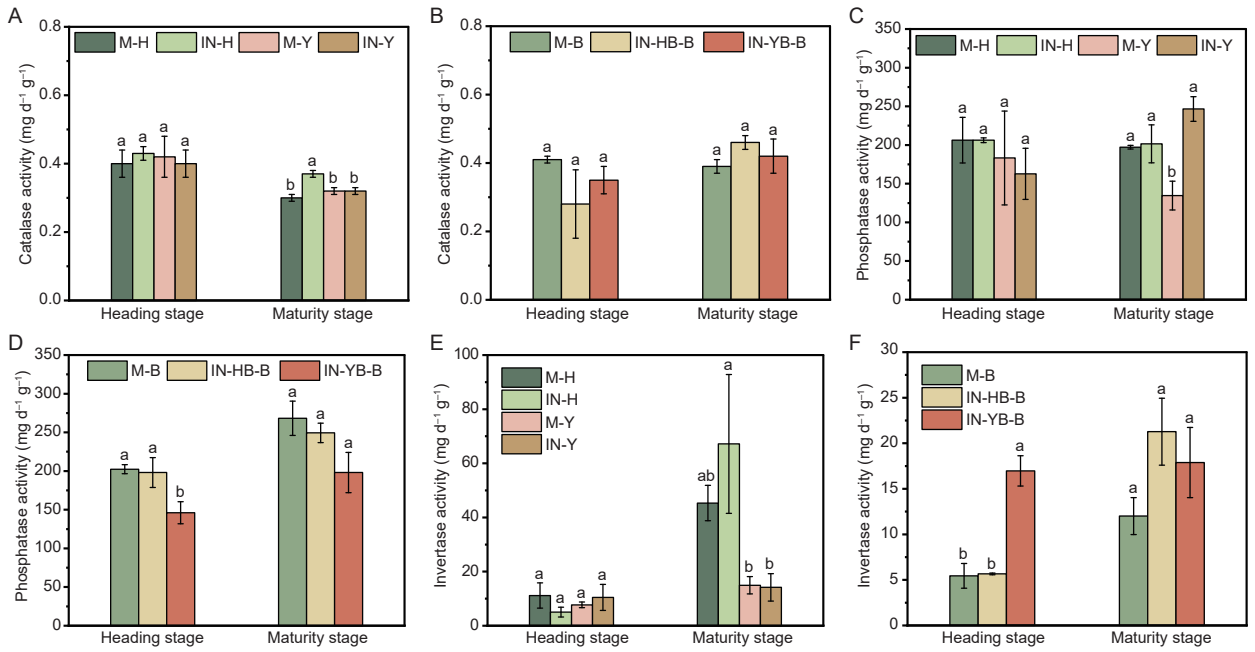


Fig. 6 The catalase, phosphatase, and invertase enzyme activities in rhizosphere soils of maize (A, C and E) and soybean (B, D and F) under different treatments. M-H, maize cultivar Huayu 1502 in monoculture; IN-H, maize cultivar Huayu 1502 intercropped with soybeans; M-Y, maize cultivar Yuetian 8 in monoculture; IN-Y, maize cultivar Yuetian 8 intercropped with soybeans. M-B, soybean cultivar Huachun 12 in monoculture; IN-HB-B, soybeans intercropped with maize cultivar Huayu 1502; IN-YB-B, soybeans intercropped with maize cultivar Yuetian 8. Different letters indicate significant differences between treatments at $P<0.05$ (mean±SE, $n=3$).

Table 3 Arbuscular mycorrhizal fungi (AMF) colorization rate (%) of maize and soybean under different treatments

Treatment[1)]	Maize		Treatment[2)]	Soybean	
	Seeding stage	Maturity stage		Seeding stage	Maturity stage
PM-H	3.69±1.87 b	3.55±1.80 b	PM-B	4.61±0.97 b	3.42±1.74 b
PIN-H	3.23±1.81 b	5.03±0.69 b	PIN-B	5.31±0.77 b	4.78±0.63 b
PIN+A-H	12.30±0.79 a	11.86±0.77 a	PIN+A-B	14.00±1.39 a	11.45±0.76 a

[1)] PM-H, maize cultivar Huayu 1502 in monoculture; PIN-H, maize intercropped with soybeans; PIN+A-H, maize intercropped with soybeans under AMF inoculation.
[2)] PM-B, soybean cultivar Huachun 12 in monoculture; PIN-B, soybeans intercropped with maize; PIN+A-B, soybeans intercropped with maize under AMF inoculation.
Different letters indicate significant differences among treatments at $P<0.05$ (mean±SE, $n=3$).

Table 4 Biomass (g/plant) of maize and soybean and land equivalent ratio (LER) under different treatments

Treatment[1)]	Maize			Treatment[2)]	Soybean			Treatment[3)]	LER
	Root	Shoot	Grain		Root	Shoot	Grain		
Seedling stage									
PM-H	1.31±0.11 a	12.61±1.40 a		PM-B	0.36±0.04 a	5.01±0.70 a			
PIN-H	0.96±0.23 a	9.88±1.79 a		PIN-B	0.34±0.10 a	3.77±0.38 a		PIN	3.26
PIN+A-H	0.83±0.17 a	6.94±2.16 a		PIN+A-B	0.37±0.03 a	4.37±0.35 a		PIN+A	3.07
Maturity stage									
PM-H	98.38±20.92 a	248.55±5.70 a	147.48±3.35 a	PM-B	3.73±0.29 a	12.16±4.66 a	1.55±0.07 a		
PIN-H	78.03±13.19 a	186.58±17.63 b	91.85±20.27 b	PIN-B	7.03±2.15 a	22.33±1.66 a	4.09±0.30 a		
PIN+A-H	78.47±7.95 a	198.61±16.63 b	106.93±14.49 ab	PIN+A-B	1.77±0.39 a	10.86±0.37 a	3.64±1.01 a		

[1)] PM-H, maize cultivar Huayu 1502 in monoculture; PIN-H, maize intercropped with soybeans; PIN+A-H, maize intercropped with soybeans under AMF inoculation.
[2)] PM-B, soybean cultivar Huachun 12 in monoculture; PIN-B, soybeans intercropped with maize; PIN+A-B, soybeans intercropped with maize under AMF inoculation.
[3)] PIN, maize–soybean intercropping; PIN+A, maize–soybean intercropping with AMF inoculation.
Different letters indicate significant differences among treatments at $P<0.05$ (mean±SE, $n=3$).

reductions of 29.62% (PIN-H) and 34.48% (PIN+A-H) *vs.* PM-H ($P<0.05$). Soybean rhizospheres displayed parallel trends though non-significant, indicating species-specific metal mobilization mechanisms.

Cadmium speciation analysis revealed significant phase transformations (Fig. 8-C). The initial soil showed a dominant residual fraction (F5: 82.35%) with minimal exchangeable Cd (F1: 1.33%). Following cultivation, soybean rhizospheres

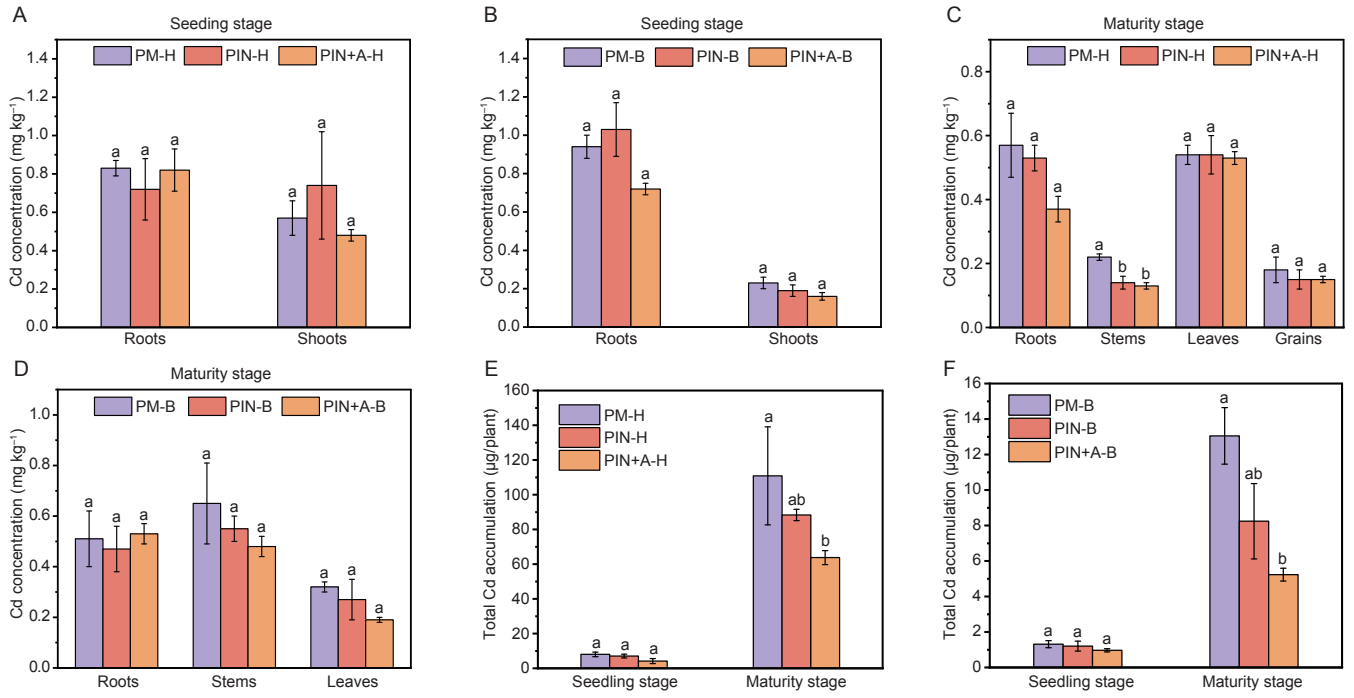


Fig. 7 Cadmium (Cd) concentrations and total Cd accumulation in maize (A, C and E) and soybean (B, D and F) under different treatments. PM-H, maize cultivar Huayu 1502 in monoculture; PIN-H, maize intercropped with soybeans; PIN+A-H, maize intercropped with soybeans under AMF inoculation. PM-B, soybean cultivar Huachun 12 in monoculture; PIN-B, soybeans intercropped with maize; PIN+A-B, soybeans intercropped with maize under AMF inoculation. Different letters indicate significant differences among treatments at $P<0.05$ (mean±SE, $n=3$).

Table 5 Bioconcentration amount (BCA; μg/plant) of maize and soybean and metal removal equivalent ratio (MRER) under different treatments

Treatment[1)]	Maize	Treatment[2)]	Soybean	MRER
Seedling stage				
PM-H	8.01±1.29 a	PM-B	1.31±0.2 a	
PIN-H	7.07±1.09 a	PIN-B	1.2±0.28 a	1.80
PIN+A-H	4.15±1.47 a	PIN+A-B	0.97±0.09 a	1.26
Maturity stage				
PM-H	110.85±28.20 a	PM-B	13.05±1.59 a	
PIN-H	88.33±3.23 ab	PIN-B	8.24±2.12 ab	1.43
PIN+A-H	63.76±4.03 b	PIN+A-B	5.23±0.36 b	

[1)] PM-H, maize cultivar Huayu 1502 in monoculture; PIN-H, maize intercropped with soybeans; PIN+A-H, maize intercropped with soybeans under AMF inoculation.
[2)] PM-B, soybean cultivar Huachun 12 in monoculture; PIN-B, soybeans intercropped with maize; PIN+A-B, soybeans intercropped with maize under AMF inoculation.
Different letters indicate significant differences among treatments at $P<0.05$ (mean±SE, $n=3$).

exhibited substantial increases in Fe/Mn oxide-bound Cd (F3) of 68.71–70.74% alongside 41.28–41.72% F5 decreases *vs.* PM-B, indicating redox-driven metal redistribution. In contrast, maize systems maintained consistent Cd speciation profiles, demonstrating distinct rhizosphere engineering capabilities between cereals and legumes.

AMF inoculation induced a systematic increase in rhizosphere pH in both crops. In maize, rhizosphere pH under PIN+A was 13.41% higher than that under PM-H at the seedling stage, and this increase reached 24.10% at maturity. A similar trend was observed in soybean, with rhizosphere pH increasing from 6.78% at the seedling stage to 24.10% at maturity (Fig. 9-A and B). Simultaneously, Eh decreased by 21.31% in AMF-treated maize rhizospheres at maturity (Fig. 9-C and D), establishing reducing microenvironments favorable for metal immobilization.

Enzyme activity profiles demonstrated AMF-mediated microbial activation. Catalase activity increased by 74.73% (maize) and 64.56% (soybean) under PIN+A at maturity, indicating enhanced oxidative stress mitigation (Fig. 9-E and F). In contrast, invertase activity decreased by 54.69–66.65% in soybean rhizospheres ($P<0.05$; Fig. 9-G and H), indicating modified carbon cycling patterns under intercropping-AMF synergy.

4. Discussion

4.1. Ecological niche complementarity drives intercropping advantages

The superior land equivalent ratios (LER>1) and metal removal efficiency (MRER>1) observed in our field trials derive from ecological niche complementarity — a process where intercropped species partition resources through spatial, temporal, or functional differentiation (Chen *et al.* 2023). Early-stage biomass competition (IN-H/Y) indicates overlapping nutrient acquisition zones, consistent with cereal-legume competition for nitrogen and phosphorus (Gou *et al.* 2018). However, distinct root architecture plasticity emerged post-tillering: sweet maize developed deep roots to access subsoil phosphorus, while common maize prioritized shoot growth to compete with soybean for light (Table 1). This

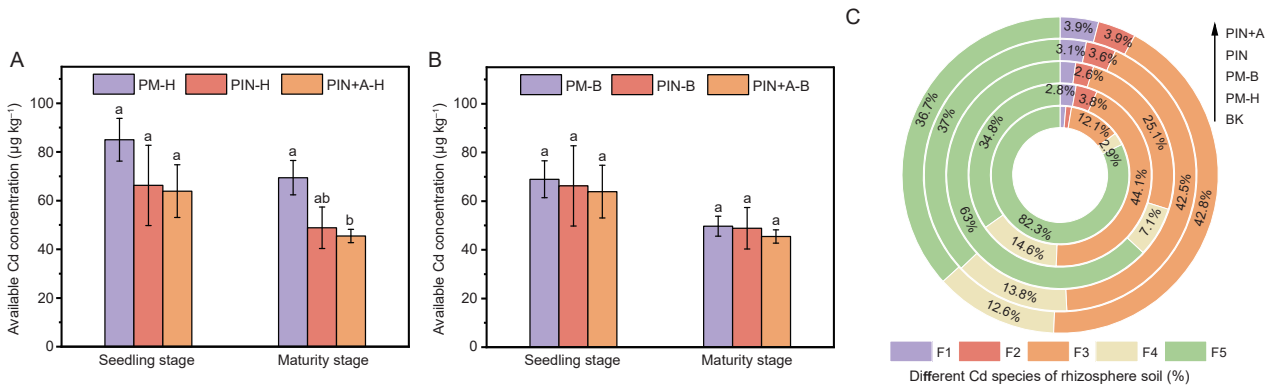


Fig. 8 Available cadmium (Cd) concentrations in the rhizosphere soils of maize (A) and soybean (B), as well as Cd species distribution under different treatments (C). PM-H, maize cultivar Huayu 1502 in monoculture; PIN-H, maize intercropped with soybeans; PIN+A-H, maize intercropped with soybeans under AMF inoculation. PM-B, soybean cultivar Huachun 12 in monoculture; PIN-B, soybeans intercropped with maize; PIN+A-B, soybeans intercropped with maize under AMF inoculation. F1, exchangeable Cd; F2, carbonate-bound Cd; F3, Fe-Mn oxide-bound Cd; F4, organic matter- and sulfide-bound Cd; F5, residual Cd. Different letters indicate significant differences among treatments at $P<0.05$ (mean±SE, $n=3$).

spatial niche differentiation reduced interspecific competition, corresponding with decreased rhizosphere phosphatase activity in soybean (Fig. 6-D) — indicating reduced microbial P-mobilization demand (Zhang *et al.* 2022).

AMF enhances this synergy through dual mechanisms: 1) extraradical hyphae expanding the phosphorus depletion zone, reducing maize–soybean competition (Qiao *et al.* 2016); 2) glomalin production improving soil aggregation, thereby enhancing water retention in Cd-contaminated matrices (Ingraffia *et al.* 2019). This microbial facilitation enables efficient land use while maintaining remediation potential.

4.2. Characteristics of rhizosphere soil

The 34.48% reduction in bioavailable Cd (EDTA-extractable) under AMF-inoculated intercropping arose from tripartite rhizosphere engineering. First, alkaline pH-driven precipitation. AMF hyphae increased rhizosphere pH (Fig. 9-A and B), promoting $CdCO_3$ precipitation over soluble Cd^{2+} (Ng *et al.* 2023). Soybean root exudates enhanced Fe/Mn oxide formation, increasing Cd adsorption capacity (Wen *et al.* 2020). AAdditionally, AMF reduced rhizosphere Eh (Fig. 9-C and D), which may favor sulfate-reducing bacteria and thereby promote the formation of sulfide, facilitating Cd immobilization through cadmium sulfide precipitation (Kang *et al.* 2021). Furthermore, enzyme-mediated organic complexation occurred. Co-inoculation enhanced catalase and sucrase activities, accelerating humic substance formation. Intercropping and AMF synergistically increased catalase and sucrase activities, indicating enhanced organic matter turnover and nutrient cycling (Curtright and Tiemann 2021). Notably, soybean-associated phosphorus solubilizers likely complement AMF by releasing iron-chelating siderophores that co-precipitate Cd^{2+} (Song *et al.* 2022).

4.3. AMF-mediated metal regulation and ecological synergy

The successful colonization of AMF in both crops (Table 1), although lower than values reported in some systems (Yang *et al.* 2021), confirms functional symbiosis under Cd stress, presumably limited by metal-induced suppression of arbuscule formation through downregulated fungal phosphate transporters (Carvalho *et al.* 2015). The AMF significantly reduced Cd accumulation in maize kernels at maturity, demonstrating its protective function in edible tissues — a finding crucial for food safety (Diagne *et al.* 2020).

The temporal reduction in AMF efficacy (MRER<1 at maturity) indicates carbon reallocation during reproductive growth, where photosynthate prioritization to grains decreased carbohydrate supply to fungi, limiting metal chelator production (Wang *et al.* 2020; Lei *et al.* 2021). This highlights the importance of synchronizing AMF activity with vegetative growth phases to maximize remediation. The intercropping-AMF system functions through tripartite regulation: microbial immobilization (hyphal biosorption), root exclusion (suberin barrier formation), and rhizosphere metal redistribution (legume-driven Fe/Mn oxide binding), illustrating its dual utility for safe crop production and soil remediation.

5. Conclusion

Maize–soybean intercropping with AMF achieved dual agricultural and remediation goals: (1) Safe crop production. Grain Cd concentration was reduced to <0.20 mg kg^{-1} (complying with GB2762-2022 standards) through root allocation plasticity, which minimized Cd transfer to edible parts. (2) Synergistic soil remediation. Cd immobilization was enhanced *via* rhizosphere alkalinization, reduction in Eh, and AMF-mediated enzyme activation, collectively decreasing bioavailable Cd by 34.5%.

The system exhibited improved yield-resource efficiency through enhanced land utilization (LER>1.6) and increased metal removal capacity (MRER>1.3). This establishes a scalable phytoremediation-with-production paradigm for Cd-contaminated soils.

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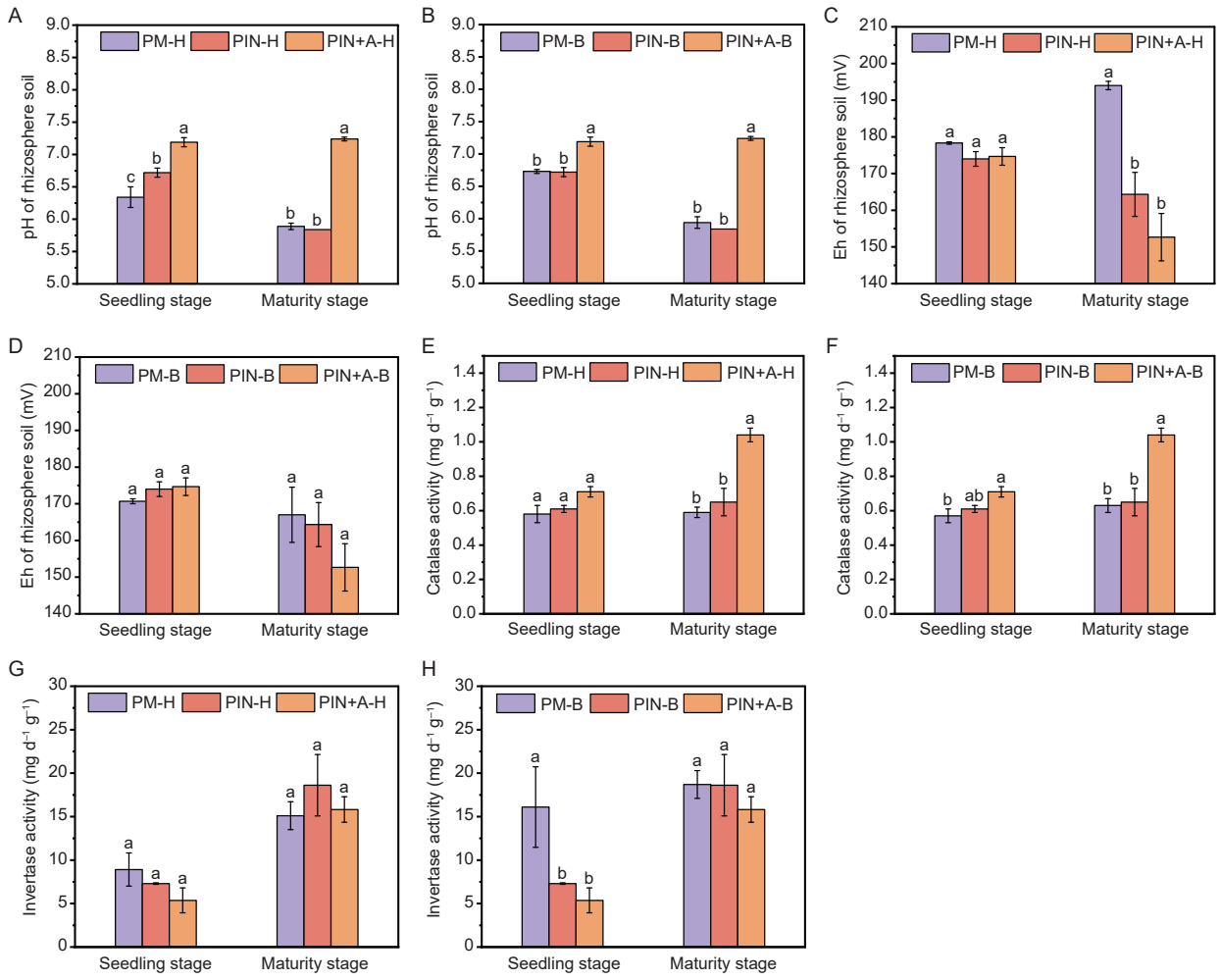


Fig. 9 The pH and redox potential (Eh) measurements in rhizosphere soil of maize (A and C) and soybean (B and D), along with the catalase and invertase activities in maize (E and G) and soybean (F and H) rhizosphere soils under varying experimental conditions. PM-H, maize cultivar Huayu 1502 in monoculture; PIN-H, maize intercropped with soybeans; PIN+A-H, maize intercropped with soybeans under AMF inoculation. PM-B, soybean cultivar Huachun 12 in monoculture; PIN-B, soybeans intercropped with maize; PIN+A-B, soybeans intercropped with maize under AMF inoculation. Different letters indicate significant differences among treatments at $P<0.05$ (mean±SE, $n=3$).

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT 4.0 to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

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