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Physiological Mechanisms Underlying the Substrate-Dependent Enhancement of Cd Tolerance in Maize by AMF and UPB Co-Inoculation

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Abstract

Background Arbuscular mycorrhizal fungi (AMF) and urease-producing bacteria (UPB) are common rhizosphere microorganisms in cadmium (Cd)-contaminated soils, but their effects on maize growth and Cd tolerance across substrates remain unclear.

Purpose To evaluate the effects of single inoculation with AMF or UPB and dual inoculation (BF) on maize growth, physiology, and Cd tolerance under sand and soil culture, and to clarify the substrate-dependent physiological mechanisms.

Methods Sand- and soil-culture experiments under 25 mg kg^{-1} Cd stress were conducted. The treatments included single inoculation with AMF or UPB and dual inoculation (BF). Maize growth, physiology, and Cd tolerance were evaluated.

Results In sand culture, BF increased plant height, shoot and belowground biomass, and stomatal conductance by 47–75%, while AMF increased root length and root surface area by 274% and 100% and reduced shoot Cd content by 57%; BF increased shoot and belowground Cd accumulation by 280% and 77%. In soil culture, UPB increased plant height by 16%, and AMF, UPB, and BF increased shoot biomass by 47–62%. UPB enhanced photosynthetic rate and stomatal conductance by 28% and 51%; BF increased stomatal conductance by 44% and decreased transpiration rate by 27%. AMF increased shoot K and belowground P by 61% and 45% and reduced shoot Cd content by 20%; BF reduced shoot Cd content by 16%; AMF increased belowground Cd accumulation by 31%. Inoculation reduced MDA content, enhanced SOD, POD, and CAT activities, and modulated IAA, ABA, ZR, and GA levels, with AMF and UPB interactions. Partial least squares structural equation modeling (PLS-SEM) indicated that in sand culture, AMF and UPB mainly affected photosynthesis by regulating endogenous hormones, thereby reducing maize Cd content; in soil culture, AMF and UPB treatments exerted direct negative effects on Cd content, whereas the hormonal pathway indirectly reduced Cd content per unit biomass, mainly through photosynthetic improvement and growth dilution.

Conclusion Overall, AMF and UPB reduce Cd and enhance maize Cd tolerance via photosynthetic physiology, antioxidant defense, and hormone modulation, with substrate-dependent co-inoculation effects.

Key words Maize; Arbuscular mycorrhizal fungi; Urease-producing bacteria; Antioxidant enzymes; Endogenous hormones; Cadmium content

0 Introduction

Soil cadmium (Cd) contamination has become a global environmental issue [1]. Cd contamination of agricultural soils is insidious, persistent, and irreversible. It not only damages soil structure and reduces soil fertility but also accumulates in crops, leading to food-chain enrichment along the soil–crop–human pathway and thereby posing a dual threat to ecosystems and human health [2-4]. Once Cd enters the human body, it is difficult to metabolize and excrete; long-term accumulation can cause renal dysfunction, osteomalacia, nervous system damage, and other diseases, severely endangering human health [5]. Therefore, the remediation and safe utilization of Cd-contaminated soils have become urgent priorities for ensuring sustainable agricultural development and food security.

Bioremediation has become a central strategy for remediating Cd-contaminated soils because it is environmentally friendly, cost-effective, and free of secondary pollution [6]. As key functional components of soil–plant systems, rhizosphere microorganisms alleviate Cd toxicity to crops by regulating the soil microenvironment, improving plant nutrient uptake, and enhancing stress-resistant physiological metabolism [7]. Plant growth-promoting rhizobacteria (PGPR) colonize the plant rhizosphere and reduce soil Cd bioavailability by secreting metabolites such as phytohormones, siderophores, and organic acids, or through biomineralization and complexation or adsorption. They also improve plant nutrient uptake and stress-resistant physiological metabolism, thereby mitigating Cd toxicity to plants [8,9]. Among these microorganisms, arbuscular mycorrhizal fungi (AMF) and urease-producing bacteria (UPB) are two widely distributed functional microbial groups in agricultural ecosystems and play key roles in mitigating heavy-metal stress.

AMF are among the microorganisms most closely associated with plants, forming symbiotic associations with over 70% of terrestrial plants [10]. By extending their extraradical hyphal networks, AMF broaden the nutrient acquisition range of plants, mobilize sparingly soluble soil phosphorus, potassium, and other nutrients, and enhance plant nutrient supply. Moreover, AMF can immobilize free Cd in soil by secreting substances such as glycoproteins, or reduce Cd translocation to shoots by regulating root morphology and enhancing antioxidant enzyme activities, thereby improving plant tolerance to Cd stress [11,12]. Similarly, under selenium (Se) stress, co-inoculation with AMF and rhizobia has been shown to significantly enhance root antioxidant activity and alleviate Se toxicity [13].

UPB are another group of functional microorganisms widely distributed in soil. Urease secreted by UPB hydrolyzes soil urea to produce carbonate ions and ammonium nitrogen, a process that not only supplies nitrogen to plants but also converts free Cd in soil into stable carbonate precipitates via biomineralization, thereby reducing Cd bioavailability [14,15]. In addition, UPB secrete plant hormones such as indole-3-acetic acid (IAA) and zeatin (ZR), directly promoting plant growth and enhancing stress resistance [16].

Maize (*Zea mays* L.) is a globally important food crop [17]. However, Cd stress markedly inhibits maize growth and development, reducing plant height, biomass, and photosynthetic efficiency while increasing the risk of Cd accumulation in grains and thereby severely compromising yield and quality [18,19]. Although single inoculation with AMF or UPB has been shown to alleviate Cd stress in maize, the effects and mechanisms of their co-inoculation remain unclear. In particular, for UPB strains isolated from Cd-contaminated farmland in lead–zinc mining areas, the mechanisms underlying co-inoculation with AMF and the associated physiological regulatory pathways have not been elucidated. The effects of AMF and UPB do not occur in isolation; their co-inoculation effects are modulated by the physicochemical properties of the substrate and the biological environment. Sand culture, characterized by limited nutrient supply, weak buffering capacity, high Cd bioavailability, and the absence of soil colloid adsorption and complex indigenous microbial interference, can amplify root–microbe interaction

signals, facilitating dissection of how inoculation treatments directly regulate maize root morphology, hormone balance, and antioxidant and photosynthetic physiology. In contrast, soil culture, with stronger nutrient buffering, Cd adsorption and immobilization, and competition from indigenous microorganisms, more closely resembles Cd-contaminated farmland and can therefore be used to test the robustness and application potential of co-inoculation for promoting growth and reducing Cd. Through parallel comparison of sand and soil cultures, the relative contributions of direct microbial physiological regulation and soil-mediated processes to maize Cd tolerance can be distinguished, thereby revealing the intrinsic mechanisms by which AMF–UPB interactions alleviate Cd stress and the substrate dependence of these mechanisms.

Accordingly, this study used a highly efficient plant growth-promoting UPB strain (*Bacillus proteolyticus*), isolated from Cd-contaminated farmland in a lead–zinc mining area, as the test strain. This strain was combined with AMF (*Funneliformis mosseae*), and sand and soil pot experiments were conducted to investigate the effects of single inoculation with AMF or UPB and dual inoculation (BF) on maize under Cd stress. Parameters included growth, photosynthetic physiology, endogenous hormone balance, antioxidant enzyme activities and MDA content, and Cd content and accumulation in shoots and roots. This study aimed to clarify the physiological mechanisms by which AMF and UPB co-inoculation alleviates Cd stress in maize, thereby providing a scientific basis and technical support for developing efficient composite microbial agents and ensuring safe maize production in Cd-contaminated farmland.

1 Materials and Methods

1.1 Experimental Materials

For sand culture, quartz sand (0.5-1 mm, mainly composed of SiO_2) was used. $CdCl_2 \cdot 2.5H_2O$ was added to prepare a 50% Hoagland nutrient solution with a Cd concentration of 25 mg kg^{-1}. After two weeks of equilibration, the mixture was used as the sand culture substrate. The test soil was collected from farmland surrounding a lead-zinc mining area in Huize County, Qujing City, Yunnan Province. The soil was classified as mountain red soil. After collection, it was naturally air-dried, passed through a 2-mm sieve, and then autoclaved. Its basic physicochemical properties are presented in Table 1. The sterilized soil was spiked with $CdCl_2$ solution to reach a Cd stress level of 25 mg kg^{-1}. After two weeks of equilibration, it was used as the soil culture substrate.

Table 1 Basic chemical properties of soil

pH	Organic matter(mg·kg^{-1})	Alkali-hydrolyzable N (mg·kg^{-1})	Available P(mg·kg^{-1})	Available K(mg·kg^{-1})	Cd content(mg·kg^{-1})
6.22	34.21	81.43	35.40	95.05	8.96

The maize cultivar used was Zhouyu 0913 (purchased from the Xiaobanqiao Seed Wholesale Market in Kunming, Yunnan Province). Seeds were surface-sterilized with 10% hypochlorous acid and 75% ethanol, rinsed thoroughly, and incubated at 28°C for 3 d in a constant-temperature incubator to promote germination. After germination and emergence of white radicles, uncontaminated seedlings with uniform growth were selected and retained for subsequent experiments [20].

The AMF strain used in this experiment, Funneliformis mosseae (BGCYN051511C0001BGCAM 0013), was provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences. The inoculum was propagated in our laboratory using potted maize and consisted

of a sand–soil mixture containing spores, mycelium, and colonized root segments, with approximately 21 spores per gram. The UPB strain was isolated and screened by our research group from maize rhizosphere soil in Cd-contaminated farmland of a lead–zinc mining area. This strain, designated W12-3, was identified as Bacillus proteolyticus. It was maintained on beef extract peptone agar slants at 4 °C and subcultured every two months to maintain viability [21].

1.2 Preparation of Bacterial Suspension and Fermentation Broth

The liquid seed medium contained the following components: peptone (5 $g \cdot L^{-1}$), anhydrous calcium chloride (0.945 $g \cdot L^{-1}$), anhydrous potassium chloride (0.506 $g \cdot L^{-1}$), ammonium nitrate (0.08 $g \cdot L^{-1}$), potassium dihydrogen phosphate (0.136 $g \cdot L^{-1}$), magnesium sulfate (0.493 $g \cdot L^{-1}$), trace element solution (5 $mL \cdot L^{-1}$), and iron salt solution (2.5 $mL \cdot L^{-1}$). All non-urea components were sterilized by autoclaving at 121 ℃ for 30 min. The urea solution (30 $g \cdot L^{-1}$) was filtered through a 0.45-μm membrane and aseptically added to the medium after cooling to room temperature.

The bacterial suspension and fermentation broth were prepared as follows. The strain preserved on an agar slant was activated on beef extract peptone medium for 48 h. Cells were collected by centrifugation at 4 °C and 7155 × g for 10 min, then resuspended and washed three times with sterile water. The bacterial suspension was adjusted to approximately 3.0×10^8 $CFU \cdot mL^{-1}$ using a McFarland standard as the turbidity reference. This suspension was inoculated into the liquid seed medium at 2% (v/v) and incubated at 30 °C with shaking at 150 $r \cdot min^{-1}$ for 24 h to obtain the fermentation broth [22].

1.3 Pot Experiment

Indoor pot experiments were conducted separately under soil and sand culture conditions. A completely randomized design was used with four treatments: control (CK), AMF inoculation (AMF), UPB inoculation (UPB), and dual inoculation with AMF and UPB (BF). Each treatment was replicated five times, yielding 20 pots per culture system and 40 pots in total.

Pots (8 cm bottom diameter, 15 cm top diameter, and 40 cm height) were disinfected, and each pot was first filled with 4.5 kg of sterilized culture substrate. Two surface-sterilized maize seeds with emerged white radicles were then planted, and microbial inoculants were applied at the planting positions according to the corresponding treatments. Subsequently, 0.5 kg of sterilized culture substrate was added to completely cover the seeds. The experiment was conducted in a greenhouse under natural light, and the pots were irrigated with deionized water to maintain soil moisture at 60–70%. After maize emergence, each pot was thinned to one seedling by removing the weaker plant (Table 2).

Table 2 Experimental design for sand and soil culture experiments

Treatment	Main applied materials
CK	100 g of sterilized AMF inoculum + 100 mL of liquid medium
AMF	100 g of AMF inoculum + 100 mL of liquid medium
UPB	100 g of sterilized AMF inoculum + 100 mL of UPB fermentation broth
BF	100 g of AMF inoculum + 100 mL of UPB fermentation broth

1.4 Sample Collection and Determination of Parameters

For both sand and soil pot experiments, maize plants were sampled after 50 days of growth. Pots were cut open on both sides with scissors, and loose soil or quartz sand was removed by gentle shaking. Rhizosphere soil tightly adhering to the roots was collected, naturally air-dried, passed through a 2-mm sieve, and stored for later use. Each maize plant was separated into shoots and roots, which were washed separately with distilled water. A portion of each fresh sample was retained for analysis, while the remaining samples were placed in a 105 ℃ oven (Model 101, Beijing Yongguangming Instrument Co., Ltd.) for 30 min to terminate enzymatic activity, then dried at 75 °C to constant weight and weighed. Finally, all samples were ground, passed through a 0.25-mm sieve, and prepared for analysis.

1.4.1 Determination of AMF Colonization Rate in Roots and UPB Colony Counts in Rhizosphere Soil

Fresh roots were selected from the samples, washed with deionized water to remove impurities adhering to the root surface, cut into segments approximately 1 cm long, and transferred to sterile centrifuge tubes. A 10% (w/v) KOH solution was added, and the samples were softened in a 90 °C water bath for 1 h. Subsequently, a 30% (w/v) H_2O_2 solution was added, and the samples were bleached for 5 min, after which they were rinsed repeatedly with deionized water to remove residual reagents. A 0.05% (w/v) trypan blue staining solution was added, and the samples were stained in a water bath at a constant temperature of 90 ℃ for 30 min. After staining, the samples were rinsed again with deionized water and then immersed in a destaining solution for 24 h. After destaining, 10 root segments were randomly selected from each sample, transferred to glass slides containing 50% glycerol, and mounted and fixed for microscopic observation [23]. The AMF colonization rate was calculated using the gridline intersect method: AMF colonization rate (%) = (number of root segments colonized by AMF / total number of observed root segments) × 100% [24].

To quantify UPB colonies in rhizosphere soil, 1 g of rhizosphere soil was mixed with 99 mL of sterile water and shaken at 150 rpm and 25 °C for 30 min to prepare a soil suspension. Using a dilution plating method, aliquots of the diluted suspension were evenly spread onto beef extract peptone solid medium containing urea, with three replicates per sample. The inoculated plates were incubated inverted at 30 °C for 72 h, after which colonies were counted ($CFU \cdot g^{-1}$) [25].

1.4.2 Determination of Plant Nutrient Concentrations

A 0.1 g portion of dried shoot or root sample was weighed into a 100 mL Erlenmeyer flask, and 5 mL of concentrated H_2SO_4 was added. The mixture was allowed to stand overnight at room temperature. The next day, 3 mL of H_2O_2 was added dropwise, and the mixture was heat-digested until the solution became colorless and transparent. After cooling, the digest solution was filtered and made up to 50 mL with deionized water in a volumetric flask for subsequent analysis. The contents of nitrogen (N), phosphorus (P), and potassium (K) were determined by the Kjeldahl method, the molybdenum-antimony colorimetric method, and flame photometry, respectively [26].

1.4.3 Determination of Photosynthetic Physiology, Antioxidant Enzyme Activities, and Phytohormone Contents in Maize

On sunny mornings (9:00–11:00 a.m.), the uppermost fully expanded leaves of maize plants were selected, and photosynthetic parameters—including photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci)—were measured using a CIRAS-1 portable photosynthesis system (PP Systems, UK) [27,28].

After sampling, fresh maize leaf and root samples (0.1 g each) were placed separately into centrifuge tubes. Subsequently, 1 mL of extraction buffer was added, and the samples were centrifuged at 12,000 rpm for 20 min at 4 ℃. The supernatant was collected, and its malondialdehyde (MDA) content and

related antioxidant enzyme activities were determined using commercial kits from Suzhou Gruis Biotechnology Co., Ltd. All procedures were performed strictly according to the kit instructions [29,30].

Fresh maize leaf and fine root samples (0.5 g each) were weighed, immediately ground into powder in liquid nitrogen, transferred into 10 mL cryogenic tubes, snap-frozen in liquid nitrogen, and stored at −80 °C in an ultra-low temperature freezer. Endogenous plant hormone contents were determined by enzyme-linked immunosorbent assay (ELISA) using kits developed by the Crop Chemical Control Research Laboratory, China Agricultural University; all procedures were performed strictly according to the kit instructions. The absorbance of the test solution was measured at 450 nm (OD_{450}), and the obtained OD values were substituted into the standard curve equation to calculate the concentrations of the corresponding hormones in the samples [31].

1.4.4 Determination of Cd Concentration and Accumulation in Maize Plants

The Cd content in plant samples was determined by HNO_3–H_2O_2 digestion and flame atomic absorption spectrometry [24]. A 0.2 g portion of dried plant powder was placed in a pressure digestion vessel, and 5 mL of concentrated HNO_3 was added. The mixture was left to stand overnight at room temperature for pre-digestion. The next day, 3 mL of H_2O_2 was added, the vessel was sealed, and digestion was performed at 140 °C for 4 h. After digestion and cooling, the digest solution was poured out, and the inner wall of the digestion vessel was rinsed repeatedly with distilled water at least three times. The digest solution was then filtered and made up to 50 mL in a volumetric flask, and the Cd concentration was determined by flame atomic absorption spectrometry using a TAS-990 atomic absorption spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.).

The Cd concentrations in shoots and roots were determined, and the Cd content was calculated to estimate Cd accumulation in the corresponding plant parts [32]: Cd accumulation in the corresponding plant part ($\mu g \cdot plant^{-1}$) = biomass of the corresponding plant part ($g \cdot plant^{-1}$) × Cd concentration in the corresponding plant part ($\mu g \cdot g^{-1}$).

1.5 Data Processing and Analysis

Raw data were organized in Microsoft Excel, and means and standard deviations were calculated for each treatment group and expressed as mean ± standard deviation. Statistical analyses were performed using SPSS 21.0. Differences among treatments were compared using one-way ANOVA followed by Duncan's test ($\alpha = 0.05$). Two-way ANOVA was used to evaluate the interactive effects of AMF and UPB inoculation on plant Cd accumulation and physiological indices. Pearson correlation coefficients were used to assess correlations among the measured indicators. Data were visualized using OriginPro 9.0. Direct and indirect regulatory pathways among key indicators were analyzed using partial least squares structural equation modeling (PLS-SEM) in R.

2 Results

2.1 Colonization Characteristics of AMF in Maize Roots and UPB in the Rhizosphere

AMF colonization occurred in maize roots under both sand and soil culture conditions. In sand culture, the AMF colonization rate was 31.9% under single AMF inoculation and 25.7% under dual inoculation (BF), while the UPB colonization rate was 29% under single UPB inoculation and 31% under BF (Table 3). In soil culture, the AMF colonization rate was 35.7% under single AMF inoculation and 36.5% under BF, and the UPB colonization rate was 42% under single UPB inoculation and 35% under BF (Table 4).

Table 3 Colonization Characteristics of AMF and UPB in Maize Roots Inoculated with AMF and UPB under Sand Culture Conditions

Treatment	Spore number (n/g)	mycorrhizal colonization rate (%)	Bacterial colonization (CFU per gram rhizosphere soil ($\times 10^5$))
CK	—	—	—
AMF	—	31.9±1.5 a	—
UPB	—	—	29±2.0 b
BF	—	25.7±1.5 a	31±3.0 a

Note: CK: non-inoculation control; AMF: single inoculation with AMF; UPB: single inoculation with UPB; BF: dual inoculation with AMF and UPB. -: indicates that the index was not detected. Different lowercase letters in the same column indicate significant differences among treatments at $P < 0.05$. The same applies below.

Table 4 Colonization Characteristics of AMF and UPB in Maize Roots Inoculated with AMF and UPB under Soil Culture Conditions

Treatment	Spore number (n/g)	mycorrhizal colonization rate (%)	Bacterial colonization (CFU per gram rhizosphere soil ($\times 10^5$))
CK	—	—	—
AMF	12±1.0 a	35.7±2.9 a	—
UPB	—	—	42±5.0 a
BF	9±1.0 b	36.5±4.7 a	35±3.0 b

2.2 Effects of AMF and UPB on Maize Growth and Root Morphology

Inoculation with AMF or UPB and dual inoculation (BF) significantly affected maize plant height, biomass, and root morphology, with effects depending on substrate type. In sand culture, compared with CK, single AMF inoculation, single UPB inoculation, and dual inoculation (BF) increased plant height by 16%, 15%, and 47%, respectively. Two-way ANOVA indicated that both AMF and UPB had highly significant effects on plant height, with no interaction between them. In soil culture, single UPB inoculation increased plant height by 16% compared with CK. Two-way ANOVA revealed a highly significant effect of UPB on plant height, with no AMF and UPB interaction (Fig. 1).

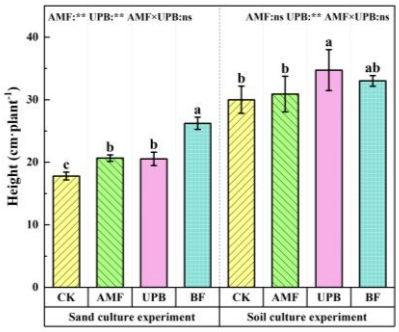


Fig. 1 Effects of AMF and UPB inoculation on maize plant height.

Note Data are presented as means±SE (n=5 independent biological replicates per treatment); error bars indicate SE, calculated as SD/√n. Different lowercase letters indicate significant differences among treatments ($P < 0.05$). CK: no inoculation; AMF: single AMF inoculation; UPB: single UPB inoculation; BF: dual inoculation with AMF and

UPB. “ns” and “**” denote the results of two-way ANOVA, indicating no significant effect and $P < 0.01$, respectively. The same notation applies hereafter.

Regarding biomass, in sand culture, dual inoculation (BF) significantly increased maize shoot biomass by 72% compared with CK, whereas single AMF inoculation and BF significantly increased belowground biomass by 60% and 75%, respectively. Two-way ANOVA indicated that AMF had a highly significant effect on shoot biomass. In soil culture, compared with CK, all three inoculation treatments significantly increased shoot biomass by 47%, 62%, and 50%, respectively. Two-way ANOVA showed that both AMF and UPB had highly significant effects on shoot biomass, with a highly significant AMF and UPB interaction for this parameter, and a significant AMF and UPB interaction for belowground biomass (Fig. 2).

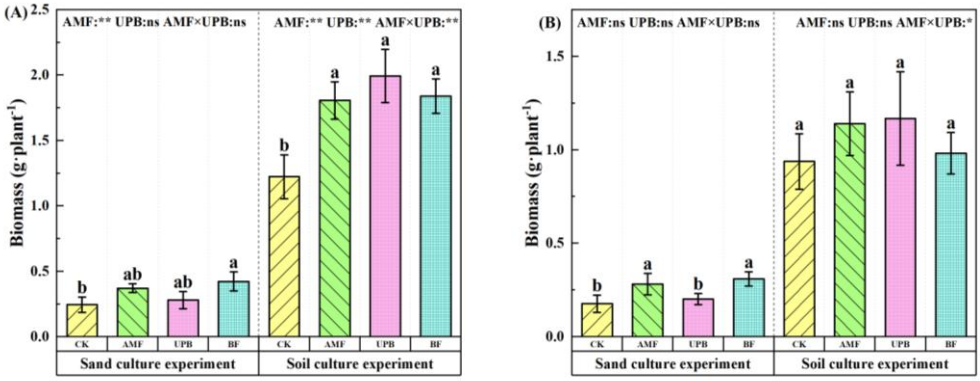


Fig. 2 Effects of AMF and UPB inoculation on maize biomass (A: shoot biomass; B: root biomass).

Regarding root morphology, in sand culture, single AMF inoculation significantly increased maize root length by 274% and root surface area by 100% compared with CK. Two-way ANOVA showed that AMF had highly significant or significant effects on root length, root surface area, and average root diameter, whereas UPB had highly significant effects on root length and root surface area; a highly significant AMF and UPB interaction was also detected for these three parameters. In soil culture, single UPB inoculation significantly increased root length by 30% compared with CK. Two-way ANOVA showed that AMF had a significant effect on average root diameter, while UPB had highly significant or significant effects on root length and root surface area (Fig. 3).

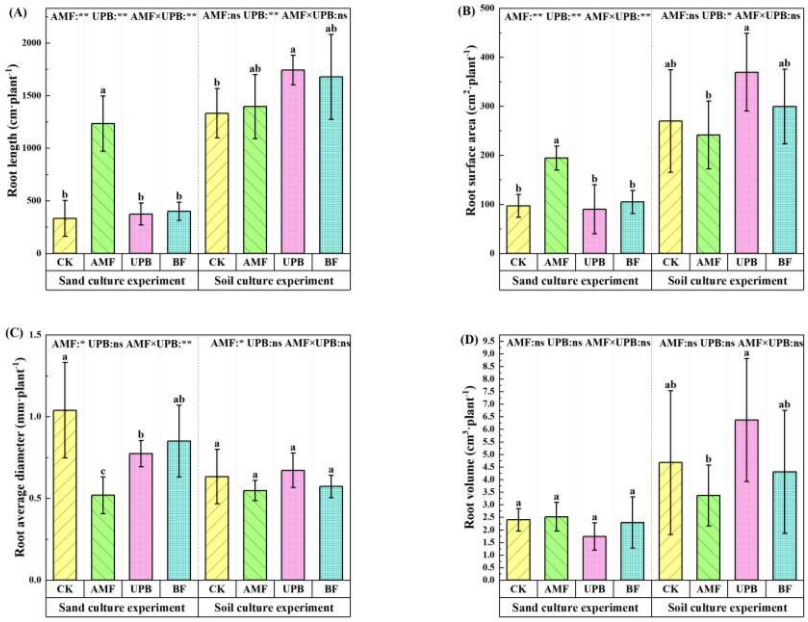


Fig. 3 Effects of AMF and UPB inoculation on maize root morphology (A: maize root length; B: maize root surface area; C: maize average root diameter; D: maize root volume).

2.3 Effects of AMF and UPB on Photosynthetic Characteristics and Mineral Nutrition of Maize

Inoculation treatments significantly regulated photosynthetic parameters and nitrogen, phosphorus, and potassium contents in maize leaves, with effects clearly dependent on substrate type. Regarding photosynthetic physiology, in sand culture, dual inoculation (BF) significantly increased stomatal conductance (Gs) by 58% compared with CK. Two-way ANOVA indicated a significant AMF and UPB interaction affecting transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) in maize leaves. In soil culture, compared with CK, single UPB inoculation significantly increased photosynthetic rate (Pn) and Gs by 28% and 51%, respectively, but significantly decreased Ci by 40%; dual inoculation (BF) significantly increased Gs by 44% and significantly decreased Tr by 27%. Two-way ANOVA showed that AMF had highly significant effects on Pn and Tr, that UPB had highly significant effects on Gs and Ci, and that a highly significant AMF and UPB interaction was observed for photosynthetic rate, transpiration rate, and intercellular CO_2 concentration in maize leaves (Fig. 4).

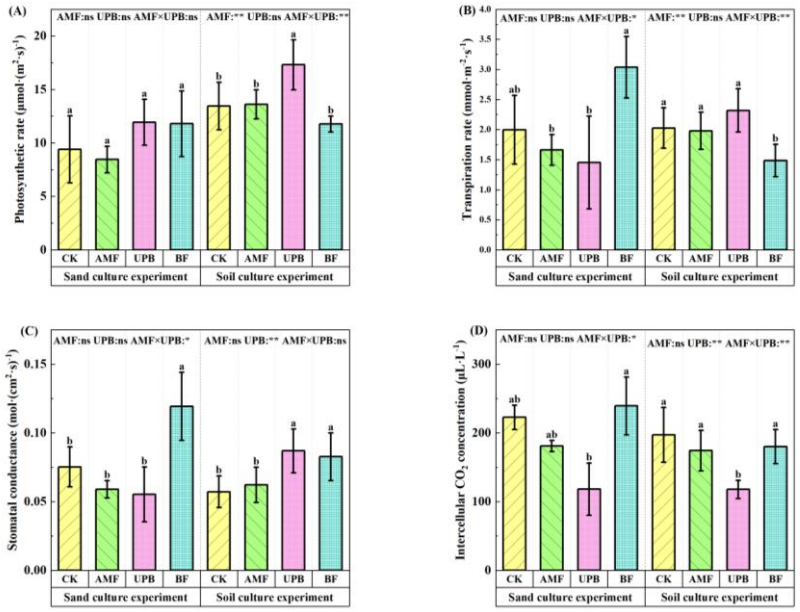


Fig. 4 Effects of AMF and UPB inoculation on maize photosynthesis (A: photosynthetic rate; B: transpiration rate; C: stomatal conductance; D: intercellular CO_2 concentration).

Regarding mineral nutrition, in sand culture, compared with CK, single AMF inoculation significantly increased belowground phosphorus and potassium contents in maize by 44% and 60%, respectively, whereas single AMF inoculation and dual inoculation (BF) significantly decreased shoot phosphorus content by 22% and 46%, respectively. Two-way ANOVA showed that AMF and UPB had highly significant effects on shoot phosphorus content and belowground potassium content, with significant interactions between them for shoot nitrogen content, belowground phosphorus content, and belowground potassium content. In soil culture, single AMF inoculation significantly increased shoot potassium content and belowground phosphorus content by 61% and 45%, respectively (Fig. 5, Fig. 6).

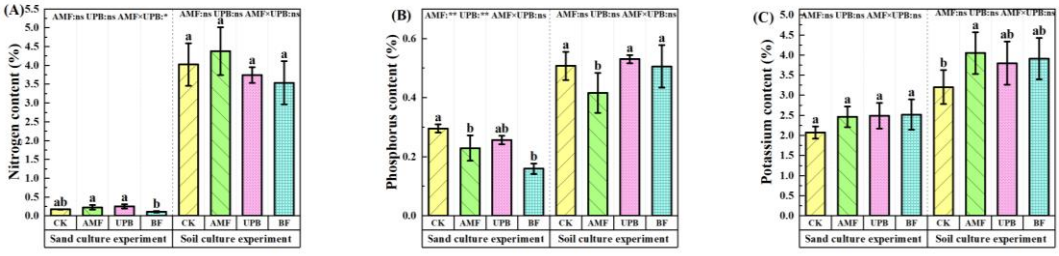


Fig. 5 Effects of AMF and UPB inoculation on shoot mineral nutrition of maize (A: nitrogen content; B: phosphorus content; C: potassium content).

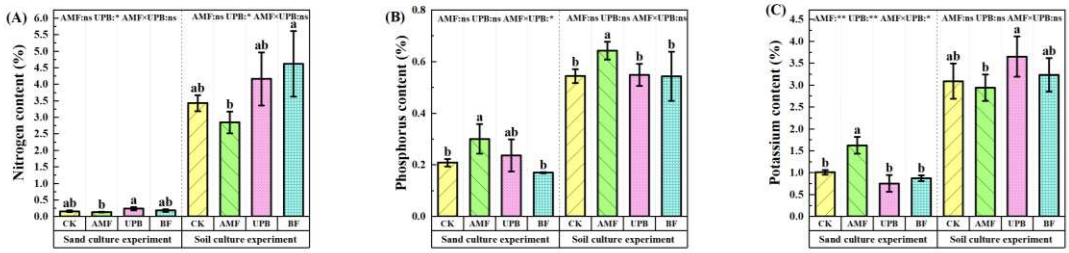


Fig. 6 Effects of AMF and UPB inoculation on root mineral nutrition of maize (A: nitrogen content; B: phosphorus content; C: potassium content).

2.4 Effects of AMF and UPB on Malondialdehyde Content and Antioxidant Enzyme Activities in Maize

Antioxidant enzyme activities and MDA content are key indicators of Cd-induced oxidative damage and plant antioxidant defense capacity. Inoculation with AMF, UPB, or both (BF) significantly affected MDA content and SOD, POD, and CAT activities in maize leaves and roots; however, the regulatory patterns differed between leaves and roots and between sand and soil cultures.

For maize leaves in sand culture, compared with CK, single AMF inoculation, single UPB inoculation, and dual inoculation (BF) significantly reduced leaf MDA content by 33%, 42%, and 43%, respectively, and significantly increased leaf SOD and POD activities. SOD activity increased by 43%, 42%, and 43%, and POD activity increased by 92%, 184%, and 151%, respectively. Two-way ANOVA showed that UPB had a significant effect on MDA content and a highly significant effect on POD activity, with a highly significant AMF and UPB interaction for POD activity in maize leaves. In soil culture, single AMF inoculation and dual inoculation (BF) significantly reduced leaf MDA content by 21% and 18%, respectively, and significantly increased POD, SOD, and CAT activities. AMF increased POD, SOD, and CAT activities by 27%, 70%, and 71%, respectively, while BF increased these activities by 26%, 49%, and 53%, respectively. Two-way ANOVA showed that AMF had a significant effect on MDA content and highly significant effects on POD and CAT activities, and that AMF and UPB had highly significant or significant interactions for SOD, POD, and CAT activities in maize leaves (Fig. 7).

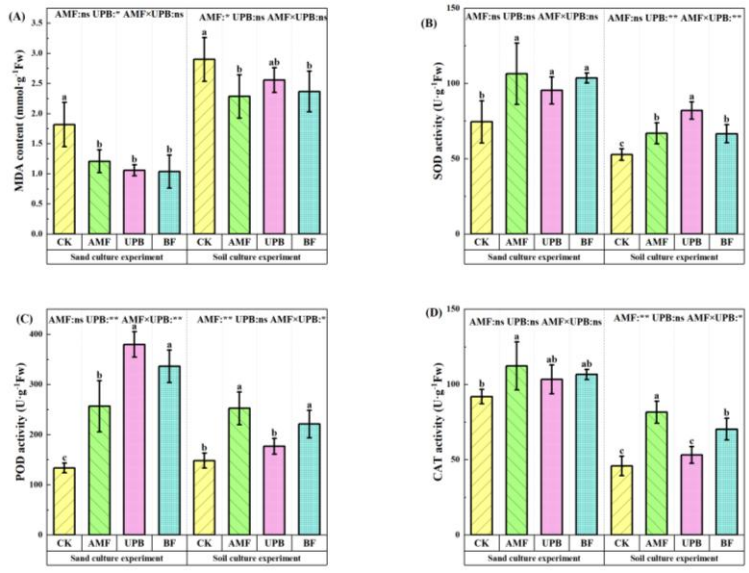


Fig. 7 Effects of AMF and UPB inoculation on shoot malondialdehyde content and antioxidant enzyme activities in maize (A: malondialdehyde (MDA) content; B: superoxide dismutase (SOD) activity; C: peroxidase (POD) activity; D: catalase (CAT) activity).

For maize roots in sand culture, compared with CK, dual inoculation (BF) significantly reduced root MDA content by 42%. Single AMF inoculation, single UPB inoculation, and dual inoculation (BF) all significantly increased root SOD, POD, and CAT activities. SOD activity increased by 35%, 62%, and 36% for AMF, UPB, and BF, respectively; POD activity increased by 49%, 50%, and 49%, respectively; and CAT activity increased by 48%, 73%, and 47%, respectively. Two-way ANOVA showed that UPB had a significant effect on root MDA content and significant or highly significant effects on SOD, CAT,

and POD activities, and that AMF and UPB had a significant interaction affecting root POD activity. In soil culture, single AMF inoculation and single UPB inoculation significantly increased root SOD, POD, and CAT activities. For AMF, SOD, POD, and CAT activities increased by 65%, 22%, and 159%, respectively; for UPB, they increased by 43%, 13%, and 196%, respectively. Two-way ANOVA showed that AMF and UPB had highly significant interactions affecting root SOD, POD, and CAT activities (Fig. 8).

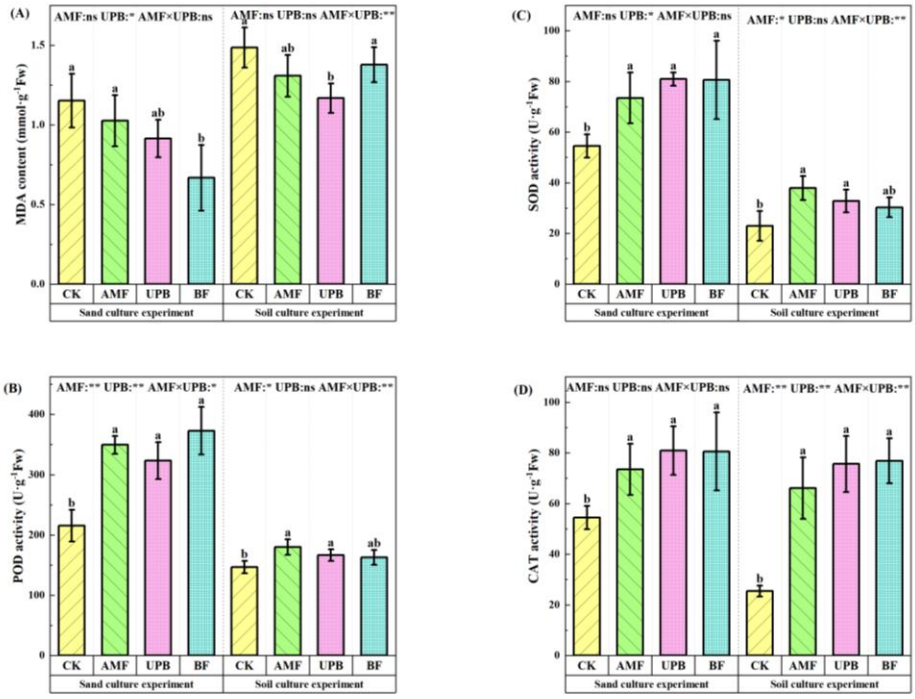


Fig. 8 Effects of AMF and UPB inoculation on root malondialdehyde content and antioxidant enzyme activities in maize (A: malondialdehyde (MDA) content; B: superoxide dismutase (SOD) activity; C: peroxidase (POD) activity; D: catalase (CAT) activity).

2.5 Effects of AMF and UPB on Endogenous Hormone Contents in Maize

Inoculation treatments significantly modulated auxin (IAA), abscisic acid (ABA), zeatin (ZR), and gibberellin (GA) levels in maize leaves and roots; however, these effects varied with tissue and substrate type.

For maize leaves in sand culture, compared with CK, single AMF inoculation and dual inoculation (BF) significantly increased ZR and GA contents; AMF increased them by 7% and 11%, respectively, while BF increased them by 8% and 9%, respectively. Single AMF and single UPB inoculation significantly increased ABA content by 19% and 15%, respectively. Two-way ANOVA showed that AMF had significant effects on ZR and GA contents, UPB had a highly significant effect on IAA content, and AMF and UPB interactions were significant or highly significant for IAA and ABA contents. In soil culture, compared with CK, single AMF inoculation significantly increased IAA, ABA, and GA contents by 20%, 7%, and 15%, respectively; single UPB inoculation significantly increased IAA, ZR, and GA contents by 4%, 5%, and 2%, respectively; and dual inoculation (BF) significantly increased IAA, ABA, and ZR contents by 13%, 3%, and 6%, respectively. Two-way ANOVA showed that AMF had significant effects on IAA, ABA, and GA contents, UPB had highly significant effects on ABA, ZR, and GA contents, and AMF and UPB interactions were highly significant for IAA and GA contents (Fig. 9).

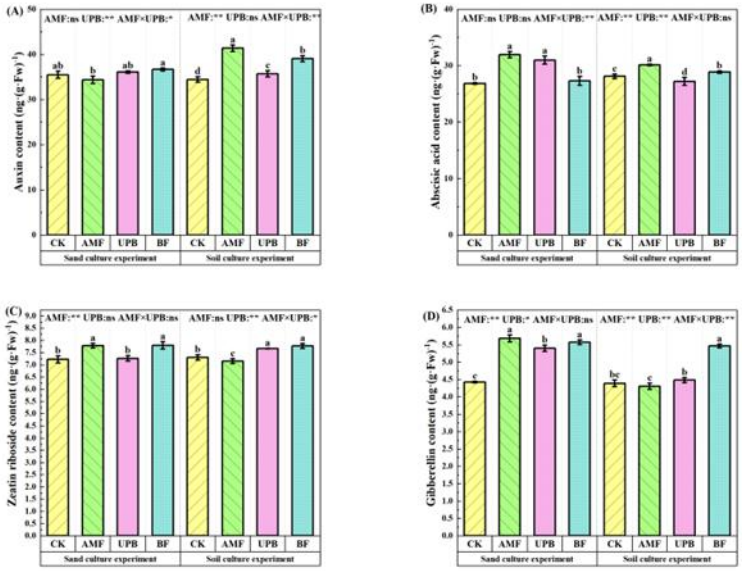


Fig. 9 Effects of AMF and UPB inoculation on shoot endogenous hormone contents in maize (A: indole-3-acetic acid (IAA) content; B: abscisic acid (ABA) content; C: zeatin (ZR) content; D: gibberellin (GA) content).

Regarding maize root hormones, in sand culture, compared with CK, single AMF inoculation, single UPB inoculation, and dual inoculation (BF) significantly increased IAA, ABA, and GA contents; the increases were 7%, 15%, and 19% for AMF; 3%, 4%, and 18% for UPB; and 28%, 22%, and 26% for BF, respectively. Two-way ANOVA showed that AMF had highly significant effects on IAA, ABA, ZR, and GA contents, UPB had highly significant effects on IAA, ABA, and GA contents, and AMF and UPB interactions were highly significant for ABA and GA contents. In soil culture, compared with CK, single AMF inoculation, single UPB inoculation, and dual inoculation (BF) significantly increased IAA and ABA contents, with increases of 19% and 5% for AMF, 27% and 12% for UPB, and 18% and 3% for BF, respectively; dual inoculation (BF) significantly increased GA content by 3%. Two-way ANOVA showed that AMF and UPB had highly significant effects on ABA, ZR, and GA contents, and AMF and UPB interactions were highly significant for ABA, ZR, and GA contents (Fig. 10).

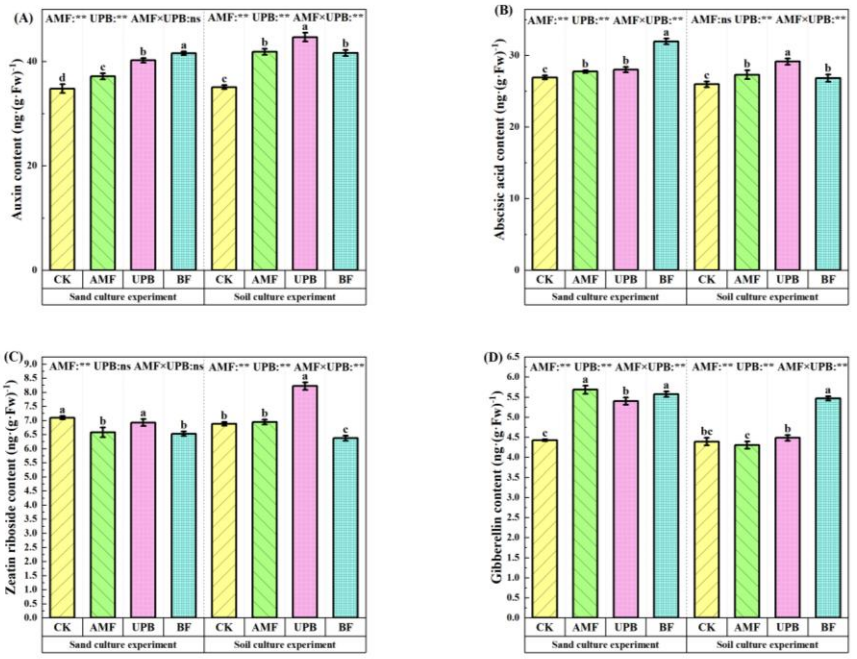


Fig. 10 Effects of AMF and UPB inoculation on root endogenous hormone contents in maize (A: indole-3-acetic acid (IAA) content; B: abscisic acid (ABA) content; C: zeatin (ZR) content; D: gibberellin (GA) content).

2.6 Effects of AMF and UPB on Cd Concentration and Uptake in Maize

Inoculation treatments regulated maize Cd content and uptake with pronounced tissue specificity and substrate dependence. Regarding Cd content, in sand culture, compared with CK, single AMF inoculation significantly reduced shoot Cd content by 57%, while dual inoculation (BF) significantly increased shoot and belowground Cd uptake by 280% and 77%, respectively. In soil culture, compared with CK, single AMF inoculation and dual inoculation (BF) significantly reduced shoot Cd content by 20% and 16%, respectively, and AMF inoculation significantly increased belowground Cd uptake by 31% (Fig. 11, Fig. 12).

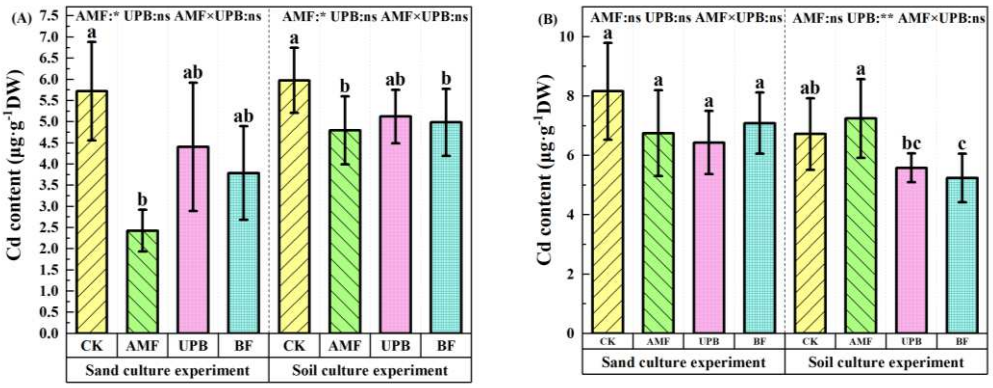


Fig. 11 Effects of AMF and UPB inoculation on Cd concentration in maize (A: shoot Cd concentration; B: root Cd concentration).

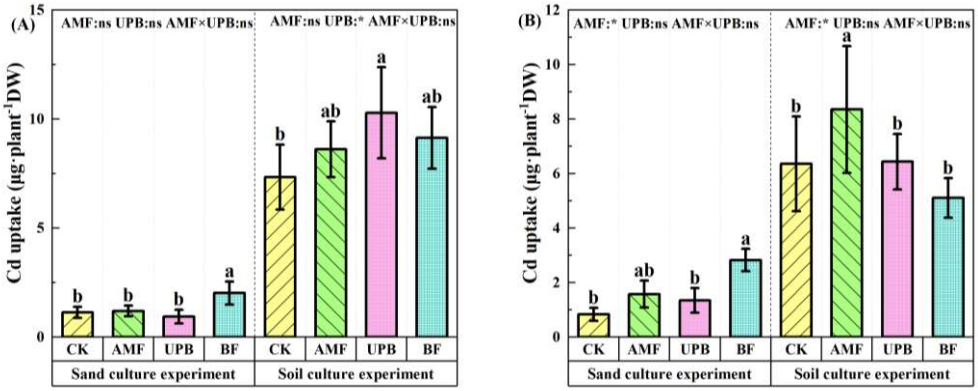


Fig. 12 Effects of AMF and UPB inoculation on Cd uptake in maize (A: shoot Cd uptake; B: root Cd uptake).

2.7 Correlation Analysis and Multivariate Path Analysis

Pearson correlation analysis revealed intrinsic associations among maize growth, root endogenous hormones, root antioxidant enzyme activities, and Cd uptake; these correlation patterns differed between sand and soil cultures. In sand culture with AMF and UPB inoculation, plant height showed highly significant positive correlations with belowground IAA, ABA, and GA contents and significant positive correlations with belowground SOD, POD, and CAT activities. Shoot biomass showed highly significant positive correlations with belowground IAA and ZR and significant correlations with SOD and POD activities. Belowground biomass showed highly significant positive correlations with IAA, ABA, and GA contents and SOD and CAT activities. Belowground Cd content showed highly significant negative correlations with IAA, ABA, and GA contents and SOD, POD, and CAT activities. Shoot Cd uptake showed highly significant positive correlations with IAA and ABA contents and SOD, POD, and CAT activities. Belowground Cd uptake showed significant or highly significant positive correlations with ZR and GA contents and SOD, POD, and CAT activities (The correlation analysis is shown in Additional file 1: Fig. S1). In soil culture, root endogenous hormones and leaf antioxidant enzyme activities showed highly significant positive correlations with plant height and shoot and belowground biomass, but highly significant negative correlations with shoot Cd content and total plant Cd uptake. MDA content showed a significant negative correlation with plant growth indicators and a significant positive correlation with Cd uptake. In addition, photosynthetic parameters were significantly correlated with biomass, Cd uptake, and antioxidant system indicators (The correlation analysis is shown in Additional file 1: Fig. S2).

Partial least squares structural equation modeling (PLS-SEM) was used to further analyze the path relationships among variables (Fig. 13). The overall goodness-of-fit (GOF) values were 0.654 (sand culture) and 0.670 (soil culture), both indicating acceptable model fit. The R^2 values for all endogenous latent variables were within a reasonable range, indicating good explanatory power for the observed data. In sand culture (Fig. 13A), path analysis indicated that AMF and UPB treatments exerted a significant direct negative effect on endogenous hormones (path coefficient = 0.860, $p < 0.01$). Photosynthesis exerted a significant direct negative effect on Cd content (path coefficient = 1.118, $p < 0.05$). Regarding measurement model loadings, the UPB loading (1.427) was markedly higher than the AMF loading (0.898), indicating a greater contribution of UPB to the treatment latent variable in sand culture. These path associations suggest that in sand culture, AMF and UPB treatments mainly regulated endogenous hormone levels, thereby affecting photosynthesis and significantly reducing plant Cd content; the antioxidant system showed a potential positive trend toward biomass, whereas Cd uptake showed a

potential inhibitory effect on biomass. In soil culture (Fig. 13B), path analysis indicated that AMF and UPB treatments exerted a significant direct positive effect on endogenous hormones (path coefficient = 0.749, $p < 0.01$) and a significant direct negative effect on antioxidant defense (path coefficient = 0.890, $p < 0.01$); endogenous hormones exerted a significant direct positive effect on photosynthesis (path coefficient = 0.814, $p < 0.01$). Both AMF and UPB treatments and endogenous hormones significantly affected Cd content, with treatments exerting a significant direct negative effect on Cd content (path coefficient = 1.068, $p < 0.01$) and endogenous hormones exerting a significant direct positive effect on Cd content (path coefficient = 1.319, $p < 0.01$). Notably, the direct path from endogenous hormones to Cd content was positive, indicating that hormones themselves did not directly reduce Cd content; instead, they mainly promoted photosynthesis and biomass accumulation, thereby reducing Cd concentration per unit biomass through the growth dilution effect. The negative direct effect of treatments on Cd content was the main pathway for Cd reduction in soil culture. Regarding the measurement model, the loadings of AMF (0.617) and UPB (−0.478) were relatively high. These path associations suggest that under soil culture conditions, AMF and UPB treatments not only exerted direct negative effects on Cd content but also positively regulated photosynthesis through endogenous hormones, indirectly reducing Cd concentration per unit biomass through photosynthetic improvement and growth dilution.

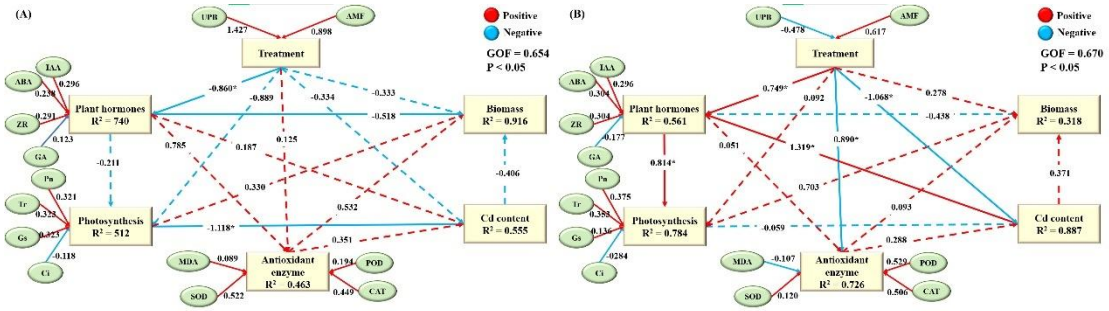


Fig. 13 PLS-SEM path analysis of maize Cd tolerance regulated by AMF and UPB.
(A) Path model under sand culture conditions (GOF = 0.654); (B) path model under soil culture conditions (GOF = 0.670).

3 Discussion

3.1 Effects of AMF and UPB on Maize Growth

Soil Cd stress inhibits crop growth by damaging root structure and limiting nutrient uptake [33], whereas plants can alleviate heavy-metal stress and promote growth by recruiting beneficial fungi and bacteria [34]. In this study, pot experiments under sand and soil culture showed that inoculation with AMF and UPB promoted maize growth; however, the magnitude of growth promotion, dominant microorganisms, and interaction patterns were strongly substrate-dependent.

In sand culture, single AMF inoculation showed a more pronounced improvement in root morphological parameters, whereas dual inoculation (BF) was generally optimal for plant height, shoot biomass, and belowground biomass. Two-way ANOVA showed that both AMF and UPB had highly significant effects on plant height but no interaction effect, whereas significant or highly significant interactions were found for root length, root surface area, and average root diameter. This indicates that the benefits of dual inoculation in sand culture were not simply additive across all growth parameters but were mainly manifested as a synergy between root architecture optimization and biomass uptake. The sand culture substrate is extremely nutrient-poor with weak buffering capacity. AMF effectively

mobilizes and transports mineral nutrients by extensively expanding the extraradical hyphal network, which is key to improving nutrient supply, promoting root architecture optimization, and enhancing shoot growth in maize [35,36]. Although UPB can secrete IAA to directly promote plant growth [14], it lacks the ability to supply nutrients via hyphae and cannot overcome the fundamental nutrient limitation in sand culture; therefore, its growth-promoting effect is limited when inoculated alone. Dual inoculation (BF) showed superior growth-promoting effects, and its mechanism involves functional synergy between the two microorganisms: AMF hyphae provide more colonization sites for UPB [37], while IAA secreted by UPB promotes fine root formation [38], forming a positive feedback loop under low-nutrient sand culture conditions. In addition, UPB hydrolyzes urea via urease, thereby increasing pH and releasing ammonium nitrogen in the rhizosphere microdomain [39]; under weakly buffered sand culture conditions, this local detoxification and nitrogen-supply effect is more effective, further creating a suitable microenvironment for AMF hyphal expansion. Similar synergistic yield-enhancing effects have been reported in other symbiotic systems; for example, co-inoculation with AMF and rhizobia greatly increased alfalfa biomass under low-nutrient conditions [40], and co-inoculation with AMF and Pseudomonas fluorescens increased plant height by more than 50% [41].

In soil culture, the growth-promoting pattern shifted. Single UPB inoculation had the most pronounced effect on plant height, and all three inoculation treatments significantly increased shoot biomass; however, dual inoculation did not show additional growth beyond single inoculation. Although two-way ANOVA indicated a highly significant AMF and UPB interaction for shoot biomass, this interaction did not translate into a positive gain from dual inoculation, suggesting that their functions may overlap under soil conditions. Soil has relatively high organic matter content and nutrient buffering capacity, providing a more suitable colonization and metabolic environment for UPB; its urease activity and sustained secretion of hormones such as IAA and ZR can be fully expressed, directly driving stem and leaf elongation and dry matter uptake by simultaneously supplying ammonium nitrogen and hormone signals [42]. Although AMF did not significantly alter root morphology in soil culture, it still improved nutrient uptake through its hyphal network and steadily increased shoot biomass. The absence of the explosive growth observed with dual inoculation in sand culture can be attributed to the stronger buffering capacity of soil, which attenuated the local detoxification effect of UPB observed in sand culture, and to the higher nutrient background, which narrowed the complementary space between AMF and UPB in nutrient supply. This also indicates that in relatively resource-rich soil, UPB can already exert near-optimal growth-promoting functions, with limited additional contribution from AMF.

The differences in growth-promoting effects between the two substrates may arise from differences in nutrient stress intensity and microbial functional expression. Sand culture is nutrient-poor and weakly buffered; AMF-mediated nutrient supply via hyphae is a prerequisite for growth recovery, and UPB-mediated detoxification and nitrogen supply are highly effective at the microdomain scale, making co-inoculation more effective. In soil culture, where nutrient buffering is strong, the nutrient-supplying role of AMF becomes secondary, whereas the nitrogen supply and hormone regulation functions of UPB are fully expressed and become the dominant factors promoting plant height and biomass uptake. Moreover, in sand culture, plants allocate more photosynthates to roots, providing a structural basis for AMF to reshape root architecture; in soil culture, root extension is constrained by physical impedance, and more resources are allocated to shoots, so inoculation effects are mainly manifested as increased shoot biomass. Integrating the results of this study on photosynthesis, mineral nutrition, endogenous hormones, antioxidant enzymes, Cd uptake, and path analysis, the regulation of maize growth by AMF and UPB is not merely a single-nutrient improvement but involves multi-pathway synergy among hormone signaling,

photosynthetic performance, antioxidant defense, and Cd uptake. These pathways differ between sand and soil cultures, further supporting the substrate dependence and functional complementarity of their growth-promoting effects.

3.2 Synergistic Regulation of Hormone Balance, Photosynthetic Efficiency, and Antioxidant Defense in Maize by AMF and UPB

Under Cd stress, endogenous hormones act as signaling molecules mediating the balance between growth and defense [43]. UPB can synthesize hormones such as IAA [39], whereas AMF regulates the levels of gibberellin (GA), abscisic acid (ABA), and other hormones in plants [44]. In this study, inoculation with AMF, UPB, and dual inoculation (BF) increased IAA, ABA, GA, and ZR contents in maize leaves and roots to varying degrees under both substrates. These hormone levels showed highly significant positive correlations with plant height and biomass, but the hormone regulation patterns differed markedly between the two substrates.

In sand culture, AMF and UPB showed significant or highly significant interactions for hormone indices such as ABA, ZR, and GA, and root IAA, ABA, and GA responded particularly strongly to inoculation treatments. This indicates that in the extremely nutrient-poor and weakly buffered sand culture environment, GA synthesis predominantly driven by AMF helps maintain cell division and root elongation [45]; AMF-induced ABA uptake can regulate stomatal aperture to cope with water and nutrient stress [46]; and IAA secreted by UPB enhances fine root formation [14]. Together, these effects amplify the growth-promoting actions of hormones. Correlation analysis further showed that in sand culture, root IAA, ABA, and GA displayed highly significant positive correlations with plant height, belowground biomass, and antioxidant enzyme activities, and highly significant negative correlations with belowground Cd content, indicating that hormone balance is tightly coupled with growth promotion and Cd uptake regulation in sand culture.

In soil culture, single inoculation with AMF or UPB and dual inoculation (BF) increased IAA and ABA levels, with interactions primarily observed for IAA and GA or for ABA, ZR, and GA. This indicates that under soil conditions with higher organic matter and nutrient buffering capacity, the nitrogen supply and hormone secretion functions of UPB were fully expressed, and sustained IAA and GA uptake was more conducive to stem and leaf elongation [43]. AMF improved root vitality and nutrient uptake, providing a material basis for plant responses to hormone signals. Notably, root endogenous hormones showed highly significant positive correlations with plant height and shoot and belowground biomass, but highly significant negative correlations with shoot Cd content and total plant Cd uptake, indicating that in soil, the hormone network tended to alleviate stress by maintaining growth and reducing Cd translocation to shoots.

Regarding photosynthetic physiology, previous studies have confirmed that AMF and beneficial bacteria enhance photosynthetic rate under Cd stress [47]. Our results are consistent with these findings, but the modes of enhancement differ by substrate. In sand culture, dual inoculation (BF) significantly increased stomatal conductance, and AMF and UPB showed significant interactions for transpiration rate, stomatal conductance, and intercellular CO_2 concentration, indicating that the co-inoculation effect in sand culture mainly involved improved stomatal behavior and gas exchange. The AMF hyphal network improved water and mineral nutrient uptake and maintained photosynthetic system integrity [48], while UPB hydrolyzed urea via urease to release ammonium nitrogen [15], delayed Cd-induced chlorophyll degradation, and secreted IAA to promote root water uptake, thereby indirectly maintaining stomatal opening [49]. In soil culture, single UPB inoculation showed a more pronounced promotion of

photosynthetic rate and stomatal conductance while reducing Ci, whereas dual inoculation (BF) increased Gs and decreased Tr. AMF and UPB showed highly significant interactions for Pn, Tr, and Ci, indicating that in soil, the direct promotion of carbon assimilation by UPB was more evident, whereas dual inoculation improved water use efficiency mainly by optimizing stomatal behavior and reducing transpiration. PLS-SEM path analysis further showed that in soil culture, endogenous hormones had a significant positive direct effect on photosynthesis, indicating that hormone-mediated photosynthetic regulation was more pronounced in soil. In sand culture, treatments mainly showed marginal direct effects on photosynthesis, whereas photosynthesis was significantly negatively associated with Cd content, indicating that improved photosynthesis in sand culture contributed to reduced plant Cd uptake.

The antioxidant enzyme system is a core defense against reactive oxygen species and a key mechanism for maintaining cell integrity [50]. In this study, all inoculation treatments reduced MDA content in leaves and roots and substantially increased SOD, POD, and CAT activities, but the activation patterns differed between the two substrates. In sand culture, leaf SOD and POD activities were significantly increased, and root SOD, POD, and CAT activities were comprehensively enhanced, with significant AMF and UPB interactions for POD activity, indicating that plants in sand culture experienced more direct Cd stress and that rapid, comprehensive activation of antioxidant enzymes served as the primary defense against oxidative damage. In soil culture, SOD, POD, and CAT activities in leaves and roots also increased substantially, and AMF and UPB showed highly significant interactions for all three root enzymes. AMF reduced the rate of reactive oxygen species production by improving mineral nutrition [51], while UPB may upregulate antioxidant enzyme gene expression [37,52]; together, they strengthened antioxidant defense in the stable soil environment. Correlation analysis further showed that in soil culture, leaf antioxidant enzyme activities showed highly significant positive correlations with plant height and shoot and belowground biomass, but highly significant negative correlations with shoot Cd content and total plant Cd uptake. MDA content was significantly negatively correlated with growth indicators and significantly positively correlated with Cd uptake, indicating that enhanced antioxidant defense not only alleviated membrane lipid peroxidation but was also closely associated with reduced Cd uptake and maintained growth. PLS-SEM further confirmed the differences in regulatory pathways between the two substrates: in sand culture, treatments mainly exerted direct regulation on hormone balance and photosynthesis; photosynthesis was negatively associated with Cd content; antioxidant defense showed a positive trend toward biomass uptake; and Cd uptake had a potential inhibitory effect on biomass. In soil culture, treatments had a positive direct effect on endogenous hormones, which in turn positively affected photosynthesis and participated in Cd uptake regulation, while treatments also exerted direct effects on the antioxidant system.

The fundamental differences in regulatory patterns between the two substrates arise from shifts in nutrient stress intensity and the relative weighting of microbial functions. In nutrient-poor, weakly buffered sand culture, hyphal nutrient supply and local detoxification act synergistically to drive rapid recovery of hormone levels, photosynthesis, and antioxidant defense. In contrast, the stronger nutrient buffering capacity of soil culture attenuates the photosynthetic enhancement by AMF; nevertheless, in this stable environment, UPB-dominated hormonal regulation and combined antioxidant defense can still reliably sustain growth and reduce Cd uptake.

3.3 Synergistic Regulation of Cd Uptake, and Translocation in Maize by AMF and UPB

Under Cd-contaminated conditions, Cd uptake and translocation by maize directly determine toxicity severity, and beneficial rhizosphere microorganisms can alleviate toxicity by altering Cd bioavailability

and root uptake and translocation efficiency [53,54]. Previous studies have shown that AMF enhance plant tolerance to heavy metals by immobilizing Cd in mycorrhizal structures and limiting its translocation to shoots [55,56], whereas UPB reduce Cd bioavailability and mobility through biomineralization and biosorption [57,58]. This study found that the regulatory patterns of AMF and UPB on Cd uptake in maize differed fundamentally between the two substrates.

In sand culture, single AMF inoculation significantly reduced maize shoot Cd content, confirming that its function of restricting Cd translocation to shoots was stably expressed in sand culture. Dual inoculation (BF), however, significantly increased both shoot and belowground Cd uptake. This is not contradictory: co-inoculation greatly promoted maize biomass, and with increased transpiration and root absorption area, the total amount of Cd passively entering the plant along with water and nutrient uptake inevitably increased, leading to a higher absolute value of Cd uptake. However, because the increase in biomass far exceeded that in Cd uptake, Cd concentration per unit biomass did not increase correspondingly, and Cd toxicity per unit plant was alleviated by the dilution effect. At the same time, Cd adsorption by AMF hyphae and compartmentalized storage of Cd in root cortical cells [59,60] remained effective, retaining large amounts of Cd in roots and limiting its long-distance translocation to shoots; therefore, shoot Cd content maintained a decreasing trend. When UPB was inoculated alone in sand culture, its effect on Cd content was limited, possibly because the sand culture system lacks soil organic matter and clay minerals. Under such conditions, the ability of UPB to reduce Cd availability by hydrolyzing urea via urease and increasing pH was markedly suppressed, as the sand culture system lacks the microenvironment required for ion exchange and precipitation reactions in soil, and carbonate ions produced by UPB metabolism can hardly form stable precipitates with Cd^{2+}.

In soil culture, inoculation effects differed: belowground Cd content was lower under single UPB inoculation and dual inoculation (BF) than under CK, indicating that the function of UPB in reducing Cd availability was effectively manifested in the soil environment. UPB hydrolyzes urea via urease, thereby increasing pH and releasing ammonium nitrogen in the rhizosphere microdomain [15]. The higher pH promoted the adsorption and immobilization of Cd^{2+} by soil colloids, while CO_3^{2-} reacted with Cd^{2+} to form sparingly soluble $CdCO_3$ precipitates, reducing the total amount of exchangeable Cd available for root uptake [57] and thus decreasing belowground Cd content. Single AMF inoculation increased belowground Cd uptake, which was related to its promotion of root growth and expansion of root contact area with contaminated soil, thereby increasing the total amount of Cd entering the roots; however, AMF simultaneously restricted most Cd to the roots through hyphal adsorption and root compartmentalization [61], so shoot Cd content still decreased significantly. The rhizosphere Cd-reducing effectiveness of UPB and the root retention and translocation-blocking functions of AMF formed a relay-based control in soil culture, resulting in significantly reduced shoot Cd content under both treatments.

Correlation analysis and PLS-SEM path results further revealed this substrate-dependent regulatory network. In sand culture, belowground Cd content was highly significantly negatively correlated with root IAA, ABA, and GA contents and SOD, POD, and CAT activities, whereas shoot Cd uptake was highly significantly positively correlated with IAA and ABA contents and antioxidant enzyme activities. Path analysis indicated that treatments affected photosynthesis by regulating endogenous hormones; photosynthesis had a significant negative direct effect on Cd content; antioxidant defense showed a positive trend toward biomass uptake; Cd uptake had a potential inhibitory effect on biomass; and UPB contributed more to the treatment latent variable. In soil culture, root endogenous hormones and leaf antioxidant enzyme activities were highly significantly positively correlated with plant height and shoot and belowground biomass, but highly significantly negatively correlated with shoot Cd content and total

plant Cd uptake; MDA content was significantly negatively correlated with plant growth indicators and significantly positively correlated with Cd uptake. Path analysis indicated that treatments had a significant positive direct effect on endogenous hormones and a significant negative direct effect on antioxidant defense; endogenous hormones had a significant positive direct effect on photosynthesis; treatments had a significant negative direct effect on Cd content, whereas endogenous hormones had a significant positive direct effect on Cd content. These results suggest that Cd regulation in soil culture is influenced both by the direct effects of inoculation treatments and by the indirect effects of hormone-mediated growth dilution, photosynthetic improvement, and antioxidant defense.

The fundamental difference in Cd regulation between the two substrates lies in the substrate-dependent expression of the Cd-availability-reducing function of UPB. Sand culture is nutrient-poor and lacks buffering capacity, which restricts UPB metabolic activity; consequently, Cd regulation relies mainly on AMF-mediated Cd immobilization and translocation restriction, as well as the growth dilution effect. In contrast, soil culture provides suitable metabolic conditions for UPB, allowing its Cd-availability-reducing function to be realized; together with AMF, UPB thereby achieves more effective alleviation of Cd toxicity.

This study was based on pot experiments; the pot and sterilized-soil conditions eliminated competition from indigenous microorganisms and differed from field conditions, so the inoculation effects may have been amplified. Only a single Cd concentration and a single cultivar were used, without covering dose effects or genetic variation. The growth period was only 50 d, and grain Cd content was not measured; therefore, the results cannot yet directly support conclusions regarding safe production. Rhizosphere pH, Cd speciation, urease activity, hyphal density, and other microbial functional indicators were not measured; consequently, the mechanisms of UPB-mediated mineralization/immobilization and AMF hyphal retention were mainly inferred indirectly. Sand culture and soil culture differed simultaneously in multiple factors, making it impossible to distinguish the contribution of each factor separately. In addition, no inactivated-microbial control was included, and the sample size for PLS-SEM was limited. Future studies should conduct field experiments with multiple Cd concentrations, multiple cultivars, and the full growth period, combined with Cd speciation, microbial functional gene analyses, and transcriptome analyses, to further verify the Cd-reducing effects and mechanisms of co-inoculation.

4 Conclusions

Under Cd stress, the regulation of maize growth, physiology, and Cd accumulation by AMF and UPB co-inoculation was markedly dependent on the indicator and substrate type. In sand culture, co-inoculation mainly improved photosynthetic performance through endogenous hormone and photosynthetic pathways and reduced shoot Cd content via AMF-mediated root retention and the growth dilution effect. In soil culture, inoculation treatments directly and negatively affected Cd content, while endogenous hormones mainly reduced Cd concentration per unit biomass indirectly by promoting photosynthesis and biomass accumulation; UPB-mediated reduction of rhizosphere Cd availability and AMF-mediated restriction of Cd translocation acted together. Overall, co-inoculation enhanced maize Cd tolerance by improving photosynthetic physiology, modulating hormone balance, and strengthening antioxidant defense; however, these effects manifested as reduced Cd concentration per unit biomass and improved growth rather than reduced total Cd accumulation, and they varied with substrate type. Future studies should integrate full-growth-period field experiments with grain Cd measurements to further elucidate the signaling mechanisms of AMF–UPB interactions and the regulatory pathways of Cd translocation.

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Author Contributions

Yuanyuan Shan: Conceptualization, Data curation, Methodology, Formal analysis, Validation, Writing-Original Draft, Writing-Review & Editing, Visualization. **Xiaoyi Li**: Validation, Investigation, Data curation. **Qian Yang**: Data curation, Visualization, Software. **Haitao Li**: Resources, Visualization, Validation. **Zhaodi Wang**: Methodology, Formal analysis, Software. **Lei Wang**: Resources, Formal analysis, Project administration. **Fangdong Zhan**: Writing-Review & Editing, Supervision, Project administration, Funding acquisition. **Yunfeng Pan**: Conceptualization, Writing-Review & Editing, Supervision, Project administration, Funding acquisition. **Yongmei He**: Writing-Review & Editing, Supervision, Project administration, Funding acquisition.

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Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed at the corresponding authors.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests

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